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# Endocrine influence on protein synthesis in the fatbodies of female red cotton bug, *Dysdercus cingulatus* Fabr

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**Abstract.** Extirpation of pars intercerebralis neurosecretory cells (PINSC) of female *D. cingulatus* significantly brought down the level of protein synthesis in the fatbodies 48, 72, and 96 hr after the operation, while implantation of active PINSC into both normal and PINSC-ablated females elevated substantially the protein content in the fatbodies. Additional supply of JHa (FME) by topical application activated protein synthesis in the fatbodies both in the allatectomised and normal females. Histochemical studies to demonstrate the protein content in the fatbodies of the above experimental insects also corroborated these findings. Probable regulatory mechanism of protein synthesis in the fatbodies of female *D. cingulatus* by the hormonal principles from PINSC and corpus allatum are discussed in the light of the above findings.

**Keywords.** *Dysdercus cingulatus*; protein; fatbody; neurosecretory cells; juvenile hormone.

## 1. Introduction

Fatbodies are the site where most of the haemolymph proteins and vitellogenin are synthesised and released in adult insects and thus it fulfils a variety of functions similar to the hepatopancreas of molluscs and crustaceans or the liver in mammals (Telfer 1965; Chen 1978; Keeley 1978). So neurohormonally dependent changes in the protein should reflect changes in the protein synthetic capacity of the fatbodies as well. As Engelmann (1979) suggests, one of the most exciting aspects and one which attracted increasing attention during the last few years, is the mechanism of control of vitellogenin biosynthesis. The synthesis of haemolymph proteins seems to be controlled, at least in part, by hormones of corpus allatum (Coles 1965a; Engelmann and Penney 1966; Lüscher 1968) and factors from the neurosecretory system (Hill 1962, 1965; Wyss-Huber and Lüscher 1966). The Ca-hormones are intimately involved in various phases of protein metabolism (Gilbert and Schneiderman 1961; Thomas and Nation 1966). Juvenile hormone is demonstrated to influence the synthesis of storage-proteins in the fatbody of *Bombyx mori* (Tojo *et al* 1981). In the American cockroach the RNA content of the fatbodies is cyclic in nature and the protein level is contributed by the fatbody (Mills *et al* 1966). In *Leucophaea maderae* fatbodies seem to react with an increased release of proteins to the changed hormonal environment during oocyte maturation (Wyss-Huber and Lüscher 1972). The brain hormone could be acting directly on the fatbody or through some other organ as the ovary itself (Hagedorn and Fallon 1973).

In the present paper, results of our experiments performed to elucidate the influence of hormonal principles from the pars intercerebralis neurosecretory cells (PINSC) and

hat from corpus allatum (JH) on the synthesis of fatbody proteins in the female red otton bug, *Dysdercus cingulatus* are presented.

## 2. Material and methods

### 2.1 *The animal*

The red cotton bug, *Dysdercus cingulatus* (Heteroptera: Pyrrhocoridae), was reared in the laboratory at 29 ∓ 3°C, r.h. 90 ∓ 3% and 12:12 LD regime. The insects were fed *ad libitum* on soaked cotton seeds. The newly emerged adults of both sexes were separated within an hour after emergence from the stock colony and fed as described earlier by Muraleedharan and Prabhu (1979) and adult females of appropriate age groups were selected from among them for experimentation.

### 2.2 *Surgical techniques*

All the instruments used for microsurgery were washed well in distilled water and sterilised in 70% ethyl alcohol. Surgical procedures for extirpation and implantation of PINSC and allatectomy were followed after Muraleedharan and Prabhu (1979, 1981). Adult donor females within 3 hr after emergence were used for extirpation of PINSC and newly emerged adults served as hosts. Sham-operated insects of corresponding age groups served as controls for each category. Pieces of gut tissue were implanted into the control instead of PINSC. Operated insects were disengaged from plasticine ribbons and after mopping off the Ringer solution sticking to them, a thin film of anti-septic powder consisting of penicillin, streptomycin and phenylthiourea in the ratio 1:1:2 was applied on the wound. Adult females, 24 hr after their emergence, were used for allatectomy. Twenty four hr after the operation such females were allowed to mix with young adult males for free mating.

### 2.3 *Protein estimation*

Fatbodies from different experimental insects were dissected out 48, 72 and 96 hr after each experimental manipulation. Pre-weighed specimen tubes containing 0·5 ml of isotonic potassium chloride solution were again weighed along with the fatbodies and the weight of fatbodies used for protein estimations were determined from the weight difference. Protein extract of fatbodies was prepared in isotonic KCl solution after homogenisation, precipitation with 10% TCA solution and subsequent centrifugation at 5000 g for 20 min. The residue dissolved in 1 ml of 0·1N NaOH served as the protein extract. Total proteins in the fatbodies were estimated according to the method of Lowry *et al* (1951), using phenol reagent of Folin-Ciocalteau. Bovine serum albumin (Sigma chemical Company, USA) was used as standard. Concentration of protein was expressed in $\mu$g protein/mg tissue. Mean values of 8 different determinations were adopted as the protein concentration in each group. Significance of the data were analysed employing student's $t$ test.

### 2.4 *Histochemistry*

For histochemical demonstration of proteins, the mercury bromophenol blue method (Pearse 1968) was followed using formalin-fixed fatbodies from different categories of experimental insects along with their respective controls.

### 2.5 *JHa treatment*

Farnesyl methyl ether (FME) (Econ. Control Inc., USA) was the juvenile hormone analogue (JHa) used. FME was dissolved in acetone for topical application; the concentration being 0·5 $\mu g/\mu l$ and 4 $\mu l$ (containing 2 $\mu g$) was applied to each animal (effective dose was determined in the preliminary experiments) with the aid of a calibrated microcapillary. FME dissolved in acetone was topically applied underneath the wings to mildly anaesthetised females. Controls were treated similarly with the same quantity of acetone.

## 3. Results

Protein concentration in the fatbodies was significantly lower in the PINSC-ablated females 48, 72 and 96 hr respectively after the operation than in the respective stages of the sham-operated control groups (figure 1). Implantation of active PINSC into normal females elevated the protein concentration to a significant level when compared with that of the operated controls ($P \leqslant 0{\cdot}01$). Substantial increase in the fatbody protein concentration was noticed in PINSC-ablated insects when they were implanted with active PINSC (figure 1). Histochemical observations corroborate these findings (figure 3; 1 to 4). Significant reduction in fatbody protein concentration was noticed in the allatectomised females as well when these were estimated 48, 72 and 96 hr after the operation ($P \leqslant 0{\cdot}05$). Topical application of FME (JHa) on the normal as well as allatectomised females (figure 2) enhanced protein concentrations to a significant level ($P \leqslant 0{\cdot}05$). However, the rise in protein concentration in allatectomised females was less than that in normal females supplied with additional JHa (figure 2). Histochemical investigations also corroborate these findings (figure 3; 5 to 8).

## 4. Discussion

Present studies show that in the female *D. cingulatus*, synthesis of protein is inhibited in the absence of PINSC while implantation of active PINSC into PINSC-ablated insects restores the level significantly; implantation into normal females enhances the protein level well above the normal level. The protocerebral neurosecretory cells have been reported to be indispensable for oogenesis in many insect species like *Calliphora erythrocephala* (Thomsen 1948, 1952; Possompes 1956), *Schistocerca gregaria* (Highnam 1962a, b, c; Hill 1962), *Schistocerca pararensis* (Strong 1965a, b), *Locusta migratoria* (Girardie 1966), *Anacardium aegyptium* (Geldiay 1967), *Dysdercus cingulatus* (Jalaja *et al* 1973). However, this does not necessarily mean that PINSC control egg maturation directly. In many insects, MNSC ablation from the brain results in a lowered

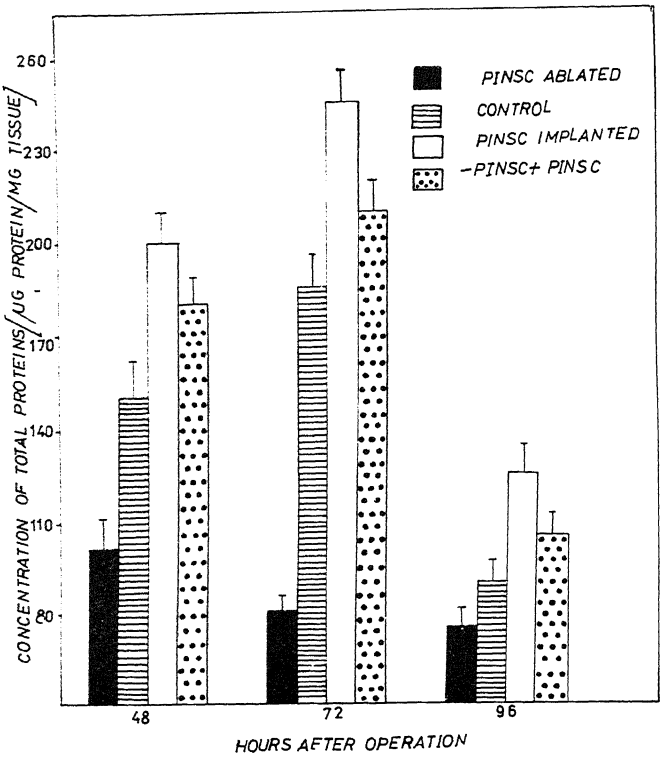


**Figure 1.** Histogram showing the effect of extirpation and implantation of PINSC on the protein content in the fatbodies of female *D. cingulatus*. Each column represents mean of 8 values and the bars denote ∓ SEM.

food intake as in *Calliphora erythrocephala* (Thomsen and Moller 1963), *D. cingulatus* (Muraleedharan and Prabhu 1979), *Hyblaea puera* (Muraleedharan and Prabhu 1981) resulting in a low protein concentration and the subsequent cessation of oocyte maturation. Therefore, the relationship between MNSC and oogenesis can only be studied properly if MNSC cauterisation has been carried out in such a way that it does not affect food intake. In the experiments performed on *Schistocerca gregaria* (Hill 1965) and *Leptinotarsa decemlineata* (de Loof and de Wilde 1970; de Loof and Lagasse 1970) this condition was fulfilled and the effect of MNSC on oogenesis was established. In *Schistocerca* this is explained by a direct effect on the synthesis of vitellogenic proteins and in *Leptinotarsa* by an effect partly on the fatbody in conjunction with the corpus allatum (vitellogenic protein synthesis) and partly *via* the corpus allatum on the terminal oocyte. Thomsen (1952) has shown that in *C. erythrocephala* females the extirpation of MNSC causes total exhaustion of the cells of fatbodies. The rate of protein synthesis in the fatbodies as well as the concentration of haemolymph proteins are reduced in pars intercerebralis-cauterised female locusts (Hill 1962, 1965). These findings by Hill corroborated a suggestion made earlier by Thomsen (1952) that a principle liberated from pars intercerebralis regulates protein metabolism. Supporting data for this may be found in the observation of low protein concentration in the haemolymph of brain operated females of *Gomphocerus rufus* (Loher 1965), *L. maderae* (Engelmann 1966; Engelmann and Penney 1966) and in young females of *T. molitor*

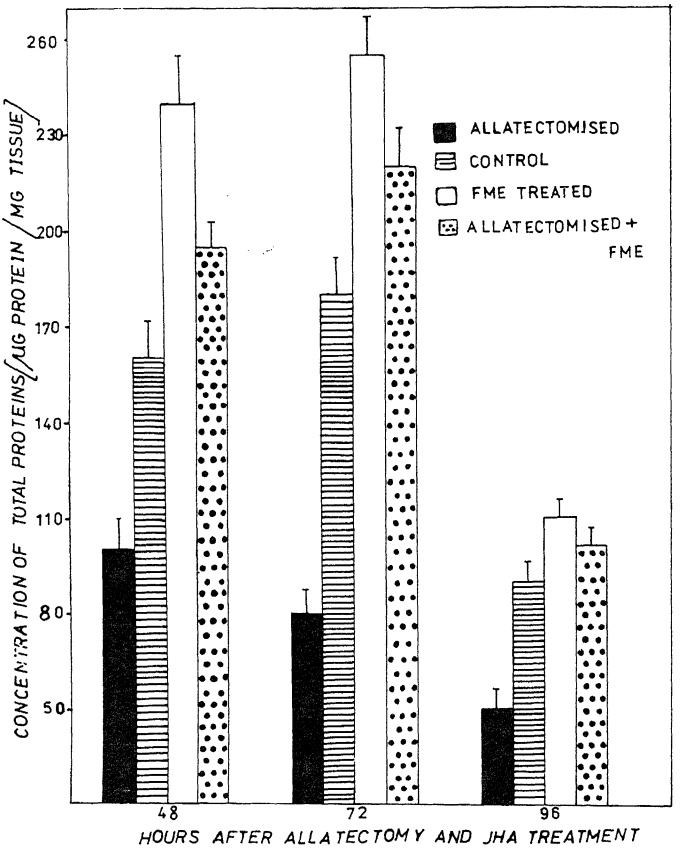


**Figure 2.** Histogram showing the effect of allatectomy and topical application of FME (JHa) on the protein content in the fatbodies of female *D. cingulatus*. Each column represents mean of 8 values and the bars denote ∓SEM.

(Mordue 1965). MNSC are known to stimulate protein synthesis in the fatbodies in many species of insects such as *S. gregaria* (Highnam *et al* 1963; Hill 1963), *M. sanguinipes* (Elliot and Gillot 1979). The content of the neurosecretory material in the MNSC of *D. cingulatus* increases steadily during the early days of the first gonotrophic cycle when active vitellogenesis is taking place and the protein build up in the haemolymph is under the control of neurosecretion (Jalaja and Prabhu 1977). A sudden decline in the haemolymph proteins is reported in the female *D. cingulatus* 72 hr after emergence which was suggested to be related to heavy yolk protein deposition in the ovaries (Jalaja and Prabhu 1971). The present studies also demonstrate a sudden decline in the protein concentration of fatbodies after 72 hr in the sham-operated controls of the PINSC-ablated insects. This indicates that the decline found in the control is due to vitellogenesis. So it is suggested that in *D. cingulatus* females hormones from PINSC stimulate protein synthesis in the fatbodies during vitellogenesis.

A highly reduced level of protein synthesis as noticed in the allatectomised females and its substantial increase when these insects are supplied with additional JHa and also the increase noticed in protein synthesis when normal females are supplied with extra titre of JH in the form of JHa, demonstrate that JH stimulates protein synthesis in the

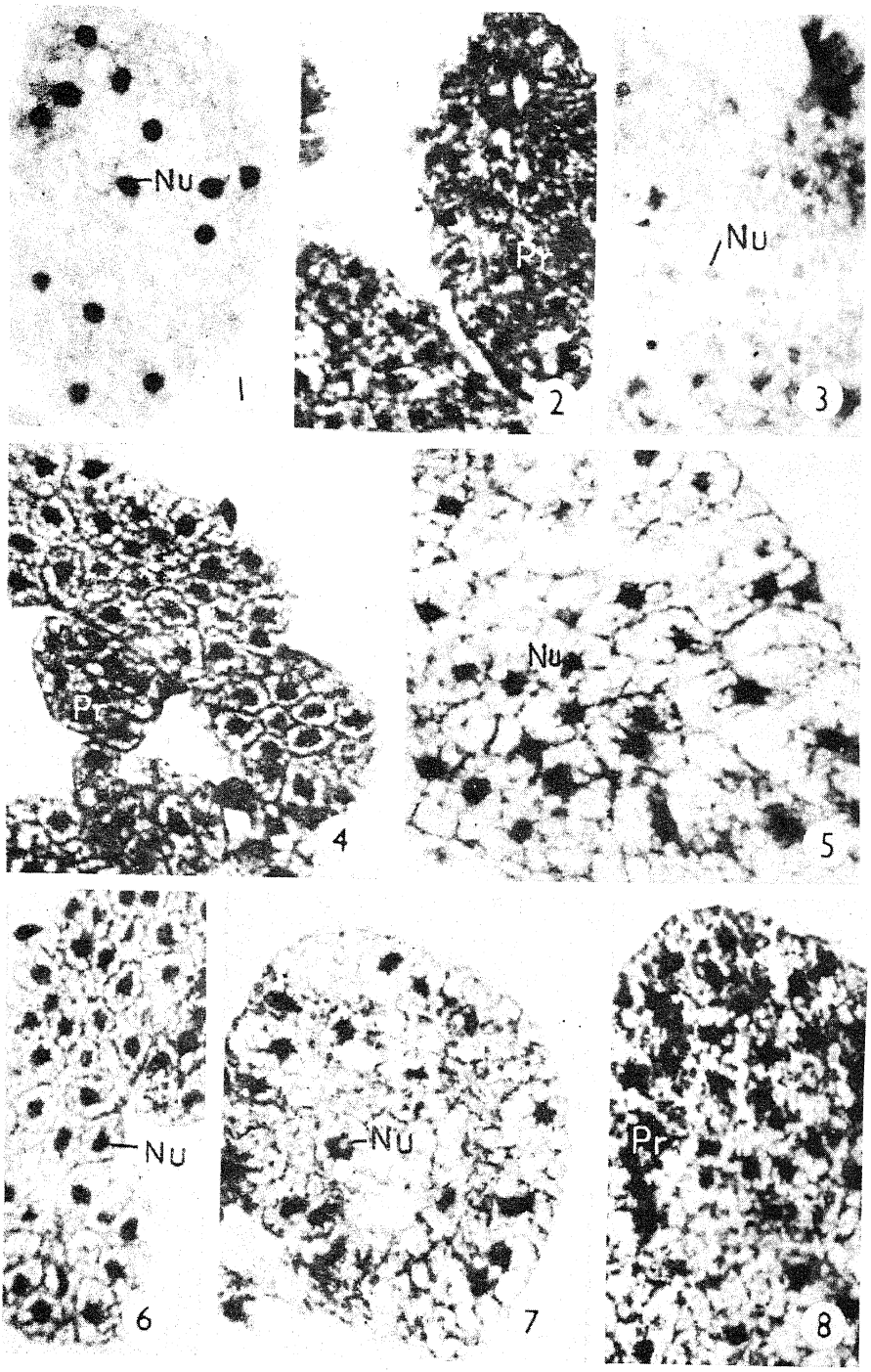


**Figure 3.** Sections of fatbodies fixed in formalin and stained for protein using mercury bromophenol blue technique (Pearse 1968). **1.** and **3.** 48 and 72 hr after PINSC ablation, **2.** and **4.** their respective controls. **5.** and **7.** 48 and 72 hr after allatectomy, **6.** and **8.** their respective controls. (Nu-Nucleus, Pr-protein) Magnifications of all figures are $\times 400$.

fatbodies of female *D. cingulatus*. The rate of incorporation of radioactive amino acids into fatbody proteins is reported to be significantly slow in allatectomised *P. americana* females (Thomas and Nation 1966). Implantation of active corpus allatum into decapitated *N. cinerea* is also reported to elevate the rate of protein synthesis in the fatbody (Lüscher 1968). However, allatectomised queens of *Apis mellifica* contain vitellogenin at a high titre (eventhough some what lower than in operated controls) and did even lay eggs when treated with $CO_2$ (Engels and Ramamurthy 1976). Repeated application of JH restored vitellogenin titres to those observed in normal queens (Ramamurthy and Engels 1977). JH stimulates the protein content in the fatbodies of a number of insects such as *Musca domestica*, *Locusta migratoria*, *Melanoplus sangunipes* and *Diatraea grandiosella* (Adams and Nelson 1969; Lauwerjat 1977; Elliot and Gillot 1978; Turnen and Chippendale 1980). Enlargement of nuclei and abundance of rough endoplasmic reticulum and golgi complexes were noticed in the fatbodies in connection with the progress of vitellogenesis during which proteins were synthesised in abundance in *L. migratoria* (Couble *et al* 1979). JH is involved in the synthesis of vitellogenins in *Rhodnius prolixus* (Coles 1964, 1965 a, b) and in *Sarcophaga bullata* (Wilkens 1969).

Muraleedharan and Prabhu (1981) have shown that in *D. cingulatus* allatectomy does not affect food consumption. So the decrease in the fatbody as noticed in the allatectomised insects cannot be attributed to deficiency of food. It was demonstrated by Jalaja and Prabhu (1977) that in *D. cingulatus* both MNC-hormone and JH are involved in vitellogenesis and MNC-hormone stimulates the process by influencing the production of JH by the corpus allatum. A neurosecretory influence is observed on the protein synthesis while a direct gonotrophic effect is with corpus allatum and a reciprocal relationship between the neurosecretory system and corpus allatum in which interference with one component of the neuroendocrine system results in interference with the other (Hill 1962; Highnam *et al* 1963).

In the light of the present findings and the pertinent available literature, it may be suggested that in adult females of *D. cingulatus* hormonal principles both from PINSC and corpus allatum have a stimulatory effect on the synthesis of proteins in the fatbody. The influence imparted by PINSC seems to be either through a trophic mechanism on the corpus allatum or by its direct effect on the fatbody while hormones from CA seem to stimulate the process directly.

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# Observations on the histology and histochemistry of *Penetrocephalus* plerocercoid (Pseudophyllidea: Cestoda)

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**Abstract.** The histology of plerocercoid of *Penetrocephalus* sp. reveal that the body surface consists of tegument, basement membrane, epidermal longitudinal musculature, parenchymatic longitudinal musculature, transverse and dorsoventral muscle fibres. Three types of glands could be recognized from the scolex of the plerocercoid. The musculature of the plerocercoid consists of glycogen, acid, sulfated, neutral and carboxylated mucopolysaccharides: basic proteins containing tyrosine, S-H and S-S groups, protein bound amino groups, sulfhydril groups, glycoprotein and lipid.

The frontal glands contain carbohydrates (1, 2 glycols, both acid and neutral mucopolysaccharides), basic protein (tyrosine, S-H group, protein bound amino group) and phospholipids. The structure, organization and histochemistry of the plerocercoid is discussed.

**Keywords.** Histology; histochemistry; *Penetrocephalus*; plerocercoid; cestoda.

## 1. Introduction

Hanumantha Rao (1960a) erected the genus *Penetrocephalus* for the form described by him earlier as *Bothriocephalus ganapatii* (Hanumantha Rao 1954), recovered from a teleost fish *Saurida tumbil* (Bloch). In his later paper (Hanumantha Rao 1960b) observations on histochemistry and egg formation were furnished. Rama Devi (1970) studied the histology and some aspects of histochemistry. But histochemical work on the plerocercoid of *Penetrocephalus* has not been carried out so far.

Histochemical investigation on pseudophyllidean cestodes probably started with the work of Takahashi (1959) on *Diphyllobothrium* (*Spirometra*) *mansoni* and then Arme (1966) on *Ligula intestinalis*. But little attention has been paid to larval stages. Ohman (1968) was the first to investigate the histochemistry of the larva of *D. detriticum* and concluded that one cannot rely solely on morphological descriptions to solve the taxonomic problems in *Diphyllobothrium*.

The present work deals with the histology and histochemistry of plerocercoid of *Penetrocephalus* collected from a number of teleost fishes.

## 2. Material and methods

Plerocercoids of *Penetrocephalus* were recovered from various locations in the body cavity of *Saurida tumbil* and 13 other species of teleosts, collected from the off-shore

fishing station, Visakhapatnam and from local fish markets. The larvae were fixed in alcoholic Bouin's, Susa, Carnoy and formal calcium, passed through grades of alcohol cleared, embedded in paraffin wax (m.p. 58°C) and sections cut at 10–12 $\mu$ thickness

Heidenhain's azan, and Mallory's triple stains were used for histological studies. The histochemical tests employed were periodic acid schiff (PAS) technique for carbohydrate containing groups, PAS saliva for glycogen, PAS after acetylation and deacetylation for 1 : 2 glycol groups. Alcian blue (AB) 1 pH and AB 2·5 pH for acid mucins, AB (1 pH)/PAS to detect sulphate free sialic acid containing mucins, AB (2·5 pH)/PAS to distinguish neutral from acid mucopolysaccharide and toluidine blue for the demonstration of acid mucopolysaccharides. Aldehyde fuchsin (AF)/AB was employed to distinguish between sulphated and non-sulphated mucosubstances and, to confirm the presence of sulphated mucins, AB/safranin was performed. For basic proteins, mercury bromo phenol blue was used and, potassium permanganate/alcian blue ($KMnO_4$/AB) for disulphide, ferric ferricyanide for sulfhydril group and Congo red for glycoprotein. To demonstrate lipids-Sudan black B and for phospholipids copper phthalocyanin techniques were employed. Most of the procedures for histochemical tests were adopted from Pearse (1968).

## 3. Observations

Live specimens were obtained from the stomach wall, liver, muscles and other regions of the alimentary canal of the hosts. The worms appeared flat, elongated and milky white in colour. The scolex was invaginated.

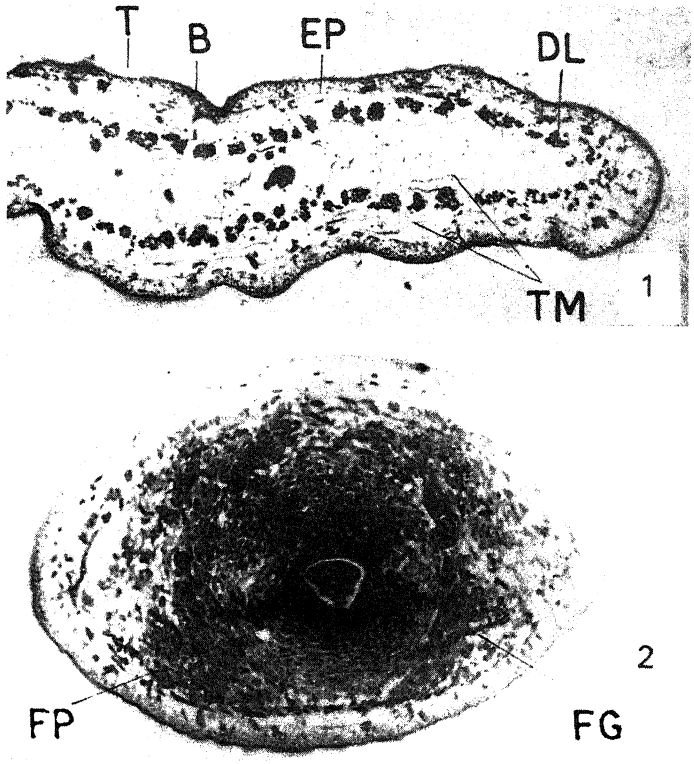
### 3.1 *Histology*

The outer layer of the larval worms of *Penetrocephalus* sp. is a thick tegument. It is smooth, but not uniform throughout the body. Beneath the tegument is the basement membrane. This layer separates the inner muscular layer. It is single layered with no vacuoles, reticulations etc. Below the basement membrane, lies the epidermal longitudinal musculature. The cells of this region are not widely spaced nor densely packed. A deeper parenchymal longitudinal musculature is also present. Transverse muscles are situated between two rows of longitudinal muscles (figure 1). They are also found in the central region running towards periphery, but are more distinct in the scolex.

In the invaginated anterior region of the larva, numerous frontal glands occur in the medular parenchyma. They are irregular and of various shapes. Three types of gland could be distinguished, though one type occupies the major part of the scolex. In the central region there is a frontal pit filled with secretions. The entire part is covered with thin fibrous parenchymal musculature (figure 2).

### 3.2 *Histochemistry*

The staining and histochemical reactions of the various anatomical regions especially the tegument and the frontal glands are summarized in table 1 and the reactions on the various regions are shown in figures 3–8.




**Figures 1–2.** **1.** Cross-section of the body showing general musculature (Azan). **2.** Cross-section through the scolex showing frontal glands (Azan).

From the ensemble of histochemical tests it could be stated that the tegument is charged with acid, sulfated, carboxylated mucopolysaccharides with hyaluronic acid, basic proteins containing tyrosine, S–S group, S–H group and lipids especially phospholipids.

The muscles display carbohydrate containing glycogen, proteins containing tyrosine, protein bound S–H and $NH_2$ groups and lipids.

The frontal glands contain carbohydrates (1, 2 glycol groups and both acid and neutral mucopolysaccharides), proteins (S–H, $NH_2$ groups and tyrosine) and phospholipids.

## 4. Discussion

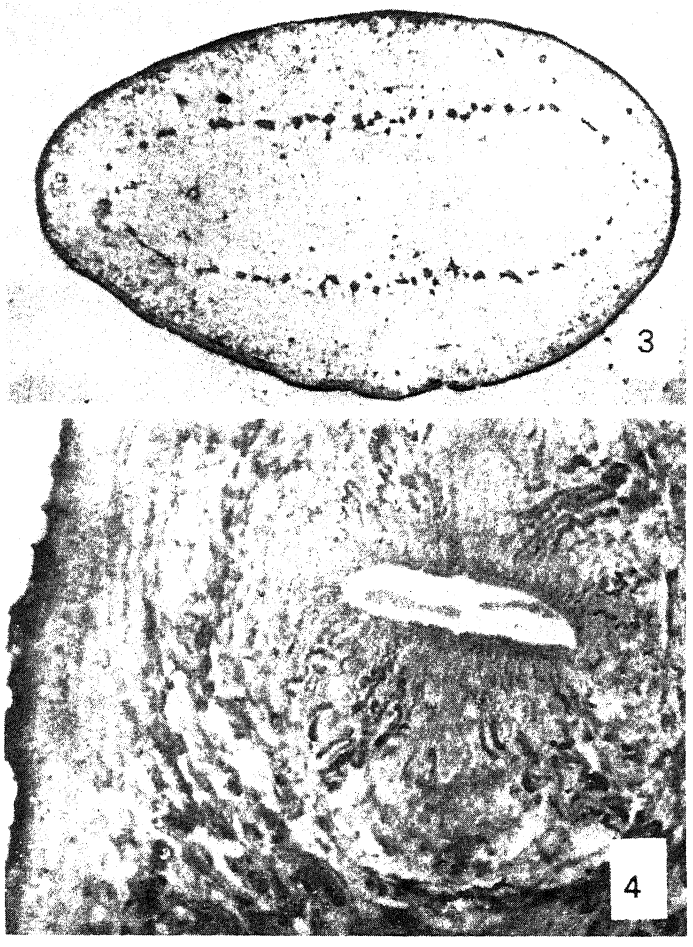
The results of the study on the histology and histochemistry of the plerocercoid of *Penetrocephalus* sp. agree to some extent with those obtained for the adult of *Penetrocephalus ganapatii.*

The histochemical composition of the tegument has been studied in detail, more in cyclophyllidean cestodes than in pseudophyllideans. Bogitsh (1963) found PAS positive material in the tegument of *Hymenolepis microstoma* and he stated that it could probably be a mucoprotein. Lumsden (1975) stated that the external limiting

**Table 1.** Results of histochemical reactions of *Penetrocephalus* plerocercoid.

| Histochemical tests applied | Tegument | Basement membrane | Epidermal longitudinal muscle | Parenchymal longitudinal muscle | Frontal glands |
|---|---|---|---|---|---|
| Periodic acid/Schiff (PAS) | ++ | + | ± | ++ | ++ |
| PAS/saliva | ± | − | ± | − | ++ |
| Acetylation | + | − | − | − | − |
| Deacetylation | ++ | − | − | − | ++ |
| Alcian blue (AB) 1 pH | ++ | − | − | − | + |
| AB 2·5 pH | +++ | − | − | − | + |
| AB 1 pH/PAS | Purple | − | − | − | Red |
| AB 2·5 pH/PAS | +++ Blue | − | − | − | Blue purple |
| Toluidine blue | γ-metachromasia | − | − | − | γ-metachromasia |
| Aldehyde fuchsin (AF)/AB | + Blue | − | − | − | Blue purple |
| AB/Safranin | Red | − | − | − | Red |
| Bromophenol blue | + | ++ | + | ++ | ++ |
| $KMnO_4$/AB | ++ | − | − | − | − |
| Ferric ferricyanide | ++ | ++ | ± | + | + |
| Congo red | +++ | ++ | + | +++ | +++ |
| Sudan black B | ++ | +++ | ++ | +++ | +++ |
| Copper phthalocyanin | ++ | ++ | + | +++ | ++ |

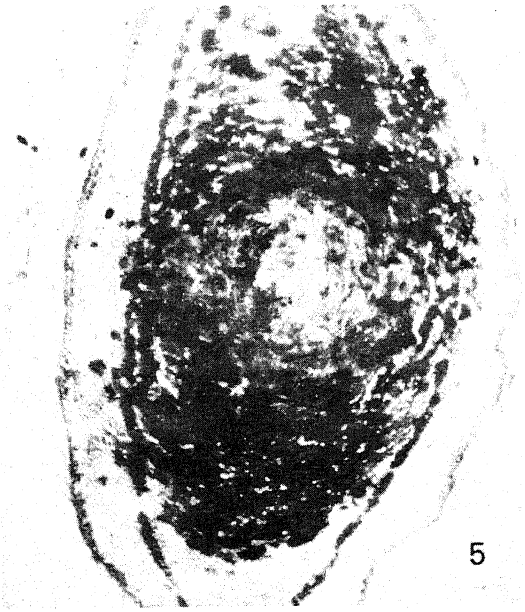
+++ intensely positive; ++ strongly positive; + moderately positive; ± faintly positive; − negative.

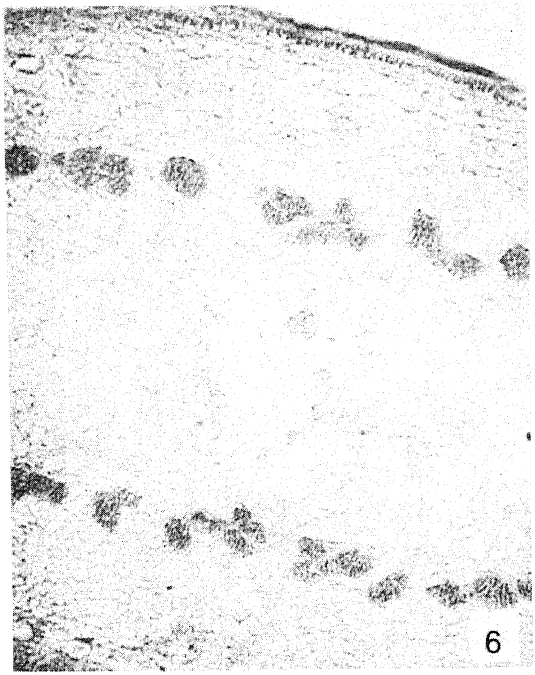



**Figures 3–4.** **3.** Tegument and the deeper longitudinal musculature showing positivity towards PAS. **4.** Frontal glands showing positivity towards PAS.

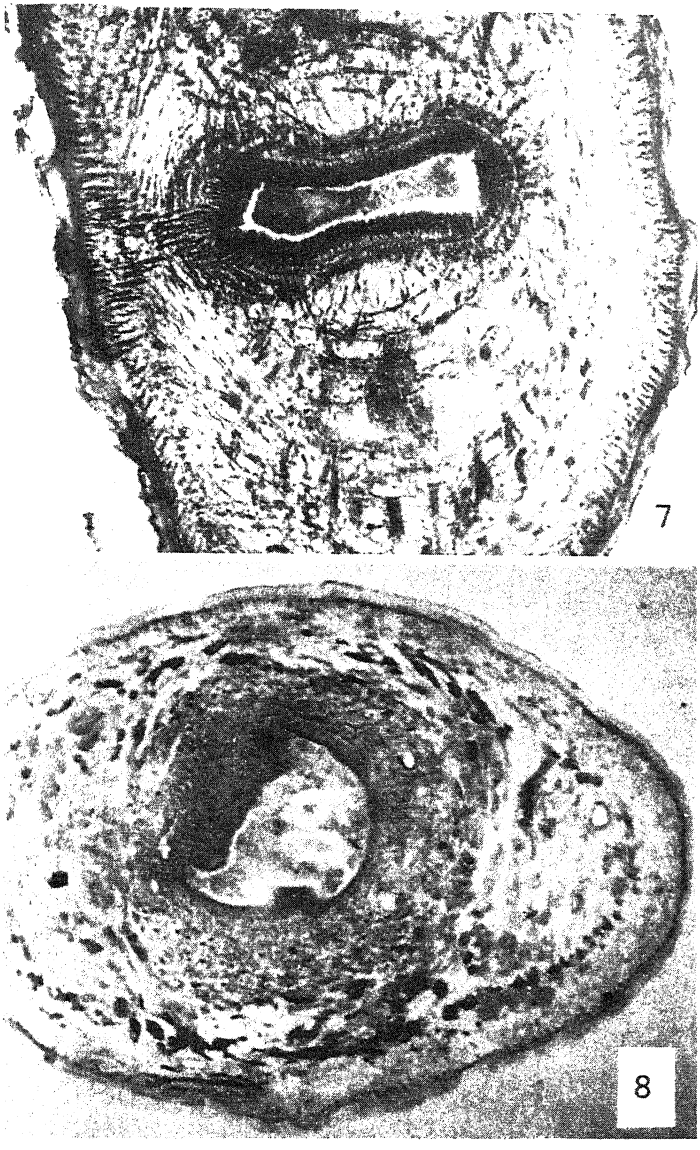
membrane is coated with a layer of carbohydrate-rich polyelectrolyte, namely, a glycocalyx which serves as a binding surface for inorganic ions and higher molecular weight organic compounds including host enzymes. Hanumantha Rao (1960a) also mentioned that the tegument of *P. ganapatii* is PAS positive and fast to saline. Trimble and Lumsden (1975) found the tegument of larval *Taenia crassiceps* to possess a surface coat rich in both neutral and acidic carbohydrates. Rama Devi (1970) worked on six species of pseudophyllidean tapeworms and reported similar findings. She also detected the presence of lipids. In *Ptychobothrium cypseluri*, *Bothriocephalus manubriformis*, *Oncodiscus fimbriatus* and *Bothriocephalus indicus* the tegument was found to possess acid mucopolysaccharides and glycogen (Rama Devi 1970). The present observations reveal the tegument to consist of basic proteins containing tyrosine, glycoprotein, S–H, S–S etc., in addition to mucopolysaccharides.

The basement membrane is composed of basic proteins containing tyrosine, S–H groups, $NH_2$ groups, protein bound amino acid groups and glycoproteins and lipids bearing phospholipids. The epidermal and the parenchymal musculature contain basic




**Figures 5–6.** 5. Frontal glands showing metachromasia with toluidine blue. 6. Basement membrane and the deeper longitudinal muscles showing the presence of basic proteins (bromophenol blue).




**Figures 7–8.** 7. Frontal glands indicating the presence of basic proteins (bromophenol blue). **8.** Frontal glands showing glycoproteins (Congo red).

*Abbreviations.* B–Basement membrane; Dl–Deeper longitudinal musculature; EP–Epidermal longitudinal musculature; FG–Frontal glands; FP–Frontal pit; T–Tegument; TM–Transverse muscle.

proteins with tyrosine, S–H groups, very little quantities of protein bound amino groups and lipids.

The presence of glycogen in the parenchyma in various pseudophyllids was demonstrated by many workers (Smyth 1946, 1947; Hanumantha Rao 1960a; Rama

Devi 1970). In the present study also glycogen was detected in the parenchyma and muscles.

In addition to glycogen, both epidermal and parenchymal musculature of the plerocercoid contain lipids especially phospholipids. Smyth (1946) reported the presence of lipid droplets of various sizes scattered throughout the parenchyma of *Ligula intestinalis*. Rama Devi (1970) also reported the presence of lipid droplets in the parenchyma of pseudophyllidean cestodes. Lipids may also be considered as break down products of metabolism (Rama Devi 1970).

Baer (1956) reported that the scolex of *Monorygma perfectum* displays a 'deep staining granular mass' which was considered to evoke a pronounced host tissue reaction. In Lecanicephalidae, a similar 'glandular complex' was described in *Polypocephalus rhinobatides* and in *P. radiatus* by Subhapradha (1951). Smyth (1964) reported the occurrence of glands in the restollum of *Echinococcus granulosus*. He stated that the secretion of the gland is PAS negative and concluded that it is probably an extremely labile lipoprotein or lipid-protein coacervate. In the present investigation on the plerocercoid of *Penetrocephalus* sp. three types of gland cells could be recognized. The glands are PAS positive. Rama Devi (1970) also recognized three types of glands in the scolex of adult *Penetrocephalus ganapatii*. Wolffhugel (1938) reported the occurrence of three types of gland cells in the scolex of *Nematoparataenia southwelli*.

Many speculations have been made as to the function of these glands. However, Smyth (1964) mentioned that the glands cause contraction of the villi which can assist the orientation of the scolex. Wolffhugel (1938) suggested that the enzymic secretion of the gland assists the cellular digestion. He also assumed that the secretion was hormonal in nature, related to the regulation of growth and maturation of the strobila. According to Rama Devi (1970) the glands of *P. ganapatii* assist in penetration activities.

Rawson and Rigby (1960) observed that in the cysticercoid of *Choanotaenia crassiscolex* these glands secrete a lubricant into the rostellar sac to help movement of the rostellum. Therefore, the gland cells in different cestodes may vary in structure, anatomy, chemical nature and function.

It is believed that in *Penetrocephalus* also the scolex glands aid in the penetration activities.

## Acknowledgements

One of the authors (KJC) is grateful to the Ministry of Education and Culture, Government of India, New Delhi, for financial assistance, the authorities of Andhra University, Waltair for facilities and also to the authorities of Bangladesh Agricultural University, Mymensingh for granting him study leave.

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Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94, No. 1, January 1985, pp. 21–23.


# Effect of starvation on acid phosphatase activity in *Gastrothylax crumenifer*

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**Abstract.** Effect of starvation on acid phosphatase activity in *Gastrothylax crumenifer* showed that activity was greater in starved individuals than in well fed ones.

**Keywords.** Starvation; acid phosphatase; *Gastrothylax crumenifer.*

## 1. Introduction

Although considerable work has been done on the subject of stress very little is known on stress and its effect on parasites or parasitism. Starvation in flukes produces increase in membraneous autophagic vacuoles exhibiting hydrolytic activity as elicited in *Megalodiscus temperatus* (Bogitsh 1973), *Schistosoma mansoni* (Bogitsh 1975), *Haematoloechus medioplexus* (Davis *et al* 1969). A study was undertaken with a view to elucidating the effects of starvation on the activity of phosphatases in *Gastrothylax crumenifer.*

## 2. Material and methods

To detect the effects of starvation on phosphatase activity in *G. crumenifer*, live parasites were obtained from the rumen wall. They were transported to the laboratory in Hedon–Fleig medium and were washed thoroughly to clean off debris. One batch of worms was taken separately and assayed for phosphatase activity and considered for normal activity at 0 hr. The second batch was incubated in Hedon–Fleig medium with 0·5% glucose (fed), one million units of penicillin and 2·5 g of streptomycin per litre as described by Thorpe (1967). To determine the effect of starvation, another batch of worms was incubated in Hedon–Fleig medium without glucose (starved). Ten to fifteen worms were placed in 200 ml experimental liquid in finger bowls and the temperature maintained at 37°C. Solutions were changed after 12 hr and thereafter at 24 hr intervals; the worms were observed twice daily. Dead worms were removed promptly. A worm was considered to be "fully alive" when moving spontaneously, "moribund" or "half alive" when sluggishly moving or responding only to mechanical stimulation and "dead" when no movement was evident or no response was obtained. Usually active worms were attached firmly to the sides of the glass jars.

Observations of the parasite's enzyme activity were made at 0 hr and at the end of 12 hr, and then at intervals of 24 hr, up to 120 hr (5 days), for both control (fed) and experimental (starved) batches. At the commencement of the 6th day, parasites in the non-nutrient medium were transferred to the nutrient (glucose) medium for measurement of any difference in activity. As no parasite survived after the 6th day, even in the

nutrient medium, the experiment had to be concluded at that stage. Phosphatase activity was assayed by the method of King and Armstrong as described by Varley (1967), using disodium phenyl phosphate (0·01 M) as substrate. The protein content of the supernatant was determined following the method of Lowry *et al* (1951), using human serum albumin as a standard.

## 3. Results

The phosphatase activity in *G. crumenifer* was estimated at 0 hr *i.e.*, prior to subjecting them to starvation for varying periods up to 120 hr. The phosphatase activity was greater in starved individuals than in the fed ones at 12, 24, 48 and 120 hr (figure 1). The phosphatase activity was greater in refed ones than in fed ones, at 144 hr.

## 4. Discussion

The results indicated that starvation/stress enhanced the acid phosphatase activity in *G. crumenifer*. No biochemical work exists describing the effects of starvation on

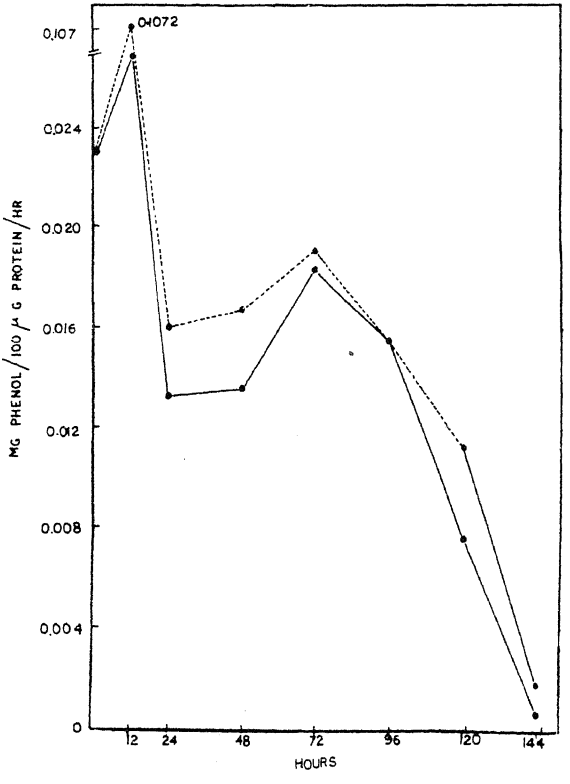


**Figure 1.** Phosphatase activity in *G. crumenifer* with glucose (fed) and without glucose (starved) at different hours in veronal buffer at pH 3 ((——)-fed, (– – –) starved).

phosphatase activity in digenetic trematodes. A few studies have been made histochemically on the effects of starvation on phosphatase activity in digenetic trematodes. The stimulus for the increased synthesis of phosphatases remains unknown. It is possible that the diminishing pressure of material in the lumen of the digestive tract is the triggering mechanism (Bogitsh 1975). As the amount of food in the lumen is reduced, the pressure is likewise lessened and an impulse is probably generated.

Bogtish (1973) suggested that starvation is a stimulus to which the gastrodermis of *M. temperatus* reacts by sequestered areas with the enclosed material subsequently being degraded. It has been reported that the golgi complexes become increasingly active in their relationship with the lysosome system in other types of organisms subjected to stress factors, such as starvation (Ericson 1969). Under conditions of stress (*e.g.*, starvation) organelle complexes are often found in increased numbers (Bogitsh 1973; Threadgold and Arme 1974). A marked increase in the number of acid phosphatase positive, membrane-bound vacuoles was reported in starved *M. temperatus* as compared to well fed worms (Bogitsh 1973).

The functional significance of this process lies in the possibility that it may represent a survival mechanism for the tissue following stress. The metabolism of the gastrodermis of *M. temperatus* may become reoriented so that the lytic rates become significantly greater than the synthetic rates (Bogitsh 1973).

It is desirable that a larger number of trematodes be investigated to determine how the stress would affect the various tissues, thus enabling a better understanding of this aspect of trematode physiology.

**Acknowledgement**

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# Four new species of trypanoplasms from the fresh water fishes of the genus *Mystus* in Maharashtra

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**Abstract.** Four new species of haemoflagellates of the genus *Trypanoplasma*, Laveran and Mesnil, 1901 *viz T. krishnamurthyi*, *T. cavacii*, *T. vidyai* and *T. seenghali* are described from the fresh water fishes of the genus *Mystus* in Maharashtra.

**Keywords.** Haematozoa; haemoflagellates; *Trypanoplasma*.

## 1. Introduction

Biflagellate organisms of the genus *Trypanoplasma* Laveran and Mesnil 1901 from fresh water fishes have been recorded from various parts of the world. The major reviews of this group are those of Pavlovaskii (1964), Becker (1970, 1977) and Lom (1979). However, this group received little attention in India. Mandal (1979) and Joshi (1982) are the only workers who have described one and two species respectively. The present contribution is the third of a series on this group in Maharashtra.

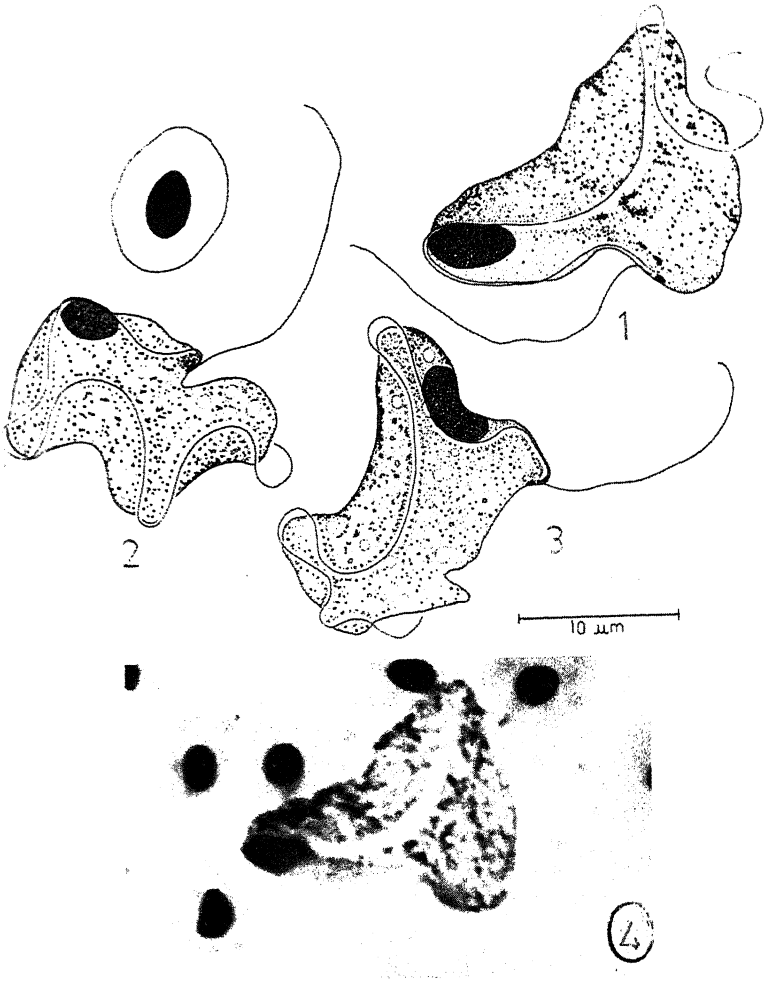
## 2. Material and methods

The material for this investigation was obtained from two rivers in two different localities, namely Purna and Aurangabad in Maharashtra. The fishes were brought alive to the laboratory for examination or were examined on the spot itself using a field microscope. Smears were made from the blood obtained from the heart and no anti-coagulent was used. The smears were air-dried, fixed in acetone-free methyl alcohol for 8–10 min and stained with Giemsa's stain diluted with phosphate buffer. The drawings were using a Leitz camera lucida at a magnification of about 2000 ×. The photo-micrographs were taken with Leica M-3 camera. The identification of the fish hosts was based on Day (1875), Shrivastava (1968) and Jhingran (1982). The slides of the type material are deposited in the Protozoology Section, Department of Zoology, Marathwada University, Aurangabad, India.

## 3. Observations and discussion

### 3.1 *Trypanoplasma krishnamurthyi* sp. nov. figures 1–4

Host: *Mystus cavacius* Hamilton.
Locality: River Purna, Parbhani Dist., Maharashtra, India.
Site of infection: Blood.




**Figures 1–4.** *Trypanoplasma krishnamurthyi* sp. nov. from *M. cavacius*. **1–3.** Camera lucida drawings. **4.** Photomicrograph.

This trypanoplasm was present in two out of the four fishes (*Mystus cavacius*) examined. The infection was moderate in both the cases.

*Morphology*

*Cell body*: The body of the trypanoplasm is short, broad and often irregular in shape.

*Cytoplasm*: The cytoplasm is vacuolated and granular, but does not show homogeneous appearance because of varying patterns of granulation in different regions.

*Nucleus*: The nucleus is dorsal and very characteristic in having a distinctly ovoidal shape (length: width = 2:1) consistently. This is the only species found during the present study having a consistent nuclear shape.

*Kinetoplast*: The kinetoplast is absent which is extremely characteristic, as no dis-kinetoplastic trypanoplasm has ever been described so far, from fresh water fishes.

*Flagella and undulating membrane*: The two flagella arise from the kinetosomes which

are distinct and rod-like. The anterior flagellum is about as long as the body. The posterior flagellum forms an 'S' configuration as it runs over the body. The free trailing portion of the posterior flagellum is long, being slightly less than the body length.

There is no clear evidence of the presence of an undulating membrane.

The details of dimensions of the trypanoplasm are given in table 1.

This is the first trypanoplasm to be described from this host species and the third from a fish of the genus *Mystus* in India, the earlier records being those of *T. indica* from *M. vittatus* and *T. mysti* from *M. aor.*

A comparison of this species with *T. indica* (Mandal 1979) shows that it is distinctly smaller in size, measuring 15·53 – 28·24 × 5·17 – 16 μm (19·89 × 10·13 μm) as against 25 – 30·5 × 6 – 10·5 μm (28·5 × 8 μm) in the present form. This form is unique in lacking a kinetoplast, while *T. indica* has a conspicuous one. It also has a much longer trailing flagellum than *T. indica.*

It is marked off from *T. mysti* Joshi, 1982, by its smaller size, (19·89 × 10·13 μm as against 28·2 × 10·9 μm) larger nucleus and distinctly longer flagella. The monomorphic nature of this trypanoplasm distinguishes it from *T. atti* Joshi, 1982 which exists in two forms. The two species are also marked off by differences in morphology and morphometrics.

The only other record of trypanoplasm from fishes of the family Bagridae is *T. pseudobagri* Chang, 1964 from *Pseudobagrus fulvidraco* in China. Unfortunately neither its description nor any information about this species could be procured by the author and hence no comparison could be made.

The only trypanoplasm without a kinetoplast recorded earlier is *T. beckeri* Burreson, 1979. That species was described from a marine fish *Scorpaenichthyes marmoratus* of family Cottidae in the United States, while the present species is from a fresh water fish of the family Bagridae in India. The body of the present form is relatively short and broad (19·89 × 10·13 μm) as contrasted with the extremely long and twisted body of *T. beckeri* (109 × 6·5 μm).

In view of its distinctness, this species is considered new to Science and designated *Trypanoplasma krishnamurthyi* sp. nov. after Dr R Krishnamurthy of the Marathwada University, Aurangabad, in grateful appreciation of his active guidance throughout the course of this work.

**Table 1.** Body dimensions of *T. krishnamurthyi* sp. nov. from *M. cavacius* (based on 50 forms).

| Particulars (μm) | Minimum | Maximum | Average |
|---|---|---|---|
| Length of cell body | 15·53 | 28·24 | 19·89 |
| Width of cell body | 5·17 | 16·00 | 10·13 |
| Length of nucleus | 3·28 | 11·29 | 6·91 |
| Width of nucleus | 1·41 | 6·12 | 3·08 |
| Length of kinetoplast | — | — | — |
| Width of kinetoplast | — | — | — |
| Length of anterior free flagellum | 10·35 | 30·13 | 22·52 |
| Length of posterior free flagellum | 11·29 | 27·30 | 18·38 |

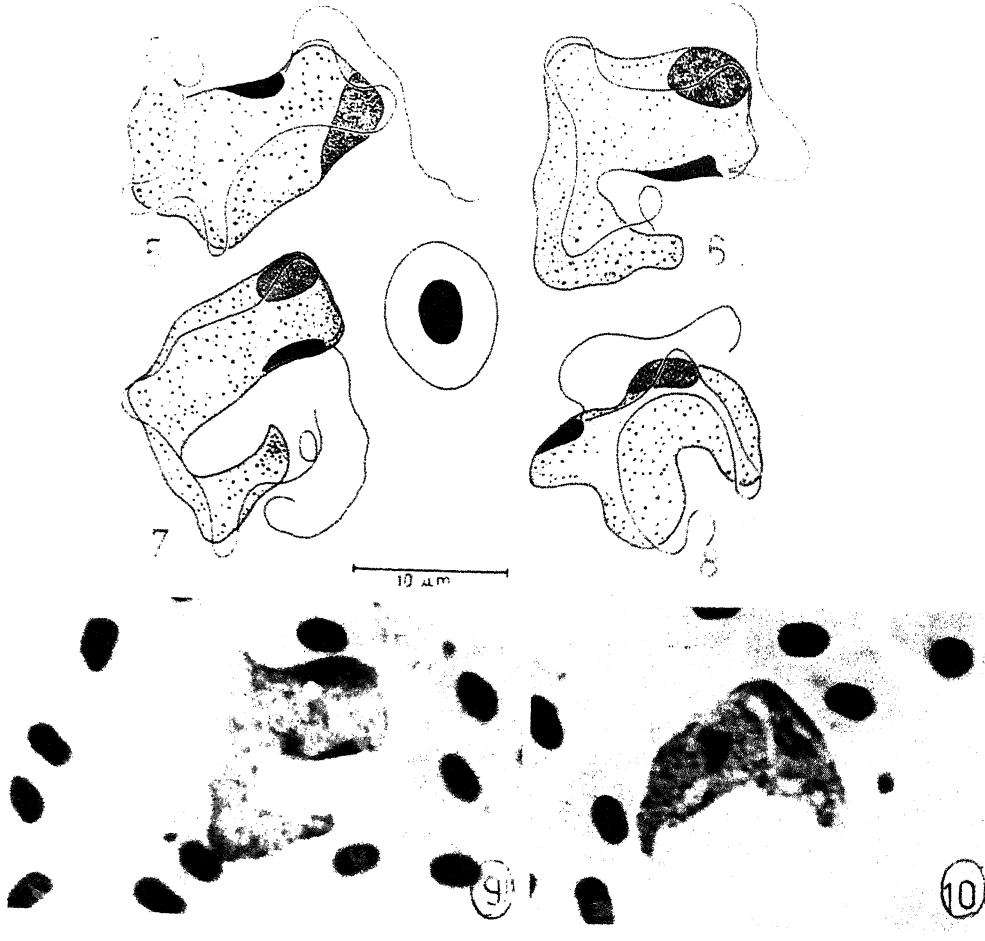
3.2 *Trypanoplasma cavacii* sp. nov. figures 5–10

Host: *Mystus cavacius* Hamilton.
Locality: Kham river, Waluj, Aurangabad, Maharashtra, India.
Site of infection: Blood.

This trypanoplasm was found as the only species in 10 out of the 51 fishes (*M. cavacius*), examined. The infection was light in all the cases and a few dividing forms were also observed (figure 10).

*Morphology*

*Cell body*: The body of the trypanoplasm is variable in shape. The elongated forms have a 'J' shaped configuration (figures 6, 7) with the anterior part being broad and forming the longer arm and the posterior third being relatively narrower and forming the shorter arm. In other cases, the body is short, broad and almost straight (figure 5).




**Figures 5–10.** *Trypanoplasma cavacii* sp. nov. from *M. cavacius*. **5–8.** Camera lucida drawings. **9.** Photomicrograph of 'Trophozoite'. **10.** Photomicrograph of dividing form.

*Cytoplasm*: The cytoplasm is vacuolated and shows distinctly coarse chromophillic granules, often abundant near the posterior end (figures 6, 7).
*Nucleus*: The nucleus is ovoid (figures 6, 7) to elongated (figure 8) and placed dorsally, close to the anterior end.
*Kinetoplast*: The kinetoplast is relatively short (L:W = 2–4:1) distinctly triangular (figure 6) or fusiform (figure 7) in the elongated specimens and slightly curved and somewhat crescentic in the short forms (figure 5). The kinetoplast is ventral in position.
*Flagella and undulating membrane*: The two flagella arise from the kinetosomes, which are placed just anterior to the kinetoplast. The anterior flagellum becomes free from the body soon after the origin, and it is about as long as body or slightly more. The posterior flagellum forms 2–3 distinct folds along the length of the body, before becoming free. The free trailing part is about as long as the body.

There is no clear evidence of an undulating membrane.

The details of the dimensions of the trypanoplasm are given in table 2.

This trypanoplasm is described from the same host (i.e. *M. cavacius*) as the preceding species i.e. *T. krishnamurthyi* sp. nov. It is easily distinguished from the earlier species by the presence of a distinct kinetoplast, its slightly smaller size (17·63 × 9·58 μm as against 19·89 × 10·13 μm) and by the presence of distinctly coarse chromophillic granules in the cytoplasm. The present species is much smaller in size than *T. indica* (17·63 × 9·58 μm as against 28·5 × 8 μm), but has a nucleus and kinetoplast which are only slightly smaller. It also has a conspicuously longer trailing flagellum (16·94 μm) compared with *T. indica* (10·5 μm).

Compared with four other species of trypanoplasms described from fresh water fishes in Maharashtra, i.e. *T. saranae*, *T. lomi* and *T. solapurensis*, Wahul (under publication) and *T. quadrii*, Krishnamurthy and Wahul (under publication) it is marked off by differences in the shape and size of the body and kinetoplast, the pattern of distribution of chromophillic granules in the cytoplasm and by a much longer trailing flagellum. Further it is distinguished from *T. qadrii* by its monomorphic nature.

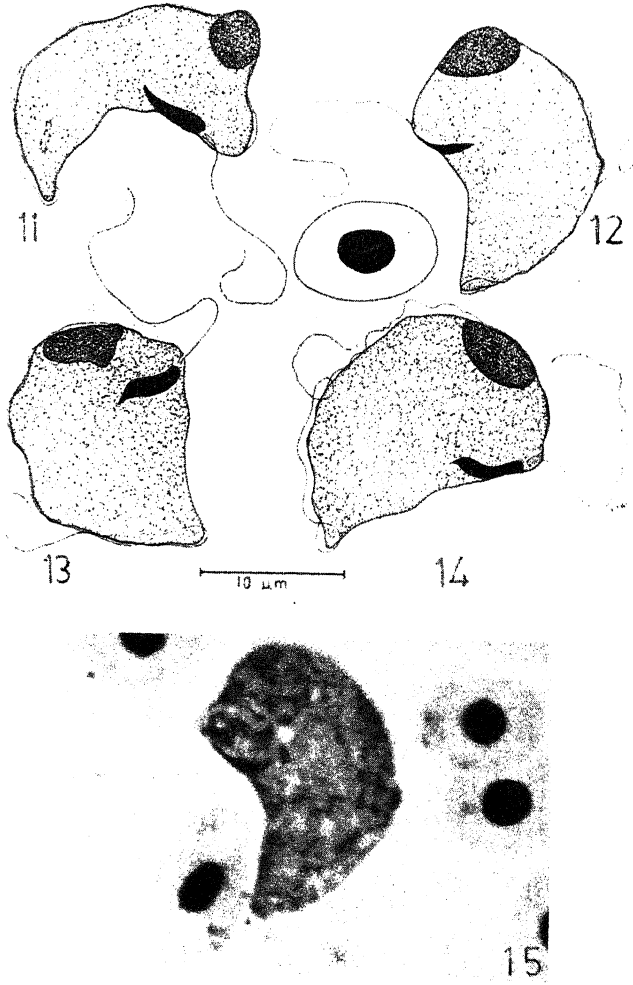
This species has distinctly smaller body dimensions than *T. mysti* but has a larger nucleus, a better developed kinetoplast and distinctly longer flagella. This species is also much smaller than *T. atti* and is typically monomorphic besides having distinctly longer flagella.

**Table 2.** Body dimensions of *T. cavacii* sp. nov. from *M. cavacius* (based on 25 forms).

| Particulars (μm) | Minimum | Maximum | Average |
|---|---|---|---|
| Length of cell body | 15·06 | 20·24 | 17·63 |
| Width of cell body | 6·59 | 14·59 | 9·58 |
| Length of nucleus | 4·23 | 8·47 | 6·25 |
| Width of nucleus | 1·41 | 4·23 | 2·65 |
| Length of kinetoplast | 2·11 | 6·12 | 4·33 |
| Width of kinetoplast | 0·47 | 3·29 | 1·43 |
| Length of anterior free flagellum | 13·65 | 23·06 | 20·41 |
| Length of posterior free flagellum | 12·17 | 21·18 | 16·94 |

It has an overlapping range in body length with *T. borelli*, Laveran and Mesnil (1901), *T. cataractae* Putz (1972), and *T. cyprini*, Plehn (1903) but is clearly much broader. It has a larger nucleus than *T. borelli* and *T. cataractae* and a smaller kinetoplast than *T. cyprini*. It has an overlapping but narrower length range with *T. varium*, Leger (1904), but is contrasted from it by its definite shape, smaller kinetoplast and longer trailing flagellum. It is marked off from all the other species by its size, being smaller than *T. abramidis* Brumpt (1906), *T. barbi*, Brumpt (1906), *T. acipenseris*, Ioff *et al* (1926) and *T. guernei*, Brumpt (1906) and larger than *T. makeevi*, Achemerov (1959), *T. salmositica*, Katz (1951), *T. markewitschi*, Schapowal (1953) and *T. pseudocaphirhynchi*, Ostroumov (1949).

In light of the above discussion, it is considered new and named *Trypanoplasma cavacii* sp. nov. after the specific name of the host in which it was found.




**Figures 11–15.** *Trypanoplasma vidyai* sp. nov. from *M. seenghala*. **11–14.** Camera lucida drawings. **15.** Photomicrograph.

3.3 *Trypanoplasma vidyai* sp. nov. figures 11–15

Host: *Mystus seenghala* Sykes.
Locality: River Purna, Parbhani Dist., Maharashtra, India.
Site of infection: Blood.

This trypanoplasm was found in 8 (*Mystus seenghala*) out of the 28 fishes examined. Out of the 8 infected fishes it occurred alongwith another species of *Trypanoplasm* (*T. seenghali* sp. nov.) and a species of *Trypanosoma* in one while in the other seven it occurred with another species of *Trypanosoma*. The infection was moderate in all cases.

*Morphology*

*Cell body*: The body of the trypanoplasm is short, broad and stumpy (L:W 2:1) with a distinctly convex dorsal margin and a straight (figures 13–14) or curved (figures 11, 12) concave ventral margin. The anterior end is broad and rounded while the posterior is bluntly conical (figure 14).

*Cytoplasm*: The cytoplasm is highly vacuolated (figure 14) and stains homogeneously and intensely.

*Nucleus*: The nucleus is spherical (figure 11) to ovoidal (figure 14) and lies along the dorsal margin in the anterior third of the body.

*Kinetoplast*: The kinetoplast is relatively short, broad and variable in shape, the L:W ratio varying from 2:1 to 4:1. The width of the kinetoplast is not uniform and the posterior end is generally pointed. In most cases it runs partly along the ventral margin and turns away from the margin into the cytoplasm (figures 11, 14).

*Flagella and undulating membrane*: The two very delicate flagella arise from the kinetosomes which are placed just anterior to the kinetoplast. The anterior flagellum is relatively long, being one and one-third times the body length. The posterior flagellum is extremely characteristic, running along or very close to the dorsal surface of the body, almost up to the posterior tip. In most cases there are hardly any undulations visible (figure 11) and where they are present, the undulations are numerous, very small and shallow attaching the flagellum to the body surface at several points (figures 12–14). It is also characteristic by its free trailing part, which in almost all cases, recurves and extends forward along the dorsal surface of the body and ends in a loop. The free part is about as long as the body.

There is no clear evidence of the existence of a distinct undulating membrane.

The details of the dimensions of the trypanoplasm are given in table 3.

**Table 3.** Body dimensions of *T. vidyai* sp. nov. from *M. seenghala* (based on 50 forms).

| Particulars (μm) | Minimum | Maximum | Average |
|---|---|---|---|
| Length of cell body | 15·53 | 30·13 | 19·16 |
| Width of cell body | 7·53 | 15·53 | 11·84 |
| Length of nucleus | 4·70 | 9·41 | 6·76 |
| Width of nucleus | 1·88 | 5·64 | 3·15 |
| Length of kinetoplast | 2·82 | 6·82 | 4·78 |
| Width of kinetoplast | 0·70 | 3·29 | 1·43 |
| Length of anterior free flagellum | 20·24 | 30·60 | 25·05 |
| Length of posterior free flagellum | 11·77 | 26·36 | 18·06 |

This is the first trypanoplasm to be described from *M. seenghala* and the fifth from fishes of the genus *Mystus*. A comparison of this species with the other four as well as the other species described from various other fresh water fishes show it to be distinct.

It has a relatively short, broad and stumpy body as contrasted with *T. indica* (19·16 × 11·84 μm as against 28·5 × 8·0 μm). The kinetoplast is shorter and broader than in *T. indica* and the flagella distinctly longer. The presence of a distinct kinetoplast and a definite body shape distinguish this species from *T. krishnamurthyi* sp. nov. Compared with *T. cavacii* sp. nov. it is distinctly larger with a more stumpy appearance because of its broad body. It also has a larger nucleus which is more spherical or ovoid and clearly longer flagella than the latter. Its smaller size, typical and almost constant body shape, much larger kinetoplast and distinctly longer and delicate flagella, distinguishes this species from *T. mysti*.

Its monomorphic nature, longer flagella and the shape and size of the kinetoplast differentiate it from *T. atti* and *T. qadrii* Krishnamurthy and Wahul (under publication). The shape and size of its body, the short and broad nature of its kinetoplast and long flagella demarcate this species from the others described from this area, namely *T. saranae*, *T. lomi* and *T. solapurensis* Wahul (under publication).

The broad and stumpy body of this species and the nature of its kinetoplast distinguish this species from all the others described so far.

The species under discussion is unique in having a posterior flagellum running very close to the dorsal surface throughout the length of the body and in having the free trailing part recurving forwards and often forming a loop.

In view of these differences it is considered new and designated *Trypanoplasma vidyai* sp. nov.

### 3.4 *Trypanoplasma seenghali* sp. nov. figures 16–20

Host: *Mystus seenghala* Sykes.
Locality: River Purna, Parbhani Dist., Maharashtra, India.
Site of infection: Blood.

This trypanoplasm was found in only one fish (*Mystus seenghala*) out of the 28 examined. This fish also harboured another species of *Trypanoplasma* (*T. vidyai* sp. nov.) and a species of *Trypanosoma*.

The infection was light in the case of the present species.

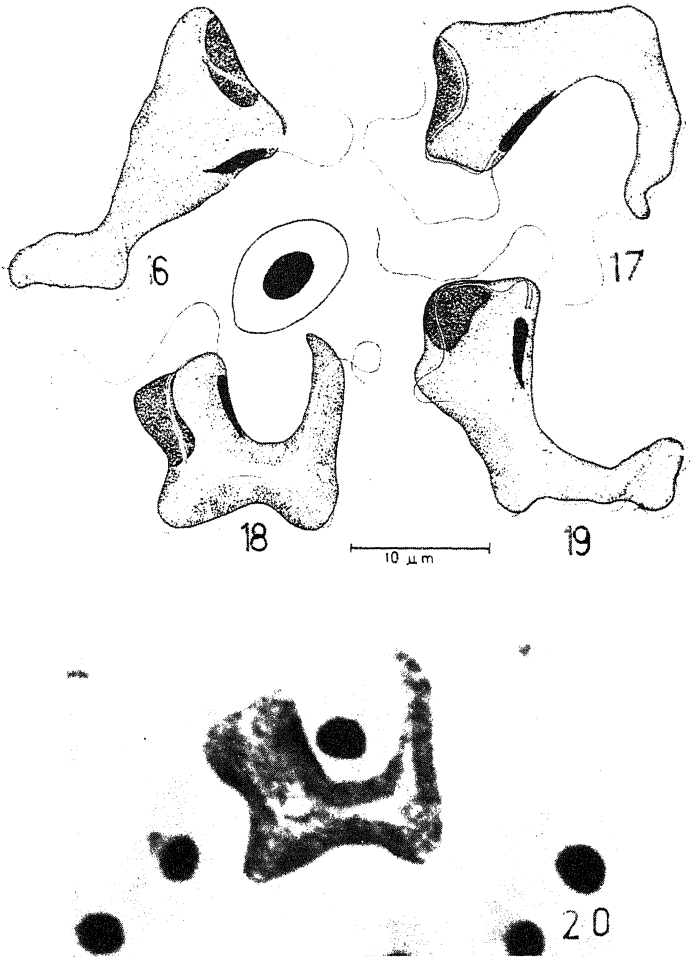
*Morphology*

*Cell body*: The body of the trypanoplasm is elongated (L:W = 3:1) with a typical 'C' (figure 19) or 'U' (figure 18) shaped configuration. The anterior third is almost twice as broad as the posterior third. The anterior end is bluntly conical and the posterior end tapering to a rounded tip.

*Cytoplasm*: The cytoplasm is vacuolated, granular and stains homogeneously.

*Nucleus*: The nucleus is oval (figure 19) to elongated (figure 16) and situated dorsally in the anterior third.

*Kinetoplast*: The kinetoplast is elongated (L:W = 6:1) apparently straight, stiff and rod like (figures 17, 18). The anterior half is broader than the posterior. The posterior end tapers to a point and runs along or close to the ventral margin.




**Figures 16–20.** *Trypanoplasma seenghali* sp. nov. from *M. seenghala*. **16–19.** Camera lucida drawings. **20.** Photomicrograph.

*Flagella and undulating membrane*: The two delicate flagella arise from the kinetosomes, which are placed just anterior to the kinetoplast. The anterior flagellum is almost as long as the body. The posterior flagellum runs along the dorsal surface and becomes free posteriorly. The free trailing part of the posterior flagellum is about three fourths of the body length. During its course the posterior flagellum is thrown into two conspicuous folds, one near the junction of the anterior and middle thirds of the body and the other near the junction of the middle and posterior thirds (figures 17, 18) and in some cases two or three very small folds in the posterior third (figures 17, 19).

There is no clear evidence of a distinct undulating membrane.

The details of dimensions of the trypanoplasm are given in table 4.

This is the second species of trypanoplasm to be described from this host species (i.e. *M. seenghala*). Though it is described from the same host as *T. vidyai* sp. nov., it is easily marked off from that species by conspicuous differences in the shape and dimensions of

**Table 4.** Body dimensions of *T. seenghali* sp. nov. from *M. seenghala* (based on 25 forms).

| Particulars ($\mu$m) | Minimum | Maximum | Average |
|---|---|---|---|
| Length of cell body | 15·53 | 32·95 | 25·27 |
| Width of cell body | 5·17 | 10·82 | 8·00 |
| Length of nucleus | 3·76 | 9·88 | 6·93 |
| Width of nucleus | 1·41 | 4·23 | 2·80 |
| Length of kinetoplast | 3·76 | 8·00 | 5·70 |
| Width of kinetoplast | 0·47 | 1·88 | 0·98 |
| Length of anterior free flagellum | 20·71 | 37·30 | 23·69 |
| Length of posterior free flagellum | 12·24 | 25·42 | 17·84 |

the body, the different nature of the kinetoplast and the pattern of undulations of the posterior flagellum. Its characteristic shape, typically elongated kinetoplast and longer flagella contrast it from *T. indica*. Its distinctly larger size and presence of a kinetoplast differentiate it from *T. krishnamurthyi* sp. nov. which is smaller and lacks a kinetoplast. It differs from *T. cavacii* sp. nov. being larger in size and having a relatively elongated and narrow kinetoplast.

It is marked off from *T. mysti* by its distinctly longer kinetoplast, larger nucleus and conspicuously longer flagella.

Its monomorphic nature marks it off from *T. atti* and *T. qadrii* Krishnamurthy and Wahul (under publication), while its characteristic shape, kinetoplast and body dimensions distinguish it from all the species described so far from this area. This is the largest of the trypanoplasms found during the present study and the only species which is close to this in body dimensions is *T. solapurensis* Wahul (under publication) which has a length range of 16·94 × 28·24 $\mu$m as compared with 15·53 × 32·95 $\mu$m here. However, the present species is much broader and differentiated from that species by the shape and size of the nucleus and kinetoplast and distinctly longer flagella.

Compared with the various other monomorphic trypanoplasms described so far, it has an overlapping length range with *T. abramidis* and *T. barbi*. However, its body is broader than that of *T. barbi* and narrower than that of *T. abramidis*. It also has a kinetoplast which is shorter than in the two species and a trailing flagellum which is distinctly much longer, besides slight differences in the size, shape and position of the nucleus.

Its body dimensions mark it off from the rest of the species.

In view of the above discussion, this species is considered new and designated *Trypanoplasma seenghali* sp. nov. after the specific name of the host.

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# Mechanism of resistance in rice varieties showing differential reaction to brown planthopper

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**Abstract.** A total of 1070 rice varieties, mainly from Assam Rice Collection, were evaluated to identify better sources of resistance to brown planthopper, *Nilaparvata lugens* (Stål). In mass screening replicated tests 17 varieties were identified as resistant. Moderate resistance was observed in 73 varieties.

All the resistant and moderately resistant varieties were relatively less preferred by nymphs and there was a positive correlation between the number of nymphs settled and the damage score. Test varieties non-preferred by adult insects for feeding and shelter were also less suitable for oviposition with the exception of ARC 13854, and ARC 14766A. On resistant varieties the nymphal survival was much lower (18·5–28·4%) and nymphal duration was prolonged by 5–7 days as against those on the susceptible check. Results of probing behaviour tests indicated that resistant varieties received more number of probing punctures (80–121) than the susceptible check (31). Further, insects caged on resistant varieties quickly lost their body weight while those on the susceptible check registered gain in weight. Honey dew excretion by brown planthopper adults on resistant varieties was 6·6 to 11·9 times less than that on susceptible T(N)1. Selected varieties showing moderate damage reaction (ARC 5918, ARC 10443, ARC 13984, ARC 14529 and ARC 14864) exhibited more feeding marks, greater amounts of excretion, and higher gain in body weight of the insects, thus confirming a moderate degree of resistance. Based on various parameters, ARC 5780, ARC 5988 and ARC 14394 were comparable to resistant check, Ptb 33 in level of resistance. No association of Lemma and Palea colour with brown planthopper resistance was observed in the rice varieties tested.

**Keywords.** Varietal resistance; rice varieties; *Nilaparvata lugens.*

## 1. Introduction

In India the brown planthopper (*Nilaparvata lugens* (Stål)) has assumed greater importance since its outbreak in Kerala during 1973–74 and subsequently in many other parts of the country. Host plant resistance as a component of pest management programme is being successfully utilized in Philippines and Indonesia in controlling this pest. In view of the more virulent biotype in India, screening for resistance to brown planthopper was carried out at various research institutes and the number of resistant donors identified have been reported (Kalode and Krishna 1979; Kalode *et al* 1983; Kalode 1983). Though there are reports from India about nymphal non-preference for certain rice varieties, no detailed information is available on adult preference. Investigations were, therefore, undertaken at the AICRIP, Hyderabad to study the reaction of selected varieties/cultures to both nymphs and adults of brown planthopper (BPH) as well as to understand the mechanism manifesting different degrees of resistance, so that better varieties (donors) with desirable characters could be utilized effectively in resistance breeding programme.

## 2. Material and methods

### 2.1 *Mass rearing and varietal screening*

BPH was reared on 30 day old T(N)1 plants inside the green house provided with coolers to maintain the temperature at $30 \pm 5°C$ to ensure uniform and steady supply of insects. The rearing cages ($70 \times 62 \times 75$ cm) were provided with glass panels with a small window on one side and fine nylon wire mesh on the other sides. Pre-mated gravid females were allowed to oviposit on plants for two days and the emerging nymphs were further maintained to get age specific insects for different experiments.

ARC cultivars (1000), 20 varieties from IRRI and another 50 cultures were screened by adopting the modified mass screening layout (Kalode *et al* 1975). Pre-germinated seeds were sown in rows in wooden flats along with susceptible and resistant check, which were then transferred to galvanized iron trays filled with water to maintain adequate humidity and to prevent ants. Seven day old seedlings were infested with a large number of 1–2 instar nymphs so as to get 5 to 10 insects/seedling and were scored for damage reaction on a 0–5 scale when more than 90% of T(N)1 seedlings were killed. Test varieties showing damage score up to 2·5 in a preliminary test were retested, replicated 3 times to confirm their reaction.

### 2.2 *Studies on preference/non-preference mechanism on selected rice varieties*

2.2a *Response of nymphs*: During the retest, the number of nymphs settled on each seedling was counted at different intervals *viz.*, 1 day, 3 days, 5 days and 7 days after infestation to assess the nymphal preference for different varieties.

2.2b *Response of adults for settling and oviposition*: About 30 selected varieties including resistant and susceptible checks were grown randomly (8 cm apart) in polythene sheet lined wooden flats. Each variety was replicated four times with seven seedlings per replication. Thirty days after sowing, each wooden flat was transferred to a suitable cage and a large number of adults were released. The counts of adult insects settled on each seedling were taken at 12, 24 and 48 hr after release. The plants were then cut as close to the base as possible and the number of eggs laid per seedling was recorded by staining in 1% erythrocin dye in an aqueous solution as suggested by Naito (1964) under a binocular microscope.

### 2.3 *Studies on antibiosis mechanism*

2.3a *Survival and development of nymphs*: Thirty six resistant and moderately resistant varieties were included along with resistant and susceptible checks Ptb 33 and T(N)1, respectively. Seeds of each variety were sown in earthen pots and each variety was replicated 6 times. Thirty days after sowing each plant was caged with 10 freshly hatched nymphs in mylar film cages ($5 \times 45$ cm) the open end of which was closed with fine muslin cloth. The counts of surviving nymphs were taken, 24 hr after infestation and thereafter once in five days till 20 days.

2.3b *Feeding response of adult brown planthopper*: Thirty varieties which were identified as resistant and moderately resistant in mass screening test were included for various investigations. The varieties were grown in wooden flats along with the resistant and susceptible checks.

2.3b(i) *Attempts of feeding—Probing marks*: Seven days after germination, the seedlings of each variety were removed from the flats and washed and then transferred individually into test tubes (2 × 17 cm) containing water. Two gravid females were released in each tube. Twelve hours later, the seedlings were transferred into 70% ethyl alcohol. These were then stained and the probing marks counted as described earlier.

2.3b(ii) *Amount of feeding—change in body weight*: Five adult insects per replication were first weighed in a small vial and then starved for 3 hr. The insects were then allowed to feed on 15-day old seedlings in a test tube for double the time of starvation i.e. 6 hr. The insects were again weighed to assess gain or loss in body weight. Each variety was replicated 5 times.

2.3b(iii) *Amount of feeding—honey dew excretion*: Each variety was replicated five times with single thirty day old plant per pot. The plant was drawn through a wooden plank which rested on the rim of the pot. Whatman No. 1 filter paper was placed on the plank by drawing the plant through a slit made in the centre. Then each plant was caged with an inverted glass funnel along with ten pre-starved adult insects (figure 1). The

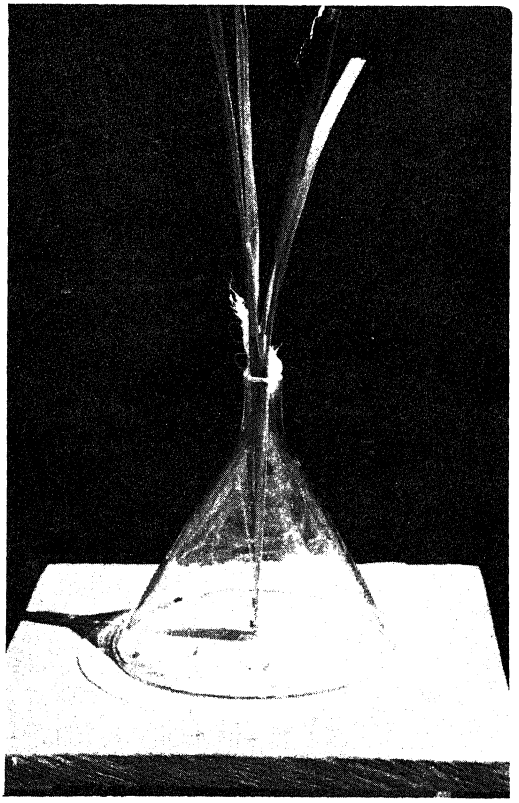

**Figure 1.** A set-up for honey dew collection.

adults were allowed to feed for 24 hr. The filter papers were removed, dried and sprayed with 0·2% ninhydrin solution, which turned the areas of honey dew excretion to pinkish violet. The coloured portions were dissolved in 80% ethanol and the amount of honey dew excreted was determined by reading the concentration in the spectrophotometer at 540 m$\mu$.

### 2.4 *Association of Lemma and Palea colour with resistance*

Lemma and Palea colour of the grains of all the varieties (1070) was noted and statistically analysed by the $\chi^2$ test to know the association of Lemma and Palea colour with BPH resistance.

## 3. Results and discussion

### 3.1 *Mass screening*

Of the 1070 varieties evaluated in the preliminary tests, 152 varieties showed damage grade up to 2·5 on a 0–5 scale. These were retested in replicated test for confirmation of reaction. ARC 5754, 5757, 5764, 5780, 5838, 5973, 5981, 5500, 5988, 13507, 12864, 13854, 13966, 14394, 14539, 14766(A) and 14903 were resistant (damage score up to 1·5) and 73 varieties indicated moderate degree of resistance recording damage score of 1·6 to 3. The rest of the entries recorded higher damage reaction indicating that these varieties might have escaped the damage in the preliminary test. About 24–36 varieties showing different degrees of resistance were selected for various tests.

### 3.2 *Studies on preference/non-preference mechanisms*

3.2a *Response of nymphs*: It is evident from table 1 that all the resistant and moderately resistant varieties were relatively less preferred as compared to susceptible check T(N)1. The nymphs could locate the feeding site within 24 hr and no distinct variation was observed between the varieties after 24 hr of release. However, on majority of the resistant varieties there was a decreasing trend in the number of nymphal population settled between 1 day and subsequent observations while on the susceptible variety T(N)1 more number of nymphs were noted. On an average, 4·2–6·5 and 4·5–7·2 nymphs were recorded on resistant and moderately resistant varieties respectively as against 12·7 nymphs on the susceptible check T(N)1. Non-preference mechanism was reported to be a factor of resistance in BPH as early as 1969 (IRRI, 1969). Kalode and Krishna (1979) and Kalode *et al* (1978) reported that Ptb 33, Ptb 21, Leb Mue Nahng, ARC 6650 and CR 57-MR 1523 had less number of BPH nymphs as compared to T(N)1 and suggested the possibility of some attractants in the susceptible variety. Absence of feeding stimulants or presence of feeding deterrants/repellents could be other possible reasons for non-preference. In the present investigation, a positive correlation was observed with regard to the number of nymphs and damage grade. Higher the number of nymphs settled greater was the damage and *vice-versa*. It appears that non-preference has a definite role in the manifestation of resistance in some of the varieties tested.

**Table 1.** Preferential response of *N. lugens* (Stål) nymphs and adults on selected rice varieties.

| | Reaction to nymphs | | | Reaction to adults | | |
|---|---|---|---|---|---|---|
| | | Average of nymphs settled after days* | | No. of adults/seedling after hours | | Av. no. of eggs per seedling |
| ARC No. | Damage score | 1 | 7 | 12 | 36 | |
| *Resistant (R)* | | | | | | |
| 5780 | 1·1 | 4·2 | 4·2 | 1·3 | 1·0 | 17·7 |
| 5973 | 1·1 | 5·7 | 4·7 | 2·2 | 2·2 | 33·2 |
| 14539 | 1·4 | 5·1 | 4·9 | 2·1 | 1·3 | 32·7 |
| 13854 | 1·4 | 5·1 | 4·8 | 3·0 | 4·3 | 66·3 |
| 5838 | 1·5 | 6·7 | 6·1 | 1·7 | 1·2 | 20·1 |
| 5981 | 1·5 | 6·6 | 5·7 | 1·7 | 1·8 | 28·0 |
| 5754 | 1·5 | 7·3 | 6·5 | 1·0 | 1·6 | 22·1 |
| 14394 | 1·5 | 4·4 | 4·1 | 1·3 | 1·0 | 34·1 |
| 14766A | 1·5 | 5·1 | 4·9 | 1·7 | 2·9 | 41·7 |
| 13507 | 1·5 | 5·1 | 5·1 | 3·3 | 1·7 | 36·3 |
| 5988 | 1·5 | 5·3 | 4·7 | 1·4 | 1·6 | 23·7 |
| *Moderately resistant (MR)* | | | | | | |
| 5913 | 1·6 | 7·3 | 6·3 | 2·2 | 2·2 | 26·8 |
| 14426 | 1·7 | 4·5 | 4·5 | 2·9 | 3·8 | 63·2 |
| 15381 | 1·7 | 5·3 | 5·3 | 2·9 | 3·9 | 38·2 |
| 5916 | 1·7 | 5·7 | 5·3 | 1·6 | 2·1 | 30·7 |
| 5912 | 1·8 | 6·2 | 6·0 | 2·5 | 3·0 | 44·7 |
| 13522 | 1·9 | 5·4 | 5·2 | 1·8 | 1·0 | 29·5 |
| 5906 | 2·0 | 8·1 | 7·2 | 1·6 | 2·0 | 39·8 |
| 5924 | 2·0 | 6·0 | 5·7 | 1·6 | 2·5 | 40·2 |
| 5918 | 2·0 | 6·1 | 5·6 | 1·8 | 2·8 | 42·3 |
| Ptb 21 | 2·1 | 5·5 | 5·2 | 2·3 | 1·3 | 28·0 |
| 14864 | 2·1 | 5·8 | 5·6 | 1·9 | 2·5 | 38·0 |
| 13984 | 2·2 | 5·7 | 5·0 | 2·0 | 1·0 | 25·0 |
| 10443 | 2·2 | 6·0 | 5·7 | 2·7 | 3·3 | 44·1 |
| Ptb 33 (Resistant check) | 1·2 | 5·0 | 4·2 | 1·3 to 1·5 | 0·9 to 1·1 | 16.5 to 25·9 |
| T(N)1 (Susceptible check) | 5·0 | 10·4 | 12·7 | 6·0 to 8·9 | 8·5 to 9·1 | 186·9 to 210·3 |

*Average of 4 observations.

3.2b *Response of adults*: Marked differences were observed in the preference of adult BPH after 36 hr, although some differences were apparent even after 12 hr of their release (table 1). ARC 5780 was least preferred by adults followed by resistant check Ptb 33 and ARC 5838, ARC 5754, ARC 5988 for settling and oviposition. Even on moderately resistant varieties the number of adults settled (1–3·9) and the number of eggs laid (25–63·2) were comparatively lower as compared to the susceptible check T(N)1 on which 8·5–9 adults settled and 186·9–210·3 eggs were laid. However, on some of the resistant varieties (ARC 13854 and ARC 14766A) comparatively more number of eggs

were deposited as compared to other resistant varieties. Choi *et al* (1979) reported that resistant varieties which were non-preferred for feeding did not exhibit the same trend towards oviposition also. The reasons could be inconsistent feeding on resistant varieties. In the present investigation varieties which were non-preferred for feeding and shelter were also non-preferred for egg laying with the exception of ARC 13854 and ARC 14766A. The ovipositional preference for these two resistant varieties, may be due to the presence of ovipository stimulants which needs further investigation.

### 3.3 *Studies on antibiosis mechanism*

3.3a *Survival and development of nymphs*: Antibiosis studies carried out with thirty six varieties indicated that some resistant varieties had adverse effects on BPH nymphs resulting in low survival of the insects (figure 2) as evident with resistant check Ptb 33 (16·5 %), ARC 5780 (18·5 %), ARC 5988 (22·5 %), ARC 5838 (25·6 %), ARC 5981 (26·5 %), ARC 5973 (27·5 %), ARC 5782 (28·5 %), ARC 14766A (26·7 %) and ARC 14394 (28·4 %) as against 90–93·4 % on T(N)1. These varieties also adversely affected the development where the nymphal period was delayed by 3–7 days as compared to that on T(N)1. BPH nymphs took 23 days on resistant check Ptb 33 and ARC 14394; 22 days on ARC 5780, ARC 14766A and ARC 13854.

It was observed that from the 6th to the 21st day there was a gradual decrease in the survival of nymphs. This might be due to nutritional deficiency in the test varieties. However, some varieties *viz.*, ARC 5780, ARC 5988, ARC 5838 had shown relatively higher antibiosis effects. Karim (1975), reported that the survival of the nymphs on resistant varieties *x*B5, Ptb 20 and Mudgo ranged from 8–17 % just 3 days after caging and only 1 and 2 % of the caged nymphs on Ptb 20 and *x*B5 reached the adult stage.

In the present study, a high level of nymphal mortality was not observed as evident by only 19·5–30 % mortality up to the sixth day. This may be either due to the ability of the

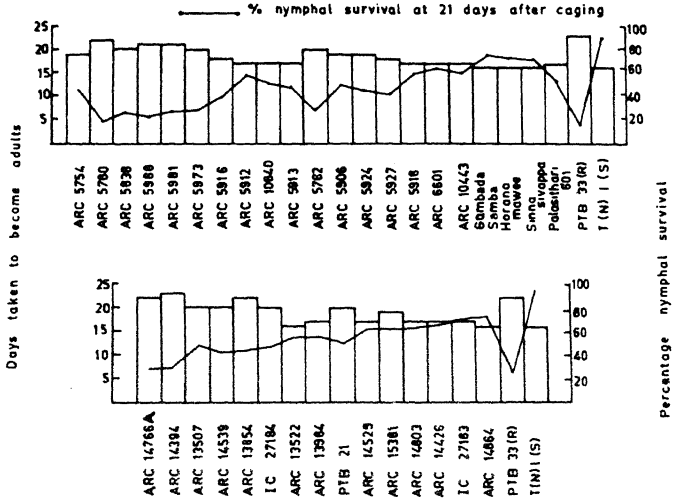


**Figure 2.** Rate of development and percentage survival of *N. lugens* nymphs on selected rice varieties.

biotype involved in the present study to tolerate antibiosis effects in earlier stages of development or the varieties involved might not be having such high concentrations of toxic components required to induce a high mortality in a short period. However, the resistant varieties could confirm their resistance by virtual low survival of BPH on them, while some moderately resistant varieties had relatively higher survival of BPH nymphs and had relatively low antibiosis effects on them. This indirectly suggests that these varieties might be tolerant to the BPH.

### 3.3b *Feeding response of adult BPH*

3.3b(i) *Attempts of feeding—Probing marks*: The results of the probing behaviour indicated that the resistant varieties received more number of probing punctures than the susceptible ones. T(N)1 the susceptible check recorded the least number of probing punctures (31·2) whereas a greater number of probing punctures was observed on resistant varieties (figure 3). Resistant varieties *viz.*, ARC 14394 (121), ARC 14766A (119·4), ARC 13507 (115·6), ARC 5780 (100·2), ARC 5838 (94·6), ARC 5754 (83·6), ARC 5988 (83), ARC 5973 (80) and moderately resistant variety ARC 14803 (100·2) received the maximum number of punctures. On the other hand, varieties *viz.*, Mudgo and ASD 7 reported to be resistant to biotype 2 in Philippines, received less number of probing marks compared to T(N)1 in the present investigation indicating that these varieties were suitable for feeding by the test insect.

It was also observed that the percentage of probing marks on the leaf blade was more on the resistant varieties than on the susceptible varieties. The results indirectly revealed that non-preference of BPH to certain varieties may be gustatory rather than olfactory or visual.

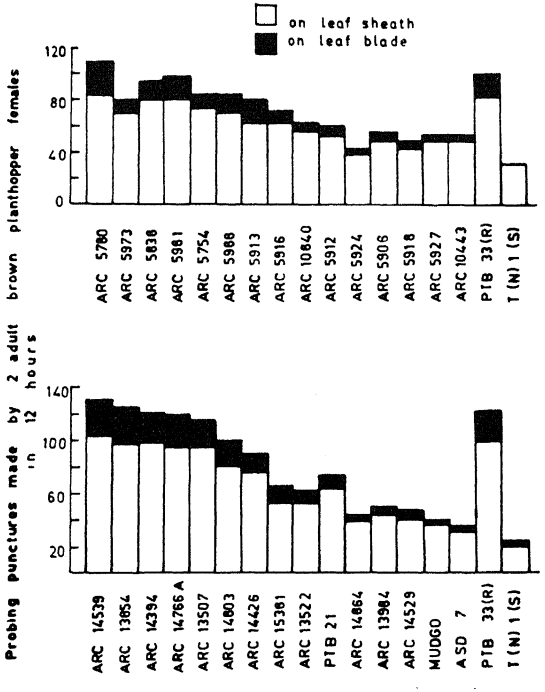


**Figure 3.** Probing punctures made by *N. lugens* adults on selected rice varieties.

Karim (1975) reported that the varieties *x*B5 and HR 12 received significantly higher number of punctures (50·9 and 48·2 respectively) which were about 2–10 times more than that received by other resistant varieties.

3.3b(ii) *Amount of feeding—change in body weight*: In earlier experiments it was observed that the insects suffered high mortality and made more feeding marks when caged on resistant varieties. In order to know whether the insects had actually fed on the test varieties or not, experiments were carried out on the amount of feeding done by the insects. The results showed that the insects caged on the resistant varieties lost their body weight; while on some of the moderately resistant varieties and susceptible check they gained weight (figure 4). Insects lost their body weight up to a maximum of over 33·3 % on ARC 5780 followed by 30 % on ARC 13854 and ARC 13507. However, insects fed on ARC 10443, ARC 6601, ARC 5918, ARC 14864, ARC 13984 and ARC 14529 gained some body weight (5·7 to 13·9 %) as compared to 27·6 % on the susceptible check.

Sogawa (1982) reported that the reduced concentration of phagostimulant amino acids might be the reason for less intake of sap and loss in body weight of the insects which fed on resistant varieties.

3.3b(iii) *Amount of feeding—honey dew excretion*: Amount of feeding by the insects was judged by the honey dew deposited when they were allowed to feed for specified period of time on selected varieties. The results indicated that the BPH adults fed very little on resistant varieties and in turn excreted honey dew in traces (table 2). On resistant varieties ARC 5780, ARC 5973, ARC 5858, ARC 5754, ARC 14539, ARC 13854, ARC 14394, ARC 14766A and ARC 13507, the hoppers excreted as little as 6·6–11·9 times less than that on susceptible T(N)1. As observed in the previous experiments, there was a higher nymphal mortality and loss in body weight on these varieties. These varieties had also received higher number of probing punctures. The less honey dew excretion while

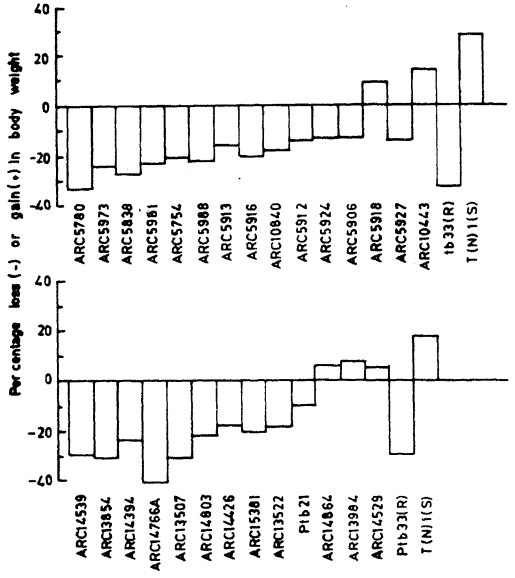


**Figure 4.** Gain or loss in body weight of *N. lugens* adults on selected rice varieties.

**Table 2.** Amount of honey dew excreted by brown planthopper adults caged on selected rice varieties for 24 hr.*

| Variety | Average | Ratio |
|---|---|---|
| *Resistant (R)* | | |
| ARC 5780 | 0·20 | 10·10 |
| ARC 5973 | 0·29 | 6·52 |
| ARC 5838 | 0·17 | 11·88 |
| ARC 5981 | 0·23 | 7·83 |
| ARC 5754 | 0·28 | 6·75 |
| ARC 5988 | 0·22 | 9·18 |
| ARC 14539 | 0·34 | 6·47 |
| ARC 13854 | 0·28 | 7·88 |
| ARC 14394 | 0·20 | 9·45 |
| ARC 14766A | 0·18 | 10·80 |
| ARC 13507 | 0·13 | 6·66 |
| *Moderately resistant (MR)* | | |
| ARC 5913 | 0·66 | 2·86 |
| ARC 5916 | 0·29 | 6·52 |
| ARC 10840 | 0·56 | 3·38 |
| ARC 5912 | 0·33 | 6·12 |
| ARC 5924 | 0·65 | 2·91 |
| ARC 5906 | 0·83 | 2·43 |
| ARC 5918 | 0·87 | 2·32 |
| ARC 5927 | 0·58 | 3·30 |
| ARC 10443 | 0·73 | 2·60 |
| ARC 14803 | 0·56 | 3·93 |
| ARC 14426 | 0·49 | 4·49 |
| ARC 15381 | 0·73 | 3·01 |
| ARC 13522 | 0·81 | 2·72 |
| Ptb 21 | 0·78 | 2·82 |
| ARC 14864 | 0·84 | 2·62 |
| ARC 13984 | 1·03 | 2·13 |
| ARC 14529 | 1·05 | 2·10 |
| Ptb 33 (Resistant check) | 0·14–0·16 | 11·78–14·42 |
| T(N)1 (Susceptible check) | 1·89–2·20 | 1·00 |

*Ten insects per plant; five replications per variety.

feeding on resistant variety may be due to the presence of feeding repellants and/or feeding deterrants or lack of feeding stimulants. It was also observed that the amount of honey dew excreted was in accordance with the degree of resistance, *i.e.*, the insects excreted comparatively little honey dew on resistant varieties than on moderately resistant varieties and susceptible check T(N)1.

Honey dew excretion was considered to be directly proportional to food intake by the insects (Maxwell and Painter 1959; Sogawa and Pathak 1970). Sogawa (1982) opined that reduced concentration of phagostimulant amino acids might be one of the reasons for the less amount of excretion of honey dew on resistant varieties. Kalode and

**Table 3.** Overall performance of selected resistant and moderately resistant varieties to *N. lugens* (Stål).

| Variety | Mass screening | Preference | | Survival of nymphs | Probing puncture | Gain/loss in body weight | Honey dew excreted | Resistance index |
|---|---|---|---|---|---|---|---|---|
| | | Nymphs | adults | | | | | |
| ARC 5780 | RR | RR | RR | RR | RR | RR | RR | 14 |
| ARC 5988 | RR | RR | RR | RR | R | RR | RR | 13 |
| ARC 14394 | RR | RR | RR | R | RR | RR | RR | 13 |
| ARC 5838 | RR | R | RR | RR | R | RR | RR | 12 |
| ARC 14539 | RR | RR | RR | R | RR | RR | R | 12 |
| ARC 14766A | RR | RR | R | R | RR | RR | RR | 12 |
| ARC 13507 | RR | R | RR | R | RR | RR | R | 11 |
| ARC 5981 | RR | R | RR | R | R | RR | R | 10 |
| ARC 5754 | RR | R | RR | R | R | RR | R | 10 |
| ARC 5973 | RR | RR | R | R | R | RR | R | 10 |
| ARC 13854 | RR | RR | S | R | RR | RR | R | 10 |
| ARC 14803 | R | RR | R | R | RR | RR | R | 10 |
| ARC 14426 | R | RR | R | S | R | R | R | 7 |
| Ptb 21 | R | R | RR | R | R | R | S | 7 |
| ARC 13522 | R | R | RR | S | R | R | S | 6 |
| ARC 10443 | R | R | R | S | R | S | S | 4 |
| ARC 13984 | R | R | RR | S | S | S | S | 4 |
| ARC 14864 | R | R | R | S | S | S | S | 3 |
| Ptb 33 | RR | RR | RR | RR | RR | RR | RR | 14 |
| T(N)1 | S | S | S | S | S | S | S | 0 |
| | | | | Grading basis | | | | |
| RR | upto 1·5 | upto 5 nymphs | upto 2 adults | upto 25% survival | above 100 | above 20% loss | upto 0·25 | |
| R | 1·6–3 | 5·1–10 | 2·1–5 | 25·1–50% | 51–100 | upto 20% loss | 0·26–0·60 | |
| S | above 3 | above 10 | above 5 | above 50 | upto 50 | gain in body weight | above 0·60 | |

Krishna (1979) reported that resistant cultivars (Ptb 33, Ptb 21, MR 1523 and ARC 6650) restricted insect feeding and only a little amount of honey dew was excreted by the insects during feeding on these varieties. They further stated that on Leb Mue Nahng and T(N)1 the insects had excreted heavily.

### 3.4 *Association of Lemma and Palea colour with brown planthopper resistance*

Lemma and Palea colour of 1070 rice varieties noted during the preliminary mass screening test was statistically analysed. The results were non-significant indicating that there was no association of Lemma and Palea colour with BPH resistance.

The overall performance of selected rice varieties based on various parameters to understand the mechanism of resistance as summarised in table 3 indicated that insects fed little on the resistant varieties *viz.*, ARC 5780, ARC 5988, ARC 14394, ARC 5838, ARC 14539, ARC 14766A, ARC 13507, ARC 5981, ARC 5754, ARC 5973, ARC 13854, ARC 14803 and lost their body weight. The nymphs also exhibited high mortality on these varieties, and their development period was also prolonged. The mechanism in manifestation of resistance to BPH appeared to be due to non-preference and antibiosis. Most of the varieties which were less preferred by nymphs were also less preferred by adults for settling and oviposition.

However, in some of the moderately resistant varieties like ARC 5918, ARC 10443, ARC 13984, ARC 14529 and ARC 14864 relatively less number of feeding marks, higher amount of honey dew excretion and gain in body weight were observed as compared to the resistant varieties indicating intermediate reaction to the pest. It is evident that the number of probing punctures, amount of honey dew excretion and gain or loss in body weight are all inter-related to the degree of resistance. Based on these parameters ARC 5780, ARC 5988 and ARC 14394 were comparable to resistant check, Ptb 33 in level of resistance.

This paper is part of the M.Sc. thesis submitted by the senior author to the Andhra Pradesh Agricultural University, AP.

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# Larval and post-larval development of *Spodoptera litura* (Fabricius) on some host plants

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**Abstract.** The tobacco caterpillar, *Spodoptera litura* (Fabricius) was reared on different host plants under laboratory conditions. This article reports the growth and development of *S. litura* on different host plants.

**Keywords.** *Spodoptera litura*; host plants; larval development; post-larval development.

## 1. Introduction

The tobacco caterpillar, *Spodoptera litura* is a serious and polyphagous pest of many economically important crops (Basu 1943; Thobbi 1961; Bhattacharya and Rathore 1977). It is a well-documented fact that food plants play a vital role in development, survival and reproductive potential of insects. In the present article we report the growth and development of *S. litura* on different host plants.

## 2. Material and methods

The eggs obtained from the moths reared as larvae on the leaves of castor, okra, groundnut and sunflower were kept in petridishes for hatching. The neonate larvae (100) were reared individually in plastic boxes (5 × 5 cm) on the respective host plants. Food was changed when required. Larvae pupated in the sieved moist soil provided in the plastic boxes. Observations on larval duration, per cent larvae pupated and larval weight on the 11th and 14th day after hatching were recorded. Sexing was made in the pupal stage. The pupal length, width and weight were recorded. The adults emerging from the respective host plants on the same day were paired and released for egg laying in plastic jars (12 × 15 cm) covered with muslin cloth held in position by rubberband. Cotton swabs soaked in 10 % honey was provided daily for adult feeding. Paper strips (7·5 × 5 cm) folded in a zig-zag fashion was provided as oviposition sites. The egg clusters laid were separated from the muslin cloth and paper strips and thus the fecundity was worked out.

## 3. Results and discussion

The host plants exhibited differential response with respect to the percentage pupation, larval duration and growth index of *S. litura* (table 1). The percentage pupation ranged from 44·33–92·21 % on groundnut and okra. The order of suitability of host plants for

**Table 1.** The mean per cent pupation, larval duration and larval weight of *S. litura* on different hosts.

| Host | Mean per cent larvae pupated (*n*) | Mean larval duration (days) | Growth index (*n*/mean days) | Mean larval weight (mg) | | Actual gain in larval weight (mg) |
|---|---|---|---|---|---|---|
| | | | | 11 days after hatching | 14 days after hatching | |
| Castor | 84·44 *a,b* | 11·50 *d* | 7·34 | 941·26 *a* | 1302·74 *a,b* | 361·48 |
| Groundnut | 44·33 *c* | 19·52 *a* | 2·29 | 31·01 *d* | 215·31 *d* | 184.30 |
| Okra | 92·21 *a* | 15·42 *b* | 5·98 | 308·76 *c* | 1107·40 *b,c* | 798·64 |
| Sunflower | 89·99 *a,b* | 12·90 *c* | 6·97 | 648·88 *b* | 1395·94 *a* | 747·06 |

Means in the same column followed by the same letter are not significantly different ($p = 0{\cdot}05$) by Duncan's multiple range test.
*a, b, c, d*—Significance of host plants with each other with Duncan's multiple range test.

pupation was okra > sunflower > castor > groundnut. The larval duration also varied significantly and ranged from 11·50 days on castor to 19·52 days on groundnut. The suitability of host plants for larvae was in the order: castor > sunflower > okra > groundnut. The mean larval weight on the 11th day of age was high (941·26 mg) on castor followed by sunflower, okra and groundnut.

A perusal of table 2 indicates that the host plants influenced the prepupal, pupal duration and mean per cent adult emergence. The mean prepupal and pupal period was longest on sunflower but pupal period on okra was similar to that of sunflower. The mean percentage adult emergence ranged from 41·11 on groundnut to 82·22 on okra. In general, females emerged earlier than males in all the host plants tested.

The results on the pupal measurements are dipicted in table 3. Significant differences were recorded in pupal length, width and weight of *S. litura* when the larvae were reared on different hosts. There was no relation between the pupal length, width or weight and the pupal duration.

The mean preoviposition and oviposition periods did not differ significantly except that on sunflower the preoviposition period was shorter (table 4). The highest fecundity was noted on sunflower (3649·4 eggs) while the lowest was on groundnut (3121·8 eggs). Reproductive index was calculated by dividing mean fecundity by average female pupal weight (Hough and Pimental 1977). On the basis of the reproductive index the host plants could be arranged in a descending order as: groundnut (10·68), okra (9·80), Sunflower (9·75) and castor (8·46). The life cycle from egg to adult emergence was shorter on castor (25·07 days) followed by sunflower (26·66 days), okra (30·03 days) and groundnut (32·80 days). In general females had a shorter life cycle than males. The females lived longer than males.

The mortality in the early instars of the larvae was 55·67 % on groundnut. Tiwari *et al* (1980) observed mortality in the early instars up to 12 days of the larval period due to less intake of leaf tissues of groundnut. The work of Thobbi (1961) and Singh and Byas (1975) also indicated heavy mortality on cotton. The mortality of larvae on okra, sunflower and castor was 7·79, 11·01 and 15·66 %, respectively. However, Singh and Hoi (1972) observed 22 % larval mortality when reared on castor. Bhattacharya and Rathore (1977) recorded 84·70, 80, 73·50 and 77·10 % pupation and growth index values of 5·39, 4·57, 2·93 and 3·97 on castor, cabbage, cotton and soybean, respectively. Bilapate and Thombre (1979) recorded 89·47 % pupation on sunflower at 26 ± 1°C temperature. A similar trend was observed in the present studies also. The differences in the larval period of *S. litura* feeding on many host plants have been reported by many researchers. Basu (1945) reported larval duration of 18–20 days on cauliflower, 14 days on castor and 14–16 days on okra (Thobbi 1961), 18 days on castor (Rattan Lal and Nayak 1963), 18·7 days on castor (Patel *et al* 1965), 15·4 days on castor (Singh and Hoi 1972), 14·18 days on castor (Aleemuddin 1979). The results obtained in the present investigations on larval period on okra and sunflower are in agreement with those of earlier workers (Thobbi 1961; Bilapate and Thombre 1979). Host plants which supported poor larval development gave smaller growth index values as in the case of groundnut. Thobbi and Srihari (1967) obtained high larval weight after 11 days on HC-6 irrigated castor variety. The differences in pupal durations of *S. litura* feeding on different host plants have been similarly demonstrated by different workers (Basu 1943; Singh and Byas 1975; Bhattacharya and Rathore 1977). Basu (1943) established a direct relationship between host plant and pupal duration. In the present investigation however, the larvae with higher growth index values do not necessarily yield pupae of

**Table 2.** The pre-pupal, pupal duration and per cent adult emergence of *S. litura* on different hosts.

| Host | Mean pre-pupal duration (days) | Mean pupal duration (days) | | | Mean per cent adult emergence | | |
|---|---|---|---|---|---|---|---|
| | | Male | Female | Both | Male | Female | Both |
| Castor | 1·14 b | 9·55 *b* | 7·37 *a* | 9·24 *a* | 27·77 *b,c* | 23·33 *b* | 51·11 *b,c* |
| Groundnut | 1·24 *a,b* | 9·68 *b* | 6·66 *a* | 8·98 *a* | 23·33 *b* | 17·77 *b* | 41·11 *c* |
| Okra | 1·05 *b* | 10·33 *a* | 8·77 *a* | 9·50 *a,b* | 38·88 *a,c* | 43·33 a | 82·22 *a* |
| Sunflower | 1·42 *a* | 9·86 *a,b* | 8·84 *a* | 9·50 *a* | 46·66 *a* | 26·66 *b* | 73·33 *a,b* |

Means in the same column followed by the same letter are not significantly different ($p = 0{\cdot}05$) by Duncan's multiple range test.

**Table 3.** The mean pupal length, width and weight of *S. litura* on different hosts.

| | Mean pupal length (mm) | | | Mean pupal width (mm) | | | Mean pupal weight (mg) | | |
|---|---|---|---|---|---|---|---|---|---|
| Host | Male | Female | Both | Male | Female | Both | Male | Female | Both |
| Castor | 19·20 *a* | 18·70 *a* | 18·97 *a* | 5·75 *a* | 6·02 *a* | 5·97 *a* | 368·46 *a* | 374·22 *a* | 373·98 *a* |
| Groundnut | 18·64 *a* | 15·44 *a* | 18·50 *a* | 5·69 *a* | 4·93 *a* | 5·76 *a* | 330·64 *a* | 292·19 *a* | 334·57 *b* |
| Okra | 18·37 *a* | 18·86 *a* | 18·64 *a* | 5·51 *a* | 5·83 *a* | 5·67 *a* | 294·74 *c* | 348·71 *a* | 321·68 *b* |
| Sunflower | 18·82 *a* | 18·71 *a* | 18·77 *a* | 5·82 *a* | 5·95 *a* | 5·86 *a* | 350·44 *a,b* | 374·22 *a* | 357·41 *a* |

Means in the same column followed by the same letter are not significantly different ($p = 0{\cdot}05$) by Duncan's multiple range test.

**Table 4.** The mean pre-oviposition, oviposition, fecundity and longevity of *S. litura* on different hosts.

| Host | Mean pre-oviposition period (days) | Mean oviposition period (days) | Fecundity | Mean life cycle (days) | | | Mean adult longevity (days) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Male | Female | Both | Male | Female | Both |
| Castor | 2·1 *a* | 4·00 *a* | 3166·8 *a* | 25·48 *d* | 20·17 *a* | 25·07 *d* | 6·00 *a,b* | 6·70 *a* | 6·35 *b* |
| Groundnut | 2·0 *a* | 3·80 *a* | 3121·8 *a* | 33·40 *a* | 26·70 *a* | 32·80 *a* | 6·30 *b* | 6·30 *a* | 6·30 *a,b* |
| Okra | 1·5 *a* | 4·10 *a* | 3420·5 *a* | 30·65 *b* | 29·46 *a* | 30·03 *b* | 8·70 *a* | 7·20 *a* | 7·95 *a* |
| Sunflower | 1·2 *a* | 4·00 *a* | 3649·4 *a* | 27·00 *c* | 25·91 *a* | 26·66 *c* | 6·10 *a,b* | 5·90 *a* | 6·00 *a,b* |

Means in the same column followed by the same letter are not significantly different ($p = 0{\cdot}05$) by Duncan's multiples range test.

shorter duration and as such a definite relationship between the food plant and the pupal duration is difficult to establish. Similar observations were recorded by Pandey and Srivastava (1967). Basu (1945) demonstrated an inverse relationship between weight of pupae and pupal duration while Singh and Byas (1975) indicated positive relationship both for length and weight with pupal period. Pandey and Srivastava (1967) did not observe such a relationship for 24 wild host plants and presumed the role of some intrinsic physiological factors to be associated with it. Bilapate and Thombre (1979) reported 1721·23 and 1855·23 fecundity at 26 and 30°C temperature. Aleemuddin (1979) observed 2650·69 eggs per female on castor. In the present findings, the fecundity is at variance with those of previous workers. These variations may be due to the substrate used, rearing techniques or intensive care during rearing. Aleemuddin (1979) reported a 25·69 days life cycle on castor. In order to understand the suitability of host plants for *S. litura*, in all thirteen characters of biology were considered. The host plants were arranged for each character and scored by giving four points for the first position and reducing one point for each subsequent position. The total score for each host plant was considered by arranging them in order of suitability. The host plants were arranged in an ascending order of characters like: larval duration, pupal duration, life cycle, pre-oviposition period, per cent larvae pupated, larval weight, growth index, per cent adult emergence, pupal weight, oviposition period, fecundity, longevity of adults and reproductive index. Thus the order of suitability of host plants with the respective total points obtained was: castor (36); okra (36); sunflower (36) and groundnut (22).

**Acknowledgement**

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# Activity-time budget in blackbuck

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**Abstract.** The activity patterns of blackbuck observed at Mudmal showed that feeding accounted for the maximum frequency (75%) with an average duration of 77 sec followed by standing with a frequency of 62% and an average duration of 19·9 sec. Lying although had only 4% frequency, showed an average duration of over 30 min.

The hourly time budgets for basic activity patterns during the day in a season varied greatly for both females and territorial males. Rhythms of feeding and lying peaks occurred alternately during the day in all seasons. The time budgets for the activity patterns showed seasonal variation. Lying time per day was more than the average time allotted to any activity. In the case of females, the average time spent for feeding per day during summer was 25% which was more than that of monsoon and winter. The time spent in lying was 39% which increased to 48% in monsoon and winter. The average time spent in walking and standing did not show any significant seasonal variation. The time budgets for the territorial males also showed the same tendency as that of the females in all seasons. During winter, however, the feeding time per day was 11% while the lying time was 57%, the former being significantly less and the latter significantly greater than the females.

**Keywords.** Activity-time budget; blackbuck.

## 1. Introduction

Few quantitative studies have been published on the daily activity patterns of blackbuck. These include the work of Schaller (1967) and Nair (1976) in India and Mungall (1978) in Texas which describe the distribution of percentage of animals engaged in basic activities in hourly classes from dawn to dusk. Based on a month's study Mungall *et al* (1981) have compared the quantitative time budgets of males and females of different age-classes in different social categories.

The activity patterns are determined by a wide range of factors, both biotic and abiotic, and information is lacking as to how blackbuck adapt and maintain themselves to seasonal changes. In the present paper, the time budgets for basic activities of blackbuck are described for both sexes based on the studies at Mudmal (16 24'N and 77 27'E) during 1979–80 and the seasonal differences discussed.

## 2. Methods

Data were recorded on all activity categorised as feeding, walking, running, standing, lying, and 'other activities' from dawn to dusk. The recordings made were as follows: The activity of 11 identified territorial males was separately recorded at different times of the day with the help of a stop watch. Recording of activity of females, however, was

difficult due to difficulty to distinguish them individually. One female was randomly selected from a herd and its activities recorded. On occasions when such an individual under observation was confused or was out of sight, another adult female was randomly selected from among the members visible. As a large number of observations thus made showed synchrony among female members, the data collected were fairly accurate. The activity patterns of the territorial males and females obtained thus represent their activities from all social categories of different herd sizes although majority of them were from mixed herds of atleast 10 head.

An activity was considered when the time spent in that activity exceeded 30 sec before changing to the next activity. The average time spent by the territorial males in each hour of daylight was calculated separately for summer, monsoon, and winter seasons and expressed in percentage. Thus the activity curves composed of a fraction of the 12 hr rhythms of the individuals recorded on different days in a season.

Most of the continuous observations lasted for 4–6 hr, although on a few occasions activity records were obtained for 10 hr continuously without external disturbance. Observations without continuous record between two consecutive daylight hours were not included in calculations.

The seasonal differences in the activity patterns of the territorial adult males and females were separately calculated for all daylight hours by employing the following formula:

$$d = \frac{(k_1 - k_2)}{\{k(1-k)(1/n_1 + 1/n_2)\}^{1/2}}$$

where $k_1 = a_1/n_1$; $k_2 = a_2/n_2$ and

$$k = (a_1 + a_2)/(n_1 + n_2)$$

$a$ = total time spent in a given activity in a daylight hour over the period of observation in a given season,

$n$ = total time spent for all activities in that hour for the period of observation in that season.

The mean time spent in an activity category in a day was calculated and comparisons made between territorial males and females and the seasonal differences within them.

Apart from the preparation of time budget, nearly 5 hr of continuous record of observation was utilized for obtaining the frequency of occurrence of the activity categories and the association between them through hierarchical cluster analysis (De Ghet 1978). Thus,

$$\text{Jaccard's association coefficient} = A/(A) + (B) + (C)$$

where, $A$ = number of times both activities (say feeding and walking) occurred,
$B$ = first activity (feeding) occurred and second (walking) not occurred,
$C$ = second activity (walking) occurred and first (feeding) not occurred.

The transition probabilities of one activity following the other, as for example feeding followed by walking and so on, was calculated using Markov models (Fagen and Young 1978). Thus, transition probability of an activity $P_{ij}$ = (Number of times activity $i$ followed by activity $j$)/(Total number of times of occurrence of activity $i$). The expected probability of an activity $i$ was calculated by the following equation:

$$\frac{\text{Sum of occurrence of an activity } i \times \text{sum of occurrence of an activity } j}{\text{Total number of times of occurrence of all activities}}$$

## 3. Results

### 3.1 *Activity frequency*

The frequency distribution of the activity categories is shown in table 1. Feeding showed highest frequency (37·69%) followed by standing (31·16%). The average duration of feeding, however, was just over a minute. Feeding was disrupted frequently either by standing or by walking. Lying which showed only 4%, on the other hand, was continuous for a longer spell with an average duration of over 30 min. The maximum duration of an individual continuously in lying activity was 2·5 hr.

### 3.2 *Activity sequence*

The association between the activity categories is shown in figure 1. Feeding and standing were in maximum association with each other with a value of 0·79. The association index of walking and feeding with standing was 0·71 followed by running (0·36). The association index of lying with the rest was 0·03.

Table 2 gives the transition probability of one activity following the other along with the expected values. The probability of standing followed by feeding was higher than feeding followed by standing. The probability of feeding followed by walking is below the expected value. On the other hand, walking followed by feeding showed a probability higher than that expected. Running had an equal probability of being followed by standing and walking. Similarly lying was either followed immediately by

**Table 1.** Transition probabilities of one activity following the other.

| Preceding acts | Following acts | | | | | |
|---|---|---|---|---|---|---|
| | *F* | *S* | *W* | *R* | *L* | *O* |
| Feeding (*F*) | 0·0 | 0·55 | 0·42 | 0·0 | 0·03 | 0·0 |
| | 0·0 | 0·51 | 0·43 | 0·02 | 0·03 | 0·02 |
| Standing (*S*) | 0·69 | 0·0 | 0·29 | 0·02 | 0·0 | 0·02 |
| | 0·27 | 0·0 | 0·18 | 0·007 | 0·01 | 0·007 |
| Walking (*W*) | 0·63 | 0·31 | 0·0 | 0·02 | 0·02 | 0·02 |
| | 0·52 | 0·43 | 0·0 | 0·015 | 0·02 | 0·015 |
| Running (*R*) | 0·0 | 0·5 | 0·5 | 0·0 | 0·0 | 0·0 |
| | 0·385 | 0·315 | 0·265 | 0·0 | 0·015 | 0·02 |
| Lying (*L*) | 0·0 | 0·67 | 0·33 | 0·0 | 0·0 | 0·0 |
| | 0·39 | 0·32 | 0·27 | 0·01 | 0·0 | 0·01 |
| Other (*O*) activities | 0·0 | 0·5 | 0·5 | 0·0 | 0·0 | 0·0 |
| | 0·385 | 0·315 | 0·27 | 0·02 | 0·01 | 0·0 |

The underlined numbers indicate the expected probabilities while the others are

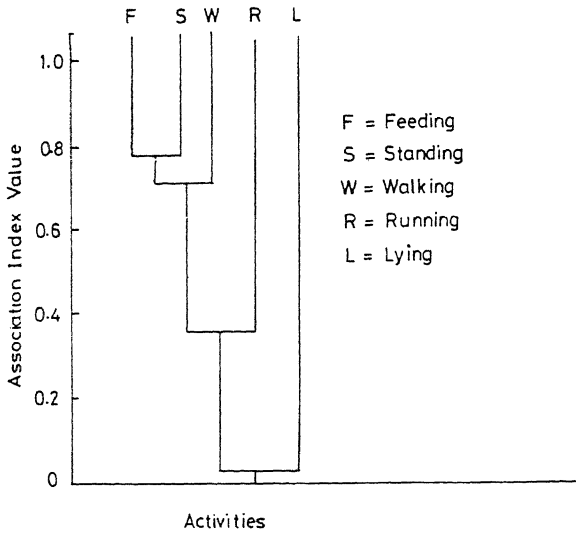


**Figure 1.** Dendrogram showing association between basic activity patterns.

**Table 2.** Frequency and duration of activity categories.

| Activity | Frequency | % Frequency | Total duration (sec) | % Duration | Average duration (sec) |
|---|---|---|---|---|---|
| Feeding | 75 | 37·69 | 5809 | 34·29 | 77·45 |
| Walking | 54 | 27·14 | 2461 | 14·53 | 45·57 |
| Running | 2 | 1·01 | 8 | 0·05 | 4·00 |
| Standing | 62 | 31·16 | 1237 | 7·30 | 19·95 |
| Lying | 4 | 2·01 | 7375 | 43·53 | 1843·75 |
| Other activities | 2 | 1·01 | 53 | 0·31 | 26.50 |

standing or walking before the start of any other activity, although the former h higher probability than the latter.

The 'other activities' usually were followed either by standing or by walking equal chance of occurrence.

### 3.3 *Activity-time budget of females*

The time spent by females in the activity categories from 7–8 hr during the three sea is shown in figure 2. The activity records between 13–17 hr were not represente summer as the observations were discontinuous. This data, however, showed animals spending most of the time lying down. Due to this reason it became too dif to locate them.

In summer bouts of feeding were noticed in all daylight hours observed with at 20% of time. In winter there were distinct peaks of feeding with a maximum of

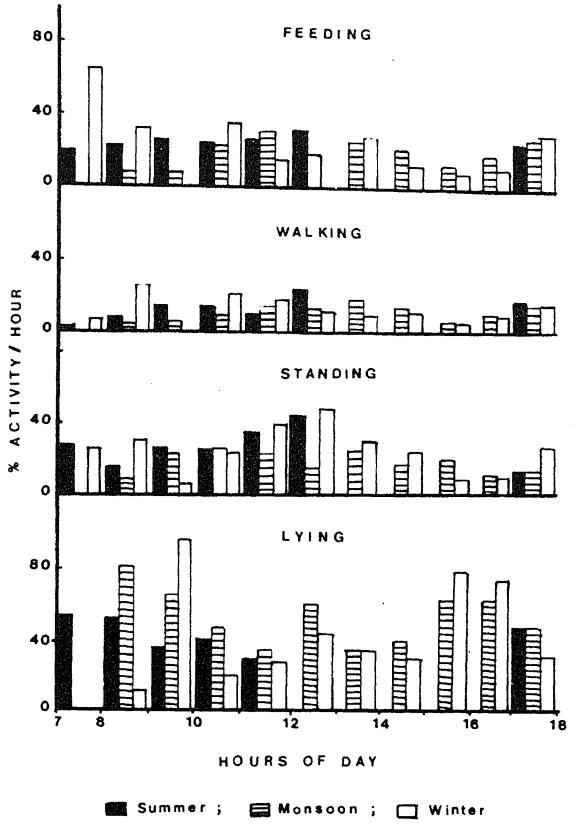


**Figure 2.** Seasonal activity patterns of females.

between 7–8 hr and 12–13 hr. During the rest of the daylight hours feeding time ranged between 8% and 15%. The time spent in feeding between 7–8 hr in winter was significantly greater than that of summer ($d = 7{\cdot}02$, $p < 0{\cdot}001$).

During monsoon, in the early hours feeding was very less (8% between 8–10 hr). However, it increased to 22% during 10–11 hr. Between 11–12 hr feeding time was 32%, the maximum noticed in the season. During the rest of the daylight hours the time apportioned for feeding ranged from 12–26%. There were no significant differences in the feeding time spent in 10–11 hr, 11–12 hr and 17–18 hr of monsoon and summer; 15–16 hr and 17–18 hr of monsoon and winter; and 8–9 hr and 17–18 hr of winter and summer. During the rest of the day hours the differences in feeding time between seasons was significant at $p < 0{\cdot}001$.

During summer and monsoon, the time for walking showed a gradual increase from 7–13 hr. Contrary to this, winter walking time decreased from 26% (9–10 hr) to 5% (15–16 hr) and again showed an increase after 17 hr.

Standing showed an increase from morning with an afternoon peak in all seasons. The maximum time spent in an hour was 44% (12–13 hr) in summer, 26% (13–14 hr) in monsoon and 48% (12–13 hr) in winter.

Lying showed definite peaks in monsoon and winter. In monsoon, when data was recorded from 8–18 hr, the maximum time spent was 80% between 8–9 hr. The rest of

**Table 3.** Average proportion of time spent per day by females and territorial males in the activity categories.

| Category | Season | % Time Feeding | Walking | Standing | Lying | Other activities |
|---|---|---|---|---|---|---|
| Territorial males | Summer | 25 | 11 | 23 | 37 | 4 |
| | Monsoon | 17 | 13 | 17 | 47 | 6 |
| | Winter | 11 | 11 | 16 | 57* | 5 |
| Females | Summer | 25 | 11 | 24 | 39 | 1 |
| | Monsoon | 19 | 10 | 23* | 48 | 0 |
| | Winter | 16* | 10 | 21* | 48 | 5 |

*Comparison between females and territorial males and allotment of significantly more time than the other at $p < 0{\cdot}001$.

the daylight hours showed 30% (11–12 hr) to 64% (9–10 hr). During winter the maximum time allotted was 94% (9–10 hr) in the morning and 64% (15–16 hr) in the afternoon. During the remaining hours of the day the amount of time spent ranged between 10% (8–9 hr) and 72% (16–17 hr).

During summer, over 50% time was spent on lying between 7–8 hr which decreased to 34% between 9–10 hr. It again increased to 40% between 10–11 hr and fell to 28% in the succeeding hour. During 17–18 hr again 45% of the time was spent in this activity.

Females spent an average of 25% of the day time for feeding, 39% for lying while walking and standing shared 11% and 14% respectively during summer season (table 3). The average feeding time decreased to 19% in monsoon and 16% in winter and the time spent in lying increased to 48% in these two seasons. Walking and standing showed a slight decrease.

### 3.4 *Activity-time budget of territorial males*

The activity-time budget of the territorial males in the three seasons is shown in figure 3. As in females, feeding and lying were the main activities to be focussed upon among all activities in all seasons. During summer two peaks of feeding were clear between 13–14 hr (58%) and 16–17 hr (61%). Between 7–8 hr and 17–18 hr about 38% of the time was spent in this activity. The minimum time spent in this activity was 14% between 8–9 hr. The rest of the daylight hours have feeding time ranging between 20 and 26%. Between 14–15 hr the data were not represented as the number of observations were very few.

During monsoon and winter there were no significant peaks in feeding activity. The maximum and minimum amount of time spent in feeding was 25% (12–13 hr) and 7% (17–18 hr) respectively. The time allotment for feeding between 7–8 hr, 13–14 hr and 15–16 hr during summer is significantly greater ($p < 0{\cdot}001$) than monsoon and winter in the corresponding hours. The total time spent in feeding per day was also higher in summer (25%) than in monsoon (17%) and (11%) which was statistically significant ($d = 8{\cdot}78$ for monsoon and summer; $d = 15{\cdot}84$ for winter and summer; $p < 0{\cdot}001$). The total time spent in feeding per day during monsoon was significantly more than that during winter ($d = 9{\cdot}17$; $p < 0{\cdot}001$).

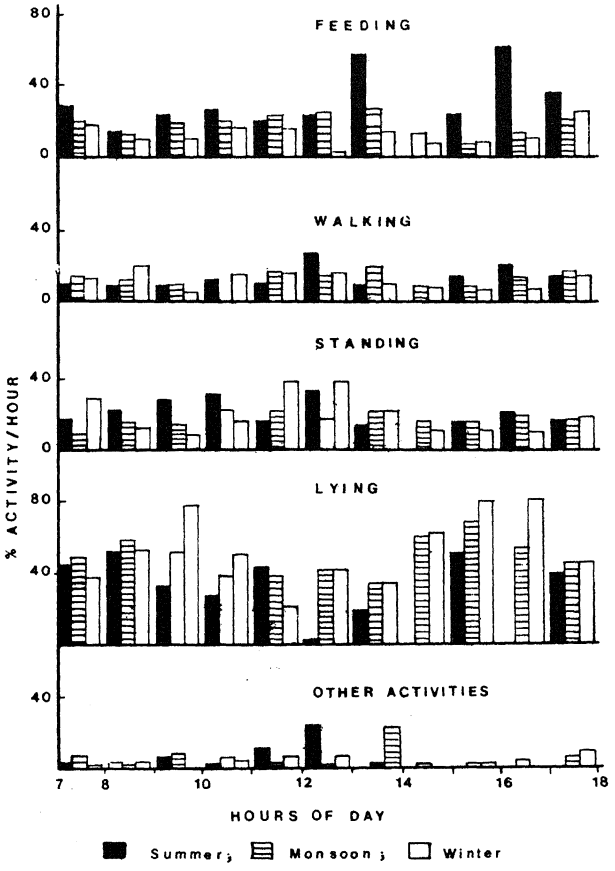


**Figure 3.** Seasonal activity patterns of territorial males.

There were marked differences in lying in the three seasons. In summer lying was more between 7–8 hr, 11–12 hr and 15–16 hr and the time spent ranged between 54–62%. Between 12–13 hr there was practically no lying. During monsoon, as in the case of summer, morning and evening hours showed more lying activity and there was an afternoon dip. Fifty to sixty per cent of the time was spent in this activity between 7–10 hr while between 10–14 hr only 34–42% time was spent. The subsequent day hours till 18 hr had 46–68% of time in this activity. During winter, there were periodical oscillations in lying. Thirtyeight per cent of time was spent between 7–8 hr which significantly increased to 78% between 9–10 hr. Lying time decreased to 21% during 11–12 hr and again increased to 42% between 12–13 hr. In the subsequent hours there was once again a decrease in this activity. But between 15–17 hr it touched a maximum of 80%. Compared to summer and monsoon seasons, winter had significantly more time in lying between 9–10 hr 11–12 hr; 13–14 hr and 15–17 hr ($p < 0{\cdot}001$). The total lying time per day was also maximum during winter (57%) followed by monsoon (47%) and summer (37%) and the differences were statistically significant ($d = 10{\cdot}37$ for summer and monsoon, and for winter and monsoon; $d = 17{\cdot}45$ for winter and summer; $p < 0{\cdot}001$).

## 4. Discussion

The maintenance of life in a mammal requires a variety of activities associated with procurement of food, shelter and protection. Each of these activities had a certain benefit and cost attached to it (Sharatchandra and Gadgil 1980). An obvious principle is that the extent of activity must be adequate to maintain the kind of life permitted by the animal's anatomic and physiological adaptations (Davis and Golley 1963). Through the time budget for basic activities of blackbuck, the following explanation emerges to show as to how they maintain themselves in different seasons.

During summer forage material in general and fresh foliage in particular was very scarce. Blackbuck being mainly grazers and preferring the tender leaf were rather forced to spend much of their time in search of food. This is the reason for their allotting a significantly large proportion of time for this purpose as compared to other seasons (table 3). During monsoon sprouting of grasses was in abundance and they could easily obtain their nourishment. During winter blackbuck take to crops, which are more readily available. The crops have more crude protein content than grasses and hence a lesser quantity may suffice their energy requirements. This probably is the cause for a further drop in feeding time during winter. This enabled them to spare more time for lying and ruminating in monsoon and winter.

Lying time was significantly more while the feeding and standing time was less in

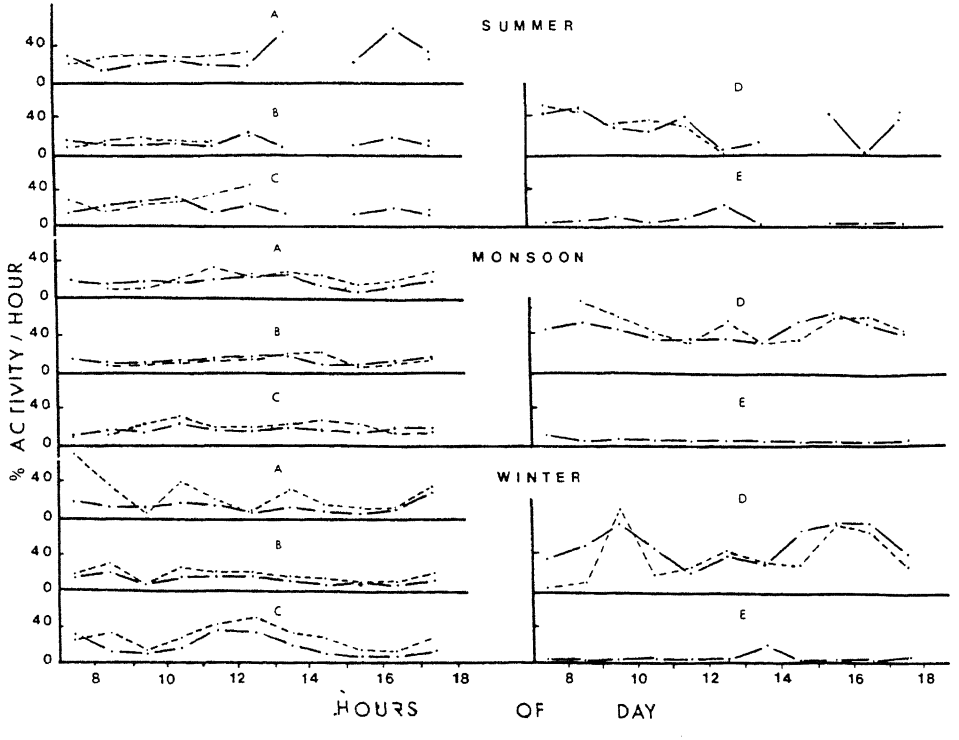


**Figure 4.** Comparison of activity patterns between females and territorial males. **A** to **E**: Feeding, Walking, Standing, Lying and other activities, respectively. Broken line indicates the activity of females while continuous line refers to the activity of the territorial males.

territorial males than in females during winter and monsoon. A major portion of lying time was spent by territorial males within or adjacent to their territories which were located near the cultivated fields. They thus had a better access for a quick bite and hence could spend the remaining time for lying and ruminating. They could also watch the activities of females and keep the bachelor associations away from their territories, which move from one area to the other.

The females showed a significantly more time for standing than the territorial males. A plausible explanation for this could be, the females in general were more cautious and watchful, both while feeding and while at rest, than the territorial males. This may be because the females mostly were seen in groups and could easily be spotted and would probably need to doubly ensure whether there would be any sort of danger before they resort to bedding.

If we look at the hourly activity-time budgets, in most cases the general pattern of curves in both territorial males and females showed a more or less similar tendency of increase and decrease (figure 4). This indicates that their response to the changing conditions in different seasons is more or less the same. The differences in some daylight hours may be due to the environmental parameters such as temperature, relative humidity, presence of shade in the area, wind speed etc. which, however, were not measured during the present study.

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# Some biochemical changes in the reproductive cycle of a hill stream teleost *Puntius chilinoides* (McClelland)

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**Abstract.** The protein content was highest in the ovaries of *Puntius chilinoides* during the maturing stage and in the testes during the mature stage. The activity of the acid phosphatase and the number of isozymes decreased in the testes during maturation, whereas in the ovaries the activity increased during the maturation and spent stages. The alkaline phosphatase activity in the testes increased during maturation phase, while in the ovaries the highest activity of the enzyme was recorded at the maturing stage and the lowest during the mature stage. Cholesterol level in the ovaries was highest during the maturing stage, while in the testes it was noticed during the immature stage. The sugar contents in the gonads were highest at the mature stage. The results are discussed in relation to the reproductive cycle in *P. chilinoides*.

**Keywords.** Biochemical changes; protein; acid phosphatase; alkaline phosphatase; cholesterol; sugars.

## 1. Introduction

It is well known that several metabolic changes occur during the development of gonads and in fact all the metabolic activities inside a developing tissue are ultimately under some biochemical control. The metabolic activities are controlled by the enzymes. Now it is clear that lysosomes are the main organelles where the acid hydrolases like the acid phosphatase are localised. The alkaline phosphatase is also much important in animal tissues. Phosphatases in general play a very important role in phosphate ($P_i$) availability in the tissues. Inorganic phosphate ($P_i$) is required in the synthesis of several metabolites during developmental stages. On the other hand, carbohydrates, fats and cholesterol also play a significant physiological role during the developmental stages in gonads.

There are several reports on the biochemical changes that occur during growth and development in fish. Lal (1963) reported decline in protein contents in the ovaries in *Cirrhina mrigala* during maturation. Contrary to this, Ehlebracht (1973) reported an increase in the protein content during maturation. Wegmann and Goetting (1971) studied a distribution of protein, polysaccharides, nucleic acids and fats in *Xiphophorous helleri*. Shaffi *et al* (1974) have reported higher alkaline phosphatase activity in the ovaries of *Clarias batrachus* during maturation. Siddiqui (1966) in *Channa punctatus*, Singh and Singh (1979) in *Heteropneustes fossilis* and Sen and Bhattacharya (1981) in *Anabas testudineus* reported the cholesterol level in different stages of maturity in the gonads.

Studies on the biochemical changes during the development of the gonads in hillstream fishes are scanty. Therefore, in addition to the seasonal morphohistological

studies of the gonads and pituitary gland, the biochemical changes in protein, acid phosphatase, alkaline phosphatase, cholesterol and sugar contents of the gonads of a hillstream minor carp *Puntius chilinoides* of Garhwal Himalaya during different stages of development were studied.

## 2. Material and methods

On the basis of detailed seasonal morphohistological changes in the gonads and pituitary gland of *P. chilinoides*, the following stages of development *viz* the immature, maturation, mature, spent and resting stage have been studied. The important morphohistological changes have been observed during immature, maturation, mature and spent stages, therefore, the present biochemical study has been conducted during these stages of maturity. Sexually mature *P. chilinoides* of each sex were collected regularly from the Khandagaad, a tributary of Alaknanda. The soluble protein contents of the gonads were determined by the method of Lowry *et al* (1951). The soluble sugar contents were estimated by the Anthrone method (Mac Cready *et al* 1950). The total cholesterol was determined by the modified method of Zlatkis *et al* (1953). For the determination of phosphatases, the homogenates were prepared in cold grinding medium consisting of 0·1 M Tris-HCl buffer (pH 7·5) and centrifuged at 2000 × g. The acid phosphatase activity was determined by the method of Baijal *et al* (1972). The assay system comprised 1 ml of 0·2 M acetate buffer (pH 5·5) 0·1 ml 0·2 M $MgSO_4$ and enzyme preparation and water, making the volume 1·9 ml. The alkaline phosphatase activity was determined following the method described by Bodansky (1932), using 1 ml 0·2 M barbitone buffer (pH 9). For both the phosphatases 0·1 ml of 0·1 M $\beta$-glycerophosphate was added as a substrate. Phosphate was determined following the method described by Fiske and Subbarow (1925). The unit of the enzyme activity was expressed as the amount which librates one $\mu$ mole of pi per minute at 37°C.

Change in protein profile and isozymes of acid phosphatase in the gonads of *P. chilinoides* were analysed for qualitative changes in their protein and acid phosphatase composition by means of disc electrophoresis. A 20% (*W/V*) homogenate was prepared by grinding the tissue in pre-chilled tris-HCL buffer (pH 7·5) at 0·5°C and centrifuged at 2000 × g. The supernatant was used for electrophoritic separation of protein and acid phosphatase using three gels for each sample. The method of disc electrophoresis in polyacrylamide gel as described by Davis (1964) was followed. 0·15 ml extract with 0·05 ml of 1 M sucrose was layered on 7·5% acrylamide gel using bromophenol blue as a tracking dye. The samples were run in cold at pH 8·3 using Tris-glycine buffer with a current of 3 mA per tube. The process was carried out till the tracking dye reached the lower end of the gel. The gels were then taken out. For proteins, the gels were stained in 0·25% Coomassie brilliant blue for 15 hr and destained in 7% acetic acid at 5 mA current per tube.

For isozymes of acid phosphatase the gels were incubated in proper incubation mixture as described by Brewbaker *et al* (1968). The zymograms of the stained gels were prepared and the transmittance of bands was measured with the help of a densitometer (Toshniwal, type CM 11).

## 3. Observations

### 3.1 *Protein*

The protein contents were higher in the ovaries in comparison with the testes during all the four stages (immature, maturing, mature and spent) (figure 1A). In the testes, the protein contents increased during the maturing stage and the highest value of protein was observed at the mature stage; and a sharp decline in the spent stage was observed. In the ovaries, the protein contents increased only during maturing stage (stage II). The protein contents then showed a slight decline in the mature period and finally a sharp decline at the spent stage.

### 3.2 *Acid phosphatase*

The activity of the acid phosphatase showed a marked decrease during maturation in the testes. The activity was highest at the immature stage and a significant decrease in

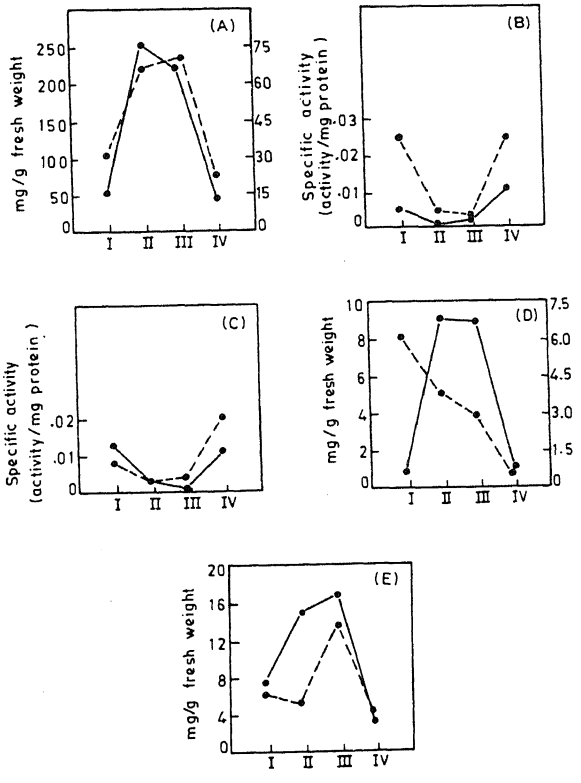


**Figure 1.** Seasonal biochemical changes in the testes (●—●) and ovary (●---●) of *P. chilinoides*. **A.** Protein; **B.** Acid phosphatase; **C.** Alkaline phosphatase; **D.** Cholesterol; **E.** Sugar. (I, II, III and IV represent immature, maturation, mature and spent phases respectively).

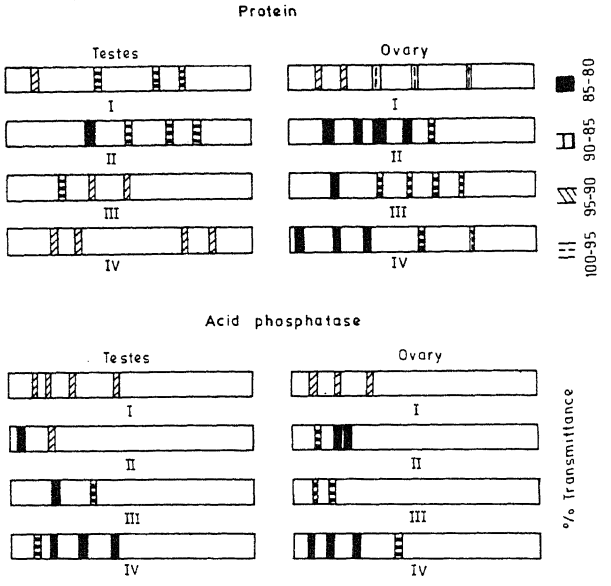


**Figure 2.** Zymograms of protein and acid phosphatase by polyacrylamide gel electrophoresis of the gonads of *P. chilinoides*. (I, II, III and IV represent immature, maturation, mature and spent phases respectively).

the activity was observed at maturing and mature stages. During the spent stage the activity increased significantly. The activity of this enzyme showed a different pattern in the ovaries. The acid phosphatase activity in the ovaries was found highest at the immature stage. At the maturing stage a significant decrease was noticed after which there was a continuous increase at the mature and spent stages (figure 1B). The activity of the acid phosphatase and the number of the isozymes decreased in the testes during maturation, whereas in the ovaries the activity increased during maturation and spent stages (figure 2).

### 3.3 *Alkaline phosphatase*

In the ovaries the highest alkaline phosphatase activity was recorded at the maturing stage which decreased sharply up to the mature stage. At the spent stage the activity again increased. In the testes it showed a different pattern. The activity was lowest at the maturing stage and then continuously increased up to the spent stage (figure 1C).

### 3.4 *Cholesterol*

The cholesterol content in the testes was highest in the immature stage. The highest cholesterol level in the ovaries was found during the maturing and the lowest during the immature stage (figure 1D).

### 3.5 *Sugars*

The amount of soluble sugars in both the gonads was highest at the mature stage as compared to the other three stages (figure 1E).

### 3.6 *Changes in protein profile and isozymes of acid phosphatase*

Five protein bands were observed at all the four stages of development in the ovary. However, in the mature stage the first band had a very low *Rf* value, whereas the band having the highest *Rf* value in the immature, mature and spent stages was not detected in the maturing stage. In the testes four protein bands were detected at all the stages except at the mature stage where only three bands were detected and the fourth band (the band of highest *Rf* value) was absent. The *Rf* value of each individual band was different at different stages.

In the ovary four bands of acid phosphatase were detected at the spent stage, only two bands at the mature stage and three bands at the immature and maturing stages. The first and the second band (I and II from origin) had the same *Rf* values at all the four stages. In the testes acid phosphatase activity appeared as four bands at the immature and spent stages with the same *Rf* values, although the *Rf* values of the third and fourth band in the spent stage was slightly less. The maturation and mature stages showed only two bands, one band in common (figure 2).

## 4. Discussion

The protein contents in the ovaries was much higher than in the testes during the annual cycle which is in consonance with previous studies. The low protein content at the spent stage in *P. chilinoides* is indicative that rapid protein synthesis is necessary only during maturation for the developing oocytes and sperms. The protein profile of the ovaries and testes also indicated that the original bands disappear during the developmental stages, indicating that the new proteins are synthesized.

The activity of the acid phosphatase and the number of isozymes decreased in the testes during maturation and spent stages. These results indicate that in the ovaries the acid phosphatase plays a significant role in the synthesis of essential metabolites by liberating Pi. However, in the testes the decline in the acid phosphatase activity is probably indicative of the fact that the enzyme apparently plays a less significant role during maturation (stage II and III), but seems to play a significant role during the spent stage as the level of the enzyme activity increases during the spent stage.

Shaffi *et al* (1974) have reported higher alkaline phosphatase activity in the ovaries of *Clarias batrachus* during maturation. *In P. chilinoides* the alkaline phosphatase activity increased in the ovaries during the maturing phase (stage II) indicating that during this period the synthesis of new proteins takes place as alkaline phosphatase has been reported to be involved in protein synthesis (Shaffi *et al* 1974). At the mature stage the activity of alkaline phosphatase was lower, showing a decline during the process of maturation. In the testes the alkaline phosphatase activity showed a different pattern. The activity was lowest at the maturing stage (stage II) and then continuously increased

up to the spent stage, suggesting that in the testes the alkaline phosphatase plays an important role during the development of the sperms.

Siddiqui (1966) recorded maximum ovarian cholesterol level in the gonads of *Channa punctatus* at the end of the maturing phase, while in *Heteropneustes fossilis* Singh and Singh (1979) observed a decline in the cholesterol level of the ovaries during the pre-spawning phase, but an increase during the spawning phase. In *Anabas testudineus* Sen and Bhattacharya (1981) reported low ovarian cholesterol level during the pre-spawning phase and high cholesterol level during the post-spawning phase.

In *P. chilinoides*, the high cholesterol level in the ovaries was found during the maturing stage and the lowest during the immature stage, while in the testes the high level of cholesterol was noticed during the immature stage and lowest during the spent stage. It is considered that the high cholesterol level in the gonads acts as a reservoir to meet the cholesterol demand of the maturing gonads and the decreased level might be due to the increase in the rate of steroidogenesis.

The sugar contents in the gonads of *P. chilinoides* were highest at the mature stage suggesting that during maturation the accumulation of sugars takes place in the ovaries and testes. The sharp decline in the sugar contents during the spent stage, confirms the previous studies (Lal 1963).

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# Effect of carbaryl on esterases in the air-breathing fish *Channa punctatus* (Bloch)

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**Abstract.** Electrophoretic analyses of liver and muscle of *Channa punctatus* revealed that they contain atleast six and eight fractions of esterase, respectively. Characterization of esterase was made on the basis of their responses towards certain inhibitors. Liver esterase consists of acetylesterase and carboxyl esterases, whereas the muscle esterase has three types namely acetylesterase, carboxylesterase and cholinesterase. The liver and muscle of *C. punctatus* subjected to maximum sublethal concentration of carbaryl were electrophoretically analysed and it was found that both liver and muscle showed only three fractions of esterase.

**Keywords.** *Channa punctatus*; esterases; characterization; liver; muscle.

## 1. Introduction

Although a number of workers have studied the effects of industrial pollutants and agricultural pesticides on the activity of hepatopancreatic enzymes in many vertebrates (Bhattacharya and Mukherjee 1976; Thomas and Murthy 1976), they have not reported the findings of the electrophoretic analysis on these enzymes. The present study deals with the electrophoretic characterization of liver and muscle esterases of a freshwater air breathing fish *Channa punctatus* and the effect of carbaryl upon this enzyme.

## 2. Material and methods

*Channa punctatus* were collected from the local pond and acclimated to laboratory condition and feeding schedule keeping them in a large glass aquarium.

For separation and characterization of the enzyme, two groups of fish were reared: one in pesticide-free water and another in a medium containing 5 ppm concentration of carbaryl (N-methyl carbamate) (Union Carbide India Ltd) (sublethal level: Arunachalam *et al* 1984) for 15 days. The liver and muscle were separated after killing the fish in each group and homogenized in 40 % sucrose solution. The homogenate was centrifuged at 5000 g for 10 min and the supernatant was used as the enzyme source.

Disc gel electrophoresis was carried out as previously described by Balasubramanian *et al* (1982). Esterases were visualized by staining the solution containing 1 % 1-napthyl acetate and 1 % fast blue RR in phosphate buffer (M/15, at pH 7) at 37°C for 15 min. For characterizing the enzymes, gels were incubated in different inhibitors of varying strength solutions for 30 min and then stained for esterase. By comparing with the control gel, different types of esterases have been identified.

## 3. Results and discussion

Electrophoretic analyses of liver and muscle of *C. punctatus* revealed that they contain atleast six and eight esterase fractions, respectively. Based on the mobility of the enzyme fractions they have been designated as LEst-1 to LEst-6 and MEst-1 to MEst-8, respectively (figure 1a, c), indicating that the esterase of both liver and muscle of *C. punctatus* exists in multiple form. This is in accordance with the findings of Varma and Frankel (1980).

Effects of certain inhibitors on esterase fractions of liver and muscle of *C. punctatus* are presented in tables 1 and 2. Characterization of esterases was made on the basis of its responses towards certain inhibitors. Among the liver esterase fractions of *C. punctatus*, LEst-1 and LEst-2 were partially inhibited by silver nitrate and other chemicals like p-CMB, EDTA, eserine sulphate and organophosphate had no effect on these two fractions which are acetylesterases (Bergmann and Rimon 1958; Dickinson and Johnson 1978; Balasubramanian *et al* 1982). LEst-3, 4, 5 and 6 were inhibited by

**Table 1.** Effect of inhibitors on various fractions of esterases in the liver of *C. punctatus.*

| Inhibitors | LEst 1 | LEst 2 | LEst 3 | LEst 4 | LEst 5 | LEst 6 |
|---|---|---|---|---|---|---|
| Control | ++ | ++ | +++ | +++ | ++ | ++ |
| p-CMB $10^{-2}$M | ++ | ++ | +++ | +++ | ++ | ++ |
| EDTA $10^{-2}$M | ++ | ++ | +++ | +++ | ++ | ++ |
| Organophosphate $10^{-4}$M | ++ | ++ | − | − | − | − |
| Eserine sulphate $10^{-4}$M | ++ | ++ | +++ | +++ | ++ | ++ |
| $AgNO_3$ $10^{-2}$M | +/− | +/− | + | + | +/− | +/− |
| | acetyl | acetyl | carboxyl | carboxyl | carboxyl | carboxyl |

− represents inhibition of enzyme activity; +25% activity; ++ 50% activity; +++ maximum activity or 100% activity.

**Table 2.** Effect of inhibitors on various fractions of esterases in the muscle of *C. punctatus.*

| Inhibitors | MEst 1 | MEst 2 | MEst 3 | MEst 4 | MEst 5 | MEst 6 | MEst 7 | MEst 8 |
|---|---|---|---|---|---|---|---|---|
| Control | +++ | ++ | ++ | ++ | ++ | +++ | +++ | ++ |
| p-CMB $10^{-2}$M | +++ | ++ | ++ | ++ | ++ | +++ | +++ | ++ |
| EDTA $10^{-2}$M | +++ | ++ | ++ | ++ | ++ | +++ | +++ | ++ |
| Organophosphate $10^{-4}$M | +++ | − | − | − | − | − | − | − |
| Eserine sulphate $10^{-4}$M | +++ | ++ | − | − | − | +++ | +++ | ++ |
| $AgNO_3$ | ++ | + | ++ | ++ | ++ | ++ | ++ | + |
| | acetyl | carboxyl | choline | choline | choline | carboxyl | carboxyl | carboxyl |

− represents inhibition of enzyme activity; + 25% activity; ++ 50% activity; +++ maximum activity or 100% activity.

organophosphate, but not by p-CMB, EDTA and eserine sulphate and these fractions are the carboxylesterase (Ahmad 1976; Payne 1978; Varma and Frankel 1980).

Regarding the fish muscle esterases, MEst-1 is partially inhibited by silver nitrate and not by other chemicals. This fraction is acetylesterase (Bergmann and Rimon 1958; Dickinson and Johnson 1978; Balasubramanian *et al* 1982). MEst-3, 4 and 5 which were inhibited by organophosphate and eserinesulphate are probably cholinesterases (Augustinsson 1961; Holmes and Masters 1968) and MEst-2, 6, 7 and 8 which were inhibited by organophosphate but not by p-CMB and EDTA are carboxylesterase (Ahmad 1976; Payne 1978; Varma and Frankel 1980).

When fishes are exposed to pollutants whether industrial or agricultural, organs like liver and kidney are affected much (Brown 1970), since most of the toxic substances passing through these organs may cause histopathological and enzymatic changes.

The esterases of liver and muscle of fish reared in sublethal concentration of carbaryl are shown in figure 1. In liver, only three fractions *i.e.* LEst-3, 4 and 6 were identified, while others (LEst-1, 2 and 5) were not exhibited (figure 1b). In the fish muscle also there are only three fractions *i.e.* MEst-2, 6 and 8 (figure 1d), and the other fractions were not exhibited. Relative mobility of enzyme fractions, LEst-3, 4 and 6 and MEst 2, 6 and 8 in comparison with that of fish reared in pesticide-free water showed that these are carboxylesterases.

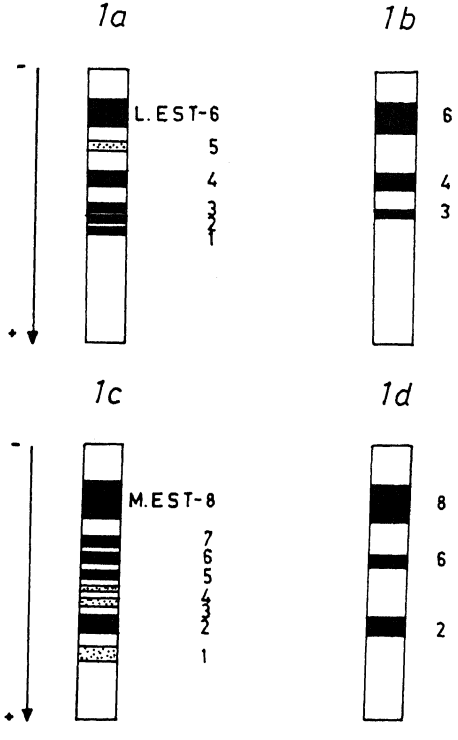


**Figure 1.** Zymogram pattern of esterases in *Channa punctatus*. (1a—Liver; 1b—Liver of treated animal; 1c—Muscle; 1d—Muscle of treated animal).

Therefore it appears that acetylesterases of liver and acetylesterases and cholinesterases of muscle in *C. punctatus* were inhibited. Such inhibition on esterases in different vertebrates due to certain pesticides has been reported (Mendoza and Hatina 1970). Industrial effluents like sodium sulphide, phenol, ammonia and copper sulphate have similar effects on liver esterase in *C. punctatus* and *Clarias batrachus* (Bhattacharya and Mukherjee 1976). The intensity of the activity of the esterases of liver and muscle of the treated fish was low when compared with that of fish reared in pesticide free-water.

Previous studies reported that carbaryl present in the medium decreased the growth rate of fishes (Arunachalam and Palanichamy 1982; Arunachalam *et al* 1984). Inhibition of acetylesterase and the consequent low activity observed in the present study may be the reasons for the decreased growth. Cholinesterase is an important enzyme in the excitable tissues of brain and muscle of teleost fishes (Nachamanson *et al* 1941; Weiss 1958; Lundin 1959). Inhibition of cholinesterase may lead to changes in the normal behaviour. This may be the reason for the erratic movements and imbalance in the fish exposed to pesticides (Arunachalam *et al* 1984).

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# Irradiation effects on the adrenal gland of rats undergoing inanition stress

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**Abstract.** The effect of total body x-irradiation was studied on rats under inanition stress. In response to irradiation an increase in the activity of cortex and medulla was noted in inanition stress administered rats rather than in the normally fed animals. Similarly, rising levels of urinary catecholamines and 5-hydroxytryptamine were observed in the starved animals after irradiation.

**Keywords.** Inanition stress; irradiation; adrenal gland.

## 1. Introduction

Earlier studies suggest that ionizing radiation decreases the production of corticoids from the adrenal cortex (Nabors *et al* 1974; Nabors 1962; Berliner *et al* 1962; Stevens *et al* 1963). Similarly, earlier investigators (Nair 1965; Hasan *et al* 1977, 1978, 1979; Veninga and Brinkman 1962; Renson and Fischer 1959; Varagic *et al* 1967) have demonstrated that the radiation elicits release of 5-HT and catecholamine in rats. Further, the response of adrenal activity is mostly dose dependent to radiation (Dougherty and White 1946; French *et al* 1955). However, no result seems to have been reported on the effect of x-irradiation on the adrenal gland of rats undergoing inanition stress. In this investigation, urinary metabolites of catecholamine and 5-hydroxytryptamine *viz* VMA and 5-HIAA have been studied in relation to inanition stress after total body irradiation.

## 2. Material and methods

Male Holtzman strain rats (110–120 g) were used in all control and experimental groups. Water was allowed *ad libitum* to each group of rats.

Group I: rats were starved for 10 days.
Group II: rats were starved for 10 days and on the 6th day of starvation they were exposed to x-rays.
Group III: rats were fed on a standard commercial diet (Hindustan Levers Ltd., India) to serve as control for groups I and IV.
Group IV: rats were fed on a standard diet as mentioned in the group III and exposed to x-rays, on the day when rats of group II were irradiated.

Total body of animals of groups II and IV were exposed to x-rays, 1000 R (80 kV; 200 MAS; 1 sec; 80 cm distance).

The control and experimental rats were killed by decapitation at intervals of 24, 48 and 96 hr after irradiation/on the 6th day after starvation. Adrenals were dissected out and fixed in Bouin's fluid. Paraffin sections (5$\mu$) were cut and stained with haematoxylin and eosin. Before the sacrifice, urine of each rat of the control and the experimental groups was collected in a specially devised box for 24 hr for the biochemical investigation of 5-HIAA (Subramaniyam and Narayanan 1973) and VMA (Armstrong *et al* 1957). Rate of mortality was also recorded during the stretch of experiments.

## 3. Results

The group undergoing inanition stress showed 29·4% mortality from the 5th to the 10th day of starvation. On the other hand the group receiving a combined treatment of starvation and irradiation exhibited nearly 33·3% mortality during this period which

**Table 1.** Chart of mortality.

| Groups | Normal control *A* | Radiation *B* | Starvation *C* | Starvation + Radiation *D* |
|---|---|---|---|---|
| Total no. of rats taken | 25 | 25 | 34 | 36 |
| 1st day of starvation | — | — | — | — |
| 2nd day of starvation | — | — | — | — |
| 3rd day of starvation | — | — | — | — |
| 4th day of starvation | — | — | — | — |
| 5th day of starvation | — | — | 2 | 2 |
| 6th day of starvation | — | — | 4 | — |
| 7th day of starvation/ 24 hr after irradiation | — | — | 2 | 4 |
| 8th day of starvation/ 48 hr after irradiation | — | — | — | — |
| 9th day of starvation/ 72 hr after irradiation | — | — | 2 | 4 |
| 10th day of starvation/ 96 hr after irradiation | — | — | — | 2 |
| Percentage mortality | — | — | 29·41% | 33·33% |

indicated an increase in the rate of mortality as compared to the non-irradiated starved rats (table 1).

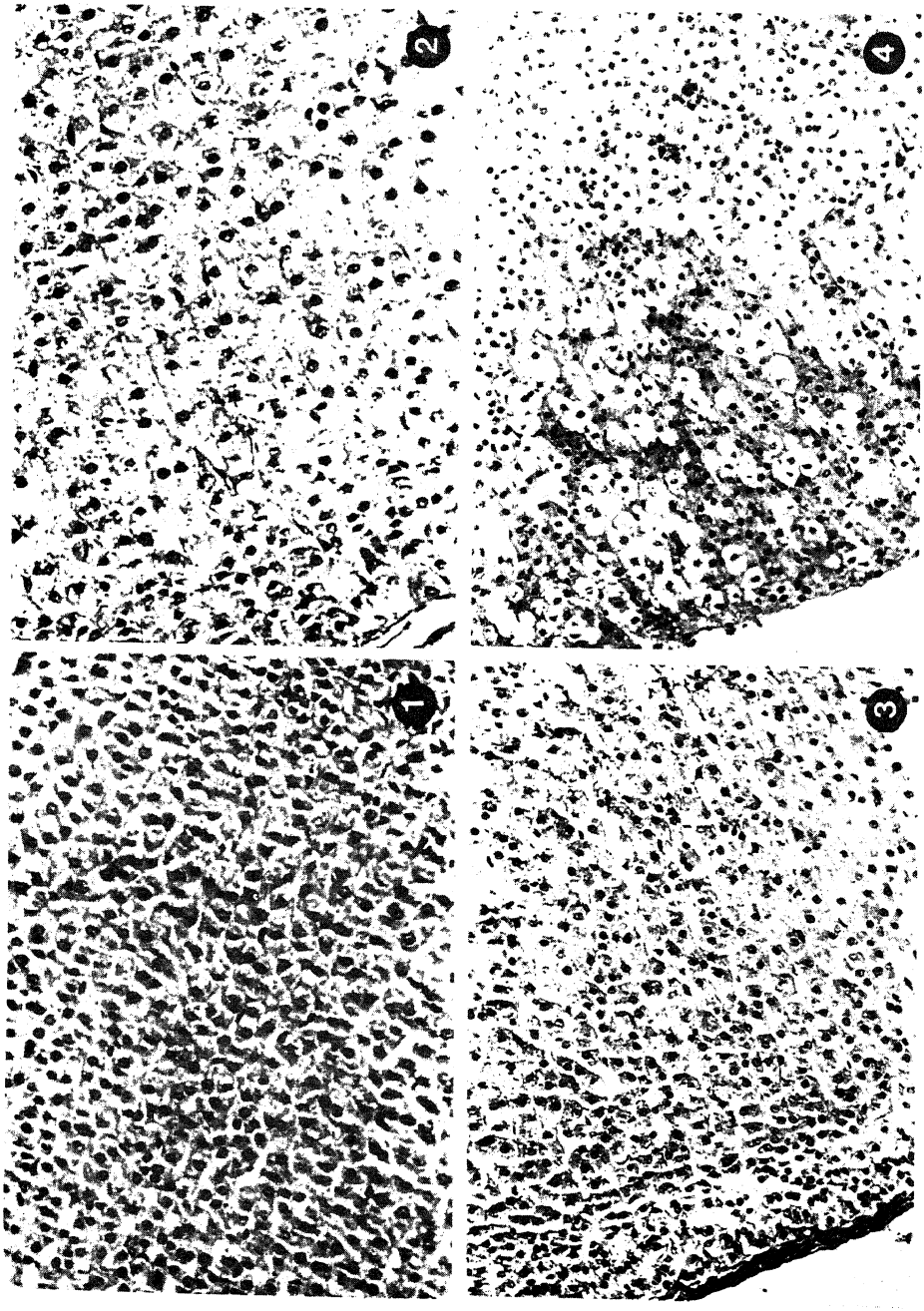
3.1a *Adrenal cortex*: Cells of the normal adrenal cortex were tightly packed and had a dense staining cytoplasm. Throughout the cortex, there was a rich vascular bed of sinusoids (figure 1). In rats undergoing inanition stress there was hypotrophy of cortical cells and most of the cells appeared vacuolated at the end of the 7th day of starvation. With increase in post-starvation period the columnar cells were widely separated by the appearance of large sinusoids which were conspicuously present in the region of zona reticularis (figure 3). Irradiation of the rats undergoing starvation brought about gradual hypertrophy of cells accompanied by degranulation of the cytoplasm; besides nuclei were markedly atrophied and had agranular nucleoplasm. By the end of 96 hr of post-irradiation most of the cells in the cortex appeared hypertrophied and showed increased depletion of granular contents of the cytoplasm indicating hyperactivity of cortical cells in response to irradiation (figure 4) as compared to the non-irradiated starved rats (figure 3). Contrary to this, normal diet fed irradiated rats showed gradual hypertrophy and vacuolization of cortical cells (figure 2) but the extent of hypertrophy and vacuolization was lesser than that observed in the starved irradiated rats (figure 4).

3.1b *Adrenal medulla*: Chromaffin cells of normal control animals contained secretory granules and the nuclei were large and prominently stained and had granular nucleoplasm (figure 5). In response to inanition stress the medullary cells got hypotrophied and the cells appeared vacuolated. With increase in post-inanition period the cells further became hypotrophied, the cytoplasm appeared almost degranulated, sinusoids were found filled with erythrocytes (figure 7). Total body x-irradiation of starved rats hastened the process of vacuolization of the chromaffin cells with nuclear atrophy and dilation of blood vessels (figure 8). In addition, sinusoids were found filled with erythrocytes (figure 8). Normal diet fed irradiated rats showed degranulation of the cytoplasm of chromaffin cells and copious secretions were observed around the nuclei indicating the secretory activity of the medullary cells (figure 6) in response to whole body irradiation.

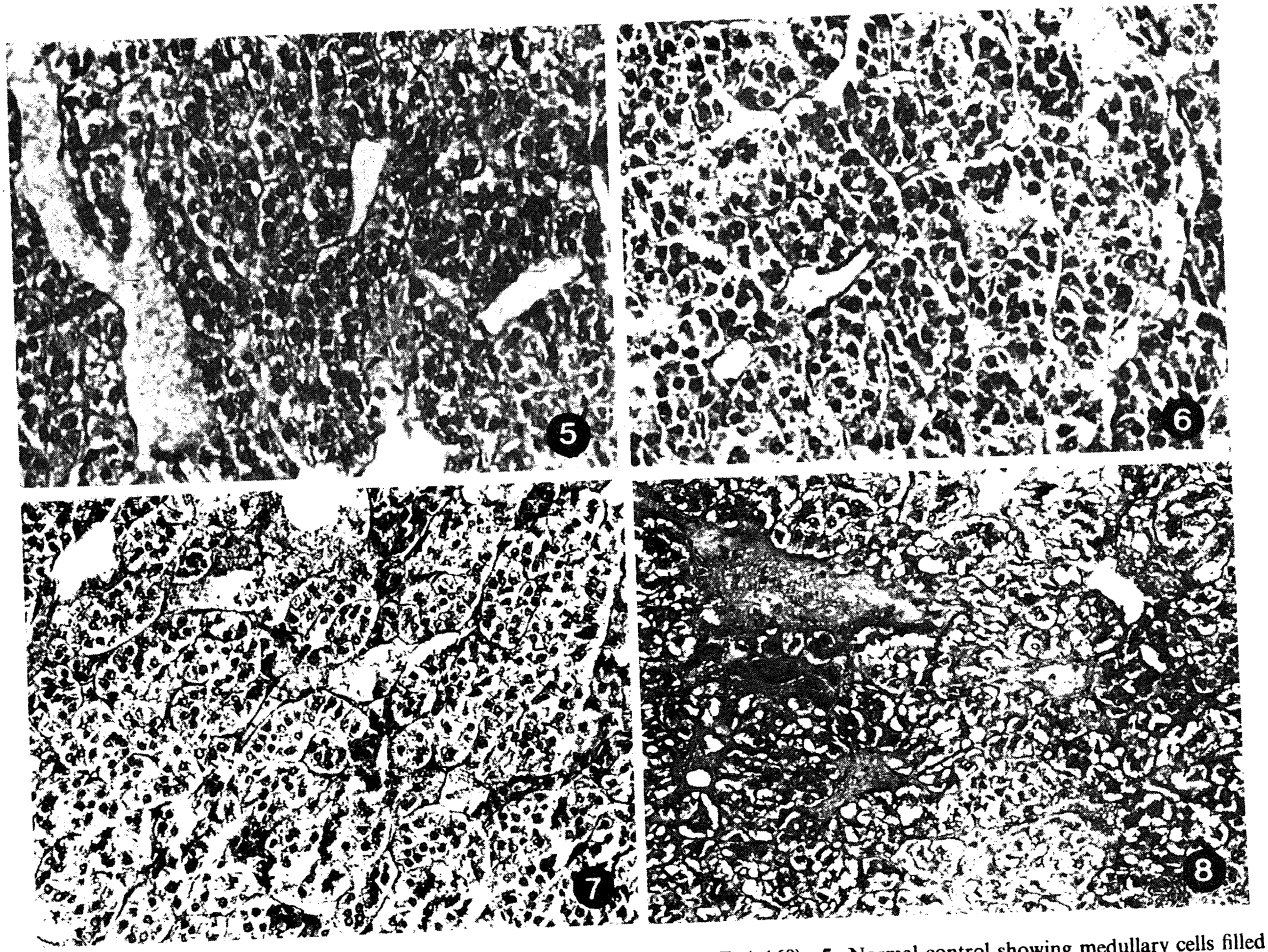
3.2 *Biochemical studies*

3.2a 5-HIAA (*table* 2): In all the three experimental groups, *i.e.*, the normal diet fed group, the inanition stress administered rats and the irradiated starved rats there was rising concentration of 5-HIAA in their urine as compared to the normal control. But the level of urinary excretion of 5-HIAA was greater in the irradiated rats undergoing inanition stress than in the normal diet fed irradiated group and the non-irradiated inanition administered group.

3.2b VMA (*table* 3): As compared to the normal control the level of urinary concentration of VMA was higher in the normal diet fed irradiated control rats and the starved irradiated rats. However, there was an increase in the concentration of VMA in the urine of rats receiving combined treatment of starvation plus irradiation compared with the levels of the normal diet fed irradiated control and the non-irradiated inanition administered rats.




**Figures 1–4.** TS of adrenal cortex of normal and experimental rats, HE (×160) **1.** normal control showing the presence of stainable granular contents in the cortical cells; **2.** 96 hr after x-irradiation showing appearance of vacuoles in the cells indicating liberation of cortical hormones from the cortical cells; **3.** 10 days after starvation showing nuclear atrophy, nuclei with agranular nucleoplasm and cortical cells being interlaced by the rich bed of sinusoids; **4.** 10 days after starvation/96 hr of post-irradiation showing increased hypertrophy of adrenocortical cells, almost complete depletion of granular material of the cytoplasm and a marked nuclear atrophy.




**Figures 5–8.** TS of adrenal medulla of normal control and experimental rats, HE, (×160). **5.** Normal control showing medullary cells filled with stainable granular contents and cells with large vesicular nuclei; **6.** 96 hr after irradiation showing nuclear atrophy, depletion of cytoplasmic granular contents and sinusoids with almost negligible amount of erythrocytes; **7.** 10 days after starvation showing hypertrophy of medullary cells, depletion of **8.** 10 days after starvation/96 hr of post-irradiation showing increased

**Table 2.** Urine- 5-HIAA (mg/24 hr) (mean ± SD).

| Time of collection of urine (hr) | Normal control *A* | Radiation *B* | Starvation *C* | Radiation + Starvation *D* |
|---|---|---|---|---|
| 24 | 0·916 ±0·125 | 1·644 ±0·106 *A*:*B* $P < 0.01$ | 1·282 ±0·051 *A*:*C* $P < 0.01$ *B*:*C* $P < 0.01$ | 1·894 ±0·245 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.02$ *C*:*D* $P < 0.01$ |
| 48 | 0·888 ±0·082 | 1·094 ±0·068 *A*:*B* $P < 0.01$ | 1·674 ±0·086 *A*:*C* $P < 0.01$ *B*:*C* $P < 0.01$ | 2·325 ±0·165 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.01$ *C*:*D* $P < 0.01$ |
| 96 | 0·799 ±0·077 | 1·087 ±0·211 *A*:*B* $P > 0.01$ | 1·826 ±0·263 *A*:*C* $P < 0.01$ *B*:*C* $P < 0.01$ | 3·539 ±0·122 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.01$ *C*:*D* $P < 0.01$ |

**Table 3.** Urine- 3-methoxy, 4-hydroxy mandelic acid (mg/24 hr) (mean ± SD).

| Time of collection of urine(hr) | Normal control *A* | Radiation *B* | Starvation *C* | Starvation + Radiation *D* |
|---|---|---|---|---|
| 24 | 0·965 ±0·195 | 1·257 ±0·089 *A*:*B* $P < 0.01$ | 1·231 ±0·134 *A*:*C* $P > 0.01$ *B*:*C* $P > 0.01$ | 1·535 ±0·143 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.01$ *C*:*D* $P < 0.01$ |
| 48 | 0·826 ±0·072 | 1·242 ±0·078 *A*:*B* $P < 0.01$ | 1·475 ±0·127 *A*:*C* $P < 0.01$ *B*:*C* $P < 0.01$ | 1·700 ±0·056 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.01$ *C*:*D* $P < 0.01$ |
| 96 | 0·832 ±0·072 | 1·098 ±0·081 *A*:*B* $P < 0.01$ | 1·769 ±0·080 *A*:*C* $P < 0.01$ *B*:*C* $P < 0.01$ | 1·956 ±0·101 *A*:*D* $P < 0.01$ *B*:*D* $P < 0.01$ *C*:*D* $P < 0.01$ |

## 4. Discussion

From the results it appears that administration of inanition stress leads to atrophy and vacuolization of cortical cells; besides there occurs dilation of vascular beds of sinusoids in the adrenal cortex. This indicates that the adrenal cortex continues to secrete a minimum amount of cortical hormone in order to meet the salt, water and glucose metabolism of the body during the course of inanition stress. The rats undergoing

inanition stress when exposed to total body irradiation showed increased hypertrophy of the cortical cells accompanied by almost complete depletion of the cytoplasm indicating enhanced hyperactivity of the adrenal cortex following total body x-irradiation during the inanition stress. In response to irradiation the non-starved rats also showed hypertrophy and vacuolization of cells in the cortex but the extent of changes were much more striking than those observed in the starved rats. Similar changes in the adrenal cortex of rats following irradiation have been reported in our earlier studies (Hasan *et al* 1977, 1978, 1979). This investigation attributes to the fact that x-irradiation induces hyperfunctioning and forced elimination of cortical hormone from the adrenal cortex in the starved animals rather than in the normally fed animals.

It is observed that under the influence of inanition stress there occurs regression in the size of medullary cells with consequential nuclear atrophy. In addition, chromaffin cells showed vacuolization and sinusoids appeared dilated suggesting increased liberation of hormones from the chromaffin tissues in the circulation. Starved rats when exposed to total body x-irradiation show an enhanced depletion of content of chromaffin cells and dilation of blood vessels with increasing number of erythrocytes in the sinusoids. Hyperactivity of the adrenal medulla is also demonstrated after $^{60}Co$ irradiation (Hasan *et al* 1977, 1978, 1979). These histological changes indicate increased hyperactivity of the medullary cells in the starved rats compared with the normally fed rats after total body irradiation.

Increased excretion of urinary catecholamine and 5-HIAA was noted after total body x-irradiation. The rise in concentration of 5-HIAA and VMA may be associated with the hyperactivity of 5-hydroxytryptamine and catecholamine in the body as the former happens to be the metabolite of the latter. Similarly, increase in the contents of 5-HT and catecholamine was reported by earlier workers after irradiation (Nair 1965; Hasan *et al* 1977, 1978; Veninga and Brinkman 1962; Renson and Fischer 1959; Varagic *et al* 1967). Rats undergoing inanition stress showed an increase in the excretion of 5-HIAA and VMA in the urine. Thus, it seems that during the period of inanition stress activity of 5-HT and nor-adrenalin gets augmented and probably this increase leads to rising levels of 5-HIAA and VMA in urine of starved rats. Further, the starved rats when exposed to x-rays showed an increase in the excretion of 5-HIAA and VMA and the concentration of these urinary metabolites were greater than in those of the non-irradiated starved rats and the normally fed irradiated rats. Further rise in the levels of 5-HIAA and VMA may be attributed to a stimulatory action of x-irradiation which possibly seems to have accelerated the already enhanced activity of 5-HT and nor-adrenalin owing to inanition stress. Thus, the biochemical studies in the present investigation further corroborate our histological observations on the adrenal gland.

The fact that x-irradiation induces more severe changes in the adrenal gland and brings about an increased excretion of urinary catecholamine and 5-HT in the starved rats rather than in the normally fed rats, favours our data recorded on the rate of mortality.

**Acknowledgement**

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# Laboratory culture of *Diaphanosoma senegal* Gauthier, (Crustacea, Cladocera) from south India

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**Abstract.** Laboratory studies on growth and reproduction of *Diaphanosoma senegal* Gauthier show that this species has a life span of 18·7 days. Three preadult and sixteen adult instars were recorded at a temperature range of 28–30°C. Maximum body size is attained at the end of its life-cycle and the growth increment is more during preadult instars. The present observations are compared with other laboratory studies on tropical South Indian cladocerans.

**Keywords.** Growth; reproduction; *Diaphanosoma senegal.*

## 1. Introduction

The various members of the zooplankton, inspite of some convergent similarities, have different types of life history (Hutchinson 1967). According to Edmondson (1955), laboratory studies of the life span, instar duration, egg production and growth are valuable sources of information for zooplankton and secondary productivity studies. Earlier studies on some tropical species of Cladocera like *Moina micrura* Kurz (Murugan 1975a), *Ceriodaphnia cornuta* Sars (Murugan 1975b), *Scapholeberis kingi* Sars (Murugan and Sivaramakrishnan 1976) and *Daphnia carinata* King (Venkataraman 1981) from the freshwater ponds have shown some important intraspecific differences. Since differences in the life histories are likely to involve different relationships, it was felt that it would be of interest to study the growth and reproduction of *Diaphanosoma senegal* Gauthier under laboratory conditions (28–30°C), recorded for the first time in South India.

## 2. Material and Methods

Ovigerous females of *D. senegal* were collected from a seasonal pond near the University campus (Lat.: 9° 53′ N; Long.: 78° E) and were acclimated to the laboratory temperature (28–30°C). Just born neonates were separated from the mothers and were reared in petridishes (50 ml) with pond water. The method used by Venkataraman (1981) was followed to study the life history. Table 1 shows the details of complete life-cycle on ten individuals.

## 3. Result

The body length of newly hatched *Diaphanosoma* which is a miniature form of adult in all respects measures about 0·59 mm. There are three preadult instars and sixteen adult

**Table 1.** Mean growth, egg number per brood and duration of instar of *D. senegal* at 28–30°C.

| Instar number | Mean body size (mm) | Mean carapace size (mm) | Mean egg number | Cumulative frequency of eggs | Mean duration of instar(hr) | Cumulative duration of instar(hr) |
|---|---|---|---|---|---|---|
| 1 | 0·59 | 0·20 | — | — | 13·2 | 13·2 |
| 2 | 0·88 | 0·26 | — | — | 18·8 | 31·0 |
| 3 | 1·22 | 0·33 | — | — | 23·8 | 54·8 |
| 4 | 1·37 | 0·45 | 3·2 | 3·2 | 37·3 | 92·1 |
| 5 | 1·47 | 0·49 | 2·6 | 5·8 | 25·6 | 117·7 |
| 6 | 1·53 | 0·52 | 2·9 | 8·7 | 23·7 | 141·4 |
| 7 | 1·56 | 0·52 | 2·8 | 11·5 | 23·5 | 164·9 |
| 8 | 1·63 | 0·55 | 3·0 | 14·5 | 24·0 | 188·9 |
| 9 | 1·70 | 0·55 | 3·5 | 18·0 | 23·2 | 212·1 |
| 10 | 1·73 | 0·55 | 2·6 | 20·6 | 21·6 | 233·7 |
| 11 | 1·73 | 0·59 | 2·7 | 23·3 | 23·4 | 257·2 |
| 12 | 1·76 | 0·59 | 2·8 | 26·1 | 23·6 | 280·7 |
| 13 | 1·76 | 0·59 | 2·6 | 28·7 | 24·8 | 305·5 |
| 14 | 1·79 | 0·59 | 3·0 | 31·7 | 24·1 | 329·6 |
| 15 | 1·83 | 0·59 | 2·2 | 33·9 | 24·3 | 353·9 |
| 16 | 1·86 | 0·62 | 1·3 | 35·2 | 23·2 | 377·1. |
| 17 | 1·86 | 0·62 | 3·0 | 38·2 | 22·6 | 399·7 |
| 18 | 1·89 | 0·62 | 2·3 | 40·5 | 24·0 | 423·7 |
| 19 | 1·89 | 0·62 | — | 40·5 | 24·0 | 447·7 |

instars. The body length of the first adult instar (4th instar) is 1·37 mm. An average maximum length of 1·89 mm is attained during the 19th instar and the mean life span is about 18·7 days (table 1). A comparison between the percentage of the preadult growth in relation to instar number of a few tropical and temperate cladocerans is shown in figure 1. It was observed that *D. senegal* has better preadult growth. The growth increment in relation to percentage of initial length and total length is shown in figure 2. Maximum number of eggs per brood of a single individual is 6 and the maximum cumulative frequency of egg production in a single individual is 56. The mean number of eggs produced and the cumulative frequency of egg production are shown in table 1. A comparison of egg production of a few tropical cladocerans in relation to instar number is shown in figure 3.

## 4. Discussion

The study shows that *D. senegal* has a total of nineteen instars (three preadult and sixteen adult) with an average life span of 18·7 days (447·7 hr) (table 1). The instar number of *D. senegal* is less than that of *Simocephalus acutirostratus* (22) and *Ceriodaphnia cornuta* (20), but greater than that of *Daphnia carinata* (18) and *Moina micrura* (13). These differences in the instar numbers may be due to hereditary factors as well as differences in the culture medium (Anderson and Jenkins 1942; Venkataraman 1983). The duration of instars in *D. senegal* varies throughout its life-cycle. Primiparous instar (4th instar, 37·3) is longer than the longest preadult instar (table 1). In this respect

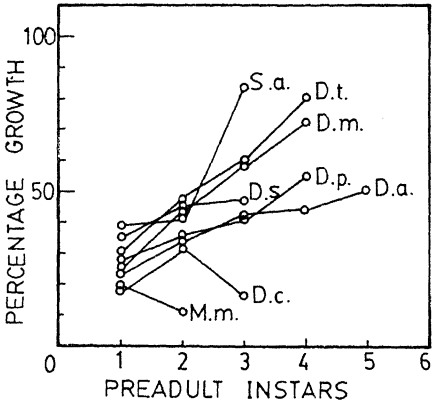


**Figure 1.** Percentage of preadult growth increment in relation to instar number of a few tropical and temperate cladocerans: S.a.— *Simocephalus acutirostratus*; D.t— *Daphnia thomsoni*; D.m—*Daphnia magna*; D.s—*Diaphanosoma senegal*; D.p—*Daphnia pulex*; D.a—*Daphnia atkinsoni*; D.c—*Daphnia carinata*; M.m—*Moina micrura*.

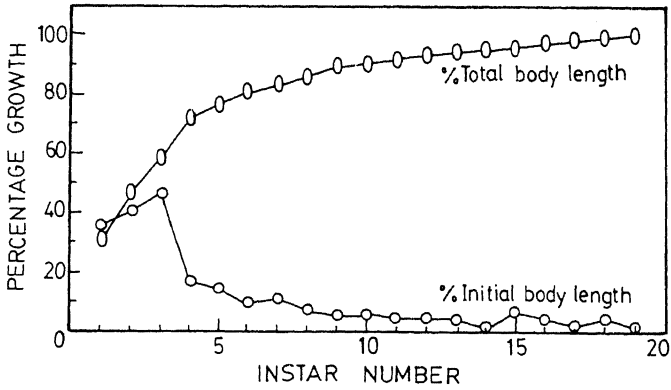


**Figure 2.** Growth increment as percentage of total body length and initial body length of *Diaphanosoma senegal*.

this species differs from *S. kingi* and *M. micrura* (Murugan and Sivaramakrishnan 1976; Murugan 1975a) where the duration of preadult and adult instars is uniform (24 hr) throughout their life cycle. *D. senegal* is similar to *S. acutirostratus* and *D. carinata* in having the longest preadult instar (Murugan and Sivaramakrishnan 1973; Venkataraman 1981).

Preadult growth increment as percentage of initial length of tropical and temperate cladocerans is shown in figure 1. The greatest growth increment does not always occur at the end of the adolescent instar or more rarely even earlier (Green 1955). In *D. senegal* the growth increment as percentage of initial length is 45·6 % initially and reaches 71·6 % at the 3rd instar (figure 2). From the 4th instar (adult instar) onwards the growth increment as percentage of initial length decreases suddenly. In the middle of its life span (at 10th instar) it attains 90 % of growth (figure 2). The body length of *D. senegal* at the 4th instar is 1·37 mm. Comparison of preadult growth increment as percentage of

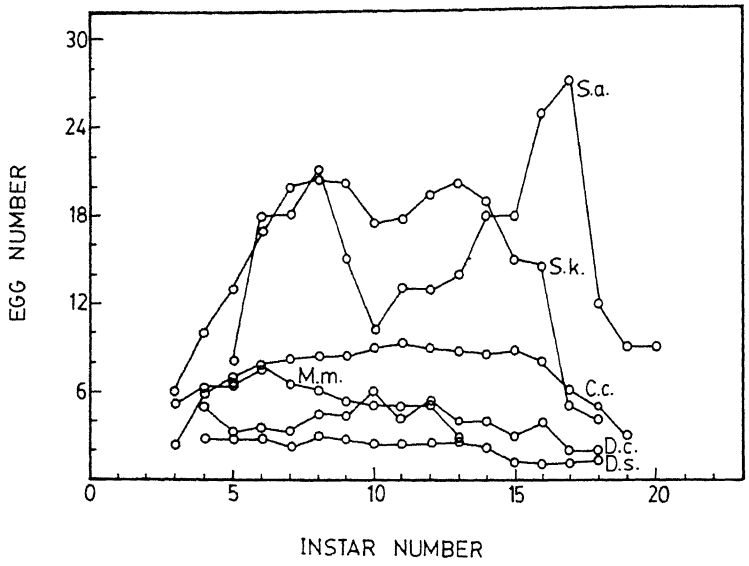


**Figure 3.** Egg production in relation to instar number of some tropical cladocerans: S.a— *Simocephalus acutirostratus*; S.k— *Scapholeberis kingi*; C.c— *Ceriodaphnia cornuta*; M.m— *Moina micrura*; D.c— *Daphnia carinata*; D.s— *Diaphanosoma senegal.*

initial length of a few temperate and tropical cladocera show that the temperate daphnids like *D. thomsoni*, *D. magna*, *D. atkinsoni* and *D. pulex* (Green 1955) have the greatest growth increment during the preadult instar. *S. acutirostratus* (Murugan and Sivaramakrishnan 1973) attains maximum growth increment in the 3rd preadult instar (84%) and this is the highest growth increment reported in any temperate or tropical species. *M. micrura* on the other hand shows minimum growth increment during the preadult instar and it recovers its growth only during the adult instars (Murugan 1975a). A study of *D. senegal* reveals that the highest rate of growth increment is seen during preadult instar 3 (45·6%).

Progressive increase in body size is a measure of growth rate of the individual (Edmondson 1955). The mean growth increment of *D. senegal* is rapid during the earlier phase and is very slow during the reproductive phase. Rapid preadult growth increment seems to be a common feature for Cladocera irrespective of physiological and physicochemical factors (Venkataraman 1983). The growth pattern of *D. senegal* shows a *s*-shaped curve. According to Hutchinson (1967) the growth rate per instar is always correlated with food supply, but growth pattern studies of *D. senegal* in relation to food or temperature has not been made.

The mean number of eggs per brood in relation to instar number of *D. senegal* is compared with a few tropical South Indian cladocerans (figure 3). The maximum number of eggs per brood under laboratory conditions (28–30°C) of a single individual is 6 and the total number of eggs produced in a life span is 56 (maximum number). Comparative study of egg production of tropical South Indian cladocerans under the same conditions reveals that *S. acutirostratus* (Murugan and Sivaramakrishnan 1973) produces the maximum number of eggs per brood (27) when compared to other tropical Cladocera. *D. senegal* produces a lesser number of eggs per brood, with a maximum of 3·5 eggs at the 9th instar (figure 3). The variation in egg

production in the species of Cladocera can be attributed to the amount of food available for the organism during its life span (Dunham 1938; Anderson and Jenkins 1942), temperature of the culture medium (Mc Arthur and Baillie 1929), and genetic makeup of the animal (Banta and Wood 1939).

An interesting aspect of this study is the record that *D. senegal* produces sexual eggs. The parthenogenetic eggs of *D. senegal* are oval but the sexual eggs are white in colour. The egg is covered by an outer leathery coat. When the eggs are dried, the outer shell shrinks and floats but when wet they sink to the bottom. The sexual eggs are entirely different from those of *D. carinata* which have a black, thick chitinous outer coat.

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# Laboratory evaluation of anticoagulant treated baits against Indian field mouse, *Mus booduga* Gray

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**Abstract.** The toxicity of warfarin (0·025%), bromadiolone (0·005%) and brodifacoum (0·005%) to the Indian field mouse, *Mus booduga* Gray was determined. A single feeding of bromadiolone or brodifacoum resulted in 83% mortality, while the same mortality was obtained with warfarin only after 6 days of continuous feeding. A single day feeding of a single dose chronic poison was effective against *M. booduga* than multiple dose chronic rodenticide.

**Keywords.** *M. booduga*; no-choice tests; mortality; susceptibility; warfarin; second generation anticoagulants; bromadiolone; brodifacoum.

## 1. Introduction

The Indian field mouse, *Mus booduga* Gray is a predominant rodent pest in irrigated lands nesting in shallow burrows with single or branched tunnels, particularly in South India and causes damage to paddy, ragi, sorghum, sugarcane and chillies at seedling, growth and harvest stages (Chandrahas 1974; Purushotham and Mohana Rao 1979). In Andhra Pradesh it breeds from August to February with a gap in summer months (Mohana Rao 1980). The field mice can be controlled either by placing baits such as cracked rice or bajra with 1% groundnut oil treated with 1–2% zinc phosphide or by using 0·025% warfarin or fumarin in the preferred bait. Of late the second generation anticoagulants emerged in view of the bait shyness developed with zinc phosphide and resistance with warfarin by a number of rodents. However, the use or value of rodenticides against *M. booduga* can be assessed basing on data from laboratory and field investigations. The present study aimed at evaluating the two second generation anticoagulants along with the warfarin (rodafarin 'C'), helps to make good the deficiency.

## 2. Materials and methods

Mice captured around Tirupati (Andhra Pradesh) were acclimatised to laboratory conditions for 2 weeks by maintaining them individually on food (Mohana Rao *et al* 1978) and water *ad libitum*. Experimental groups consisted of equal number of males and females.

The mice were provided with anticoagulant treated baits for 1, 2 and 3 days in case of second generation anticoagulants; 2, 4 and 6 days for warfarin separately. Daily intake of poisoned bait was recorded by offering freshly prepared bait every day, besides

noting down the day of mortality of the animal. The mortality due to poison was confirmed by autopsy and signs of anticoagulant poisoning.

Technical grade bromadiolone (1%) and brodifacoum (0·25%) were mixed with cracked bajra (*Pennisetum typhoides*) to give 0·005% concentration. Warfarin (0·5% technical grade) was mixed with the bait to give 0·025% concentration. The methods provisionally recommended by WHO (1976) were followed for determining the susceptibility of rodents to anticoagulant rodenticides.

## 3. Results

Data on the no choice tests with two anticoagulants *viz*, bromadiolone and brodifacoum (tables 1 and 2) show that both these poisons caused complete mortality within two weeks. A single feeding of bromadiolone and brodifacoum mixed baits resulted in 83% mortality; while 100% mortality was obtained after two and three feedings respectively. Warfarin is the least effective giving 83% kill after 6 days of continuous feeding (table 3). The mean days taken for causing 83% mortality using bromadiolone, brodifacoum (in single dose) and warfarin (in multiple dose) was 9·2, 9·4 and 8·6 days respectively. Mortality occurred after day 3, on consumption of bait containing either bromadiolone or brodifacoum, whereas, death occurred only after day 5 when fed on warfarin treated bait. Bait intake in no-choice tests was high up to 4–5 days which later declined possibly due to the development of symptoms of anticoagulant poisoning. In case of all the three rodenticides, increase in the number of feedings resulted in the lowering of the time taken to die.

## 4. Discussion

A single day feeding of second generation anticoagulants resulted in 83% mortality (in 9 days) in *M. booduga*. Mathur and Prakash (1980) noted 66% mortality (in 7·6 days) in *Funambulus pennanti* with a single feeding of brodifacoum mixed bait. The combined sex mortality for brodifacoum (in 6·25 days) on *Gerbillus gleadowi* was 50% (Soni and Prakash 1981). A similar study by Renapurkar and Kamath (1982) revealed 75, 80 and 100% mortality (in 10 days) for *Rattus rattus*, *R. norvegicus* and *Bandicota bengalensis*. Fifty and 35% mortality (in 7·4 and 5·6 days) were obtained from a single feeding of 0·002% brodifacoum and 0·005% bromadiolone respectively in cotton rat, *Sigmodon hispidus* (Gill and Redfern 1980). Meehan (1978) reported 100% mortality (in 6·8 days) in *R. norvegicus*. The mortality (83% in a single dose) seen in *M. booduga* using bromadiolone and brodifacoum indicate that these chemicals are effective in single dose against test species. Since there is no appreciable attenuation, in the time taken for death between 1 and 2 feeding periods and since the maximum mortality was evidenced in single dose feeding itself, control of *M. booduga* with single day feeding of either of these two poisons may be expected in the crop fields. Although the average time to elicit 83% mortality for all the three poisons was around 9 days, the use of single dose anticoagulants saves a lot of bait material and the manual operations involved as against the warfarin, a multiple dose poison.

The evaluation of warfarin against *R. rattus* (Agarwal *et al* 1979); *R. argentiventer* (Buckle *et al* 1980); *B. bengalensis* (Brooks *et al* 1980); and a variety of desert rodents (Mathur and Prakash 1981) has been made. The range and mean days to death using

**Table 1.** Toxicity of 0·005% bromadiolone to Indian field mouse, *Mus booduga* Gray.

| Feeding period (days) | Body weight (g) Mean ± SD | Poison bait consumed (g) Mean ± SD | | Bromadiolone consumed (mg/kg) Mean ± SD | | Mortality | Days to death | |
|---|---|---|---|---|---|---|---|---|
| | | Dead | Survived | Dead | Survived | | Mean | Range |
| 1 | 9·78 ± 0·6 | 2·23 ± 0·19 | 1·70 | 11·39 ± 1·36 (10·26–13·77) | 8·09 | 5/6 | 9·20 ± 4·86 | 6–17 |
| 2 | 11·41 ± 1·6 | 3·62 ± 1·48 | 2·90 | 15·74 ± 6·00 (11·03–25·87) | 13·06 | 5/6 | 7·60 ± 4·21 | 5–15 |
| 3 | 11·00 ± 1·0 | 6·80 ± 1·22 | — | 31·31 ± 7·59 (24·44–43·93) | — | 6/6 | 7·00 ± 3·63 | 4–14 |

**Table 2.** Toxicity of 0·005% brodifacoum to Indian field mouse, *Mus booduga* Gray.

| Feeding period (days) | Body weight (g) Mean ± SD | Poison bait consumed (g) Mean ± SD | | Brodifacoum consumed (mg/kg) Mean ± SD | | Mortality | Days to death | |
|---|---|---|---|---|---|---|---|---|
| | | Dead | Survived | Dead | Survived | | Mean | Range |
| 1 | 8·49 ± 0·28 | 1·72 ± 0·26 | 1·50 | 10·22 ± 1·58 (8·43–12·12) | 8·42 | 5/6 | 9·40 ± 3·04 | 6–14 |
| 2 | 10·40 ± 0·77 | 4·90 ± 0·38 | — | 23·92 ± 3·33 (21·26–30·00) | — | 6/6 | 7·00 ± 3·43 | 5–13 |
| 3 | 10·75 ± 1·40 | 5·78 ± 1·48 | — | 27·20 ± 7·38 (17·03–37·14) | — | 6/6 | 6·66 ± 3·44 | 4–13 |

**Table 3.** Toxicity of 0·025% warfarin to Indian field mouse, *Mus booduga* Gray.

| Feeding period (days) | Body weight (g) Mean ± SD | Poison bait consumed (g) Mean ± SD | | Warfarin consumed (mg/kg) Mean ± SD | | Mortality | Days to death | |
|---|---|---|---|---|---|---|---|---|
| | | Dead | Survived | Dead | Survived | | Mean | Range |
| 2 | 9·92 ± 1·05 | 5·15 ± 0·22 | 4·73 ± 2·08 | 126·51 ± 9·26 (115·88–132·90) | 124·40 ± 17·20 (104·65–136·11) | 3/6 | 11·3 ± 4·04 | 7–15 |
| 4 | 9·89 ± 0·56 | 8·25 ± 0·68 | 7·37 ± 0·32 | 208·39 ± 22·62 (176·61–228·64) | 188·09 ± 4·04 (185·23–190·95) | 4/6 | 9·0 ± 4·69 | 6–16 |
| 6 | 10·09 ± 1·27 | 10·38 ± 0·95 | 10·50 | 266·86 ± 27·12 (232·91–297·75) | 246·47 | 5/6 | 8·6 ± 1·95 | 6–11 |

warfarin in *M. booduga* can be comparable to the other species. In 2, 4 and 6 days feeding of warfarin, mortality started from day 6 and lasted up to day 16 and the maximum kill occurred between 6 and 10 days. Similar mortality rates were noted for bromadiolone and brodifacoum when used for 1 day only (tables 1 and 2). Comparing susceptibility of warfarin to *M. booduga* with other species, it is revealed that they are less susceptible than *Tatera indica*, *Meriones hurrianae* (Mathur and Prakash 1982) and *B. bengalensis* (Sridhara 1979; Brooks and Bowerman 1974). Thus it can be concluded that 0·005% bromadiolone and brodifacoum is more toxic and active against *M. booduga* than warfarin, giving a satisfactory mortality with single day feeding period.

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# Effects of starvation on respiration and major nutrient stores of the prosobranch snail *Bellamya bengalensis* (Lamarck)

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**Abstract.** The effects of starvation on the metabolic rate and the glucose, glycogen and total lipid contents in the freshwater prosobranch snail *Bellamya bengalensis* (Lamarck) have been investigated. Starvation influenced the metabolic rate of *B. bengalensis*. Although there was an initial increase, the metabolic rate of both the sexes decreased in the later stages of starvation. There was a marked difference in the utilization of nutrient stores between male and female snails starved for 55 days.

**Keywords.** *Bellamya bengalensis*; metabolic rate; nutrient stores; starvation effects.

## 1. Introduction

Studies on the effect of starvation on the oxygen consumption of molluscs are sparse. Berg and Ockelmann (1959) studied the effects of starvation on the oxygen consumption of *Ancylus fluviatilis*, starved for 96 hr. Stickle and Duerr (1970) in *Thais lamellosa*, Widdows (1973), Bayne (1973) and Bayne *et al* (1976) in *Mytilus edulis*, Mane (1975) in *Katelysia opima* and Mane and Talikhedker (1976) in *Donax cuneatus* studied respiration in relation to starvation. Heeg (1977) observed the oxygen uptake during starvation and aestivation in the pulmonate snail *Bulinus africanus*.

Not only the metabolic rate but also the nutrient reserves decreased during starvation in a number of molluscs. von Brand (1931) found that in *Helix pomatia* the oxygen uptake and also the carbohydrate reserves decreased during starvation. Emerson (1967) found that the metabolism of the aquatic pulmonate *Planorbis corneus* is carbohydrate oriented because the snail utilized 95% of the original carbohydrate during 58 days of starvation. Emerson and Duerr (1967) found the herbivorous prosobranch *Littorina planaxis* and Stickle and Duerr (1970) found the carnivorous prosobranch *Thais lamellosa* to have lipid oriented metabolism, since these snails utilized lipid during starvation.

It appears that carbohydrate and/or lipid are the main nutrient reserves of molluscs. Many lamellibranchs and pulmonates utilize glycogen (von Brand *et al* 1948; 1957; Martin 1961; Martin and Goddard 1966) whereas, amphineurans utilize lipid (Giese 1966). Some gastropods appear to have carbohydrate oriented metabolism while others have lipid oriented metabolism.

In the present study an attempt has been made to investigate the effects of starvation on metabolic rate and glucose, glycogen and lipid reserves of the herbivorous prosobranch *Bellamya bengalensis* (Lamarck).

## 2. Material and methods

Animals were collected from a pond at Vengalayapalem village (16°24 N: 80°33′ E). After clearing the encrustations on the shells, the snails were acclimatized in the laboratory at 27 ± 2°C for 96 hr.

### 2.1 *Respiratory measurements*

Respiratory measurements were made by the method of Ganapati and Prasada Rao (1960) and the dissolved oxygen was estimated by Winkler's method (Golterman 1970). Metabolic rates were measured on individual snails at day 4, 8, 16 and 20 of starvation. Experiments were run for 2 hr and the time of the experiment (1100–1300 hr) was kept constant to avoid the effect of time of day on the respiration of snails. Each snail was used only once and the oxygen consumption for 1 hr was taken into consideration. After the experiment the snails were shelled and the wet weight of the soft parts was determined to the nearest 0·1 mg. The allometric equation $y/x = aX^{(b-1)}$ (Davies 1966; Newell 1970) was used to express the results. All experiments were carried out at constant temperature (27 ± 1°C) and pH (8·9 ± 0·1). Experiments were performed on 10 males and 10 females at each period of starvation except at day 4 where 40 males and 60 females were used.

### 2.2 *Statistical procedure*

Regression lines were fitted by the method of least squares. The slopes and intercepts (elevations) of the regression lines are compared separately between males and females at each period of starvation and between successive periods of starvation in males and females using analysis of co-variance (Snedecor and Cochran 1967).

### 2.3 *Biochemical methods*

The glucose, glycogen and total lipid contents of snails prestarved and starved for 55 days were also estimated. The glucose and glycogen contents were estimated by the method of Kemp *et al* (1954) and total lipid by the method of Pande *et al* (1963). Immediately prior to chemical analysis, the animals were weighed, removed from the shells and sexed. They were then dissected, foot and visceral mass separated and their wet weight determined to the nearest 0·1 mg. Experiments were performed on 20 animals and the results are expressed as mg per 100 mg of wet weight.

## 3. Results

Few of the experimental animals died during the beginning weeks of starvation, only 10% mortality was observed on day 20. Most mortality occurred between days 40 and 50. Approximately 40% of the experimental animals were alive on day 55.

Figures 1 and 2 show the relationship of the metabolic rate and weight at different

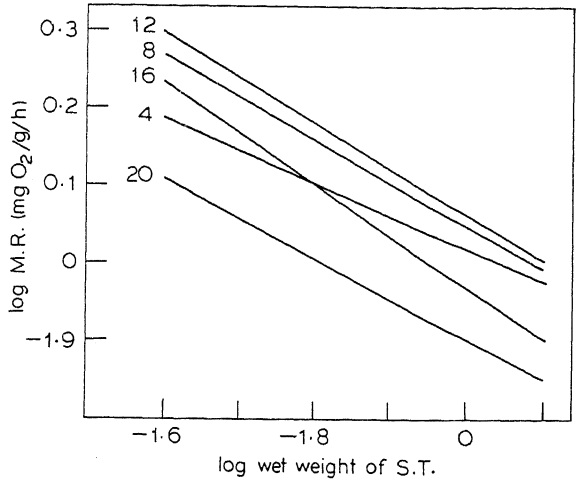


**Figure 1.** The relationship of log metabolic rate and log wet weight of soft tissues (ST) in males on different days of starvation.

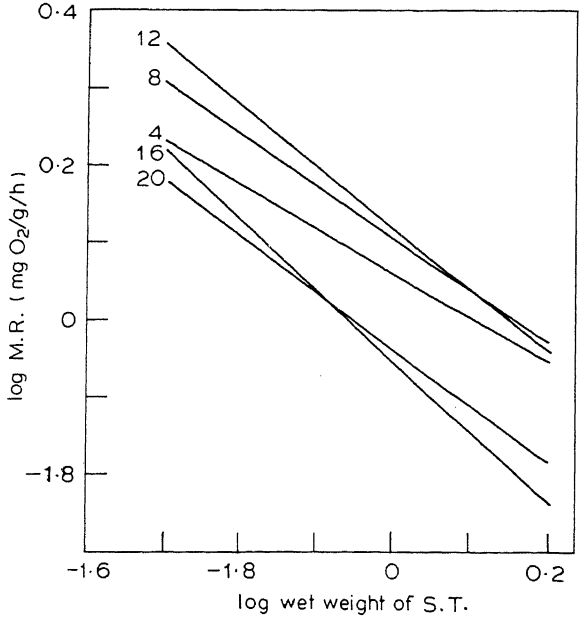


**Figure 2.** The relationship of log metabolic rate and log wet weight of soft tissues (ST) in females on different days of starvation

periods of starvation in males and females, respectively. The metabolic rate of both the sexes at all periods of starvation is negative linear in relationship with weight when plotted logarithmically. The regression equations are given in table 1. The wet weight of the soft parts and the metabolic rate ranged from 0·33 to 1·24 g and 0·63 to 1·94 mg $O_2$/g/hr in males and 0·47 to 1·88 g and 0·52 to 2·18 mg $O_2$/g/hr in females, respectively.

**Table 1.** Equations of the regression lines: $\log MR = \log a + (b-1) \log W$, relating weight ($W$) in g. to metabolic rate (MR) of *B. bengalensis* at different periods of starvation.

| Period of starvation (days) | Regression equations | |
|---|---|---|
| | Male | Female |
| 4 | $\log MR = 0{\cdot}0224 - 0{\cdot}4142 \log W$ | $\log MR = 0{\cdot}0639 - 0{\cdot}5718 \log W$ |
| 8 | $\log MR = 0{\cdot}0506 - 0{\cdot}5487 \log W$ | $\log MR = 0{\cdot}1098 - 0{\cdot}6718 \log W$ |
| 12 | $\log MR = 0{\cdot}0657 - 0{\cdot}5838 \log W$ | $\log MR = 0{\cdot}1225 - 0{\cdot}7842 \log W$ |
| 16 | $\log MR = -0{\cdot}0292 - 0{\cdot}6630 \log W$ | $\log MR = -0{\cdot}0508 - 0{\cdot}9271 \log W$ |
| 20 | $\log MR = -0{\cdot}0953 - 0{\cdot}5134 \log W$ | $\log MR = -0{\cdot}0348 - 0{\cdot}7245 \log W$ |

$n = 10$ for each sex except at the period 4 days where 40 males and 60 females were used.

Tables 2–4 show the results of ANCOVA comparing the slopes and elevations of the regression lines for various sex-starvation combinations. The results presented in table 2 suggest common slopes and also common elevations for the two sexes for the 4th and 16th day of starvation, thereby indicating no marked differences in the metabolic rate of the male and female snails on these periods of starvation. Further, it is also clear that common slopes or elevations could not be assumed for the two sexes for the remaining periods of starvation (8, 12 and 20 days) suggesting that the metabolic rate of males and females differs significantly on these days.

The position of the regression lines in figures 1 and 2 shows that the metabolic rate in both the sexes is highest on 12th day and lowest on 20th day of starvation. The results of ANCOVA (tables 3 and 4) indicate common slopes amongst different periods of starvation in both the sexes. However, the elevations were significantly different between 4th and 12th day, 12th and 16th day and 16th and 20th day in males (table 3) and between 4th and 12th day and 12th and 16th day in females (table 4). The elevations also were not significantly different between 4th and 8th day and 8th and 16th day in both the sexes and between 16th and 20th day in females.

The above observations suggest that the metabolic rate between days 4 and 8, 8 and 12 in both the sexes and 16 and 20 in female is not significantly different. The difference in the metabolic rate between 4th and 12th day, however, is significantly different in both the sexes.

The effects of starvation on the metabolic rate of different standard weights, calculated from the regression equations presented in table 1, are shown in figures 3 and 4. It is evident from these figures that the general pattern of increase or decrease is same in both the sexes, but the rate at which the metabolic rate increased or decreased is different in different sizes of males and females. The percentage increase from 4th to 12th day in the young (0·5 g) and adult (1·25 g) males was 26 and 6 and in the young (0·5 g) and adult (1·5 g) females was 32 and 6, respectively.

The metabolic rate in both the sexes decreased after 12th day. In males it decreased by 10 to 20% and in females 25 to 43% from 12th to 16th day for different sizes. The metabolic rate showed further decrease of 17 to 25% from 16th to 20th day in males of different sizes while in females it remained more or less constant.

Emerson (1967) while studying the metabolism of *Planorbis corneus* stated that "in order to estimate the weight loss of dry soft parts during starvation, it is necessary to calculate the prestarved weights". Therefore, the wet weight, dry weight and entire

**Table 2.** Results of analysis of covariance: comparison of the slopes ($b$) and elevations ($a$) of the regression lines of males and females of *B. bengalensis* at different periods of starvation.

| Period of starvation (days) | Variable (sex) | d.f. | $Sxx$ | $Sxy$ | $Syy$ | $(b-1)$ | Variation from regression S.S. | Variation from regression M.S. | Variance ratio ($F$) | | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Male | 39 | 0·7950 | −0·3294 | 0·5307 | −0·4143 | 0·3939 | 0·0082 | $F_{slopes}$ | 0·9208 | NS** |
| | Female | 59 | 0·7081 | −0·4048 | 0·8074 | −0·5717 | 0·5760 | 0·0099 | $F_{elevations}$ | 2·7030 | NS** |
| 8 | Male | 9 | 0·1185 | −0·0652 | 0·0391 | −0·5502 | 0·0032 | 0·0004 | $F_{slopes}$ | 0·5417 | NS* |
| | Female | 9 | 0·2899 | −0·1948 | 0·1654 | −0·6720 | 0·0345 | 0·0043 | $F_{elevations}$ | 12·0000 | HS* |
| 12 | Male | 9 | 0·1402 | −0·0819 | 0·0590 | −0·5842 | 0·0112 | 0·0014 | $F_{slopes}$ | 2·2353 | NS* |
| | Female | 9 | 0·3147 | −0·2467 | 0·2089 | −0·7839 | 0·0155 | 0·0019 | $F_{elevations}$ | 21·0000 | HS* |
| 16 | Male | 9 | 0·0758 | −0·0504 | 0·0973 | −0·6649 | 0·0638 | 0·0080 | $F_{slopes}$ | 0·3750 | NS* |
| | Female | 9 | 0·2343 | −0·2171 | 0·3036 | −0·9266 | 0·1024 | 0·0128 | $F_{elevations}$ | 0·1700 | NS* |
| 20 | Male | 9 | 0·0493 | −0·0253 | 0·0343 | −0·5132 | 0·0213 | 0·0027 | $F_{slopes}$ | 0·7895 | NS* |
| | Female | 9 | 0·1076 | −0·0779 | 0·0656 | −0·7240 | 0·0092 | 0·0012 | $F_{elevations}$ | 18·2105 | HS* |

NS = Not significant; HS = Highly Significant; *d.f. = $F_{slopes}$: 1,16 and $F_{elevations}$: 1,17; **d.f. = $F_{slopes}$: 1,96 and $F_{elevations}$: 1,97.

**Table 3.** Results of analysis of covariance: comparison of the slopes ($b$) and elevations ($a$) of the regression lines of males of *B. bengalensis* between successive periods of starvation.

| Variable (period of starvation days) | d.f. | $Sxx$ | $Sxy$ | $Syy$ | $(b-1)$ | Variation from regression | | Level of comparison (days) | Variance ratio ($F$) | | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | S.S. | M.S. | | | | |
| 4 | 39 | 0·7950 | −0·3294 | 0·5307 | −0·4143 | 0·3939 | 0·0082 | 4 and 8 | $F_{slopes}$ | 0·2209 | NS** |
| | | | | | | | | | $F_{elevations}$ | 2·8706 | NS** |
| 8 | 9 | 0·1185 | −0·0652 | 0·0391 | −0·5502 | 0·0032 | 0·0004 | 8 and 12 | $F_{slopes}$ | 0·1111 | NS** |
| | | | | | | | | | $F_{elevations}$ | 2·5555 | NS** |
| 12 | 9 | 0·1402 | −0·0819 | 0·0590 | −0·5842 | 0·0112 | 0·0014 | 12 and 4 | $F_{slopes}$ | 0·3864 | NS** |
| | | | | | | | | | $F_{elevations}$ | 4·8736 | S** |
| | | | | | | | | 12 and 16 | $F_{slopes}$ | 0·0638 | NS* |
| | | | | | | | | | $F_{elevations}$ | 7·5000 | HS* |
| 16 | 9 | 0·0758 | −0·0504 | 0·0973 | −0·6649 | 0·0638 | 0·0080 | 16 and 20 | $F_{slopes}$ | 0·1321 | NS* |
| | | | | | | | | | $F_{elevations}$ | 7·8400 | HS* |
| 20 | 9 | 0·0493 | −0·0253 | 0·0343 | −0·5132 | 0·0213 | 0·0027 | | | | |

NS = Not significant; S = Significant; HS = Highly Significant; *d.f. = $F_{slopes}$: 1,16 and $F_{elevation}$: 1, 17; **d.f. = $F_{slopes}$:1,46 and $F_{elevations}$: 1,47.

**Table 4.** Results of analysis of co-variance: comparison of slopes and elevations of the regression lines of females of *Bellamya bengalensis* between successive periods of starvation.

| Variable (period of starvation days) | d.f. | *Sxx* | *Sxy* | *Syy* | $(b-1)$ | Variation from regression | | Level of comparison (days) | Variance ratio ($F$) | | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | S.S. | M.S. | | | | |
| 4 | 59 | 0·7081 | −0·4048 | 0·8074 | −0·5717 | 0·5760 | 0·0099 | 4 and 8 | $F_{slopes}$ | 0·2258 | NS** |
| | | | | | | | | | $F_{elevations}$ | 1·9121 | NS** |
| 8 | 9 | 0·2899 | −0·1948 | 0·1654 | −0·6720 | 0·0345 | 0·0043 | 8 and 12 | $F_{slopes}$ | 0·6129 | NS* |
| | | | | | | | | | $F_{elevations}$ | 1.0000 | NS* |
| 12 | 9 | 0·3147 | −0·2467 | 0·2089 | −0·7839 | 0·0155 | 0·0019 | 12 and 4 | $F_{slopes}$ | 1·0889 | NS** |
| | | | | | | | | | $F_{elevations}$ | 3·6800 | NS** |
| 16 | 9 | 0·2343 | −0·2171 | 0·3036 | −0·9266 | 0·1024 | 0·0128 | 12 and 16 | $F_{slopes}$ | 0·3784 | NS* |
| | | | | | | | | | $F_{elevations}$ | 17·4507 | HS* |
| | | | | | | | | 16 and 20 | $F_{slopes}$ | 0·4429 | NS* |
| 20 | 9 | 0·1076 | −0·0779 | 0·0656 | −0·7240 | 0·0092 | 0·0012 | | $F_{elevations}$ | 0·3582 | NS* |

NS = Not significant; HS = Highly Significant; *d.f. = $F_{slopes}$: 1, 16 and $F_{elevations}$: 1, 17; **d.f. = $F_{slopes}$: 1, 66 and $F_{elevations}$: 1, 67.

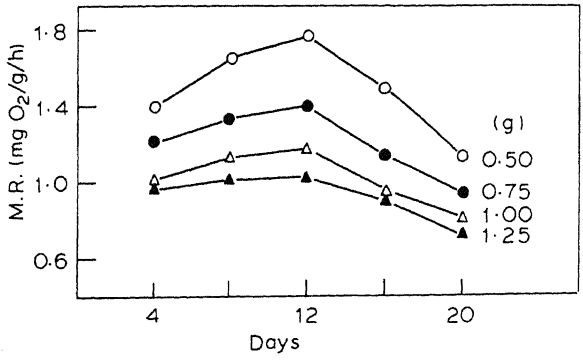


**Figure 3.** Metabolic rate-starvation curves for male *B. bengalensis* of different body weights.

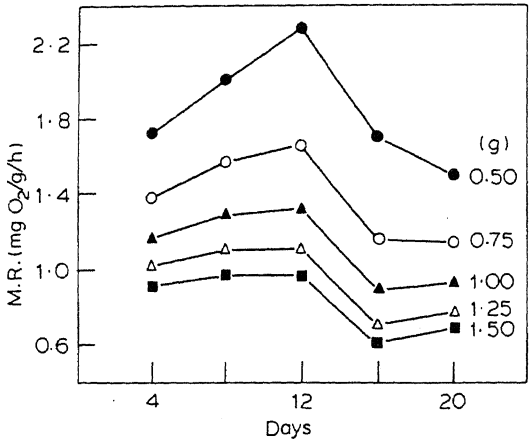


**Figure 4.** Metabolic rate-starvation curves for female *B. bengalensis* of different body weights.

weight relations are also calculated and a summary of the results is presented in table 5. From this table it is clear that the dry weight of the soft parts of animals starved for 55 days is 87 % of the prestarved weights indicating a loss of 13 %. This is considerably less than the weight loss of 23 % reported for the carnivorous prosobranch *Thais lamellosa* starved for 53 days (Stickle and Duerr 1970).

Few observations were also made on the metabolic rate of snails starved for 55 days. The average metabolic rate ($n = 5$; shell height range 1·8 to 2·5 cm) in males was 0·96 mg $O_2$/g/hr and in females 0·71 mg $O_2$/g/hr on 55th day of starvation.

The glucose, glycogen, total lipid contents and their percentage decrease in foot and viscera of male and female snails starved for 55 days are shown in table 6. The glucose, glycogen and total lipid in males and glucose and total lipid in females decreased more in foot than in viscera. Further, the glucose and glycogen contents decreased more in males than in females whereas, the lipid content decreased more in females than in males starved for 55 days.

**Table 5.** Weight loss of *Bellamya bengalensis* starved for 55 days.

| | Control group (average of 20 animals) | Animals starved for 55 days (average of 10 animals) |
|---|---|---|
| Wet weight of soft parts of animals expressed as percentage of entire weight | 31·00 (±0·7417) | 34·36 (±1·8663) |
| Dry weight of soft parts of animals expressed as percentage of wet weight | 18·19 (±0·5764) | 13·99 (±1·1706) |
| Dry weight of soft parts of animals expressed as percentage of entire weight | 5·64 (±0·2399) | 4·89 (±0·6096) |
| Dry weight of soft parts of animals after starvation expressed as percentage of original dry weight | — | 87·00 |
| Factor | | 1·15 |

(Figure in parenthesis indicates standard error).
Factor = Reciprocal of 87%.

**Table 6.** Glucose, Glycogen and total lipid content of males and females of *Bellamya bengalensis* starved for 55 days.

| | Male | | Female | |
|---|---|---|---|---|
| | Foot | Viscera | Foot | Viscera |
| Glucose (%) | | | | |
| Control | 23·75 | 29·30 | 21·50 | 29·30 |
| Starved | 13·13 | 22·50 | 13·75 | 22·76 |
| % Decrease | 44·72 | 23·21 | 36·05 | 22·32 |
| Glycogen (%) | | | | |
| Control | 43·75 | 46·25 | 23·75 | 41·25 |
| Starved | 16·56 | 26·25 | 16·56 | 21·88 |
| % Decrease | 62·14 | 43·24 | 30·27 | 46·96 |
| Total lipid (%) | | | | |
| Control | 0·51 | 11·19 | 0·83 | 17·44 |
| Starved | 0·40 | 9·82 | 0·55 | 14·86 |
| % Decrease | 20·80 | 12·20 | 34·04 | 14·79 |

## 4. Discussion

Decrease in the metabolic rate has been reported in a number of starved molluscs. Lomte and Nagabhushanam (1971) found that the oxygen consumption of the freshwater mussel, *Parreysia corrugata* was reduced to nearly 50% after starving the

animals for 10 days. Three-fifths reduction in the initial value of oxygen uptake of the limpet, *Ancylus fluviatilis* was recorded after 96 hr of starvation (Berg and Ockelmann 1959). There was 50% reduction in the metabolic rate after a period of 8 days starvation in the clams, *Katelysia opima* (Mane 1975) and *Donax cuneatus* (Mane and Talikhedker 1976). In *Bellamya bengalensis* also starvation affected the metabolic rate. In both the sexes the metabolic rate showed an initial increase of about 15% from 4th to 12th day of starvation. The metabolic rate decreased from 12th day onwards, but the rate of decrease was different in males and females. The metabolic rate of males, from 12th to 16th day, decreased by about 15% and from 16th to 20th day by another 15%; whereas the metabolic rate of females, from 12th to 16th day, decreased by about 30% and from 16th to 20th day it remained more or less constant. Thus ultimately there is only 15% reduction, from the initial value, in the metabolic rate of both the sexes of *Bellamya bengalensis* indicating that this decrease (15%) is considerably less than that reported for bivalves, *Parreysia corrugata*, *Katelysia opima* and *Donax cuneatus* (50%) and *Ancylus* (60%).

It is of interest to note that, whereas the oxygen consumption of the pulmonates (von Brand *et al* 1948; Duerr 1965) and bivalves (Lomte and Nagabhushanam 1971; Widdows 1973; Bayne 1973, 1976; Mane 1975; Mane and Talikhedker 1976) decreased during starvation, the oxygen consumption of the prosobranch, *Bellamya bengalensis* increased during the initial days of starvation. This is in agreement with the previous work on the carnivorous prosobranch *Thais lamellosa* which also shows an increased oxygen consumption during starvation (Stickle and Duerr 1970).

After 12th day the metabolic rate of both sexes of *B. bengalensis* decreased. Stickle and Duerr (1970) stated that "a decreased oxygen consumption could indicate a lowered metabolic rate or it could indicate a switch in emphasis from a lipid oriented metabolism to a carbohydrate or protein oriented metabolism". von Brand *et al* (1948) found that in pulmonates the lowered oxygen consumption is an adaptation to conserve food stores. The increasing metabolic rate of *B. bengalensis* up to the 12th day of starvation indicates that this snail does not possess this adaptation in the initial stages of starvation. However, decrease in the metabolic rate after 12th day suggests conservation of food reserves or it may indicate a switch in the metabolism as suggested by Stickle and Duerr (1970). Calow (1975) has shown that on starvation, animals may have one of two responses; they may decrease their metabolism immediately as a means of saving energy or they may initially increase their metabolism due to increased activity caused by searching for food. The present study on *Bellamya bengalensis* indicates the possibility of both these responses in starving animals.

Twenty to 40% of glucose, 30 to 60% of glycogen and 12 to 34% of total lipid is lost in snails starved for 55 days. This indicates that the snails utilize more glucose and glycogen than lipid during starvation. The decrease in the lipid content may be due to its direct utilization or it may be converted into carbohydrate during starvation. The results suggest that probably the metabolism of the herbivorous prosobranch, *B. bengalensis* is carbohydrate oriented like the other freshwater snails *Helix pomatia* (von Brand 1931) and *Planorbis corneus* (Emerson 1967). It is possible that some of the protein might have also been utilized during starvation, but no measurements of protein were made in the present study.

It is also interesting to note that, whereas the metabolic rate of males continued to decrease from 16th to 20th day of starvation, the metabolic rate of females remained constant during this period, thereby indicating that the ultimate decrease in the

metabolic rate of both the sexes remains the same (15%) at the end of 20 days starvation. Further, when the metabolic rate of males decreased by only about 2%, the metabolic rate of females decreased by about 12% from 20th-55th day of starvation. Furthermore, there was also a marked difference between the male and female snails in the utilization of glucose, glycogen and total lipid stores. These observations suggest that there may be differences in the basic physiological and biochemical processes of the two sexes during starvation and it would be of interest to examine these processes in detail.

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# Influence of distillery effluent on growth and metamorphosis of *Rana malabarica* (Bibron)

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**Abstract.** Increase in effluent concentration reduced the period of limb bud emergence and tail resorption; it also produced elevated values for length of limbs, tail and body weight of adult *R. malabarica*. Analysis of variance (Anova test) confirmed that time (week) has pronounced effect on morphological parameters ($P < 0.05$) than treatment ($P > 0.05$).

**Keywords.** Distillery effluent; *Rana malabarica*; growth; metamorphosis.

## 1. Introduction

To assess the effects of various pesticides and industrial effluents on aquatic life, toxicologists generally prefer fish and invertebrates and omit amphibians considerably. A few publications are available on amphibians especially for the effect of pesticides (Cook 1971; Dial 1976; Greenhouse 1976). Except one or two reports (*e.g.* Ghate *et al* 1978) no information is available on the impact of industrial effluents on amphibian tadpoles. The above authors have reported tail abnormalities, eye defects and odema in *Microhyla ornata* exposed to dye factory effluent but omitted the aspects of growth and metamorphosis in their studies. Since amphibians are the components of food webs in both terrestrial as well as aquatic communities, Porter and Hakanson (1976) stressed that preference should be given to amphibians for bioassay studies. The present investigation is a preliminary report dealing with the effect of distillery effluent* on growth and metamorphosis of *Rana malabarica*.

## 2. Material and methods

The tadpoles of *R. malabarica* (premetamorphic stage**) were collected from their natural habitat (Mundanthurai, Tamil Nadu) fed on boiled leaves of *Amaranthus spinosus* for 3 days and acclimatized to laboratory conditions. Twenty test individuals of equal body length and weight were recruited from the stock and divided into 4 series, each with 5 individuals. The first group of individuals were reared in dechlorinated tap water as control, while the remaining groups were exposed to different sublethal concentrations (0·03, 0·06 and 0·12%) of distillery effluent (Barnabas Xavier 1983). During the experimental period, fresh concentrations of effluent were prepared in 31 of

* Courtesy Trichy Chemicals and Distilleries Ltd., Tiruchirapalli.

** Before the hind limb bud emergence.

water by mixing the required quantity of the effluent with tap water and supplied to the test individuals daily (Haniffa and Sundaravadhanam 1984). Boiled *A. spinosus* leaves were supplied *ad libitum* to the tadpoles every day. Weekly observations for the change in length of body, tail, hindlimb and forelimb in cm were made. Every week the tadpoles were weighed to 0·001 mg after blotting on a cloth towel (Hota and Dash 1981). All measurements were analysed to standard deviation whereas analysis of variance was attempted after Zar (1974). Calculations were made for correction factor, total sum of squares, summation due to week, summation due to treatment and mean square. $F$ values were separately estimated for time effect ($F_1$) and treatment effect ($F_2$) on the length of limbs, tail and body and weight of *R. malabarica*. $F$ value probability was taken from the Anova table (table 3, Snedecor and Cochran 1968).

## 3. Results and discussion

*R. malabarica* tadpoles reared in tap water took 84 days to complete metamorphosis. Among the effluent concentrations, individuals exposed to 0·12% took the shortest period of 50 days to complete metamorphosis (table 1). Body length and tail length increased during progressive metamorphic stages and after that the body length remained almost constant whereas tail showed a gradual decrease and was finally resorbed. Peak values of body length were noticed on 29th, 36th, 36th and 15th day in *R. malabarica* reared in tap water, 0·03%, 0·06% and 0·12% effluent respectively. Among all the test individuals, maximum body length of adults (4·8 cm) was noticed for the tadpole exposed to 0·03% followed by those reared in 0·06% (4·7 cm), 0·12% (4·4 cm) and tap water (4·4 cm). During the progressive metamorphic period, the tail length of tadpoles reared in tap water, 0·03%, 0·06% and 0·12% effluent increased from 4·9 to 5·4 cm, 5·2 to 6·2 cm, 5·5 to 5·8 cm and 5·5 to 6·2 cm respectively. Increase in effluent concentration produced a decrease in the period of tail resorption. Tail resorption was noticed much earlier (50 days) for tadpoles exposed to 0·12% followed by those reared in 0·06% (64 days), 0·03% (71 days) and tap water (85 days). Total length (body and tail) rapidly increased and reached the peak during the progressive metamorphic stage and after gradual decrease (retrogressive period) attained a constant value (table 1).

Hind limb bud emergence was much earlier (15th day) for tadpoles exposed to 0·12% followed by those reared in 0·06%, 0·03% (22nd day) and tap water (29th day). Increase in effluent concentration produced elevated values for the final length of hind limb, but the period to attain the maximum length was constant (35 days) at all concentrations except in tap water. Fore limb bud emergence was also quicker (29th day) for tadpoles exposed to 0·12%, when compared with those reared in 0·06% (43rd day), 0·03% (57th day) and tap water (71st day). The difference in fore limb length was rather perceived high as a function of effluent concentration taking almost the same duration (28 days) at the respective concentrations (table 2).

Figure 1 shows the change in body weight of *R. malabarica* reared in tap water and effluent. The control test individuals increased from an initial weight of 7·1 g to 8·5 g on the 36th day and after that slowly decreased to 6·3 g on the 78th day. The corresponding changes during the progressive metamorphic period for those exposed to 0·03, 0·06 and 0·12 effluent were from 7·7 to 9 g, 6·8 to 8·5 g and 7·1 to 7·6 g on the 29th day respectively. The final body weight of *R. malabarica* reared in tap water was much less

**Table 1.** Influence of distillery effluent on body length and tail length of *R. malabarica*.

| | Body length (cm) | | | | Tail length (cm) | | | | Total length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Control | 0·03% | 0·06% | 0·12% | Control | 0·03% | 0·06% | 0·12% | Control | 0·03% | 0·06% | 0·12% |
| 1 | 3·8± 0·15 | 4·1± 0·17 | 4·2± 0·25 | 4·1± 0·51 | 4·9± 0·15 | 5·2± 0·21 | 5·5± 0·15 | 5·5± 0·51 | 8·7± 0·30 | 9·3± 0·38 | 9·7± 0·40 | 9·6± 1·02 |
| 8 | 3·8± 0·15 | 4·2± 0·27 | 4·3± 0·25 | 4·3± 0·56 | 5·0± 0·15 | 5·2± 0·17 | 5·6± 0·13 | 5·7± 0·15 | 8·8± 0·30 | 9·4± 0·44 | 9·9± 0·38 | 10·0± 0·71 |
| 15 | 4·0± 0·15 | 4·3± 0·21 | 4·4± 0·26 | 4·4± 0·26 | 5·2± 0·12 | 5·8± 0·25 | 5·7± 0·20 | 6·2± 0·21 | 9·2± 0·27 | 10·1± 0·46 | 10·1± 0·46 | 10·6± 0·47 |
| 22 | 4·2± 0·15 | 4·4± 0·21 | 4·5± 0·26 | 4·4± 0·26 | 5·4± 0·12 | 6·0± 0·25 | 5·8± 0·20 | 5·7± 0·23 | 9·6± 0·27 | 10·4± 0·46 | 10·3± 0·46 | 10·1± 0·49 |
| 29 | 5·0± 0·13 | 4·6± 0·25 | 4·6± 0·62 | 4·4± 0·51 | 5·2± 0·30 | 6·2± 0·15 | 5·4± 0·70 | 3·7± 0·15 | 10·2± 0·43 | 10·8± 0·40 | 10·0± 1·32 | 8·1± 0·66 |
| 36 | 4·4± 0·13 | 4·8± 0·30 | 4·7± 0·15 | 4·4± 0·51 | 4·4± 0·58 | 5·7± 0·74 | 4·1± 1·60 | 2·0± 0·21 | 8·8± 0·71 | 10·5± 1·04 | 8·8± 1·75 | 6·4± 0·72 |
| 43 | 4·4± 0·15 | 4·8± 0·39 | 4·7± 0·53 | 4·4± 0·15 | 3·8± 0·31 | 4·2± 0·23 | 3·1± 1·10 | 0·9± 0·02 | 8·2± 0·46 | 9·0± 0·62 | 7·8± 1·63 | 5·3± 0·17 |
| 50 | 4·4± 0·25 | 4·8± 0·39 | 4·7± 0·26 | Adult | 3·1± 0·61 | 3·2± 0·20 | 0·8± 0·14 | Adult | 7.5± 0·86 | 8·0± 0·59 | 5·5± 0·40 | Adult |
| 57 | 4·4± 0·15 | 4·8± 0·36 | 4·7± 0·15 | | 2·4± 0·81 | 2·0± 0·23 | 0·6± 0 | | 6·8± 0·96 | 6·8± 0·59 | 5·3± 0·15 | |
| 64 | 4·4± 0·45 | 4·8± 0·50 | Adult | | 1·6± 0·25 | 0·9± 0·01 | Adult | | 6·0± 0·70 | 5·7± 0·51 | Adult | |
| 71 | 4·4± 0·51 | Adult | | | 1·2± 0·24 | Adult | | | 5·6± 0·75 | Adult | | |
| 78 | 4·4± 0·56 | | | | 0·8± 0 | | | | 5·2± 0·56 | | | |
| 85 | Adult | | | | Adult | | | | Adult | | | |

Each value represents the average performance of 5 individuals and data reported as ± indicate the standard deviation.

**Table 2.** Influence of distillery effluent on length of hind limb and fore limb of *R. malabarica*.

| | Hindlimb length (cm) | | | | Forelimb length (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| Day | Control | 0·03% | 0·06% | 0·12% | Control | 0·03% | 0·06% | 0·12% |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0·5± 0·06 | 0 | 0 | 0 | 0 |
| 22 | 0 | 1·1± 0·23 | 0·8± 0·20 | 1·2± 0·32 | 0 | 0 | 0 | 0 |
| 29 | 1·0± 0·14 | 1·8± 0·40 | 1·3± 0·26 | 1·9± 0·35 | 0 | 0 | 0 | 0·2± 0·05 |
| 36 | 1·2± 0·15 | 2·0± 0·06 | 2·1± 0·05 | 2·5± 0·26 | 0 | 0 | 0 | 0·6± 0·10 |
| 43 | 1·9± 0·12 | 2·6± 0·13 | 2·4± 0·25 | 2·8± 0·05 | 0 | 0 | 0·1± 0 | 1·1± 0·06 |
| 50 | 2·5± 0·06 | 2·7± 0·05 | 2·7± 0·05 | 2·8± 0·06 | 0 | 0 | 0·6± 0·15 | 1·7± 0·10 |
| 57 | 2·5± 0·06 | 2·7± 0·06 | 2·7± 0·04 | | 0 | 0·1± 0 | 1·1± 0·12 | |
| 64 | 2·5± 0·06 | 2·7± 0·06 | 2·7± 0 | 0 | 0 | 0·6± 0·06 | 1·7± 0·10 | |
| 71 | 2·5± 0·06 | 2·7± 0·06 | 0 | | 0·3± 0·07 | 1·1± 0·06 | | |
| 78 | 2·5± 0·22 | 2·7± 0·32 | | | 0·8± 0·87 | 1·7± 0·05 | | |
| 85 | 2·5± 0·04 | | | | 1·8± 0·17 | 1·7± 0·06 | | |

Each value represents the average performance of 5 individuals and data reported as ± indicate the standard deviation.

(6·3 g) when compared with those reared at 0·03% (8·2 g), 0·06% (6·8 g) and 0·12% (6·8 g; figure 1). At 0·03% maximum body weight was noticed as two peaks on the 29th and 50th day whereas the same was noticed as only one peak on the 29th day and 22nd day for those exposed to 0·06% and 0·12% effluent respectively. These two peaks correspond to the times of necrosis of old and build up of new tissues in relation to formation of gut and fore and hind limbs and lungs and resorption of gills, old gut and tail. The reason for the elevation of the peak from the control in relation to 0·03% and its depression in the case of 0·06% and 0·12% is under separate investigation.

According to Hota and Dash (1981) body size in poikilotherms is controlled by differences in environmental conditions such as food availability and larval density. The body size and growth rate of *Rana* larvae are functions of amount of available food (Wilbur 1977), density (Brockelman 1969; Wilbur and Collins 1973; De Benedicts 1974) and temperature (Hota and Dash 1981). As already cited, most of the reports on growth and metamorphosis of amphibians deal with influence of food limitation and density. The few reports which are available on the impact of pesticides (*e.g.* Cook 1971; Greenhouse 1976) or industrial effluents on amphibian larvae (*e.g.* Ghate *et al* 1978) mainly deal with teratogenic and embryological properties and do not reveal any information on growth and/or metamorphosis. The toxic agents which inhibit or

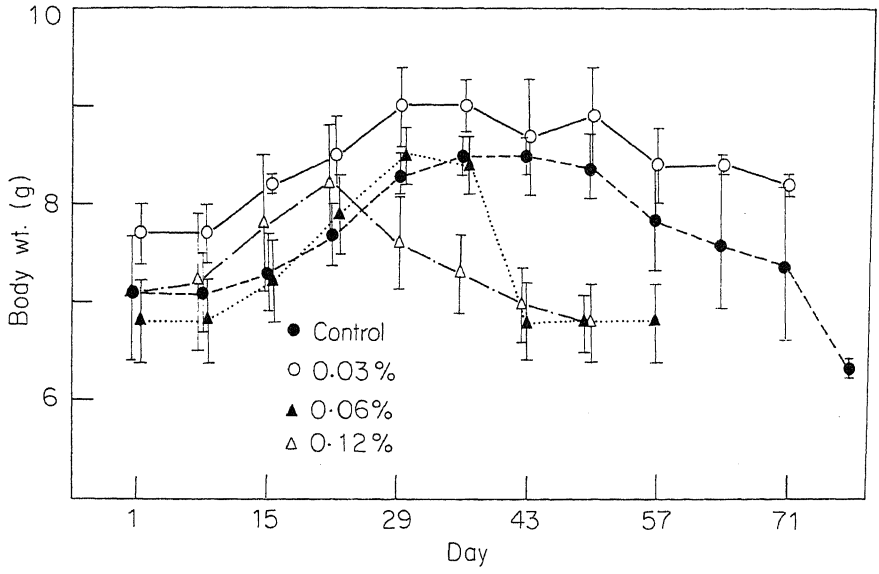


**Figure 1.** Influence of distillery effluent on body weight of *R. malabarica*. Each value represents the average performance of 5 individuals.

**Table 3.** Analysis of variance: Influence of time (week) and treatment (effluent concentration) on length of limbs, tail and body and weight of *R. malabarica*

| | Time effect | | Treatment effect | |
|---|---|---|---|---|
| Character | *F*1 value | Probability | *F*2 value | Probability |
| Adult weight | 2·917 | $P < 0·01$ | 0·434 | $P > 0·05$ |
| Body length | 5·857 | $P < 0·01$ | 10·857 | $P < 0·01$ |
| Tail length | 11·729 | $P < 0·01$ | 0·436 | $P > 0·05$ |
| Fore limb | 0·857 | $P > 0·05$ | 0·357 | $P > 0·05$ |
| Hind limb | 2·165 | $P < 0·05$ | 0·410 | $P > 0·05$ |

$P < 0·05$ significant
$P > 0·05$ not significant.

modify the development of the animal, are likely to be detrimental even if the adults of a particular species are apparently unaffected.

Limb bud emergence and tail resorption occurred earlier in tadpoles exposed to 0·12% effluent (tables 1 and 2). According to Haniffa and Sundaravadhanam (1983), *Barbus stigma* exposed to lower concentrations of distillery effluent showed more food consumption and growth than those exposed to tap water. The above authors suggested that the chemical constituents at lower concentrations enhanced the growth through food consumption. Barnabas (1983) also confirmed this by reporting decrease in the duration of metamorphosis and an increase in body weight of *R. malabarica* exposed to lower concentrations (up to 0·12%) and vice versa, at higher concentrations (0·15% and above). Hence it is possible to suggest that the increase in body weight of

*R. malabarica* at lower concentrations (up to 0·12%) could be due to more food consumption.

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# Bait preferences of rodents in their natural habitat

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**Abstract.** Preferences of rodents toward cereal baits have been studied in relation to the availability of food from their natural habitat in crop fields of groundnut (*Arachis hypogaea*) and lentil (*Lens culinaris*). The experimental area was infested by three rodent species—*Bandicota bengalensis*, *Tatera indica* and *Mus* sp. At the podding stage of groundnut crop they showed a poor response towards plain bait of whole wheat grains, the consumption of which increased significantly after addition of arachis oil at 1 % concentration. The withdrawal of oil from the bait had no significant effect on its daily consumption by the rodents. In paired bait tests in podding groundnut crop, the addition of oil significantly increased the bait consumption of wheat and millet grains. The differences between daily consumption of millet grains became more significant when the bait station pairs were shifted to growing lentil crop which reflect the effect of environment on the feeding responses of rodents. Laboratory tests with *B. bengalensis* and *T. indica* trapped from the experimental fields confirmed the results of field studies that addition of oil in the cereal bait enhance bait consumption.

**Keywords.** Arachis oil; bait; crop fields; groundnut; lentil; millet; rodents; wheat.

## 1. Introduction

Previous laboratory studies have revealed that quality of the oil in the cereal baits significantly affect the bait preferences of rodents (Barnett 1966; Durairaj and Rao 1975; Kamal and Khan 1977; Kumari and Khan 1978; Ramana and Sood 1982). But no information is available about the effects of oil on the bait consumption by wild rodents in their natural environments in relation to the availability of food from the crops. Therefore, the same have been studied in groundnut and lentil crop fields. Such information would be useful in improving poison baiting programmes by attracting larger number of rats to the baits and increasing poison bait consumption.

## 2. Materials and methods

### 2.1 *Experimental fields*

The field experiments were conducted in the following fields of Punjab Agricultural University, Ludhiana (30°56′ N, 75°52′E).

2.1a *Field A* (*figure* 1*a*): Groundnut (*Arachis hypogaea*) crop field (podding stage) including a 3 m broad strip of non-cropped land full of weeds was selected. It was surrounded by groundnut crop fields on the North and East, a brick-walled water channel on the west and a field road on the south. A heterogeneous and almost stable population was observed in the experimental fields by weekly live burrow counts (see

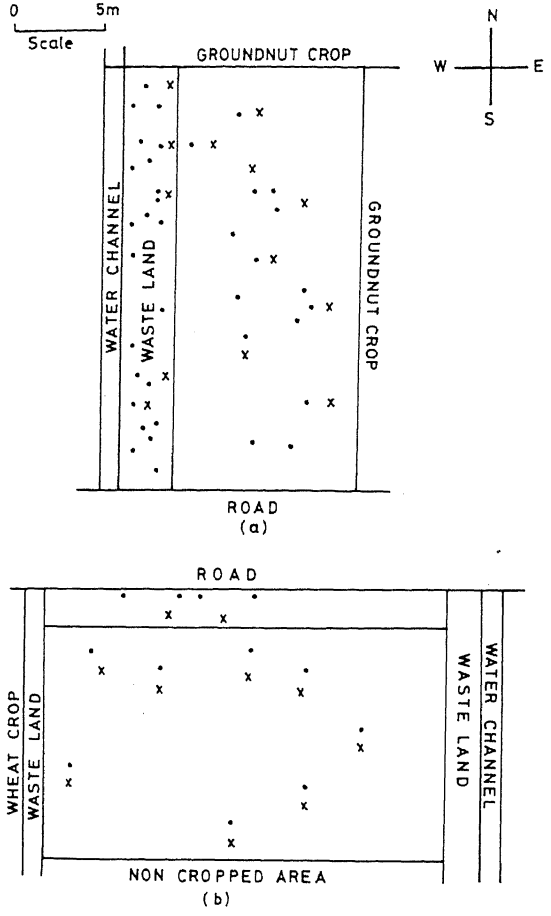


**Figure 1.** Diagrammatic plans of the **a.** groundnut crop field and **b.** lentil crop field showing the locations of burrow holes (●) of rodents and of bait stations (×).

figure 1). Trapping of rodents in this area showed the occurrence of three species namely *Bandicota bengalensis*, *Tatera indica* and *Mus platythrix*.

2.1b *Field B* (*figure* 1*b*): This field was adopted to study the effects of change of crop and availability of food material on the bait preferences of rodents. Here, lentil (*Lens culinaris*) was sown after harvesting of groundnut crop. This field was almost similar to field A (figure 1b) and the same three species of rodents occurred here.

## 2.2 *Field trials*

In the above fields six trials were carried out one after the other, the details of which are given below. The number and duration of placement of bait stations during each trial are given in tables 1 and 2. At each baiting point 50 g bait was placed in wooden boxes (26 × 10 × 11 cm) near the burrow openings (figure 1).

**Table 1.** Mean daily bait consumption (g per bait station) of rodents in single bait tests in the podding stage of groundnut crop[a].

| Trial No. | Bait | Number of bait stations: Total | Number of bait stations: Showing consumption (Mean ± SE) | Days | Mean consumption (± SE) |
|---|---|---|---|---|---|
| 1. | Wheat grains | 13 | 2·2 ± 0·66 | 5 | 0·48 ± 0·13* |
| 2. | Wheat grains and oil (99:1) | 13 | 10·3 ± 1·06 | 7 | 2·46 ± 0·6[b] |
| 3. | Wheat grains | 10 | 9·4 ± 0·6 | 5 | 2·64 ± 0·54 |

[a]Though no marked change in burrow counts has been observed during the single bait test but slight changes in rodent population during different trials can not be excluded.

[b]The differences between mean daily consumption of wheat grain bait (*) with other baits are significant ($P < 0·05$).

**Table 2.** Mean daily bait consumption (g per bait station) of rodents in paired bait tests in maturing groundnut and growing lentil crops.

| Trial No. | Crop | Number of bait station pairs[a]: Total | Showing consumption Mean ± SE | Days | Mean consumption (± SE) | |
|---|---|---|---|---|---|---|
| | | | | | Wheat | Wheat and Oil |
| 4. | Maturing groundnut | 10 | 9·5 ± 0·29 | 4 | 1·095 ± 0·21 | 3·46 ± 0·15[b] |
| | | | | | Millet | Millet and Oil |
| 5. | Maturing groundnut | 10 | 7·57 ± 1·51 | 7 | 2·5 ± 0.62 | 3·39 ± 0·88[b] |
| 6. | Growing lentil | 10 | 7·29 ± 1·04 | 7 | 3·14 ± 0·15 | 7·05 ± 0·20[b] |

[a]The bait stations were exposed to the same population.

[b]The differences between mean consumption within a pair are significant ($P < 0·05$).

*Trial* 1: To determine the initial response of rodents towards the plain cereal bait without oil, wheat grains were placed at 13 baiting points in field A for 5 days. The bait consumption per 24 hr was recorded.

*Trial* 2: To study the effect of oil on bait consumption, whole wheat grains smeared with 1% arachis oil were offered to rodents as in 'trial 1'.

*Trial* 3: After enhancing bait consumption with oil in trial 2, 'trial 1' was repeated to study the effect of withdrawal of oil on plain bait consumption.

*Trial* 4: Assuming that baits placed at two adjoining baiting points would be available to the same population of rodents, the box pairs with plain wheat grains and wheat with

arachis oil (99:1) separately were placed in fields as shown in figures 1 a, b. The distance between the two boxes of the pair was about 15 cm. The position of the bait boxes was altered after an interval of 24 hr to eliminate any side preference.

*Trial* 5: To study the effects of change of cereal on the preference behaviour of rodents towards the oily bait, this trial was carried out as 'trial 4' by replacing wheat with millet (*Pennisetum typhoides*) grains.

*Trial* 6: This trial was carried out in lentil crop (*L. culinaris*) similar to trials 4 and 5 using millet as the cereal.

### 2.3 *Laboratory experiments*

For the laboratory test, specimens of *B. bengalensis* and *T. indica* were trapped from the experimental fields and caged individually in $92 \times 30 \times 25$ cm size cages. After acclimatization for 15 days in the laboratory they were offered in choice, 50 g each of wheat grains with and without 1 % arachis oil in separate food cups. The side of the food cup was altered daily to eliminate effects of site preferences of rodents on food consumption.

For comparing the bait consumption between the trials and between two baits within the same trial, *t*-test of the difference between means was applied (Sokal and Rohlf 1973).

## 3. Results

Comparisons of mean bait consumption between the baits with and without oil are given in tables 1 and 2 and the daily pattern of the mean cumulative bait consumption is illustrated in figure 2. The rodents showed a poor response (trial 1, table 1) towards plain wheat grain bait at the podding stage of the groundnut crop in which signs of considerable damage of developing pods by rodents were also observed. Addition of 1 % arachis oil increased the feeding of wheat grain bait in more number of bait boxes and resulted in a significant increase in bait consumption (trial 2). After enhancing bait consumption with oil its withdrawal had no significant effect on the bait consumption by rodents during the subsequent five days (cf trials 2 and 3). The damage to groundnut pods continued to occur during trials 2 and 3, as well as during subsequent trials.

When the bait stations were placed in pairs, one containing cereal grains without oil and the other with oil, the rodents preferred the oily bait (trials 4 and 6, table 2). The nature of cereal had no effect on the preference of rodents for oily baits as in both cases they preferred oily cereals over the respective plain cereals. However, more millet grains were consumed than wheat indicating its preference by rodents. Preference of rodents for millet with oil over grains without oil (trial 6, table 2) continued in the lentil crop which was sown after groundnut harvesting. These results indicate that the crop had no effect on the choice of rodents for cereals containing the oil. However, the differences between the mean daily consumption of millet grains with and without oil were more significant in lentil fields than in groundnut fields indicating the effects of the crop on the feeding responses of rodents. Though rodent damage occurred in lentil crop but no sign of nibbling or eating of any part of the plant was observed.

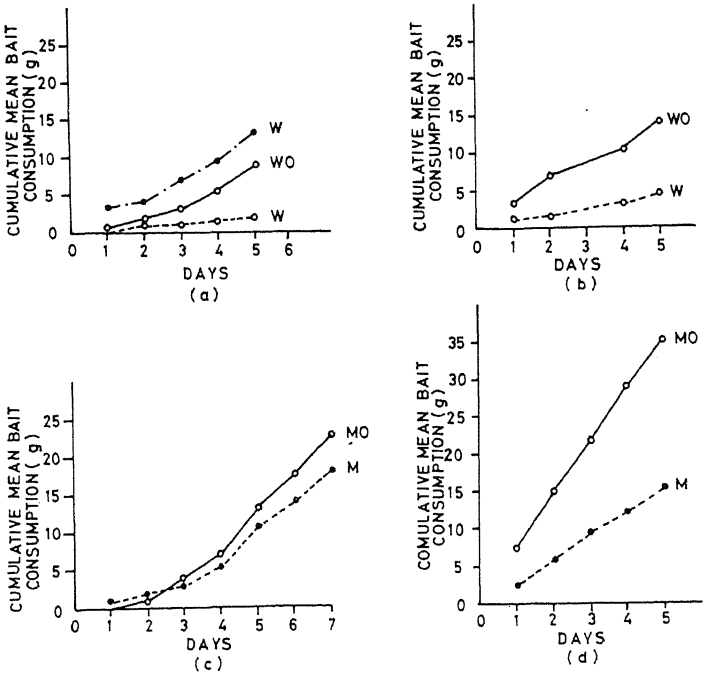


**Figure 2.** Comparisons of daily bait consumption of rodents in experimental fields between the cereal baits without oil (W, wheat; M, millet) and with oil (WO and MO, indicates wheat and millet grains with 1 % arachis oil) **a.** cumulative bait consumption in single bait trials in groundnut crop fields, **b.** in paired bait trial (trial 4) in groundnut crop field where wheat grains were used as the bait, **c.** in paired bait trial (trial 5) in groundnut crop where millet was used as the bait, and **d.** in paired bait trial in lentil crop field where millet was used as a cereal bait.

**Table 3.** Mean daily bait consumption (per 100 g body weight) of rodents in bi-choice test in laboratory.

| No. of test | Species | No. of individuals | Days | Mean consumption ± (SE) Wheat | Wheat and Oil (99:1) |
|---|---|---|---|---|---|
| 1. | *B. bengalensis* | 4 | 6 | 3·30 ± 0·13 | 4·20 ± 0·15 |
| 2. | *T. indica* | 6 | 6 | 3·96 ± 0·16 | 5·15 ± 0·18* |

*The differences between mean consumption within a pair are significant ($P < 0{\cdot}05$).

Specimens of two species *B. bengalensis* and *T. indica* captured from the experimental fields showed preference (table 3) for wheat grains containing arachis oil over the grains without oil in a laboratory experiment; thus confirming the results of the field trials.

## 4. Discussion

The results of the present field studies have shown that addition of 1 % arachis oil in the bait significantly increases bait consumption of two cereal baits (wheat and millet) by

rodents in field situations of groundnut and lentil crops. Similar response of rodents (*B. bengalensis* and *T. indica*) toward oily grains was observed in laboratory experiments in the present as well as in previous studies (Kamal and Khan 1977; Ramana and Sood 1982). The possibility cannot be excluded that the increased consumption of cereal grains containing oil in single bait tests may be due to some population change in the crop fields but the results of feeding during paired bait tests confirm that arachis oil enhances bait consumption.

Poor response of rodents towards the wheat bait during initial trial at the podding stage of the crop may be due to their preference for juicy and sweet pods of groundnut, as field observations showed high damage of developing pods. Inspite of the availability of the preferred food material from the groundnut crop, the addition of oil in the dry grains, both in single and paired bait tests, enhanced the consumption of wheat grains. This response may also be related to the familiarity of the odour or taste of arachis oil from the bait with that of groundnut pods from the field. Food flavour familiarity forms an important factor controlling the food preference behaviour of wild rodents (Barnett 1975; Shumake 1978). No significant change in bait consumption after withdrawal of the oil (cf. trials 2 and 3), further indicate that the rodents continue to eat the familiar food material.

In lentil crop also the rodents preferred the oily bait and the total bait consumption was significantly higher than in the groundnut crop in the same field. This may be due to the absence of preferred food material from the crop as no sign of its nibbling and eating by rodents was noticed.

## Acknowledgements

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# Biochemical correlates of agonistic behaviour in *Bandicota bengalensis*: Hepatic cholesterol and ascorbic acid

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**Abstract.** Social stress was induced in *Bandicota bengalensis* by staging 10 min encounters in neutral area between male-male, male-female and residents- intruder male for 9 continuous days. On the 10th day changes in the weight, ascorbic acid, and cholesterol levels of liver were estimated. In submissive males, females of male-female encounter and intruders, adrenals hypertrophied. Liver weight remained unaltered. Ascorbic acid levels increased in both members of heterosexual pair, residents and intruder but decreased in subordinates compared to controls. Cholesterol increased in subordinate males and stressed females.

**Keywords.** Social stress; *Bandicota bengalensis*; adrenals; liver; ascorbic acid; cholesterol.

## 1. Introduction

Amongst small mammals increased adrencorticotrophic hormones (ACTH) are implicated in crowding and other social stressful situations leading to hyperadrenalism, increased adrenalin and noradrenalin content of the adrenal glands and enhanced secretion of adrenal steroids (Barnett 1969). Increased steroid production by adrenals involves cholesterol as substrate and the reaction is dependent on ascorbic acid. Both cholesterol and ascorbic acid are synthesized in the liver and hence the levels of these can be considered as an index and reflection of social stress in rodents. Archer (1969) regarded adrenal cholesterol and ascorbic acid levels to reflect crowding stress in mice. This paper examines the significance of changes in hepatic cholesterol and ascorbic acid content during social stress in *Bandicota bengalensis.*

Based on inter-species conflict *B. bengalensis* is considered as the most aggressive wild rodent in India (Spillet 1968; Sridhara *et al* 1980). The species also exhibits high levels of intraspecies strife (Sridhara and Krishnamoorthy 1983). Adrenal hypertrophy was shown to accompany such interspecific agonistic interactions (Sridhara *et al* 1983). The present paper reports hepatic levels of cholesterol, the precursor for adrenal steroid synthesis and ascorbic acid levels in *B. bengalensis* exposed to different kinds of social stress.

## 2. Materials and methods

Wild *B. bengalensis* were collected and maintained according to Sridhara and Krishnamoorthy (1983). Behavioural stress was induced by three ways (i) by allowing

interactions between two adult males for 15 min daily (ii) by staging male-female encounters for 15 min daily (iii) by introducing an intruder male into an all male resident cage for the same duration daily. The paired interactions were staged in a behaviour chamber described elsewhere (Sridhara and Krishnamoorthy 1983). It consisted of a 100 × 50 × 50 cm galvanized iron chamber with a glass front, glass sides of sliding type to facilitate introduction of animals and a wiremesh top. A slot in the centre of the roof enabled insertion of a thin metal sheet thus dividing the chamber into two equal portions.

Prior to induction of stress the animals were kept in isolation for 15 days. From days 16–24 members of hetero- and iso-sexual pairs were introduced into either side of the behaviour chamber and allowed to habituate to the test environment for 10 min. Later the metal partition was removed and the two animals allowed to interact for 10 min daily over a nine day schedule. Similarly three adult males of differing weights were kept in a single cage (35 × 35 × 50 cm) for 15 days. Such animals were considered residents in contrast to single males kept in isolation. The latter were assigned intruder status as they were put into resident cages from 16th to 24th days for 10 min each day for 9 continuous days.

Rats of both sexes, kept in isolation served as controls.

On the 25th day both experimental and control animals were weighed and sacrificed by decapitation. Adrenals, both left and right and whole liver were excised, cleaned of blood and weighed. The levels of ascorbic acid, and cholesterol from livers of differentially stressed rats were determined according to Omaye *et al* (1979) and Sperry and Webb (1950) respectively.

Adrenal: body weight and liver: body weight ratios were calculated and compared for different stress situations to see if hypo- or hypertrophy of the organs ensued consequent to social stress. A total of six male-male, six male-female and six resident-intruder interactions were staged, each over a nine day schedule. Statistical significance were arrived at by student $t$ tests.

## 3. Results

The behavioural aspects of the three different social situations have been reported elsewhere (Sridhara *et al* 1983). Briefly one male dominated the other in male-male encounters. The latter was considered submissive. In heterosexual encounters males dominated females although females too exhibited aggressive behaviour. Residents were agonistic towards intruders.

Adrenals hypertrophied in submissives, females of male-female conflict and intruders (table 1). Females kept in isolation had heavier adrenals than their male counterparts. The sexes did not differ in the weight of liver. Similarly social stress did not affect liver weight in any of the experimental situations. Only submissive males and females of heterosexual conflict registered significantly lower hepatic ascorbic acid levels compared to dominants and males of male-female pair respectively (table 1). Compared to control males the liver ascorbic acid levels decreased only in subordinate rats ($P < 0{\cdot}01$) but rose considerably in males of heterosexual conflict ($P < 0{\cdot}01$) and in both residents ($P < 0{\cdot}05$) and intruders ($P < 0{\cdot}02$). Similarly stressed females had higher ascorbic acid levels compared to female controls ($P < 0{\cdot}01$).

Amongst controls the two sexes did not differ in cholesterol levels of liver (table 1).

**Table 1.** Changes in weight of adrenal, hepatic, ascorbic acid and cholesterol levels due to social stress.

| | Control | | | male *vs* male | | | male *vs* female | | | resident *vs* intruder | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | $P<$ | dom | sub | $P<$ | M | F | $P<$ | res | int. | $P<$ |
| Body weight (g) | 178 ± 3·3 | 173 ± 4·02 | NS | 181 ± 6·26 | 203 ± 3·12 | NS | 201 ± 6·75 | 174 ± 8·56 | NS | 225 ± 7·52 | 231 ± 3·83 | NS |
| Adrenal weight (mg) | 103·4 ± 2·31 | 397·7 ± 6·75 | 0·001 | 126·7 ± 7·21 | 243·6 ± 3·28 | 0·001 | 311·5 ± 2·58 | 690·01 ± 3·82 | 0·001 | 517·5 ± 8·95 | 866·25 ± 10·72 | 0·001 |
| Adrenal wt/body wt ($10^{-3}$ otherwise mentioned) | 5·8* ± 2·19 | 23·0 ± 2·33 | 0·001 | 7·00* ± 1·24 | 12·00 ± 1·3 | 0·001 | 15·5 ± 2·45 | 39·66 ± 4·42 | 0·001 | 23·00 ± 1·95 | 37·5 ± 2·63 | 0·001 |
| Ascorbic acid (mg/g) | 12·7 ± 1·07 | 13·64 ± 2·07 | NS | 13·33 ± 1·17 | 6·66 ± 1·38 | 0·001 | 28·15 ± 2·22 | 22·03 ± 2·61 | 0·001 | 23·04 ± 2·37 | 24·94 ± 4·07 | NS |
| Cholesterol (mg/g) | 2·26 ± 0·85 | 3·84 ± 1·03 | NS | 2·61 ± 0·23 | 5·72 ± 0·7 | 0·001 | 9·65 ± 0·94 | 8·75 ± 0·36 | NS | 3·83 ± 0·43 | 3·41 ± 0·33 | NS |

* $10^{-4}$.
NS: not significant.

Heterosexual and resident-intruder conflict did not affect cholesterol content of liver while male-male encounters resulted in enhanced levels in subordinate rats (table 1). Social stress elevated cholesterol levels in the liver of submissive males ($P < 0{\cdot}05$). Similarly females of heterosexual interaction had higher hepatic cholesterol levels compared to control females ($P < 0{\cdot}001$).

## 4. Discussion

Hyperactivity of adrenals and accompanying enhanced corticosteroid production has been correlated with density and territoriality (Christian and Davies 1964; Andrews *et al* 1972), disorganization of social behaviour (Benton *et al* 1978) and agonistic interactions (Bronson and Eleftheriou 1964; Archer 1969): Subordinate and defeated mice had heavier adrenals (Brain 1972; McKinney and Pasley 1979; Sridhara *et al* 1983). Adrenal hypertrophy was also reported in intruders and females of *Bandicota bengalensis* during resident-intruder and male-female confrontations respectively (Sridhara *et al* 1983). The elevated corticosteroid levels in such situations are believed to affect population dynamics in small mammals (Christian *et al* 1965). Cholesterol is the source of steroid hormones formed in adrenal cortex (White *et al* 1978). Increased social stress was correlated with depleted adrenal cholesterol during male-male, resident-intruder and male-female encounters (Sridhara *et al* 1983). Since liver is the major site of cholesterol synthesis, hepatic levels of cholesterol possibly reflect changed rates of corticosteroid synthesis. The present finding of higher hepatic cholesterol in submissive and female rats compared to dominants and males of heterosexual pairs lends credence to the theory of adrenal hyperactivity during social stress.

Barnett (1969) postulated that social interactions of mammals induces physiological changes which resemble those resulting from adverse conditions like cold or infection. Ascorbic acid content of adrenals, testis, liver and kidney of rats increased significantly during cold exposure (Dugal and Therien 1955). In several mammals ascorbic acid administration was shown to increase tolerance to cold acclimation and acclimatization (Lloyd and Sinclair 1953). Additionally ascorbic acid is essential for the synthesis of adrenal steroids from cholesterol. Liver is the site of ascorbic acid synthesis. In the current study the hepatic levels of ascorbic acid were higher in subordinates compared to dominants and females compared to males during male-male and male-female conflict respectively. Such enhanced synthesis of ascorbic acid may contribute towards coping with stress and also reflect increased demand for ascorbic acid due to elevated corticosteroid synthesis during behavioural stress.

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# Topography of nervous system in two pouched paramphistomes

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**Abstract.** Using the indigogenic technique for localizing the non-specific esterases, the complete nerve arrangement in *Fischoederius elongatus* and *Gastrothylax crumenifer*, the paramphistome parasites in the rumen of cattle, has been visualized. In *F. elongatus*, of the three pairs of anterior nerves given off from the cerebral ganglia, two are ventral and one is dorsal in disposition. The nerves running posterior from the cerebral ganglia include two pairs of ventral nerves and one of dorsals. Throughout their course the two ventrals of either side are joined to each other by several loop-like connectives. In *G. crumenifer*, the overall nerve arrangement is somewhat similar to that in *F. elongatus*; the connectives joining the posterior ventrals of each side are not loop-like. The course of all the nerves and the innervation to the various parts of the body is traced in both the species.

**Keywords.** Nervous system; paramphistome; trematoda; digenea; *Fischoederius elongatus*; *Gastrothylax crumenifer*.

## 1. Introduction

In recent years, the association of non-specific esterases (NSE) with the nervous system has been successfully exploited by many workers for the demonstration of the fine nerve arrangements and other components of the nervous system in several trematode species (Mishra and Tandon 1984). Based on the localization of the esterases, the nervous organization has been described in some more digenetic flukes, namely, *Encyclometra colubrimurorum*, *Echinostoma revolutum*, *Schistosoma spindalis*, *Isoparorchis hypselobagri*, *Singhiatrema najai* and *Euparadistomum herpestesi* (Kishore and Shyamasundari 1980; Krishna 1981; Rao *et al* 1982; Simha and Fernandez 1982; Fernandez *et al* 1982; Kishore *et al* 1982).

Among the paramphistome flukes most of the available accounts on the nervous system are based on histological observations (Gupta and Dutta 1967; Lee 1971). However, the pattern of nerve distribution has been visualized in toto in *Fischoederius cobboldi*, a pouched paramphistome of bovines (Mishra and Tandon 1984). This study revealed significant deviations from the studies on the sectioned material.

The present communication also deals with the topography of the nervous system in entire in two more pouched paramphistome species, namely, *Fischoederius elongatus* (Poirier 1883) Stiles et Goldberger, 1910 and *Gastrothylax crumenifer* (Creplin 1847) Poirier 1883.

## 2. Material and methods

Live specimens of *F. elongatus* and *G. crumenifer* were collected in 0·9 % saline from the rumen of cattle slaughtered at the local abattoirs. The procured material was fixed and processed for localization of NSE as described elsewhere (Mishra and Tandon 1984).

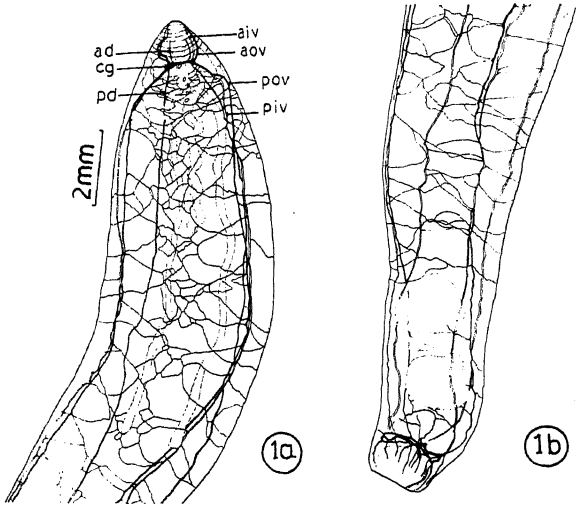
## 3. Observations

Conforming to the commonly observed pattern in many other trematode species, in *F. elongatus* also three pairs of nerves run cephalad and three pairs caudad from the brain mass which comprises two cerebral ganglia connected with a thick band-like cerebral commissure and lies immediately posterior to the pharynx and dorsal to the oesophagus (figures 1, 2).

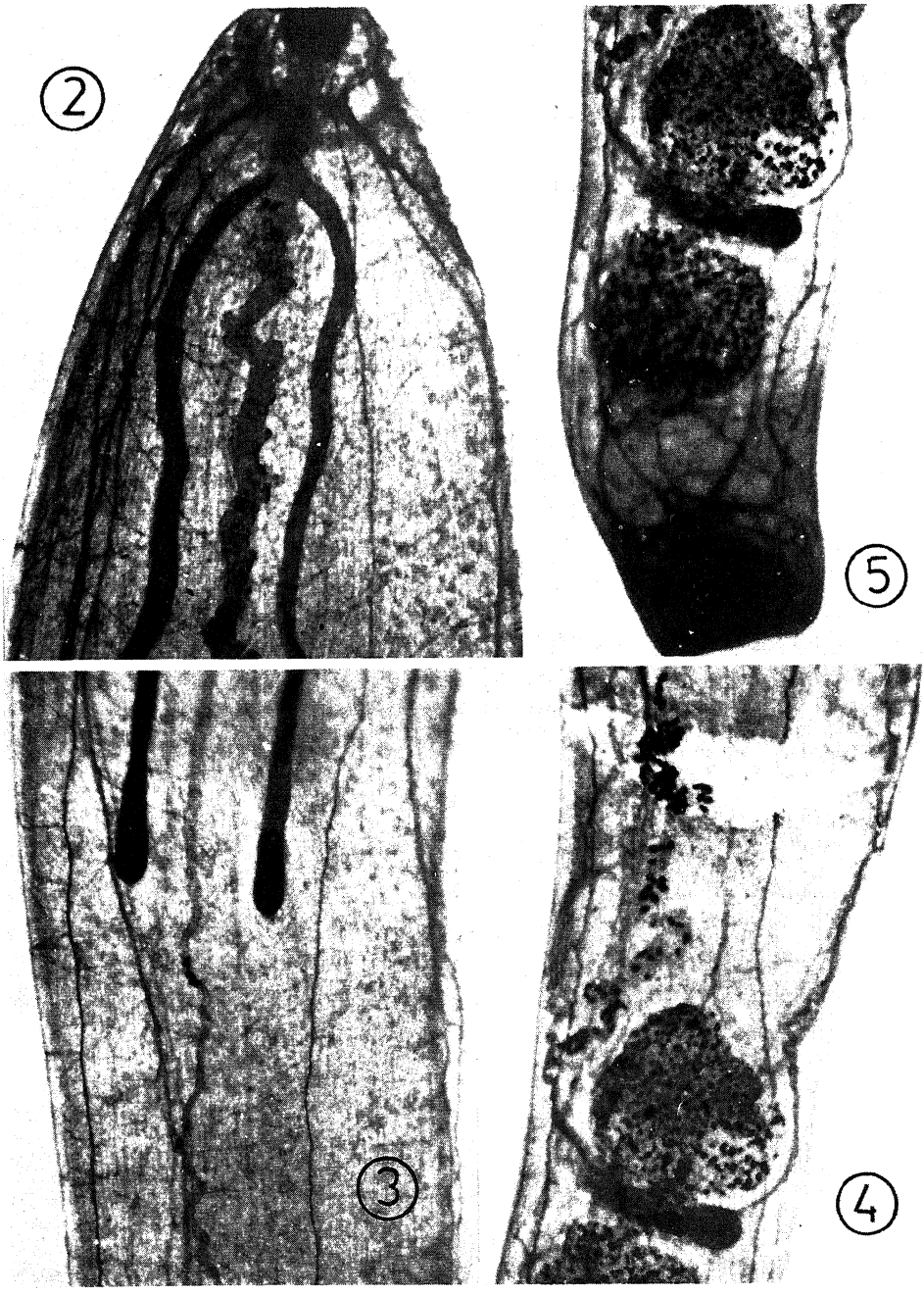
Of the anterior nerves, there is one pair of dorsal nerves and 2 pairs of ventral nerves. The dorsal nerves arise from the inner border of the cerebral ganglia and supply the oral tip and the dorsal surface of the pharynx and tegument. The lateral walls of the pharynx are supplied by the antero-inner ventral nerves that arise from the antero-median facet of the ganglia. The antero-outer ventral nerves, arising from the outer lateral side of the cerebral ganglia, innervate the whole ventral surface of the pharynx. Thin transverse connectives, completely encircling the pharynx, join all the anterior nerves with one another. Very fine branches from these are observed petering out into the circum-pharyngeal tegument.

The post cephalic longitudinal nerves comprise a pair of postero-dorsals and two pairs of postero-ventrals (figures 3, 4). The postero-dorsals originate from the inner facet of the cerebral ganglia and are superfically placed nerves. Fine branches of these nerves innervate the gut, reproductive system and excretory bladder. The postero-dorsals of the two sides are joined with each other by thin transverse connectives throughout their course; the connectives, in turn, are connected to one another by still thinner, 2–3 longitudinal connectives, thus forming a nerve net under the dorsal surface of the body.

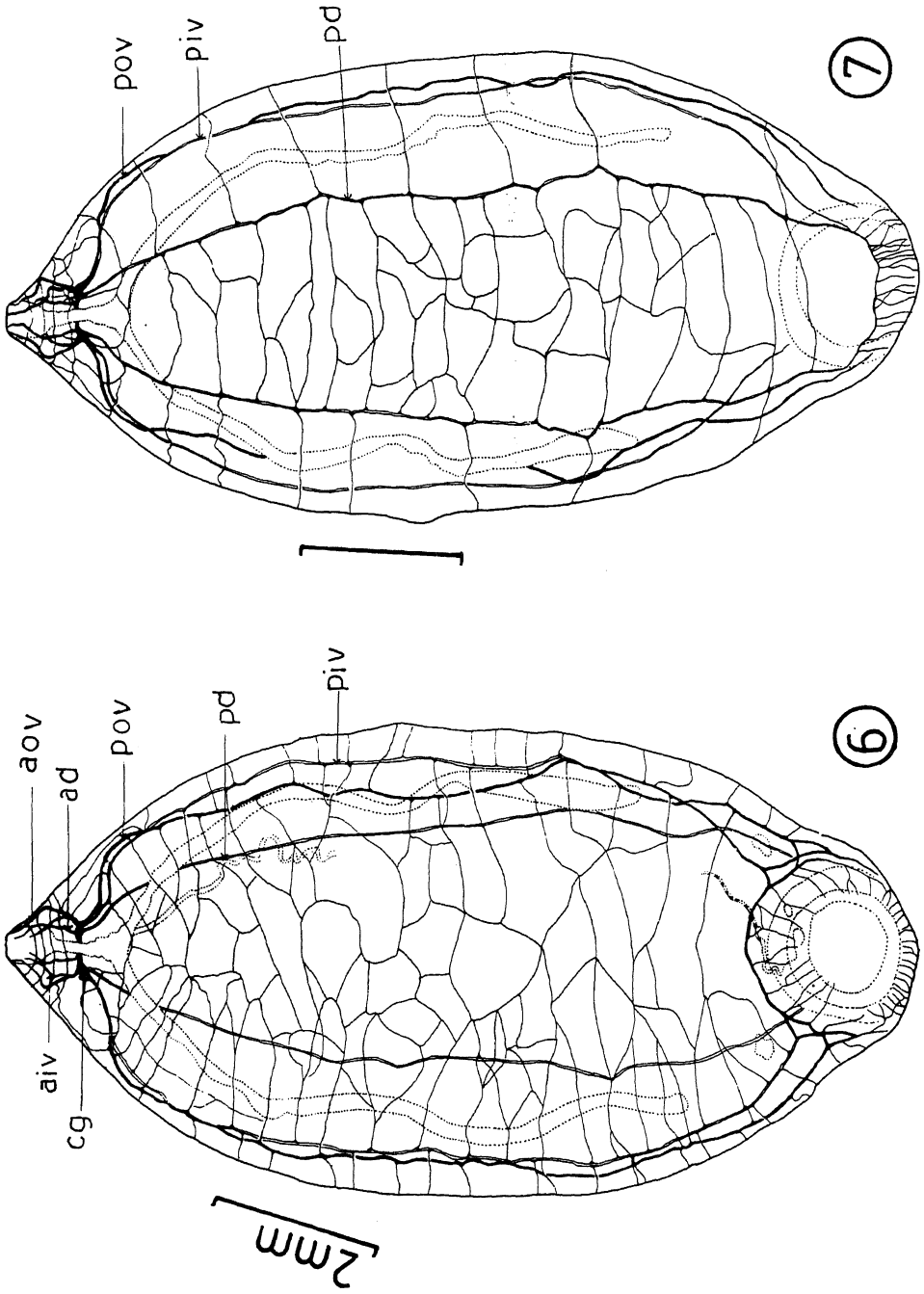
The postero-inner ventral nerves arise from the postero-ventral border of the cephalic ganglia and terminate in the region of the acetabulum. The postero-outer ventral nerves arise from the lateral aspects of the brain. Initially they run lateral to the inner ventrals for some distance but thereafter are seen changing their course and running inner to the latter at places. A thin, longitudinal lateral connective joins the




**Figures 1a, b.** For caption, see next page.




**Figures 1–5.** *Fischoederius elongatus* **1.** Camera lucida sketch of the whole mount of the worm (ventral view); **a.** anterior half; **b.** posterior half. For clarity sake only the nerve net of postero-ventral nerves has been shown. **2.** Brain mass and the main anterior and posterior nerves ( × 12·3). **3.** Posterior longitudinal nerves in the midbody region ( × 12·3). **4.** Posterior longitudinal nerves in the gonadal and pregonadal region ( × 12·3). **5.** Terminal course of the posterior nerves and innervation of the acetabulum.




**Figures 6–7.** For captions, see page no. 138.

antero- and the postero-outer ventral nerves of the same side. Along most of their length the two postero-ventrals (*i.e.*, outer and inner) of either side are joined with each other by many, rather conspicuous but thin, laterally placed loop-like connectives, which appear C- or tilted V-shaped. Fine branches from these loops constitute a nerve net on the midventral side and innervate the tegument and the wall of the ventral pouch. The tributaries of the postero-ventrals also innervate the reproductive system, vitellaria and the opening of the ventral pouch. The postero-dorsals are connected only to the postero-outer ventrals, and not to the inner ventrals, by means of lateral connectives. While the postero-inner ventrals are deep seated in the parenchyma, the postero-outer ventrals are superficially lodged.

All the posterior longitudinal nerves join with one another and form a conspicuous ring-like nerve just in front of the anterior border of the acetabulum. Gradually tapering branches given out from this nerve and further secondary fine branches of these innervate the sucker (figures 1b, 5).

Neuroanatomy of *Gastrothylax crumenifer*, also having three pairs each of anterior and posterior nerves, follows the same pattern as that of *F. elongatus* (figures 6–8). The origin, position and naming of the anterior and posterior nerves are the same as described for *F. elongatus*. All the anterior nerves of both the sides are joined with one another by means of thin connectives encircling the pharynx. However, a conspicuous circumpharyngeal nerve basket is not formed.

The postero-outer and inner ventral nerves send branches towards the median axis, which ramify and anastomose with one another, thus constituting a nerve net on the ventral surface (figure 9). Both these nerves also send branches towards the lateral body wall.

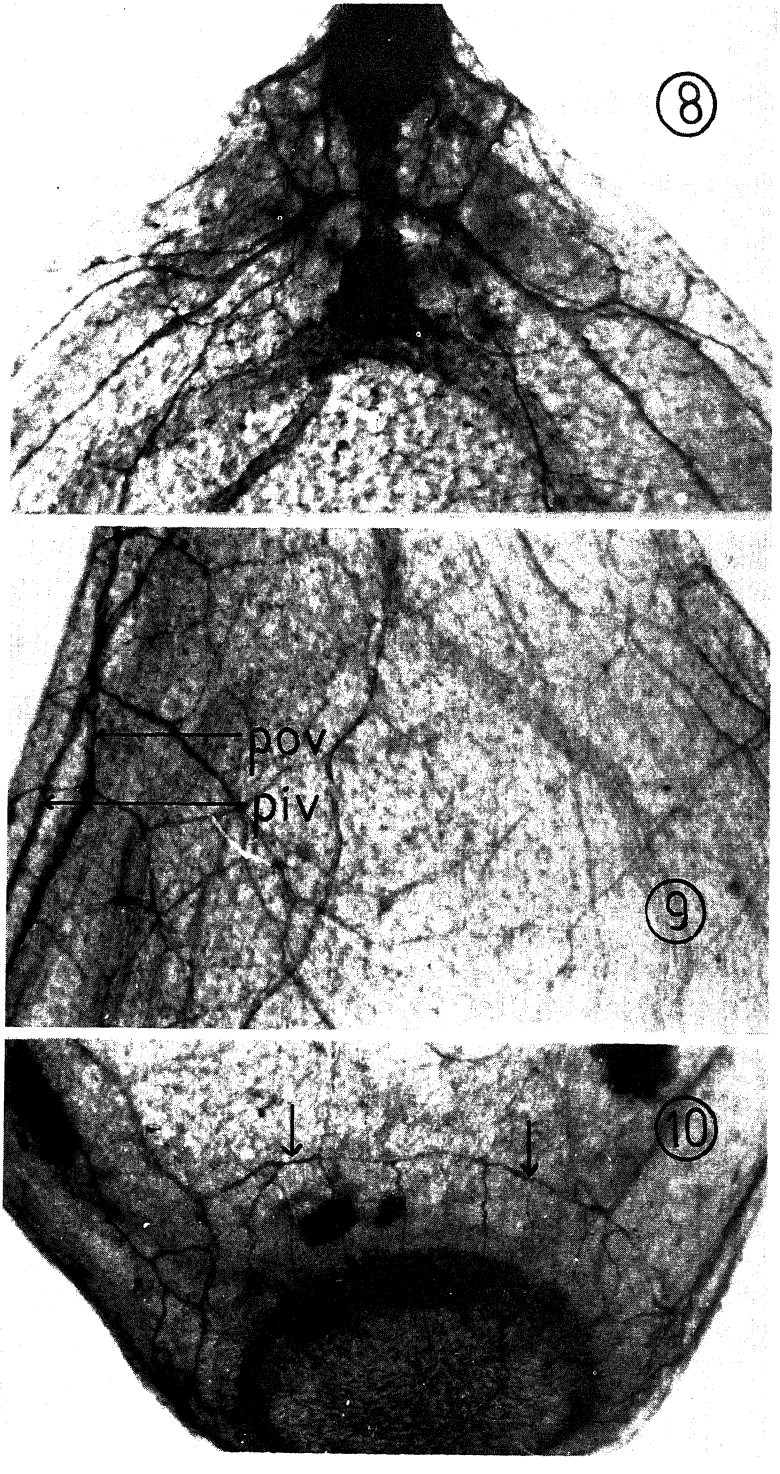
Each postero-outer ventral nerve bifurcates just a little anterior to the acetabulum: the inner branch meets its fellow of the other side, making a thin transverse connective (figure 10) many fine branches from which supply the rim of the sucker; the outer branch innervates the lateral walls and the floor of the latter. Likewise, the postero-inner ventral also bifurcates, its inner branch joining the transverse connective and the outer branch joining its counterpart from the postero-outer ventral.

A dorsal nerve net is also constituted by the transverse and longitudinal nerve connectives of the postero-dorsals (figure 11). Both the postero-dorsals of either side join with each other posteriorly and innervate the middle and lower edges of the sucker (figure 12).

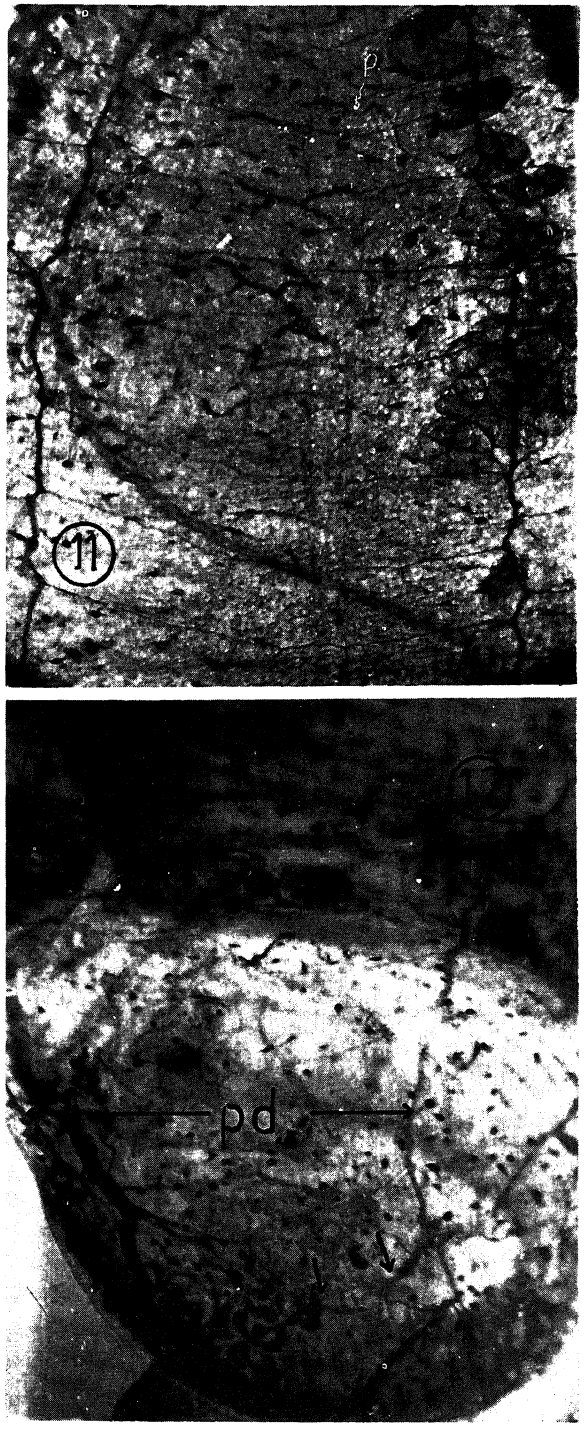
The innervation to the various regions and organs of the body is the same as observed in *F. elongatus*.

## 4. Discussion

The neuroanatomy of *F. elongatus* and *G. crumenifer*, as revealed by the indigogenic technique for localization of non-specific esterases, shows many deviations from the description given for these species by Lee (1971) and Brandes (1898). In the pre-cerebral region there are three pairs of nerves in both these species. The additional pairs of lateral and pharyngeal nerves referred to by Lee and Brandes could not be traced in the present study. Brandes has reported 2 accessory (dorsal and ventral) anterior nerves in *F. elongatus* and 3 accessory anterior nerves in *G. crumenifer*. However, no such nerves were observed in the present study. Lee has stated the splitting of one of the anterior




**Figures 8–10.** For captions, see page no. 138.




**Figures 11–12.** For captions, see page no. 138.

ventral nerves into two, thus mentioning the occurrence of 3 ventral branches on both sides. In the present study all the anterior nerves are found running singly up to the tip; ring-like transverse connectives, joining the anterior nerves of both the sides of the system and encircling the pharynx, are conspicuous. Lee has mentioned only a few poorly developed anterior ventral commissures. Fukui (1929) also mentioned some transverse commissures in an unspecified Japanese amphistome but did not state which nerve they connect. Anterior commissures were not figured by Brandes (1898).

The occurrence of three pairs of posterior longitudinal nerves is the most constant feature of the system in the species investigated. In our findings there is one pair of posterior dorsal nerves and two pairs of posterior ventral nerves proceeding singly up to the acetabulum; the posterior inner and outer ventral nerves correspond to the ventral and lateral nerves in Lee's description. Lee has reported fusion of the lateral and ventral nerves in the pre-acetabular region and also of the ventral and dorsal nerves in the acetabular region. Union between the posterior nerves was also reported by Brandes in *G. crumenifer* and *F. elongatus*.

In both these paramphistomes the presence of an additional pair of collateral nerves resulting from the splitting of the ventral cord has earlier been described (Brandes 1898; Otto 1896; Lee 1971). However, no such nerves were observed in the present study. Fukui (1929) also did not refer to the existence of collateral ventral nerves. In *F. elongatus* all the posterior longitudinal nerves join in the immediate pre-acetabular region to form a thick circular plexus from which many branches emerge in the posterior direction, supplying the whole wall and floor of the sucker. The presence of posterior transverse connection between the nerves of the two sides was also reported by both Brandes and Lee in *F. elongatus*. According to Brandes, the ventral and dorsal nerves in the acetabular region split into several branches, some entering the acetabulum while others participate in the formation of a complete commissural ring around the acetabulum. Contrary to Lee's (1971) observations that in *F. elongatus* the acetabular nerve is solely derived from the ventral cord, ours are in conformation with those of Brandes in that the acetabulum is supplied by both the posterior ventrals and dorsal nerves. In the post-cerebral region, conspicuous transverse connectives connect the three pairs of nerves with one another and with their counterparts establishing a direct communication between the two sides of the system. Lee has mentioned only a few ventral commissures and 1 or 2 poorly developed dorsal commissures. Under the present investigation, the pair of oesophageal nerves (Lee 1971) or a single unpaired oesophageal nerve (Brandes 1898) were not present.

The genital atrium is innervated by branches of both the posterior ventral nerves. A pair of transverse genital nerves reported by Lee could not be traced here. Our findings agree with those of Brandes who stated that these nerves do not occur in the paramphistomes he examined.

Otto (1896), in his study of amphistomes, found only three pairs of anterior nerves. The same author stated that the anterior dorsal and ventral nerves of *G. gregarius* (*Carmyerius gregarius* (Loose 1896)) Stiles et Goldberger 1910 occur immediately below the surface and are probably connected with pharyngeal nerves at the anterior end. He further maintained that these nerves send off branches posteriorly to connect with the posterior cords. Fukui 1929 also reported that the same nerves in some Japanese unspecified amphistome give off paired branches which turn caudad. In the present study the anterior nerves are situated slightly deeper in the parenchyma and do not give off branches to join the posterior nerves. Our observation tally with those of Lee (1971) for *F. elongatus* and *Parorientodiscus magnus*.

A ventro-lateral connective between the antero- and the postero-outer ventrals in *F. elongatus* and *G. crumenifer* seems to correspond to the accessory nerve of anterior lateral and posterior lateral nerves as reported in *P. magnus* (see Lee 1971). The ring-like anterior commissures described in *P. magnus* show a similarity to those observed by us in *F. elongatus* and *G. crumenifer.*

A comparison of the nerve pattern among the pouched paramphistomes, *F. elongatus*, *G. crumenifer* and *F. cobboldi*, brings out some notable variations. The nerve distribution in the pre-cerebral region of *F. elongatus* and *G. crumenifer* is simple in not possessing a conspicuous circumpharyngeal nerve basket which is a prominent feature in *F. cobboldi* (see Mishra and Tandon 1984). The C- or tilted V-shaped (= >) connectives of the postero-outer and postero-inner ventrals in *F. elongatus* are conspicuous by their absence in *F. cobboldi* and *G. crumenifer.* In *F. elongatus* all the posterior nerves join and form a circular connective anterior to the acetabulum and thus do not terminate individually in the posterior sucker as in *F. cobboldi* and *G. crumenifer.* An interesting aspect of the present study as compared to other trematodes is the presence of a dorsal and ventral nerve net, thus providing a direct communication system between all the posterior nerves.

Simha and Rao (1977) and Fernandez *et al* (1982) have mentioned about the ganglionated nature of nerves in *Singhiatrema longifurca* and *S. najai*, respectively. No ganglionated thickening were observed in the parasites under the present investigations. Nevertheless all the anterior and posterior nerves are well developed.

The present observations as well as the so far available descriptions on the nervous system in paramphistomes clearly reveal that the system cannot be regarded as a primitively developed one.

## Acknowledgements

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**Figures 6–12.** *Gastrothylax crumenifer.* **6–7.** Camera lucida sketches of the nervous system in the whole mount of the worm in ventral and dorsal view, respectively. **8.** Brain mass and the main anterior and posterior nerves (× 72). **9.** Postero-ventral nerves and their nerve net as seen in the bifurcal region (× 72). **10.** Postero-ventrals bifurcating and forming a transverse connective (arrow) just anterior to the acetabulum (× 72). **11.** Postero-dorsals and their nerve net (× 23). **12.** Postero-dorsals joining medially (arrow) in the acetabular region (× 23).

(*Abbreviations.* ad—antero-dorsal nerve; aiv—antero-inner ventral nerve; aov—antero-outer ventral nerve; cg—cerebral ganglion; pd—postero-dorsal nerve; piv—postero-inner ventral nerve; pov—postero-outer ventral nerve.)

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# Effect of hypoxia on tissue metabolism of midgut gland of the scorpion *Heterometrus fulvipes*

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**Abstract.** The function of the midgut gland of the scorpion *Heterometrus fulvipes* has been investigated in relation to hypoxia. Regional differences in the midgut gland became apparent, one part being more active metabolically than the other. It is concluded that the midgut gland might be serving as the liver, as gluconeogenesis is predominant.

**Keywords.** *Heterometrus fulvipes*; midgut gland; hypoxia; tissue metabolism; gluconeogenesis.

## 1. Introduction

The gland which performs the function of a 'liver' in crustaceans (Vonk 1960) is referred to as the "hepatopancreas" inspite of lack of enough evidence to support its hepatopancreatic function (Phillips *et al* 1977). Van Weel (1974) has questioned the usage of the word hepatopancreas for this gland and called it the midgut gland (MGG).

The phylogenetic origin of MGG in the members of the phylum Arthropoda is puzzling. The presence of a conspicuous digestive gland in the non-tracheate Arthropods of classes Crustacea and Arachnida, its replacement by a few to many digestive caecae (hepatic or midgut caecae) in the tracheate insects and its total absence in the tracheate Myriapods prompted us to investigate the nature and function of this MGG in the scorpion *Heterometrus fulvipes* in relation to respiration.

## 2. Material and methods

Active *H. fulvipes* females (4·5–6·5 g) in the non-breeding season were used. The MGG was divided into three parts—anterior (I), middle (II) and posterior (III) in order to know the regional differences if any. The oxygen consumption and carbon dioxide production in all parts was studied using the conventional Warburg apparatus as given by Umbreit *et al* (1959) and their RQ values calculated.

Fuels, metabolites and end products (glycogen, phospholipids, reducing sugars, lactate and pyruvate) were estimated in the anterior and posterior parts of the MGG and the in vitro effect of hypoxia (30 min) on their levels evaluated at 37°C.

Cyanide method of Park and Johnson (1949) was used to estimate reducing sugars. Glycogen was estimated after ethanol precipitation by the method of Good *et al* (1933); Barker and Summerson (1941) method was adopted for lactate estimation. The method

of Youngburg and Youngburg (1930) for phospholipid and the method of Lu (1939) as given by Umbreit *et al* (1959) for pyruvate were employed. Total carbohydrates were estimated by the colorimetric method of Carrol *et al* (1956).

## 3. Results and discussion

Although there appears to be no difference in oxygen consumption or carbon dioxide production in the three parts of the MGG, RQ values appear to be markedly different, (table 1). Therefore RQ has a different value for each of the major food components and serves to determine what substances are being burned (Oser 1954). MGG I with RQ value of more than 1 is involved probably in the interconversion of carbohydrates and fats.

**Table 1.** Regional differences in $O_2$ consumption and $CO_2$ production in the midgut gland.

| Regions of midgut gland | $O_2$ uptake ($\mu$l/gm wet wt/10′) | $CO_2$ liberation ($\mu$l/gm wet wt/10′) | RQ value |
|---|---|---|---|
| MGG I | 71·51 (12) ±24·89 | 91·31 (12) ±36·42 | 1·28 |
| MGG II | 68·99 (12) ±20·52 | 71·57 (12) ±19·71 | 1·04 |
| MGG III | 108·38 (12) ±59·34 | 99·21 (12) ±34·95 | 0·915 |
| | $t$ = 1·99 (MGG I *vs* III) | $t$ = 0·54 (MGG I *vs* III) | |

$t$ value from table = 2·07 (5%).
Numbers in parentheses denote the number of samples studied.

**Table 2.** Water and protein contents in the midgut gland.

| Regions of midgut gland | Percentage of water | Protein content (mg/g wet wt) |
|---|---|---|
| MGG I | 53·50 (6) ±5·45 | 72·26 (6) ±47·16 |
| MGG II | 56·95 (6) ±4·78 | 56·13 (6) ±15·80 |
| MGG III | 60·49 (6) ±16·60 | 155·33 (6) ±62·25 |
| | $t$ = 0·98 (MGG I *vs* III) | $t$ = 2·61 (MGG I *vs* III) |

$t$ value from table = 2·23 (5%).

**Table 3.** Levels of glucose, glycogen, lactate, total carbohydrate and phospholipids in different regions of the midgut gland under different conditions.

| Particulars | Prior to incubation | | | | After incubation (30″) | | | |
|---|---|---|---|---|---|---|---|---|
| | MGG I | | MGG III | | MGG I | | MGG III | |
| | Normal | Hypoxic | Normal | Hypoxic | Normal | Hypoxic | Normal | Hypoxic |
| Glucose (mg/g wet wt) | 31·66 ±15·87 | 32·68 ±13·75 | 35·84 ±2·76 | 27·35 ±5·70 | 27·28 ±11·73 | 13·51[d] ±6·36 | 19·52[f] ±9·44 | 26·70 ±11·198 |
| Glycogen (mg/g wet wt) | 5·51 ±3·17 | 4·73 ±3·19 | 4·48 ±1·79 | 5·19 ±2·50 | 4·33 ±2·72 | 6·52 ±3·25 | 2·70 ±1·79 | 7·70 ±5·66 |
| Lactate (mg/g wet wt) | 10·93 ±9·005 | 6·97 ±4·83 | 7·35 ±5·20 | 8·96 ±6·70 | 5·89 ±4·17 | 5·09 ±3·08 | 6·28 ±3·75 | 5·00 ±3·94 |
| Total carbohydrates ($\mu$ mol of glucose/g wet wt) | 240·62 ±53·29 | 367·78 ±107·01 | 527·07[a] ±101·14 | 538·67[b] ±94·99 | 404·92[d] ±105·79 | 581·59[e] +198·67 | 640·30[c] ±119·79 | 634·89 ±72·42 |
| Phospholipids (mM of phosphorus/g wet wt) | 81·77 ±16·08 | 83·56 ±24·37 | 65·53 ±15·59 | 82·51 ±19·10 | 82·81 ±15·95 | 94·48 ±15·03 | 85·98[f] ±16·09 | 85·04 ±13·10 |

Symbols indicate that the samples are significant at 5% level.
Number of samples studied = 6.

[a] Prior to incubation MGG I *vs* MGG III Normal
[b] Prior to incubation MGG I *vs* MGG III Hypoxic
[c] After incubation MGG I *vs* MGG III Normal
[d] Preincubation *vs* Post incubation MGG I Normal
[e] Preincubation *vs* Post incubation MGG I Hypoxic
[f] Preincubation *vs* Post incubation MGG III Normal

MGG II with RQ value of 1 might be carbohydrate oriented in its metabolism. MGG III with RQ value of 0·9 might be metabolising non-carbohydrates or mixed fuels. MGG III with a significantly high protein content (table 2) might be the seat of high synthetic or secretory activity.

Table 3 shows that MGG I loses glucose under hypoxia whereas MGG III maintains more or less the same level as under normal conditions. Apparently under hypoxia, glucose production is continuing in MGG III, but not in MGG I. The drop in glycogen levels in MGG III under normal conditions and the higher than the normal hypoxic levels of glycogen probably further suggests the presence of glycogen synthetic activity in MGG III. Phillips *et al* (1977) are of the opinion that the hepatopancreas of the crustacean *Homarus gammurus* may not be the site for gluconeogenesis. In vivo studies reveal gluconeogenesis taking place from lactate in *Cherax destructor* (Phillips *et al* 1977). Munday and Poat (1972) suggested MGG as a possible site for gluconeogenesis. Giles *et al* (1975) have also expressed a similar view. Maintenance of more or less the same glucose level under hypoxic conditions in the scorpion MGG might be due to the gluconeogenetic role of MGG III and gluconeogenesis is therefore one of its important functions as in the vertebrate liver.

The absence of any change (table 3) in phospholipid content on incubation and lack of any difference in MGG I and MGG III might indicate the membranous nature of the gland and these lipids may get involved only on prolonged starvation of the animal (Reddy and Selvarajan 1975; Sinha and Kanungo 1967). The unexpectedly high levels of these phospholipids in MGG as compared to the levels of total carbohydrate, glycogen, glucose or lactate, probably suggest the role of the MGG as a storage organ for these lipids. Similar situation seems to prevail in other arthropods as well (Ravindranath Gupta 1971; Satyam 1976; Venkata Reddy 1976). The liver mainly functions as a storage organ for glycogen and lipids. The digestive gland of malacostracan crustaceans contains glycogen and fat. The stored glycogen is said to be utilized during the formation of new chitinous substances (Scheer 1957) and during exercise in insects (Clements 1955). But storage of glycogen alone cannot justify the term 'liver' for this organ. However, since it also has a gluconeogenetic role it could be suggested that the MGG of the scorpion serves as a liver.

The higher levels of glucose, glycogen and phospholipid in MGG I when calculated per milligramme protein (tables 2 and 3) together with the low protein value and lack of difference in their levels between MGG I and III when calculated on wet weight basis may indicate synthesis of these fuels in the protein rich MGG III and probably their storage in the protein deficient MGG I.

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# Male reproductive system of some digenetic trematodes

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**Abstract.** A histological study of the male reproductive system undertaken in the present study has been concentrated on trematodes with a cirrus sac. Special attention has been focussed on the terminal genitalia. Five species of trematodes, *Fasciola gigantica*, *Acanthocolpus liodorus*, *Stephanostomoides dorabi*, *Rhynchocreadium singhia* and *Prosorhynchus manteri* have been considered for the present study.

**Keywords.** Trematodes; cirrus sac; prostate glands; cirrus.

## 1. Introduction

The trematode fauna being parasitic has attracted the attention of scientists ubiquitously. Since time immemorial various aspects of this parasitic group have been studied in detail. Although the biology of this group has attracted the attention for a few years, presently physio-pathology, histopathology and life cycle studies are in vogue, but systematics is the only aspect left unimpeded. One particular aspect that should cause concern to the biologists and systematists, is the most neglected, apparently most simple, male genitalia of trematodes. Even though one finds a wide array of orientation in this system, this has not caught the needed attention. It is with this intention a special attention has been focussed to bring to light the true significance of this system. Another point of interest is that only anatomical characteristics were observed from the whole mounts for this system to a large extent. The present study throws light especially on the importance of histological study of this system. The details which escape the scrutiny of anatomical study, turn out in many a case to be of systematic importance. The orientation of the system can also be better understood histologically. In this study attention has been focussed on trematodes in which the male terminal genitalia are separate from the female, enclosed in a cirrus sac.

## 2. Material and methods

The parasites on collection from different hosts were fixed immediately in different fixatives to accomplish both anatomical and histological details. Some of these parasites were fixed in FAA under appropriate cover glass pressure and later stained with alum caramine to study the anatomical aspects. Other parasites were fixed in Susa and Bouin's fixatives. After dehydration, through graded series of alcohols, infiltration and sectioning, Heidenhain's Azan and hematoxylin stains were applied to study the histology of the male genitalia. All the parasites of the present study are of common

occurrence and atleast 15 specimens have been examined on an average for each of the above species. Measurements are in millimetres unless otherwise mentioned.

## 3. Observations

### 3.1 *Fasciola gigantica Cobbold*, 1890 (figure 1)

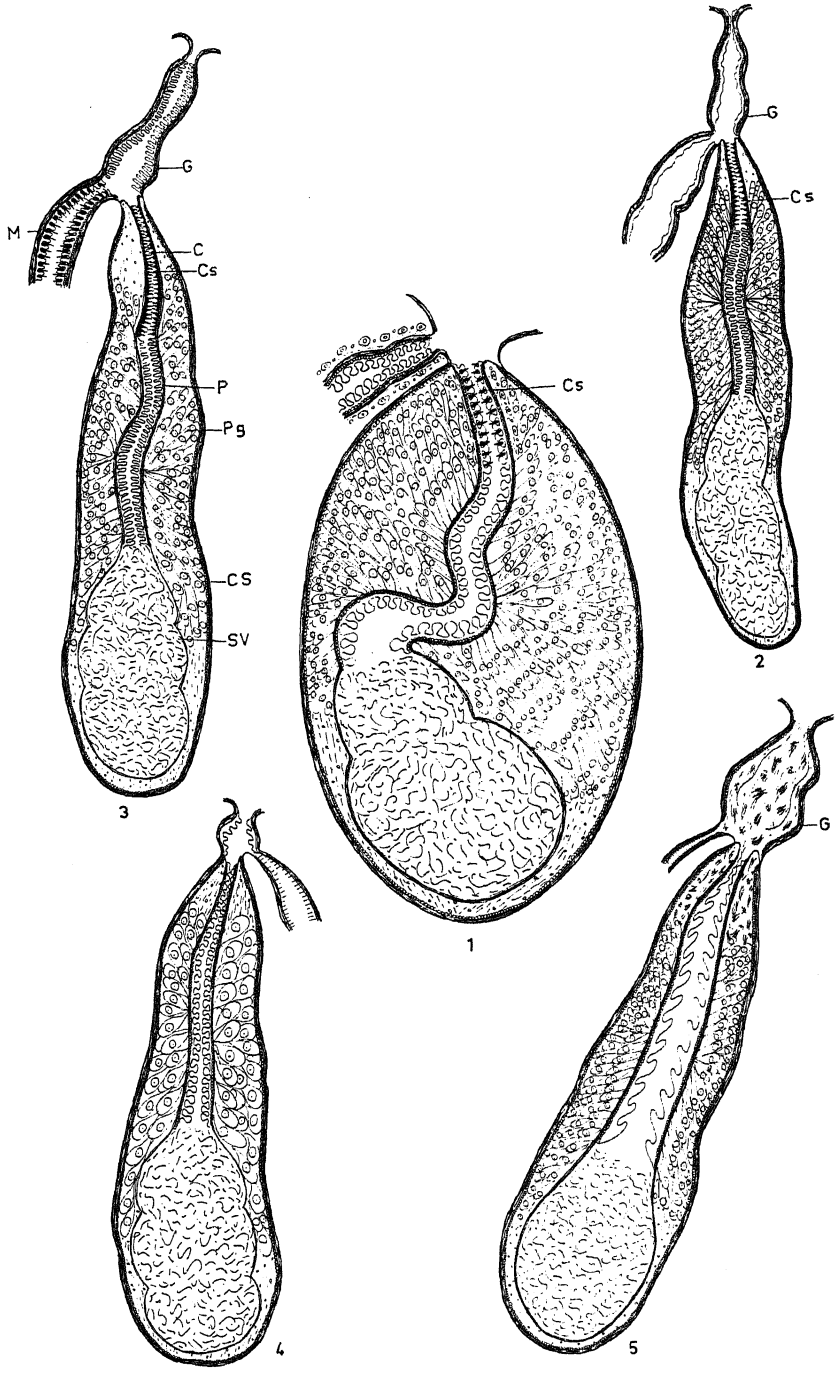
This parasite was collected from the bile duct of *Bos indicus*.

The two testes are highly branched, situated in the middle of the body, post-acetabular, post-ovarian and tandem in position.

The two vasa efferentia which come from the testes run anteriorly and join each other above the acetabulum and below the cirrus sac. The common duct, the vas deferens enters the cirrus sac leading into a bipartite saccular seminal vesicle. The epithelium of the seminal vesicle lies on a dense, granular basement membrane, below which a thin layer of interstitial tissue containing circular and longitudinal muscles is present. Surprisingly in one specimen of *F. gigantica* a large number of spermatogonial cells have entered the seminal vesicle. The seminal vesicle narrows and leads into a tubular, sinuous duct, the pars prostatica which is covered by a syncytial tegument that lies on a dense, granular basement membrane, below which a thick layer of interstitial tissue is present containing a single layer of circular muscles and a thick layer of longitudinal muscles. The base of the syncytial tegument is folded. It is aspinose. Prostate gland cells open into the pars prostatica, although they are present around the seminal vesicle and cirrus to a certain extent. Prostate gland cells are pear-shaped and long, with their narrow duct like anterior part passing through the interstitial tissue and then into the syncytium of pars prostatica. Nucleus of the cells is conspicuous and round in shape containing a single nucleolus. Each prostate gland cell is 0·014–0·017 broad and the nucleus measures 0·007. The prostate secretions are of two types, globular and granular. Pars prostatica leads into a highly muscular, tubular, spiny, eversible, cirrus. The cirrus is lined by a syncytial tegument, which lies on a thick, granular basement membrane, which is thrown into a number of papillae, the syncytial tegument following the pattern of the basement membrane. Below the basement membrane lies a thick layer of interstitial tissue containing a single layer of circular muscles and a thick layer of longitudinal muscles. A number of conspicuous, stout spines are present embedded in the syncytial tegument of the cirrus. On either side of the cirrus sub-tegumental cells are present. The cirrus opens independently into the genital atrium. The cirrus, pars prostatica and seminal vesicle are enclosed in a muscular, cirrus sac which is covered by a syncytial tegument. The cirrus sac measures 0·268–0·272 × 0·136–0·138 and is pre-acetabular. Metraterm is a muscular, saccular and multi-lobed structure which leads independently into the genital atrium. It is covered by a syncytial tegument, and is devoid of spines and is surrounded by subtegumental cells. The genital atrium is simple and is covered by a syncytial tegument, the nature of which resembles the body tegument. The genital pore is median.

*Discussion*

Threadgold (1975a) gave ultrastructural details of the prostate gland of *F. hepatica*. In the present study it has been observed that *F. gigantica* mostly resembles *F. hepatica*.




**Figures 1–5.** Cirrus sac. **1.** *F. gigantica*, **2.** *A. liodorus*, **3.** *S. dorabi*, **4.** *R. singhia*, **5.** *P. manteri.*

*Abbreviations*: C—cirrus; Cs—cirrus spines; CS—cirrus sac; G—genital atrium; P—pars prostatica; Pg—prostate gland cells; SV—seminal vesicle.

Threadgold (1975b) described the duct surrounded by prostate gland cells as the ejaculatory duct. In the present study this duct has been referred to as pars prostatica. He also described that the cirrus and cirrus sac are covered by a modified syncytial tegument. Such a nature has also been reported by Wittrock (1976) in *Quinqueserialis quinqueserialis*. Even in the present study it has been observed that the pars prostatica, cirrus, and cirrus sac are covered by a modified syncytial tegument. Threadgold (1975b) observed spines in the syncytium of both cirrus and cirrus sac. But in *F. gigantica* spines are observed only in the syncytium of the cirrus. Cirrus sac is aspinose. Subtegumental cells are observed on either sides of the cirrus. These subtegumental cells are of similar to the subtegumental cells of the general body surface as was observed by Threadgold (1975b).

### 3.2 *Acanthocolpus liodorus Luhe*, 1906 (figure 2)

This parasite has been collected from the intestine of the marine fish, *Chirocentrus dorab*.

Testes are elongated, rod-like, situated in the posterior region of the body, post-acetabular, pre-ovarian, intercaecal, tandem in arrangement, median in position. The testes are unequal in size, the anterior testis is smaller (0·359–0·373 × 0·097–0·152) than the posterior testis (0·414–0·483 × 0·097–0·138).

Seminal vesicle is saccular, thin-walled and tripartite. The tripartite nature is well pronounced with deep intrusion of the walls into the lumen. Pars prostatica is covered by a syncytial tegument, the underlying interstitial tissue is thick. The base of the syncytial tegument is much folded. Prostate gland cells are pear shaped, each gland cell is 10 $\mu$m broad and the nucleus measures 3 $\mu$m. The cirrus is muscular, covered by a syncytial tegument, the base of the syncytial tegument is folded. The cirrus is spiny and the spines are rose thorn shaped; pointed end of the spine is very long and the base is bulbous. Spines are numerous and present on either side at the inner surface of the cirrus touching the base of the syncytial tegument and directed towards the lumen of the duct. The cirrus leads into a tubular, conspicuous genital atrium. The cirrus sac is moderately thick and is aspinose and is prominent, elongated, commences above the ovary and extends anteriorly. The metraterm is saccular and aspinose. The genital atrium is tubular, muscular and aspinose. Genital pore is in front of the acetabulum. The syncytium of genital atrium and metraterm closely lines the underlying musculature and is not folded.

#### *Discussion*

In the present study it has been observed that spines are present only in the cirrus. But Luhe (1906) described spines in the metraterm and the terminal portion of the hermaphroditic duct. Further the term hermaphroditic duct seems to be vague, as in the histological study it has been observed that the cirrus and the metraterm open separately into a common chamber, which is more appropriately called the genital atrium. Further the cirrus is spinose indicating its involvement during copulation. If we consider that the metraterm and cirrus unite to form a common hermaphroditic duct, it has to be considered that the terminal portion of the hermaphroditic duct as eversible.

It is not justifiable to think that both cirrus and hermaphroditic duct as eversible. Therefore it has been considered that the cirrus and the metraterm open into a long, tubular, weakly muscular, aspinose genital atrium. Whenever a hermaphroditic duct is present, it has been observed that the male genitalia lie free in the parenchyma. So it can be concluded that since the cirrus sac is also present in *Acanthocolpus*, it would seem more appropriate to consider that both cirrus and metraterm open into a common genital atrium rather than the hermaphroditic duct.

Yamaguti (1971) considered that if the seminal vesicle is bipartite in *Acanthocolpus liodorus* and *A. luhei*, it might serve to differentiate *Acanthocolpus* from *Tormopsolus*, in which the vesicle is definitely unipartite. In the present study it has been observed that the seminal vesicle is tripartite in *A. liodorus*.

### 3.3 *Stephanostomoides dorabi Mamaev and Oshmarin*, 1966 (figure 3)

This parasite has been collected from the intestine of *Chirocentrus dorab*.

Testes, two in number, situated in the posterior region of the body, post-acetabular, post-ovarian, intercaecal, median, tandem in position, smooth elongated and rod-like.

The anterior testis is smaller (0·814–0·820 × 0·220–0·224) than the posterior testis (0·952–0·958 × 0·179–0·182).

A conspicuous, saccular and tripartite seminal vesicle is present, occuping the basal region of the cirrus sac. Pars prostatica is covered by a syncytial tegument, the base of which is much folded. The interstitial tissue of pars prostatica is thick. Prostate gland cells are pear shaped. Nucleus is round with a single nucleolus. Each gland cell is 10 $\mu$m broad and the nucleus measures 3 $\mu$m. The cirrus is muscular, sinuous, and spiny. The base of syncytial tegument covering the cirrus is much folded. There are numerous spines on either sides at the inner surface of the cirrus, the base of the spine touches the base of syncytial tegument. The spines are rose thorn shaped. The cirrus leads into a tubular and broad genital atrium. The cirrus sac is sinuous and is thin-walled and commences above the ovary and measures 0·207–0·210 × 0·152–0·156. The metraterm is long, sinuous, broad and spinose. The spines of metraterm are similar to that of the cirrus spines. The musculature of the genital atrium is not very thick. The genital pore is in front of the acetabulum. The syncytium of metraterm and genital atrium is folded.

#### *Discussion*

It has been observed that the cirrus and metraterm are spiny and there are no spines in the genital atrium. This observation agrees with the description of Mamaev and Oshmarin (1966). But they have described the presence of a long hermaphroditic duct. In the histological study, it has been observed that cirrus and metraterm open separately into a common genital atrium as in *Acanthocolpus*.

It has been observed that the seminal vesicle is tripartite and not bipartite as reported by Mamaev and Oshmarin (1966).

### 3.4 *Rhynchocreadium singhia Pershad*, 1965 (figure 4)

This parasite has been collected from the intestine of *Macrognathus aculeatus*.

Testes, two in number, situated in the posterior region, post-acetabular, post-ovarian, intercaecal, tandem in position, smooth and oval in shape. Testes are unequal in size. The two testes are widely separated. The anterior testis measures 0·276–0·324 × 0·180–0·240 and posterior testis 0·252–0·360 × 0·192–0·300.

Seminal vesicle is conspicuous, saccular, tripartite and thin-walled. The partitions of the seminal vesicle are clearly demarcated. Pars prostatica is covered by a syncytial tegument the base of which is much folded. The interstitial tissue of pars prostatica is thick. The lumen of the duct is narrow. Prostate gland cells are pear shaped. Nucleus is round, with a single nucleolus. Each gland cell is big and broad (20–24 $\mu$m). The nucleus measures 7–10 $\mu$m. The cirrus is muscular. Both cirrus and cirrus sac are covered by a syncytial tegument. The cirrus sac is situated on the right side, starting in the lateral field just above the level of the acetabulum. It runs anteriorly up to a short distance and takes a bend towards the acetabulum in the middle of the body. The wall of the cirrus sac is relatively thin. The metraterm is muscular, tubular, leading into a muscular, small genital atrium. Genital papillae are present inside the genital atrium.

*Discussion*

Srivastava (1962) in his generic diagnosis of *Rhynchocreadium* reported that the seminal vesicle is bipartite. Pershad (1965) also observed a similar condition, but in the present histological study it has been observed that the seminal vesicle is tripartite. The cirrus is found to be eversible together with the terminal portion of the cirrus sac. The prostate gland cells are very big in size in this trematode.

3.5 *Prosorhynchus manteri* Srivastava, 1938 (figure 5)

This parasite has been collected from intestinal caeca of *Trichuris trichuris*.

Testes two, situated in the posterior region. Testes are posterior to the stomach, post-ovarian, situated in the lateral field on the right side. Tandem in arrangement, smooth and round in shape. Testes are unequal in size. The anterior testis measures 0·193–0·196 × 0·179–0·183 and the posterior testis is 0·166–0·172 × 0·190–0·193.

Seminal vesicle is prominent and bulb shaped. Below the epithelium of the seminal vesicle there is a slightly thick layer of interstitial tissue. Pars prostatica is covered by a syncytial tegument, the base of which is much folded. The interstitial tissue of pars prostatica is thick. The lumen of pars prostatica is narrow. Prostate gland cells are pear shaped. Nucleus is round with a single nucleolus. The gland cells are small in size, arranged in a few rows only and are 7 $\mu$m broad. Nucleus is also small and measures 3 $\mu$m. The cirrus and cirrus sac are muscular and covered by a syncytial tegument. The cirrus sac is prominent, it is vertical in position and directed posteriorly. It commences very close to the posterior testis. The base of the sac is slightly broader and it is in this region a bulb shaped seminal vesicle is seen. The cirrus sac measures 0·179–0·183 × 0·089–0·097. The seminal vesicle is 0·069–0·072 × 0·028–0·032.

The interstitial tissue of the cirrus sac is thick. The metraterm is narrow. The genital atrium is very conspicuous and highly muscular and very complicated in nature. Muscles are present throughout the genital atrium, there being no conspicuous space inside the genital atrium.

*Discussion*

In the members of the family Bucephalidae the genital atrium is highly muscular and very complicated. As the Bucephalidae lack powerful adhesive organs, the highly muscular genital atrium helps in powerful union during copulation and also aids in adhesion when needed. In this trematode the space inside the cirrus sac for the prostate gland cells is very narrow, whereas the pars prostatica is with a broad lumen.

## Acknowledgement

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# Extraretinal photoreception involved in photoperiodic effects on gonadal activity in the Indian murrel, *Channa (Ophiocephalus) punctatus* (Bloch)

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**Abstract.** The effects of blinding on *Channa punctatus* exposed to LD 14:10, 12:12, 9:15, continuous dark (DD) and continuous light (LL) was studied during the winter quiescent phase of the annual reproductive cycle. Ovarian weights of blinded fish exposed to LD 14:10, 12:12 or LL were higher than those exposed to LD 9:15 or DD. However, no effects of blinding on testicular recrudescence under any photoperiodic regime were observed. Studies suggest that in addition to the eyes, other extraocular photoreceptors are also involved for gathering information on day length.

**Keywords.** *Channa punctatus*; photoreception; extraretinal; extrapineal; blinding; gonadal response; photoperiod.

## 1. Introduction

Seasonally changing daylength and temperature have been shown to regulate the reproductive cycles of many a teleost species (Htun-Han 1977; Sundararaj 1981; Vivien-Roels 1981). However, at present it is not known whether the effects of light on reproductive cycles are mediated *via* retinal pathways and/or by extra-retinal photoreceptors.

The effects of retinal and extraretinal photoreception in photoperiodic effects on reproduction has been studied extensively in birds and reptiles (see Menaker and Underwood 1976; Underwood 1979). However, very little information is available on their role in the regulation of reproduction in teleosts (Urasaki 1973, 1976; Dalahunty *et al* 1979; Hontela and Peter 1980).

*Channa punctatus* is a seasonal breeder. Gonadal recrudescence in this species is greatly influenced by the changing photoperiod and temperature (Garg and Jain 1985), but the role of eyes in gonadal response to photoperiod has not been studied. Therefore, present studies were undertaken to investigate the role of eyes in gonadal response to daylength in fish exposed to different photoperiodic regimes during the winter quiescent phase of the annual reproductive cycle.

## 2. Material and methods

Specimens of *C. punctatus* were obtained from the fish dealers of Hissar (Lat. 29° 10'N; Long. 75° 46'E) and were maintained in the laboratory under constant temperature of 25 ± 1° C and a lighting schedule at 12 hr of light (0800–2000 hr) alternating with 12 hr of darkness (2000–0800 hr). Fish were acclimated in the above mentioned conditions

**Table 1.** The effects of blinding on GSI and ovarian histology in *C. punctatus* exposed to LD 14:10, 9:15, 12:12, LL and DD during the post-spawning and preparatory periods (1981–82).

| Photoperiod | Days of exposure | Treatment | GSI* | Stages of oocytes I | II | III | Percentage of fish with atretic oocytes |
|---|---|---|---|---|---|---|---|
| Initial control | | | 0·30 ± 0·01 (6) | 100 (6) | — | — | — |
| LD 14:10 25°C | 30 | Intact | 1·00 ± 0·1 (7) | 84·1 ± 9·8 (7) | 14·8 ± 1·1 | 1·1 ± 0·04 | — |
| | | Blind | 0·90 ± 0·06 (6) | 88·0 ± 6·6 (6) | 12·0 ± 4·6 | — | 16.7 |
| | 45 | Intact | 1·00 ± 0·08 (7) | 83·0 ± 8·7 (7) | 10·8 ± 8 | 6·2 ± 0·8[a] | — |
| | | Blind | 0·80 ± 0·1 (5) | 93·2 ± 6·2 (5) | 4·6 ± 1·8 | 2·2 ± 1.0[b] | 20·0 |
| LD 9:15 25°C | 30 | Intact | 1·00 ± 0·04[c] (7) | 86·6 ± 10 (7) | 13·4 ± 1·1 | — | — |
| | | Blind | 0·60 ± 0·07[d] (6) | 94·4 ± 6·8 (6) | 5·7 ± 2·2 | — | 33·1 |
| | 45 | Intact | 0·63 ± 0·06[e] (6) | 94·0 ± 10·8 (6) | 5·2 ± 1·6 | 0·8 ± 1·1 | 33·1 |
| | | Blind | 0·62 ± 0·03 (4) | 95·8 ± 7·8 (4) | 4·2 ± 1·1 | — | 25·0 |
| LD 12:12 25°C | 30 | Intact | 0·75 ± 0·1 (5) | 89·8 ± 4·3 (5) | 10·2 ± 2·1 | — | — |
| | | Blind | 1·00 ± 0·07 (7) | 85·2 ± 5·6 (7) | 14·8 ± 2·2 | — | — |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LD 12:12 25°C | 45 | Intact | 1·00 ± 0·01 (5) | 83·6 ± 8·8 (5) | 14·2 ± 1·8 | 2·2 ± 2·2 | — |
| | | Blind | 1·10 ± 0·1 (5) | 86·0 ± 7·7 (5) | 11·8 ± 1 | 2·2 ± 1·1 | — |
| LL 25°C | 30 | Intact | 0·90 ± 0·1 (6) | 87·0 ± 8 (6) | 13·0 ± 1 | — | — |
| | | Blind | 0·80 ± 0·06 (4) | 90·2 ± 9 (4) | 9·8 ± 2·2 | — | — |
| DD 25°C | 30 | Intact | 0·50 ± 0·05 (5) | 95·5 ± 6·6 (5) | 4·5 ± 2 | — | — |
| | | Blind | 0·67 ± 0·08 (5) | 90·8 ± 7·8 (5) | 9·2 ± 1·8 | — | — |

*Mean ± SE of mean. Figures in parentheses indicate number of fish.
[a] vs [b] $P < 0{\cdot}001$.
[c] vs [d] $P < 0{\cdot}001$.
[c] vs [e] $P < 0{\cdot}001$.

for a minimum of seven days prior to the initiation of experimental treatments. Fish were fed on alternate days with fresh meat and liver of lamb and the water in the aquaria was changed daily.

After acclimation the fish were divided into two groups, one group was blinded and the other served as control. A blinded and an intact (control) group each of fish was assigned to each of the photoperiodic treatments at 25°C (LD 14:10, 12:12, 9:15, DD and LL). Specially made light proof glass aquaria (60 × 30 × 30 cm) were used to keep the fish. For blinding, the fish were anaesthetized in an aqueous solution (1:4000) of tricaine methane sulphonate (Sandoz). The complete eye ball was removed with a pair of iris scissors. To prevent bleeding a small bud of cotton was then inserted into each empty orbit. Fortified procaine penicillin was occasionally added to the aquaria water (35000 units/l) as a prophylactic against skin infection. The duration of photoperiod in each aquarium or chamber was regulated by time switches and the light was provided by 20 W Philips fluorescent cool daylight tubes. Feeding of fish and changing of water was always done during the day, except the fish maintained in DD, where it was done in dark using a dim red light.

Fish sacrificed at the beginning of the experiment served as the initial control. Thereafter, fish subjected to various photoperiod-temperature regimes were sacrificed at the end of 30 and 45 days (tables 1 and 2). The gonads were removed and weighed to

**Table 2.** Effects of blinding on testicular recrudescence in *C. punctatus* exposed to LD 14:10, 9:15, 12:12, LL and DD during the post-spawning and preparatory periods (1981–82).

| Days of exposure | Photoperiod | GSI* Intact | GSI* Blind |
|---|---|---|---|
| Initial control | | 40·1 ± 6·1 (6) | |
| 30 | LD 14:10 25°C | 46·2 ± 10·3 (5) | 40 ± 6 (9) |
| 45 | | 76·5 ± 6 (8) | 66 ± 4 (5) |
| 30 | LD 9:15 25°C | 85 ± 10·2 (4) | 69 ± 12 (6) |
| 45 | | 59 ± 7 (6) | 54 ± 12 (5) |
| 30 | LD 12:12 25°C | 42 ± 8 (5) | 62 ± 10 (5) |
| 45 | | 70 ± 10·4 (6) | 67 ± 7 (7) |
| 30 | LL 25°C | 84·2 ± 9·6 (5) | 71 ± 10 (6) |
| 30 | DD 25°C | 63 ± 4 (6) | 73 ± 5 (7) |

*Mean ± SE of mean, Figures in parentheses indicate number of fish.

the nearest 0·5 mg and fixed in Bouin's fixative for histological studies. For comparison of data, all gonadal weights were calculated on a 100 g body weight basis (GSI: gonosomatic index). *P* values between the experimental and control groups were calculated by students *t* test (Snedecor and Cochran 1971).

Three types of primary oocytes were identified from stained ovarian section of murrel (Garg and Jain 1985) on the basis of nuclear and cytoplasmic characteristics; stage I (figure 1A), primary oocytes (diameter 20–130 $\mu$m, mean, 100 $\mu$m) the non-yolky oocytes present in the ovary during all seasons of the year; stage II (figure 1B) primary oocytes (diameter 140–240 $\mu$m, mean: 170 $\mu$m) characterized by the presence of a ring of cortical alveoli, an indication of the onset of vitellogenesis and stage III (figure 1C), primary oocytes (diameter 360–720 $\mu$m, mean: 510 $\mu$m), the fully formed yolky oocytes.

## 3. Results

At the initiation of the experiments gonads were totally regressed, ovaries had only stage I primary oocytes, while the testes possessed primary spermatogonia and residual spermatozoa. Significant differences in ovarian weights or their histology between blinded and intact fish exposed to LD 14:10 were not observed after 30 or 45 days of treatment (table 1), however, lower percentage of yolky oocytes ($P < 0{\cdot}001$) and higher number of atretic oocytes were observed in the ovaries of the blinded females under LD 14:10 at the end of 45 days. Under LD 9:15 ovarian weights of blinded group was significantly lower ($P < 0{\cdot}001$) than the intact group after 30 days of treatment, while at the end of 45 days GSI of blinded fish did not increase appreciably but the ovaries of the intact fish regressed significantly ($P < 0{\cdot}001$). Atretic oocytes were also observed in both intact and blind fish. No significant differences in the ovarian weights of blinded and intact fish under LD 12:12, LL or DD was observed. However, the GSI of blinded fish under DD was slightly higher compared to the intact control (table 1).

Testicular recrudescence under different photoperiodic regimes though followed almost similar patterns as that observed in the ovarian recrudescence, statistical comparisons were not possible (table 2). Also no differences in testicular histology among different groups were observed.

A review of the results of different treatments indicate that GSI of blinded fish exposed to LD 14:10, 12:12 or LL was higher than that of fish exposed to LD 9:15 or to DD.

## 4. Discussion

The presence of photoperiodic effects on gonads even after blinding demonstrates the involvement of extraretinal photoreceptors. The effects of blinding on gonadal development in *C. punctatus* are broadly consistent with the findings of Urasaki (1973, 1974), Vodicnik *et al* (1979) and Borg (1982) that gonadal stimulation can take place in response to photoperiod even in the absence of eyes. The results obtained on exposure to short photoperiod however, are in contrast with those obtained by Urasaki (1976) on *Oryzias latipes* and by Garg (1981) on *Heteropneustes fossilis*, where the females exposed to short photoperiod had higher GSI than that of the intact fish exposed to similar conditions. It is not clear whether these effects are due to species difference or due to some other reasons.

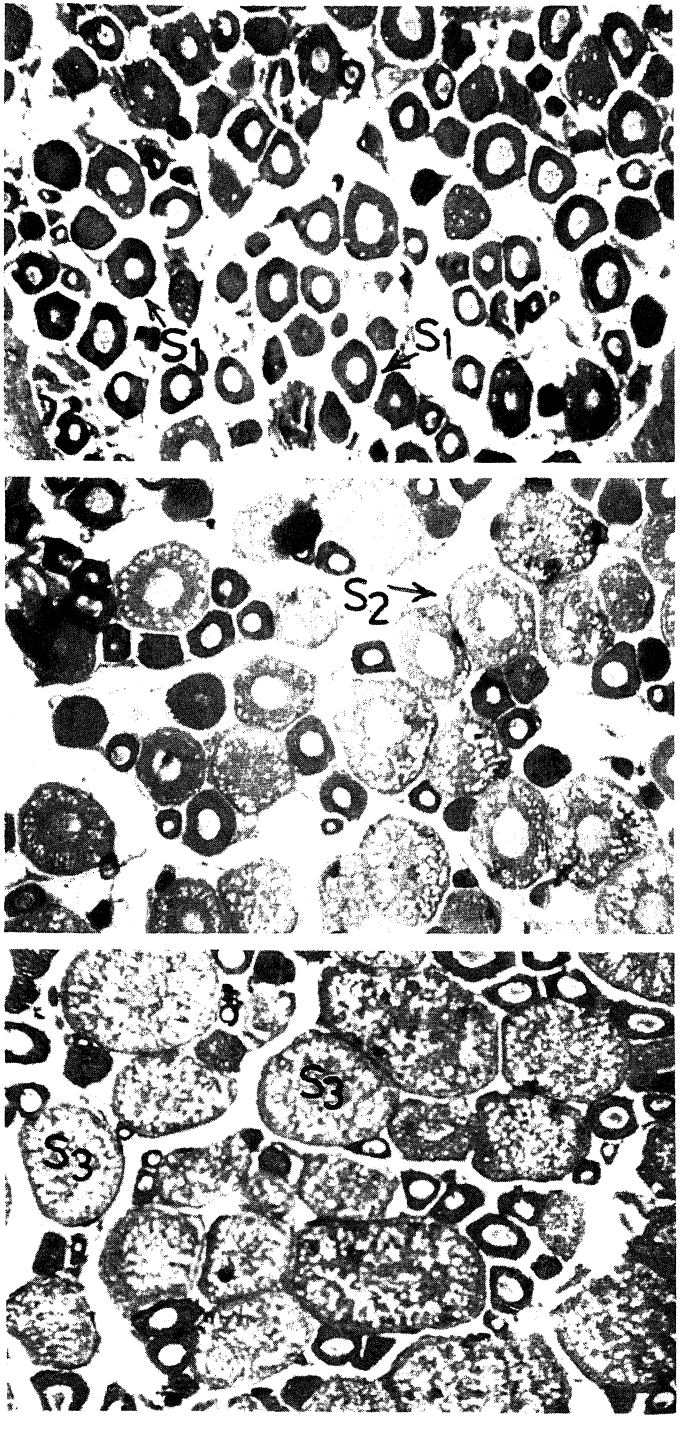


**Figure 1.** Photomicrograph of transverse sections of the ovary of the Indian murrel, *Channa punctatus* (Bloch). (**A**). Stage I ($S_1$) oocytes, (**B**). Stage II ($S_2$) oocytes, note the presence of peripheral ring of cortical alveoli, (**C**). Stage III ($S_3$) yolk-laden oocytes ( × 200).

The pineal organ must be given a primary consideration as a site for the extraretinal photoreception involved in photoperiodic control of reproduction, since the pineal organ of teleosts contains well-developed photoreceptors (see Oksche and Hartwig 1979 for references) and the removal of this organ affects reproduction in several species of teleosts (see Matty 1978; de Vlaming and Olcese 1981). However, the effects of pinealectomy do not necessarily prove that it is the pineal photoreception which is involved in gathering information on daylength, as the photic information from the eyes might reach the pineal organ (Hafeez *et al* 1978).

Saxena (1980) and Delahunty *et al* (1979) reported that it is primarily the retinal pathways which mediate the effects of increasing daylength on ovarian development in goldfish. In *H. fossilis*, the rate of ovarian recrudescence has been reported to be more rapid in fish with intact eyes, however, blinding did not prevent gonadal maturation and this was attributed to the fact that light penetrates the skin and skull and affects the hypothalamic centres (Sehgal and Sundararaj 1970), suggesting that eyes are not essential for gonadal development in this fish. In the present studies a higher GSI under LD 14:10 than that of the intact or blinded fish exposed to LD 9:15, indicate that the retinal pathways are responsible for gathering information on daylength, but the other photoreceptors like the pineal or the extrapineal photoreceptors located in the brain may also be responsible for modulating the seasonal gonadal recrudescence through the photoperiodic information. The studies of Vodicnik *et al* (1979) on *Carrasius auratus* had suggested that both retinal pathways and the pineal organ are involved in the gonadal response to the increasing daylength of spring. Hontela and Peter (1980) have hypothesized that both the pineal and eyes interdependently stimulate ovarian recrudescence in goldfish under long photoperiod.

The possible involvement of extraretinal and extrapineal photoreception in teleost reproduction has not been clearly demonstrated, although the presence of such photoreception in some teleosts has been shown to be present (Urasaki 1976; van Veen *et al* 1976), which respond to light stimulus directly and may be involved in gonadal response to photoperiod in certain teleosts. Lower gonadal weights in blinded *C. punctatus* in the present studies perhaps is an indication of the importance of eyes in the gonadal response to photoperiod and differential response under different photoperiodic regimes reveal that photoreceptors other than eyes are also involved. Although these results indicate the involvement of extraretinal photoreception in the photoperiodic effects on gonads, however, further studies on pinealectomized *C. punctatus* are needed to confirm these findings.

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# Influence of food plants on the food utilization and chemical composition of *Henosepilachna septima* (Coleoptera: coccinellidae)

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**Abstract.** Influence of three food plants, *viz Memordica charantia*, *Luffa acutangula*, and *Trichosanthus anguina* on the food utilization and chemical composition of *Henosepilachna septima* has been studied. The rate of conversion and conversion efficiencies were higher in *T. anguina* fed beetles. The rate of conversion was positively correlated with the protein content of the food plants. The organic constituents of *H. septima* specially protein and lipid increased when fed on the different food plants. Greater increase of all the organic constituents occurred when the insect was fed on the nutrient rich *T. anguina* than when fed on other food plants.

**Keywords.** Food plants; food utilization; chemical composition; *Henosepilachna septima.*

## 1. Introduction

Studies on the consumption, digestion and utilization of food plants by insect pests are important both from fundamental and applied points of view. They provide information on the quantitative loss brought about by the pests. Several workers have made such quantitative study of food consumption and assimilation by insects (SooHoo and Fraenkel 1966; Waldbauer 1968; Latheef and Harcourt 1972; Bailey and Mukerji 1976; Muthukrishnan and Rajeeya 1979; Ganga and Meenakshi Nagappan 1983). These studies reveal that the quantity of food consumed is very much influenced by the type of food. The different feeding activities of insects on different food plants are influenced by the chemical composition of the diets. Direct relationship between the protein, carbohydrate, lipid and water content of the food plants and the growth and conversion efficiencies have been noted in many herbivorous insects (Keller *et al* 1963; Dhandapani and Balasubramanian 1980; Manoharan *et al* 1982) and also the chemical composition of the insect tissues are modified according to the composition of the diet (Subbiah *et al* 1981). Such studies seem to be less in coleoptera and the present work is designed to relate the influence of food plants on the feeding budget and biochemical composition of a coleopteran, *Henosepilachna septima.*

## 2. Material and methods

The lady bird beetle, *Henosepilachna septima* were collected from the infected crops of bitter gourd from the local gardens of Madurai, and maintained on the leaves of bitter gourd *Memordica charantia* at 30 ± 1°C and 70 RH. Freshly emerged adults were used for feeding experiments. Since the individual beetles were small and consumed little

food, a group of 10 insects (5 males + 5 females) was used for the feeding experiment (Ganga and Meenakshi Nagappan 1983). The experimental groups were reared in perforated plastic containers (10 × 8 × 6 cm). The food plants used in this feeding study were *M. charantia* (bitter gourd), *Luffa acutangula* (ridge gourd) and *Trichosanthus anguina* (bottle gourd). The experimental groups were fed *ad libitum* on weighed quantities of their respective food for 10 days, and triplicate groups for each food were maintained. Unfed leaves and faeces were collected daily and dried at 60°C to find out the percentage of water content. The scheme of feeding budget followed is the slightly modified IBP formula (Petrusewicz and Macfadyen 1970) represented as $C = P + R + (F + U)$, where $C$ is the total food consumed, $P$ the growth (conversion), $R$ the energy spent on metabolism and $F + U$ the energy loss *via* faeces including nitrogenous excretory products; it has been described in detail elsewhere (Muthukrishnan *et al* 1978). The total protein, carbohydrate and lipid contents of the food plants, freshly emerged adults and adults after being fed on different food plants for 10 days were estimated following the methods of Lowry *et al* (1951), Seifter *et al* (1950) and Bragdon (1951) respectively.

## 3. Results and discussion

### 3·1 *Composition of the leaves*

Percentage composition of protein, carbohydrate, lipid and water content of leaves of the food plants are presented in table 1. The results of the biochemical analysis of the leaves of food plants reveal that organic constituents and water content are higher in *T. anguina* suggesting that the nutritive value of this plant is more than that of the other food plants used.

### 3·2 *Feeding budget of H. septima*

The feeding budget of *H. septima* on three food plants is given in table 2.

3.2a *Consumption*: The total amount of food consumed by a group of 10 beetles for 10 days on three food plants used showed significant variations. The total consumption amounted to 2210·33, 1043·33 and 674·00 mg when fed on *L. acutangula*, *T. anguina* and *M. charantia* respectively. The rate of food intake of *H. septima* significantly increased from 153·05 mg/g live wt/day on *M. charantia* to 286·51 mg/g live wt/day on *T. anguina* and 532·27 mg/g live wt/day on *L. acutangula*. Hence the potential damage that this insect can cause is maximum on *L. acutangula* and minimum on *M. charantia*.

**Table 1.** Composition of the food plants (in % of dry weight except water) ($\bar{X}$ + SD).

| Food plants | Protein | Sugar | Lipid | Water |
|---|---|---|---|---|
| *L. acutangula* | 15·76 ± 0·38 | 0·70 ± 0·00 | 7·73 ± 0·05 | 75·2 ± 0·37 |
| *T. anguina* | 17·75 ± 0·36 | 1·11 ± 0·02 | 10·98 ± 0·11 | 81·1 ± 0·42 |
| *M. charantia* | 2·90 ± 0·00 | 0·82 ± 0·01 | 7·56 ± 0·00 | 78·4 ± 0·26 |

**Table 2.** Feeding budget of *H. septima* when fed on three different food plants. ($\bar{X}$ + SD).

| Food plants | Consumption (mg) | Assimilation (mg) | Production (mg) | Metabolism (mg) | Consumption rate* | Assimilation rate* | Production rate* | Metabolic rate* | Assimilation efficiency (%) | Gross conversion efficiency (%) | Net conversion efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. acutangula* | 2210·33 | 2180·33 | 69·06 | 2111·27 | 532·27 | 525·08 | 16·60 | 509·11 | 98·78 | 3·15 | 3·20 |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 139·34 | 143·71 | 5·08 | 148·78 | 40·26 | 41·50 | 1·03 | 42·89 | 0·29 | 0·43 | 0·45 |
| *T. anguina* | 1043·33 | 1007·00 | 61·73 | 945·27 | 286·51 | 276·56 | 16·88 | 259·69 | 96·52 | 5·90 | 6·10 |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 45·11 | 42·71 | 5·77 | 37·22 | 15·07 | 15·00 | 0·25 | 14·99 | 0·32 | 0·32 | 0·34 |
| *M. charantia* | 674·00 | 607·67 | 37·00 | 570·67 | 153·05 | 138·06 | 8·40 | 129·67 | 90·18 | 5·50 | 6·09 |
| | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| | 15·56 | 9·02 | 2·45 | 10·06 | 8·19 | 8·24 | 0·64 | 7·97 | 0·78 | 0·41 | 0·44 |

*mg/g live wt/day.

Differences in food consumption may result from a variety of factors. Feeding is governed by passiveness of food, water content and other physico-chemical properties of food material (Bhat and Bhattacharya 1978). Continuation of a feeding response in a feeding behaviour scheme which inevitably, would lead to a greater amount of food intake by an insect is governed by certain chemical feeding stimulants in its diet (Beck 1965). This possibility of such chemical feeding stimulant in *L. acutangula* is ruled out by studies on food orientation behaviour by *H. septima* (Mary Saroja 1982). The higher feeding rate on *L. acutangula* may be due to some nutritional deficiency due to which, *H. septima* have to consume more food to fulfil the nutritional requirements or that the food may be nutritionally unbalanced which increased the feeding rate (Babu *et al* 1979).

3·2b *Assimilation*: The total amount of food assimilation was also higher in *L. acutangula* (2180·33 mg) than that of *T. anguina* (1007 mg) and *M. charantia* (607·67 mg) in ten days. Of these three plants, *L. acutangula* has a lower water content (75·2 %). Maximum assimilation of food having low water content was also reported by Waldbauer (1968). Total food assimilation was directly related to the total amount of the food consumed. In *Periplaneta americana* and *Pieris brassicae* also assimilation is found to be proportional to the amount of food consumed (Muthukrishnan and Rajeeya 1979; Yadava *et al* 1979). The relationship between the approximate digestibility and the palatability of food reported by Mathavan and Baskaran (1975) probably accounts for this direct relationship between consumption and assimilation. The rate of assimilation of *H. septima* feeding on *L. acutangula*, *T. anguina* and *M. charantia* were 525·08, 276·56 and 138·06 mg/g live wt/day respectively. Any two mean values obtained for total assimilation or for assimilation rate of *H. septima* on the three different food plants were significantly different from each other at 1 % probability level. Assimilation efficiency represents the ability of an insect to digest the food. The insect fed on *L. acutangula* has a high assimilation efficiency (98·78 %) than that fed on *T. anguina* (96·52 %) or on *M. charantia* (90·18 %). The lowest assimilation efficiency of *H. septima* when fed on *M. charantia* appears to be related to the lesser nutrient content present in this food, as nutrient imbalance has been implied to be a factor inversely affecting digestive efficiency (Waldbauer 1964). However, statistical analysis has revealed that no significant correlation exists between the amount of any of the organic constituents of the food and assimilation by *H. septima*.

3·2c *Conversion*: The conversion by *H. septima* fed on different food plants are in the following decreasing order. *L. acutangula* (69·06 mg) > *T. anguina* (61·73 mg) > *M. charantia* (37 mg). Total production seems to be directly related to the amount of food consumed and assimilated on the different food plants. Such direct relationship between growth and consumption has been observed in other insects also (Mukerji and LeRoux 1969; Latheef and Harcourt 1972; Sing *et al* 1975).

The order of food plants in their ability to promote the rate of food conversion *T. anguina* (16·88 mg/g live wt/day) followed by *L. acutangula* (16·6 mg/g live wt/day) and *M. charantia* (8·4 mg/g live wt/day). The rate of production is positively correlated with the protein content of the leaves which is significant at 1 % level (table 3). Corresponding relationship between the protein content of diet and growth rate has already been reported in many herbivorous insects (Taylor and Bardner 1968; Schramm 1972; Onuf 1977; Slansky and Feeny 1977).

**Table 3.** Correlation (*r* values) between the organic constituents of the three different food plants and feeding parameters of *H. septima*.

| Feeding parameters | Protein | Lipid | Sugar |
|---|---|---|---|
| Consumption | 0·6842 | −0·1250 | −0·4661 |
| Assimilation | 0·6891 | −0·1184 | −0·4596 |
| Production | 0·9956* | 0·5620 | 0·2798 |
| Metabolism | 0·6770 | −0·1353 | −0·4757 |

*Significant 1 % level.

The report of Waldbauer (1964) that low growth rate may be due to low rate of feeding, nutritional inadequacy of food or a combination of the two, explains the low production rate of *H. septima* on *M. charantia.*

Gross conversion efficiency (ECI) indicates the efficiency with which the ingested food is converted to body matter. Net conversion efficiency (ECD) represents the efficiency with which the digested food is converted into body matter. Both ECI and ECD are highest in *T. anguina* (5·9 and 6·1) which are due to high nutritious food (SooHoo and Fraenkel 1966). But surprisingly both ECI and ECD are lowest in *L. acutangula* fed groups (3·05 and 3·2 %) and low ECD is due to metabolization of greater digested material which are not used for structural purposes (Hoekstra and Beenakkers 1976). Singhal *et al* (1976) also suggested that the high consumption and assimilation with lower amount of ECD indicates a greater respiratory consumption in these insects. In *M. charantia*, which is the least consumed food and on which the assimilation efficiency is also lowest, the conversion efficiencies are very high (5·5 and 6·09 %) and comparable to those found on *T. anguina.* This may be explained on the basis of the existence of compensatory mechanism suggested by Duodu and Biney (1981). Poor consumption and poor digestion of *M. charantia* is compensated by high conversion efficiencies.

### 3.3 *Influence of food plants on the biochemical composition of the insects*

Organic constituents of newly emerged adult *H. septima* and *H. septima* fed on three different food plants are given in table 4. Feeding of the freshly emerged adult on different food plants resulted in an increase in the biochemical constituents of the insect to different degrees. In all the adults, the carbohydrate content was uniformly lower than the other organic constituents. Ramdev and Rao (1979) suggested that carbohydrates are utilized by the insects either for maintenance or for conversion to body lipid, rather than being stored. Carbohydrates have been reported to contribute to the building up of protein in *Phormia regina* (Tate and Wimer 1974). The level of carbohydrate increased (11·11 %) when the insects were fed *T. anguina* but there was no increase of sugar when the insects reared on the other food plants. The increase of carbohydrate content when fed on *T. anguina* seems to be influenced by the carbohydrate content of the food plant. In *Heliothis zea* also the carbohydrate content of the diet directly influenced the carbohydrate content of the insect (Nettles *et al* 1971).

There was an increase of protein and lipid in all the fed insects, but the percentage of increase differed according to the nutritive value of the food plant.

**Table 4.** Organic constituents of adult *H. septima* (in % of dry weight).

| Samples of animal tissue analysed | Protein | Carbohydrate | Lipid |
|---|---|---|---|
| Newly emerged adult | 20·49 ± 0·24 | 0·09 | 18·77 ± 0·14 |
| Adults fed on *L. acutangula* for 10 days | 24·96 ± 0·35 | 0·09 | 18·91 ± 0·00 |
| Adults fed on *T. anguina* for 10 days | 32·74 ± 0·61 | 0·10 | 25·38 ± 0·30 |
| Adults fed on *M. charantia* for 10 days | 22·11 ± 0·00 | 0·09 | 19·87 ± 0·18 |

The percentage of increase of protein was minimum when fed on *M. charantia* (7·1 %) and maximum when fed on *T. anguina* (59·79 %). The percentage of increase of protein appears to be related to the percentage composition of protein in the food plants. The protein content of the different tissues of *P. americana* showed an increase when fed on protein rich diet (Senthamil Selvi 1982). Maximum increase in lipid content is observed when fed on *T. anguina* (35·22 %) and minimum when fed on *L. acutangula* (0·74 %). Increase of lipid in *H. septima* is not directly correlated with the lipid content of the food is suggested by the fact that though *L. acutangula* and *M. charantia* have almost equal amounts (7·73 % and 7·56 % respectively) of lipid, the insects fed in the former showed an increase of only 0·74 % while those fed on the latter, had an increase of 5·86 %. The higher food conversion efficiency of *H. septima* when fed on *M. charantia* is probably responsible for this.

From the results it is evident that all the organic constituents of the insect increased to a greater degree when fed on *T. anguina*. In the light of these observations, it is suggested that *T. anguina* is the most suitable host plant for *H. septima*, because of its nutritional value with high protein and water content which allow the insects to convert the food materials into body tissues.

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# Advances in insect behaviour

## Foreword

Behavioural studies form an integral part of the biology of insects and one of the major aspects involved in the concept of integrated control of insects is behavioural approach. Neural integration, forming as it does an important parameter, naturally involves the role of chemosensory and other structures which are known to adequately respond to a variety of chemical substances, whether attractants or repellants. It is in this light that diverse approaches are being made to study various components of insect behaviour with reference to phytophagous or haematophagous insects, not to mention of predators or parasites. The growing tendency for an indiscriminate use of several categories of insecticides has so visibly affected the behaviour of many insects, that a proper understanding of behavioural studies becomes imperative. The recognition of the significance of biological rhythms in the behavioural activities of several insects, as well as an understanding of energetics involved in such activities have opened up new avenues of research. Social insects also offer many clues to an understanding of the complexities of the behavioural repertoire, and further studies in this area have gone a long way in enabling us to understand the true significance of the genetics and evolution of social behaviour. Papers presented in this volume relate to behavioural rhythms in insects, bioenergetics and behavioural mechanisms in insects, hormones in insect behaviour, insects and behaviour-modifying chemicals, insect toxicology and behaviour, analysis of behavioural trends in social insects, host-switching mechanisms and searching behaviour and behavioural analysis of feeding and breeding in insects.

Communication signals play a salient role in the social interactions of various animal groups. Despite the prevalence of diverse signals such as visual, acoustic, tactile and olfactory among higher animals, olfactory cues have certain specific advantages over other modes of communication. During the recent past considerable attention has been focussed on chemical signals in animals especially in economically important insects. Sex pheromones, aphrodisiacs, trail markers, aggregating and alerting pheromones have been isolated in different insects and diverse factors regulating sex pheromone behaviour have been discussed.

Hormones may directly control the behaviour of insects, or they may modulate the nervous system in integrating the behavioural repertoire. In neurally-modulated hormonal control of behaviour, hormones may either switch-on new neural patterns of behavioural activity, or by evoking neurophysiological activity, or hormones may increase or decrease the threshold of stimuli modifying the behavioural pattern of the animal. Pheromones are of utmost importance in the hormone-behaviour interaction in insects. Hormones also affect adult behaviour, including male sexual behaviour, receptivity as well as oviposition behaviour. Insect migration and orientation as also related behavioural patterns in insects like locusts, is controlled by hormones.

Different types of behavioural, developmental and physiological rhythms have been identified in many species of insects. Daily cycles of activity in endocrine and nervous systems of insects have attracted attention because of the probable importance of these systems, in the control of overt rhythms of physiology and behaviour. Many developmental events in insects have long been known to occur in a specific part of the day to manifest a population rhythm. Although some information is available regarding endocrine rhythms in varied groups of insects, studies on different physiological and metabolic rhythms in relation to the hormonal activity pattern in insects remain scanty and available information in this regard is presented. Biological clocks in relation to the eclosion rhythms of *Drosophila*, the problem of on-and-off rhythms and their simulations by appropriate high/low or low/high intensity transfers are also discussed.

Food acquisition behaviour in insects includes such energy requiring activity components as location, gathering and processing. In most insects oviposition is preceded by a precise estimation of the presence of minimum food supply for successful completion of developmental stages and emergence. Discussion is presented on the energy-requiring behavioural activities associated with courtship, mating and oviposition in some insects. Like the phytophagous insects, haematophagous species are also known to possess chemoreceptors that can detect host specific factors (odours) and non-specific or group factors. Closely associated with this behavioural aspect is the capacity of the haematophagous arthropod to 'taste' the blood meal, where contact chemoreceptors come into play and these aspects have been discussed in detail. The feeding behaviour and the patterns of host selection in phytophagous insects are conditioned not only by their ecological requirements but also the general behaviour of the insects concerned. Though they are generally polyphagous, they are not indiscriminate feeders and the food plant range is often correlated with their behaviour which is conditioned by their sensory perception.

Social insects often behave in ways that appear to lower their genetic fitness while increasing the fitness of other conspecifics. The most extreme examples of such altruistic behaviour is provided by the sterile workers of social insects, and the evolution by natural selection of social or altruistic behaviour has long been recognised as a problem and an incisive discussion is provided on the evolution of social behaviour. Lamellicorn beetles in particular, the passalids and scarabaeids are also known to exhibit social behaviour, sound production by stridulation in both the larvae and adult passalids being attributed to social behaviour permitting gregariousness. Interesting aspects relating to the whole sequence of bisexual co-operation in the nesting behaviour of scarabaeids are also discussed.

As conventional methods of control developed for agricultural pests are often not suitable for forest pests because of the large area covered by the crops, recent investigations have shown the potentiality of using behavioural studies to greater advantage in the management of forest insect pests. Information provided on the teak defoliator, particularly on moth immigration, a common occurrence in tropical forests, other behavioural characteristics such as crowding of caterpillars on tree trunks and aggregation, appear to be useful in the development of suitable management strategies.

The papers published in this volume were presented at the National Workshop sponsored by the Department of Science and Technology on 'Advances in Insect

Behaviour', held at the Entomology Research Institute, Loyola College, Madras from December 14–16, 1984. I wish to express my deep sense of gratitude to the Department of Science and Technology for sponsoring this Workshop. It is hoped that this publication comprising as it does diverse aspects of insect behaviour would stimulate further interest in this upcoming, inter-disciplinary field, of considerable significance in applied entomological studies.

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# Recent advances in animal behaviour

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**Abstract.** Ethology, a fast developing field of animal sciences has considerable relevance in animal husbandry, agriculture, control of animal populations, pest control, medicine, wildlife biology, etc. It has made vast strides of progress during the past few decades and some of these trends are reviewed.

Communication signals play a salient role in sociobiology of animal groups. Animals deploy visual, acoustic, tactile and olfactory signals during their social interactions. Among these, olfactory cues have certain specific advantages over the other modes concerned. Recently considerable attention has been focussed on chemical signals in animals, especially those of economically important forms such as insects, fishes and mammals.

Regarding insects, sex pheromones, aphrodisiacs, trail markers, aggregating and alerting pheromones have been isolated in various insectan orders. The factors controlling sex pheromone behaviour and impact of pheromones on control of insect population have been elaborated.

Investigations on chemical cues of lower vertebrates indicate that fishes, amphibians and reptiles deploy them in their social interactions. Pheromones modulate the schooling, reproductive and alarm response behaviour in fishes.

Among mammals, urine, fecal pellets, saliva and secretions of specialised skin glands function as sources of olfactory cues. Data on histophysiology, and ultrastructure of specialised skin glands, biochemistry of their secretions have been collected. Osmetrichia, scent marking patterns and flehmen responses and their hormonal control have been elucidated. The neuroendocrinological basis of scent marking has been made explicit.

Relatively only very few of the mammalian pheromones have been isolated. The role of Primer pheromones in modulation of reproductive processes in some of the rodents and signalling pheromones in social interactions of some mammals have been elaborated.

Data on olfactory cues in human social interactions indicate the presence of social pheromones.

Visual signals of some insects and their role in reproductive activities have been investigated. Social postures in some rodent pests and their behavioural relevance have been studied. Acoustic signals in insects facilitate congregation, sexual attraction, aggregation and alarm responses. Further various aspects of vocalisations in birds and mammals have been investigated. Reproductive investment patterns and sex ratios in insects and parental investment in birds have been elucidated. Play behaviour and their role in behavioural development has been investigated. Ethological analysis of drug action in aggressive behaviour in certain mammals has been made.

**Keywords.** Ethology; applied zoology; communication signals; olfactory cues; pheromones; aphrodisiacs; vertebrates; chemical signals; schooling and alarm responses; amphibians; reptiles; secretions of specialised skin glands; histophysiology; osmetrichia; scent marking; hormonal control; flehmen response; visual and vocal signals; social postures in mice; vocalisation in birds and mammals; reproductive investment in insects; ethological analysis of drug action.

## 1. Introduction

Ethology, the scientific study of animal behaviour is a relatively recent field of animal sciences, integrating animal ecology, neurophysiology, endocrinology, sensory physiology, etc., and it has made considerable strides of progress during the past few decades. The contributions of Lorenz (1971), Tinbergen (1951), Frisch (1967), Thorpe (1979),

Hinde (1970), Muller-Schwarze (1969) to mention only a few, have widened our concepts and opened up new frontiers in the ever expanding horizons of ethological investigations.

Communication signals play a salient role in the sociobiology of various animal groups. In fact a clear insight into the diverse modes of communication is absolutely essential for having a better understanding of the biology of the animal group concerned. Generally higher animals deploy diverse modes of communication such as visual, acoustic, tactile and olfactory (chemical) during their social interactions. Among these the olfactory signals exhibit certain specific advantages over others in as much as that they are effective over longer distances, they can be deployed in darkness, their fade-out time is longer and the presence of the signalling animal is not necessary at the site of emanation of the signal. In the last mentioned trait, they are comparable to the written language of human beings.

Considerable attention had been focussed on the communication systems of animals, especially economically important forms such as insects, fishes and mammals during the past few years. Due to the limitation of time attention may be focussed only on some aspects of chemical communication in some of these animal groups.

Ever since Butler (1967) introduced the term aphrodisiac pheromone, it has been widely used and it pertains to a substance produced by one or the other sex, usually by a male and often as a part of the complex pattern of courtship behaviour, preparing the partner for copulation after being brought together by olfactory sex attractants or other means. According to Shorey (1973) aphrodisiac should influence that part of the nervous system (NS) of a female which controls her mating behaviour, thereby increasing her chances of accepting a male in copulation.

Many insect species deploy sex pheromones acting as stimulants when two sexes come together (Jacobson 1972; Shorey 1973). However only a few experimental studies have been made on this aspect.

## 2. Honey bee queen substances

One of the most versatile pheromone, the queen bee substance of the honey bee, *Apis mellifera* is 9-oxydec-trans-2 enoic acid and is produced by the mandibular glands of the queen and not the workers. Data suggest that it can function as an attractant for workers in colony, cohesion, swarming, inhibition of queen cell construction and ovary development in workers, sex attractant and mating stimulant for drones (Butler 1967; Gary 1970). Experimental studies of Butler (1967) had revealed that both open sting chamber and odour of the queen bee substance are necessary for the drones to mount a queen in flight and the queen substance functions as an aphrodisiac.

Apart from the queen bee substance yet another substance released from the abdominal tergites may induce mounting and copulation. In fact much more remains to be investigated regarding the queen bee behaviour.

A hierarchy of behaviour in response to stimulation by female sex pheromones has been demonstrated in a large number of insects belonging to diverse orders (Shorey 1973). For *e.g.* in *Trichophusia ni* a quantitative increase in concentration of female sex pheromone alone is sufficient for the initiation of each successive step in response sequence including the release of male copulatory behaviour (Shorey and Gaston 1970). Such a pheromone is found in many other insects. However it would be much more

logical to confine the term aphrodisiac to substances released after the sexes have been brought together.

## 3. Gustatory aphrodisiacs

Sex pheromones acting through the gustatory sense require that the male and female make contact and hence may possibly have an aphrodisiac function. In many Orthopteran species virgin females produce sex pheromone which attracts the male. The courting males themselves produce a pheromone which stimulates the female to mount and feed on secretion thus attaining the correct position for copulation. Such male pheromones have been found in *Blatta germanica*, *Blatta orientalis* and *Periplanata americana*.

Roth and Dateo (1966) had isolated a pheromone from the males of *Nauphoeta cinerea* which elicits typical behaviour of a female attracted to a courting male's tergum. This pheromone is a polar neutral lipid of low volatility, named seducin for its role in releasing sexual behaviour.

Regarding Lepidopterans, main female response to male producing aphrodisiac pheromone tends to be either inhibition of the female's natural tendency to fly or cessation of flight.

In Noctuidae it has been shown that volatile secretions from the male scent brushes of moths are used in courtship (Birch 1970). The noctuid males have scent brushes either on the 8th abdominal segment of *Plusia gamma* or a pair of brush organs at the anterior end of the abdomen *e.g. Apamea*. The male brushes have compounds, generally simple terpenoids and aromatics—carboxylic acids (Birch 1972; Grant *et al* 1972). Electrophysiological studies of Grant *et al* (1970, 1972) reveal that male scent brushes do not elicit antennal responses which are specific to species or sex.

## 4. Sex pheromones

The complexities of describing species specific pheromone blends have brought the challenge of pheromone identification from the chemistry laboratory to the field. Despite the fact that chemical characterisation should still be continued to unravel the intriguing sex pheromones used by many other species, especially those in families for which no identification has yet been made, the final duplicating of any natural pheromone blend can only be accomplished by analysis of various ratios and release rates under field conditions. Infact an exact reproduction of the pheromone input should enable man to attract males very efficiently. On the contrary one should bear in mind the long term use of fairly exact blends could merely serve as an artificial pressure enabling the insect to further modify its chemical systems.

Reproductive isolation with one or multiple compound systems have been exemplified by many species changing the functional moieties, (acetate, aldehyde or alcohol) the double bond position (7–11), configuration (*cis* or *trans*) or number (1 or 2 sites of unsaturation) or carbon chain length (12–14 carbons). Apart from these different release rates the specific circadian rhythms facilitate reproductive isolation.

## 5. Aggregating pheromones

According to Shorey (1973) aggregating pheromones cause other members of the same species to aggregate in a particular area. They occur ubiquitously in Coleoptera, family Scolytidae (Borden and Stokkink 1971). The pheromone functions as a population aggregation pheromone.

## 6. Trail markers

Diverse types of odour trails are deployed by a wide variety of hymenopteran species as an effective means of coordinating the movements of individuals. Seven glands have been identified as the source of this pheromone and the chemistry and specificity of these pheromones have been identified.

Ant trail pheromone, methyl-4-methyl pyrrole-2-carboxylate of myrmycine, *Atta texana* is the only ant trail pheromone which has been identified (Tumlinson *et al* 1972). The functional aspects of this pheromone especially its role in transmitting cues about distance and direction have been identified.

## 7. Alarm pheromones

Considerable amount of data had been collected on the alarm pheromones of ants. The mandibular glands of meliponine bees are the sources of all alarm pheromones so far characterised (Blum *et al* 1970). Many species of ants and bees signal alarm with a large variety of ketones which function as chemical releasers.

## 8. Environmental and physiological control of insect sex pheromone behaviour

It has been shown that certain environmental factors *viz* temperature, intensity of light, velocity of wind etc. control sex pheromone communication in insects by diverse means (Bartell and Shorey 1969). Regarding physiological variables, circadian rhythms, age, mating, previous exposure to pheromones, population density, hormones etc, are also important.

## 9. Pheromonal control of insectan population

The pheromones play a vital role in control of insectan population. However the true potential of pheromones as a part of survey devices and as control agents cannot be easily realised without input of commercial technology. This necessitates a combination of established and new techniques along with coordination of efforts of industrial concerns and research centres concerned. Such an exchange is necessary before educating the end users.

Along with transfer of information, the commercial pheromones developed should update data, often requiring alterations in protection standards. If pheromones are to be used directly or indirectly in insect control programmes a registration protocol has to be established for permitting such use.

In consumers, pheromones and related substances are used in a variety of problems facing agriculture and forestry. As a survey and detection device, they have already become an important tool in the overall management of certain pests such as cabbage looper (*Trichoplusia ni*), pink boll worm, boll weevil, gypsy moth, spruce bud worm and bark beetles.

## 10. Vertebrates

Considerable amount of data have been collected on pheromones in fishes especially those concerned with attraction and recognition of other sex, the offspring or the parents, maintenance of schooling behaviour and during anadromous migration. However the exact nature of most of these olfactory signals have not been established.

In fact the only fish pheromone which has been well established is the alarm substance. The fright reaction was first discovered by Frisch (1938) in the minnow, *Phoxinus phoxinus*. The epidermal cells (club cells) concerned with the production of alarm substance have been localised (Pfeiffer 1960). The alarm substance of the minnow is a pterin.

## 11. Amphibians

The fright reaction has been described in amphibians too. Kulzer (1954) reported this in tadpoles of *Bufo bufo*. The source of these olfactory cues have been traced to the giant cells in the epidermis (Pfeiffer 1966).

## 12. Reptilia

It has been shown that chemical signals play a salient role in the sociobiology of lizards and snakes. Snakes have many advantages for chemosensory research in as much as they rely heavily on chemical senses and most of their responses are regulated by chemical cues. However very little is known about their reproductive biology. The role of chemical cues in orientation in chelonians, alarm reaction in snakes, territorial marking, predation warning and nocturnal behaviour also could be shown.

## 13. Mammals

Investigations on chemical communication in mammals have revealed that olfactory cues are deployed by these forms quite frequently. The major sources of body odour are the urine, fecal pellets, saliva and the secretions of the specialised skin glands. The specialised integumentary glands have an ubiquitous distribution among various mammalian orders with over 16 of them exhibiting these glands which are mainly of two types, holocrine sebaceous and apocrine sudoriferous.

It has been shown that monotremes like Platypus have femoral glands. Some of these glands produce poisonous secretions which could even kill a dog. Marsupials have cloacal (anal) glands, frontal and sternal glands.

Studies conducted at the School of Mammalian Ethology, Department of Zoology, University of Kerala have revealed the presence of specialised skin glands in a large number of South Indian mammals such as the Indian Musk shrew, rodent pests such as the wild house mouse, Indian field mouse, South Indian gerbil, common house rat, palm squirrel and common Indian mongoose (Balakrishnan and Alexander 1984b). Diverse aspects of histophysiology and hormonal control of these specialised skin glands have been elaborated. Despite the fact that most of these glands are hypotrophied by castration and reactivated by hormone administration some of these glands such as the tarsal glands exhibit a converse effect.

Despite the reports by Stoddart (1976) regarding the absence of behaviourally relevant specialised skin glands in *Mus* sp. our studies on the social interactions of these wild house mouse and Indian field mouse indicate the presence of specific glands at eyelid, oral angle, perineal and preputial regions in these forms (Alexander *et al* 1982). Further the studies on social postures of these forms indicate specific behavioural responses concerned with olfactory investigations of specific body regions of olfactory relevance (Santhi and Alexander 1979). The role of gonadectomy in altering the olfactory status of various interacting conspecifics had also been elaborated.

## 14. Scent marking

Scent marking in mammals have been reviewed by Ewer (1968), Johnson (1973), Thiessen and Rice (1976) and Balakrishnan and Alexander (1984). Generally most of the mammals disseminate their body odour with the aid of specific behavioural responses known as scent marking, which could broadly be categorised into two, passive and active.

The scent marking patterns of many of the above mentioned mammals have been studied. The hormonal control of scent marking in musk shrew had been elaborated (Balakrishnan and Alexander 1976; Alexander *et al* 1984). The role of dominance hierarchy in scent marking of certain artiodactyles such as the spotted deer and the black buck had also been elucidated (Pillai and Alexander 1984).

## 15. Functions

It has been shown that chemical signals, similar to other sensory modalities tend to influence physiology and behaviour and ultimately regulate spacing between individuals and populations and thus contribute the adjustment of a species to its resources and general environment.

It has been shown that these olfactory signals generally function in the following functional contexts: maternal, agonistic, social, recognition, physiological state, recognition of sex, species, sexual attraction and alarm.

Diverse pathways of dissemination of odour has also been studied: (i) Direct release into air (ii) Secretions may be left on a substrate (iii) Odoriferous substance smeared on to various parts of the body (iv) Secretions of the body rubbed on other conspecifics.

Scent marking has both individual and social uses for the donor. Regarding the former, an animal scent marks a novel area for olfactory reassurance, orientation or self advertisement. As for the latter, *viz* social uses, it could signal identity or presence of

conspecifics and provide valuable data regarding sex, age, identity, reproductive status etc. of the donor.

Investigations on the olfactory inhibition of scent marking has been conducted on musk shrew (Balakrishnan and Alexander 1980). Further the effect of cage surface odours especially urine and fecal pellets on behavioural responses of some rodents have also been investigated (Nair and Alexander 1984).

## 16. Ultrastructure and osmetrichia

The fine structure of the flank gland of the Indian musk shrew had been reported (Balakrishnan *et al* 1984b) recently. Further the specialised scent hair had also been investigated in certain N. American and Indian mammals. It has been shown that these special tuft of hair, such as flank gland hair of the musk shrew exhibit specific structural modifications on its scaly surface for holding the odourous molecules (Balakrishnan and Alexander 1984a). These osmetrichia are used by the musk shrew as scent brushes for painting the areas to be marked with the odourous secretions of the flank gland, thereby facilitating olfactory communication.

## 17. Biochemical assay of glandular secretions and pheromones

Biochemical investigations on the glandular secretions of various mammals such as wild rabbit, mongolian gerbil, black tailed deer, musk shrew, common Indian mongoose etc. have been conducted.

However relatively very few mammalian pheromones have been isolated as yet. In fact, only in seven species of mammals, specific pheromones have been identified. These are as follows: domestic boar, *Sus scrofa* (3 alpha hydroxy-5 alpha androst-16-ene) which stimulates female's sexual behaviour, rhesus monkey (*Macaca mulatta*) vaginal secretion, 'copulin' (5 fatty acids, acetic, propionic, isobutyric, *n*-butyric, isovaleric acid), prong horn, (*Antilocapra americana*) sub auricular gland (isovaleric acid), Mongolian gerbil, (*Meriones unguiculatus*) ventral gland (phenyl acetic acid), golden hamster (*Mesocricetus auratus*) vaginal secretion (dimethyl disulphide), dog (*Canis familiaris*) vaginal secretion (paramethyl hydroxybenzoate), black tailed deer (*Odocoileus hemionus columbianuss*) (urine in tarsal scent) (*cis*-4-hydroxy dodec-6-enoic acid lactone).

## 18. Primer pheromones

Very interesting data have been collected on the primer pheromones of laboratory rodents and their role in modulation of reproductive behaviour and processes. The Bruce effect (pregnancy block), Whitten effect (oestrus suppression in all female groups) and Vanderbergh effect (induces precocial puberty) are excellent examples of the role of these priming cues. Vandenbergh (1983) has also demonstrated acceleration of puberty in heifers by exposure to bull urine.

## 19. Signalling pheromones

These are frequently used in evocation of many specific hormones modulated and reproductively related social behaviour such as those occurring in sexual aggression and maternal behaviour. Mouse signalling pheromones facilitate identification of individual, species, age, sex, sexual state, presence of fear (alarm pheromone carried in urine) etc.

## 20. Neural mechanisms of scent marking

It has been shown that in mongolian gerbil, *Meriones unguiculatus*, testosterone implants in the preoptic area, reinstate ventral gland scent marking in castrated males, whereas cholesterol implanted at the same site has no effect. Testosterone implants in the cortex, caudate nucleus, amygdala, hippocampus, reticular formation and septum have also no effect in scent marking. The anterior area of the median preoptic area is the most androgen sensitive area for stimulating scent marking in the mongolian gerbil. Recent studies on South Indian gerbil, *Tatera indica* also indicate similar pattern.

Regarding ovariectomised female mongolian gerbils, they exhibit scent marking when oestrodiol benzoate is implanted in the anterior hypothalamus preoptic area, septum but implants in hippocampus, amygdala, thalamus or olfactory nucleus are not effective.

## 21. Role of chemical signals in human social behaviour

The probable occurrence of functional human pheromones has been both asserted (Wilson 1963) and denied (Gleasoen and Reynierse 1969) both without experimental evidences. However the observation of Michael and Keverne (1970) on 'copulin' in monkeys and menstrual synchronisation among close friends (McClintock 1971; Russel *et al* 1980) has opened up new vistas in this field. It is quite relevant as regards primer control of human endocrine cycles and reproduction generally and simultaneously opens up new prospects in reproductive pharmacology.

Observations reveal that odour plays an important part in psychosexual development in infants (Kalogerakis 1963).

Since human male sexual behaviour is non-cyclical and not dependent on female receptivity, the female-male influence may be a releaser only, except possibly in infancy.

Curtis *et al* (1971) noted that even a synthetic mixture of acetic, propionic isobutyric, *n*-butyric and isovaleric acid evoked a response in the male. The same set of substances are found in the human vagina and contribute to its attractant odour. The sexually excitant component of human genital odour is complex involving both musk like notes and odour similar to trimethyl amine, and is enhanced by alkaline fixatives. An alkaline component of male genital odour resembles another amine, 1,5, diamino pentane (cadaverine).

The probable primer and releaser substance in man are all musk odours (steroid, large ring cycloketones and lactones) (Sink 1967).

The most likely areas of olfactory relevance in man are skin, axillary and pubic apocrine glands and their tufts. Axillary secretion is most likely the site of human social

pheromone. Prostaglandins also may function as nonolfactory pheromones being secreted in large amounts in the semen and cause uterine contractions and facilitate the sperm transport.

It has been shown conclusively that infants can identify their own mothers after the second week of birth (McFarlane 1975). Humans can also recognise other individuals by smell. Porter and Moore (1981) have shown that infants are able to identify odours of siblings and that parents can identify the odours of the T-shirts of their children.

It has also been observed that infants scent-mark blankets, clothes and toys with their own odour and carry this marked object with them (Passman and Weisberg 1975). These scent-marks reduce their anxiety.

Studies on menstrual synchrony (Russel *et al* 1980) of college women indicate the existence of some types of pheromones in human beings. In fact detailed investigations are warranted to replicate and extend this work, especially to evaluate the correlation between menstrual synchrony and ovulation.

## 22. Reproductive investment patterns and sex ratios in insects

Regarding insects, commitment of resources for reproduction can be dichotomised in various ways, such as mating *vs* rearing, pre *vs* post birth but none of these are mutually exclusive. The commitment of genetic material *per se* is trivial in terms of investment but defines the interests of various individuals on each other in terms of relatedness. The provision of resources is altruistic if it reduces the ability to produce future young; parental care is thus altruistic. Relatedness can be assymmetric among individuals and this together with the facts that parents need not be the main investors in the production of new individuals leads to the particular complexities of insect societies with reproductive division of labour. Such complexities can lead to markedly different preferred sex ratios by workers and reproductives. The effort to determine the level at which selection acts to set sex ratios of investment in hymenopteran societies in which males are haploid and which thus have intrinsic assymmetries has run into technical difficulties and competition for explaining female based sex ratios between the worker-control and local mate competition hypothesis. Recent findings indicate that the local mate competition can yield either female or male based ratios, depending on the dispersal traits of the 2 sexes and that depending on the mode of inheritance, worker control can yield a variety of sex ratios, not just 3 : 1 in favour of females (Owen 1983).

## 23. Visual communication in insects

Relatively ethologists have focussed more attention on visual communication. Many insects emit light signals by virtue of their capacity for bioluminescence, described mostly in beetles (Coleoptera) fireflies, adult lampyrid and elateid beetles. The morphological and biochemical aspects of insect bioluminescence had been reviewed by De Luca *et al* (1974). Lloyd (1977) has stated the diverse uses for which it is employed, such as a lure for prey, pair formation, mate identification and location. In fact in insect courtship the dynamic properties of visual signals are often critically important in eliciting responses from the opposite sex. One of the excellent examples is the reproductive behaviour of fireflies.

Considerable work has been done on the sequence of courtship in N. American fireflies, *Photuris* and *Photinus*. In the main sequence of courtship, the male initiates flashes during flight at a species specific frequency in well defined habitat areas, with the female remaining stationary but relies on the flashes of the male and later follows the male after a brief species specific pause. It has been shown that repeated flashes bring the two sexes together (Lloyd 1971). It has been shown that flash signals of sympatric species differred significantly, but species which are not located in nearby areas exhibit similar flash pattern. The mimicking of flash signals of *Photinus* females by *Photuris* females result in the devouring of the former male, by the latter female. According to Lloyd (1975) female *Photuris* is very versatile that it can simulate the flash pattern of at least 4 different species.

It has been shown that dispersion of insects occur by visual signals. Insects direct signals at each other and these are important in territorial behaviour. Dragon flies are good examples. They are selective about their breeding sites, with males arriving earlier than the females and defend their territories by ritualised aggressive displays supported by physical combat. During aggressive displays the bright silvery abdomen is directed towards the opponent. Preying mantis also displays territorial behaviour.

## 24. Alarm signals

Although visual alarm signals are not well developed in insects, the butterflies (lepidopterans) and paper wasp, *Polistes annulans* exhibit this reaction.

## 25. Sexual signals

Visual signals also play an important part in sexual behaviour. These visual signals mediate a chain of stimuli interactions. It may be possible that in some of the lepidopteran butterflies the well designed wings may be used as visual signals in reproductive displays. In certain cases such as the queen butterfly both visual and chemical signals are involved.

## 26. Acoustic communication in insects

Despite the fact that acoustic communication has evolved hundreds of times in insectan species, hearing has been demonstrated only in 5 orders which produce sounds of higher frequency *e.g.* Orthoptera, Homoptera, Lepidoptera, Coleoptera and Diptera. The true nature of insect songs have not been fully understood by human beings, although the advent of precision recording equipments and sophisticated sound analysis instruments has facilitated the bridging of the gap between the occurrence of the insect sounds and their significance.

It has been shown that most of the insects produce sound of communicative value at some stages of their life cycle, displaying a wide variety with the frictional methods predominating.

Generally most insect sounds have fewer dimensions than vertebrate sounds and the insects cannot carry a tune. Their individual song components are called phonatomes (all sounds produced during one cycle of movement).

Insect sounds function as sexual signals facilitating attraction, courtship, copulation and post courtship pair formation and also for chorusing and aggregation. Special courtship signals have been reported for male orthopterans for cicadas and also for males of many other arthropodan groups, from crabs to Drosophila, occasionally these insect sounds function as social signals also *e.g.* wingless cockroaches and gromphadia of Madagascar, hiss when their culture boxes are disturbed.

## 27. Vocalisation in birds

Recent studies on acoustic communication in birds had effected an acoustic analysis, supplemented by x-ray cinematography which resulted in the formulation of a coherent theory of avian speech (Scanlan 1983). The warbling of the budgerigars (*Melospittacus undulatus*) is acoustically similar in many respects to human speech. In fact only slight alterations are necessary to transform the warbling sound into a speech-like sound. The nature and extent of this transformation differ among 'talking' species and can be related to the vocal behaviour and ontogeny of each species. Such transformations are possible because the birds can best produce just those acoustic cues necessary for human perception of speech. Thus an important cue for human discrimination of vowels is the relative formation frequencies and analysis of avian vowels which showed that the Indian hill Mynah (*Gracula religiosa*) and several species of Psittacidae can reproduce these patterns. Temporal cues because of this bird's excellent capability for time resolution are precisely produced. Some of these acoustic features may be related to movements of suprasyringeal vocal tract during speech imitation. These movements have been observed and analysed during x-ray cinematography.

## 28. Vocalisation in mammals

Very little is known about vocal imitation in mammals. An adult male harbour seal kept in captivity in New England Aquarium, near Boston, USA has been reported to reproduce several english words and phrases (Ralls and Gish 1983). This harbour seal was originally brought in as an orphan pup in 1971. There were no adult male harbour seals around for it to serve as acoustic models. It was observed that in 1978 it started imitating its own name "Hoover" and later on a number of other words such as 'hello', 'how are you' etc.

Adult females rarely vocalise. The rate of male vocalisation increases during breeding season. Although harbour seals are considered as non-vocal pinnipedes, it has been shown that they produce a number of growls, groans, hums etc. The quality of their vocalisation apparently seems to be more superior to that of parrots. A comparison with human spectrogram showed that Hoover's imitation contained key acoustic features that the humans use for discriminating lightest vowels sounds.

## 29. Ethological analysis of drug action on aggression and defence

It has been possible to develop an ethopharmacological analysis of aggression. Specific attention has been focussed on biologically relevant situations and events engendering a broad repertoire of species specific agonistic behaviour (Miczek 1983).

The sequential and multicomponent nature of aggressive interactions standardised means to detect potentially pathological forms of aggression. It also affords the opportunity to characterize neural and behavioural processes relevant to an organism's social and environmental adaptations.

In one of the experiments, catecholamine antagonists such as amphetamines enhanced threat behaviour under certain conditions in rodents but mainly disrupted integrated aggressive and social behaviour patterns. In attacked animals amphetamines disengages defensive and flight reactions from their prompting social stimuli. Amphetamine as well as hallucinogen treated animals are more frequently targets of attacks than control animals. It appears that engaging in aggressive or defensive behaviour in the past and at the time of pharmacological intervention alters the functional state and dynamics of brain catecholamine systems which in turn determines the nature of drug action. The significance of the profound neurochemical changes owing to specific behavioural events is further illustrated with experiments on the role of endorphins in defeat. Mice which are frequently attacked will ultimately emerge in a typical physiological and behavioural pattern of defeat. Defeated animals become immune to pain concurrent with significant changes in brain beta endorphins. This analgesia is reversed by centrally acting opiate antagonists. Complete cross tolerant to morphine analgesia further suggests that defeat experience readily activates brain endorphins.

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# The *Drosophila* circadian clock*

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**Abstract.** The circadian rhythm in the process of eclosion of the fruitfly *Drosophila* is the best investigated with regards to properties such as entrainment, freerun and phase shifts. The system has been the basis of an important coupled oscillator model, several hypotheses, landmark papers and a monograph. The PRC for this rhythm has been extensively used in experiments designed to test the kinetics of the basic clock. The singularity point, signifying a stimulus that can 'stop' the clock, was also predicted and discovered in this rhythm. Fittingly the first clock mutant was also discovered in *Drosophila*.

**Keywords.** Circadian rhythm; *Drosophila*; phase response curves.

## 1. Introduction

The time course in the eclosion process of *Drosophila* represents perhaps the most intensively studied and best understood circadian rhythm (Saunders 1976). The system has stimulated the publication of several landmark papers (Pittendrigh and Minis 1964; Pittendrigh 1966; Engelmann 1966; Winfree 1970), postulation of several hypotheses (Pittendrigh and Bruce 1957; Chandrashekaran 1967b) and the writing of a most stimulating monograph (Winfree 1980). Work on this rhythm has also helped to analyse the formal properties of circadian rhythms, their response features and kinetics of responses. Interestingly one of the diagnostic features, the temperature compensation of circadian clocks, was first elucidated in 1954 for the *Drosophila* rhythm (Pittendrigh 1954). One of the earliest phase response curves (PRCs) was also worked out for this system (Pittendrigh 1960). The PRC of the *Drosophila* rhythm has also been used as a tool to analyse the process of entrainment (Pittendrigh and Minis 1964) and to understand the time constants involved during phase shifts ($\Delta\phi$) of the basic oscillation by light flashes (Chandrashekaran 1967a). Perhaps fittingly the first 'clock' mutant—a single locus—was also isolated for the *Drosophila* circadian rhythm (Konopka and Benzer 1971). This paper is a brief review of the author's own contributions to the gradual unravelling of the formal properties of the *Drosophila* circadian clock.

## 2. The eclosion rhythm

In nature, much as other insects do, the fruitflies eclose during the early hours of the morning and the last of the flies for the day to eclose would have done so by noon. The entire eclosion of a group of flies lasts over *ca* 8 hr. Thus the 'eclosion gate' is one third

*Dedicated to Prof. Dr (multi) h c Erwin Bünning, teacher and exemplar to the author, on the occasion of his eightieth birthday.

the circadian span. If the flies are raised under light dark (LD) conditions in the laboratory eclosion peaks some 3 hr after the light comes on. Earlier it was believed that this eclosion peak appeared in response to the light-on stimulus but it became apparent that the flies take their cue from the last light-off information. In other words emergence (eclosion) of flies would start 12 hr after the L/D transition and peak (median value) 15 hr after L/D. Flies raised in continuous light (LL) or constant darkness (DD) for one or more generations do not show any rhythmicity. Adult flies eclose at all hours of the day and night. Nearly all the *Drosophila* experiments referred to in this short review were carried out at 20°C. At this temperature it takes the flies about 20–22 days to

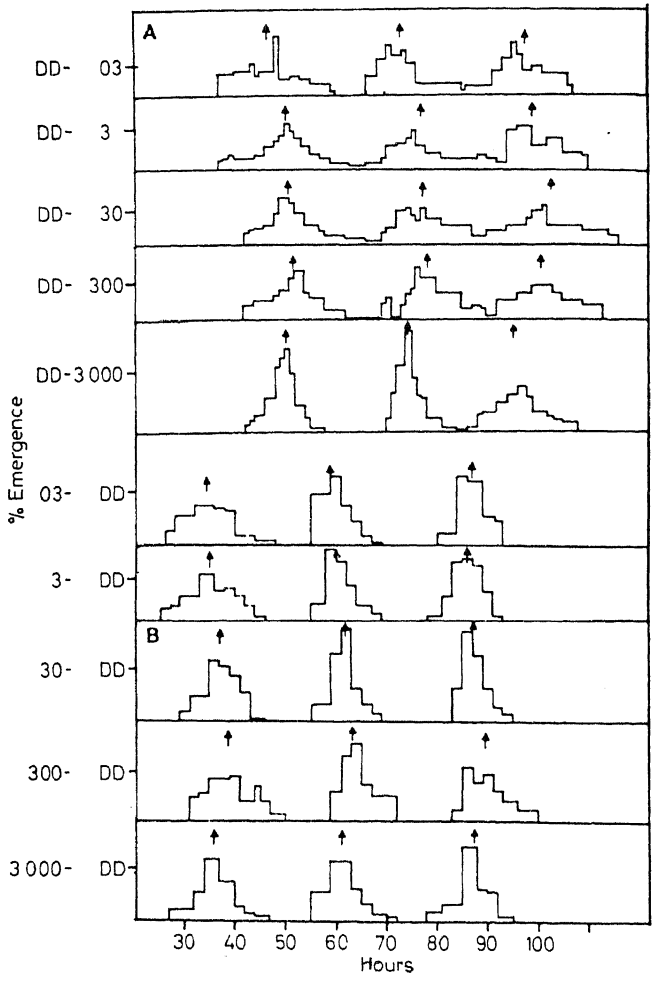


**Figure 1.** 'On' and 'off' rhythms in the eclosion of pharate adult *Drosophila pseudoobscura* flies. The several populations were raised either in DD (upper panel) and then transferred to LL of intensities in lux indicated or were raised in LL of varying intensities (lower panel) and then transferred to DD. Time of transfer was on day 20 of the cultures arbitrarily and designated hour 0. First eclosion peak not shown since synchronization was generally poor in all cases. The 'on' rhythms illustrated in the upper panel and the 'off' rhythms illustrated in the lower panel show time courses *ca* 180° apart relative to each other.

progress from the egg stage, through the instars to the pupal stage and eclosion. These conditions refer to *Drosophila pseudoobscura* PU301 captured by C S Pittendrigh and Th Dobhzhansky and used by Pittendrigh and his colleagues (Pittendrigh and Minis 1964; Pittendrigh and Bruce 1957; Pittendrigh 1954, 1960; Engelmann 1966; Chandrashekaran 1967a, b; Winfree 1970).

In potentially arrhythmic pupal populations of *Drosophila pseudoobscura* raised in LL or DD it is possible to induce rhythms by a DD/LL transfer ('on' rhythms) or LL/DD transfer ('off' rhythms). The so called 'on' or 'off' rhythms are illustrated in figure 1. The two rhythms are approximately 12 hr apart (180° displacement) relative to each other. This has prompted Honegger (1967) to talk of *two* oscillators, one set in motion at sunrise and the other set in motion by sunset, and interpret that LD entrained rhythms resulted from an interaction of these 'on' and 'off' rhythms. It turned out later (Winfree 1970) that the 'off' rhythm was for all practical purposes very much like LD 12:12 entrained rhythms, so much so that LD entrainment before allowing the circadian rhythm to freerun in DD (with a period $\tau$ of 24·3 hr) was dispensed with. It was sufficient to raise the pupae in LL and transfer the populations a day or two before the first flies eclosed. Another difference, was that the 'on' rhythm waned after 4–5 days. Light intensities of just 0·0001 lux could attenuate the rhythms. Interestingly it was found that the 'on' and 'off' rhythms could be 'simulated' with appropriately higher/lower or lower/higher intensity transfer of pupae. Figure 2 describes the time course of simulated 'on' and 'off' rhythms. On the right side the magnitude of intensity differences between $I_1$ (rearing light intensity) and $I_2$ (transfer light intensity) are shown. These rhythms seemingly contradict a dictum of Pittendrigh (1966) that the *Drosophila* circadian rhythm is held

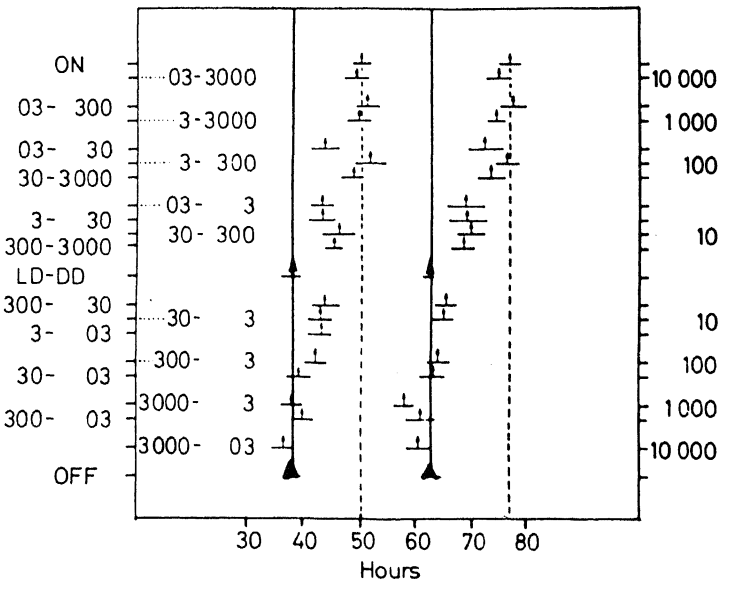


**Figure 2.** Illustrates the time course by means of indicating 'median' values of 'simulated on' and 'simulated off' rhythms. Appropriately high and low intensities whose actual value is given on the left and the factor of simulation is given on the right. All data above LD/DD line are simulated 'on' rhythms and those below simulated 'off' rhythms. Real 'on' and 'off' rhythm median values are given in the uppermost and lowermost lines respectively.

'fixed' at CT 12 phase by $L$ extending beyond the customary 12 hr and will be released into further motion only by the restoration of $D$. Thus peaks would follow on a pattern of $n \times \tau + 15$ hr in all pupal populations when light was maintained beyond 12 hr. On the other hand the simulated 'on' and 'off' peaks may describe the behaviour of a slave oscillator(s) or may be themselves 'transients'.

## 3. The *Drosophila* PRC

The circadian rhythm in the eclosion rhythm of *Drosophila* is sensitive to light perturbations of 0·5 msec administered against a background of darkness. The responses of the rhythm assume the form of displacement of the peaks along the time axis. The peaks either advance or delay relative to unperturbed controls. The direction of $\Delta\phi$ s and their magnitude are direct functions of the phase being perturbed. Figure 3 describes the standard PRC for the *Drosophila* rhythm first worked out in all its details by Pittendrigh and Minis (1964). CT 0 hr represents sunrise, CT 12 hr sunset, CT 0–12 hr subjective day and CT 12–24 hr subjective night. The rhythm is refractory to light stimuli given during the subjective day but responds with increasingly dilating delays during the first half of the night. At midnight the system switches the quality of its responses from massive delays to massive advances. The magnitude of advances

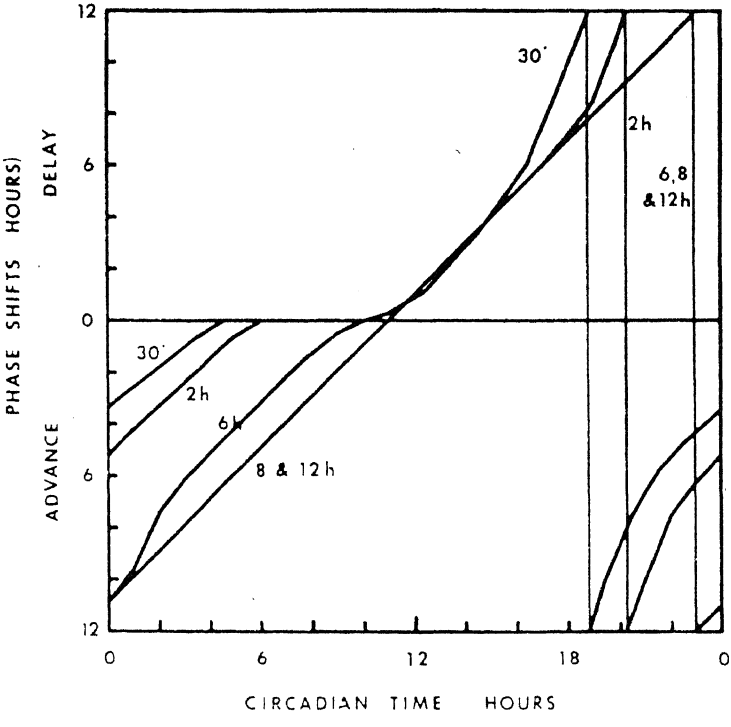


**Figure 3.** PRCs for 30 min, 2 hr, 6 hr, 8 hr and 12 hr light pulses of 1000 lux plotted against 'onset' of perturbation. The 30 min light pulse PRC is similar in all details to the standard 15'1000 lux PRC of Pittendrigh and Minis (1964). $\Delta\phi$ s are given in hr. Values above 0 line (control) indicate delay $\Delta\phi$ s and below the line advance $\Delta\phi$ s.

diminish in the course of the second half of the subjective night. This is one of the best worked out PRCs and was somewhat picturesquely described to represent the 'time course and waveform' of the basic oscillation gating eclosion.

## 4. Transients and the coupled-oscillator model

The $\Delta\phi$s that follow light perturbations do not express themselves in the same cycle or in the one after that. It takes the rhythm 3–4 cycles until the altered steady state with the stable $\Delta\phi$ is achieved. The 'creeping' fashion in which the $\Delta\phi$s express themselves has been the subject of much discussion and interpretation. One interpretation is given by a coupled-oscillator model proposed by Pittendrigh and Bruce (1957). This model postulated an *A* oscillator which was the pacemaker, light sensitive, temperature compensated and suffered *instantaneous* $\Delta\phi$s and a *B* oscillator which was the slave, light insensitive but sensitive to temperature. *A* influenced *B* but *B* had no feedback influence on *A*. The transients represent the efforts of a *B* oscillator trying to regain original phase angle ($\Psi$) coupling properties and phases with an instantaneously phase shifted *A* oscillator. Even though the model appears in retrospect fanciful and the transients can be explained even in terms of a single oscillator (Chandrashekaran 1980) at the time it was postulated it appeared to be an elegant way to explain the phenomenon of transients. Furthermore one of the main postulates of the model was stated without ambiguity and lent itself to direct experimentation. The main postulate was: the instantaneous resettability of the basic light sensitive *A* oscillator.

Soon after the coupled oscillator model was postulated Bünning and Zimmer (1962) gave a different interpretation to transients. They concluded from their studies on the petal movement rhythm of the crassulacean plant *Kalanchoe blossfeldiana* that the transient oscillation of petal movement following light signals reflects the behaviour of the underlying oscillator. They found the several phases of the transients to respond to a second light signal in a manner similar to the movement phases of the original rhythm.

This author designed critical experiments with *Drosophila* to critically test the two views of transients. In planning the experiments on graph paper *i.e.* gedanken experimental phase, the classical phase response curve (Pittendrigh and Minis 1964) was assumed to really characterize 'the waveform and time course of the basic oscillation'. The rationale was to administer light pulse 1 at a given phase and then follow up with light pulse 2 soon after to check if $\Delta\phi$ had already occurred. $\Delta\phi$s would be large and to scale of the PRC on the assumptions made by the coupled oscillator model, but small and nearly undetectable according to the assumption of Bünning and Zimmer (1962).

Figure 4 illustrates the results of an experiment where pulse 1 (15′1000 lux) was given at 15·5 CT and pulse 2 at 22 CT. Both pulses given individually to two different populations would have induced 5 hr delay $\Delta\phi$ and 5 hr advance $\Delta\phi$ respectively. According to the Bünning–Zimmer interpretation pulses 1 and 2 should have mutually counter-acted each other's influence and no $\Delta\phi$s should have shown up. The results indicate a larger than PRC-postulated delay $\Delta\phi$ which indicates a *summative* effect. This would happen if light pulse 1 had indeed shifted the basic oscillation *instantaneously* by an amount postulated by the PRC.

Figure 5 illustrates results of an experiment where it was assumed that light pulse 1 indeed shifts phase instantaneously and light pulse 2 was given to effect then an advance

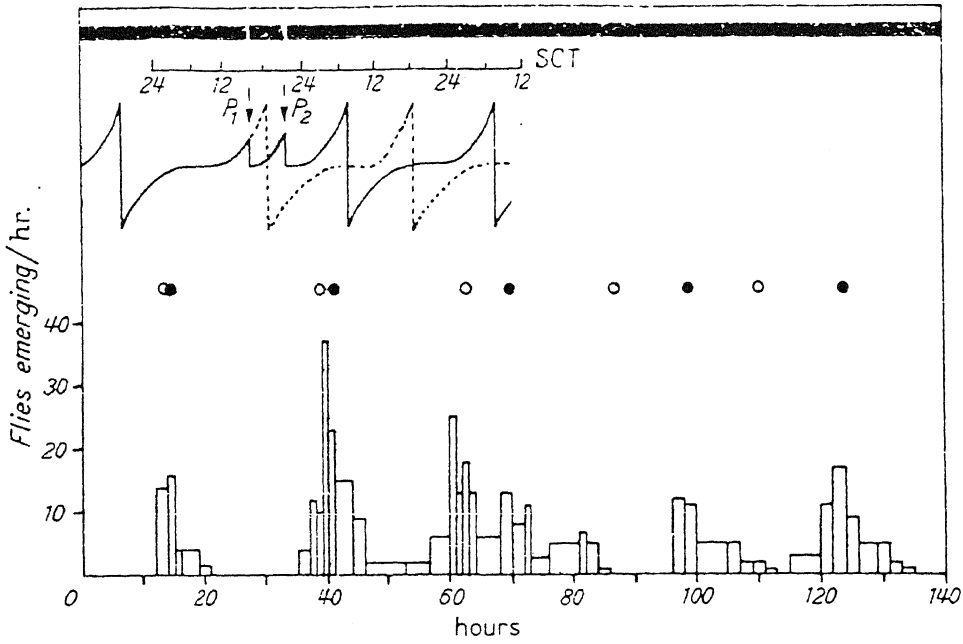


**Figure 4.** The effect of two light pulses ($P_1$ and $P_2$) of 15 min duration each and 300 lux intensity. $P_1$ was given at 15·5 CT and $P_2$ at 22 CT. The curve above the raw data of eclosion depicts schematically the instantaneous $\Delta\phi$ s effected by pulses. The dotted line indicates the time course of unperturbed controls. Open circles are calculated medians of peaks and solid circles indicate positions of medians of experimental populations.

$\Delta\phi$ of 5 hr *i.e.* at phase 22 CT + 5 hr = 3 CT. Now pulse 2 was seen to counteract the effects of pulse 1 with the result eclosion peaks of experimental and control pupal populations show the same time course. Figure 6 contains data of a two pulse experiment whose rationale was the same as in the first two pulse experiment except that the second pulse was administered in the second cycle. The results are unequivocal. It was concluded that the assumption of the coupled oscillator model that the basic oscillator gating eclosion in *Drosophila* is phase shifted instantaneously by light pulses holds at least for the *Drosophila* clock. But it must be mentioned in this context that no concrete proof has been forthcoming for the so-called *B*-oscillator (Chandrashekaran 1980). It is now generally assumed that most organisms, especially animals, may possess several rhythms generally coupled to each other in a hierarchical fashion (Moore-Ede *et al* 1982).

## 5. Dawn and dusk effects

In the course of experiments with *Drosophila* certain data tended to indicate that the first half of the subjective night of the system may shown qualitatively different responses of light on and light off transitions than the second half. In other words the first half seemed to respond only to the light 'off' *component* of a light pulse, the second half seemed to respond only to the light 'on' *component* of a light pulse. This is the gist of the

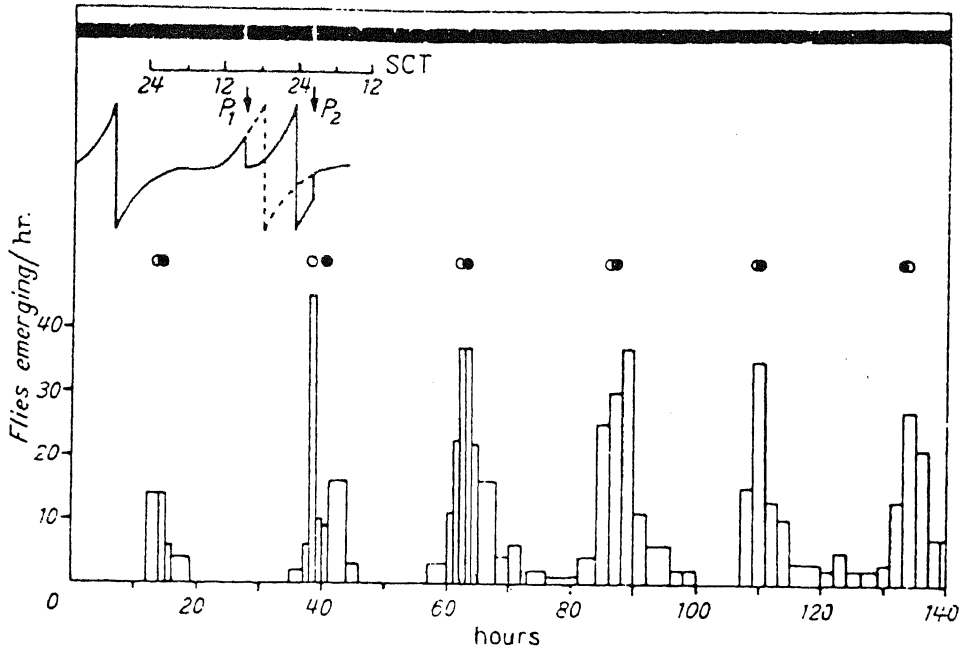


**Figure 5.** The effect on the rhythm of two pulses $P_1$ and $P_2$ of 15 min duration and 10000 lux intensity. $P_1$ at 15·5 CT, $P_2$ at 02·5 CT. Other details as in figure 4.

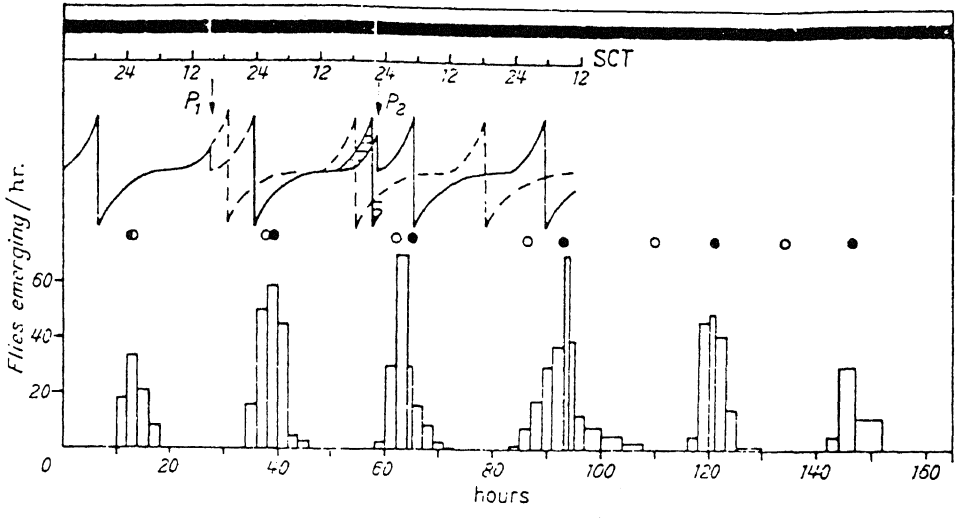


**Figure 6.** The effect of two light pulses of 15 min and 3000 lux. $P_1$ was given at 15·5 CT in the first cycle and $P_2$ at 22 CT of the second cycles. In practice $P_1$ falls 27·5 hr after LD/DD and $P_2$ 58 hr after LD/DD. Other details as in figure 4.

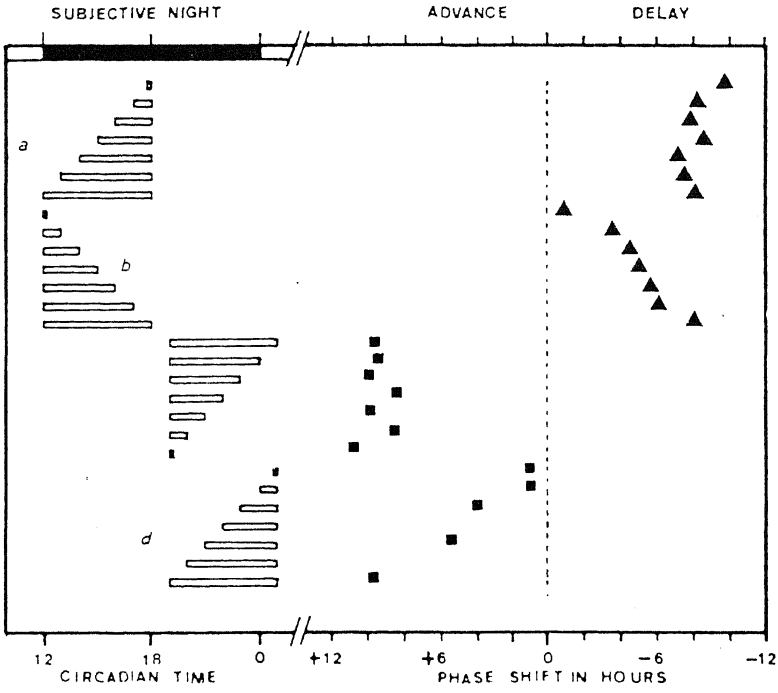


**Figure 7.** Shifting the phase of the *Drosophila pseudoobscura* eclosion rhythm with light pulses of 1000 lux and varying duration given in the first and second halves of the subjective night. The light pulses are represented by the unfilled bars and are arranged in 4 batches on the 'circadian time scale' of Pittendrigh and Minis (1964). In batch 'a' the different populations experienced the 'on' transition of pulses at different hours but experienced the 'off' transition at the same phase (18 CT). In batch 'b' the populations experienced the 'on' transition at the same circadian hour (12 CT) but the 'off' transition occurred for each population at a different hour. The pulses of batches 'a' and 'b' scan the first half of the subjective night. Batches 'c' and 'd' scan the hours of the second half of the subjective night. In batch 'c' the 'on' transitions of all the pulses were in alignment (at 19 CT) with the 'off' transition occurring at a different hour for each population. In batch 'd' on the other hand the 'on' transitions were systematically staggered and the 'off' transitions of pulses aligned. The filled triangles represent averaged median values of eclosion peaks of experimental populations 4–5 days after light treatment, which responded with delay phase shifts. The filled squares represent averaged median values of peaks 4–5 days after light treatment showing advancing phase shifts. Apparently the 'off' transitions of light pulses determine direction and degree of phase shifts during the first half of the night and the 'on' transitions determine phase shifts during the second half of the night.

dawn/dusk effect model postulated by Chandrashekaran (1967b). The light 'off' information simulates a dusk or sunset and the light 'on' information acts like dawn or sunrise. Figure 7 illustrates results obtained in a later series of experiments (Chandrashekaran *et al* 1973). The design was to compare the results of $\Delta\phi$ s evoked by light pulses given during the first half of the night such that they were of varying durations started at differing phases but went off at the same (18 CT) phase. If 'off' is indeed the discrete component recognized by the system then all $\Delta\phi$ s must be of equal magnitude regardless of the duration of pulses evoking them. This is the case. The opposite design was used for the second half of the subjective night. Pulses of varying duration started at the same phase (19 CT) but ended at varying phases. Since 'on' is the

discrete component implicated all $\Delta\phi$ s evoked by the pulses must be of comparable magnitude. This again is the case. Reciprocal experiments were also performed and the results further fortified these findings.

## 6. How to stop the *Drosophila* clock

Pavlidis (1967) predicted that the *Drosophila* clock must have a point of singularity on theoretical considerations. Drawn in the form of a phase plane limit cycle diagram the singular status will be achieved by a pulse of critical strength $S^*$ given at a critical time $T^*$. Winfree (1970) discovered the values for these two parameters. $T^*$ was 6·8 hr after an LL/DD transfer (18·8 CT) and $S^*$ was 100 erg/cm$^2$/sec light of 460 nm given over 50 sec. If this treatment is indeed given to pupal populations, eclosion becomes arrhythmic. Total arrhythmicity according to the formula: number of flies outside the gate ÷ number of flies inside the 'gate' × 100 would be 200.

## 7. The *Drosophila* clock in contemporary research

Konopka and Benzer (1971) isolated a clock mutant for *Drosophila*. The clock could even be surgically transplanted (Handler and Konopka 1979). Engelmann and Mack (1978) showed that the PRC for the locomotor activity rhythm of *Drosophila pseudoobscura* looked very different from the PRC for the eclosion process. This is indicative of two different oscillators controlling the different rhythms. Work presently in progress in the laboratory of Engelmann in Tübingen (personal communication) indicates that the oscillatory pacemaker governing the locomotory activity in *Drosophila* is *not* situated in the optic lobes. The optic lobes are provenly the sites of the pacemakers in circadian rhythms of locomotion in the cockroach and crickets (Saunders 1976). It is to be hoped that the real nature of the elusive circadian clock in *Drosophila* might soon be unravelled using the modern techniques of gene cloning and recombinant DNA.

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# Hormones in insect behaviour

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**Abstract.** Hormones play an important role in insect behaviour. These hormones are mainly the neurohormones of the brain and of the corpus cardiacum, the juvenile hormone of the corpus allatum and the ecdysone of the prothoracic glands. These produce either releaser effects or modifier effects. Hormonal modulation of neurophysiological activity controlling various aspects of behaviour, hormonal influence of reproductive behaviour in the male and the female insects, their role in migration, as well as hormonal influence of caste determination and behaviour of social insects, have been discussed.

**Keywords.** Insect hormones; insect behaviour; insect reproduction; social insects; migration; neurophysiological activity.

## 1. Introduction

Insects occupy a unique position in the animal kingdom in that hormones are actively involved in the control of behaviour in this group more than in any other group of animals, either invertebrates or vertebrates. This is apparently due, on the one hand, to the rich behavioural repertoire shown by insects and on the other to the accessibility of blood containing the hormones, to the neuronal elements of the nervous system. Ever since the discovery by Bounhiol (1938) that extirpation of the corpus allatum from the penultimate larvae of the silk worm *Bombyx mori* led to precocious, miniature cocoon formation, and especially during the last twenty years there has been a surge of evidence implicating hormonal control of various behavioural aspects in a variety of insect species, like courtship, mating, oviposition, circadian rhythm, autogeny and anautogeny, diapause, caste determination in social insects, feeding behaviour, migration etc. It is in fact preposterous to attempt to cover all aspects of hormonal control of insect behaviour in this brief paper and hence it is intended here to touch upon only a few of the points which are considered rather important.

## 2. The insect endocrine glands

The insect endocrine system itself consists chiefly of the neurosecretory cells, the corpora cardiaca, corpora allata and the prothoracic glands, of which the latter degenerate during adult metamorphosis and hence are present only in the larval and the pupal instars. In addition, there is considerable mass of evidence implicating ovaries in ecdysone production in many insects though the exact role played by the ovarian ecdysone is far from clear. There are also perivisceral neurohaemal organs associated with the various ventral ganglia (Nayar 1973; Highnam and Hill 1979) whose exact role is known only very little. The neurosecretory cells are scattered in the cerebral ganglia,

but mainly in the pars intercerebralis, distinguishable usually into a median group of "Gomori positive" cells and the lateral group of phloxinophil cells. There may be other groups also. The suboesophageal ganglion and the other ventral ganglia also contain occasional neurosecretory cells. The corpus cardiacum serves as a storage-cum-release centre for the secretory material elaborated in the brain neurosecretory cells; it also elaborates its own hormone. As the brain as well as the cardiacum contain heterogeneous hormones from different sources, removal of these organs resulted in removal of more than one hormonal principle and it was difficult to pinpoint the role of individual hormones until recently when many of these hormones have been isolated and characterized.

## 3. General effects of hormones on behaviour

The effects of hormones on behaviour have been divided into releaser effects and modifier effects (Truman and Riddiford 1974). Accordingly, a releaser effect of the hormone on behaviour is relatively rapid, and is directly triggered by the hormone. Modifier effect is, on the other hand, slower to appear and results in a change in responsiveness of the nervous system. Hormones may have either only releaser effect, or primer effect or both.

An excellent example of the releaser effect of a hormone is the motor effect to phallic nerve-stimulating hormone, demonstrated by Milburn *et al* (1960) and Milburn and Roeder (1962). It is known that in *Periplaneta americana*, the suboesophageal centres normally inhibit motor activities involved in copulatory movements. Decapitation however removes the sub-oesophageal centre from this inhibition resulting in copulatory movements. When extracts of the corpora cardiaca are injected into male cockroach, it caused rhythmic movements of the abdomen as characteristic during copulation, and when the extract is applied to the nerve cord, evoked rhythmic activity in the phallic nerve. Apparently, the phallic nerve-stimulating hormone acts on the suboesophageal ganglion to remove the inhibition of the motor centres. Though these hormones thus perform the same functions as neural pathways from the brain centres to the lower motor centres, these have the advantage of being sustained in its action, unlike nervous action.

A good illustration for modifier effect of hormone is the effect of juvenile hormone on sexual maturation behaviour. For example, in the female grasshopper *Gomphocerus rufus* (Loher and Huber 1966), juvenile hormone is necessary for development and maintenance of receptivity. The immature female responds to the courting male by primary defence reaction, involving kicking the male and other escape reactions. Corpus allatum induces maturation resulting in copulatory readiness, the female now stridulating and moving towards him, resulting in mounting and copulation. Allatectomy of newly emerged adult female or of sexually mature female results in maintenance in the animal, or its reversion, to defensive behaviour respectively. Primer pheromones bring about secretion of a modifier hormone resulting in a change in behaviour as in the case of maturation pheromone facilitating maturation in male *Schistocerca* by JH release, or long term effects of changing environmental stimuli such as migratory behaviour in *Oncopeltus* under the stimuli of decreasing day length due to juvenile hormone secretion or again, co-ordinating behaviour with developmental or physiological changes of the animal.

## 4. Hormonal modulation of neurophysiological activity

Neurophysiological basis of hormone action has started receiving attention in recent years. As reported earlier, the effect of the extract of the corpus cardiacum on some of the inhibitory centres in the nervous system of the cockroach which normally repress motor programme resulting in copulation, have been demonstrated neurophysiologically.

Activation of the adult behaviour in wild silk moths is another interesting example. Development and differentiation of the adult nervous system is completed in the wild silk moths (*Antheraea pernyi*, *A. polyphemus* and in *Hyalophora cecropia*) in the pharate adult even though the pharate adult does not show adult type movement even if the pupal skin is peeled off, before the normal time of eclosion. That its nervous system is comparable to that of the adult is shown by the fact that the peeled pharate male exposed to female sex pheromone under dim illumination shows normal electroantennogram, although it does not show any behavioural response characteristic of the normal adult male, indicating the inhibition to be likely of central nervous nature. This behavioural deficiency of the peeled pupa extends to other aspects of behaviour like lack of tonus, even simple righting reflexes etc. It shows only occasional spasmodic movements of the legs, abdominal twitches and pupal-like rotary movements of the abdomen. However, at the time of the day when eclosion normally occurs, the peeled moth shows pre-eclosion behaviour consisting of a 1·25 hr long programme of abdominal movements, followed by eclosion and spreading of the wings. This switch over to adult behaviour is hormonally controlled. An eclosion hormone is found in the brain and corpus cardiacum of the pharate moth; when homogenates containing this extract is injected into pharate animals, normal eclosion and precocious adult behaviour was observed. At normal eclosion, the eclosion hormone appears in the blood. Its function thus turns on the adult behaviour. The eclosion hormones turns off certain other parts of the nervous system, for example those motor centres which control the dense bands of intersegmental muscles of the abdomen used in eclosion. The motor neurones which supply these muscles become silent shortly after eclosion, thus leading the muscles to degeneration. When eclosion hormone is injected into isolated abdomen of pharate moths, the hormone triggers muscle degeneration, thus turning off the motor neurones to the muscles.

In *Hyalophora cecropia*, the pre-eclosion abdominal movements of the pharate adult consists of three phases with reference to the type and relative frequency of movements (i) a hyperactive period involving mainly abdominal rotations extending to 30 min; (ii) a rather quiescent period of about 30 min; and (iii) a period of hyperactivity involving strong peristaltic waves moving anteriorly along the abdomen. These series of movements begin 10–30 minutes after injection of extracts containing eclosion hormone into pharate moth or into abdomens isolated from pharate moth. Truman and Sokolove (1972) showed that the timing and patterning of these movements are built into the circuitry of the abdominal gangila, by recording the motor output to the inter segmental muscles from the deafferented abdominal nerve cord. This preparation normally showed very low level of firing. On the other hand, addition of eclosion hormone evoked strong and active motor output, beginning 20–40 minutes after addition of hormone. This was followed by a decline and then by new frequent bursting of volleys. The timing and the bursting pattern was such as would result in the pre-eclosion behaviour of the pharate adults, were the efferent fibres connected to the

muscle bundles. The above motor programmes are hence encoded in the abdominal ganglia, and the eclosion hormone activates the programme.

The flashing of the firefly beetle *Luciola* is an example to illustrate a case of peripheral hormone action. Light-induced inhibition of flashing is due to a central mechanism acting on the pacemaker, and a peripheral mechanism acting on the lantern itself. That a hormone produced by the testis is involved in this peripheral inhibition has been shown by connecting the haemocoel of two fire fly beetles and covering the eyes of one partner by opaque paint. When the other partner is illuminated, flashing by the "opaque" partner was also inhibited; when the testis of the illuminated partner is removed, the inhibition of flashing was abolished (Brunelli *et al* 1968). When the light organ was electrically stimulated directly, the intensity of the flashing elicited was not abolished either by decapitation or by denervation of the light organ. On the other hand, if the eyes of a denervated fly were illuminated, the intensity of the electrically induced flashing was inhibited, which however was abolished if testis was removed (Bagnoli *et al* 1970). Transection of the nerve cord anterior to the ganglion which innervates the testis, destroyed the inhibitory response to illumination, whereas electrical stimulation of the nerve to testis led to inhibition of electrically induced lantern flashing. The flash inhibiting substance stored in and released from the testis is noradrenalin (Bagnoli *et al* 1972). The noradrenalin appears to act peripherally, as application of this substance inhibits flashing, but does not affect the size or frequency of efferent volleys coming to the lantern.

## 5. Hormonal influence of reproductive behaviour

Hormonal effects on reproductive behaviour are many and varied; there are vast species difference also. On the whole, it may be said that in the male, influence of hormones on reproductive behaviour is either non-existant or unknown in many species; where they exist, these influences are comparatively simple. Many adults like silk moths display full sexual behaviour immediately after emergence and remains so for the rest of their adult life, there being no opportunity for hormones to play any part in the adult life. However, in some males like grasshoppers and locusts the corpora allata are required for the maturation of adult sexual behaviour. Perhaps endocrine control of male sexual behaviour has been best studied in grasshoppers and locusts (Pener 1974). In this group, information appears to be reasonably thorough in *Locusta migratoria migratorioides*, but rather fragmentary in the other species. Pener concludes that the C-cells of the pars intercerebralis completely control the sexual behaviour, their effect being direct and not mediated through the corpus allatum. The C-cells also activate the corpus allatum completely controlling yellow colour, which exert a secondary effect on mating behaviour; the corpus allatum influences the intensity of sexual behaviour, but does not exercise a complete control since their removal does not completely inhibit mating behaviour.

The corpora allata may be controlled by nervous means by the brain; or the allata may be controlled by the pars intercerebralis neurosecretory cells of the brain. In the cockroach, the neurosecretory hormone as reported earlier, elicits abdominal movements acting on the phallic nerves.

In the female, generally speaking the young virgin becomes receptive to male under the influence of juvenile hormone. This has a direct effect on the behaviour of the female toward courting male, as opposed to the indirect effect of JH on sex pheromone

production and thereby attraction of the male. The control may again be nervous or neurosecretory, as in the male. In the wild silk moths, the sexually receptive female, in response to proper environmental stimuli, assumes, calling posture involving protrusion of the last two abdominal segments which expose the pheromone glands permitting pheromone release. This calling behaviour is under the control of the release of corpus cardiacum's calling hormone under the neural influence of the brain. Injection of blood from calling female into virgin in the absence of proper stimuli, induces in them, calling behaviour.

Apparently, under the neurosecretory hormones released by courtship, copulatory behaviour ensues. Generally, mating is followed by termination of male receptivity and the females thus become refractory which may however be in some temporary, whereas in others, permanent. This may be due to a variety of stimuli, like mechanical stimulus, presence of spermatophore in the female bursa copulatrix or due to the sperms themselves, which might secrete a "bursa factor" acting on the central nervous system. As in the mosquito, accessory gland substances of the male, like matrone, may also be involved. These influences may be coupled with neural influences. Refractoriness may also be due to withdrawal of juvenile hormone or due to factors from maturing ovaries. The spermatheca or the bursa of the female may release hormonal factors which may exert their effect through the central nervous or neuroendocrine system of the female.

There is considerable evidence now that pheromone production in insects is under hormonal control in many species. Whereas in some insects the corpus allatum stimulates pheromone secretion, in some the corpus cardiacum is involved in this activity. However, an inhibitory role for juvenile hormone in pheromone production is now emerging from some recent studies. For example, it has been known for a long time that large quantity of juvenile hormone accumulated in the male saturniid moth abdomen. This store of juvenile hormone is now known to be transferred from the accessory gland of the male to the bursa copulatrix of the female during mating (Shirk *et al* 1980). Webster and Carde (1984) propose that in *Platynota stultana* and probably in other similar moths also, this exogenous juvenile hormone transferred in the seminal fluid to the female might be involved in the switch from virgin to mated behaviour in the female. Mating in this species resulted in termination of calling, and gradual reduction of pheromone in the glands comparable to decapitation of virgin females. Mating apparently terminated neural and hormonal stimuli required for pheromone production; exogenous juvenile hormone treatment in virgin females also gave similar results.

Closely connected with pheromone production is pheromone perception; Schafer (1977) and colleagues have demonstrated that the male adults of *Periplaneta* have nearly twice as many olfactory sensillae as female adults; this sexual dimorphism of adult antennal sense organs appeared only during the adult stage. Treatment of terminal instar with JH mimics resulted in supernumerary larvae lacking antennal sexual dimorphism. Inhibitory action of JH prevented the appearance of antennal sexual dimorphism during normal larval development. Adult males with larval antennae produced by bilateral treatment with exogenous juvenile hormone mimic do not respond to the pheromone although they are completely adult in other respects. Electrophysiological studies involving single unit and electroantennogram recording confirm that a portion of the receptors added at the adult ecdysis are sex-attractant receptors, which are not present in the larval or in the adult female antennae in large numbers, and that topical application of JH mimics to male antennae during terminal larval instar inhibits their development.

In the mated female, release of the neurosecretory material appears to take place, resulting in not only myotropic activity leading to oviposition, but to oviposition behaviour as well (Nayar 1958). When ovarian extract or blood from the female during the oviposition is injected into partially gravid mating females, depletion of neurosecretory cells occurred, followed by oviposition. If median neurosecretory cells are implanted into young females lacking mature oocytes in the ovaries, quivering movements of the genital plates, simulating oviposition behaviour, occurred. Further work on ovulation/oviposition has been reported recently by Davey (1984) who found that in *Rhodnius*, ovulation is stimulated by a peptide neurohormone originating from ten large identifiable neurosecretory cells of pars inter-cerebralis. This neurohormone is released in response to feeding and the other in response to mating. The latter has been investigated in detail in this animal. On mating, female *Rhodnius* releases a spermathecal factor, which is only one of the two factors involved in the release of myotropic hormone from the neurosecretory cells, as mating precedes ovulation by some days. A second stimulus, which is provided by the ecdysteroids from the ovary, also appears to be involved. It has been found that injection of ecdysteroids into ovariectomized female results in an increase of myotropic activity of the haemolymph of mated females, but not in virgins. Bursts of action potentials recorded from the corpus cardiacum during ovulation have been associated with the ten pars intercerebralis neurosecretory cells, the source of myotropin. Isolated brain retrocerebral complex from mated female have shown the characteristic action potentials *in vitro* under the influence of ecdysterone. Ecdysterone action on the neurosecretory cells appear to be mediated through aminergic neurons of the pars intercerebralis.

## 6. Hormone in migration

The case of locusts is one of those which has been studied fairly well especially in *Locusta migratoria* and *Schistocerca gregaria.* Young ones (hoppers) reared in isolation, show moderate level of activity; the adults tend to be solitary and do not perform long flights. On the other hand, when crowded, they become highly active, show marching behaviour and oriented locomotion; their adults become gregarious and undertake migration. This difference is subsequently shown to be due to the better developed prothoracic glands in solitary forms. Experiments involving transplantation of prothoracic glands or injection of their extracts have shown that the gregarious hoppers or adults could be converted to solitary individuals (Carlisle and Ellis 1963). Haskell and Moore (1963) have substantiated these findings by demonstrating that ecdysone reversibly reduced the spontaneous motor output from the metathoracic ganglion of adult locusts. On the other hand, the corpus allatum has been shown to stimulate spontaneous locomotor activity as well as sexual activity by a direct action on the nervous system (Odhiambo 1966).

According to Johnson (1969), migration was triggered when ecdysone was absent and JH titre was low. Subsequent rise in JH level brought about cessation of migratory behaviour and onset of oogenesis. Rankin (1974) analysed in detail the causative factors and hormonal control of flight in the milkweed bug *Oncopeltus fasciatus*, using a series of elegant experiments. In this insect, flight is post-teneral and pre-reproductive. It undergoes an adult reproductive diapause in response to short photoperiods making available longer time for flights of greater duration. Long photoperiods on the other

hand afford favourable breeding conditions, and so it undertakes reproductive activity. A skillful manipulation of corpus allatum and ovaries of the animal, and by starvation as well as topical application of JH or by implantation of corpora allata and by combination of some of these experiments, indicated that the corpus allatum can stimulate flight behaviour and the effect can be duplicated by application of juvenile hormone analogue. The flight system may respond to lower titer of juvenile hormone than does the reproductive system. Rankin and Riddiford (1977) subsequently confirmed that JH was the primary hormone responsible for stimulation and co-ordination of migration and reproduction in *Oncopeltus* by JH bioassay and exogenous application of JH to experimental animals. It is to be noted that in *Dysdercus* sp where starvation stimulates flight, and feeding brings about ovarian development, flight muscle degeneration and vitellogenesis are induced by corpus allatum which is activated by feeding and mating (Edwards 1970; Nair and Prabhu 1984a,b) where the situation is opposite to that of *Oncopeltus fasciatus*.

The migratory behaviour of cockchafer beetle *Melolontha melolontha* is characterized by reversal of the sense of direction of flight in the female (but not in the male) which is closely connected to the maturation of oocytes. Stengel (1974) and his colleagues have shown that the migratory behaviour of this beetle is a good example of neurohormonal regulation of behaviour which depends upon the existence of two types of neurosecretory activity separated in time. During the life above ground, the female adult undergoes two or three ovarian cycles each of which is characterized by oriented migrations which leads her towards feeding areas consisting of the edge of forest, a thicket or an isolated tree, constituting "pre-feeding flight" during which the ovaries are immature; and then back to the egg-laying sites after feeding and mating in the reverse direction, to the fields whence she came, constituting the "oviposition flight" with fully mature ovaries, to lay eggs.

The male, however, upon leaving the soil makes an oriented flight to the forest, but is not capable of reversal in the sense of direction of flight, but moves only in the adjacent feeding area where it feeds and mates. During the reversal flight the corpus allatum releases its hormone, which releases the reversal mechanism for the flight sense and at the same time, blocks oogenesis. The corpus allatum of the pre-oviposition female can release the reversal mechanism even in the male which does not normally reverse the flight direction. The corpus allatum of the pre-oviposition female containing oostatic hormone, if implanted into prefeeding female whose corpus allatum is active in secreting gonadotrophic hormones, is capable of suppressing its activity and blocking oogenesis. So it appears, two hormones secreted by corpus allatum are involved here. Stengel and his colleagues (see Stengel 1974) have also shown that neurosecretory cells of pars intercerebralis secreted the hormone and they are released by the corpora allata.

## 7. Hormonal control of social behaviour

According to Lüscher (1975), in termites and in the honey bees, the societies use pheromones which influence juvenile hormone production, which in turn influence caste development. However, considerable differences exist in the mode of action of these principles. In termites and honeybees, the development of reproductives is inhibited by the pheromone of the queen. However, the queen substance of the honey bee inhibits the corpora allata whereas the pheromone of the termite reproductives

stimulates the allatum. Hence the stimulation of replacement reproductives in termites, by the honeybee queen substance. It has also to be noted that the termite pheromone acts on the developing larva whereas the queen substance of the bee acts on the adult worker bees. In bees the pheromone produced by the workers acts upon the larvae, whereas in termites the queen pheromone acts on them. In both cases the corpora allata are stimulated to produce more juvenile hormone, causing queen development in bees and inhibiting development of reproductives in termites.

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# Hormonal rhythm and behavioural trends in insects

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**Abstract.** Circadian rhythmicity in the timing of secretion and release of many of the neurohormones appears to be a common phenomenon in insects. Involvement of hormonal components in the locomotor activity rhythm in cockroaches, crickets etc. has not yet been proved unequivocally eventhough some of the findings along these lines support this. Many of the physiological events in insects occur only once in each individual's life-time-gated events. Release of eclosion hormone in insects is determined both by a circadian clock and by the developmental competence of the insect. Periodic release of PTTH which influence the moulting process in larvae has been established to be gated. Induction of prodromal signs of pupation as a result of gated release of PTTH in some insects have been confirmed. Intrinsic neurosecretory cells of CC release a hormone (calling hormone) in a rhythmic fashion which affect the pheromone release and subsequent initiation of calling behaviour in some of the lepidopteran virgin females. Production of proctodone by the epithelial cells of hindgut also follows a rhythm bringing about diapause in some of the insects.

**Keywords.** Rhythm; endocrines; eclosion; hatching; moulting, bursicon; calling hormone; diapause.

## 1. Introduction

As in many other animals, insects are also known to possess internal clocks which can be synchronised and entrained by daily environmental periodicities. Since many of these clocks follow a periodicity of about 24 hr, these are designated as 'circadian clocks' and diverse types of behaviour in insects have been implicated to follow this cyclic pattern. The performance of behaviour is often directly coupled to the output of the clock with little or no influence from other stimuli. In many instances, the coupling between the driving circadian clock and the behaviour appears to be hormonal. Daily, seasonal and even annual phenomena are apparently linked to biological clock functions. Daily and seasonal responses are the results of the effects of photoperiodic stimulation on the clocks of the neural and endocrine cells that control the behavioural, physiological or developmental processes (Beck 1980).

Circadian rhythms have a major influence on insectan endocrine activity. Daily fluctuations of the titers of circulating hormones may reflect daily alterations in the activity of endocrine organs themselves or they may reflect daily alterations in specific or general metabolic pathways that serve to clear hormonal activity from the blood (Truman 1978). Monitoring the daily cycles of activity in the endocrine system of insects have attracted attention because of the probable importance of this system in the control of overt rhythms of physiology and behaviour.

## 2. Endocrine rhythms

### 2.1 *Rhythms in the brain neurosecretory cells*

The involvement of circadian rhythmicity in the determination of timing of secretion of insect neurohormones now appears to be a general phenomenon and a precise

knowledge of the nature of the clock that controls hormone release is a prerequisite for a thorough understanding of any developmental problems related to hormone action. This circadian clock of timing of release of insect neurohormones was demonstrated unequivocally for the eclosion hormone of silkmoths, a hormone which triggers their adult eclosion (Truman and Riddiford 1974; Truman 1971a, b).

The first report of a circadian cyclic pattern of activity in the neurosecretory cells was that by Klug (1958) in the brain of a beetle, *Carabus nemoralis*. Rensing (1964, 1966) used a sort of microspectrophotometer to compare the absorption of neurosecretory material in the region around the nucleus with that in the axon 'hump' of brain neurosecretory (NS) cells of *Drosophila melanogaster*. A maximum accumulation was noticed near the nucleus around 'dawn' and 'dusk' i.e. some 3 hr after their peak nuclear size. Larvae also showed similar but less marked changes.

Dutkowski *et al* (1971) made some ultra-structural investigations to demonstrate the circadian cycle of neurosecretory activity. Brain NS cells of *Acheta domesticus* recovered after sacrificing them 30 min after 'dawn' and 30 min after 'dusk' (i.e. at the time of minimum and maximum locomotor activity) showed marked differences between the two groups. The cells of the inactive animals contained extensive ER and secretory vesicles in the golgi region and in the axon only and nuclei with smooth membranes. On the other hand cells of the active ones housed only fragmented ER, apparently quiescent golgi, secretory vesicles in the perikariyon, but not in the axon and nuclei with undulating membranes. It was therefore concluded that the brain cells of the inactive animals were synthesising and releasing neurosecretion whereas the cells of the active animals had ceased (temporarily) to synthesise actively or to release the secretion and thus accumulated secretion in the perikaryon. The potential value of such EM studies is revealed from these investigations in elucidating the circadian secretory cycles.

We have noticed a distinct circadian rhythmic pattern of secretory activity in the PINSC (pars intercerebralis neurosecretory cells) *A* cells of the late instar larvae (4th and 5th) of castor semilooper, *Achoea janata*. During the early hours of the day, the activity was minimum and around mid-day at peak level and with a gradual drop towards evening and night. Topical application of an anti-allatotropin, Precocene-II, completely upsets this rhythm of neurosecretory activity and the level of secretory activity as well (Mohanakumar and Muraleedharan 1985).

Autoradiographic techniques have been used as another tool in the elucidation of cyclical metabolic activities of endocrine cells. Cymborowski and Dutkowski (1969) used this technique in *Acheta domesticus* to relate neurosecretory cell function to the control of locomotor activity. They showed that there is a sharp diel rhythm in RNA synthesis (as indicated by $^3H$ incorporation) in the median NS cells of the brain and the NS cells of the suboesophageal ganglion of crickets.

Fowler and Goodnight (1966) succeeded in cluturing isolated brains from an opilionid, *Leiobunum longipes* for 80 days in L:D at the end of which there was a distinct residual rhythm of 5-hydroxy tryptamine accumulation. Rensing (1969) also studied the 24 hr rhythmic pattern of secretory activity in the salivary glands by measuring the size of nuclei of the gland cells of *Drosophila melanogaster* which were cultured.

### 2.2 *Rhythms in the NS cells of ventral nerve cord*

In the ventral nerve cord of almost all insects studied, four major categories of NS cells

are to be noticed. However, the pathways and release sites of these NS cells-products have not yet been completely elucidated (Raabe 1983). Naturally, very few attempts have been made to study the activity rhythms of these cell types as well. A reasonably clear diel change in the nuclear size of NS cells of the SOG (suboesophageal ganglion) was demonstrated in *Drosophila melanogaster* (Rensing 1964, 1966; Rensing *et al* 1965). In the cockroach *Periplaneta americana* cyclic changes in the SOG NS cells were noticed (Cymborowski and Flisinka–Bojanowska 1970). A similar rhythm was also noticed in the 'C' cells of SOG of the stick insect, *Clitumnus extradentatus*. Harker (1960a, b, c) traced the rhythm inducing factor of the SOG as two pairs of NS cells located on the lateral aspects of SOG, one pair on each side. During the active period 'A' type neurosecretory cells in the ventral ganglia of *Leucophaea maderae* contained greater quantities of NS granules (De Besse 1965). In *Acheta domesticus* a bimodal rhythm in the SOG NS cells was noticed with a maximum in the midphotophase and midscotophase (Cymborowski and Dutkowski 1969). Protein synthesis in the NS cells were also found to be rhythmic. In the light of all the above findings they suggest that in response to photoperiod, the brain begins RNA synthesis at the onset of light followed by protein synthesis and subsequent elaboration of neurohormone which is being translocated to the SOG where it stimulates RNA synthesis and neurosecretion.

### 2.3 *Rhythms in corpus allatum and prothoracic glands*

A daily rhythm of nuclear and nucleolar size in the cells of corpus allatum (CA) and prothoracic glands (PTG) was noticed in the larvae of *Drosophila melanogaster* (Rensing 1964, 1966; Rensing *et al* 1965). Ecdysteroid titers in the different tissues like the ovaries, fatbody and haemolymph of the cricket, *Gryllus bimaculatus* were monitored by Hoffmann *et al* (1982) and it was noticed that ecdysteroid was present in small quantities in all young organs which increased markedly during ovarian maturation and decreased again during the last days of adult maturity. Two peaks of haemolymph ecdysteroids during larval-pupal development, one at the transition from the feeding stage to post-feeding prepupa and the other in association with pupal cuticle formation were demonstrated in *Manduca sexta* by Bollenbacher *et al* (1975) and the same observations were later confirmed in *Calpodes ethius* by Dean *et al* (1980). Later Fujishita and Ishizaki (1982) have demonstrated that in the larvae of *Samia cynthia ricini* haemolymph ecdysteroid titer begins to rise at 1800 hr of the day preceding the gut purge under LD 12:12 to reach a maximal level 4·5 hr before the purge.

Eventhough certain amount of work has already been done in investigating the activity rhythms of endocrines in some of the selected insect species, we are still unaware of the exact rhythmic activity patterns (whether it is daily modulation of hormone titers, gating of hormone secretion or photoperiodic control of hormone release) of most of these hormones especially juvenile hormone, ecdysone etc. in most of the insects. Even attempts made on neurohormones along these lines are mostly observations made on the basis of daily changes in the histological appearance of NS cell groups or endocrine organs. In certain cases rhythmic changes in the target tissues have provided evidence for rhythmic endocrine activity. Knowledge about the factors initiating release of JH, ecdysone, PTTH, JH esterase or proctodone and the time of their release are prerequisites for the proper interpretation of events controlling metamorphosis in insects. One of the methods which can be relied upon is by quantitatively

monitoring the titer of a particular hormone by using radioimmunological, mass-spectrometric and other techniques.

## 3. Hormones and circadian rhythms

Morphogenesis in insects are known to be controlled by different hormones. Hence it is not very surprising to see that the timing of larval-larval moulting, pupation, adult emergence etc. are also effected *via* hormones. The circadian control of gated—once in a lifetime—programmes and the more typical daily, repeated, ongoing behaviour of the type that is measurable in individual insects appears different. For gated rhythms, control is in the form of a 'single shot' hormonal release of each unique and largely fixed behaviour pattern. For ongoing behaviour continuous control must be exerted right round the clock.

### 3.1 *Locomotion*

Harker (1954) reported that rhythmic activity could be induced in arryhythmic *Periplaneta americana* by parabiosing them to rhythmic animals. Later Cymborowsky and Brady (1972) demonstrated in both crickets (*Acheta demesticus*) and cockroaches (*Periplaneta americana*) that headless animals take up the rhythm of the intact animal stuck on their backs, but significantly more rhythms are induced if the haemocoels are interconnected than if they are not. Thus some sort of influence affecting rhythmicity in locomotor activity apparently passed *via* the haemolymph from the intact donor to the headless recipient. Harker (1956) showed that the transplantation of SOG from the rhythmic donor induced rhythm in a headless arrhythmic cockroach recipient. Subsequent experiments implied that if two pairs of lateral NS cells of SOG are destroyed, no rhythms were induced even when the ganglia were implanted. So it was inferred that these four lateral NS cells of SOG act as an autonomous hormonal clock (Harker 1960c, 1961, 1964). However subsequent work by many others failed to confirm the induction of activity rhythm in the cockroach itself by any sort of SOG transplant (Leuthold 1966; Roberts 1966; Brady 1967a). Observations made in the grasshopper, *Romalea microptera* and in the beetle, *Blaps mucronata* also failed to support the above finding by Harker (Fingerman *et al* 1958; Thomas and Finlayson 1970). The implicated NS cells could be successfully removed by microcautery without impairing the periodicity of activity from the cockroach and even the removal of a great bulk of cell bodies and neuropile from SOG ventral region—leaving little more than the thorough tracts—did not stop the rhythm (Brady 1967c; Nishiitsutsujii-Uwe and Pittendrigh 1968). So it becomes almost clear that SOG NS cells are in no way essential to the timing or control of cockroach locomotor activity.

Many of the experiments conducted by different workers to establish the role of CC-CA complex also demonstrated that CC (corpus cardiacum) may be involved in judging the amount of locomotor activity (Shepard and Keeley 1972; Michel 1972) but appear not to be involved with its periodicity (Roberts 1966; Brady 1967b; Weber and Gaude 1971; Brady 1971). Attempts were also made to demonstrate the effect of hormonal principles from the brain NS cells on the cockroach locomotor activity rhythms (Harker 1956; Brady 1967b; Nishiitsutsujii-Uwo *et al* 1967) and the results showed no influence

as such on the timing of activity rather than destroying the behavioural integration.

If the compound eyes are severed from the optic lobes, the activity in *Leucophaea maderae* remained normally rhythmic but became uncoupled from the environment. In effect the animals became blind and their rhythm freeran unentrained by light/dark cycle in which they were kept. On the other hand, if the optic tracts were cut between the optic lobes and the protocerebrum, the animals became arrhythmic even in LD (Nishiitsutsujii-Uwo and Pittendrigh 1968). Later this finding was confirmed by Roberts (1971) by removing the optic lobes along with the compound eyes which resulted in arrhythmicity. However, as Brady (1974) suggests a possibility still exists for the involvement of hormonal components in the cockroach locomotor activity rhythms since it has already been demonstrated in the NS cells in the optic lobes (Beattie 1971) and also their axons in the circumoesophageal connectives of locusts (Michel 1972).

Experimental work on crickets (*Acheta domesticus*) by Cymborowski (1970) demonstrated hyperactivity and superficial arrhythmicity due to pars intercerebralis ablation eventhough autopsy of operated animals showed equivocal stainable material in the pars intercerebralis region. So it appears premature to conclude that brain NS cells ablation in crickets necessarily disrupts its rhythm at least in LD. In some of the noctuid moths, ablation of median NS cells has a different effect; the normal night disappears, but is replaced by a burst of activity for an hour or two after dawn.

### 3.2 *Gated events*

There is a whole class of physiological events that occur once only in each individual's life time, but which are nevertheless timed by a circadian rhythm. This sort of phenomenon cannot be detected as a rhythm in an individual; it becomes apparent only in mixed-age populations. Here the individual completes the morphogenetic aspects of its development at random with respect to time of the day for its emergence. Thus although individuals become ready to emerge at all times, they only do so through a narrow span of time each day, when a so-called circadian gate is open.

3.2a *Eclosion*: The concept of gating events by a circadian clock grew out of the studies of the rhythm of adult eclosion in *Drosophila* by Pittendrigh and Skopik (1970). In these flies eclosion is restricted to a specific temporal gate, the time of which is determined by an interaction between the photoperiod and the fly's circadian clock. Experimental evidence for a triggering effect of hormone on adult eclosion in the moth *Manduca sexta* was given in detail (Truman 1970, 1971a, b; Reynolds 1977). When blood was removed from eclosing animals and injected into pharate moths prior to their normal gate, the recipients showed precocious eclosion. So also extracts from the brains or CC of pharate moths contained eclosion stimulating activity which was depleted during eclosion. This 'eclosion hormone' proved to have a number of actions on the pharate moth including the behaviour release involved in emergence and wing spreading, the triggering of the break down of the intersegmental muscles and plasticising of the wing cuticle. The time of appearance of 'eclosion hormone' was determined by bleeding pharate *Manduca sexta* at various times of the day and assaying each sample for hormonal activity (Reynolds *et al* 1979). The hormone appears in the blood only at a restricted time of day about 2·5 to 3 hr before the moth subsequently emerges. At the gate, eclosion hormone was released as a rapid pulse which is then

gradually cleared from the blood. The appearance of the circulating hormone is complemented by an 85 to 90% depletion of activity stored in the CC.

Truman (1978) concludes in the light of earlier findings, that the gating of the 'eclosion hormone' release assumes that the time of release is determined both by a circadian clock and by the developmental competence of the insect. Therefore eventhough the proper circadian time has arrived secretion will not occur during that gate if development has not been completed. But when the ability of *Manduca* to respond to the eclosion hormone was examined, it was found that receptivity appeared only about 4 hr before the hormone was actually secreted. Thus eventhough the proper circadian time is arrived at, hormone release will not occur if the animal is not in the proper developmental state.

3.2b *Hatching*: Since other developmental events are clearly gated by circadian clocks, it might have been expected that egg-hatch would also be so. The possibility have been examined thoroughly only in the pink bollworm, *Pectinophora gossipiella* (Minis and Pittendrigh 1968). In this particular species, the hatch rhythm is initiable until the 12th day of embryogenesis when the first cephalic pigmentation coincides with some essential link-up in the central nervous system. In *Aedes* mosquitoes, hatching occurs as a direct response to environmental amelioration, related only to the effects of temperature on embryogenesis and the presence of water after some sort of delayed developmental period (Gillett 1955) and unrelated to any rhythm (Corbet 1966). Pre-conditioned *Aedes taeniorhynchus* eggs hatch at any time of day within 15 min of emersion in de-oxygenated water (Nayar 1967).

3.2c *Larval moulting*: The release of PTTH to induce the periodic larval moults in *Manduca sexta* was established to be gated (Truman 1972). From an analysis of quantitative and qualitative differences in the responses of neck-ligatured *Manduca* larvae at various times of the day, it was found that the PTG needed contact with the brain for at least 1·5 hr before they were fully activated. Thus a minimal time interval was necessary for PTTH secretion by the brain. With the opening of the first gate, the larvae were apparently not competent to release PTTH but some gained this competence before the gate subsequently closed. The remainder attained competence during the succeeding day and were able to release PTTH as soon as the next gate opened. Thus the distribution in the first gate identified the closing of the gate and that in the second gate identified the time of the opening of the gate. Also the gates for the release of PTTH for the various instars occurred at essentially the same time of the day and the duration of gates tended to narrow as the animals grew.

Fujishita and Ishizaki (1981) demonstrated that in *Samia cynthia ricini* an endogenous circadian clock controls the timing of larval ecdysis and PTTH secretion preceding it. The clock upon reaching a specific phase point causes the brain to secrete PTTH provided that the brain has acquired the secretory competence. Full secretion of ecdysone occurred 6 hr after PTTH secretion and ecdysis ensued 34 hr thereafter to complete the ultimate sequence of ecdysis.

3.2d *Pupation*: The puparium formation in *Drosophila* is induced by the moulting hormone, ecdysterone. The process of metamorphosis starts with the puparium formation and can be regarded as a closed system. In *Drosophila lebabnonensis* puparium formation is a rhythmic process which can be characterised as a circadian

rhythm. The circadian oscillation regulates the timing of the ecdysterone mediated process of puparium formation. Jan Eeken (1978) opines that the influence of the circadian oscillation is not at the level of the ecdysterone concentration itself since no endogenous ecdysterone nor a changed ecdysterone degrading system is present at different phases of the circadian oscillation. So he suggests the interference system, coupled with circadian oscillation which determines whether or not the puparium formation can take place, seems to enforce its action at a level between transcription and translation.

The release of PTTH by the fifth instar larvae brings about the start of metamorphosis in *Manduca sexta*. Ligation experiments showed that the brain was required until an average time of 1600 hr in order to trigger the start of metamorphosis. However, a careful study of various ecdysone dependent epidermal changes in the larvae indicated that PTTH release probably began as early as 0100 hr on the preceding night. Thus the PTTH gate in the fifth instar appears to start at a time similar to that for other instars but the hormone release in this last case is greatly prolonged over about 15 hr (Truman 1978).

Considering the tropic action of brain on the PTG, one might expect that PTTH rhythm would result in the rhythmic secretion of ecdysone. Using the time of appearance of an ecdysone sensitive puff in *Drosophila*, Rensing (1966) postulated a rhythm of ecdysone release prior to pupariation.

In the last instar larva of *Samia cynthia ricini*, the initiation of development towards pupation as visualised by overt events such as gut purge and wandering occur with circadian rhythmicity (Ishizaki 1980; Fujishita and Ishizaki 1982), and the involvement of an innate circadian clock has been demonstrated. Fujishita *et al* (1982) have demonstrated that the timed surge of ecdysteroids is responsible for the gated occurrence of gut purge and that 18 hr before gut purge, larvae acquire the competence to undergo gut purge in a gated fashion provided that they are exposed to a sufficient surge of ecdysteroids. A gated release of PTTH was confirmed in the induction of prodromal signs of pupation in Manduca as well (Truman and Riddiford 1974).

3.2e *Bursicon release*: Another hormone whose appearance is gated is the tanning hormone, bursicon. In *Manduca sexta* this hormone is produced in the abdominal nerve cord and released from the periviceral organs. Bursicon release occurs during wing inflation by newly emerged moths and it triggers the tanning of the freshly expanded wing (Truman 1973). Under normal conditions wing inflation behaviour is well under way by 15 min after eclosion and thus bursicon secretion was estimated to occur about 3 hr after the eclosion hormone peak. The time course of bursicon appearance was followed in individual cannulated *Manduca* (Reynolds *et al* 1979) by means of an isolated wing assay that responded to bursicon by tanning the wing veins. Within one to two minutes after eclosion, no hormonal activity was detected and 2 min later substantial increase in bursicon level was noticed in the blood and a peak titer was reached within 5 to 10 min. Thus the secretion of bursicon also occurs as a large pulse. The secretion of bursicon is dependent on the prior release of eclosion hormone (Truman 1973). When moths were induced to emerge early by eclosion hormone injection, they also showed early bursicon secretion. In fact the release of bursicon by neurones in the abdominal ganglia of *Manduca* appears to be a part of the complex neural programme that is triggered by the eclosion hormone. The gated appearance of

bursicon in newly emerged moths is a consequence of the prior gating of eclosion hormone release.

3.2f *Calling hormone*: This hormone in moths also show a daily pattern of secretion. The hormone differs from those discussed above in that its release is not gated but presumably occurs on a daily basis as long as the female is unmated. In virgin females of silkmoths, the behaviour involved in pheromone release—calling behaviour—shows a rhythmic occurrence (Riddiford and Williams 1971) and is triggered by a hormone released from the intrinsic NS cells of the CC. Blood from calling females when injected into non-calling individuals readily induced the characteristic behaviour. At other times of the day, this activity was reduced or absent. Consequently, the rhythmic display of calling behaviour is apparently triggered by the rhythmic secretion of this hormone from the CC.

Truman (1978) is of the opinion that in the moth, the cerebral lobe area contains the gating centre for at least one hormone. Further, the rhythm of hormone secretion could be a direct or indirect result of an interaction with a circadian clock. The rhythm of ecdysone and bursicon release are secondary gated rhythms since they result only from the tropic actions of PTTH and eclosion hormone respectively. By contrast, the latter two hormones and the calling hormone appear to be primary gated rhythms, the rhythm is enforced through direct association of the circadian clock(s) and the endocrine courses. Most likely the rhythmic centres which control the release of the other two hormones discussed above also reside in the same region of the brain. Whether these centres are distinct from one another is unknown at this time. Also, there is no evidence to indicate whether the respective endocrine cells contain the entire rhythmic system.

### 3.3 *Diapause*

The epithelial cells of part of the hindgut (ileum) of mature larvae of the European corn borer were implicated in the production of a hormone (proctodone) involved in the physiology of diapause (Beck and Alexander 1964). Part of the evidence offered in support of the postulated endocrine function of the ileal cells was the appearance of secretory granules within the cytoplasm. The secretory rhythm of these granules had an 8 hr periodicity with phase setting being effected by photoperiod. The cells released their secretory products shortly after the beginning of the scotophase, after which cytoplasmic granules would again accumulate and again disappear at 8 hr intervals. Although the postulated hormonal function of these cells remains questionable, the rhythmic secretory cycle is striking.

## 4. Conclusion

As is evident from the foregoing discussion, research work already done on the endocrine rhythms in insects have been mainly restricted to only a few species of insects. A thorough understanding about the rhythmic activity patterns of the entire array of hormones in insects and the controlling centres for the release of these hormones are immensely useful. Once we know the exact activity patterns of all these developmental, metabolic and other hormones, one can artificially manipulate the hormone titer at the wrong time in the insecton life resulting in its maldevelopment promising a novel approach in pest management.

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# Behavioural energetics of some insects

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**Abstract.** Foraging behaviour of insects includes the following energy-requiring processes: (i) location and (ii) gathering. Some insects do incur additional energy cost on transporting and storing food. Energy cost of foraging ranges from 2 to 5% of the energy gained in bees and wasps. Initiation of flight, in large and insulated insects obligatorily requires 'warming-up' of muscle temperature and maintenance of endothermy by over 20°C above the ambient. Overheating is avoided by pumping the cooler abdominal blood into the hot thorax. Pollinating insects include (i) hovering high-energy foragers, which expend more energy and visit more flowers per unit time and (ii) walking low-energy foragers, which expend less energy and visit few flowers per unit time. Decreasing of "wing loading" is another strategy adopted by saturniids, which do not feed as adults. Most bees forage, when flowers are just blooming, and when they have maximum nectar reward to offer. From the model study on energy cost of oviposition, it has been shown that *Sceliphron violaceum* makes greater and greater effort to complete the process of food provisioning and sealing the larval nest, when it has invested more and more energy on foraging and provisioning spiders to the larviposited young ones.

**Keywords.** Behavioural energetics; foraging behaviour; *Sceliphron violaceum*; high-energy foragers; walking low-energy foragers.

## 1. Introduction

A survey on pertinent literature reveals that there is a large number of publications concerning energetics (Waldbauer 1968; Scriber and Slansky 1981; Muthukrishnan and Pandian 1983) and behaviour (Saunders 1976) of insects. However, only a few publications are available on behavioural energetics of insects. Hence, it is chosen to highlight in this paper only the following aspects: (i) Energy cost of foraging and (ii) Energy cost of oviposition in selected insects.

## 2. Energy cost of food acquisition

In all insects acquisition of food involves a series of behavioural responses; while these responses are related, and perhaps inter-dependent, they are separate processes each under the control of a set of physical and chemical co-ordination. The processes are (i) energy cost of maintaining food supply (*e.g.* ants which maintain aphid population); (ii) energy cost of locating food supply, (iii) energy cost of gathering or catching food, (iv) energy cost of processing of food (*e.g.* conversion of nectar into honey), (v) energy cost of eating food and (vi) energy cost of transporting and storing food (*e.g.* bees) (see Lawton 1973). Of these, processes related to the energy costs of locating and gathering food are important; some insects such as bees and wasps do invest energy on transporting and storing food. Interestingly, much work has been done on the energy cost of transporting and storing food by bees and wasps (Heinrich 1979).

### 2.1 *Endothermy and initiation of flight*

Mechanical efficiency of the flight mechanism of insects is approximately 10–20% (Weis-Fogh 1972). More than 80% of the energy expended during flight is necessarily degraded into heat. Curiously those insects, which are large and insulated, retain most heat in the thorax during flight, also require the highest muscle temperature in order to maintain sufficient power output to continue flight. The minimum muscle temperature required to initiate flight varies over the relatively narrow range of 40–45°C (Kammer and Heinrich 1978). For instance, when the sphinx moth *Manduca sexta* vibrates its wings at the rate of 40 times/sec, and produces about 1 J/min of energy, its thorax is heated to 38°C, and the moth is ready for a take-off (Heinrich and Barthelonew 1971). Flight activity and endothermy are thus invariably linked in several insects, and endothermy in flight is a large part an obligatory phenomenon (Heinrich 1974). Temperate insects such as *Bombus* sp. invest quite a lot of energy (2·18 kJ/g thorax/hr) to elevate the thoracic temperature to about 40°C from the ambient temperature of 3–16°C, tropical insects such as *Schistocerca* sp. may require far less energy to elevate its thoracic temperature to over 35°C from the ambient temperature of 20–25°C. Information on the energy cost of endothermy and initiation of flight for tropical insects is almost totally wanting and a comparative study of this aspect for tropical and temperate insects will be rewarding.

### 2.2 *Thermoregulation during flight*

Most insects are small and uninsulated, so that over-heating of the flight musculature is not a general problem. However, build-up of heat is rapid in the flight muscle of some of the large, uninsulated insects. In these insects, the over-heating is avoided by transferring the hot blood from the thorax to the abdomen, when the abdominal heart (dorsal vessel) beats rapidly and pumps the cool blood through the heated thorax. For instance, the thoracic temperature of *Manduca sexta* never exceeds 40°C, and the excess heat is passed into the cool abdomen (26°C), by adjusting the rate of heat beat. More than pre-flight heating and endothermy, cooling and thermoregulation during flight should pose a major problem to the tropical insects. However, no publication is available on this subject for tropical insects.

### 2.3 *Energy cost of foraging*

Measured and calculated energy cost of flying for insects vary over a large continuum. In general most values fall between 418 and 2090 J/g/hr. They represent 50 to 100 fold increases over the resting metabolic rate (Kammer and Heinrich 1978). Necessarily, an insect may forage by hovering at high energy cost for a shorter duration or by walking at low energy cost for a longer duration. Table 1 shows the foraging cost of some bees and wasps, for which information is available. The report by Southwick and Pimentel (1981) is by far the most complete one for the estimation of foraging energetics of insects. A colony consisting of 50000 bees (*Apis mellifera*) is estimated to collect 259 kg nectar worth 1590680 kJ and 24 kg pollen worth 339066 kJ annually by flying a cumulative distance of about 13 million km. At an energy cost of foraging as 13·8 J, *i.e.* 4·6 J/km

**Table 1.** Foraging costs in some insects.

| Predator | Prey | Foraging cost (% acquired food energy) | Reference |
|---|---|---|---|
| *Bombus vagans* | Nectar | 8·2 | Heinrich (1972a, b) |
| *Apis mellifera* | Nectar and pollen | 3·7 | Southwick and Pimental (1981) |
| *Delta conoideus* | Caterpillar | 2·7* | Muthukrishnan and Senthamilselvan (1985) |
| *Trypoxylon rejector* | Spider | 1·6* | Muthukrishnan and Senthamilselvan (1985) |
| *Sceliphron violaceum* | Spider | 5·2* | Pandian and Marian (1985) |

*Considering energy cost of flight as equivalent to 418·6 J/g/hr, a value reported for the wasp *Vespa crabro* by Weis–Fogh (1967).

(Tucker 1970; Dade 1977; Schaffer *et al* 1979), a bee travels over 3 km to collect 370·7 kJ worth nectar and pollen, *i.e.* the energy cost of foraging is 3·7% (Southwick and Pimentel 1981). Similar calculation for the estimation of foraging cost of the bumble bee *Bombus vagans* shows that it spends about 8% of the food energy on acquiring it (Heinrich 1972a, b). Estimations on energy cost of foraging in walking and swimming insects are totally wanting.

Several species of wasps forage on caterpillars or spiders and transport them to the nest to provide food for their larvae. Flying a distance of about 68·4 km, *Trypoxylon rejector* (Sphecidae) predates and transports 190 spiders (7–22 mg each) worth 24·1 kJ in about 11 hr and 36 min for providing food for larvae developing in 9 cells in a nest. Covering a distance of 0·64 km in 4 trips *Delta conoideus* forages and transports 4 caterpillars (71–182 mg each) worth 4·2 kJ in about 2 hr and 57 min for provisioning one cell with a single larva (Muthukrishnan and Senthamilselvan 1985). Investing 1·04 kJ on flight for 2 hr and 30 min, *Sceliphron violaceum* transports spiders worth 13·32 kJ to provide food for its larva developing in an unused hole of electrical socket. Energy cost of foraging in these wasps amounts to 1·6, 2·7 and 5·2% whereas *T. rejector* and *D. conoideus* have to invest another fraction of their respective food energy on nest building activity, *S. violaceum* has avoided the investment of nest building by choosing unused holes.

## 2.4 *Metabolic strategies of flower foragers*

In the extremes there are two basic metabolic strategies of harvesting food energy from flowers: (i) Hovering high-energy foragers, which expend more energy and visit more flowers per unit time and (ii) Walking low-energy foragers, which expend less energy and visit few flowers per unit time. Hovering flight places heavy energy demands on insects (836 J/g/hr; Weis-Fogh 1972). This mode of foraging increases the rate of intake of food energy. For example, hovering flies *Bombilius* spp visited 21 flowers of *Houstania caerulea*/min; whereas *Syrphus* spp which do not hover at flowers, visited

only 5/min. Hovering moths *Hemaris* spp. visited 50 *Kalmia angustofolium* flowers/min, whereas non-hovering *Bombus* spp visited only 15/min. The food rewards of a composite inflorescence for example, are generally individually too small to be economically harvested by hovering. But they can be gathered by a butterfly or a bee that lands on the flowers and reduces its energy expenditure. Low-energy food sources can generally not be harvested by high-energy foragers, which can make much more rapid energy profits from high-energy food sources (Kammer and Heinrich 1978).

Large wings allow insects to fly with a low wing beat frequency and allow some insects to initiate flight without prior endothermic warming-up and to continue flight by gliding; the energy expenditure of locomotion is considerably reduced in such insects. Thus, the third strategy of reducing the energy cost of flying is to decrease 'wing loading' by increasing the wing area per unit body weight. Some saturniid moths and sphinx moths, which do not feed as adults, represent the extreme examples of this kind of strategy. Having relinquished energy intake, and having to rely only on the fixed energy reserves, they have minimized the energy cost of flying by decreasing the 'wing loading' as much as possible (Nachtigall 1966; Pringle 1974).

### 2.5 *Blooming times*

Flower density is another important factor that affects the energy cost of foraging. Although the flower density is ultimately determined by population density of the plants; it is altered by the time and duration of blooming. Synchronous blooming of the flowers of a species in a given plant population would minimize the temporal and energy costs of flying between plants (Heinrich and Raven 1972). Besides, flower density, (i) daily time of blooming, (ii) amount of energy reward provided, (iii) type of flower product (nectar or pollen or both), and (iv) structures affecting access nectar or pollen are some factors that may modify the energy cost of foraging. Thus, *Bombus* spp., which can forage at ambient temperatures of 5°C or less, forage at an energy cost two or three times greater than that at 26°C (Heinrich 1972a, b). Hence, flowers which are pollinated at low temperatures should either provide more energy rewards than those blooming at high temperatures (perhaps one reason for the low efficiency of honey production in tropical bees) or be denser so that they can be visited in rapid succession (Heinrich and Raven 1972).

While foraging in the early morning at an ambient temperature of 2°C from flowers of manzanita *Arctostaphylos otayensis*, *Bombus edwardsii* (0·1 g) has a thoracic temperature near 37°C. The energy cost of maintaining this thoracic temperature is 3·3 J/min. Each flower of *A. otayensis* provides nectar equivalent to 6·3 J in the early morning and the nectar reward dwindles to 1·3 J by noon. Thus, it is energetically advantageous for *B. edwardsii* to forage in the morning, when there is little competition for nectar, or the rate of nectar production is high (Heinrich and Raven 1972). Likewise, *Chilopsis* flowers provide the largest amount of nectar (2·4 ml/flower) in the early morning and as the result of foraging by *Bombus*, nectar volume declined to 0·3 ml/flower by 0930 hr. By taking into account the time required to suck up nectar and the energy cost of foraging at different times of the day, Witham (1977) calculated that in the early morning *Bombus* that took only the pool nectar was making a net foraging profit of 51 J/min, whereas that which went for both groove and pool nectars, could make a profit of only 41·4 J/min. In a country like India, where oil-seeds are in short supply, research work on pollination ecology of legumes deserves priority.

## 3. Energy cost of oviposition

For want of pertinent publication, the presentation on the energy cost of oviposition has been restricted to information collected by Pandian and Marian (1985) for *Scelipheron violaceum*. Male *S. violaceum* predates, stings, paralizes spiders belonging to *Argiope pulchella*, *Cyrtophora cicabrosa* and *C. citricola* and deposits them into unused holes of electrical sockets. When the male has deposited spiders equivalent to $68 \pm 9$ mg in about 35 min the female oviposits a single egg. Subsequently the male continues the process of spider deposition and seals the hole. From experimentations and observations lasting over 3 years, Pandian and Marian (1985) noted that the wasp deposits spiders equivalent to $200 \pm 10$ mg. The male brings spiders weighing 3–60 mg/trip, and within 10–30 trips, he deposits 200 mg. Marian *et al* (1982) reported that spiders equivalent to 68 and 110 mg are the minimum requirements for the successful completion of larval and pupal stages respectively.

To test the ability of the wasp (i) to recognize its own prey, (ii) to add more prey and (iii) to keep in memory the quantity of prey provided at any stage, the process of spider deposition was interfered by Pandian and Marian (1985) by way of adding or removing

**Table 2.** Interference with the deposited spiders and response of the wasp *Sceliphron violaceum* (from Pandian and Marian 1985).

| Deposited spider wt (mg) | Addition (+) or removal (−) of spider (mg) | Wasp response |
|---|---|---|
| | | Before oviposition |
| 18 | $+58 \pm 14$ | All the wasps recognized and removed the added spiders; 60% wasps continued depositing spiders up to 200 mg but the others abandoned the hole |
| 42 | $+19 \pm 3$ | All the wasps recognized and removed the added spiders; 70% wasps continued depositing spiders up to 200 mg but the others abandoned the hole |
| | | After oviposition |
| 122 | $+83 \pm 3$ | 100% wasps recognized and removed the added spiders; 80% wasps closed the hole but the others abandoned the hole |
| 112 | $+21 \pm 3$ | 100% Wasps recognized and removed the added spiders; 80% wasps sealed the hole; but the others abandoned the hole |
| 106 | +19, 63* | 100% Wasps recognized and removed the added spiders but abandoned the hole |
| 149 | $-38 \pm 3$ | 50% Wasps added spiders up to 200 mg and sealed; the others, which have seen the interference, abandoned the hole |
| | | Nearing the sealing |
| 182 | $+74 \pm 22$ | 50% Wasps identified and removed the added spiders and sealed the hole; others abandoned the hole |
| 193 | $+17 \pm 4$ | 100% Wasps removed the added spiders and sealed the hole |
| 203 | $-53 \pm 6$ | Ignored and sealed the hole |
| 201 | −201 | Ignored and sealed the hole |

*These spiders were added accommodating them in between the originally deposited spiders.

spiders. Their intention was to study the response of the wasp (i) before oviposition, when spiders weighing less than 68 mg were deposited, (ii) after oviposition, when spiders weighing more than 110 mg were deposited and (iii) before the closure of the hole, when spiders weighing about 180–200 mg were deposited. From their observations presented in table 2, the following may be inferred: (i) an individual wasp is able to recognize its own prey from that of others, (ii) with increasing energy cost of spider deposition and hence food provisioning, a higher percentage of the wasps makes a greater effort to continue and to complete the process of spider deposition, and seal the hole, (iii) the wasp was capable of doing addition and its memory lasted atleast for one day and (iv) the wasp was not capable of realising the removal of spiders from the hole. Briefly, greater the energy cost of providing food for its larva, greater is the effort by the wasp to successfully complete oviposition and provision of food for its larva. Energetics of oviposition behaviour is a woefully neglected area and requires immediate attention atleast for those pests, which are being considered for biological control.

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# Behavioural analysis of feeding and reproduction in haematophagous insects

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**Abstract.** The only common factor the haematophagous arthropods share among themselves is the blood sucking habit. This habit which ties them down to an unnatural assemblage, confers on them certain parallelism even in their natural diversities. Behavioural activities of haematophagous arthropods, like those of many other animals, centre around 3 major aspects: searching for a suitable host and feeding on it; meeting of the sexes and finding a suitable place for oviposition.

Behaviour of blood sucking insects assume importance because these insects act as vectors of many blood-borne infections of man and animals. In this article, feeding and reproductive behaviours of haematophagous insects are analysed on certain hierarchy of events like: motivation; search and consummation.

**Keywords.** Haematophagous insects; behaviour; feeding; reproduction.

## 1. Introduction

Feeding and reproduction are largely interconnected performances. This would become clear from the flow diagram (figure 1) which shows in a very general and simplified way how a hungry, motivated insect searches for a host and after finding a suitable one, takes a blood meal (consummation) leading to satiation. This further leads to ecdysis and/or ovarian maturation and egg laying (in hemimetabolous insects like bugs and lice which are blood feeders during all the stages of their life cycles, feeding results in ecdysis during immature stages, while in adults, feeding results in egg laying). This brings our insect back to the beginning of the cycle *i.e.*, searching for a host. The flow diagram is qualified as 'metabolic homeostasis' because the whole cycle deals fundamentally with the energy flow into and within the arthropod (see Gelperin 1971a, for the terminology).

Treating feeding and reproduction as separate entities would be an over simplification. However, in the present context these are treated as independent performances each having motivational activities leading to search of a host or a mating partner; sign stimuli from a host or a mating partner which releases a behavioural reaction and finally the consummatory act. In this article, analyses of both functions and mechanisms are dealt together emphasising the influence of endogenous and exogenous stimuli.

Study of the feeding and breeding behaviours of haematophagous arthropods assumes importance in two respects. While on the one hand the study is important for its own sake, on the other it is of relevance from epizootiological/epidemiological and disease or insect control angles. It is an established fact that many of the haematophagous arthropods act as transmitters (vectors) of blood-borne pathogens of vertebrates including humans. The type of cycle given in figure 1 becomes epizootiologically/epidemiologically involved, when it is repeated several times during the life of

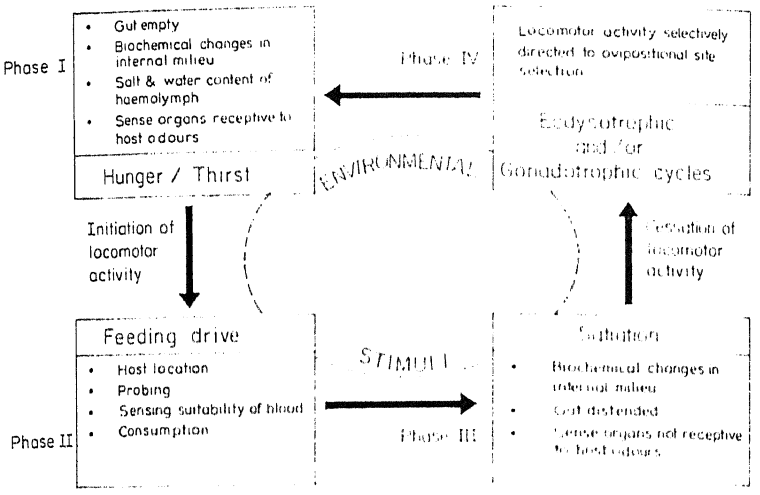


**Figure 1.** Metabolic homeostasis and its relationship to behaviour of haematophagous insect.

a vector. Many of the factors such as: whether the vector is zoophilic or anthropophilic; whether it rests outside the house or inside it; whether it is the 1st feed or subsequent ones; whether the time lag between phase III and phase I (through phase IV) (see figure 1) is sufficient for the growth and development of the pathogen in the vector and so on and so forth, are important in deciding the vectorial status of a blood sucking arthropod. A proper understanding of the behaviour of the vertebrate host, the vector and the pathogen—the three elements involved in vector borne diseases—is a must for effective vector/disease control operations. For example, effective vector or disease control operations can be directed only if host preferences, resting habits, choice of breeding areas etc., of vectors are known.

## 2. Feeding behaviour

In feeding behaviour the chain of events can be described as: motivation leads to the search for a host and sign stimuli from the host help in host recognition; probing, sampling of blood and sustained feeding which further leads to termination of feeding (Chart 1).

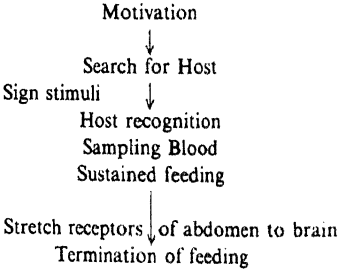


**Chart 1.** Sequence of events in feeding behaviour.

One of the most interesting yet poorly understood aspects of feeding behaviour is the causes which motivate the insect to search for a blood meal (induction of biting drive). Both external and internal factors can motivate the insect to feed. "The internal motivational factors can either be endocrinal or nervous. Nervous excitation may have its origin in excitation of enteroreceptors sensitive to the internal conditions of the animal and/or in spontaneous activity of nervous centres" (Markl 1974). Only limited studies have been made on the biochemical changes of the haemolymph which induce feeding drive. Garcia (1959) has presented a unique observation, which states that a mosquito seeks a blood meal to satiate the lowering of the levels of serotonin and norepinephrine in its haemolymph. Experiments on blood avidity of mosquitoes have shown that presence of developing oocytes inhibit biting (Khan and Maibach 1970; Edman *et al* 1975). However Armstrong (1968) suggested that ovaries have a secondary role in the control of feeding activity in that they remove protein from the haemolymph; the primary control is the protein reserves (as measured by the amino acid level) and high energy phosphate bonds (AMP). He showed that when the total free amino acid level in the haemolymph was raised above that found in the unfed female there was no feeding. He also suggested that there exists a system (probably neuroendocrine in nature) to measure amino acid level and high energy phosphate bond availability and to regulate feeding activity. Such a correlation between the haemolymph protein concentration and blood avidity was detected in rat fleas *Xenopsylla cheopis* and *X. astia* also. Injection of 50% bovine albumin into the haemocoel of fleas reduced blood intake of teneral females, but injection of 10% albumin was sufficient to cause a reduction in blood intake of 7-day old female fleas (Geetha Devi and Prasad 1980). The concentration of haemolymph protein of 7-day old fed female fleas was found to be at least two times greater than that of newly emerged unfed female fleas (Narayana Pillai 1983). Klowden (1981) suggested the involvement of 2 humoral factors in the inhibition of host-seeking behaviour of gravid *Aedes aegypti*. The first factor produced by the ovaries which causes the release of a second, from another site (site to be identified), which is responsible for the inhibition of host seeking. Involvement of 20-hydroxyecdysone in the development of host-seeking inhibition in *Anopheles freeborni* was proposed by Beach (1979). But Klowden (1981) does not believe the substance involved in host-seeking inhibition in *A. aegypti* to be ecdysone. Meola and Petralia (1980) suggested juvenile hormone to be involved in post emergence development of biting in *Culex pipiens* and *Cx. quinquefasciatus*. Though in most cases biting drive is directly related to gonotrophic cycle, in certain diapausing mosquitoes a process called 'gonotrophic dissociation' results in no egg development and laying eventhough the mosquito continues to feed (Washino 1977). Maybe in such cases the reserves built up in the haemolymph as a result of blood meal are channelised for build up of fat body reserves rather than for oocyte maturation. The end result in both cases, as one should guess, would be the depletion of reserves from the haemolymph. The actual link between the haemolymph changes and the endocrinal or nervous controlling mechanism still remains to be fully elucidated in haematophagous insects. Extensive work done on the feeding behaviour of the blowfly *Phormia regina* has thrown light on the various internal components controlling feeding drive. The components involved are identified as: the locomotor centre of the thoracic ganglion and the stretch receptors of the foregut and abdomen. The foregut stretch receptors monitor peristalisis of a restricted region of the foregut (and so control gut filling) whereas the abdominal stretch receptors monitor crop volume. The activity of these receptors regulates

centrally the threshold of acceptability for food by setting taste threshold. The locomotor centre of the thoracic ganglion which inhibit feeding is under the control of a humoral factor from the corpus cadiacum, which in turn is controlled by the stretch receptors monitoring foregut filling. Food uptake in *Phormia* can only begin when the internal inhibitory stimulus ceases (Dethier and Bodenstein 1958; Dethier and Gelperin 1967; Gelperin 1966a, b; 1967; 1971a, b; Gelperin and Dethier 1967; Getting 1971; Getting and Steinhardt 1972; Green 1964a, b; Nunez 1964; Omand 1971).

Motivation is followed by search for a host which may be called 'appetitive behaviour'. This may use simple or complicated stereotyped movement patterns together with release controlling mechanisms. It is possible to categorise haematophagous insects into three groups (i) those that live as permanent ectoparasites on their hosts (*e.g.* lice), (ii) those that live in close quarters of the host and visit them frequently to obtain blood meal (*e.g.* fleas) and (iii) those that are free-living in one sense of the term, yet are dependent on the host for blood meal and visit the host only occasionally as and when the need for a blood meal arises (*e.g.* mosquitoes). Sensory equipment that aid in distant perception of host show maximum development in the third group and the least in the first. The cues that can release a 'host-attacking reaction' with reference to haematophagous arthropods are: host specific odours; non-specific or group factors like $CO_2$, convection currents, water vapour etc., and visual cues. Visual cues are important for day-biting insects (Gatehouse 1972; Gillies 1972). As a matter of fact Gatehouse (1972) found that when tsetse flies were not presented with any visual stimulus, their response to calf odours was low. Convection currents of IR emanations from the body of the warm blooded vertebrate would be important for close range host detection. Non-specific factors are universal features of all vertebrates, but quantity of $CO_2$ given by large and small-sized hosts would vary. In general it may be stated that odours provide the best information possible about the host at all ranges (Gillies 1972). The insect recognises the presence of the host when it gets into a 'host-stream' (the plume of host-conditioned air drafting downwind from the host) during its random dispersal flight from the breeding areas. Once they are in the host-stream, the flight becomes oriented towards the host (Daykin *et al* 1965; Gillies and Wilkes 1969). The efficacy of various substances—$CO_2$, water vapour, fatty acids and derivatives, ammonia, amines, amino acids etc.—have been tested on mosquitoes for release of host-attack reaction (Brown 1966). $CO_2$ activates the insect to fly while water vapour and warmth induce target orientation (Kellogg and Wright 1962). As mentioned earlier these three stimuli emanate from all warm blooded vertebrates and so would be non-specific. Then what causes specific host selection? Discrimination lies, it appears, with fatty acids and their derivatives. Among fatty acids, lactic acid plays an important role and this was shown to be an attractant of female *Ae. aegypti* in the laboratory by Acree *et al* (1968) and Muller (1968). The degree of attractiveness of mosquitoes varies with the quantity of lactic acid secreted by each individual host. Three types of olfactory sensillae are recognised in mosquito antennae; the sharp trichoid (type A1), short blunt trichoid (type A2) and smaller, thorn-like basiconic (type A3). Lacher (1967) showed that the type A1 sensillae of *Ae. aegypti* are sensitive to fatty acids but essential oils depress their activity. A2 are excited by higher fatty acids but are depressed by lower fatty acids. According to him the type A1 sensillae should be considered as odour specialists. Once the insect has located and settled on the host, it starts probing for blood. Several phagostimulants have been discovered in the blood of vertebrates which trigger sustained feeding. Many nucleotides and amino acids have been shown to act as

phagostimulants for mosquitoes, tsetse flies, Rhodnius, fleas and ticks (Friend 1965; Friend and Smith 1971, 1975, 1977, 1982; Galun 1966, 1967; Galun and Kindler 1968; Galun and Margalit 1969, 1970; Galun and Rice 1971; Galun *et al* 1963, 1969; Hosoi 1958, 1959). Contact chemoreceptors are of importance in sampling the blood meal. These are distributed on the tarsi, mouth parts and cibarium. Electrophysiologically these contact chemoreceptors are little known. A review on the chemoreceptors of haematophagous insects is presented by Lewis (1972).

Stretch receptors of the gut play an important role in termination of feeding (Klowden and Lea 1979). Messages sent to the brain from stretch receptors of the abdomen via the ventral nerve cord would tell the insect when to stop feeding (Maddrell 1963; Jones 1978). Gwadz (1969) showed that hyperphagy can be induced in mosquito by cutting the ventral nerve cord in the 2nd abdominal segment. Maybe termination of feeding is also effected at a critical level of back pressure from the abdomen as suggested by Bennet–Clark (1963) in *Rhodnius*. Hyperphagia was shown to be induced in the blowfly by transecting the recurrent nerve posterior to the brain (Dethier and Bodenstein 1958; Dethier and Gelperin 1967; Green 1964b).

## 3. Reproductive behaviour

It is possible to describe reproductive behaviour also in the same sequence of events as described for feeding behaviour (Chart 2).

A sexually motivated insect searches for a receptive partner leading to mating (consummation); feeding (may or may not precede mating) and oviposition. As to motivation, endogenous factors motivating the insect to search for a mate are not fully understood. As suggested by Anderson (1974) they may include; (i) prior feeding on carbohydrate/protein; (ii) maturation of sperm in the male and partial or full maturation of primary follicles after feeding and the resulting receptivity of the female and (iii) secretion of specific hormones. In any event sexual maturity and the reproductive physiology of the two sexes must be synchronised.

Excepting for the cases where parthenogenesis is seen (lice, *Bovicola bovis*; certain mites and certain strains of tick spp.) all other haematophagous insects are bisexual and the sexes are to meet somewhere. With regard to haematophagous insects which live on the host itself or in the near vicinity of its nesting quarters (in other words, those like lice, fleas and pupipara, where the individuals of a population are not widely scattered as in mosquitoes or other free-flying blood-sucking diptera) meeting of the sexes offers little problem. In the flea *Ceratophyllus gallinae*, for example, an accidental collision

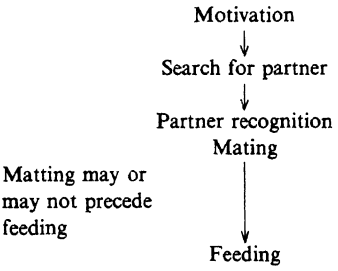


**Chart 2.** Sequence of events in reproductive behaviour.

between the male and the female results in mating (Humphries 1967), whereas in dipterous insects an aggregation or assembly of males called swarming into which females occasionally enter serves as one of the means of meeting of the sexes. In swarming, the assembly typically builds up over a visual land mark which is recognised by both sexes (Downes 1955; 1958a, b; 1969) or males wait in a zone which is likely to be traversed by females. For example, above a food source as some simuliids the males of which swarm over cattle and catch the females as they come to feed (Wenk 1965). Not that all diptera swarm before mating. An unusual mating system among mosquitoes is that of *Deinocerites cancer*. Adult male of *D. cancer* walk over water and locate female pupae and stand guard over it and mate with the female as soon as it emerges. The antennae are used to locate female pupae. Pupal attendance in *D. cancer* may be triggered by chemical cues emanating from the pupa (Conner and Itagaki 1984). The only other mosquito which shows pupal attendance is *Opifex fuscus*, but this has not evolved the ability to distinguish the sex of the pupa as *D. cancer* has. Such species of mosquitoes which do not swarm have lost the long hairs with auditory function which characterise the male antennae of most species of mosquitoes (Clements 1963). It was originally thought that the females in a swarm are recognised in close range by their wing sound in many culicids, but researches have shown the presence of contact pheromone which are active only over short distances (a few centimeters). Such pheromones are thought to be involved in sex recognition of *Culiseta inornata* (Kliewer *et al* 1966), *Culex tarsalis*, *C. pipiens* and *C. quinquefasciatus* (Gjullin *et al* 1967); *Aedes aegypti*, *Ae. mascarensis*, *Ae. albopictus* and *Ae. polynesiyensis* (Nijhout and Craig 1971). Presence of contact pheromones active over short distances have also been suggested in *Ceratophyllus gallinae* (Humphries 1967); *Rhodnius prolixus* (Baldwin *et al* 1971); *Amblyomma americanum*, *A. maculatum* and *Dermacentor variabilis* (Berger 1972; Berger *et al* 1971; Chow *et al* 1975; Gladney 1971).

Mating may take place exclusively on the host; on and off the host or exclusively off the host depending on the blood sucking insect's life style. For example, anoplura and pupipara which are permanent residents on the host, mate exclusively on the host, whereas mating of free-flying forms like mosquitoes and bugs takes place off the host. Strain variations are seen in laboratory colonies of mosquitoes with regard to their requirement of space for mating. Eurygamous strains require large space for mating because copulation is initiated in flight, while males of stenogamous strains will approach and mate with resting females and can do so in confined areas such as a small cage or a test tube.

In most cases of haematophagous diptera (Nematocera and Brachycera) the hierarchy of behavioural responses would be: host seeking, mating and blood feeding. But in certain cases blood feeding may precede mating (Teesdale 1955). Females of most Cyclorrapha take a blood meal before mating. Similar variations are also seen in fleas, ticks and bugs. In some cases like ticks, mating may take place while the female is feeding.

Oviposition behaviour is rhythmic in nature and finds a correlation with feeding cycle. In the case of permanent ectoparasites like lice which do not leave the body of the host and all the stages are blood suckers, eggs are laid on the body of the host itself and eggs are cemented to the hair/feather. In those like fleas, eggs are laid in the nest of the host. In the event of laying while the flea is on the host, eggs will roll down into the nest of the host and would not stick to the body of the host as in the case of lice. Free-flying dipterous blood-sucking insects lay off the host in sites suitable for the development of

their immature stages. Oviposition site preference is a ritual in most mosquitoes. Two phases may be recognised—a general reaction to the environment and final selection. Site selection not only involves "finding the water and laying eggs, but also selection of environment, whether shaded or open, stream, rice field, pond, tree hole or artificial container, whether water is moving or still, polluted, saline or fresh" (Clements 1963). Vision and chemoreceptors of the legs are thought to play important roles in site selection for oviposition in these cases. Some mosquitoes even 'taste' the water to assess suitability.

## 4. Rhythms in adult activities

Locomotor activity is normally accompanied by other behavioural manifestations like feeding, mate seeking and oviposition. All these behavioural manifestations are known to be rhythmic in nature. In addition to these, pupation, adult emergence, erections of antennal fibrillae in the males of some spp of mosquitoes; the start of migration of some spp., of mosquitoes, nectar feeding and unspecific flight activities are all known to be rhythmic. These rhythms, as typical of biological rhythms, are entrained by or synchronised to a cyclical environmental cue called Zeitgeber.

The diel pattern of adult emergence of a given species tends to be correlated with the locomotory activity rhythms and the reproductive behavioural pattern of that species (Beck 1968). Adult emergence rhythms depicts a synchrony of developmental processes among individuals belonging to a population. This synchrony, possibly has a basis in oviposition rhythms. As has been mentioned before, adult emergence is closely followed by swarming and finding a mate in diptera on which extensive research has been made. Swarming is a circadian endogenous phenomenon, entrained by environmental photoperiod. Light intensity plays a role of releaser of swarming habit, though temperature, wind etc., do play important roles. Swarms were induced only when transition from light to dark (or vice versa) was gradual. The possible role of visual adaptation in swarming of *C. tarsalis* was shown by Harwood (1964). He used 'eye index'—defined as the average cornea diameter: iris diameter ratio—to study visual adaptation. According to him fully light adapted mosquito has an eye index of 21 whereas in fully dark adapted ones it was only 4 and swarming occurred when the eye index reached 5.

One of the few rhythmic biochemical changes studied in mosquitoes is the synthesis of glycogen in *Cx. pipiens* (Takahashi and Harwood 1964). Peak glycogen level is seen towards the end of photophase and this gets depleted as the insect becomes active during the scotophase. Photoperiod has some influence on the synthesis of glycogen which in turn is under the control of neurosecretory system of the insect (Handel and Lea 1965).

Both embryonic and adult diapause have been described in mosquitoes. The former is caused by the photoperiod experienced by the parental generation rather than the eggs themselves with one exception of the case of *Ae. triseriatus* in which case diapause occurred in response to short-day photoperiods experienced by the embryos from 5 to 8 days after egg deposition (Kappus 1965). With regard to larval diapause, the studies on *An. barberi* and *Ae. triseriatus* showed that short-day photoperiods induced diapause and long-day photoperiods terminated it. Photoperiod sensitive stages varies widely among different species. Larval diapause may be determined in most cases by the

photoperiod to which the larval stages have been exposed but in the case of *Ae. triseriatus*, larval diapause appeared to be determined by the photoperiod experienced by the parental generation (Love and Whelchel 1955). Similarly, pupal diapause was determined in response to short-day photoperiods experienced by the female progenitor (Depner 1962).

In-depth studies have been made on feeding rhythms of mosquitoes but not in other haematophagous insects. Each mosquito species has a preferred feeding time and only at such times would it become receptive to host odours. This is true for both day-biting and night-biting mosquitoes. Feeding and egg laying are correlated. For e.g., *Ae. aegypti* which shows a feeding peak late in the afternoon and egg laying peak almost at the same time 3 days after. Inborn endogenous 24 hr rhythms of activity and rest which use onset of darkness as an external time-cue for the timing of the cycle have been detected in mosquitoes. Such circadian rhythms not only serves to brief the mosquito on its take-off time, but determines the duration of flight period keeping it within the normal fuel range. Temperature, light and wind can inhibit activities normally controlled by circadian rhythms, but very little is known about the interaction of climatic factors and endogenous rhythms in determining the population activity. Most behavioural patterns require releasing stimuli from the environment. Light intensity may be such a releaser for mosquito biting behaviour, although other exogenous stimuli perhaps host-borne may also be involved. Photoperiodic entrainment may play a role in determining the responsiveness of the insect to the releasing stimulus.

Another synchronised rhythmic behaviour between the reproduction of the host rabbit and its flea ectoparasite *Spilopsyllus cuniculi* was described by Rothschild (1965). The flea starts its reproductive activities only when the host becomes pregnant and the level of ACTH in the blood rises. Mating of these fleas takes place on the newly born young rabbits. Thus there is a rhythmic synchronisation of the breeding of rabbits and that of their flea ectoparasites.

## 5. Behavioural genetics

In general it may be said that genetics of behaviour of haematophagous arthropods is a very poorly studied aspect. Mattingly (1962) states that "despite its great importance, this difficult field is one of the most neglected in contemporary biology". Mattingly (1967) draws out the few studies carried out under genetics of: mating behaviour; host choice; irritability and photoperiodism; oviposition behaviour and environmental selection.

The recessive autosomal gene causing yellow eye in *Ae. aegypti* enhances mating efficiency (Adhami and Craig 1965). Tate and Vincent (1936) found stenogamy expressed in $F_1$ progeny of cross between eurygamous *Cx. pipiens* and stenogamous *Cx. p.* var. *molestus* and this was maintained in the subsequent generations also. Bates (1941) showed heritable changes in swarming behaviour of hybrids between members of the *An. maculipennis* complex. Mating vigour is another aspect on which some studies have been made among members of *Cx. p.* complex and found marked differences (Rozeboom and Gilford 1954; Parker and Rozeboom 1960). Gillies (1964) was able to select distinct zoophilic and anthropophilic strains of *An. gambiae*. Heritability of enhanced irritability and increased positive phototropism of mosquitoes under insecticidal pressure was demonstrated by Gerold and Laarman (1964) in *An. atroparvus* in the laboratory. Wood

(1961, 1962) was able to show a possible genetic variation in oviposition site preference and variation in the length of time elapsing between the blood meal and oviposition among different strains of *Ae. aegypti*. Strain variations have also been reported in *Ae. aegypti* with regard to their requirement of mating for egg laying (Gillett 1955, 1956).

## 6. Evolution of haematophagy

Haematophagy is exhibited by Ixodidae (hard ticks), Argasidae (soft ticks) and a few families of Mesostigmatid mites among Acarina; Anoplura, Thysanoptera and Hemiptera among the Hemimetabola; Lepidoptera, Diptera and Siphonaptera (all the three belonging to the Panorpoid complex-Hinton 1958) among the Holometabola (Hocking 1971). Blood sucking behaviour can probably be traced back to the Permian when land vertebrates emerged. Hoogstraal (1965) has concluded that ticks have arisen in association with reptiles in the late Paleozoic or early Mesozoic. By Triassic Psychodiform, Culiciform and Tabaniform have been well differentiated and insects in a form nearest to Psychodiform appear during the Permian (table 1) and these were contemporaries of Theromorpha—the warm blooded reptiles (Downes 1971).

This would mean: (a) that blood-sucking habit originated much before the emergence of mammals as an ecologically important group; and that (b) the blood sucking forms had the whole lot of land arthropods and vertebrates to choose as hosts. Feeding on poikilotherms is still a habit noticed among many of the blood sucking insects and arachnids. Hocking (1971) states that "the most readily available blood initially was probably arthropod blood, that many different groups today feed on this suggests that it may have been an early development". There is even a report of *Aedes* mosquitoes feeding on mantis and laying viable eggs in the laboratory (Mathews and Mathews 1978). Hocking (1971) concludes that "in the Acarina and more or less contemporaneously in the Hemimetabola and Holometabola, trends toward fluid feeding seem to have developed. It may be supposed in each instance the initial adaptation was to feeding on exposed fluids, but that as plants and animals generally became better adapted to life on really dry land, by developing substantially impermeable skins and cuticles, a taste for nutrient fluids demanded a combination of suction with cutting or penetration". Among diptera a dichotomy exists in feeding habits *i.e.*, sugar meal *vs* blood meal. Evidences strongly support the assumption that blood sucking at least in most cases is a secondary development. This duality of feeding habit has a foundation in Mecoptera—the probable ancestors of Diptera and Siphonaptera. But insects like Siphonaptera, which are more advanced in parasitic habits, are exclusive blood suckers. Animal tissue feeding appears to have evolved independently in different groups through four routes. In mites, lice and fleas this could have evolved through lair or nest associations. Scavenging on debris within lairs, burrows or nests of vertebrates could have developed into feeding on blood. For example psocids which are found in large numbers on animal habitations. Hopkins (1949) believes that lice were derived from psocid-like ancestors. Fleas in their larval stages, still continue to be scavengers, in the nests of rodents, feeding on debris. A second possible mode would be predation. Blood sucking triatomine bugs illustrate how predatory habit could have developed into blood feeding on vertebrates. A similar situation may be seen in the dipteran family Rhagionidae. Some of them are blood suckers, attacking mammals, while some others are predaceous on insects. It may be

**Table 1.** Appearance of blood sucking arthropods in relation to their host.

| Era | | Period | | Dominant life | Blood sucking forms |
|---|---|---|---|---|---|
| Cenozoic | | Pleistocene | | | |
| | | Tertiary (60) | Pliocene | | |
| | | | Miocene | | |
| | | | Oligocene | | |
| | | | Eocene | | |
| | | | Paleocene | Mammals | |
| Mesozoic | Late (120) | Cretaceous | | | |
| | Early (155) | Jurassic | | | All the main Insect orders known today except Lepidoptera |
| | Early (155) | Triassic | | Birds and flying reptiles | Psychodiform Culiciform Tabaniform |
| Palaeozoic | Late (215) | Permian | | Theromorpha Land vertebrates | Forms nearest to Psychodiform. |
| | | Carboniferous | | First Insects | Ticks |
| | Middle (350) | Devonian | | Arthropoda, land plants, amphibia | |
| | | Silurian | | brachiopoda, scorpions | |
| | Early (550) | Ordovician | | First fish | |
| | | Cambrian | | Mollusca, Trilobite | |

noticed that predation in the cases cited consists of feeding on liquid contents of the prey rather than consuming the whole prey. Feeding on proteinaceous secretions could as a further step lead to haematophagy. The feeding habits of the eye frequenting lepidoptera *Arcyophora* and *Lobocraspis* spp. which feed on the lachrymal secretion of cattle, sambar and other large mammals are examples to this. Secondary haematophagy-feeding on blood oozing out from wound—could be a fourth route to development of more purposive blood sucking habit (James and Harwood 1969).

## 7. Conclusion

In conclusion it may be stated that the little that is done on the behaviour of haematophagous insects centre round mosquitoes, leaving out the several other groups practically untouched.

Factors which induce feeding drive in the majority of haematophagous arthropods still remain unknown. Host and oviposition site preferences need further attention. It is

known that kairomones emanating from the water bodies attract mosquitoes to reach a suitable oviposition site. It is also known that micro-organisms especially bacteria have a role to play in the production of such attractants. The feasibility of altering sites suitable for egg laying by genetic or other manipulations of the bacterial flora has not yet been experimented.

Very few studies have been made on human influence on behaviour of haematophagous arthropods, an aspect which has importance from epidemiological angle. One of the few studies made show that exophilism and exophagy are induced among mosquitoes as a result of spraying insecticides in houses. As a result of spraying the walls, endophilous mosquitoes seldom remain on the walls inside houses and show positive phototropism. Such an induced exophilism would result in deviation to alternate host especially cattle. This would bring in a dangerous situation with regard to zoonotic diseases (Trapido 1952).

Much emphasis need be laid on genetics of behaviour especially those of host selection, feeding (exophagy, and endophagy), mating, oviposition site selection and such other behavioural aspects. The full impact of gene manipulations on the above mentioned behavioural aspects is yet to be fully explored. With the development of gene splicing and gene transfer technologies several alterations at population levels can be thought of.

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# Feeding and ovipositional behaviour in some reduviids (Insecta-Heteroptera)

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**Abstract.** Feeding behavioural studies of many exclusively predatory species exhibit clearcut stimuli-response mediated sequences and these can be categorised into distinct sub-units like: search and location of prey → approach and attack of prey → immobilisation of prey → transportation of prey to safe place → consumption of prey. These feeding behavioural activities differ among reduviids particularly with respect to prey types. These bugs are endowed with many structural, physiological and behavioural adaptations for efficient predation.

The ovipositional behaviour of reduviids in different habitats also shows considerable variation and their reproductive strategies include selection of suitable sites to assure successful emergence and development of young ones and so far very few egg predators and egg parasites have been reported for these terrestrial insects.

**Keywords.** Feeding behaviour; ovipositional behaviour; reduviids.

## 1. Introduction

The family Reduviidae is one of the terrestrial groups of bugs well represented in tropical and subtropical regions of the world. They are known to colonise a wide variety of habitats, such as from under stones, on low herbage or lower foliage or on trees, to the most unusual ones like ant-hills, termitaria, cobwebs, bird nests, rat holes and human dwellings. Naturally, they exhibit a wide range of structural, physiological and behavioural adaptations for an exclusive predatory habit, feeding on a variety of arthropods, including millipedes, termites, bugs, beetles, caterpillars, ants, bees etc. Members of the Triatominae alone have specialised for haematophagy, engorging the blood of birds and mammals.

## 2. Feeding behaviour

Feeding behaviour of reduviids, as it is true for several other predatory insects, shows many distinct events, and these stimuli-response mediated sequences can be conveniently divided into: location of prey → approach and pounce on prey → immobilisation of prey → transportation of prey to safe place → feeding.

### 2.1 *Location*

Being exclusive predators, visual stimuli appear to be of primary importance to these and with the possession of well-developed compound eyes and ocelli they easily locate

and capture various prey types. Visual stimuli from the moving prey initiate subsequent predator-prey interactions. The importance of visual stimuli for predatory reduviids has been well documented (Odhiambo 1958a; Edwards 1962; Parker 1969, 1971, 1972; Livingstone and Ambrose 1978a; Louis 1974; Haridass and Ananthakrishnan 1980a). Species like *Haematorrhophus nigroviolaceus* (Reuter), *Guionius nigripennis* (Fabr.) and *Ectrychotes pilicornis* (Fabr.) as well as other members of the subfamily *Ectrichodiinae* feed exclusively on millipedes (Cachan 1952; Miller 1971; Haridass 1978) and these predators are aroused from a state of akinesis only after receiving the stimuli from the moving millipedes. Even artificial baits like paper, rolled like millipedes, or dead and dried millipedes, also initiated feeding responses in them. Artificial objects or dead bodies of their prey dragged in front of piratine species like *Pirates affinis* Serville, *Ectomocoris tibialis* Distant, *E. ochropterus* Stal, and *Catamiarus brevipennis* Serville, elicited similar responses. Arousal of feeding responses by optic stimuli has also been noticed in several other reduviids, including termite feeding *Rhaphidosoma atkinsoni* Bergroth (figure 1F) and ant feeding *Acanthaspis pedestris*, Stal (figure 1D) *A. siva* Distant, and caterpillar feeding *Sycanus collaris* Fabr (figure 1E), *Rhinocoris marginatus* Fabr and *Sphedanolestes rubicola* Distant. Exceptions to this are the members of Triatominae, majority of which have taken to haematophagy and in forms like *Triatoma rubrofasciata* De geer (figures 1G, H), *Linschcosteus costalis* Ghouri (Haridass 1978; Haridass and Ananthakrishnan 1980a) and *Rhodnius prolixus* Stal (Friend and Smith 1977), it is the temperature gradient from the vertebrate hosts that aroused the starved insects. Blinding of their eyes does not deter them from locating their correct hosts and in these haematophagous insects the antenna are the primary sense organs.

### 2.2 *Approach and pounce on prey*

Successful location is followed by the quick approach to the prey located and pouncing on them for subsequent immobilisation. Several species exhibit a definite preference for a particular prey type. Ectrichidiinae show a preference for spirostreptid millipedes (figure 1A) and never attempt predation on polydesmid species. Similarly *P. affinis* prefers carabid *Omphora pilosa* Klug and *O. atrata* Klug than any other ground beetles and *E. tibialis* and *E. ochropterus* prefer gryllids to any other insects (figure 1C). Fast runners like Piratinae, Ectrichodiinae, Acanthaspidinae, Reduviinae etc., quickly reach the prey and pounce on them gripping them tightly with tibial pads (figures 1A, B). The presence of tibial pads on the fore- and/or mid-tibiae is a characteristic feature of this family (Gillette and Wigglesworth 1932; Miller 1942; Edwards 1962; Bahadur 1963; Haridass and Ananthakrishnan 1980b). These tibial pads with their oil secreting tenet hairs, enable the predators to increase the gripping efficiency during prey capture. The tibial pads enable the insects to withstand static tension, on rough and smooth surfaces to an extent of 20–27 g (Haridass and Ananthakrishnan 1980b). In contrast to the fast running habits, members of Harpactorinae, Emesinae, and Rhaphidosomatinae exhibit slow gait and use the long legs and rostrum to reach the prey and gripping of the latter is never involved (figures 1G, H). Some reduviids like the species of Apiomerinae and Ectinoderinae make use of the resin coated legs to capture prey like fly-paper (Miller 1971). The fore-legs of the thread-legged emesine bugs are also raptorial (Wygodzinsky 1966). The camouflaging of the body surfaces with particles of mud and debris as seen in

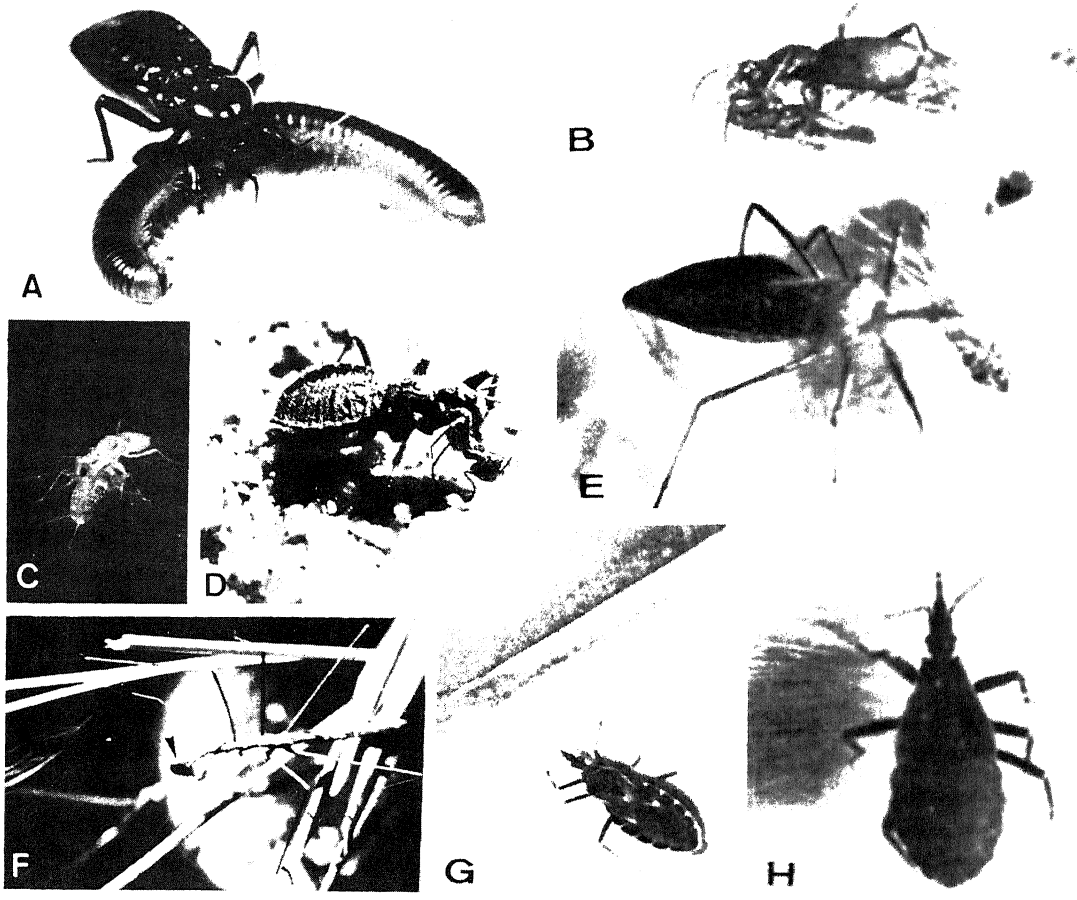


**Figure 1.** *Feeding behaviour in some reduviids.* **A.** *Haematorrhophus* [illegible] feeding on millipede prey. **B.** *Pirates affinis* (5th nymph) immobilising carabid beetle. **C.** [illegible] *tibialis* feeding the gryllid prey. **D.** *Acanthaspis pedestris* feeding the [illegible] **E.** *Sycanus collaris* dragging the immobilised caterpillar prey. **F.** [illegible] with termite worker (arrow) hanging from the rostral tip. **G.** *Triatoma* [illegible] orienting towards the hand of a sleeping person. **H.** *Triatoma* [illegible] engorging blood from the body of a dog.

many Acanthaspidinae and Triatominae (Odhiambo 1958b; Livingstone and Ambrose 1978a, b; Zeledon *et al* 1973), the cryptic body colouration and mimicry of dry twigs and grass stems as in Rhaphidosomatinae and Emesinae are some of the other adaptations of this family for efficient predation.

## 2.3 *Immobilisation of prey*

After prey capture, the behaviour of these bugs is to search for a suitable site for stylet insertion and injection of toxic salivary secretions. The maxillary and mandibular stylets are usually inserted in the pleural membrane of the neck region or at the base of

the anterior legs or at the antennal bases (Haridass and Ananthakrishnan 1980a). When small, like termites and young caterpillars, the prey is lifted off the ground by the rostral tip of the predators to escape the violent encounters with the prey (figure 1F). But in most cases, involving larger prey species like carabid beetles, millipedes and caterpillars, the predation always entails a violent reaction and following unsuccessful salivary injection, the prey often escapes. Under these conditions, predation is attempted quickly for a second time, invariably with success. Often in Ectrichodiinae, improper gripping of the millipede always resulted in the entwinning of the prey around the predator, and the latter allows itself to be dragged for long distances. It is noteworthy that a majority of them, particularly those of Ectrichodiinae, Piratinae, Acanthaspidinae and Reduviinae that encounter such violent reactions from their preys are endowed with tough body coverings to withstand the rough treatment meted out to them during predation. In all cases of successful insertion of stylets and injection of salivary toxins, the prey including larger ones like millipedes, caterpillars and beetles, become totally paralysed and killed within 20–30 sec. The caterpillar feeding Harpactorinae immobilise and kill their prey by stabbing the stylets and by injecting the saliva two to three times in quick succession.

The stylet structure of Reduviidae exhibit a wide range of modifications and evolutionary progression, involving elaborate barbs, teeth and tubercles in their mandibular and maxillary stylets (Cobben 1978). The salivary system of reduviids is also very complex (Baptist 1941; Edwards 1961; Southwood 1955; Haridass 1978) and the anterior lobes of the main glands are concerned with the secretion of neurotoxic substances involved in the paralysis and death of the prey (Haridass and Ananthakrishnan 1981b).

While succumbing to death, millipedes, caterpillars, beetles and ants secrete copious secretions of obnoxious, and irritant exudations like formic acid, *p*-benzoquinones, and other phenolic compounds (Eisner *et al* 1962, 1963; Roth and Eisner 1962). When such repellants are secreted, the immobilisation of the prey is followed by a distinct behaviour of the predators, where they spend considerable time in cleaning the body and antennae, using the fore legs and by rubbing the body surfaces on the ground (Haridass and Ananthakrishnan 1981a).

### 2.4 *Transportation of prey*

Transporting the immobilised prey to a safe place for consumption is yet another distinct unit of the feeding behaviour. By inserting the stylets at suitable places, usually at the bases of the mouth parts, or antennae or anterior legs, the prey is dragged beneath the body as the predator walks, or by pulling the long bodied prey while the predators move backwards (figure 1E).

### 2.5 *Feeding*

Feeding on the immobilised prey is the last unit of the behavioural sequence and it lasts for about $1\frac{1}{2}$ to 2 hr. Since the mouth parts are of the stylet-type, the predigested food from the prey body is flushed out by the watery secretions of the accessory glands, while the posterior lobes of the main glands secrete enzymes for the digestion of the prey

(Miles 1972; Miles and Slowiak 1976; Haridass and Ananthakrishnan 1981b). The salivary glands of haematophagous triatomine bugs secrete anticoagulants to facilitate sucking of large quantity of vertebrate blood without the danger of the blood being clotted (Baptist 1941; Haridass and Ananthakrishnan 1981a, c). While blood feeders engorge from the same feeding site, the predatory forms manipulate the body of the prey with the fore legs and change the feeding sites as and when a particular part of the prey's body contents are emptied (figure 1A) and in this fashion they completely suck out everything leaving behind only the rectal regions and the empty exoskeleton.

Sharing of the food is not observed in any of the adults, though smaller nymphal stages of Ectrichodiinae and Harpactorinae not only share the same millipede or the caterpillar respectively, but also jointly attempt predation of larger prey. Cannibolism is very prevalent in reduviids, the males often succumbing to females and small nymphal stages falling victims to large ones. The feeding behaviour of Triatominae is comparatively less complicated and the host location is by the temperature gradients emanating from the vertebrate host (figure 1G). On locating a suitable feeding site, considerable time is spent in probing with rostral tip and in the sampling of blood. The mechano- and chemo-receptors of the rostrum are believed to get signals from nucleotides of the host's blood about the suitability of food source (Pinet 1968; Bernard *et al* 1970; Friend and Smith 1977). Once selection is made feeding continues from the same site and terminates only after satiation, due to the stretching of the abdominal stretch receptors affecting critical abdominal volume (Maddrell 1963; Anuzel 1972). Nymphs of triatomine bugs also exhibit cannibalism, fully fed older nymphs are attacked by younger ones, the latter pierce the swollen blood filled abdomen of the latter and feed. Such victims do not suffer from any ill effects (Ryckman 1951; Haridass and Ananthakrishnan 1981c).

## 3. Ovipositional behaviour

A wide variety of habitats are colonised by reduviids and an important aspect of their reproductive strategy is the selection of a suitable site for oviposition to ensure successful emergence and development of young ones. Sexually mature females resort to multiple matings, exhibiting either an-end-to-end or a riding type of copulatory posture characteristic of several Heteroptera (figures 2 A, E, G, H and K). Ground dwelling piratine species like *P. affinis*, *E. tibialis*, *E. ochropterus*, *E. cordiger* and *C. brevipennis*, deposit their eggs in the soil (Readio 1926; Miller 1953, 1971), using the plate-like ovipositors. The gravid female assumes a slanting posture with raised head and thorax and with the apex of the abdomen alone touching the ground makes side-to-side, twisting, and downward thrusting movements to insert one egg (figure 2D). The exposed part of the egg (figure 2F) is then covered with small particles of sand and mud by the manipulation of the hind legs. Ectrichodiinae like *H. nigroviolaceous*, *Guionius nigripennis* and *Ectrychotes pilicornis* deposit their eggs in clusters. While the latter two species glue the eggs in the crevices of bark of trees (figure 2C), the females of the former dig slanting tunnels (7–8 cm) in the ground to deposit eggs loosely (figure 2B). After oviposition they spend considerable time to refill the tunnel with excavated mud using mid- and hind legs, and finally press the closed tunnel with the abdomen. Unlike the plate-like ovipositors of the species that insert their eggs in the soil or in crevices, these structures of Ectrichodiinae are very much reduced and stublike. Members of

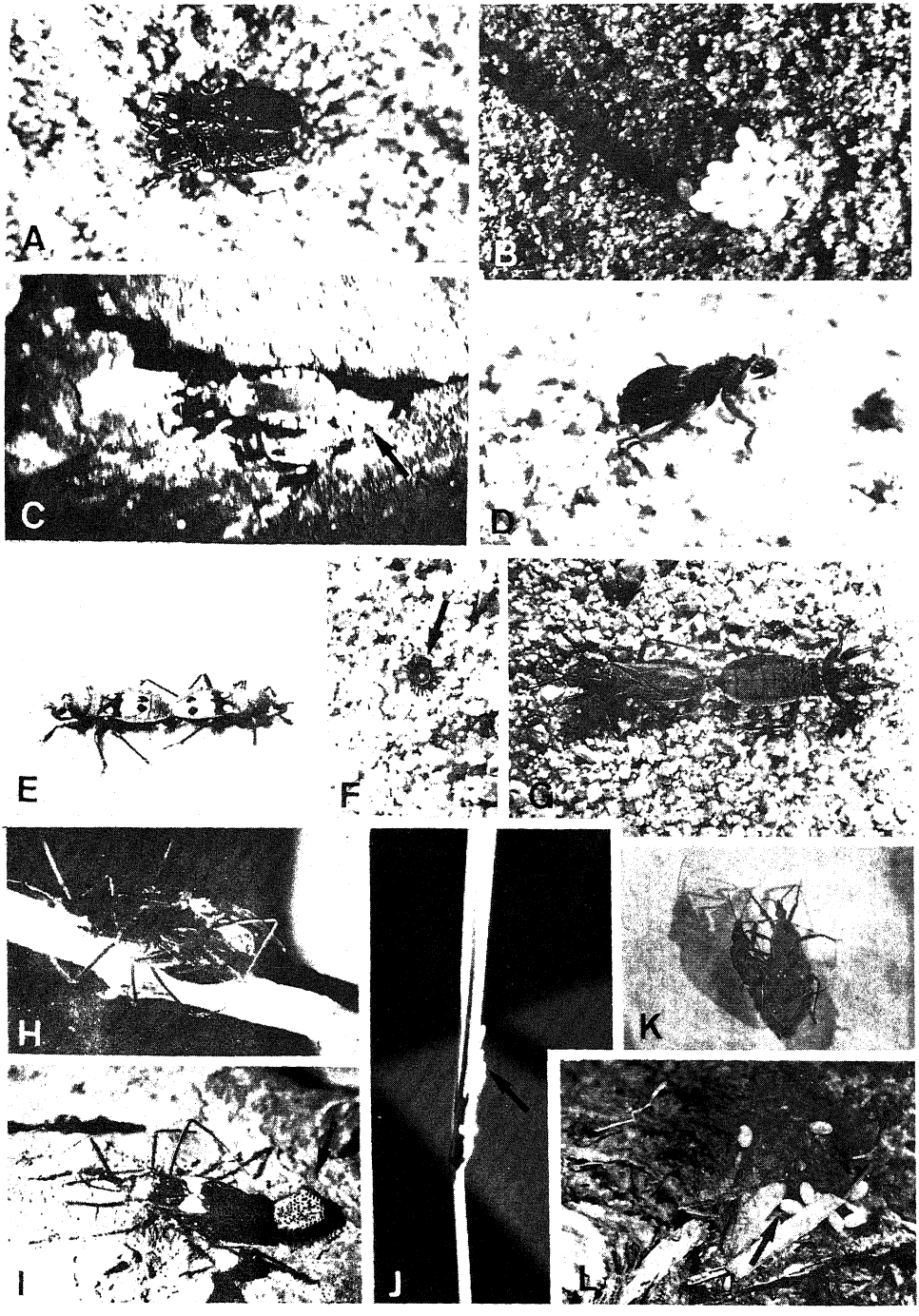


**Figure 2.** *Ovipositional behaviour in some reduviids.* **A.** Copulating adults of *Haematorrhophus nigroviolaceus.* **B.** Loose egg cluster of *H. nigroviolaceus* inside a tunnel excavated in the ground. **C.** *Guionius nigripennis* attaching eggs to the crevices on tree trunk. **D.** *Ectomocoris tibialis* inserting single egg into the ground. **E.** Copulating adults of *Catamiarus brevipennis.* **F.** Oviposited egg of *Pirates affinis.* **G.** Copulating adults of *P. affinis.* **H.** Copulating adults of *Sycannus collaris.* **I.** Compact egg mass of *S. collaris* glued to the surface of tree trunk. **J.** Eggs of *Rhaphidosoma atkinsoni* attached to grass stem. **K.** Copulating adults of *Triatoma rubrofasciata.* **L.** Eggs of *T. rubrofasciata* inserted into the crevices of dry cow-dung cakes

Acanthaspidinae like *Acanthaspis siva*, *A. pedestris*, *A. quinquispinosa* (Fab.) and those of Salyavatinae like *Lizarda annulosa* Stal and *Petalochirus indicus* as well as these of Triatominae like *T. rubrofasciata* and *L. costalis* oviposit the spheroidal or sub-ovate eggs loosely scattering them on the ground, under stones or in crevices (figure 2L). *Rhaphidosoma atkinsoni* found usually among grasses, glue the bases of the flask shaped eggs to the stems, the eggs projecting at an angle (figure 2J). This oviposition is very similar to those already described for other Rhaphidosomatinae (Miller 1953).

The most elaborate ovipositional behaviour is exhibited by species of Harpactorinae and this is known for a large number of forms (Kershaw 1909; Muller 1937; Cheriyan and Kylasam 1939; Bose 1951; Wallace 1953; Miller 1953, 1971; Odhiambo 1959; Edwards 1962, 1966; Parker 1969; Nyiira 1970; Swadner and Yonke 1973a, b). The gravid females of *Rhinocoris marginatus*, *Sycanus collaris* and *Sphedanolestes bowringi* Distant attach a cluster of large number of eggs on the under surface of big boulders, or on the stems of trees and plants. While placing the eggs the females work from the margins to the centre of the egg mass always in precise 'chevron' pattern, gluing the eggs in vertical but oblique rows. Each egg is attached to the substratum as well as to the previously laid one giving a polygonal shape to the completed egg mass (figure 2I). The females cover such egg masses with copious secretions from their accessory glands transforming the egg masses into almost an ootheca (Southwood 1956; Miller 1971; Hinton 1981). Though parental care has been reported in many harpactorine bugs (Bequart 1912; Odhiambo 1959; Parker 1965; Miller 1971; Ralston 1977) this has not been noticed in any of the species observed.

The success of the ovipositional behaviour of Reduviidae is evident from the total absence of egg predators for this group as well as from the fact that so far very few egg parasites have been reported for reduviid eggs (Odhiambo 1959; Swadner and Yonke 1973a; Masner 1975; Sankaran and Nagaraja 1975).

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# A behavioural assessment of the impact of some environmental and physiological factors on the reproductive potential of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)

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**Abstract.** Results of a study of certain specific environmental and physiological variables affecting the reproductive activity (specially egg yield and egg hatchability characteristics) of *Corcyra cephalonica* (Stainton) (a pyralid pest damaging a variety of stored edible commodities) are considered, from a behavioural point of view, in this contribution. The environmental cues examined are (a) light, (b) population density, (c) space availability and (d) host presence. The physiological factors tested are (a) age, (b) sex ratio, (c) nutrition and (d) time of mating. The findings obtained in this investigation provide a basis to comprehend more meaningfully the complex, delicate and varied effects produced by these factors on the reproductive performance of this moth in relation to its establishment on jowar, one of the stored products naturally infested by this pest.

**Keywords.** Rice moth; mating; oviposition; egg viability; environmental factors; physiological factors. *Corcyra cephalonica.*

## 1. Introduction

It is well-known that the degree of establishment of an insect on its host is determined not only by its competency to survive and grow but also by its ability to breed on that host. In the rice moth, *Corcyra cephalonica* (Stainton)—a major pest of stored commodities (Piltz 1977)—, despite availability of appreciable amount of useful information on various aspects of nutrition (review of Bhattacharya and Pant 1965; Srivastava and Krishna 1976, 1978), our knowledge concerning the reproduction of this insect, specially with regard to the influence of environmental and physiological factors on mating and ovipositional programmes of the female, is still far from adequate and is mainly derived from the accounts published by a few workers (Seshagiri Rao 1954; Mammen and Vishalakshi 1973; Singh and Sidhu 1975, 1976a, b; Krishna and Narain 1976; Sehgal and Chand 1978; Mishra and Krishna 1979, 1980, 1981; Russel *et al* 1980; Chakravorty and Das 1983a, b). The acquisition of more information in these areas becomes important and necessary for a proper appreciation of the ecological and physiological relationships between the pest and its host. The present communication, based on this objective, specifically includes our observations on the moth's mating, egg laying and egg fertility correlated with photoperiod, space availability, population density, host presence (all environmental), time, age, sex ratio and nutrition (all physiological).

## 2. Material and methods

Newborn caterpillars, obtained from eggs collected from a laboratory stock culture of the rice moth maintained at 23–27°C and 90–95% (relative humidity) on coarsely

ground jowar (*Sorghum vulgare* Pers.) containing 5% (w/w) powdered yeast (Krishna and Narain 1976), were allowed to develop singly, unless otherwise stated, inside muslin-capped glass vials (10 mm diameter; 50 mm height) on similar dietary medium (except in certain specifically arranged trials described herein) into moths for eventual utilization as experimental animals in the various tests included in this investigation.

The basic set up of an oviposition test conducted here consisted of pairing a freshly emerged male and a female individual, both associated with the same experimental regimes, inside a glass container (35 mm diameter; 100 mm height) serving as the oviposition chamber whose top open end was covered by a piece of black muslin held by elastic bands. Number of eggs deposited inside this chamber was monitored daily for 5 days (when the females were generally prolific in their egg laying) and the hatchability of these eggs was also ascertained. All experiments were adequately replicated and were performed within the temperature and humidity ranges selected for maintenance of culture of this insect. Wherever desirable, the data were subjected to appropriate statistical analysis (Paterson 1939) for interpretation. Since these lepidopterans do not feed as adults, no food was provided to them during experimentation.

Specific features of the experimental outlay connected with each variable considered in this enquiry are outlined separately below:

### 2.1 *Effect of photoperiod*

The following light regimes were arranged for a study of this aspect:

(a) 24 hr L : 0 hr D (The illumination provided by a 15 W electric bulb was either white light or blue, green, yellow or red light rendered possible by employing appropriately coloured bulbs of similar wattage)
(b) 0 hr L : 24 hr D.

### 2.2 *Effect of variation in space availability*

Two types of experiments were designed to examine this issue. In the first type, adults of either sex belonging to a pair enclosed in an oviposition chamber were raised singly inside one of the 3 kinds of glass containers whose diameter and height were (a) 10 mm and 50 mm or (b) 35 mm and 100 mm or (c) 70 mm and 90 mm respectively. In the second type, males and females, individually reared in glass vials (10 mm diameter, 50 mm height), were single paired within glass containers (functioning as oviposition chambers in this set up) whose height measured up to 50, 100, 150 or 200 mm although its diameter always remained 35 mm.

### 2.3 *Effect of population density*

Here the moths tested were developed from individually reared caterpillars or from those maintained in batches of 5, 20 or 40 individuals per batch for the first 25 days of larval life in glass containers (70 mm diameter, 90 mm height) and later isolated to enable each larva to continue its growth solitarily into an adult in the same container.

### 2.4 *Effect of host presence*

Consideration of this aspect entailed the conduct of two different series of experiments. The first series of tests were arranged with single paired freshly emerged males and females held inside oviposition chambers (as described in the basic set up) within each of which was already placed a small glass tube (15 mm diameter; 50 mm height) covered at the top with black muslin and half filled with normal or water- or ether-extracted jowar supplemented with yeast (host material).

Extraction of jowar, carried out in distilled water or in diethyl ether, was performed separately employing a soxhlet extraction assembly. For each extraction, 500 g of jowar was first properly ground in dry form in an electric grinder to yield the flour. This was later wholly transferred to the soxhlet assembly containing 500 ml of one of the above mentioned solvents and subjected to repetitive extractions within the apparatus to ensure removal of all soluble constituents. The extracted flour was subsequently taken out of the assembly and air-dried to eliminate completely the odour of the employed organic solvent or any excess moisture (in case of water-extracted flour) present in them before placing them as host material inside the glass tube.

Number of eggs deposited by the mated females on the muslin cloth piece covering the main oviposition chamber and on that closing the inner glass tube having yeast-added normal jowar were determined separately. Also, egg output recorded on the cover of this glass tube was compared with egg yields obtained on the same site when the tube contained water- or ether-extracted jowar mixed with yeast. As in previous tests, egg hatchability was ascertained in every case.

The second series of trials, with normal jowar enriched with yeast placed inside the small glass tube, was planned along similar lines to compare the moth's egg output and egg viability at the two oviposition locations between mated females possessing both antennae and those devoid of these head appendages. The technical procedure concerning removal of antennae and time allowed for the females to overcome the post-operational trauma were identical to that reported earlier (Krishna and Sinha 1969).

### 2.5 *Effect of time of mating*

Information into this area of the reproductive biology of this moth was obtained by confining single pairs of newly eclosed male and female adults inside oviposition chambers for one of the three 4 hr periods (1830 through 2230 hr—first quartet; 2230 through 0230—second quartet or 0230 through 0630 hr—third quartet) on the first day and continued similarly for 4 successive days. The reason for choosing the nocturnal part of the daily time-cycle to hold these insects in "couples" is because of the observed pronounced high sexual activity of both sexes leading to mating, like in a number of other lepidopteran members (Engelmann 1970), during the night hours. For the remaining part of the normal day-night rhythm, when the sexes were not paired, they were, however, allowed to enjoy the company of each other only through a metallic wire-mesh partition installed in a manner basically identical to that reported for *Earias fabia* (Shahi and Krishna 1979) or for *Tribolium castaneum* (Singh and Krishna 1980). In addition to procurement of egg output and egg viability data, continuous observation was made to record the number of matings and the length of each copulatory act per female during a quartet of every experimental day when the insects were in the paired state.

### 2.6 *Effect of age*

Mating potential and subsequent reproductive performance of females were assessed by pairing a newly emerged male or female moth with an adult individual of the opposite sex belonging to one of the following ages (expressed in days counted from emergence): 0, 3 and 6.

### 2.7 *Effect of sex ratio*

There were 6 distinct sex ratio groups with two densities of 1 : 1 sex ratio (1 male : 1 female; 1 male : 5 females; 2 males : 4 females; 3 males : 3 females; 4 males : 2 females and 5 males : 1 female) on which this study was based. In all the tests conducted here, the glass container functioning as oviposition chamber was a relatively larger one (40 mm diameter; 200 mm height).

### 2.8 *Effect of nutrition*

The reproductive competency of this moth was evaluated separately in relation to its single rearing on (a) yeast-added normal jowar or (b) one of the two different strains of rice IR 8 or IR 20 enriched with yeast or (c) normal jowar fortified with yeast up to the first 15 days of the caterpillar's life and later on jowar extracted with water, 100% ethanol, chloroform or diethyl ether and then supplemented with yeast. Extraction of jowar with one of these solvents was carried out as mentioned earlier.

## 3. Observations

### 3.1 *Effect of photoperiod*

Complete darkness or coloured lights providing red or yellow illumination in place of only blue facilitated *C. cephalonica* females, subjected to variable light experiences, to release significantly higher number of total and viable eggs at the same time ($P < 0{\cdot}01$ or $< 0{\cdot}05$) (table 1). Nevertheless, a 24 hr scotophase situation specially favoured, amongst all the light regimes tested here, a marked augmentation in egg hatchability in these females ($P < 0{\cdot}01$).

### 3.2 *Effect of variation in space availability*

Females produced from larvae and pupae raised individually on jowar supplemented with yeast inside glass containers (70 mm diameter; 90 mm height) laid significantly greater number of total and viable eggs than single-reared counterparts whose post embryonic development occurred in relatively smaller containers (35 mm diameter; 100 mm height or 10 mm diameter; 50 mm height) ($P < 0{\cdot}01$) (table 2).

From the account given above, it is clear that provision of greater accommodation to the rice moths during their postembryonic development enables emergence of females

**Table 1.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* held on various light regimes during their adult lives (data pooled from five females)*.

| Light regimes | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| DD | 216·6 a | 172·8 a |
| LL | | |
| (i) Red | 223·0 a | 104·2 b |
| (ii) Yellow | 204·8 a | 92·6 b |
| (iii) White | 183·6 ab | 73·8 bc |
| (iv) Green | 152·2 b | 73·0 bc |
| (v) Blue | 150·4 b | 45.0 c |
| Mean | 188·4 | 93·6 |
| LSD (1%) | 71·3 | 44·8 |
| (5%) | 52·6 | 33·1 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the least significant difference (LSD) test.

DD = Continuous Darkness; LL = Continuous Light.

**Table 2.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* raised on jowar supplemented with yeast in glass containers of varying dimensions (data pooled from five females)*.

| Dimensions of glass container (in mm) | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| Diameter × Height | | |
| 70 × 90 | 499·0 a | 433·6 a |
| 35 × 100 | 388·4 b | 320·6 b |
| 10 × 50 | 336·8 b | 272·0 b |
| Mean | 408·1 | 342·1 |
| LSD (1%) | 72·6 | 81·2 |
| (5%) | 51·8 | 57.9 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% level by the LSD test.

whose egg output and egg hatchability become decidedly greater. Will alterations made in spatial accommodation only during the adult lives of these moths affect the reproductive capacity and, if so, to what degree? Answers to these questions were also obtained in this investigation. Males and females housed in glass containers having maximum vertical space facility (200 mm height) resulted in females depositing only slightly higher mean number of total and viable eggs which, based on aggregate egg scores relating to both these determinations, were about 12% and 10% respectively more than the values obtained similarly for females coupled with males and enclosed in containers whose height measured up to only 50 mm (figure 1).

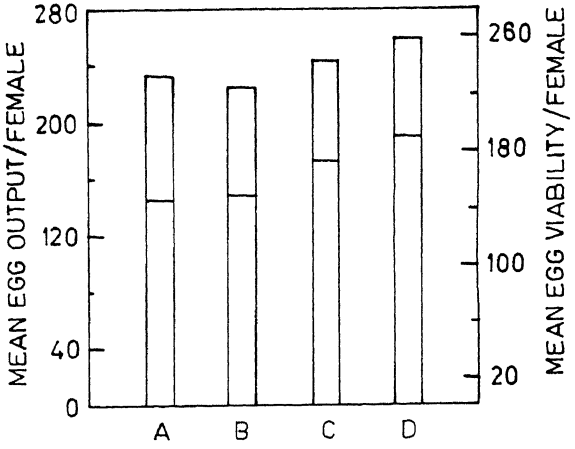


**Figure 1.** Histogrammic representation of mean total and viable eggs laid by *C. cephalonica* in relation to confinement of both sexes during their adult lives in glass containers having uniform diameter (35 mm) but of varying heights (data based on 5 replicates per test): A – 50 mm; B – 100 mm; C – 150 mm; D – 200 mm. Full height of each bar in the figure represents mean number of total eggs laid/female. Level of horizontal line within the body of each bar shows the value pertaining to the mean number of viable eggs laid/female.

### 3.3 *Effect of population density*

Egg deposition and egg hatchability progressively decreased when these pyralids, instead of being reared singly all through, were allowed to develop, even in the midst of abundant food provisions, in groups of 5, 20 or 40 larvae per batch for the first 25 days of the caterpillars' lives and subsequently individually up to the eclosion of adults (figure 2).

### 3.4 *Effect of host presence*

Number of total and viable eggs laid by *C. cephalonica* on the cloth covering the small glass tube holding the larval food and kept within the larger glass container (oviposition chamber) were significantly higher than what were recorded on the muslin roofing the latter ($P < 0{\cdot}01$) (table 3). However, oviposition by these moths on the cloth of the diet tube and fertility of these eggs sharply fell when the tube contained ether- instead of water-extracted or normal jowar ($P < 0{\cdot}01$ or $< 0{\cdot}05$), though between the latter two test conditions egg hatchability values were significantly different ($P < 0{\cdot}05$) (table 4).

The selection of the cloth binding the diet-filled glass tube for laying more viable eggs by the rice moth is evidently a behavioural phenomenon exhibited by females whose antennae were intact. It is likely that these head appendages might bear certain chemoreceptors, as in *Plodia interpunctella* (Deseo 1976), guiding the females to arrive at the site nearest to food odour source for greater oviposition. This aspect was also considered in the present enquiry. Removal of antennae in females resulted in their

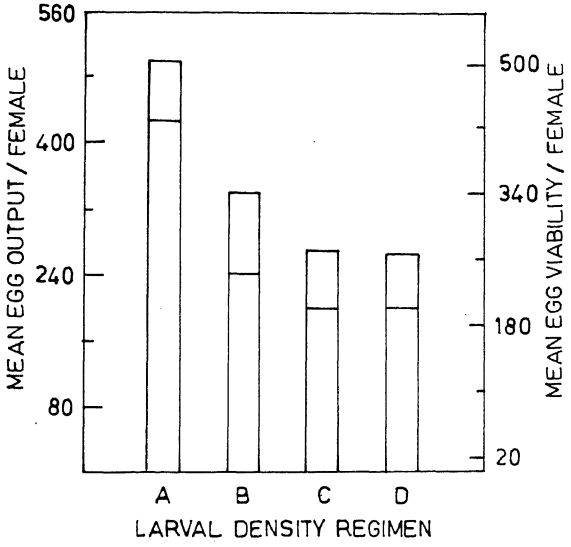


**Figure 2.** Histogrammic representation of mean total and viable eggs laid by *C. cephalonica* in relation to certain rearing regimes (data for each bar pooled from 5 females). A – Adults of both sexes developed from single-reared larvae. B, C and D – Adults of both sexes developed from larvae kept together in lots of 5, 20 and 40 members per group respectively for the first 25 days after which the caterpillars were always allowed to grow individually. For explanation of other features pertaining to the histograms, see legend for figure 1.

**Table 3.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* on muslin cloth pieces covering separately the larger glass container and the diet-containing (normal jowar supplemented with yeast) small glass tube kept within the former (data pooled from five females).

| Oviposition site | Mean number of total eggs laid (± SE) | Mean number of viable eggs laid (± SE) |
|---|---|---|
| On muslin cloth piece covering the larger glass container | 18·8 ± 9·81* | 11·6 ± 6·36* |
| On muslin cloth piece covering the small glass tube holding diet | 124·4 ± 20·58 | 80·8 ± 15·68 |

*Significantly different at 1% level (*t*-test) from the value just below in the column.
SE = standard error.

having no specific preference for deposition of total and fertile eggs on the cloth covering the diet tube or on the top muslin closing the larger oviposition chamber ($P > 0{\cdot}05$) (table 5).

### 3.5 *Effect of time of mating*

Results concerning the effects of time-related copulations during the scotophase part of the daily light-dark cycle on oviposition and egg hatchability are summarized in table 6.

**Table 4.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* on muslin cloth piece covering the small glass tube containing normal or variously extracted jowar supplemented with yeast (data pooled from five females)*.

| Yeast-supplemented diet within small glass tube | Mean number of total eggs laid | Mean number of viable eggs deposited |
|---|---|---|
| Water-extracted jowar | 168·2 ab | 124·4 a |
| Normal jowar | 124·4 b | 80·8 b |
| Ether-extracted jowar | 54·0 c | 34·8 c |
| Mean | 115·5 | 80·0 |
| LSD (1%) | 72·6 | 53·0 |
| (5%) | 51·8 | 37·8 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the LSD test.

**Table 5.** Estimates of oviposition and hatchability of eggs in antennectomised females of *Corcyra cephalonica* on muslin cloth pieces covering separately the larger glass container and the diet-containing (normal jowar supplemented with yeast) small glass tube kept within the former (data pooled from five females).

| Oviposition site | Mean number of total eggs laid (± SE) | Mean number of viable eggs laid (± SE) |
|---|---|---|
| On muslin cloth piece covering the larger glass container | 59·8 ± 14·59 NS | 35·4 ± 10·95 NS |
| On muslin cloth piece covering the small glass tube holding diet | 123·6 ± 23·60 | 83·2 ± 16·56 |

NS—Mean values in a column not significant from one another.
SE = standard error.

**Table 6.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* subjected to different time-related mating schedules (data pooled from five females).

| Time period (hr) | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| 1830–2230 | 119·4 | 63·6 |
| 2230–0230 | 175·2 | 132·2 |
| 0230–0630 | 143·6 | 79·2 |

Females which mated during the 4 hr interval (2230 through 0230 hr) falling in the late night period, interestingly, deposited the highest number of total and fertile eggs, while those which completed this sexual function in the early scotophase (1830 through 2230 hr) were least productive with respect to both yield and viability of eggs.

A female was also often found capable of mating, within the 5-day tenure of the experiment, twice with the same male paired with her and, occasionally, even thrice (as in the case of one individual) (figure 3). The duration of an individual mating act was quite brief and ranged between 2 and 8 min. For each quartet, there were varying proportions of matings between pairs possessing different temporal lengths (table 7). Majority of the coitus sessions lasted for 2 or for 5 min.

### 3.6 *Effect of age*

Males or females as old as 6 days, except in very few instances, courted and mated with freshly emerged individuals of the opposite sex. Newborn mated females showed an almost equal competency to unload eggs when copulated with 0, 3 or 6-day old males although the proportion of fertile eggs released by them was largest when such individuals were sexually united with freshly enclosed males (table 8).

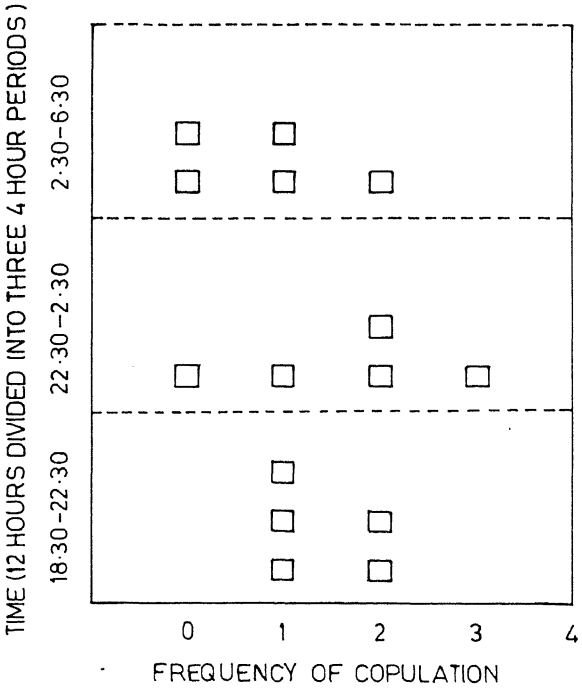


**Figure 3.** Schematic diagram showing the number of matings during each of the three quartets of a 12 hr period in 5 separately arranged pairs of males and females of *C. cephalonica* in a 5-day test period. Each squarish symbol within a segment above the corresponding mating number (given in abscissa) in the diagram represents the datum concerning frequency of copulation of a female individual during the entire test period.

**Table 7.** Frequency distribution for length of mating period in total matings *C. cephalonica**.

| Experimental day (counted from pairing day) | Total matings | Length of mating period (in min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | 4 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| 1 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| | 3 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 |
| 2 | 4 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 |
| | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Entire data based on observations collected from 15 mated pairs (5 tested for each quartet separately), a single pair constituting an independent replicate.

Note: (i) Upper number of each entry for every experimental day refers to mating(s) in the 5 pairs tested during the 1st quartet (1830–2230 hr).
(ii) Middle number of each entry for every experimental day refers to mating(s) in the 5 pairs tested during the 2nd quartet (2230–0230 hr).
(iii) Lower number of each entry for every experimental day refers to mating(s) in the 5 pairs tested during the 3rd quartet (0230–0630 hr).

**Table 8.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* subjected to different age-related mating schedules (data pooled from five females).

| Age (in days) at the commencement of mating | | Mean number of total eggs laid | Mean number of viable eggs deposited |
|---|---|---|---|
| M | F | | |
| 0 | 0 | 263·4 | 142·4 |
| 0 | 3 | 23·2 | 3·8 |
| 0 | 6 | 7·8 | 3·3 |

### 3.7 *Effect of sex ratio*

Total egg output and egg viability estimates reached highest levels when the sex ratio between males and females was maintained at 1:5 and the mean values significantly surpassed all others obtained in tests conducted with males and females held in varying numbers ($P < 0{\cdot}01$) (table 9).

### 3.8 *Effect of nutrition*

Females raised completely on normal jowar fortified with yeast laid significantly more number of total and viable eggs than those whose postembryonic development took place on yeast-mixed normal jowar up to the first 15 days and later on jowar extracted with water, chloroform or 100% ethanol and then reinforced with yeast ($P < 0{\cdot}01$) (table 10). Egg hatchability further pronouncedly increased if these females reared initially on normal jowar were continued to grow from the 16th day of their larval lives on yeast-supplemented ether- instead of water-, chloroform- or 100% ethanol-extracted jowar ($P < 0{\cdot}01$ or $< 0{\cdot}05$). If, on the other hand, rice of a particular strain IR 20 in place of IR 8 supplemented with yeast became the food instead of normal jowar in the rearing medium of these moths since their birth, the productivity of females in terms of egg output and egg viability became significantly poorer ($P < 0{\cdot}01$ or $< 0{\cdot}05$) (table 11).

## 4. Discussion

This investigation has brought to the fore several unexplored or insufficiently known and, nonetheless, important environmental and physiological factors affecting mating, oviposition and egg viability in the reproductive biology of *C. cephalonica*. They are

**Table 9.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* held on different sex ratio regimes (data pooled from five sets of experiments for each sex ratio)*.

| Sex ratio M F | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| 1 : 5 | 1321·2 a | 866·8 a |
| 2 : 4 | 850·8 b | 548·4 b |
| 3 : 3 | 605·6 c | 421·4 bc |
| 4 : 2 | 573·2 c | 468·4 bc |
| 1 : 1 | 398·0 d | 321·2 c |
| 5 : 1 | 333·8 d | 275·6 c |
| Mean | 680·4 | 483·6 |
| LSD (1%) | 196·2 | 299·6 |
| (5%) | 144·8 | 221·1 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the LSD test.

**Table 10.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* maintained on certain prescribed dietary schedules during the insect's postembryonic development (data pooled from five females)*.

| Diet + 5% yeast | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| Normal jowar all through | 410·8 a | 364·0 a |
| Normal jowar for the first 15 days followed by: | | |
| (i) ether-extracted jowar | 369·0 ab | 333·0 a |
| (ii) 100% ethanol-extracted jowar | 309·0 bcd | 252·2 b |
| (iii) water-extracted jowar | 291·6 c | 175·6 c |
| (iv) chloroform-extracted jowar | 269·8 cd | 152·8 c |
| Mean | 330·0 | 255·5 |
| LSD (1%) | 96·5 | 95·6 |
| (5%) | 70·8 | 70·1 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the LSD test.

**Table 11.** Estimates of oviposition and hatchability of eggs in *C. cephalonica* raised completely on normal jowar or on one of two different strains of rice fortified with yeast (data pooled from five females)*.

| Diet + 5% yeast | Mean number of total eggs laid | Mean number of viable eggs laid |
|---|---|---|
| Rice IR 8 | 430·8 a | 369·0 a |
| Normal jowar | 410·8 a | 364·0 a |
| Rice IR 20 | 330·8 b | 284·4 b |
| Mean | 390·8 | 339·1 |
| LSD (1%) | 98·4 | 99·2 |
| (5%) | 70·2 | 70·8 |

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the LSD test.

now considered at length here for a comprehensive appreciation of the problem in relation to the establishment of this pest on stored materials, specially on jowar.

Oviposition and fertility of eggs were conspicuously greater in females experiencing total scotophase or a complete photophase condition where red or yellow light prevailed instead of blue. This fascinating finding, apart from motivating further explorations into the role of photoperiod in regulating the intrinsically lodged mechanisms involved in the behaviour and physiology associated with reproduction in *C. cephalonica*, provides a basis to hint at the possibility of achieving appreciable measure of success in reducing the evolution of fresh populations of this pest if stored commodities harbouring a heavy infestation of these moths are exposed to continuously illuminated environment lit by blue light.

Caterpillars raised for the first 25 days of their lives in groups of 5, 20 or 40 individuals per batch even under congenial nutritional conditions in glass containers yielded adult females which, following copulation, were less fecund than counterpart mateds reared individually all through. Presumably availability of relatively more space

for uninterrupted movement within and/or outside the diet medium in the glass containers during the growth period of caterpillars reared singly was a positive factor contributing to their development into healthy moths exhibiting higher reproductive potential. This postulation, emphasizing the importance of the extent of space availability during postembryonic development in relation to the reproductive efficiency of these moths, is further strengthened by the record of greater egg yield and egg viability values from females associated with such individually grown larvae in bigger glass containers. However, imposition of limitation in the availability of space for reproductive males and females, during their adult lives, within the oviposition chamber by progressively lowering only its height did not severely affect the moth's egg deposition or egg viability unlike in the case of another lepidopteran species, *Earias fabia* (Mani and Krishna 1984).

There was pronounced increment in the number of eggs laid and in the hatchability of these eggs when the females of *C. cephalonica* oviposited on a site nearest to the larval food (normal jowar supplemented with yeast). Apparently, the odour of some volatile compounds emanating from this diet seems to have exerted a stimulating influence on the ovipositional behaviour of these lepidopterans—a point missed by Seshagiri Rao (1954) in his observations—favouring them to select the substratum closest to the food. The fact that egg output and egg fertility declined significantly on the same oviposition site when the food comprised ether-extracted jowar enriched with yeast clearly shows the ether-soluble chemical nature of these volatile compounds playing the role of ovipositional stimulants for these insects. The inability of the mated females to continue laying greater number of total and viable eggs, consequent to removal of both antennae, on the cloth (oviposition site) proximal to the larval diet is enough evidence to infer that the smell of these volatile constituents present in the food is olfactorily perceived by chemoreceptors located on these cephalic appendages as in another stored grain moth, *Plodia interpunctella* (Deseo 1976).

A male and a female individual were often successful in entering into sexual union more than once during the 5-day experimental period. Nevertheless, within a prescribed quartet on any single day they performed only one coition. The existence of this multiple mating phenomenon in the reproductive behaviour of the rice moth, while contradicting the observations of Chakravorty and Das (1983a), was, to some extent, similar to that already reported for this species by Sehgal and Chand (1978) and to that observed in certain other stored products insects like *Tribolium confusum* (Good 1933), *Tribolium destructor* (Reynolds 1944), *Trogoderma granarium* (Karnavar 1972) and *Tribolium castaneum* (Singh and Krishna 1980). But the fact that the duration of a single mating act in *C. cephalonica* was short, though not as brief as that recorded in *T. castaneum* (Singh and Krishna 1980), testifies the inherent incapability of these insects to remain in coition for extended periods, notwithstanding provision of facility for these moths to accomplish such a task associated with their reproductive activity. Evidently, these findings raise interesting questions concerning the not yet elucidated extrinsic and intrinsic conditions regulating frequency and duration of mating in this insect.

Highest number of total and fertile eggs were laid by females which were paired with males for mating between 2230 and 0230 hr daily during the 5-day test period. Presumably copulations which occurred during this quartet of the nocturnal period afforded the best opportunity for the females to get maximally stimulated for impregnation upon receipt of the largest amount of viable sperms from the males and

for oviposition. Admittedly, this calls for more detailed investigations in future to examine into the relationship between time and spermatozoa content (quality as well as quantity) in the males of the rice moth and its implication on the reproductive potential of the females subjected to mating at different periods during scotophase of a 24 hr day-night cycle.

Age of male or female insect prolonged up to 6 days from eclosion did not prohibit these moths from mating with freshly emerged individuals. Plausibly in such combination of sexes the ability of the females to produce and release sex pheromones and the competency of the males to perceive these chemical agents remain unaffected by advancing age in the reproductive life of the rice moth. But the occurrence of high levels of egg output by mated females when they were associated from the day of their emergence with newborn or 3- or 6-day old males and the laying of maximum number of viable eggs by these females coupled with just eclosed males suggest that oviposition and egg viability in this lepidopterous pest are possibly regulated by endogenously-based mechanism(s) possessing an interestingly intricate operational relationship with age of males and females at the time of their mating.

Considering the effect of variation in sex ratio in the adult population on the reproductive capacity of *C. cephalonica* females, it was found that a 1:5 ratio between males and females resulted in maximum egg output and egg hatchability by the mated females. This characteristic feature in the reproductive behaviour of the rice moth is quite unlike that reported in another, though taxonomically unrelated, stored grain pest, *Tribolium castaneum* (Lee and Smith 1976) in which, however, egg deposition and egg fertility values associated with the first oviposition period of more or less same duration did not show great disparity among different sex ratios. Egg yield and hatchability also pronouncedly shot up if 4 females were housed with 2 males rather than a single female enjoying the company of a lone male or 5 individuals of this sex. All these observations clearly indicate that the lower the proportion of males the greater would be the reproductive potential of these moths which, in turn, would increase their population levels during a given period of time. This relationship between the population density of males and productivity in *C. cephalonica* could be of applied significance from the standpoint of pest management if a pesticide was used that killed a higher proportion of females than males—a hypothesis different from that put forth by earlier workers (Shorey 1970; Otake and Sukuratani 1972; Otake and Oyama 1973; Kehat and Gordon 1975, 1977) wherein the necessity of drastic reduction in male population for achievement of economic control of the female's reproduction in lepidopterous pests was emphasized.

With regard to the impact of varying larval nutrition on the reproductive potential of the rice moth, it is clear from the results that normal jowar or rice of a particular strain IR 8 enriched with yeast provided right from the first day during the caterpillar stage facilitated the mated females to be more productive than all other prescribed dietary schedules though females reared initially on normal jowar combined with yeast up to the first 15 days and subsequently on yeast-supplemented ether-extracted instead of water- or chloroform-extracted jowar also showed marked augmentation in their egg output and egg hatchability. The precise nutritional factors resident in jowar and IR 8 rice strain and their involvement in regulating the physiological mechanisms (most likely neuroendocrinal as in several other insects (Wigglesworth 1960; Engelmann, 1970)) connected with reproduction are worth examining in follow-up studies in this insect.

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# Behavioural analysis of feeding and breeding in Orthopteran insects

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**Abstract.** Various aspects of the feeding and breeding behaviours in Orthoptera with special reference to Acridoidea and Tetrigoidea are discussed. The changes in the incisor and molar mandibular surfaces, laciniae and galeae of the maxillae, in relation to graminivory, herbivory and omnivory are cited as specific manifestations of the feeding behaviour. Similarly, in sharp contrast to Acridoids the rather poor foregut armature and small and compact feculae in Tetrigoids is suggested as an evidence indicating the correlation between food and feeding habits. While describing the breeding behaviour a generalized comparison of the utilization of the acoustic sexual signals in crickets and grasshoppers causing attraction and copulation or otherwise is made. Differences in the ovipositors, mode of egg-laying and the types of eggs in Acridoids and Tetrigoids are stated as characteristic features of reproductive behaviour. Factors influencing these behaviours in Orthoptera as well as the behaviours bringing about succession and changes in the patterns of life-forms are mentioned.

Tools of behavioural investigations leading to the formulation of ethograms are briefly stated. Methods and techniques generally adopted in studying these aspects of behaviours are referred to as application of such ethological studies. The causative effect of feeding and breeding behaviours is depicted by proposing the adaptive radiation diagrams for the order Orthoptera.

The article, in conclusion, points out certain areas related to these behaviours on which, work would seemingly be useful. For example, determination of the cues that bring about mating in grouse-locusts in the absence of stridulatory and tympanal organs; the energy budget on account of their peculiar diet; and diapause are few such areas. The possibility of these forms turning out to be good models for experimental, lab-oriented studies is suggested. Since, as compared to Acridoids very little studies in the areas of economic and ecological impact in terms of population dynamics have been made on the Tettigonioids and Tetrigoids, it is further suggested that these if undertaken, would also furnish valuable information.

**Keywords.** Ethology; ethogram; trophic and reproductive behaviour; methodology; adaptive radiation.

## 1. Introduction

Feeding and breeding behaviours are of fundamental importance though highly complex in nature. Feeding represents a form of maintenance activities and is, therefore, of an individualistic nature, while breeding constitutes a type of communicatory activity. Together they represent ways of interaction with environment through adjustment: feeding providing the energy source and breeding ensuring survival and continuation of the species.

An attempt is made here to present an overview of the feeding and breeding behaviour of a very large and economically important insect-order, Orthoptera. The *modo et forma* of how this has been done is outlined below.

There are a number of related aspects, such as the role of visual, chemoreceptory, olfactory, gustatory cues; that are essential in finding and recognition of the food before the actual act of feeding. Similarly the nervous, hormonal and pheromonal factors are known to particularly influence the insect breeding behaviour and also phase

polymorphism in locusts. Together with abiotic factors, the hormones also probably influence the phenomenon of diapause. The manifestation of feeding and breeding behaviours in these Orthopteran insects is outlined through some representative structural and functional adaptations. In doing so the term Orthoptera, *sensu stricto* means Acridoids (grasshoppers and locusts) in general and the Tetrigoids (grouse-locusts) in particular. While referring to Tetrigoids four species of family Tetrigidae; namely, *Euscelimena harpago* Serville, *Eucriotettix flavopictus* Bolivar, (Subfam. Scelimeninae); *Euparatettix personatus* Bolivar (Subfam. Tetriginae) and *Potua sabulosa* Hancock (Subfam. Cladonotinae) are considered representative forms (figures 1A–C). These none too studied but, very interesting forms allied to Acridoids, are being investigated at this Centre. While describing the spacio-temporal manifestations of these behavioural aspects through proposed adaptive radiation and interrelationships, the term Orthoptera is used *sensu lato*. The account of related methodology and adaptive radiation has been presented in the form of schematic representations both for brevity and clarity.

## 2. Review of literature

### 2.1 *Food and feeding behaviour*

Studies on food habits and biology of Acrididae by Gangwere *et al* (1976); on food selection in Orthoptera together with feeding behaviour by Freeland (1975), Gangwere (1961), Gangwere and Agacino (1973), Gangwere and Ronderos (1975) and Mulkern (1967, 1969); on effect of specific food on growth by Bajoi and Knutson (1977); on food preferences by Lambley *et al* (1972); on regulation of food intake by Bernays and Chapman (1974); on host finding and food availability modifying the feeding behaviour, as well as studies on succession in grasshoppers by Gangwere (1972); on host related responses by Browne (1977) and Mitchell (1975); on chemosensory responses by Schoonhoven (1977); on the observations of a monophagous grasshopper by Knutson (1982); on the structural adaptations of the mouthparts by Gangwere (1965), Isely (1944) and Muralirangan (1978); on foregut morphology, its armature as an adaptation to food preference, as well as the taxonomic significance of foregut armature by Muralirangan (1980) and by Muralirangan and Ananthakrishnan (1974, 1981); on control through feeding with use of deterrents by Munakata (1977) and wheat bran bait with chemical and biological agents by Onsager *et al* (1980, 1981); on population ecology and energetics by Delvi and Pandian (1971, 1972, 1979), Hoekstra and Beenakkers (1976), Khan and Aziz (1976), Muralirangan and Ananthakrishnan (1981), Muthukrishnan and Delvi (1973, 1974) and Onsager (1983) are quite informative.

### 2.2 *Different aspects of breeding behaviour*

Studies on the life history strategies in insects by Dingle (1974); on endocrine research in Orthopteran insects by Penner (1983); on sequential analysis of insect behaviour by Richard (1974); on hormones and insect behaviour by Riddiford and Truman (1974); on acoustic and courtship behaviour by Otte (1972) and Loher and Chandrashekaran (1972); on chorusing flight by Willey (1979) are of interest. Observations by Cantrall (1979), Gangwere (1964–65), Mores (1904) and Otte (1979) also appear useful.

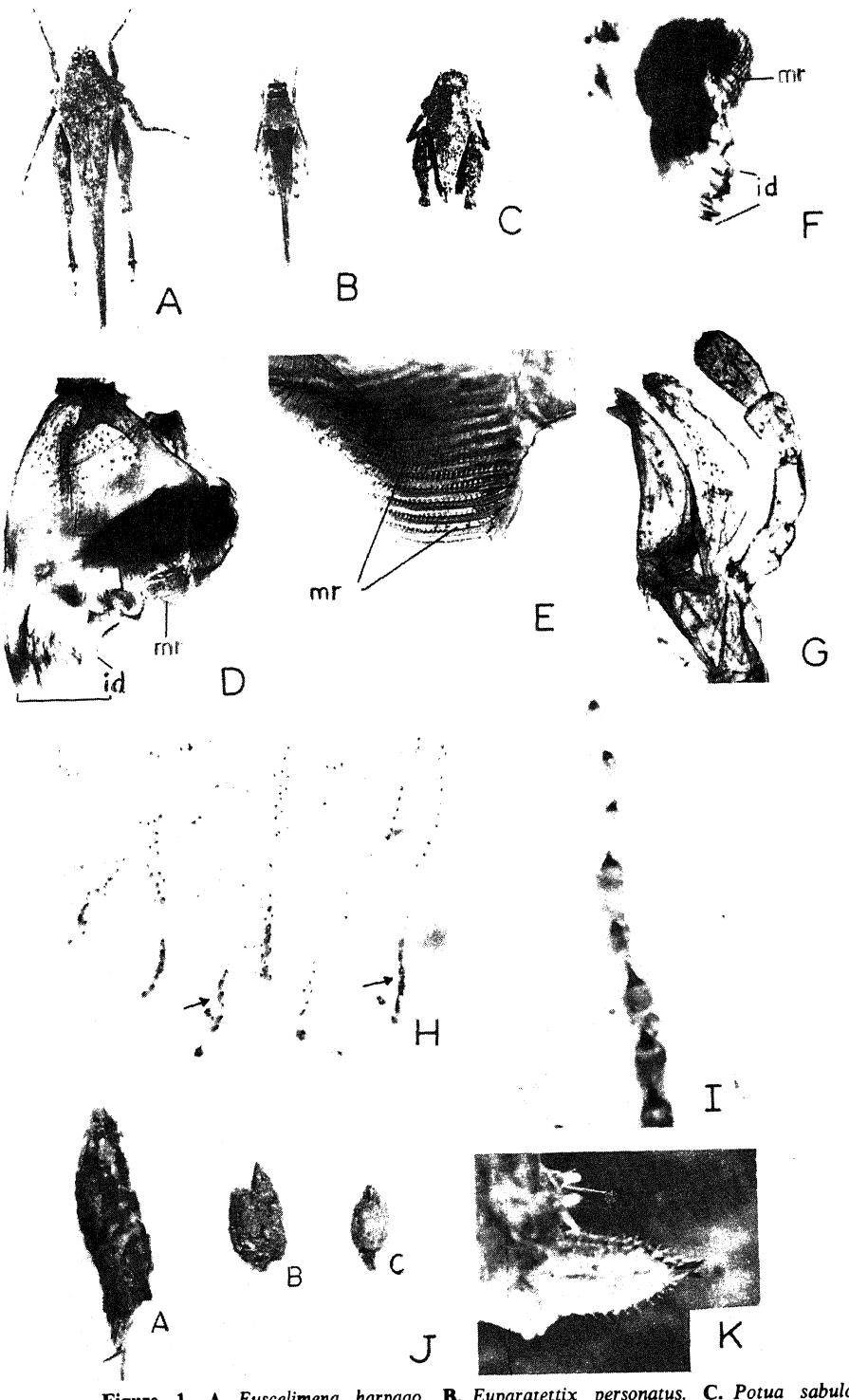



**Figure 1.** **A.** *Euscelimena harpago.* **B.** *Euparatettix personatus.* **C.** *Potua sabulosa.* **D.** Mandible of *Euscelimena*, with blunt incisor dentes (id) and the molar ridges (mr). **E.** Details of molar ridges in *Euscelimena*. **F.** Mandible of Tetriginae showing sharp incisor dentes (id). **G.** A typical Tetrigoid maxilla. **H.** Foregut armature in *Euparatettix*. **I.** Details of the foregut armature. **J.** Feculae of an Acridoid (A), *Euscelimena* (B) and *Potua* (C). **K.** A typical Tetrigoid ovipositor.

### 2.4 *Books*

Those of topical interest as far as ethology is concerned are by Eibl-Eibesfeldt (1970), Huntingford (1984), Grzimek's encyclopedia of Ethology (1977), Tavolga (1969) and Wallace (1979). The books referring to the various aspects related to feeding and breeding behaviour of insects in general and Acridoids in particular by Bei-Bienko and Mischenko (1951), Chapman (1973), Cummins *et al* (1965), Friedlander (1978), Frost (1959), Gillot (1980), Matthews and Matthews (1978), and the two volumes on grasshoppers and locusts by Uvarov (1966, 1977) are invaluable.

### 2.5 *Work on Tetrigoidea*

Compared to grasshoppers and locusts there are relatively few studies carried out on the different aspects of grouse-locusts, including feeding and breeding behaviour. However, those referred herein are by Bhalerao and Paranjape (1982, 1984), Hodgson (1963), Hancock (1898, 1902, 1906–1916), Hartley (1962), Karandikar and Paranjape (1964), Kevan (1982), Nabours (1919), Nabours and Stebbins (1950), Paranjape (1976), Paranjape and Bhalerao (1984), Poras (1979), Rehn and Grant (1961) and Sabrosky *et al* (1933). Some, so far unpublished data of this Centre is also included in this article.

## 3. Material and methods

The feeding and breeding behavioural studies involve a variety of approaches. These in turn contribute in ways more than one, to the development of an ethological profile or ethogram for a particular species. A representative form of various steps is presented in table 1.

## 4. Discussion

### 4.1 *Feeding behaviour*

The Orthopteran biting-chewing mouthparts in general and the mandibles and maxillae in particular show structural adaptations in consonance to the feeding habit and have been an object of study indicating the feeding behaviour of these insects (table 1). The degree of asymmetry allowing the left mandible to overlap the right one when closed, the molar and incisor surfaces together with their teeth (dents or dentes) of the mandibles and the laciniae together with their recurved teeth (dents or maxadentes) and galeae of the maxillae are particularly important in this connection.

As the acridoids are known not to be polyphagous, but at the same time showing considerable, species-related selection and variation in the range of phytophagy, these tend to show some broad groups of mandibular patterns indicating adaptive radiation in relation to the feeding behaviour (table 5). The nomenclature of the patterns is, however, not unanimous and furthermore, while discussing Acridoid feeding behaviour the term omnivorous is used by some workers synonymous to polyphagous, meaning thereby feeding on 'everything green'. However, as the Orthoptera is under

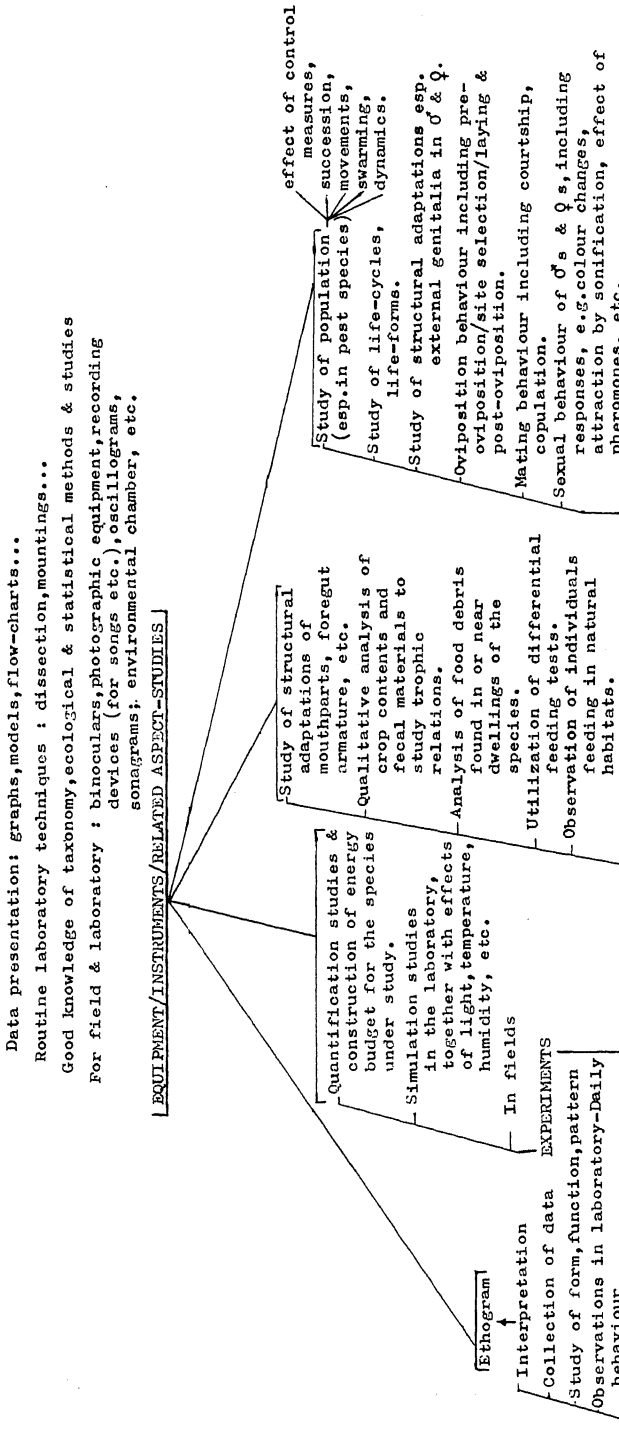



**Table 1.** Tools/methods/techniques necessary in feeding and breeding behavioural studies (ref. Orthoptera).

discussion as a group, the terms herbivorous (feeding on plants) and omnivorous (*i.e.* feeding on plants as well as animals) are used in a broader sense, unless specified otherwise. Thus those feeding mainly on grasses and similar type of plants show graminivorous type of mandibles (figure 2A). These overlap but slightly and have the incisor regions represented typically by prominent parallel ridges (cusps or teeth) which often tend to fuse to develop a cutting edge; while the molar regions show somewhat flattened ridges and furrows useful for grinding the food. The forms that feed mainly on broad-leaved dicotyledonous plants show herbivorous (= forbivorous according to

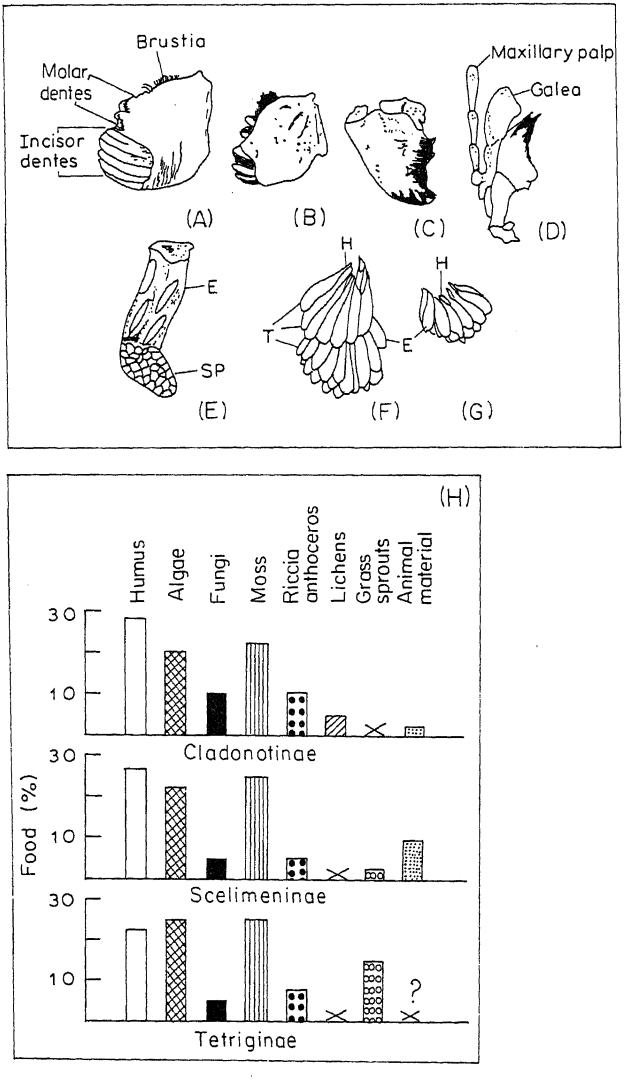



**Figure 2.** **A.** Graminivorous mandible. **B.** Herbivorous mandible. **C.** Carnivorous mandible. **D.** Maxilla of a marginal feeder. **E.** Partly exposed piece of a spongy (SP) egg-pod of an Acridoid. **F.** Eggs (E) of *Euscelimena* arranged in tiers (T) Note prominent horns (H). **G.** Eggs of *Potua* arranged in a cluster. **H.** Relative acceptance of foods by subfamilies of Tetrigoidea.

some workers) type of mandibles (figure 2B). These overlap to a greater degree and show dentes instead of ridges; those of the incisor regions are pointed while those of the molar surfaces are subconical. The forms that are capable of feeding both on grasses and herbaceous plants show a range of mandibular patterns of intermediate nature. These are called ambivorous (= herbivorous according to some) types of mandibles. In some forms, the feeding is mainly on shrubs, woody shrubs and on foliage of trees. The mandibles in such cases are stouter, with sharp dentes and each molar surface bearing varying degree of concavity for grinding. Such arborivorous mandibles are variously called forbivorous and dendrophagous types representing modified herbivorous condition. The carnivorous Orthoptera, e.g. the mantis, possess mandibles that are elongated, hook-like and bearing sharp dentes (figure 2C). On the other hand omnivorous forms possess mandibles that are equipped with somewhat sharp dentes, mostly uniform in length on the incisor surface, while the molar surface (figure 1D) is without dentes but bearing characteristic ridges. The ridges are near parallel, transverse, closely placed, delicately denticulate, and alternating with grooves (figure 1E). The mandibles of grouse-locusts are of omnivorous type. Our observations on the three subfamilies namely Scelimeninae, Cladonotinae and Tetriginae reveal interesting variations when compared amongst each other on one hand and the Acridoids on the other. For example, in *Euscelimena* the incisor dentes are not sharp but in *Eucriotettix* they are sharp. Further the incisor dentes are more sharp and somewhat irregular in Tetriginae (figure 1F) than in Cladonotinae. However, in all the types, the molar region bears the parallel 'ridge-groove' pattern mentioned earlier and is very unlike the 'ridges and furrows or dentes' pattern of the Acridoids in general.

The maxillae also show adaptations in relation to the feeding habits. These features are, however, less predominant and variable as compared to those of the mandibles. The lacinial dentes are rather blunt in graminivorous and dendrophagous Acridoids. The dentes are however long, sharp and somewhat curved in forbivorous, carnivorous and omnivorous types. The galeae in 'margin-feeding' graminivorous forms are more flattened than tubular (figure 2D). On the other hand, the galeae are of lobular type in forms that are 'centre-feeders'. The Tetrigoid maxillae have somewhat long, curved, pointed lacinial dentes giving the lacinia a dinner fork-like appearance and lobular galeae helping while feeding on pulpy food and fluids (figure 1G).

The diet of Tetrigoids is basically different and varied as compared to that of Acridoids. The grouse-locusts feed mainly on algae, mosses, molds, lichens, humus, detritous material and ingest superficial soil too along with the food. The grouse-locusts are observed to frequent marshy places, paddy fields and to feed on grass and other cereal sprouts in the fields as well as in the laboratory. Tough-textured leaves of monocot and dicot plants are, however, not preferred by the grouse-locusts. *Euscelimena* is observed to feed even on tender *Eichhornia* leaves. Some species from Scelimeninae and Cladonotinae also show scavenging tendencies and feed on soft parts such as the abdomen of dead and decaying insects including their own species. Thus some grouse-locusts show necrophagy but cannibalism has not been observed. Another interesting peculiarity observable in grouse-locusts feeding on green carpets of moss is that, the mode of feeding tends to be similar to grazing. This results into almost complete wiping out and cleaning of the small patch of substratum on which the moss is seen before feeding.

It is for these reasons that these insects have the peculiar well-developed denticulate ridges and grooves on the molar region of the mandibles. The incisor dentes of the

mandibles help in biting, cutting and nibbling whereas during feeding molar surfaces of the two mandibles and the surface of the left overlapping on that of the right mandible, help in cutting further the algal filaments together with such other food, into smaller fragments. At the same time the mandibles presumably help in pressing, packing and filtering the pulpy food, humus, and fluid matter rather than acting as jaws that chew or grind the food, as in most Acridoids. The sharp, fork-like lacinial dentes and lobular galeae help in feeding and centrally pushing the food into the pre-oral cavity. Regarding the food and feeding behaviour of Tetrigidae, it can be said that, as compared to Acridoidea, the grouse-locusts show herbivory through omnivory, in the general sense of these terms as indicated earlier. The relative acceptance of the food in the three subfamilies of Tetrigidae is given in figure 2H. Other methods of study of the feeding behaviour (table 1) such as, the study of crop contents and feculae also support these observations. The difference in the usual pattern of feculae in the adults of graminivorous Acridoids and ominivorous-herbivorous Tetrigoids can be easily made out from figure 1J.

The foregut armature which is widely studied in locusts and grasshoppers such as *Locusta*, *Schistocerca*, *Eyprepocnemis*, *Cyrtacanthacris* is yet another feature that has been considered mainly from taxonomic point of view and is also believed to be mechanically helping the processing of food in Acridoidea. Our studies on grouse-locusts indicate absence of spines as well as cuticular folds in *Euscelimena* but presence of scanty longitudinal rows of sclerotized, spiny elements in the posterior region of the crop in *Eucriotettix* and *Euparatettix* (figures 1H and I). But in all grouse-locusts the proventicular valve bears numerous, delicate and minute teeth. This poor development of foregut armature observable in grouse-locusts is presumably because, the soft, pulpy, food present in the shortened foregut might not be requiring the mechanical treatment as required in locusts and grasshoppers.

There are a number of factors that influence the feeding behaviour of grasshoppers in general. These can be briefly enumerated as under:

Abiotic factors such as light, humidity, wind, air, ground-level heat and seasonal variations influence feeding behaviour. Similarly the type of habitat, the nature and density of vegetation, the changes in pattern of vegetation due to topographic changes, man's activities and such other factors can not only alter food habits on the basis of availability but also lead to changes in grasshopper associations and succession of which the feeding behaviour forms an important basis. The feeding behaviour is also influenced by physical characteristics of food material such as toughness and turgidity of the plant tissues and chemical constituents that cause the food to be a stimulant or deterrent. The physiological state of the insect such as of hunger, thirst, satiety, injury, disease as well as its state of development such as hatching, moulting, maturation of gonads and the state of reproduction can also influence or modify the feeding behaviour. As compared to the extensive observations and work carried out on Acridoidea, much remains to be understood as far as the influence of these aspects on the feeding behaviour in Tetrigoidea is concerned.

There are certain other aspects that are related to the food and feeding behaviour. For example, the types of food-plants can have profound effect on the fecundity, the rate of completion of the life-cycle, number of instars and also on the growth of wings as has been indicated by studies on *Locusta*, *Schistocerca*, *Melanoplus* species. Generally, a mixed diet, rather than a single plant diet, has a better effect leading to faster development and lower mortality. The feeding behaviour can lead to diet-based

variation in phytophagous forms resulting into the development of either polyphagous, steno- or oligophagous or even monophagous species. The latter condition is, however, very rare in Orthoptera. Another interesting aspect is the quantification of feeding in the different instars and/or adults as well as in the somatic growth period or gonotrophic cycle of a particular species. For these studies, various parameters such as the food consumed during a certain period or on per day basis, weight of a single meal, ratio of food per body weight and the increase or the decrease in daily food consumption depending upon the sexual maturation in the male or in the female, are used. Similarly the amount of green food consumed by an individual Acridoid in its life-time, its economic and ecological impact, importance and effect of biomass and energy transfer, population dynamics and energetics are also ultimately related to the feeding behaviour. The literature, reviewed indicates that much remains to be done in this area as far as the Acridological studies in India are concerned and that practically no related information is available on the Tetrigoids.

### 4.2 *Breeding behaviour*

As compared to feeding, the breeding or reproductive behaviour is still complex a phenomenon influenced by a variety of internal and external factors. The breeding behaviour works internally through the maturation of gonads, while it manifests externally, mainly through the mating and oviposition behaviour.

The maturation of genital products depends on the nervous, hormonal and also to a certain extent on the trophic factors. The gonidial state of the reproductive system in the male and female Orthopteran insects, in turn, triggers the development and visible expressions of the breeding behaviour. In most of the Orthoptera, especially the crickets, long and short-horned grasshoppers, acoustic sexual signals mark the beginning of the mating behaviour. The devices to produce songs differ in the crickets and the grasshoppers (table 3). The patterns of songs essentially differ in 'time' distribution of the pulses of sound; the songs also differ in their meanings as per the changing conditions. As far as the mating behaviour is concerned, it can be said that the songs are sung mostly by the males to attract the receptive females of the same species and to disuade indulgence, territorial intrusion, to indicate dominance and so on, between members of similar sexes. The songs, based on the purpose they serve are variously called as calling songs, courtship songs, post-copulatory songs, or rival's song suggesting threatening. The courtship songs aid the copulatory behaviour while the post-copulatory songs are mainly meant to ward off further attempts by other males and thus prevent any interference with the physiological events to follow in the inseminated female. The rival's song in crickets invariably ensues a fight, while in grasshoppers it is not so much for threatening but a suggestion to 'keep away' or avoid, a result that is generally achieved. It is known at least in the crickets, that the songs are innate means of communication and that they have genetical basis. The various songs are also species specific. There are no stridulatory and tympanal organs in the grouse-locusts and may be that certain other sensory cues initiate the mating behaviour in Tetrigoids.

The next stage in mating behaviour leads to act of copulation through the courtship phase. The copulatory behaviour is also dependent on variety of stimuli and mutual reactions. This culminates into copulation, an act which differs in duration and posture,

the latter depending upon the difference in the relative sizes of the rather small male and the larger female of the copulating pair and the type of concealed copulatory apparatus in the male. The completion of copulation tends the now non-receptive female to undertake the last of the phases in the breeding behaviour, namely, the oviposition behaviour culminating into the act of egg-laying. The oviposition behaviour is both complex and highly specific. Many factors such as habitat, vegetation, soil conditions, temperature, humidity, crowding, etc. play an important role in the oviposition behaviour.

The act of oviposition also depends upon the structure of the ovipositor, the site of oviposition etc. (tables 3, 4). The eggs in Acridoidea are laid mostly in the soil, in the form of an egg-pod (figure 2E) and are either arranged regularly as in *Acrida* or irregularly as in *Nomadacris*. The egg-pod is formed of frothy secretion. The depth at which the eggs are laid is dependent on the soil humidity; drier the habitat deeper are the eggs laid. On the other hand in progressively humid to wet habitat the eggs are very near or even on the surface. In some Acridoids eggs are laid at peculiar sites such as in a leaf-stalk or under a floating leaf of an aquatic plant, while some show group-laying, e.g. *Dociostaurus*. The ovipositor, oviposition and the eggs of Tetrigoids markedly differ from those of the Acridoids in general. The ovipositor has serrated valves ending in a rather straight or slightly curved spine-like structures (figure 1K). It is unlike that of Acridoids, wherein the valves are generally smooth (except the serrate ones in *Oxya*) and terminally bearing a hook-like structure. In the grouse-locusts, the eggs are laid in richly humid soil or in marshy habitat about 1–3 cm deep and the valves therefore are presumably more useful in separating the vegetation and digging a superficial rather than a deeper hole. Unlike Acridoids such as *Locusta, Melanoplus, Gomphocerippus*, the Tetrigoid females do not cover the hole after oviposition. However, as observed in *Euscelimena* and *Potua* since the holes are dug in moss carpets or such other low vegetation, these many a times are concealed. Our observations on *Euscelimena* under experimental conditions also indicate that the development gets completed and the eggs can hatch even if kept completely under water. The structural modification of the ovipositor in grouse-locusts is thus in accordance with their oviposition behaviour. The eggs in Tetrigoids are laid not in egg-pods but in clusters that may or may not be showing a tier-like arrangement (figures 2F and G). Furthermore, the Tetrigoid eggs are loosely glued together and each carries a chorionic horn or filament at the anterior end. In a cluster all the horns point upward. At the posterior end, the egg has a hydropyle that facilitates water-uptake and the resulting increase in size is partly accommodated because of the horn mentioned above. The horn of the egg is longer in the species that are semiquatic or inhabiting marshy habitat while it is shorter or even knob-like in the species that are more terricoles in nature. Tetrigoids show nymphal or adult diapause, tendency to hibernate and also show univoltine or biannual cycles. Our studies on grouse-locusts indicate some interesting features of the breeding behaviour of the tropical forms. For example, the widely distributed semiaquatic *Euscelimena* breeds in natural conditions practically throughout the year, except the summer months as evidenced by the presence of all nymphal instars and gravid females throughout the year. On the other hand the pigmy locusts, *Potua* shows an altitude limited distribution, inhabits cooler places and feeds predominantly on moss (*Funaria* sps.). The pigmy locusts are therefore observed to be aestivating as adults by remaining buried 5–6 cm below the soil surface along the fence-walls, without feeding, to tide over the summer months. The aestivation period terminates with the onset of rains when these adults

come out, copulate and lay eggs in, by then newly developed moss carpets. Some Tetrigoids are reported to show parthenogenesis.

Both the feeding and breeding behavioural aspects are capable of influencing each other. Furthermore, although dependent on many factors, both possess some instinctive basis and exhibit some stereotype, sequential pattern. An attempt has been made in table 2 to analyze these events in the reproductive behaviour with reference to the food and feeding behaviour in particular.

### 4.3 *Ecological features and adaptive radiation*

These studies in Orthoptera in general and Acridoidea in particular form the basis of various important aspects. For example, the phase polymorphism is now known to be due to changes both in the behaviour and in the endocrines. The studies also throw more light on the economic aspects and also help understand the possible trends in the adaptive radiation and phylogeny. Acridoid families show some broad grouping based on food predominance and preference. For example, subfamilies Acridinae, Oxyinae of the family Acrididae are graminivorous; Eyprepocnemidinae is herbivorous and ambivorous; while families Pamphagidae and Pyrgomorphidae are herbivorous. Secondly herbivorous, dendrophagous and forbivorous types are generally considered as less advanced as compared to the graminivorous types that are believed to be more progressive ones. The habitats such as terricoles (geophilous/geophiles/geobionts by some workers), planticoles (phytophilous/phytophiles/phytobionts by some) and its modifications, e.g. the herbicoles, graminicoles etc. and these behaviours have also given rise to different life-forms. For example, herbicoles represent the typical body-shape of the grasshoppers, while terricoles being restricted for feeding and breeding to the ground only have strongly depressed body form, and graminicoles have laterally compressed body and more oblique face. The feeding and breeding behaviours through course of time have established patterns that indicate different lines of adaptive radiation shown by Orthoptera in general (tables 3–4) and Caelifera in particular (table 5).

## 5. Conclusions

(i) As compared to Acridoids, more such studies need to be undertaken on Tettigonioids and Tetrigoids. The bio-ecological studies related to these aspects of behaviour could further contribute in the designing of control measures on the economically important order Orthoptera.

(ii) As far as the grouse-locusts are concerned, the detailed study of the mouthparts, quantification of feeding and energy budget studies, the role of sensillae in the feeding and breeding behaviours (this important area in relation to the feeding behaviour of Acridoids is being studied at Entomology Research Institute, Loyola College, Madras), determination of the cues that bring about mating, in the absence of stridulatory and tympanal organs generating and receiving the acoustic signals respectively, the life-cycle and diapause would furnish valuable information.

(iii) *Euscelimena*, *Eucriotettix* species of the Tetrigoids seemingly have the potential as good models for experimental, lab-oriented test-studies.

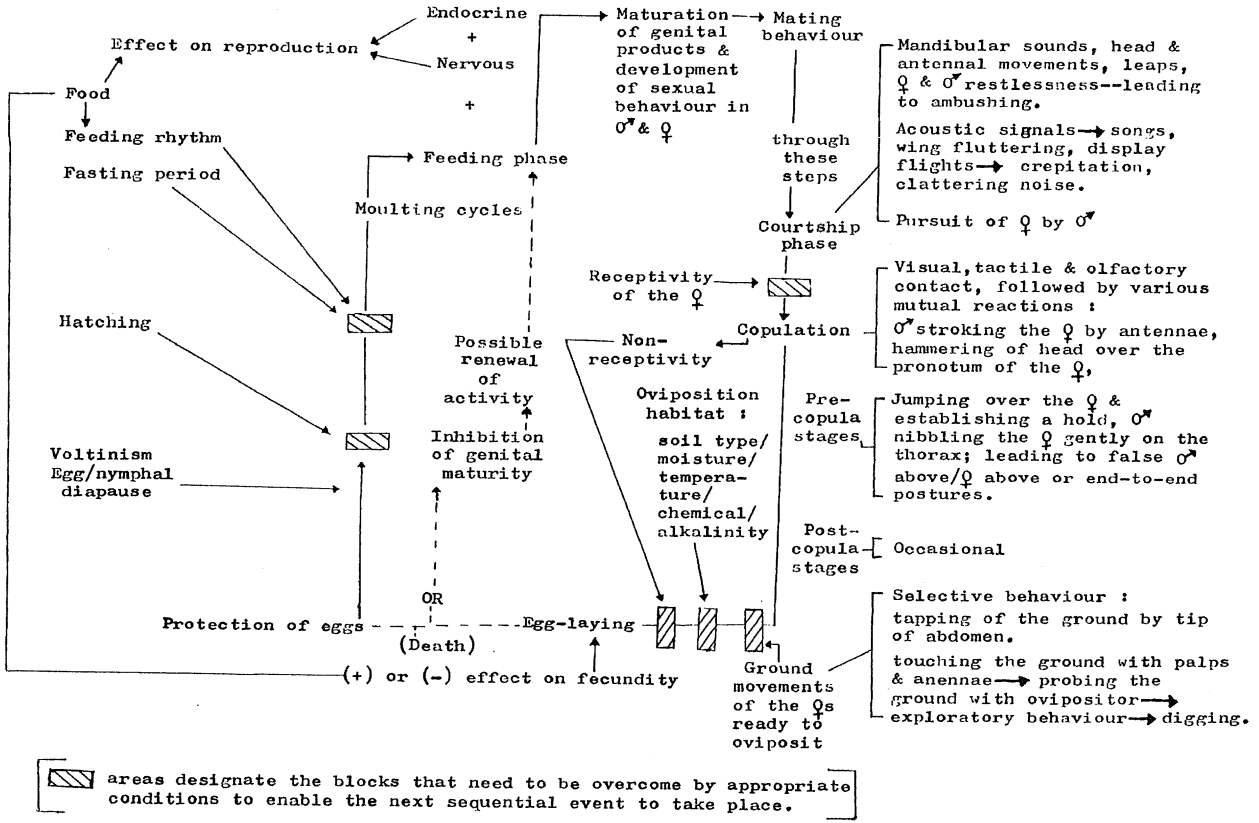
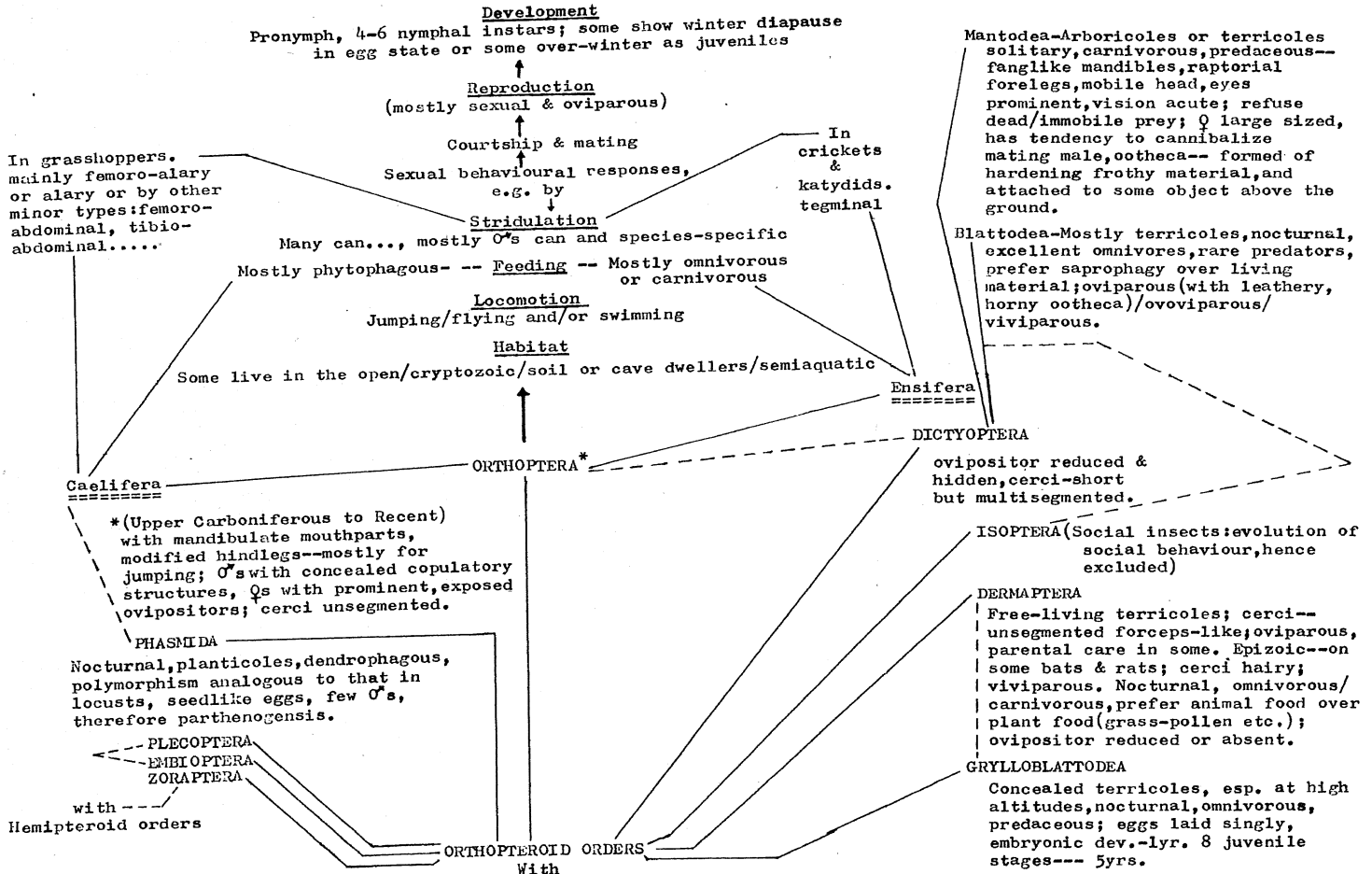



**Table 2.** Schematic representation and sequential analysis of events in reproductive behaviour in Orthoptera with special reference to food and feeding (other important biotic factors such as population density, interaction between the individuals are excluded).


Development
Pronymph, 4-6 nymphal instars; some show winter diapause in egg state or some over-winter as juveniles
Reproduction
(mostly sexual & oviparous)
Courtship & mating
Sexual behavioural responses, e.g. by
Stridulation
In grasshoppers. mainly femoro-alary or alary or by other minor types:femoro-abdominal, tibio-abdominal.....
In crickets & katydids. tegminal
Many can..., mostly ♂s can and species-specific
Mostly phytophagous- -- Feeding -- Mostly omnivorous or carnivorous
Locomotion
Jumping/flying and/or swimming
Habitat
Some live in the open/cryptozoic/soil or cave dwellers/semiaquatic
Ensifera
ORTHOPTERA*
Caelifera
*(Upper Carboniferous to Recent) with mandibulate mouthparts, modified hindlegs--mostly for jumping; ♂s with concealed copulatory structures, ♀s with prominent, exposed ovipositors; cerci unsegmented.
PHASMIDA
Nocturnal, planticoles, dendrophagous, polymorphism analogous to that in locusts, seedlike eggs, few ♂s, therefore parthenogensis.
PLECOPTERA
EMBIOPTERA
ZORAPTERA
with Hemipteroid orders
ORTHOPTEROID ORDERS
With biting-chewing mouth-parts, presence of cerci, complex wing venation & so on...
Mantodea-Arboricoles or terricoles solitary, carnivorous, predaceous--fanglike mandibles, raptorial forelegs, mobile head, eyes prominent, vision acute; refuse dead/immobile prey; ♀ large sized, has tendency to cannibalize mating male, ootheca-- formed of hardening frothy material, and attached to some object above the ground.
Blattodea-Mostly terricoles, nocturnal, excellent omnivores, rare predators, prefer saprophagy over living material; oviparous (with leathery, horny ootheca)/ovoviparous/viviparous.
DICTYOPTERA
ovipositor reduced & hidden, cerci-short but multisegmented.
ISOPTERA (Social insects: evolution of social behaviour, hence excluded)
DERMAPTERA
Free-living terricoles; cerci--unsegmented forceps-like; oviparous, parental care in some. Epizoic--on some bats & rats; cerci hairy; viviparous. Nocturnal, omnivorous/carnivorous, prefer animal food over plant food (grass-pollen etc.); ovipositor reduced or absent.
GRYLLOBLATTODEA
Concealed terricoles, esp. at high altitudes, nocturnal, omnivorous, predaceous; eggs laid singly, embryonic dev.-1yr. 8 juvenile stages--- 5yrs.
( @ indicated by broken lines)

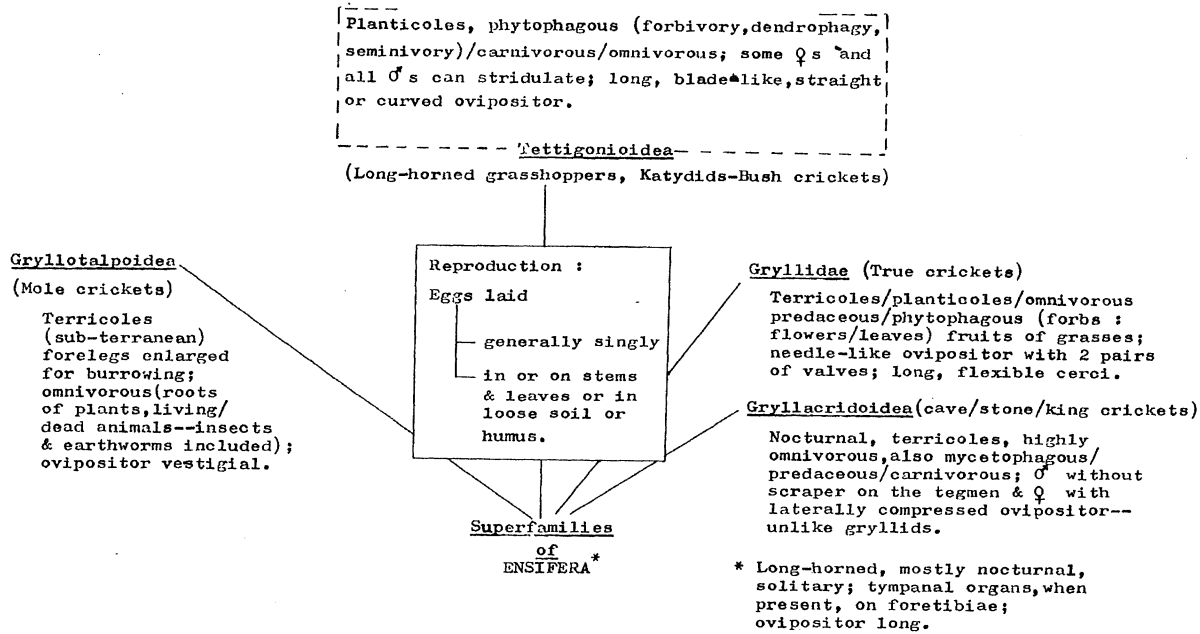



**Table 4.** Habitat, feeding and breeding behaviour—based adaptive radiation in Ensiferan Orthoptera.

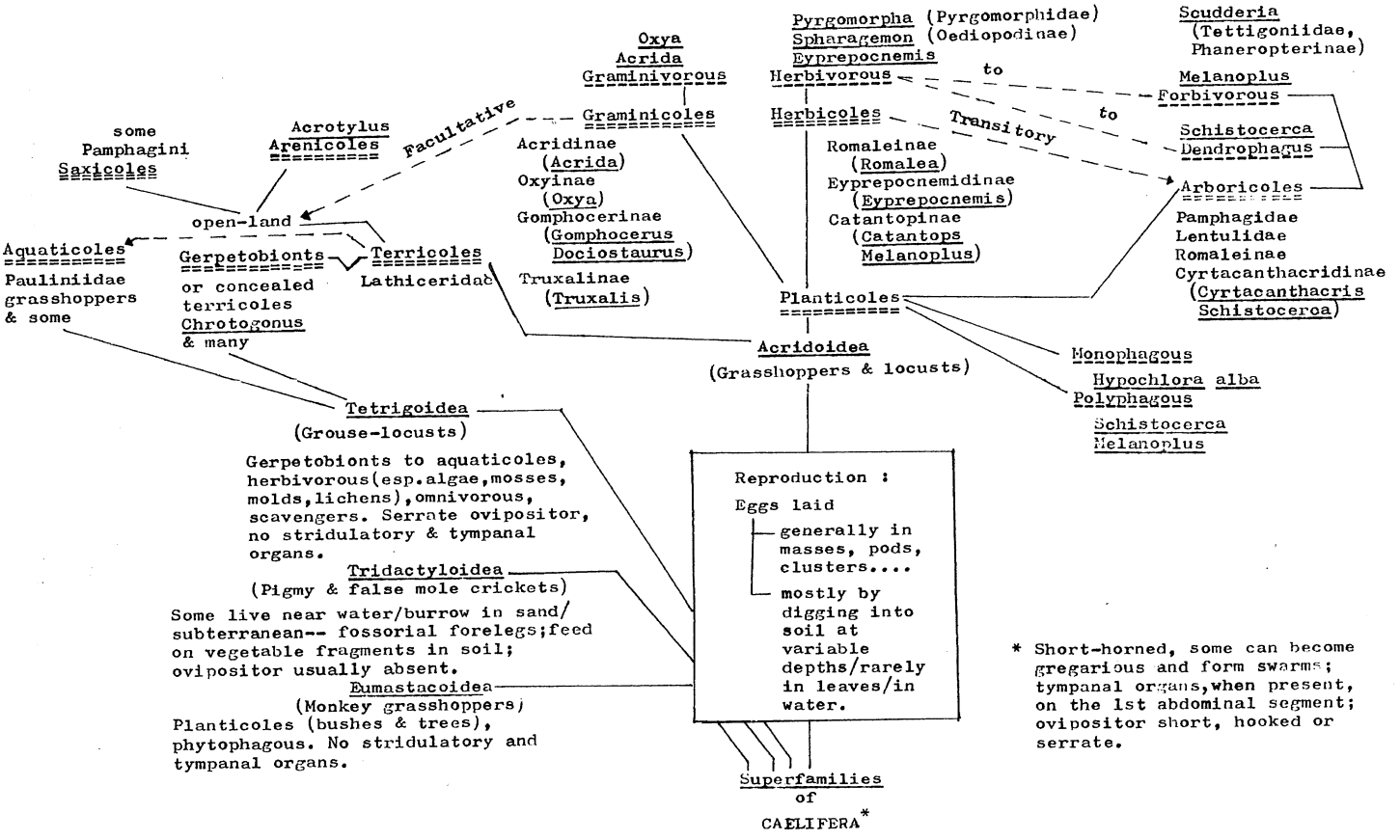



**Table 5.** Habitat, feeding and breeding behaviour—based adaptive radiation in Caeliferan Orthoptera.

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# Physico-chemical factors in the acridid feeding behaviour (Orthoptera: Acrididae)

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**Abstract.** Though acridids are generally polyphagous, they are not indiscriminate feeders as is observed, on the basis of extensive studies on several species of grasshoppers like *Eyprepocnemis alacris alacris* (Serv.), *Oxya nitidula* (Walker), etc. The feeding behaviour of such herbivorous insects in general is of immense importance because of their direct relevance to applied ecological problems. These insects live in environments with abundance of food, but their suitability is differently related to each available plant species in the environment. Hence the feeding behaviour patterns are seen to be generally influenced by several factors such as the morphological correlates of the insect as well as the physico-chemical factors of the host plant.

In this context, consideration is being given to, (a) physical factors of the host plant such as the thickness of the leaf lamina, the presence of trichomes, the position of the leaf blade, the general colour pattern and the effect of blinding, (b) morphological correlates of the insect such as mandibular modifications in relation to the host, its changes during post-embryonic development and their role in the shift of the host, the foregut armature and its influence on host selection, (c) the influence of chemicals like silica, phagostimulants and deterrents of the hosts on the insect feeding behaviour and (d) the role of sensory structures of the insect in the detection and discrimination of the host.

In addition to the discussion on the general pattern of feeding, the factors responsible for the initiation, continuation and termination of feeding are also analysed.

**Keywords.** Phagostimulant; deterrent; sensilla; palpation; acridids.

## 1. Introduction

The feeding behaviour and the pattern of host selection in acridids are known to be conditioned not only by their ecological requirements but also by their general behaviour. Though they are generally polyphagous, they are not indiscriminate feeders and the range of host plants is often correlated with natural taxonomic plant groupings, but the host plants may be randomly distributed among different plant families as evident in several species of acridids. As such the effective allocation of grazing privilages to herbivorous insects like acridids therefore tends to lead to diversity of host selection patterns. Generally the distribution of grasshoppers is related to the composition of the vegetation, as the movement of grasshoppers is much less in areas of favourable vegetation, which accounts for the concentration of several species of acridids in a particular habitat. However, the existence of many plant species and grasshoppers in a given habitat may not indicate the host specificity of the insects as the general behaviour of the insect is also involved. Feeding behaviour in acridids therefore revolves around specific feeding patterns, proximate cues influencing the plant choice.

This paper highlights some aspects of the feeding behaviour on the basis of the studies on *Eyprepocnemis alacris alacris* (Serv.) and *Oxya nitidula* (Walker) in relation to

accrued information on other acridid species, both endemic and exotic, focusing on the physico-chemical factors which influence the pattern of feeding and feeding behaviour. The following aspects of the feeding behaviour are discussed:

Orientation behaviour of acridids towards the food source; Feeding behaviour of acridids in the host selection; General feeding patterns in acridids.

## 2. Orientation behaviour of acridids

Visual attraction, mechano- and chemoreception involving gustatory and olfactory receptors are associated with the orientation behaviour of acridids towards the food source.

### 2.1 *Visual attraction and light*

Attraction of an activated insect towards the host plant, involves a number of factors such as visual and olfactory cues and continuation of the feeding activity, an essentially light controlled reaction (Chapman 1954) mainly rests on the receipt of favourable stimuli from the host. The movement of grasshoppers towards the food source in reaction to light involves visual cues. Though feeding and other activities also take place in the dark, most species are diurnal. Pielou (1948) showed in *Nomadacris septemfasciata* (Serville) that they reacted positively to light. Kaufmann (1968) in *Melanoplus differentialis* Uhler showed that it preferred *Taraxacum* to *Poa* grass in an uniformly lighted environment, but if *Poa* was kept in the light and *Taraxacum* in the shade, the insect preferred *Poa* to *Taraxacum*. The importance of visual attraction and the role of vision in perception and identification of the hosts have also been investigated in experiments involving the painting of eyes; total blinding results in random feeding as observed in several grasshoppers (Mulkern and Mongolkiti 1977; Meera 1982).

### 2.2 *Position of the leaf blade*

Many of the acridids tested in the laboratory showed a preference for upright blades over those lying on the floor (Williams 1954; Hjelle and Mulkern 1964; Ba-Angood 1977; Meera 1982). In *Zonocerus variegatus* (Linn.), Chapman (1955) and Kaufmann (1965) demonstrated that the insect was attracted to the vertical objects within 7 cm and could identify the leaf form from a distance of 10–15 cm. Hjelle and Mulkern (1964) projected the leaf pattern on ground glass and found that *Melanoplus femur-rubrum* (De Geer) was attracted to the vertical patterns, readily ascending the projected pattern. Both in the laboratory as well as in the field, Meera (1982) observed a similar behaviour in *Oxya nitidula*. Hence the upright leaf blades are always appreciated by the climbing forms of acridids, although the leaf on the floor may be selected when there is no choice.

### 2.3 *The colour of the host plant*

The colour of the host plants also influences the food selection behaviour in grasshoppers, though each grasshopper is believed to be attracted only to green colour,

grasshoppers like *M. differentialis* (Cresitelli and Jahn 1939) have been found to be more sensitive to blue/green wave-lengths, though generally showing a preference to the bright lush-green blades of the host. Most of the North Dakota grasshoppers studied by Mulkern are responsive to a range of wave-lengths including green, and hence it may be suggested that colour might also play a role in the feeding behaviour, especially in the location of the host.

## 3. Tactile and chemoreception

Chemical stimuli may play the most important role in the detection, discrimination and selection of host plants. It is likely that these insects may use combined visual and chemical information to locate the potential host. In this connection, the structure and functions of the receptors of the antennae and mouth parts are of considerable importance in the various behavioural aspects connected with feeding by acridids.

The sense of smell plays a significant role in host selection and in feeding behaviour as each insect responds only to smell which is relevant to it. It has been shown in all acridids studied (mostly Locust sp.) that the surface of the antennae is crowded with sensory hairs and tiny pits concerned with olfaction, useful in testing the host plant during feeding (Goodhue 1963). In *Melanoplus differentialis differentialis*, *M. mexicanus mexicanus* and *Romalea microptera* (Beauvois), Slifer *et al* (1957) has demonstrated that the thin-walled basiconic pegs of the antennae are the major olfactory sensilla used in testing the host for its palatability. Similarly, the maxillary palps also have a group of sensilla which are responsive to volatile chemicals.

Many tactile and olfactory receptors on the tips of the maxillary and labial palps also provide the insect with considerable amount of information, much of it being related to feeding behaviour (Blaney and Chapman 1969a, b). Thurm (1965) and Nicklaus *et al* (1967) associate the palp-tip sensilla with the mechano receptive function. On the basis of his study on *Locusta migratoria* L., La Berre *et al* (1967) concluded that these sensilla are capable of functioning as mechano- and chemoreceptors while the structure of others suggest a purely mechanoreceptor function. In *Locusta migratoria* and *Schistocerca gregaria* (Forsk.) it has been shown that they have a circle of mechanoreceptors surrounding about 400 chemoreceptors. The chemoreceptors are of three types *viz* (a) short-peg sensilla with pores and olfactory in function, (b) another group of sensilla that reacts to solutions and volatile chemicals and (c) crested sensillae with a single pore at the tip which are contact chemoreceptive and mechanoreceptive in function. Such sensilla are also identified on the inner surfaces of *Poekilocerus hieroglyphicus* (Klug) (Abushama 1968) and *M. differentialis* (Frings and Frings 1949). Haskell and Schoonhoven (1969) proved the domes of the maxillary palps of *L. migratoria* to be mechanoreceptor for testing the hardness of the grass, or contact chemoreceptors which play an important role in food selection when the insect is not starved for long (Blaney and Chapman 1970; Blaney *et al* 1973). Blaney (1974) has shown that the individual neurons respond to a wide range of chemicals and are capable of responding to more than one type of chemicals. The property of mechanoreception was observed only in 47% of the sensilla tested. Blaney and Chapman (1969) and Blaney *et al* (1971) found similar sensilla in *S. gregaria.*

In addition, to the sensilla of the palps, the laciniae and the galeae also carry campaniform and trichoid sensilla on their surfaces (Louveaux 1973; Chapman and

Thomas 1978). These sensilla are believed to be directly concerned with the feeding behaviour as they are found to be fewer in graminivorous species than herbivorous species.

On the inner surface of the epipharynx lining the cibarial cavity is a closely-packed group of sensilla which are regarded as chemoreceptors because of their characteristic position (Viscuso 1974; Chapman and Thomas 1978). In *S. gregaria* and *L. migratoria*, the inner surfaces of the clypeo-labrum contains very fine sensilla. Simple behavioural experiments conducted by Cook (1972, 1976) with several chemicals have established that the hexose sugars are the most powerful phagostimulants, stimulating these sensilla. In *Zonocerus variegatus*, Chapman and Thomas (1978) have identified several types of scattered chemoreceptors.

Uvarov (1977) had postulated that the sensilla on the surface of the mandibles may play an important role. Though it has been identified in certain species, it appears that they are useful as secondary structures acting as chemo- or mechanoreceptors and they may not play as important a role in host-selection as the palp sensilla. In addition, before the actual testing of the host is initiated, acridids are found to tap the host with their tarsi. Kendall (1971) showed that there are some chemoreceptors in the tarsi of *S. gregaria* with which the insect, to begin with, is able to perceive and discriminate the hosts.

## 4. Physical factors of the host and the morphological correlates of the insect

Many physical factors of the host have been observed as influencing the feeding behaviour and host selection in acridids.

### 4.1 *Leaf thickness*

The leaf thickness has been shown to decide the nymphal feeding pattern with regard to the gap of the mandibles and has been correlated with the inability of the early instars to open their mandibles wide. Bernays and Chapman (1970) and Meera (1982) have attributed the failure of the early nymphs of *Chorthippus parallelus* (Zett.) and *Oxya nitidula* to feed on *Festuca* sp. and the crop plants like *Oryza sativa*, *Panicum maximum* respectively to this factor. In *O. nitidula* the adults feed on the mid-lamellar region while the young ones prefer the apices of the leaves of *Cyperus rotundus* and *Cynodon dactylon*.

### 4.2 *Trichomes*

In addition, the trichomes on the leaf lamina have also been found to influence the nymphal feeding behaviour and food selection in *O. nitidula* and *C. parallelus*. In *O. nitidula*, the early instars are unable to feed on the crop plants like *Oryza sativa* and *Panicum maximum* because of their long trichomes which may hinder feeding of the early instars either physically or through their secretion and modify the general behaviour of the insect.

### 4.3 *Morphological correlates of the insect*

The morphological correlates of the insect are also important as the physical factors of the host. Observations of *Eyprepocnemis alacris alacris* (Serv.) and *O. nitidula* (Muralirangan and Ananthakrishnan 1978; Meera 1982) have shown that there seems to be a difference in the feeding behaviour of the early instars and the late instars and adults. The post embryonic development of the mandibles, especially the molar region, if analysed, clearly exhibits a transformation of the morphological structure. As a result of the transformation, the molar ridges becomes more complex during the post embryonic development. If proper studies are undertaken, such observations could be made even in other species as well.

Similarly, difference in the pattern and complexity of the foregut armature could also be correlated with the feeding behaviour and the food habits of the grasshoppers and this could be considered an ecological adaptation of the grasshopper concerned. (Uvarov 1966). Muralirangan and Ananthakrishnan (1974, 1978) have analysed the foregut armature pattern of 30 species of south Indian acridids and of the five zones recognised in the foregut region, Z II and Z IV exhibit a marked developmental differentiation during post embryonic development. Such a differentiation in the foregut morphology has been observed from the very early instars, thus accounting for the variation in the food habits. Based on the observations of *E. alacris alacris* and *O. nitidula* the morphological adaptation in the foregut structure is manifested in an increase in the number of ridges, the complexity of teeth arrangements and the basal chitinisation of the Z IV teeth from the III instar onwards, an adaptation that enables the insect to process the tougher leaves of the crop plants with high silica content.

All these factors, *viz.* the physical factors of the host as well as the morphological correlates of the insect are responsible for the absence of certain hosts in their diet, especially in the I and II instars. Greater complexity of the grinding surfaces of the mandibles with well-differentiated and well-chitinised foregut armature is the morphological adaptation not only to the silica content of the host but also to the thickness of the leaves, when the nymphs are not able to open their mandibles wide. As a result of these factors, a change in the feeding behaviour occurs, resulting in the shift of the host from III instar, thereby increasing the total amount of food ingestion. Perhaps this may be the reason for a sudden increase in the body weight of nymphs gained from III to IV instar.

## 5. Chemical factors of the host influencing feeding

Though the feeding activity could be initiated by the sensory receptors of the insect, the activation of these receptors requires a stimulatory factor to evoke normal feeding behaviour. The leaf surface chemicals play an important role in determining the feeding activity and acridids have been known to identify the phagostimulatory ones from the deterrents by palpation and recognise the leaf form from the leaf surface attributes (Chapman 1977).

Many substances stimulate feeding and such phagostimulants have been identified and established for *Schistocerca* sp. and *Locusta* sp. (Bernays and Chapman 1977; Cook 1977; Uvarov 1977). The substances which may stimulate feeding are specific and the occurrence of suitable phagostimulants may, in part, effect feeding. Several such

phagostimulants have been found to evoke a striking feeding activity in the older nymphs and adults of *Melanoplus bivittatus* Say and *Camnula pellucida* (Scudder) (Thorsteinson and Nayar 1963); *S. gregaria* (Goodhue 1963) and *L. migratoria* (Mehrotra and Rao 1966). Some aminoacids (like L. proline and L. serine), hexose sugar and disaccharides are found to be phagostimulatory for *L. migratoria* (Cook 1977), and Mulkern *et al* (1978) have analysed the experimental results on the attractants and phagostimulants used for the control and estimation of grasshopper populations.

Some hosts are rejected because of the presence of certain chemicals that are deterrents to feeding and such deterrent chemicals seem to play an important role in the feeding behaviour of acridids. Combinations of feeding deterrents have also been shown to be additive in their effects on the feeding behaviour of *Locusta migratoria* (Adams and Bernays 1978). The release of HCN from the non-deterrent cyanogenic glycosides has been shown quantitatively to play an important role with the increasing maturity in the unpalatability of *Sorghum bicolor* to *L. migratoria* (Woodhead and Bernays 1978). Probably all the plant species contain a combination of nutritional substances, some of which tend to promote feeding activity while others inhibit it.

Some secondary compounds, deterrents for *Locusta* are ineffective as deterrent for *Schistocerca* and some which are deterrent at high concentrations stimulate feeding at lower concentrations (Bernays and Chapman 1978). Thus the sensory system of forbivorous *Schistocerca* must receive more information on the nature and concentration of secondary compounds than by an oligophagous *Locusta*. Rowell (1978), while discussing the feeding strategy in relation to other aspects of life cycle, presumed that the high diversity of secondary plant chemicals makes a general strategy impractical. This is because acridid species have evolved a diversity to recognise only a small number of phagostimulants rather than a larger number of feeding inhibitors. Hence feeding depends on the balance between the phagostimulants and the feeding deterrents and the response varies in different species so that a plant may be acceptable to some but not to other species, or the rejection of hosts by these insects may be owing to the presence of one or more chemicals in amounts which inhibit feeding; Poaceae and few other plants are readily accepted only because of the absence of deterrent chemicals in sufficient quantities to limit feeding (Bernays 1978; Bernays and Chapman 1977).

In order to differentiate these chemicals, a number of sensilla located on the mouth parts, initiate the feeding behaviour on being favourably excited. Evidence suggests that each of these sense cells in the chemoreceptors are particularly sensitive to one class of chemical substances, perhaps because of the form of its receptor membrane (Chapman 1974a). With the excitation of different cells, it is able to differentiate between some classes of chemical compounds, as between inorganic salts and sugars, and to identify certain 'key' chemicals to which the cells are specially sensitive. Chemically similar complex substances are differentiated, possibly by producing responses in several sensory cells in each sensillum, the pattern of response of each cell varying in a particular way so that the overall pattern from all the cells combined produces a characteristic effect which could be interpreted within the central nervous system. As the sensory inputs of some cells sensitive to feeding deterrents is not qualitatively different from that of positively stimulating cells, differentiation between positive stimulation and inhibition is presumably a function of the central nervous system (Chapman 1974a, b). Only after elaborate exploratory behaviour of the insect—first by the sensilla of the antenna, then by those of the maxillary and labial palps, test-biting takes place. As a result, the group of sensillae i.e. contact chemoreceptors found on the

inner surface of the labrum are excited and they decide the palatability of the food before it is engulfed; Chapman (1977) has estimated that at least 4000 sense cells and 12000 neurons are involved in the feeding behaviour of acridids.

## 6. General feeding patterns in acridids

### 6.1 *Basic pattern of feeding*

Feeding does not start until 6–12 hr after eclosion and no feeding occurs for the first few hours after ecdysis. But the first one or two feeds are much longer. In the acridids so far studied, as in several *Locust* sp., *Oxya nitidula*, *Eyprepocnemis alacris alacris* etc. the first two feeds last over 20–30 min followed by a short resting period of 5–7 min. The first instar generally feed on the apices of the leaf lamina while the late instars and the adults are marginal feeders. In *O. nitidula*, the leaves, leaf bases and the stems of *C. rotundus* and *O. sativa*, and occasionally those of *P. maximum* are consumed entirely during shortage of food, while the stem and leaf bases of *C. dactylon* are never consumed, even during shortage of food. Feeding commences after the test bite from the margins of the leaves (both in *Oxya* and *Eyprepocnemis*), continuing downward towards the mid-rib in a semi-circle. Further cutting is inner to the first site, starting from an anterior region and proceeding downwards. On the basis of 50 such observations we have concluded that a semi-circle is formed either inward or above the previous one. Such a definite pattern of feeding behaviour has been observed in most of the graminivorous acridids so far studied.

With different types of food habits, non-graminivorous acridids deviate from the graminivorous pattern though the feeding sequence remains the same. The forbivorous species generally eat ovoid, net-veined leaves rather than the linear and parallel veined leaves. Because of the venation, it is unable to remove morsels by a combination of incision and splitting between veins. The cut made—by incision only—is necessarily escalloped and irregular.

The classification of acridids into herbivorous, forbivorous and graminivorous types, is associated closely with the structure and the shape of the mandibles. Mandibles of the forbivores (Catantopinae) have an armature of irregular and sharp incisors; those of the graminivores (Truxalinae and Acridinae) have incisors in parallel ridges often fused or worn into a semi-continuous cutting edge; the herbivores have incisors intermediate between the above two types. The three kinds of cutting patterns of the leaves exist as a result of the three types of mandibles; frilled margin of leaf by forbivores; even margined cutting by graminivores and intermediate one by herbivores (Gangwere 1972).

The initial choice of the host by adults is made by visual stimulus followed by tapping of the leaves by their antennae and tarsi. The over-all picture of the feeding behaviour seems to be similar in all acridids (Gangwere 1972; Mordue (Luntz) 1979; Meera 1982). While selecting a host plant, both maxillary and labial palps are repeatedly moved. This "palpation" (Blaney and Chapman 1970) continues, when the animal is in search of food as well as during feeding; after the interfeed period, again palps are projected. On coming into contact with the host plant, the head is lowered with the hypognathous mouth parts pushed outward touching the food. Upto this point, the behavioural pattern is considered as 'exploratory behaviour' (Mordue (Luntz) 1979). The head first

moves in a backward and downward path to bring the incisor cusps of the mandible in contact with the food from its margin. The continuous palpation during feeding is also attributed to their mandibular movement. Depending upon the acceptance of the test-bite the host plant is either well consumed, nibbled or rejected altogether, rejection taking place while probing, palpating or test-biting.

Thus, palpation is a step towards the selection of the host plant, the final choice or rejection being made only after the test-bite by which the sensory inputs are produced from the sensilla; and if the reaction is favourable, then the initiation of feeding begins.

The role of maxillary palps in feeding has been assessed in *O. nitidula* through palpectomy and antennectomy and these experiments have shown that (i) without both the maxillary palps, the adults are able to identify their hosts with their antennae to a certain extent, but even an inert crape paper strip is bitten immediately; (ii) with only one palp, *O. nitidula* palpates and feed normally, but the rate of feeding is slower than in normal-control insects. In the absence of the palps, the labrum is brought into contact with the food often and in *S. gregaria* exploratory behaviour is significantly reduced in insects without palps. Mordue (Luntz) (1979) has shown in *S. gregaria* that the ability to perceive gustatory stimuli is reduced by removal of maxillary and labial palps, because single chemical like sucrose is not recognised easily in the absence of palps. (iii) without both antennae but with both the palps intact, the insects are much slower in the location of the food than the palpectomised insects, the adults palpating slowly on the food plants as well as on the inert media (Meera 1982). Antennectomised grasshoppers do not behave normally in feeding as they fed even on the non-preferred host. This is more pronounced when both antennae and palps are removed.

### 6.2 *Initiation and continuation of feeding*

If the grasshoppers are not deprived of food for a long time, feeding is normally initiated by chemical stimulation. If starved, they bite indiscriminately and only after a full meal, they revert to the normal feeding behaviour as reported in several locust sp., *O. nitidula* and *Eyprepocnemis a. alacris*. In acridids no specific chemical has been identified initiating biting and swallowing (Goodhue 1963). In acridids, feeding is generally initiated by sugars, by sucrose in particular (Bernays and Chapman 1978), which are perceived by the sensilla of the palps, the epipharynx and the hypopharynx, and the stimulation of any of these sensilla initiate feeding. Immediately after a full meal, the grasshopper generally exhibits little or no feeding and shows a post-prandial rest for about 40–70 min, after which feeding once again commences but this process of starting again is sudden. The increase in potential reponsiveness after the post-prandial rest can be envisaged as a result of a progressive decrease in the various inhibitory inputs which lead to the cessation of feeding. As a result, the insect starts to feed again. The mechanism which causes the feeding behaviour even in the absence of any changing external conditions is unknown (Chapman 1982) but Simpson (1981) indicates the rhythmic changes occurring in the central nervous system as a possible factor.

For continuation of feeding, a continuous and sustained sensory input is necessary. Feeding stops in *Locusta*, if they are provided with an inert substrate (Chapman 1982). So in a normal meal the chemosensory inputs are continuous, but the effects of sensory inputs are not limited to the immediate response; but according to Barton Browne (1975) there is also a sustained preservation effect, which has been demonstrated in

acridids like *Locusta migratoria* (Bernays and Chapman 1974) and *Chorthippus terminifera* (Barton Browne *et al* 1975). It is probable, therefore, that these inputs influence the duration of the meal and continue to have their effects on the sensilla. Perhaps the initial stimulation sets the level of the central excitory stage (see Barton Browne 1975; Dethier 1966) which then persists in the presence of chemosensory inputs signalling the existence of a suitable host even after the original stimuli have ceased to be effective.

### 6.3 *What causes them to terminate feeding?*

According to Chapman (1982) two types of phenomena are involved in the termination of feeding, *viz* volumetric feed-back and chemosensory inputs. The feeding stops when the foregut gets filled with food. The last part to get filled is the anterior-most end of the foregut. If the post-pharyngeal nerve which runs from this region to the frontal ganglion is cut, it results in an excessively large and prolonged meal (Rowell 1963; Bernays and Chapman 1973). If the inner oesophageal nerve is cut, the food is retained in the front part of the foregut distending the crop, and the feeding stops although relatively little food has been ingested. So it seems certain that the inputs from the stretch receptors from the pharyngeal region may have an inhibitory effect on feeding. The implication in such cases, as suggested by Sinoir (1968) and Bernays and Chapman (1974), is that the distension of the foregut is the major importance in the termination of the feeding behaviour.

While the volumetric feed-back possibly imposes an absolute limit in the meal size, there is evidence for its interaction with other inputs from the peripheral chemoreceptors. For example, there is a difference in the meal size of nymphs of *L. migratoria* when fed on mature grasses. If they are fed on *Agropyrun* sp. previously, they consume more of this grass in one meal than *Poa* grass but after pre-feeding or habituation on *Poa* the converse is true (Bernays and Chapman 1972). A similar observation has been made in *O. nitidula* (Meera 1982) and *S. gregaria* (Azzi 1975) as well. Chemical stimulation of receptors of the mouth parts before feeding leads the insect to take a larger meal.

The importance of chemosensory inputs and the termination of feeding have been clearly demonstrated by the experiments of Blaney and Duckett (1975); continuous stimulation of the sensilla of the maxillary palps of *L. migratoria* with feeding deterrents have been observed to reduce the length of the meal even though the other sensilla are exposed to the whole meal in the normal way. In contrast, a sucrose extract on the palp lengthens the duration of the meal. Cook (1977) and Bernays and Chapman (1978) have shown in *L. migratoria* and *S. gregaria* respectively that the meal size is dependent on the concentration of sucrose. Hence it may be concluded that the effect of stretch receptors is modulated by the chemical inputs.

Normally the duration of the feeding may be longer, often lasting more than 10 min. During this period, the adaptation of the sensilla on the tips of the palps is offset to some extent by the rapid vibration of the palps, which makes sure that they are brought in physical contact with the host ten times for a very brief period of about a second (Bernays and Chapman 1970). As a result of this, the information is transmitted to the central nervous system (Blaney and Duckett 1975). But Mordue (Luntz) (1974) has observed that even in palpectomised nymphs of *S. gregaria*, duration of feeding is

normal. In such circumstances it is suggested that the cibarial receptors as well as the antennal sensilla might overtake this function.

The work of Bernays and Chapman (1974) indicated that the sensory adaptation is of little importance in the regulation of meal size in acridids feeding on normal food. Then how does the chemical input influence the meal size? The experiments of Blaney and Duckett (1975) have shown that the inputs from the sensilla have some lasting effect, resulting in a decrease in the meal size. Since the palps provide information on the meal size intermittently during feeding, it is likely that the closure of the sensilla, which deprives the insect of the stimulatory inputs, may lead to the cessation of feeding. But the meal lengths do not differ in controls as well as in palpectomised insects (Chapman 1982).

Bernays and Chapman (1973) have reported that the distension of the crop by a full meal leads to the release of a hormone from the storage cells of corpora cardiaca. Bernays and Mordue (1973) have suggested that the hormone effects the tips of the palps by closing the pores of the terminal palp sensilla which then become non-functional. This hormone also decreases the locomotory activity after feeding (Bernays 1980). It is suggested (Cazel 1969) that they might enhance the movement of the foregut so that the food moves out and the volumetric feedback from the crop to stop feeding is removed.

It may be concluded that the first information from the stretch receptors of the foregut is relayed to the brain so that feeding behaviour is continued or switched off. More long term effect is brought about by the release of one or more hormone from the carpora cardiaca. This effect persists for an hour or more during the post-prandial rest, but after 2 hr or more they become fully functional (Bernays *et al* 1972; Bernays and Chapman 1973). This closure of the terminal sensilla decides the end of the meal and prevents further feeding.

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# Behavioural response (feeding preference and dispersal posture) of *Aphis gossypii* Glover on brinjal crop

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**Abstract.** During late July to early August (kharif) and late November to late December (rabi), alates of *Aphis gossypii* Glover appear on the old leaves of the tender brinjal plants. Population build up is quite gradual with a sigmoid growth and the pest reaches peaks during late December (kharif) and late February (rabi) when plants become mature. In course of incidence they distribute from old to young to tender leaves and the aphid incidence on these leaves is always followed by the migration of alate immigrants. Thereafter, the aphid prefers mature tissues of the crop and in course of crop maturity its dispersing trend follows from old to the other leaves as well as plants in a sigmoid posture in a latero-angular spectrum.

**Keywords.** Brinjal; *Aphis gossypii*; sigmoid growth rate; matured tissue preference; latero-angular sigmoid dispersal contour.

## 1. Introduction

Aphids appear to have evolved the ability to profit from the environmental cues (*e.g.* mutual tactile stimulation, photoperiod, properties of different parts or ages of the host plant or of different plant species) to develop the morphs most suited to successful exploitation. They are found to disperse to the host plant as immigrant alates to exploit it if it is suitable and if not they tend to be away from there as emigrants. Thus the dispersive behaviour of alate migrants for better niche facilities is directly dependent on crop infestation. However, several factors for the development of the said form have been studied by several authors (Wadley 1923; Evans 1938; Dickson and Laird 1962; Johnson 1966; Lees 1967; Dadd 1968; Mittler and Kunkel 1971; Raccah *et al* 1971; Schaefers and Judge 1971; Ghosh and Mitra 1979; Rajagopal and Kareem 1979) in different species. Kennedy and Booth (1951) categorised the factors and flavour stimuli and nutrient stimuli to enhance alate migration. Muller and Unger (1951) also proposed a funnel-like locomotory path of alate immigrant of *Aphis fabae* Scop. in intercrop (*Vicia faba*) migration. The present study aims at gathering knowledge about the feeding preference and intracrop-dispersal posture of *Aphis gossypii* Glover on brinjal.

## 2. Material and methods

In a plot (20 m × 20 m) in Hooghly district (W.B.) twenty plants of brinjal (Krishnanagar cluster in kharif and Pusha purple long in rabi seasons) were planted. Observations were made on randomly selected three leaves (old, young and tender) per plant at 15 days interval. During observations adults and nymphs (3rd and 4th instars)

of both forms (alate and apterous) were separately counted along with the counting of colonies. Simultaneously the rate of infestation of plant and leaves were also recorded. Observations on kharif (1980–82) and rabi (1981–83) crops were recorded separately.

## 3. Results

### 3.1 *Incidence pattern*

The data on mean population (table 1) shows that after the initiation of colony aphids (*A. gossypii* Gl.) on brinjal plant followed a gradual increasing trend to reach the climax during late December (kharif) and late February (rabi). In both the seasons the aphid retained colony over the crop up to its harvest. Further, *A. gossypii* Gl. appeared on 2 month old hosts and the individuals in the population pyramid of the same increased with the rise of the host age (figures 1 C and F). Again, initiation of population was

**Table 1.** Kharif and rabi incidence of aphids and correlation coefficient between the increasing change of plant infestation with the decrease of alate adult incidence from old leaves.

| Month | Incidence of aphid (%) | Incidence of alate immigrants (%) | Increasing change of infested plant (%) ($x$) | Decreasing change of alate adults (%) from old leaves ($y$) | Correlation coefficient ($r$) between $x$ and $y$ |
|---|---|---|---|---|---|
| Time | | | Kharif | | |
| $Jl_2$ | 18·33 | 13·66 | 0 | 0 | |
| $A_1$ | 57·77 | 18·94 | 8·35 | 8·33 | |
| $A_2$ | 68·33 | 13·86 | 12·20 | 9·96 | |
| $S_1$ | 113·88 | 10·22 | 3·35 | +2·00 | |
| $S_2$ | 98·33 | 4·16 | −5·00 | 6·79 | |
| $O_1$ | 126·66 | 4·11 | 5·00 | +5·74 | * |
| $O_2$ | 156·10 | 6·15 | 5·00 | 8·88 | 0·605 |
| $N_1$ | 123·33 | 6·47 | 5·00 | 8·60 | |
| $N_2$ | 199·99 | 10·87 | 1·65 | 3·98 | |
| $D_1$ | 168·88 | 15·30 | −3·30 | 3·52 | |
| $D_2$ | 199·99 | 17·06 | 0·80 | 5·58 | |
| | | | Rabi | | |
| $N_2$ | 13·88 | 4·00 | 0 | 0 | |
| $D_1$ | 71·11 | 13·98 | 10·00 | 35·77 | |
| $D_2$ | 80·22 | 10·33 | 1·70 | 12.63 | |
| $J_1$ | 108·33 | 20·54 | 1·30 | +7·20 | |
| $J_2$ | 151·66 | 11·40 | 7·00 | +1·50 | |
| $F_1$ | 188·88 | 9·21 | 3·35 | 7·63 | ** |
| $F_2$ | 247·22 | 8·90 | 3·35 | 15·19 | 0·689 |
| $M_1$ | 244·99 | 9·75 | 1·65 | 0·05 | |
| $M_2$ | 152·77 | 13·79 | −10·00 | 4·79 | |
| $A_1$ | 118·88 | 19·11 | −5·55 | 4·95 | |
| $A_2$ | 175·55 | 26·06 | −0·55 | 2·91 | |

Data plotted here, mean of three years' observation.
* Significant at 1%.
** Significant at 5%.

mostly restricted to the old leaves and within a short period, aphid incidence could be traced on old, young and tender leaves. In course of aphid incidence on the old leaves it was marked with a gradual decrease whereas it was reverse on the young leaves. But on the tender leaves no such trend could be traced though there was a relatively higher incidence on it just prior to the harvest. Interestingly, the old, young and tender leaves had higher incidence values during early, middle and late sessions of the crops respectively in both the seasons but in no case incidence on old leaves could be surpassed by the others.

Another interesting feature was the incidence of the alate adults which could be marked with a higher percentage during early and late sessions of the crop seasons. The incidence of the said morph was relatively lower during the mid session. Like the trend of aphid incidence, the higher values of alate adults were marked successionally on old, young and tender leaves during early, middle and late sessions of the crop respectively (figures 1A and D; figures 2 B and E).

### 3.2 *Growth rate*

The growth curves (figures 1 B and E) show a modified sigmoid form where the upper asymptote level was marked during late November (kharif) and late February (rabi) when the plants were fully mature (5–7 months old).

### 3.3 *Infestation percentage*

Maximum incidence of *A. gossypii* Gl. infested plants and leaves in maximum frequencies (figures 2 A and D). Furthermore, the leaf infestational trend was distributed from old to young to tender leaves in course of its incidence (figures 2 C and F). Again it could be seen (table 1) that decrease of alate immigrant incidence from old leaves positively correlated ($r$ = 0·605/kharif and 0·689/rabi) with the increase of plant infestation.

## 4. Discussion

It has often been shown that the aphids (*A. gossypii* Gl.) favour winter with low temperature and optimum rh (Bodenheimer and Swirski 1957; Agarwala and Raychaudhuri 1979; Roy and Behura 1979). Here too the aphids showed abundance on

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**Figures 1 A–F.** Preferential behaviour of aphid to the host tissues. **A, D.** Population distribution on different leaves **B, E.** sigmoid (s-shaped) population growth form and **C, F.** population pyramid of *Aphis gossypii* Gl. in kharif and rabi crops of brinjal. These show that the aphids always favour the mature tissues of the brinjal crop.

**Figures 2 A–G.** Intra crop dispersal-trend of aphid (*Aphis gossypii* Gl.) on brinjal. **A, B.** Incidence of aphid on the crop in relation to plant and leaf infestation. **C, D.** Distribution of alate adult at the different levels of the leaves. **E, F.** Rate of infestation of different leaves during pest incidence. These suggest that the aphids tend to be dispersed from old to young to tender leaves. **G.** Model showing intra crop dispersal contour of aphid (*Aphis gossypii* Gl.) in brinjal.

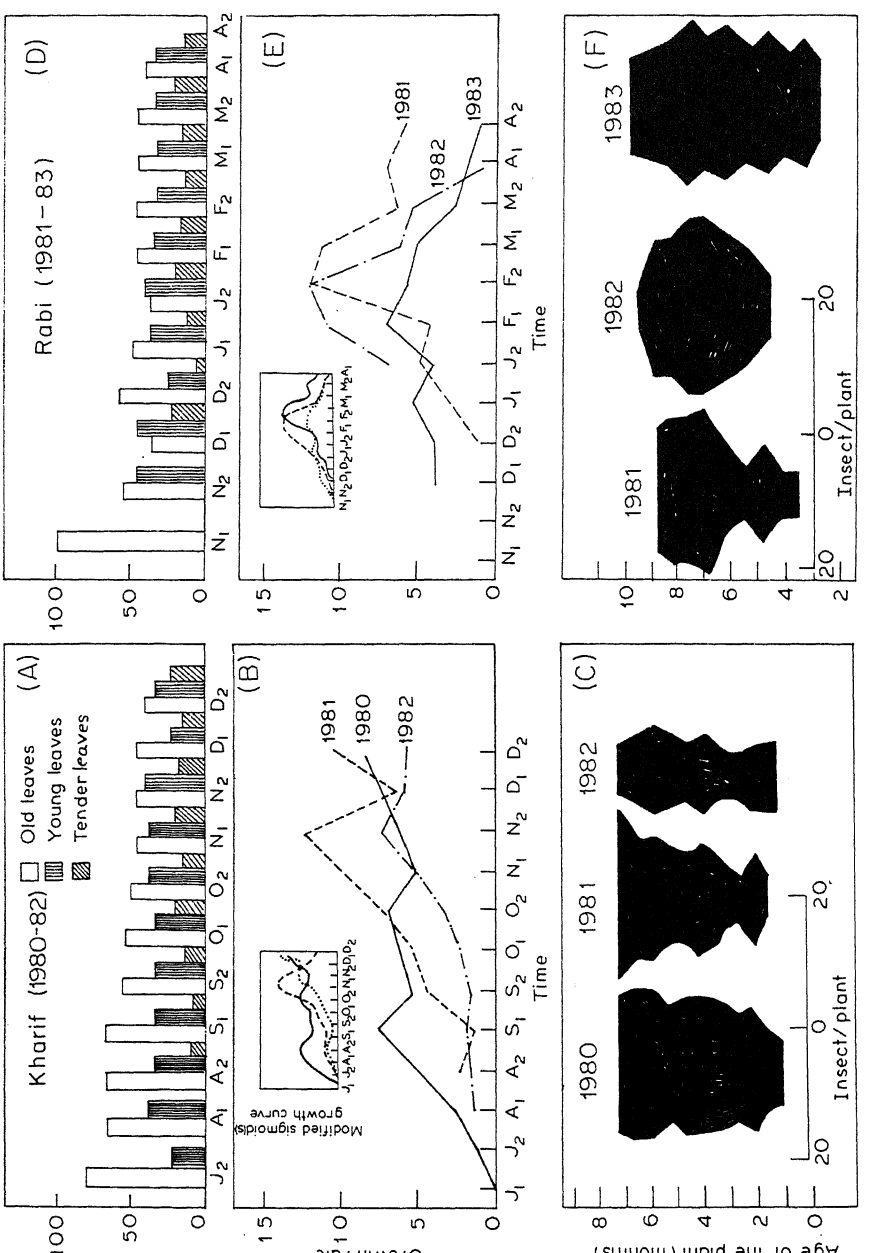


**Figure 1.** For caption, see page 297.

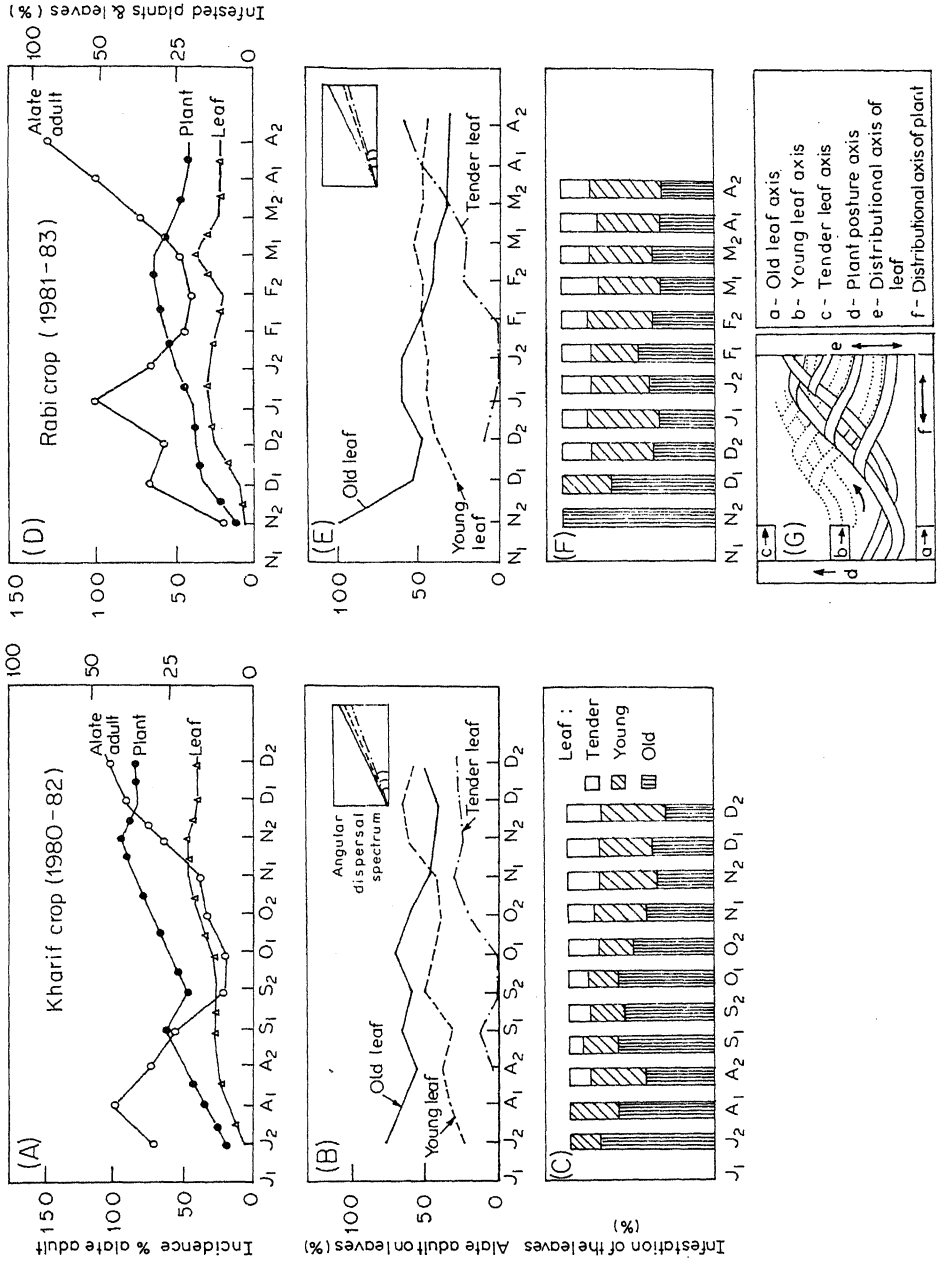


**Figure 2.** For caption, see page 297.

mature brinjal plants. Nevertheless, the forms of population pyramids diverged towards old age, as they were active during that period. Again, the trends of aphid incidence (colony as well as alate immigrants) from old to young to the tender leaves and the retention of the same over old leaves during harvest reflect a behavioural affinity of the aphids towards the older hosts. Furthermore, the higher growth rate with sigmoid form on the old plants during late session, indicates the host status where the aphids find normal situation in which population size deviates less from the asymptote (Odum 1971). This could be placed as a supplementary factor for the former reflection. Again to pay emphasis over aphid-host interaction in relation to matured tissues several factors including emergence of largest veins (Gibson 1972), intermittent water stress (Wearing 1972), nitrogen saturation (van Emden *et al* 1969) have been referred. Moreover, Turner (1971) found that the growth of *A. gossypii* Gl. was haltered in the absence of methionine. Though the present study does not deal with any of these factors, it is obvious that the aphid prefer to feed on older tissues rather than the young or tender.

Based chiefly on alate incidence and the relative infestation of the plants by them the speculation has often been advanced that the initiation of aphid colony is being made by the alate adults. Subsequently, factors like feeding competitiveness (Ghosh and Mitra 1979), crowding effect (Mittler 1973) and reduced moisture content of the host (Ratanlal 1951) enhance aphid incidence thereby increasing the rate of host infestation. But it is obvious that throughout the season alate persisted in all the leaves though the relative percentage varied during different periods. It is noteworthy that in the observation, the preabundance of alate adults led the abundance of aphids in general along with the peak percentage of infestation. It is quite clear from the correlation between the decrease of alate immigrant incidence and increase of plant infestation, that the aphids tried to avail latero-angular dispersal than vertical or irregular. Thus the projectile of alate percentage on different leaf level, a sigmoid dispersal contour (Wolfenbarger 1946; Odum 1971) appears (figures 2 B, E and G).

## Acknowledgements

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# Behavioural analysis of feeding and breeding in Lamellicorn beetles

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**Abstract.** Social behaviour is recognised in nine families of Coleoptera. The Lamellicorn beetles, in the families Passalidae and Scarabaeidae exhibit varying types of social behaviour.

Sound production by stridulation in both the larvae and adult passalids is attributed as a social behaviour to hold the families together.

Some South American scarabs live very close to the anus of sloths and monkeys in order to oviposit on their dung. Many have association with ant nests either for food, shelter or breeding.

The dung beetles present a whole sequence of bisexual cooperation in the nesting behaviour, excavation and ball-rolling. Parental care is exhibited to a varying degree.

An attempt has been made to review the feeding and breeding behaviour of Lamellicorn beetles in the light of available Indian literature including studies made by the authors.

**Keywords.** Behavioural analysis; Lamellicorn beetles; feeding and breeding behaviour.

Feeding and breeding habits seen in many Lamellicorn beetles are fundamental features of their biology which determines the characteristics of their behaviour, distribution, morphology and development. Their food habits are varied but most of the free living scarab adults and larvae are saprophagous or phytophagous. In addition many members belonging to the six subfamilies, the Scarabaeinae, Aphodiinae, Ceratocanthinae, Melolonthinae, Dynastinae and Cetoniinae are found in the nests of ants, termites, honey-bees, wasps etc.

Although "Truly Social" or "Eusocial" species which meet three criteria like living in groups as adults of different generations, with co-operative activity and different individuals performing different roles for the success of the colony, are not found in scarabs, yet varying types of social behaviour are encountered especially in Passalidae and Scarabaeinae.

The hind legs of larvae of the scarabaeoid families Lucanidae and Passalidae and the subfamily Geotrupinae often have stridulatory organs on the coxae working against those of the middle legs. In Passalidae the great reduction and modification of the hind legs has little effect on locomotion but are believed to be of great help in communication by stridulation to hold the family together which is attributed as a social behaviour.

The Passalids are considered to have a primitive society. They are gregarious living in the same tunnel system but during reproduction, each beetle maintains its own tunnel, lays eggs. The developing young feed on the material prepared by the adults. The wood consuming species lack digestive symbionts and mix fecal pellets and frass which act as a substrate for bacterial and fungal development, as was found by Matthews and Matthews (1978) in *Odontotaenius disjunctus* whose larvae cooperate with the adults in the construction of the pupal chamber. Parental care is also exhibited in passalids.

Two species of passalids are commonly found in the decaying, wet logs in the evergreen forests of the Western ghats in Karnataka. These two species *Episphenus indicus* (Stol.) and *Plaurarius brachuphyllus* Stol. are often in the same log, side by side

but will have distinct tunnels in which their own larvae are lodged separately, thus exhibiting a niche behaviour. The larvae, like adults, are gregarious, 3 cm long, with well developed first two pairs of legs, the hind legs being greatly reduced and stubby. These larvae live very close to each other in the tunnel and move quickly to come together when separated.

In Cetoniinae, the adults are usually phytophagous and the larvae feed on dung, humus or decaying wood. However some species become adapted to the nests of ants and termites. *Potosida cuprea* spends the larval and pupal stage in the nests of *Formica rufa* in Europe (Wheeler 1910). Larval cases are generally ignored by the ants as they look like lumps of earth. Other cetoniids like *Potosia cuprea* (Fab.) and *P. lumgarica* Herbst feed as adults upon honey in bee hives while the larvae feed on decaying organic matter (Caron 1978).

In South Africa the larvae of the green protea beetle (*Trichostetha fascicularis*) live on termite droppings for two years in the mound of the termite *Amitermes hastatus*, whereas the adults are flower feeders (Skaife 1955).

A cetoniid beetle *Coenochilus taprobanicus* Westwood and a valgine scarab *Oreoderus argillaceus* (Hope) are commonly found in the nest of *Odontotermes wallonensis* in South India. They feed on the fungus reared by the termites (Rajagopal and Veeresh 1981). According to Kistner (1982) the principle adaptation here seems to be the conditioning of the wood by the termites rather than social interactions with similar eating habits.

The Cetoniiae beetles having predaceous food habit is reported from North America and India. Adult *Cremastocheilus stathamae* Cazier (Cetoniinae) are obligate predators of the ant larvae in the nests (Cazier and Mortenson 1965). Another interesting feeding behaviour of a cetoniid beetle *Spilophorus maculatus* (Gory and Percheron) has been reported by Ghorpade (1975) from Southern India. These beetles feed on the nymphs of the treehopper *Oxyrhachis tarandus* Fab. occurring on *Acacia concinna*. Cremastocheilini feed on a variety of insects but it is not known whether they became predaceous before or after their invasion of ant nests.

The two genera *Chaetopisthes* and *Corythoderus* of Aphodiinae, are found with *Odontotermes* sp. in India. *Chaetopisthes assmuthi* Wasmann is quite common in the nests of *Odontotermes obesus* (Rambur) (Wasmann 1903). Although these beetles are normally found in the fungus gardens, they may also occur in the royal cells. The termite workers find the trichomes attractive and carry the beetles from place to place and the beetles feed on the fungus (Kistner 1982) but how this help the termites is not known.

Melolonthines are rarely reported from ant and termite nests. A species of *Diplotaxis* is reported from the nest of *Pogonomyrmex occidentalis* (Idaho, USA) and a species of *Maechidias* in ants and termites nest is reported from Australia (Lea 1910).

The Dynastid, *Coelosis bilobata* Linn. found with *Atta sexdens* in Brazil is supposed to be the largest of all the myrmecophilous arthropods (Eidmann 1931). The adult beetles lay eggs in the leaf mulch which are carried to the fungus gardens where they live in oval earthen holes and feed on fungus.

Nest making reaches its epitome among dung and carrion feeding Scarabaeinae (Eickwort 1981). Since the food source, dung or carrion, is ephemeral and randomly scattered it should be removed and protected from desiccation and has to be buried before egg laying. Both sexes frequently participate in food provisioning, defence and prevention of fungus contamination resulting in parental care and true subsocial behaviour.

The peak of subsocial behaviour is seen in *Cephalodesmius* and *Necrophorus* in which larvae are provided food by regurgitation (Wilson 1971). In *Cephalodesmius* the male is responsible for foraging and the female molds the food into 'cake' and allow it to ferment for two weeks adding adult feces to it. This "home made dung" is thus partitioned by the female into six to ten brood balls. The male and female remain in the nest till their offspring emerge as adults (Halffter 1977).

According to Halffter and Matthews (1966). "Scarabaeine beetles live in a world of smell and touch almost exclusively and that a suitable ambient temperature is the first requisite for activity". Olfaction seems to be the dominant sense of scarabaeines, image perception not being that dominant due to poor vision. Light is used perhaps only for orientation. Sound production although exists in most scarabaeinae, auditory stimuli is very less. Tactile perception seems to be highly developed particularly in ball-rolling beetles.

Considering the Scarabaeinae as a whole, the food used by the majority of the species both for the larvae and for adult is the excrement of large animals, particularly of mammals and man, suggesting that these beetles are coprophagous. However other types of food habits, among the scarabaeines are not very rare.

Necrophagy is found in one genus *Onthophagus* in South America and India. A number of species of *Onthophagus* are known to be carrion feeders in India. *Onthophagus igneus*, *O. unifasciatus*, *O. pygmaeus* and *O. kchatriya* were found in the carcasses of crows and frogs in Bangalore (Veena Kumari 1984).

Saprophagous scarabaeids are not uncommon. There are different types of saprophagous Scarabaeinae feeding on leaf litter, vegetable debris, decaying fruits, fungi etc.

Predatory habits among the scarabaeines are rare except a Brazilian species of *Canthon* which attacks ants of the genus *Atta* (Navajas 1950).

In addition, there are several special ecological niches where the scarabaeines are found, although their food habits are not well defined.

There are reports of scarabaeines on ectocommensals of mammals like monkeys in Brazil, sloths in America, kangaroos and wallabies in Australia.

As endoparasites of mammals there are reports of scarabs causing 'Scarabiasis' in India among human beings resulting in recurrent intestinal illness accompanied by bloody diarrhoea due to *Onthophagus bifasciatus* (Fab.) and *Caccobius vulcanus* (Fab.) (Senior-white 1920; Iyengar 1923).

In recent years there are reports of Scarabaeinae, mainly *Onthophagus*, occurring in nests and burrows of vertebrates, particularly in rat burrows.

Examples of termitophily and myrmecophily in Scarabaeinae are many. Arrow (1931) has reported presence of *Sisyphus longipes* in the nest of *Pheidole rhombinoda* in Madras.

Largest number of Scarabeinae are known from Grassland biomes and Forest ecosystems. High mountain colonization of these beetles is known from the Himalayas. Various species of *Copris* are known to climb high mountains between 2000 and 2500 m. *Caccobius himalayanus* Jekel has been collected frequently at 3000 m and *Onthophagus tibetanus* Arrow lives between 3000–4200 m in Sikkim and Tibet. The highest locality known for any scarabaeid is that of *O. cupreiceps* which is found at 5200 m and the same has not yet been collected below 4000 m (Arrow 1931; Balthasar 1963). Feeding and breeding behaviour of these high altitude scarabs are not known clearly.

Fossilized scarab brood balls have been described from various tertiary deposits in South America, which demonstrate nidification behaviour at a fairly advanced level in Scarabaeinae, as early as in the lower Oligocene (Balthasar 1963).

Detection of food and approach behaviour like search flight, altitude of flight, distance at which the smell of food is first perceived in the Scarabaeinae are not fully understood. These behaviours differ from species to species and place to place depending on the source of food.

Most of the dung beetles land a little away from the pat and crawl towards the food. Some, like most Coprini come to semiliquid cow dung immediately after deposition, and utilise it in that state. In the case of human excrement many species come a few minutes after deposition of the feces. The Eucraniina dung beetles habitually go to dry excrement under semi desert conditions. *O. tritinctus* is found attracted to dry dung in Bangalore (Veena Kumari 1984).

Feeding behaviour differ from group to group. Adult Scarabaeinae and Geotrupinae nearly always bury the food both for themselves and for their larvae directly beneath or beside the food source. In the genus *Gymnopleurus* most of the species feed at the surface. *G. miliaris* and *G. spilotus* recorded from Bangalore fall under this category.

Overland transportation of food without formation of ball, is done in three ways: (i) carry food with forelegs and walk backward towards the burrow e.g. *Copris* spp. and *Onthophagus* spp. (ii) pieces of food rolled away from the source without making balls and walking forward and pushing with its head and forelegs. This "butting" technique is seen in *Onthophagus tritinctus* (iii) the beetle grasps the food with the forelegs and head, and elevating the fore body it runs rapidly forward on the remaining four legs as found in Argentine subtribe Eucraniina (Kolbe 1905).

Overland transportation with formation of ball is common to the tribe Scarabaeini with a few exceptions. Ball rolling behaviour of dung beetles has been studied in detail in various parts of the world (*Scarabaeus* spp., *Gymnopleurus* spp., *Sisyphus* spp. and *Canthon* spp.)

The biological advantage of ball rolling is that the ephemeral food source, scattered randomly has to be protected from competition from other insects and desiccation.

The ball rolling behaviour seems to have originated with the habit of carrying more or less spherical pellets such as those of rodents, lagomorphs and caprines and later they might have developed other techniques for rolling (Halffter and Matthews 1966).

Ball making behaviour, initiation of ball rolling, the role of sexes in ball rolling, the direction in which balls are rolled, distance rolled and burial of the ball have all been well documented for several species from many parts of the world and the same is beautifully summarised by Halffter and Matthews (1966). The above behaviours are well exhibited in *Gymnopleurus miliaris*, and *Gymnopleurus geoffroyi*.

Nidification behaviour in Scarabaeinae may be classified into four groups:

(i) Egg laid directly in the food mass packed into the blind end or branch or a burrow dug near or under food source e.g. *Onthophagus* spp., *Onitis* spp.

(ii) Egg laid in a pear shaped shell covered with soil, constructed under the food source e.g. *Catharsius* spp.

(iii) Spacious underground chambers are constructed near or under the food source, a large mass of dung is compacted and then divided into several brood ovoids containing one egg each not enveloped in a clay shell. Male and female remain in the nest till the larvae develop. e.g. *Copris*, *Synapsis*, *Catharsius* spp.

(iv) Formation of ball of food on the surface and rolling away on the surface and

laying an egg in it. e.g. *Gymnopleurus, Scarabaeus, Sisyphus* etc.

Male and female cooperation in ball formation and burying the dung, combat and parental care have been reported from a number of species like *Copris hispanus, Copris lunaris, Heliocopris dilloni* etc.

The larval behaviour of some of the Scarabaeinae are interesting. In *Copris repertus* the larva repairs the breech in the brood ball if it is damaged. Also the larvae make a scratching noise when the ball is touched. This noise is a result of scratching the inner wall of the ball with its mandible. Melolonthine beetles emerge at a particular intensity of light (foot candle) in the evening after the first summer rains and get back to the soil early morning at the same intensity of light. Although rain is a must for adult emergence yet unless the gonads are well developed, the young ones will not emerge inspite of the rains. *Holotrichia serrata* needs a minimum of 23°C soil temperature for its gonads to mature (Veeresh 1983).

Among the phytophagous lamellicorn beetles some species show strong tendencies towards a particular host plant, the presence of which decides the distribution of the pest. Adults of *Holotrichia serrata* F. goes only to neem plants *Azadirecta indica* in the midst of several of its host plants. Likewise adults of *Holotrichia nilgiria* Arrow has decided preference to *Ficus racemose* and the pest distribution is confined around the adult beetle's, host plants, in coffee plantations. Similarly *Holotrichia reynondi* Bl. concentrates around *Moringa oleifera* to which it is highly attracted.

Concentration of *Holotrichia* to a particular patch of field seems to be guided by the adults egg laying behaviour, which in turn is influenced by the first few adults going to a particular side of a host tree or a particular patch of a field for egg laying. There is a strong tendency of the beetles following a pheromone trial (Veeresh 1983).

The *Leucopholis* spp. have no attractive adult host plants. *Leucopholis coneophora* female attracts the male while it is still in the process of emergence and more often it gets back into the soil from where it has emerged, for egg-laying.

These are but a few scattered reports of behavioural analysis of feeding and breeding in this vast and widely distributed group of Lamellicorn beetles. Except for a few reports, nothing is available from India on the behaviour of Lamellicorn beetles. Many more fascinating accounts of behaviour particularly of Scarabaeinae will come to light if more and more attempts are made on these abundantly available beetles.

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# Evolution of insect sociality—A review of some attempts to test modern theories

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**Abstract.** An important feature of insect societies is the presence of a sterile worker caste that makes it possible for the fertile queens to produce a large number of offsprings. The mechanism of evolution by natural selection of such sterility and similar, though less extreme, forms of altruism has long been considered as a paradox. In recent years a large body of theoretical ideas has accumulated that purports to explain altruistic behaviour within the framework of the theory of natural selection. With special reference to insect sociality three theories namely kin selection, parental manipulation and mutualism have been suggested. Some attempts have now been made to empirically test the mutually exclusive predictions arising out of these alternative theories. A somewhat different approach to empirically distinguishing between kin selection and parental manipulation is to measure sex-investment ratios. This approach was at one time believed to have provided overwhelming support in favour of the theory of kin selection. It has now been realised that several complicating factors such as local mate competition and multiple mating have to be considered before arriving at appropriate theoretical predictions of the two rival theories. I argue in this paper that rigorous quantitative studies on inter-individual variations in behavioural strategies in primitively eusocial insects constitutes yet another approach that is likely to help in understanding the forces that mould the evolution of insect societies.

**Keywords.** Social insects; kin selection; parental manipulation; mutualism; altruism; quantitative ethology; sex-investment ratios.

## 1. Introduction

Living organisms present a fantastic diversity of structure, function and behaviour unparalleled by anything in man's experience. This diversity has kept generations of biologists so busy in describing and cataloguing life phenomena that biology has sometimes been compared to stamp collecting. It is perhaps no exaggeration to say that Darwin along with Wallace changed this scene and converted biology into a 'Real Science' by his unifying theory of evolution by natural selection. Darwin set the tradition of asking such questions as why do peacocks have those incredible feathers?, why is sickle cell anemia more common in certain parts of Africa?, why do Hanuman Langurs commit infanticide?

## 2. The theory of evolution

The theory of evolution by natural selection is as pretty as it is simple. Living organisms normally produce many more offsprings than can be supported by the environment. This results in intense competition for survival. The individuals in each generation are not all identical but show a whole range of variation in their structure, function and behaviour that leads to differences in efficiencies of survival and reproduction; differences both from one variant to another as well as from one environment to

another. If these features are inherited, as they commonly are, it follows that in any given environment some kinds of organisms survive and reproduce better than others and thereby come to dominate the population. The variants that dominate in any given environment are often described by biologists as being 'adapted' to or being 'fittest' in that environment and the efficiency of survival and reproduction is called the fitness. Darwin put together a lifetime's experience in natural history to amass evidence for his theory (Darwin 1859). The result was overwhelming but there was one odd fact that did not fall in place. It is a testimony to Darwin's perceptive mind that he did not fail to notice this anamoly.

## 3. The puzzle

Many animals ranging from slime molds to man live in societies of varying degrees of organisation. Individuals in these societies sometimes behave as if they are not maximising their own fitness but lowering it in order to maximise somebody else's fitness. At the approach of a predator a squirrel gives an alarm call that warns off its neighbours but the individual that gives the alarm call itself attracts the attention of the predator and increases its chances of becoming prey. The grand finale in the evolution of such altruistic behaviour is the case of the worker honey bee that never reproduces on its own but rather spends its entire life-time working for its colony. Nor does this superaltruist hesitate to sting an approaching predator notwithstanding the fact that stinging is suicide. Every honey bee that stings dies within the next few minutes because its barbed sting as well as a part of its intestines are pulled out as it tries to fly away. It was this example of sterile workers in social insects that attracted the attention of Darwin.

## 4. The social insects

Understanding the forces that mould the evolution of social behaviour is one of the most challenging areas of modern biology. A whole new field of sociobiology has developed to meet this challenge (Wilson 1975; Barash 1982). Social insects, especially the ants, bees and wasps have been the focus of special attention in this context (Wilson 1971). The reasons for this have been three fold. Firstly, social insects show the most extreme forms of altruistic behaviour such as the case of the sterile worker bee. Secondly these insects exemplify a series of stages in the course of evolution from the solitary to the highly eusocial. Thirdly the ants, bees and wasps are characterized by a peculiar kind of genetics known as haplodiploidy that introduces asymmetries in genetic relatedness between siblings on the one hand and between parents and offsprings on the other thereby predisposing them towards sociality. (see below).

## 5. The theory of kin selection

### 5.1 *Statement of the theory*

Hamilton (1964a, b) proposed what has now come to be known as the theory of kin selection. Hamilton argued that social or altruistic traits are selected for by natural

selection because, although they decrease the classical individual fitness of an animal, they serve to increase the number of copies of the genes coding for such behaviour. This is because altruism is often directed towards genetic relatives who are also likely to carry the same genes. Thus there is really no altruism from the point of view of the genes. Altruism at the level of an individual animal is simply the genes' way of making more copies of itself. Thus animals that behave altruistically may still be maximising their 'inclusive fitness' which is the sum of their direct contribution through their offspring to the gene pool and their indirect contribution through their relatives. This argument can be stated precisely as follows:

As altruistic act will be favoured if

$$\frac{b}{c} > \frac{1}{r}$$

where $b$ is the benefit to the recipient, $c$ the cost to the donor and $r$ the coefficient of genetic relatedness between donor and recepient, benefit and cost being measured in fitness units. In other words an altruistic act will be favoured if the benefit to the recipient devalued by the probability that he carried the gene in question is greater than the cost to the donor. Altruistic traits will therefore spread rather easily either if the benefit to cost ratio is high or if the recipient and donor are very closely related.

## 5.2 *Haplodiploidy*

Consider a hypothetical diploid organism that gives up producing its own offspring and instead helps its parents to produce more of its siblings. This behaviour, if genetically coded, will spread in the population only if our hypothetical organism can raise more siblings than it gives up offspring. This is because both siblings and offsprings are equally related to it ($r = 0{\cdot}5$). On the other hand if the siblings were more closely related to it than its own offspring, then the behaviour would be selected even if less siblings were produced than offspring given up. This is the kind of situation that occurs in many social insects. The only truly social (with sterile castes) animals are among ants, bees, wasps, termites and a single example from higher animals, the naked mole rat. Of these ants, bees and wasps belong to the insect order Hymenoptera a group characterised by haplodiploidy. Males develop from unfertilized eggs and are consequently haploid. Females develop from fertilized eggs and are diploid. This introduces asymmetries in genetic relatedness (table 1). For example a female hymenopteran is more closely related to her sister ($r = 0{\cdot}75$) than her own daughter ($r = 0{\cdot}5$).

**Table 1.** Coefficients of relatedness under haplodiploidy assuming complete outbreeding.

| | Daughter | Son | Sister | Brother | Mother | Father |
|---|---|---|---|---|---|---|
| Female | 0·5 | 0·5 | 0·75 0·25 (Av = 0·5) | | 0·5 | 0·5 |
| Male | 1·0 0·0 (Av = 0·5) | | 0·5 | 0·5 | 1·0 0·0 (Av = 0·5) | |

### 5.3 *Implications of haplodiploidy*

The concept of inclusive fitness together with a knowledge of the asymmetries in genetic relatedness lead to a number of predictions summarised by Wilson (1971) as follows:

(i) True sociality should be more common in haplodiploid organisms than in diploid ones.
(ii) Queens should not be mated by more than one unrelated male.
(iii) Males should be more selfish than females.
(iv) Females should be more altruistic towards their sisters than towards their brothers or neices.
(v) Workers should prefer their own sons over their brothers.

Wilson (1971) also summarised the evidence supporting these predictions and showed that most of the evidence is in qualitative agreement with the theory of kin selection. As pointed out by him this qualitative agreement means that 'the factor of haplodiploid bias should be taken into account in future evolutionary interpretations and as a guideline in planning some further empirical research' but not as proof of the correctness of the theory. What then should we do in order to ascertain the validity of kin selection theory? First we ought to put the theory to a critical test by generating quantitative predictions that can potentially falsify the theory and perform the appropriate experiments to see if the predictions are borne out. This alone is sometimes considered inadequate and what we need therefore is also a comparison of the predictions of two or more competing theories (see Lakatos and Musgrave 1970 for a detailed discussion of the methodology of scientific research). In recent years some attempts have been made in providing both these requirements and in the remaining pages some of these studies are reviewed.

## 6. Alternative theories

### 6.1 *Parental manipulation*

The theory of parental manipulation advanced by Alexander (1974) states that altruistic behaviour could evolve even if it does not increase the inclusive fitness of the altruistic animal because selection can act on the parent to manipulate some of its offspring to be altruistic towards the rest. Ecological conditions could be imagined under which, an animal that produces a certain fraction of sterile offspring which in turn help the remaining fraction of fertile offspring to survive and reproduce better, could leave behind more grandchildren than an animal that produces all fertile, selfish offspring.

### 6.2 *Mutualism*

Lin and Michener (1972) emphasizing the ecological factors involved in the evolution of altruistic behaviour argue that at least in the early stages of the evolution of sociality, when complete sterility had not yet evolved, mutual advantage in defence against predation for example, might have been an important force. This idea might be more important in present day primitively eusocial insects than has been hitherto suspected (see below).

## 7. Testing the theories

In this section three major approaches that are being pursued in making quantitative tests of the theories are discussed. The first is the direct measurement of inclusive fitness, the second tests predictions of optimal sex investment strategies and the third involves quantitative studies on the ethology of primitively eusocial insects. I will discuss the logic behind each of these approaches and then very briefly review illustrative examples of empirical investigations representing these approaches, making however no attempt to provide a comprehensive review of the literature. The interested reader may consult several excellent reviews dealing both with theoretical ideas as well as empirical studies on social insects (Evans and West–Eberhard 1970; Wilson 1971; Hamilton 1972; Spradbery 1973; Michener 1974; West–Eberhard 1975; Hermann 1979–82; Starr 1979; Edwards 1980; Jeanne 1980; Barash 1982; Charnov 1982; Michod 1982; Brian 1983).

### 7.1 *Computation of inclusive fitness*

This approach has mainly been applied to primitively eusocial wasps of the genus *Polistes*. Typically these wasps follow one of two strategies. Sometimes a single female initiates a colony on her own and raises the first brood of offsprings unaided by any other wasps and later the daughters from her first brood become workers and help her to raise reproductive male and female offsprings. Alternatively a group of females of the same generation, typically sisters, jointly found a nest and again produce a first brood of workers which help them raise reproductive offspring. Here all the cofoundresses do not contribute equally to the production of reproductive offspring. Sometimes only a single dominant ($\alpha$) female lays all the eggs while the other cofoundresses remain subordinate and behave like workers. Even if the subordinate ($\beta$) cofoundresses lay some eggs this number is usually less than that laid by the $\alpha$ foundress. In such a situation it is possible to calculate the productivities of the single foundress colonies and the multiple foundress colonies. With a knowledge of the genetic relatedness between cofoundresses and, the exact number of offsprings produced by each cofoundress one can compute the fitness of the solitary foundress as well as the inclusive fitness of the dominant cofoundress and each subordinate foundress in multiple foundress colonies.

For the sake of simplicity let us consider two full sisters $\alpha$ and $\beta$, each having mated with any one male, in a diploid population (table 2). When nesting solitarily (table 2A) let them produce 10 offspring each thus having an individual fitness of 5 (10 offspring $\times$ 0·5, the coefficient of genetic relatedness to each offspring) and an inclusive fitness of 7·5 each (individual fitness of 5 + indirect contribution of 2·5 as a result of 10 neices or nephews $\times$ 0·25, the coefficient of relatedness to each nephew or neice). Note that the term *individual fitness* is used for the direct contribution to the gene pool and the term *inclusive fitness* for the *sum* of the direct and indirect contributions. When they nest together (table 2, B) and if their summed productivity does not increase but merely $\alpha$ produces all the 20 possible offspring and $\beta$ produces none of her own but helps her sister, $\alpha$ has a fitness as well as inclusive fitness of 10 while $\beta$ has a fitness of 0 but an inclusive fitness of 5. This situation is advantageous to $\alpha$ but not to $\beta$. $\beta$ will therefore not be selected to accept this subordinate role. Nor will selection acting on the parents of $\alpha$ and $\beta$ favour their manipulating $\beta$ into remaining subordinate and helping $\alpha$.

**Table 2.** Conditions for the evolution of sterility in a diploid population where $\alpha$ and $\beta$ are full sisters.

| | | $\alpha$ | $\beta$ | Remarks |
|---|---|---|---|---|
| | Number of offspring | 10 | 10 | |
| A | Classical individual fitness | 5 | 5 | Solitary |
| | Inclusive fitness | 7·5 | 7·5 | Nesting |
| | Number of offspring | 20 | 0 | Sterility |
| B | Classical individual fitness | 10 | 0 | Will not be selected |
| | Inclusive fitness | 10 | 5 | |
| | Number of offspring | 21 | 0 | Parental |
| C | Classical individual fitness | 10·5 | 0 | manipulation |
| | Inclusive fitness | 10·5 | 5·25 | |
| | Number of offspring | 31 | 0 | Kin selection |
| D | Classical individual fitness | 15·5 | 0 | |
| | Inclusive fitness | 15·5 | 7·75 | |
| | Number of offspring | 21 or 0 | 0 or 21 | Classical |
| E | Classical individual fitness | 10·5 or 0 | 0 or 10·5 | Individual selection |
| | Inclusive fitness | 10·5 or 5·25 | 5·25 or 10·5 | |

Sterility on the part of $\beta$ will thus not spread by any of the mechanisms under consideration, parental manipulation, kin selection or individual selection. On the other hand, when nesting jointly (table 2, C), if there is even a slight increase in the total productivity such as $\alpha$ being able to produce 21 offspring then the situation is quite different. Although $\beta$ still has a lower inclusive fitness than when she nested solitarily, $\alpha$ and $\beta$ together have done better than when they were nesting solitarily. Thus it pays the parents of $\alpha$ and $\beta$ to manipulate $\beta$ into being subordinate and sterile because now the parents of $\alpha$ and $\beta$ have more grandchildren. Parental manipulation can thus promote the spread of sterility on the part of $\beta$ although $\beta$ loses in the process. If the productivity increases substantially (table 2, D) such that $\alpha$ produces, say, 31 offspring then $\beta$ gets an inclusive fitness of 7·75 although she does not produce a single offspring. Now kin selection acting on $\beta$ will promote the spread of subordinate behaviour as $\beta$ now contributes more to the gene pool of the population than when she was nesting solitarily and producing 10 of her own offspring. The exact amount by which productivity under joint nesting should increase would depend upon the degree of relatedness between the altruist and those she individual raises. Under haplodiploidy, if a female worker raises sisters in place of daughters then the productivity need not even increase because the workers are more closely related to their sisters than to their daughters (0·75 *vs* 0·5). If they raise equal numbers of brothers and sisters of course they gain no more fitness than they would, had they raised sons and daughters instead. The asymmetries in genetic relatedness created by haplodiploidy could however be capitalised on if the workers produce more sisters and less brothers. This by itself is a prediction of kin selection theory whose verification has also been attempted (see below).

It is of course possible that both $\alpha$ and $\beta$ produce offspring when nesting jointly and

that both produce more offsprings than when they were nesting solitarily. This will lead to the evolution of joint nesting by classical individual selection but there will be no sterility. Such a situation is envisaged by Lin and Michener (1972) in the early stages of the evolution of sociality. Ecological conditions can be sufficiently harsh and it may for example be impossible for a single female to guard her nest against predators as well as forage for food. Thus by nesting jointly both $\alpha$ and $\beta$ may increase their offspring production. Once such joint nesting gets established in the population it could give scope for the evolution of parental manipulation or kin selection. Another interesting case of evolution of sterility is possible through classical individual selection (mutualism) which may be more important in present day primitively eusocial insects. For example, let $\alpha$ and $\beta$ nest jointly but who will lay the eggs and who will remain sterile be decided by chance (table 2, E). If $\alpha$ and $\beta$ each have on the average an equal chance of egg laying then, even if there is a slight increase in productivity due to joint nesting, classical individual selection will promote such sterility. When nesting, solitarily $\alpha$ and $\beta$ each get an inclusive fitness of 7·5. Here each gets on the average $10{\cdot}5 + 5{\cdot}25 = 15{\cdot}75/2 = 7{\cdot}875$. In *Polistes exclamans* the $\beta$ foundress remains subordinate and waits for the $\alpha$ foundress to produce a batch of workers. When it is time to lay eggs that will mature into reproductives the $\beta$ foundress suddenly becomes very aggressive and challenges the $\alpha$ foundress. In a substantial number of cases the $\beta$ foundress succeeds in driving away the $\alpha$ foundress and then lays all the eggs. (Alan MacCormac personal communication). This would be a situation analogous to the example described above and here subordinate behaviour and sterility with a certain probability could be brought about by classical individual selection.

Perhaps the most complete study attempting to distinguish between different theories by the computation of inclusive fitness is that of Metcalf and Whitt (1977) on *Polistes metricus*. They used enzyme electrophoresis to determine the genetic relatedness between different individuals in the colony and knowing the productivity of single and multiple foundress colonies they were able to calculate the inclusive fitnesses of a solitary foundress, the dominant $\alpha$ foundresses and the subordinate $\beta$ foundresses of joint nesting colonies. Their results are as follows:

In the population studied by Metcalf and Whitt, 83% of the colonies were solitary foundress colonies. In multiple foundress colonies, $\alpha$ foundresses had a relative inclusive fitness $1{\cdot}83 \pm 0{\cdot}57$ times that of a solitary foundress while the $\beta$ foundress had a relative inclusive fitness $1{\cdot}39 \pm 0{\cdot}44$ times that of a solitary foundress. A female's expected number of grandchildren from two of her daughters jointly founding a nest is 1·55 times what she would achieve by the two daughters acting as two solitary foundresses.

Since the $\beta$ foundress does not have an inclusive fitness significantly greater than that of the solitary foundress, neither kin selection theory nor mutualism (classical individual selection; table 2, E) can be accepted as the selective force responsible for the altruistic behaviour of $\beta$ although the authors themselves conclude that their results are in accordance with the predictions of kin selection theory. Parental manipulation could explain this behaviour on the part of $\beta$ provided the figure of '1·55 times more grandchildren' is statistically significant (no standard deviation is provided by the authors). Besides one does not know how to interpret the fact that 83% of the colonies were single foundress colonies (see below). A very similar study was conducted on a related species *P. fuscatus* (Noonan 1981). Not having used electrophoresis Noonan did not know the exact values of genetic relatedness but made her computations of inclusive fitness for different possible values of relatedness between the dominant and

subordinate females on a nest (full sisters, half sisters etc.). These results showed that subordinate females lay some eggs (and gain some individual fitness) but not enough to make subordinate roles better than solitary nesting. Because foundresses are often sisters the subordinates have inclusive fitness values greater than their individual fitness values. Noonan's data however do not permit distinction between parental manipulation and kin selection models because of the high variances associated with her mean values of inclusive fitness. Thus we find that the best empirical studies using the approach of computing inclusive fitness does not permit us to draw any definite conclusions.

I would also like to argue that there are difficulties with this approach itself. Firstly the kind of analysis described above compared wasps which are naturally nesting either solitarily or jointly. Implicit in such a comparison is the assumption that the two females being compared are reproductively equivalent and that any difference in productivity is only because of solitary versus joint nesting. We have no information that might help decide whether this assumption is valid or not. However, if solitary and joint nesting females have different reproductive potentialities, then the effects of the intrinsic differences in productivity and the effects of nesting strategy will be confounded in the analysis. The second problem with this approach is that the frequency of solitary versus joint nesting varies widely in different situations. No attempt has so far been made to take this into consideration. Ideally, the theory should be able to predict the frequency distribution of foundress size associations. Even if we find that the inclusive fitness of the subordinate foundress is much higher than a solitary foundress, there would still remain a puzzle if it turns out that most of the females prefer to nest solitarily.

### 7.2 *Sex investment ratios*

We argued earlier that haplodiploidy predisposes the hymenopterans to the path of sociality because a female is more closely related to her sister than to her daughter. But this asymmetry exactly cancels out because a female is less closely related to her brother than to her son. Her average relationship to her offspring of 0·5 is exactly the same as her average relationship to her siblings $\left(\frac{0{\cdot}75+0{\cdot}25}{2}\right)$. Trivers and Hare (1976) argued therefore that workers in hymenopteran societies should be capitalizing on the asymmetries created by haplodiploidy by investing differentially in their sisters and brothers, in fact in the ratio 3 : 1, which is the ratio of their genetic relatedness to their sisters and brothers respectively. Since it is the workers who feed the larvae, it should be easy enough for them to feed their sisters and brothers differently and achieve the maximum possible inclusive fitness. Notice however that on the Fisherian argument (Fisher 1930) the mother queen who is equally related to her sons and daughters would prefer an equal investment in brood of the two sexes (assuming on outbreeding, random mating population). In other words there is a conflict of interests between the queen (who prefers a 3:1 ratio of investment) and the workers (who prefer a 3:1 ratio of investment). Notice that this approach too makes a precise quantitative prediction and also contrasts kin selection theory with the parental manipulation theory. If the parental manipulation theory is correct, then it means that the mother queen should be able to have her way and manipulate the workers to invest equally in the two sexes although it is not the optimum strategy for them. If kin selection theory on the other hand is

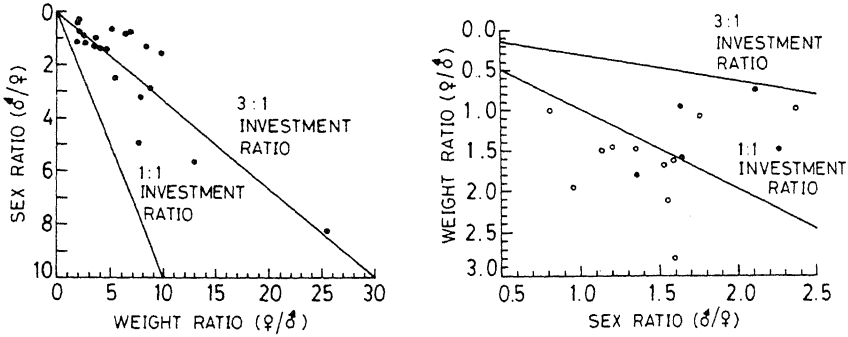


**Figure 1.** **A.** Relationship between the sex ratio and weight ratio of reproductive caste ants. The two lines show 1:1 and 3:1 investment ratios. **B.** Relationship between sex and weight ratios for solitary bees (open circles) and solitary wasps (closed circles) compared for 1:1 and 3:1 investment ratios (modified slightly from Trivers and Hare 1976, after Barash 1982. Reprinted with permission).

correct, then workers behave altruistically only because this is the strategy that maximises their inclusive fitness. But it would not maximise their inclusive fitness if they invested equally in their brothers and sisters. Workers should therefore have their way and invest in the ratio 3:1.

Trivers and Hare (1976) weighed the total male and female reproductive brood in a large number of species and appeared to provide overwhelming support for kin selection theory. In a large number of monogynous ant species the weight ratio of the reproductive brood was significantly close to the 3:1 prediction. Besides, in solitary bees and wasps, and termites the investment ratio as expected was close to 1:1.

Soon after the publication of this study, Alexander and Shermann (1977) pointed out that there are serious problems with the interpretations of Trivers and Hare. The predictions used by Trivers and Hare are valid only if the queens have mated only once and only if the populations are completely outbreeding. If the queens mate more than once then the workers are not necessarily full sisters of the reproductive siblings and therefore they would not be expected to invest in the ratio 3:1. If the population inbreeds then according to the theory of local mate competition proposed by Hamilton (1967) even the queens would prefer a female biased sex investment ratio. According to Alexander and Shermann, Trivers and Hare's data thus may not represent the triumph of kin selection theory over parental manipulation theory but may simply be a response to local mate competition. Supporting this argument is a 1:1 ratio of investment demonstrated in *Polistes fuscatus* in a situation where local mate competition was known to be absent (Noonan 1978). One obvious difficulty here is the lack of good empirical data on multiple mating and local mate competition. Multiple mating has long been realised to be very common in social insects (Wilson 1971) but it has often been ignored because the sperms from different males have been assumed not to mix in the spermatheca (Orlove 1975, for example). Page and Metcalf (1982) have recently demonstrated that at least in the honey bee, sperms from different males do mix and that the average genetic relatedness between workers and the reproductive sisters they rear can be quite low. In any case it is clear that multiple mating and local mate competition both influence the predictions of optimum sex investment ratios in the framework of kin selection theory and parental manipulation theory. Information on the extent of multiple mating and local mate competition in social insects is thus urgently needed.

Given certain levels of multiple mating and local mate competition, the prediction of optimum sex investment ratios by kin selection theory and parental manipulation theory would be different from those used by Trivers and Hare. Recently Joshi and Gadagkar (1985) have modelled this phenomenon and computed the optimum sex investment ratios under different levels of multiple mating and local mate competition (figure 2). This has been done by considering a haplodiploid population with monogynous colonies where a certain fraction of the reproductive offspring disperse and outbreed while the remaining fraction undergo brother-sister mating. Our results can be summarised as follows (figure 2). In the absence of multiple mating and local mate competition, parental manipulation theory (queen control) predicts a 1:1 investment ratio while kin selection theory predicts a 3:1 ratio (female:male). Local mate competition biases the investment ratio in favour of females in the frame work of both the theories. For any given value of local mate competition however, the investment ratio predicted by parental manipulation theory is more male biased than that predicted by kin selection theory. Multiple mating does not affect the prediction of the parental manipulation theory at all but makes the optimum investment ratio under kin selection theory more male biased than the single mating case. We hope that the exact predictions of sex investment ratios in the framework of these two theories provided by us will be used in a fresh attempt to distinguish between these theories in future studies that should attempt to measure not only the sex investment ratios but also the levels of multiple mating and local mate competition.

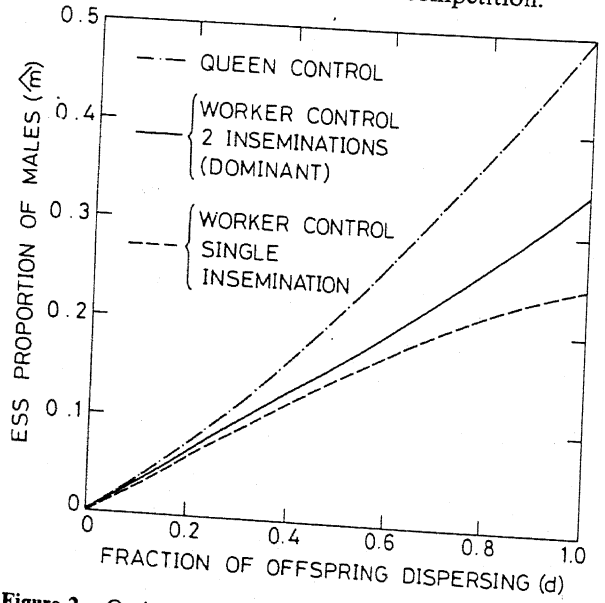


**Figure 2.** Optimum sex ratio at different levels of local mate competition. Evolutionarily stable proportions of male offspring (m) are plotted as a function of the fraction of offspring dispersing to outbreed (d) for three cases. (——·——)when queens control the ratio of investment (parental manipulation). (---) when workers control the ratio of investment but the queen has mated only once i.e., the workers are rearing their brothers and full sisters. (——) workers control the ratio of investment but the queen has mated with two unrelated males so that, the workers are rearing their brothers and a combination of full and half sisters. In the model investigated, the workers cannot distinguish between their full and half sisters. (From Joshi and Gadagkar 1985. Reprinted with permission.)

## 7.3 *Quantitative ethology*

In the last few years I have been studying the behaviour of adults on colonies of primitively eusocial wasps such as *Ropalidia* in India and *Polistes* in America with the hope that these studies will in the long run provide a third approach to understanding the forces that mould the evolution of social behaviour (Gadagkar 1980; Gadagkar and Joshi 1982a, b, 1983, 1984, 1985). Taking *Ropalidia marginata* in India as an example I shall now illustrate this approach. Colonies of *Ropalidia marginata* are initiated by one or a group of females (foundresses) at any time of the year (Gadagkar *et al* 1982a, b). One of these foundresses assumes the role of the queen while the others remain subordinate to the queen and assume the role of workers. Of the female offspring produced in such a colony many remain at the parent colony and become workers although some leave it to found or join other colonies. Males disappear from the colony within a few days after their emergence. The lack of a very severe winter in peninsular India permits these colonies to be perennial. A single colony can therefore survive for many years. This makes it possible for queens to be replaced (Gadagkar unpublished observations). When an existing queen dies or is driven away, one of the other females takes over and begins to lay eggs. In addition, females sometimes leave their parent colonies to found their own colonies either alone or in small groups where one of them again becomes the queen. These events can occur any time in the year and therefore every female must have a fair chance of becoming a queen.

I argue that in such a situation these colonies cannot simply consist of a queen and a bunch of willing workers but each colony must be a highly competitive association of female wasps each trying to maximise its chances of becoming a queen either by leaving the colony at the appropriate time and perhaps with the appropriate company or to challenge the existing queen at the appropriate moment and inherit the nest, its brood and the workers. Using two monogynous colonies of *R. marginata*, I sought to discern the competitive strategies of these wasps by carefully studying and quantifying their behaviour. Instead of following the classical method of concentrating either on species specific behaviour patterns or on certain kinds of behaviours that appear to be important from our point of view, I decided to study the patterns in which the wasps allocated their time between different behaviours. Since time must be a very limiting resource, I considered those behaviours important in which the wasps spent more time. Besides, my emphasis was on the differences between different individuals within a colony. Sitting, sitting with raised antennae (alert to external disturbance), sitting with raised antennae and raised wings (a state of alarm), walking on the nest, being in the cells, and being away from the nest turned out to be the activities in which the wasps spent most of their daylight hours. For these six behaviours I constructed time-activity budgets for a number of individually identified animals. As it turns out, all animals studied spent about 95 % ($95{\cdot}9 \pm 4{\cdot}4$) of their daylight hours in these six behaviours but the manner in which they allocated their time between these behaviours was highly variable (figure 3).

These time-activity budgets were analysed by multivariate statistical techniques such as principal components analysis and hierarchical cluster analysis with the aim of understanding the difference between individuals. The wasps could be classified into three distinct behavioural castes (figure 4) with sitting, sitting with raised antennae and being absent from the nest as the main attributes of the three clusters respectively (figure 5). I had also collected data on the frequencies of certain rare behaviours such as

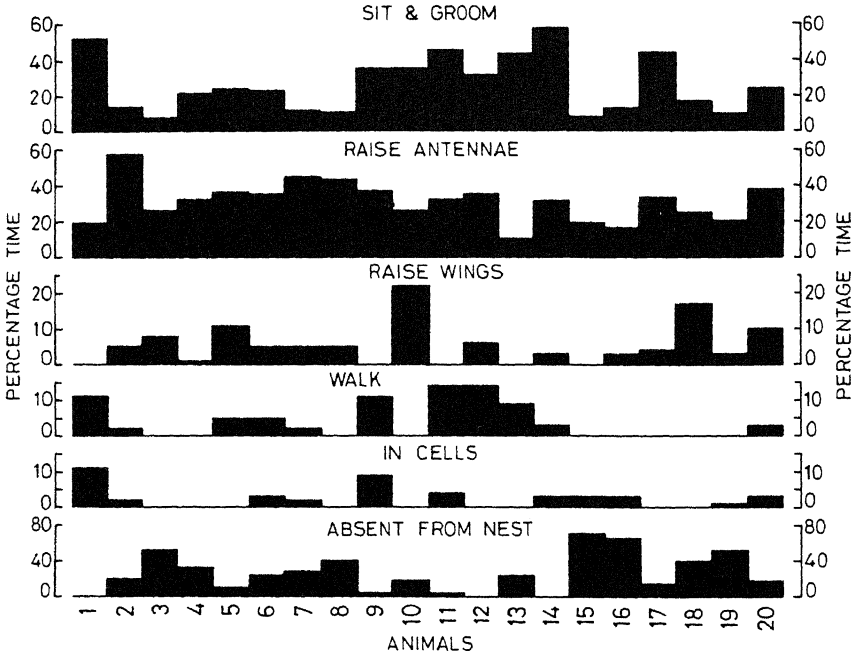


**Figure 3.** Time-activity budgets of 20 individually identified animals from 2 monogynous nests for 6 behaviours. Animals 1 and 14 are the queens of nest 1 and 2 respectively.

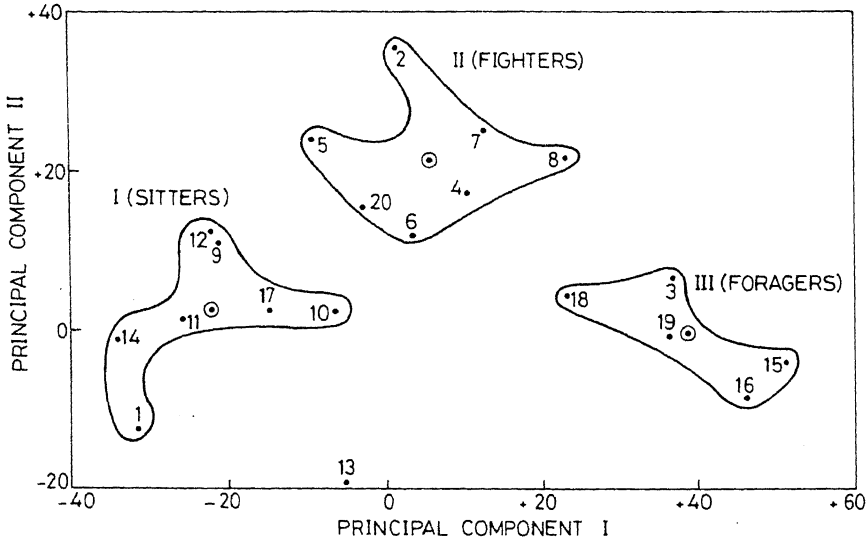


**Figure 4.** Behavioural castes of *R. marginata*. Twenty wasps from 2 different nests are shown as points in the coordinate space of the amplitudes associated with the first two principal components. The points fall into three clusters (or castes) by the criterion of nearest centroid. Circled dot = centroid. (From Gadagkar and Joshi 1983a. Reprinted with permission).

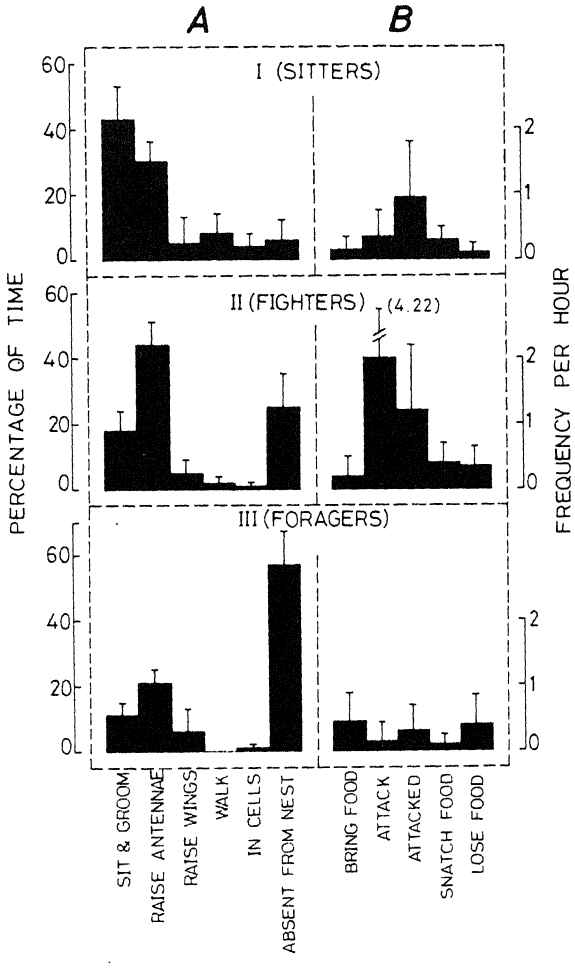


**Figure 5.** Mean behavioural profiles of the clusters obtained in figure 4A, mean percentages of time spent in each of the six activities that were used in obtaining the clusters are shown for sitters, fighters and foragers. **B.** mean frequencies per hour of the five activities that were not used to obtain the clusters are shown for sitters, fighters and foragers. (From Gadagkar and Joshi 1983a. Reprinted with permission).

dominance behaviour, egg laying etc. which were not used in the analysis. Sitting with raised antennae is positively correlated with dominance behaviour ($P < 0{\cdot}01$). Wasps absent from the nest often return with food loads (figure 5B). The three clusters were thus named Sitters, Fighters and Foragers (although it is possible that wasps absent from their nests spent some of their time looking for other nests to join or new nest sites to start their own nests).

We can now begin to interpret the biological significance of this behavioural caste differentiation. Note that the two queens (individuals 1 and 14) are among sitters (queens are recognised by their egg laying behaviour). Thus the queens do little other than sitting and grooming because it is perhaps the best strategy to conserve their

energy and maximise their egg laying capacity. But this also means that the queens face little competition from their nestmates who can therefore be 'trusted' to do all the work for the colony. This seemed to be the situation in the colonies of *R. marginata* that were studied. Significantly when some polygynous colonies of another species *R. cyathiformis* were studied, the queens were not sitters but fighters. In the latter case the queens do not just sit but they were the most active individuals in the colony. In *R. marginata* however, there were many sitters who were not queens. These we hypothesise are 'hopeful queens' who still have some chances of becoming queens. On the other hand the fighters could also be hopeful queens who are following an alternative strategy of maximising their chances of becoming queens. These hypotheses assume significance because they can be readily tested. Experiments are in progress where I am studying a number of colonies, classifying the wasps and then removing the queens to see who takes over. Take over somebody does, but preliminary results indicate that either a Sitter or a Fighter could be the replacement queen (Gadagkar unpublished observations). The foragers are interpreted as having the least chance of becoming replacement queens. This interpretation holds whether the foragers really spent all their time away from the nest in foraging or attempted to join or initiate new nests. The fighters very frequently behave dominantly towards other members of the colony. Apart from establishing their claim to the position of the next queen this fighting could have other functions. For example it may serve to keep the foragers active. I have often seen foragers leave the colony after a series of attacks by a dominant wasp. That the queens could be sitters or fighters depending on the extent of reproductive competition they face from their nest mates seems to be supported by our results. As mentioned above queens of monogynous colonies of *R. marginata* were sitters while queens of polygynous colonies of *R. cyathiformis* were fighters. Even in *R. cyathiformis* when a single foundress was attending a colony and thus had no one to compete with, she was a Sitter *i.e.* she did not spend more time with raised antennae. Furthermore in *Polistes versicolor* in Panama, I had an opportunity to study both pre-emergence colonies as well as post emergence colonies. Pre-emergence colonies are associations of females of the same generation. In such a situation, the closest possible relationship between the workers and the brood they rear is 3/8 (neices or nephews). These colonies are therefore likely to be much more competitive than post-emergence colonies consisting of a mother queen and her daughter workers; the latter caring for their siblings. The queens in two pre-emergence colonies studied were Fighters while the queen in the post-emergence colony studied was a Sitter (Gadagkar and Joshi in preparation).

The behaviour of the wasps is very highly variable and seemingly unpredictable. But I believe that in this variability lies the clue to the understanding of the evolution of sociality. Wasps that all look morphologically similar may behave as sitters, fighters or foragers, they may be queens or subordinate workers, they may challenge an existing queen and chase her away to take her position, they may leave the colony alone or with a submissive group to start new colonies. Kin selection theory predicts that the wasps should behave so as to maximise their inclusive fitness. Parental manipulation would predict that the animals should at least sometimes behave not according to 'their own optimum criteria' but according to what is best for their parents to produce the largest number of grandchildren. If we know what factors influence the chances of a wasp becoming an egg layer in a given circumstance it should be possible to predict the behaviour of a given animal within the framework of a particular theory. With this long term aim in mind experiments are in progress in my laboratory to determine the relative

contributions of body size, age, prior social experience, hormone levels etc. to the egg laying capacity and the capacity to win in an encounter with a conspecific.

It is in the context of such present day primitive insect societies that I have been discussing above that mutualism or classical individual selection of the kind considered in table 2E might be important. For instance a wasp may stay on at the parent colony and work as a subordinate individual if it has a fair chance of becoming the next queen. There may be a certain probability of not succeeding but if more inclusive fitness is gained on the average by taking the risk than by solitary nesting, classical individual selection will favour this behaviour and we will see certain individuals remaining sterile even if that particular individual does not have higher inclusive fitness than its solitary counterpart.

## 8. Conclusions

In conclusion it may be said that today we have a very attractive body of theoretical ideas concerning the forces that might be responsible for the origin and maintenance of social behaviour especially in social insects. Several simple minded attempts have been made to test these theoretical ideas but none has yielded unambiguous results. This is primarily because the social insects live in rather complex societies and pursue complex strategies. We need much more information on the details of the lives of these insects, especially in areas such as breeding structure of populations and the factors that influence the chances of an animal's success in an encounter with a conspecific before we can launch more sophisticated empirical tests of the theories.

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# An analysis of the superparasitic behaviour and host discrimination of chalcid wasps (Hymenoptera: Chalcidoidea)

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**Abstract.** Superparasitism is frequently met with in chalcids. The actual mechanism of suppression of the supernumerary individuals is by mutual combat though exceptions to this general rule may also be seen rarely. Many chalcids are known to discriminate between parasitised and healthy hosts. It is an interesting phenomenon that superparasitism occurs even when a female is capable of discriminating parasitised and unparasitised hosts. Several factors play prominent roles in causing superparasitism and the avoidance of superparasitism by a chalcid is the result of maximisation of its reproductive success.

**Keywords.** Superparasitism; host discrimination; analysis; chalcids.

## 1. Introduction

The chalcid wasps are well known for various salient features of their ethology. The majority of chalcids are solitary, developing singly upon their hosts. Superparasitic behaviour is frequently exhibited by many species of chalcids. Superparasitism is the parasitisation of an individual host by more larvae of a single parasitic species than can mature in that host. In superparasitism usually a single parasite individual survives or all may die or the brood may produce undersized weaker adults. When a parasite superparasitises a host it usually condemns its own progeny to death thus resulting in a wastage of its own eggs. To avoid such a contigency it must be able to discriminate between parasitised hosts and unparasitised hosts. Such avoidance of superparasitism is an interesting aspect of insect behaviour. This paper presents an analysis of some of these interesting aspects of the ethology of chalcids.

## 2. Ethology and analysis

Supernumerary individuals are usually suppressed by destruction by mutual combat between the first instar larvae. They attack each other with their mandibles and finally only one survives. However if an egg is laid in a host that already contained an advanced larva then the younger of the two dies due to oxygen starvation and apparently no fighting occurs in most cases, though autoparasitism and hyperparasitism are occasionally met with in chalcids. A different method of suppression of supernumeraries is seen in an *Elachertus* species (Eulophidae) which is a solitary ectoparasite of caterpillars of *Artona*. In this case when more than one egg is laid on a host, the eclosion of one egg causes immediate cessation of development of the remaining ones (Clausen 1940).

When a chalcid finds a host a sequence of behavioural patterns follow (figure 1).

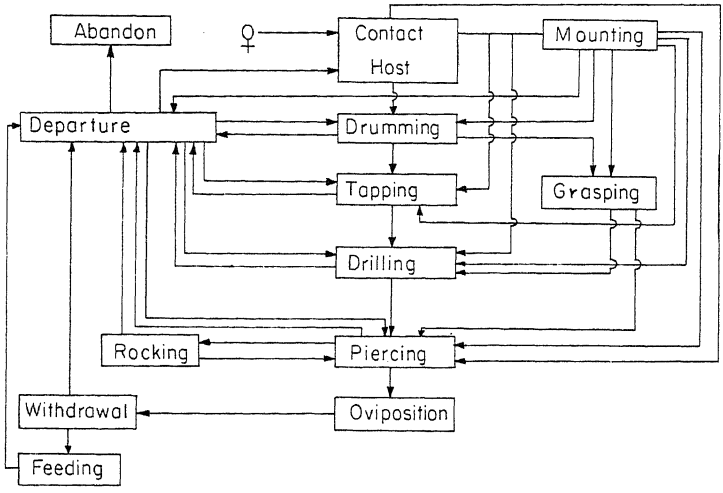


**Figure 1.** Behavioural patterns of chalcids when they come across different types of hosts (suitable and unsuitable hosts).

Fiske (1910) recognised that "the prevalence of superparasitism depends entirely upon whether or not the female parasite is gifted with a prescience which will enable her to select healthy hosts for her offspring". The same author pointed out that the total absence of such an instinct would make the prevalence of superparasitism wholly dependent upon the laws of probability. Fiske found that a vast majority of parasites studied by him showed no such instinct and oviposition therefore, occurred at random with a consequent incidence of superparasitism. Thompson (1924) accepted Fiske's theory of 'random distribution' and gave a brief mathematical interpretation to the problem by providing the formula:

$$Y = N\left(\frac{1-e-x}{N}\right)$$

where $N$ is the number of hosts, $x$ is the number of parasite eggs distributed, $Y$ is the number of hosts parasitised and $e$ is the Napierian logarithmic base. Stoy (in Salt 1932) also believed in the random distribution of eggs and explained that the probable number of hosts that will receive a given number of parasites can be calculated by using the formula:

$$Z = Nx_{C_p}(1/N)^p\ (1-1/N)^{x-p}$$

where $N$ is the number of hosts, $x$ is the number of parasites distributed, $Z$ is the number of hosts containing $p$ parasites. Explaining this formula Askew (1971) pointed out that "A binomial distribution would be approached when several eggs are distributed over a limited number of hosts, but the rate of parasitism is not too high a Poisson series, which is probably more easily calculated, may be used to obtain values for random distribution. The probability of occurrence of 0, 1, 2, 3 etc parasites per host is then

$$e^{-z},\quad ze^{-z},\quad \frac{z^2e^{-z}}{2!},\quad \frac{z^3e^{-z}}{3!}\quad \text{etc}$$

where $e = 2{\cdot}72$ (natural logarithm base), and

$$Z = \frac{\text{total number of parasites}}{\text{total number of hosts}}$$

If the actual distribution of parasite eggs differs significantly from the calculated random distribution in the direction of more hosts than expected supporting only one parasite, and fewer than expected remaining unparasitised then it can be said that the parasite exercises discrimination." There are several reports which show that many chalcids have the ability to discriminate parasitised and healthy hosts. *Melittobia acasta* Walker (Eulophidae) will not oviposit in puparia of Diptera which contain either their own larvae or pupae or those of *Pteromalus* (Pteromalidae) or *Dibrachys* (Pteromalidae) (Thompson and Parker 1927). The female *Trichogramma evanescens* Westwood (Eulophidae) is capable of discriminating parasitised hosts from unparasitised hosts (Salt 1934, 1937; Flanders 1937). With her antennae the female can recognise the residual odour of the tarsal gland's secretion left on the eggs that have been walked on by another female of the same species. This was subsequently termed by Flanders (1951) as the 'spoor effect". Parasitised hosts may be thus detected by the female *Trichogramma evanescens* initially by the antennal 'drumming' on the surface of the host. If this initial examination of the host with the antennae fails to indicate parasitism due to the washing away of the odour by rain or by other means, the female tested the hosts by inserting its ovipositor into the host and withdrawing it immediately (Salt 1934, 1937). The present author in his observations has noted that several species of *Brachymeria* (Chalcididae) were unable to discriminate between parasitised hosts and healthy hosts in the beginning stages and superparasitism was a common occurrence. However the females were found taking a longer time than usual for 'drumming' if they happened to meet hosts which were parasitised for the first time by another female of the same species or by the same female, 4 to 6 days earlier. In such instances the female either abandoned the hosts after a thorough antennal 'drumming' or pierced the host with its ovipositor just to withdraw it immediately and then left the host. It is suggested that changes in the physical or chemical condition of the parasitised hosts might be responsible for providing the stimuli for discrimination between potential hosts (Salt 1938; Wylie 1965; Fisher 1971; Narendran 1975; Narendran and Joseph 1977). In those cases where a parasitised host is detected only after the penetration of the ovipositor, it is evidently the sense organs of the ovipositor that are believed to be responsible for detecting the hosts. Such sense organs especially chemosensory "pores" are seen in several species of chalcids such as *Eurytoma tibialis* Boheman (Eurytomidae), *Brachymeria lasus* Walker (Chalcididae), *Tetrastichus rapo* (Walker) (Eulophidae), *Nasonia vitripennis* (Walker) (Pteromalidae), *Microterys flavus* (Howard) (Encyrtidae), *Aphytis* sp. (Aphelinidae) and in several other species of chalcids (Fulton 1933; Varley 1941; Copland and King 1971a, b, 1972a, b, c; Fisher 1971; King and Rafai 1970; Jackson 1966, 1969; Edwards 1954; Wylie 1958; King and Fordy 1970; Bartlett and Lagace 1961; Quendnau and Hubsch 1964; Weseloh 1969; Narendran 1975; Askew 1971). It is suggested by Fisher (1971) that biochemical changes of the host's haemolymph are likely to act as sign stimuli for discrimination between parasitised and unparasitised hosts. Mouthparts and tarsi are also reported to play a role (probably a minor one when compared to antenna and ovipositor) in the host detection behaviour of certain chalcids of the family Aphelinidae (Viggiani 1984).

In *Spalangia drosophilae* Ashmead, either the smell of the host's haemolymph clotted around the oviposition puncture or the odour of the fluid left by the ovipositor provided the stimulus for the detection of parasitised hosts from healthy hosts (Simmonds 1954). The discharge of a 'venom' presumably from the poison apparatus of the ovipositing female was suggested (Jackson 1966) to be responsible for providing the stimulus for discrimination between parasitised and unparasitised hosts by *Caraphractus cinctus* Walker (Mymaridae). The vibrations produced by the palpation of the host provided the stimulus for discrimination between parasitised host and healthy host in the case of *Microplectron fuscipennis* Zett (Eulophidae) (Ullyett 1936). Similarly movement or lack of movement by the host might contribute part of the stimulus for detecting healthy and parasitised hosts by *Spalangia drosophilae*, and *Nasonia vitripennis* (Simmonds 1954; Wylie 1965). In certain chalcids like *Lasiochalcidia igiliensis* Steffan, (Chalcididae), active movement of the host is an essential requisite for oviposition. This interesting chalcid parasitises the larvae of ant-lion. The female provokes its host to come out from its burrow and to seize the leg of the chalcid whereupon the chalcid inserts its ovipositor into the membrane between the head and thorax of the host. Among the chalcid parasites of the knapweed (*Centaurea nigra* L.) gall fly (*Urophora jaceana* Hering) only the endoparasitic *Eurytoma tibialis* is able to discriminate parasitised and healthy hosts by avoiding superparasitism whilst the four ectoparasitic chalcids species either distribute their eggs randomly or even in an aggregated manner (Varley 1941). In perilampids and eucharitids the planidium or the first instar larva upon emergence undergoes a freeliving period during which it must find its hosts. In these cases this is "more exactly a waiting period rather than a searching period; for relatively little movement takes place and the greater portion of the time is passed in the erect position awaiting the arrival of a host or carrier" (Clausen 1940). In these cases, the instincts of the planidium are not sufficiently developed to enable it to discriminate parasitised and unparasitised hosts and in several instances the planidium responds to virtually any moving object that approaches its immediate vicinity.

In chalcids, superparasitism is not always caused by the failure of the discriminative ability. In *Trichogramma evanescens*, *Encarsia formosa* Gahan (Aphelinidae), *Pachycrepoides vindemmiae* Rondani (Pteromalidae) and in several other species, superparasitism occurs although the females were capable of discriminating parasitised and unparasitised hosts. One of the possible reasons for this is the breakdown of the 'restraint' of the ovipositing females when there is a scarcity of healthy hosts. Another possible suggested explanation is that the female has to learn to discriminate between parasitised and unparasitised hosts if it is an inexperienced one (Salt 1934; Van Lenteren and Bakker 1975; Van Lenteren *et al* 1978). It is known that the 'restraint' exercised by the chalcid *Caraphractus cinctus* Walker in avoiding superparasitism of water-beetle eggs was best developed in old females and inexperienced young females would superparasitise the hosts (Jackson 1966). In the case of *Ooencyrtus kuwanae* (Howard) (Encyrtidae) the female tends to retain her eggs rather than deposit them if she finds only parasitised hosts and the exercise of this 'restraint' in this case is shown to be related to the developmental stage of the parasite in the parasitised hosts, the age and condition of the ovary of the female and the number and nature of the hosts available (Lloyd 1940). There are several other possible causes for superparasitisation such as, when a female lays more than one egg after the first oviposition within the period which is needed for building up the factor which causes avoidance of superparasitisation and

when two or more females oviposit simultaneously in one host (Van Lenteren and Bakker 1975).

Gregarious parasitism and polyembryonic parasitism are also met with in the case of some chalcids. In these cases two or more individuals can develop in one host but the number is often limited so that the danger of superparasitism is present. Hence in such cases the parasite would have to distinguish not only parasitised hosts from unparasitised hosts but also hosts already bearing a full compliment of parasites from those not yet fully supplied (Salt 1934). This capacity to discriminate parasitised hosts with different number of eggs is found in some species of parasitic hymenopterans including chalcids especially when there is a failure of the 'restraint' to oviposit due to scarcity of healthy hosts. However it is not undoubtedly established how exactly the parasite recognise the hosts with different number of eggs and the only fact so far known clearly is that such an ability does exist atleast in some species. Hence the avoidance of superparasitism in a sense is not only by discriminating parasitised hosts from unparasitised hosts but also by discriminating hosts with different number of eggs.

## 3. Conclusion

Superparasitism and avoidance of superparasitism are commonly found among chalcids. The stimuli for the discrimination of parasitised and healthy hosts varied from species to species. It may be based on the changes of the physical and chemical properties of the hosts due to parasitisation or based on the odour of the fluid left by the ovipositor or tarsal glands. It can be based on the odour of the haemolymph clotted around the oviposition puncture or based on any other causes. Whatever may be the stimulus, the primary and final detection of hosts are done by chalcids mainly by using antennae and ovipositor respectively, though occasionally either of the two alone is used by certain species.

In chalcids superparasitism is not always caused by the lack of ability to discriminate parasitised hosts and unparasitised hosts. Breakdown of the 'restraint' due to scarcity of healthy hosts, inability to learn to discriminate between parasitised and unparasitised hosts by inexperienced parasites, simultaneous oviposition by more than one or two females on a host, laying more than one egg at an act of oviposition etc, are some of the main possible causes for superparasitism. Avoidance of superparasitism by chalcids, in *sensu lato*, is not only by distinguishing parasitised hosts from unparasitised hosts bu also by discriminating hosts with different number of eggs. The avoidance o superparasitism by a chalcid shows the maximisation of its reproductive success an this trait is one of the important attributes of an effective biological control agent.

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# Application of sex pheromones in sugarcane pest management

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**Abstract.** Seven species of moth borers are known to cause heavy losses in sugarcane production in different parts of the country. Because of their concealed habits, the control of these borers becomes complex and hence a number of methods have been tried to suppress their field population. The sex behaviour of four species of these borers *viz.*, internode borer, stalk borer, shoot borer and top borer have been studied in recent years and among these the internode borer and stalk borer have been found to have potent sex attractants.

Mass trapping may be useful in the management of internode borer while disruption technique is likely to be useful in the case of stalk borer. In both the borer species, the synthetic pheromones will be highly useful in monitoring their activity.

**Keywords.** Sex pheromones; sugarcane; moth borers.

## 1. Introduction

Moth borers of sugarcane are major pests causing heavy recurring losses in cane yield which has been estimated to be 15·8 to 41·8% due to shoot borer (Parthasarathy *et al* 1953), 40 to 55·4% due to internode borer (David *et al* 1979), 20 to 30% due to stalk borer (Gupta and Avasthy 1954) and 8·8 to 34·8% due to top borer (Kalra and Chaudhary 1964). These borers are concealed within the tissues of cane stalk and are not exposed for parasitisation or predation by natural enemies and for contact with insecticides. Hence the suppression of their population becomes difficult. This warrants different methods of suppression strategies which have to be integrated harmoniously in order to manage them below the economic injuring level. Among the modern technologies for pest suppression, use of sex pheromones offers scope for monitoring the activity of these borers and also in reducing the fertility of wild females thereby bringing down the fertile egg population of the borers. This method can also be conveniently integrated with other methods in the pest management programme. Preliminary investigations on this strategy have been carried out in four species of moth borers and these are summarised in this paper.

## 2. Sexual activity of the borer moths

These moths are nocturnal in habit. Mating occurs during a specific period in different species. Most of the females mate only once though some of them mate more than once. Mating has been observed to take place between 1000 hr and 0200 hr in stalk borer (Kalra and David 1971), during the early hours of the night in shoot borer (Usman *et al* 1957), during the late hours of the night in internode borer (David and Kalra 1965) and

throughout the night in top borer (Kalra *et al* 1978). Prior to mating the virgin female moths assume a 'calling position' by raising their abdominal tips and during this process the pheromone is released. The male moths downwind take up the scent and fly towards the female. A large number of male moths are attracted simultaneously towards the virgin female and they fly excitedly around it and then mating takes place. This chemical communication between the two sexes in these moths can be taken advantage of for either mass trapping the male moths or for disruption of the communication by suitable contrivances so that mating and fertility of wild females can be reduced.

## 3. Studies with virgin females

Studies have been conducted on the potency of attraction of male moths by virgin females of stalk borer (Kalra and David 1971), top borer (Kalra *et al* 1978), internode borer (David and Chandra 1972) and shoot borer (David *et al* unpublished). The virgin females collected from pupae which are separated and kept in isolation from the male pupae, when placed in sticky/water traps attracted male moths. The number of male moths attracted ranged from 0 to 107 in stalk borer, 0 to 5 in top borer, 0 to 10 in internode borer and 0 to 33 in shoot borer. In stalk borer, the number of male moths trapped was more during the first 24 hr and it declined subsequently. It was also observed that four-day old virgin females or mated females did not attract male moths. Abdominal extracts of virgin females taken in solvents like ether also attracted males of stalk borer and the number trapped ranged from 0·1 to 4·2 moths/day/trap. These experiments have indicated the presence of potent sex pheromones in the females of stalk borer, internode borer and shoot borer.

## 4. Identification of sex pheromones

The fresh pupae of stalk borer and internode borer were air freighted to UK and the pheromones were isolated and identified by Dr B F Nesbitt at the Tropical Development and Research Institute. The pheromone of stalk borer consists of the following four components, *viz.*,

(i) (*Z*)-7-dodecynyl acetate,
(ii) (*Z*)-8-tridecenyl acetate,
(iii) (*Z*)-9-tetradecenyl acetate and
(iv) (*Z*)-10-pentadecenyl acetate.

while that of the internode borer consists of two components, *viz.*,

(i) (*Z*)-13-Octodecenyl acetate, and
(ii) (*Z*)-13-Octodecenyl alcohol.

The synthetic products of these pheromones have been prepared in different combinations and were evaluated in the field. The sex pheromones of shoot and top borers are yet to be chemically characterised.

## 5. Comparative performance of synthetic pheromone with virgin females

The comparative efficacy of the virgin female and synthetic pheromone in water traps was assessed for 42 days in the stalk borer. The number of moths trapped/trap/day was

significantly more in synthetic pheromone traps (7·95) than in virgin female traps (0·39). In the internode borer, the comparative efficacy was evaluated for 17 days. The mean number of moths trapped was significantly high in water traps baited with synthetic pheromone (5·35) compared to virgin females (2·35).

## 6. Mass trapping

### 6.1 *Stalk borer*

Two combinations *viz.*, 1:1:1:1 and 4:8:4:1 of the components (*Z*)-7-dodecenyl acetate, (*Z*)-8-tridecenyl acetate, (*Z*)-9-tetradecenyl acetate and (*Z*)-10-pentadecenyl acetate at two different doses, *viz.*, 0·1 and 1 mg were tried at Shamli in 1982 and the results are given in table 1. The combination of 4:8:4:1 at the dose of 1 mg was found to be more potent than the three other treatments. During 1983, eight more combinations (table 2) were tried and it was observed that the moth catch was more in traps baited with vials containing a 2:1 ratio of (*Z*)-8-tridecenyl acetate and (*Z*)-7-dodecenyl acetate and an 8:1 ratio of (*Z*)-8-tridecenyl acetate and (*Z*)-10-pentadecenyl acetate.

Between 25.2.1983 and 8.4.1983, mass trapping was attempted with 20 water traps baited with 1 mg of 4:8:4:1 combination. A total of 2863 moths were trapped with a mean of 7·95 moths/trap/day. The maximum number of moths trapped was 259/trap/day.

### 6.2 *Internode borer*

The efficacy of different combinations of acetate and alcohol *viz.*, 1:1; 3:1; 5:1; 7:1 and 9:1 was assessed by conducting field experiments at Sakthi Sugars Ltd., Sakthi Nagar and Deccan Sugars Ltd., Pugalur. The experiment had five treatments and each treatment was replicated six times. The distance between the traps was 30 m. Observations on moth catch was recorded at weekly intervals for 11 weeks from the

**Table 1.** Evaluation of different components in the mass trapping of *C. auricilius.*

| Components | Dosage (mg) | Total male moths caught | Mean moths/ trap/day |
|---|---|---|---|
| 1:1:1:1 $Z_7:Z_8:Z_9:Z_{10}$ component | 1 | 31 | 0.70[B] |
| 1:1:1:1 $Z_7:Z_8:Z_9:Z_{10}$ component | 0·1 | 25 | 0·60[B] |
| 4:8:4:1 $Z_7:Z_8:Z_9:Z_{10}$ component | 1 | 1643 | 16·5[A] |
| 4:8:4:1 $Z_7:Z_8:Z_9:Z_{10}$ component | 0·1 | 54 | 1·21[B] |

Figures followed by the same letters are not statistically different.

start of the experiment. It was found that the average moth catch was more at Pugalur (6·23 moths/trap/day) than at Sakthi Nagar (3·44) and the difference was statistically significant. Among the combinations tested, though the catch was more in 5:1 (acetate:alcohol) combination, the differences between the different combinations were not statistically significant (table 3). The catch was more and on par during fourth to eleventh weeks indicating the persistence of the material under field conditions for longer duration.

Another experiment was conducted to find out the optimum height of the water trap for maximum moth catch. The water traps were set at 60, 90, 120, 150 and 180 cm height and the treatments were replicated five times. The mean moths trapped/trap/day was more in traps set at 60 cm height (12·88) compared to 7·10 to 8·72 moths/trap/day in other treatments.

After fixing up the optimum combination and height of traps, the synthetic pheromone of the borer was used for mass trapping of male moths under field conditions in two centres *viz.*, Coimbatore and Pugalur during 1982. The area trapped was about three hectares at Coimbatore and seven hectares at Pugalur. In both the centres suitable control of about one hectare at a distance of 0·5 km was earmarked for observations. The distance between the traps was 33 m. The traps were set up in about five to six months old crop and two changes of the pheromone vials were given after two

**Table 2.** Evaluation of different components in the mass trapping of *C. auricilius.*

| Components | Total moth caught | Mean moths/ trap/ day |
|---|---|---|
| 4:8:4:1 mixture CA 83/35 | | |
| $Z_7$:$Z_8$:$Z_9$:$Z_{10}$ component | 751 | $10{\cdot}80^{BC}$ |
| $Z_7$ component CA 83/86 | 21 | $0{\cdot}31^{C}$ |
| $Z_8$ component CA 83/37 | 38 | $0{\cdot}55^{C}$ |
| $Z_9$ component CA 83/38 | 39 | $0{\cdot}56^{C}$ |
| $Z_{10}$ component CA 83/39 | 275 | $3{\cdot}94^{C}$ |
| $Z_8$:$Z_7$ mixture 2:1) CA 83/40 | 536 | $12{\cdot}70^{B}$ |
| $Z_8$:$Z_7$ mixture 2:1) CA 83/41 | 1585 | $22{\cdot}75^{A}$ |
| $Z_8 + Z_{10}$ mixture 8:1) CA 83/42 | 1524 | $21{\cdot}78^{A}$ |

The dosage used was 1 mg for all the cases.

**Table 3.** Evaluation of different components on the mass trapping of *C. sacchariphagus indicus* (K).

| | Mean moths trapped | |
|---|---|---|
| Components | Sakthinagar | Pugalur |
| 1:1 Acetate:alcohol | $3{\cdot}28^{A}$ | $5{\cdot}87^{AB}$ |
| 3:1 Acetate:alcohol | $3{\cdot}58^{A}$ | $5{\cdot}80^{B}$ |
| 5:1 Acetate:alcohol | $3{\cdot}57^{A}$ | $6{\cdot}57^{A}$ |
| 7:1 Acetate:alcohol | $3{\cdot}39^{A}$ | $6{\cdot}41^{A}$ |
| 9:1 Acetate:alcohol | $3{\cdot}38^{A}$ | $6{\cdot}49^{A}$ |

months. Observations on the moth catches were made weekly. Observations on the progressive incidence and intensity of infestation of the borer, both cumulative and fresh attack, were made at periodic intervals. Detailed observations were made at harvest on incidence and intensity of the infestation, larval population, yield and quality of canes at Pugalur.

A total of 9822 male moths were collected at Pugalur with a mean catch of 0·83 moths/trap/day. The mean borer incidence was 93·63 and 87·17% in the treated and control plots and the difference was not statistically significant. There was no significant difference in the intensity of borer infestation and larval population, yield of cane and CCS% between the treated and control plots.

At Coimbatore, a total of 1122 male moths were collected with a mean of 0·59 moth/trap/day. The details of observation on incidence and intensity of borer infestation are given in table 4. It was observed that there was significant difference in the incidence of borer at harvest but not with respect to the intensity of borer infestation.

During 1983, mass trapping was attempted in an area of five hectares at Pugalur. Suitable control of one hectare at a distance of 0·5 km with the same variety and age of the crop was earmarked for observations. The distance between the traps was 16·5 m in one set and 24·8 m in another. The traps were set up in about four months old crop and three changes of pheromone vials were done.

A total of 74697 male moths were trapped with an average catch of 3·4 moths/trap /day. The mean moth catch was 2·95/trap/day in the traps set up at 24·8 m distance and it was 3·8 /trap/day in the traps set up at 16·5 m distance and the difference was not significant. The data collected at harvest revealed that there was significant difference in incidence (table 5). The mean incidence in treated plots was 68·36% and in control plots

**Table 4.** Internode borer incidence and intensity of attack at Coimbatore.

| Period | Treated plots | | Control plots | |
|---|---|---|---|---|
| | Per cent incidence | Per cent intensity | Per cent incidence | Per cent intensity |
| Before setting up of traps | 8·73 | 4·07 | 13·65 | 3·39 |
| 30 days after setting up of traps | 13·44 | 5·27 | 19·18 | 5·08 |
| 60 days after setting up of traps | 17·16 | 4·90 | 31·36 | 5·57 |

**Table 5.** Yield data from the pheromone treated and control plots.

| Parameters | Treated | Control |
|---|---|---|
| Per cent incidence (plot basis) | $68·36^{B}$ | $76·85^{A}$ |
| Per cent intensity—Old | $2·86^{A}$ | $3·26^{A}$ |
| Fresh | $1·49^{A}$ | $1·29^{A}$ |
| Plot weight (kg) | $564·41^{A}$ | $473·26^{B}$ |
| Per cent purity | $84·38^{A}$ | $82·73^{B}$ |
| CCS per cent* | $9·63^{A}$ | $9·00^{B}$ |

*Commercial cane sugar per cent

it was 76·85 %. But the differences observed in the intensity of the borer infestation were not significant. The cane yield was significantly more in treated plots and also there was improvement in purity and ccs % due to mass trapping of internode borer male moths.

## 7. Communication disruption

### 7.1 *Stalk borer*

The scope of disruption of the pheromone communication system in stalk borer was assessed by saturating the atmosphere near the pheromone trap with the optimum combination of 4:8:4:1 at the dose of 1 mg/vial during 1983. The surround vials used for saturating the atmosphere contained either (*Z*)-7-dodecenyl acetate, (*Z*)-8-tridecenyl acetate, (*Z*)-9-tetradecenyl acetate, (*Z*)-10-pentadecenyl acetate or 4:8:4:1 combination of the above components at the dose of 1 mg. Maximum reduction (85·02 %) in moth catch was recorded when (*Z*)-7-dodecenyl acetate was used in the surround vials. The reduction in other treatments was 1·63, 0·0, 0·0 and 54·07 % respectively.

During 1984, two more experiments were conducted with the most effective component *viz*, (*Z*)-7-dodecenyl acetate. The result obtained confirmed the earlier finding and the per cent reduction observed was 76·18 % in one experiment and 94·96 % in another.

### 7.2 *Internode borer*

The experiments conducted on the same lines as in stalk borer, did not give much encouraging results. The reduction observed ranged only from 24·87 to 38·58 % when different combinations were used in the surround vials.

## 8. Discussion

The observations on the sexual attraction in these species of moths showed that there is potent sex pheromone in the females of stalk borer, internode borer and shoot borer while it is apparently feeble in the case of top borer. Intensive studies on the former two species indicate that these two vary with regard to the population density. The population density is low in internode borer and high in stalk borer, especially in the later broods and this is reflected in the number of moths trapped in the water traps with a maximum of 259. Moreover, in the stalk borer, the emergence of moths is apparently not continuous, being restricted to specific broods under subtropical conditions whereas in internode borer, it is continuous throughout the year under tropical conditions. Because of the continuous emergence, male moths of internode borer can be trapped throughout the year using water traps. The traps are to be set up with the commencement of internode formation with only two changes of the synthetic pheromone vials at trimonthly intervals. In the case of stalk borer, the initial two broods have low population and the setting up of the traps will be highly useful for identifying the initial foci of infestation which is otherwise normally difficult to identify in the field.

The results of the communication disruption experiment have shown clearly that it may be highly useful for field application in the case of stalk borer while it may not be much effective for the check of internode borer.

These studies have indicated promise for field application of mass trapping and disruption techniques using synthetic pheromones and hence further detailed information on cheap and durable dispensers, simple traps for field use, optimum number of traps/unit area and scope of combining this method with other methods in the integrated pest management of these highly destructive borers may be obtained.

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# Role of behavioural studies in the development of management strategies for forest insect pests

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**Abstract.** Under forestry conditions, management techniques aimed at maintenance of pest populations at moderate levels have greater chance of success than conventional methods of pest control. Simple behavioural observations can sometimes be used to great advantage in the development of such methods, some examples of which are given. Although there has been considerable excitement over the past two decades on the possibility of using behaviour modifying chemicals for control of pests through mass trapping or disruption of the insect's normal communication systems, no significant practical achievement has so far been reported. Difficulties in the use of these chemicals include inadequate information on the biological responses of natural populations of insects; utilization by most insects of a complex pheromone system involving several chemical components; non-reproducibility of laboratory results under natural conditions due to several modifying factors; high cost of the development and deployment of pheromonal control systems, particularly for low value forestry crops; inadequacy of pheromonal control methods for coping with the high epidemic densities of most forest pests; and the possibility of development of pheromone resistance. Behaviour-modifying chemicals, such as food lures, sex pheromones and population aggregating pheromones, however, are useful in pest management as tools for survey and ecological research. Populations generally exhibit properties that cannot be understood by studying individual insects; study of the behaviour of populations is therefore more important than study of the behaviour of individuals for developing management strategies.

**Keywords** Forest pest management; pheromones in insect control; insect behaviour; food plant chemicals; *Hyblaea puera.*

## 1. Introduction

The major insect pest problems of natural and man-made forests (forest plantations) have been summarised by several authors (Pant 1974; UNESCO 1978; Mathur 1964; Chatterjee and Sen-Sarma 1968; Sen-Sarma and Thapa 1981; Nair 1980). In the tropics, most pest problems are noticed in man-made, as against, natural forests. Experience in Kerala has shown (Nair 1980) that all forest tree species grown in a sufficiently large area for at least one tree generation have brought up with them at least one or two serious insect pests. Although the economic damage caused by most forest pests have not been adequately assessed, many of them are considered serious enough to merit application of control measures. The present paper will examine the unique problems of forest pest control in the tropics and the relevance of behavioural studies in developing control measures. Some examples of successful use of behavioural observations for practical pest management will be discussed and a critical evaluation made of the prospects of using behaviour-modifying chemicals for direct control of pests.

## 2. Unique problems of forest pest control

Although forests have fewer pest problems than agricultural crops, the problems entomologists must face in devising control measures for them are much greater. Conventional methods developed primarily for agricultural crops often turn out to be unsuitable for forest crops for several reasons – (i) The extensive area under forest plantations, often in inaccessible hilly terrain, impose serious restrictions on the practical implementation of surveillance and control operations, in spite of theoretical feasibility. (ii) Again because of the extensive land area covered, the risk of adverse environmental impact of control operations, particularly the use of insecticides, is large (iii) The large size of the trees makes special demands on techniques of applications of control measures – whether it be spraying of a chemical or microbial insecticide or hand-picking of insect larvae. (iv) The greater permanence of the forest crop (for example, the harvesting period of teak is 60 years even in a good quality site) preclude certain types of control measures like crop rotation. (v) Above all, the lower economic returns from forest crops (compared to agricultural crops) limits the expenditure that can be incurred to prevent insect damage.

The appropriateness of control measures for forest pests must be judged in the light of the above considerations. Obviously, methods acceptable for use in forests must be effective, cheap and environmentally "safe", a combination of qualities that is difficult to accomplish in practice although it sounds simple and ideal. What is usually achieved is something less than ideal: compromise usually involves tolerance of a certain degree of damage (i.e., no control or partial control), acceptance of a high cost of treatment, or neglect of minor environmental damage. Methods which make use of silvicultural manipulations, natural enemies (including predators, parasitoids and pathogens) and other population management techniques based on behavioural peculiarities of pests and their natural enemies to keep the pest populations at reasonable levels are the most suitable for dealing with forest pests.

If there are unique difficulties in dealing with forest pests, there is also at least one unique advantage. Because the forests are owned by governments, indiscriminate use of insecticides can be prevented by policy decisions of the governments, even if a certain degree of damage is sustained. In agriculture, on the other hand, the profit motive of individual farmers often works against the larger interests of society. The chances of practising pest management, as distinct from direct kill of insects, is therefore greater in forestry than in agriculture. Behavioural studies that could turn out useful in the development of management strategies may be considered under two major heads – simple behavioural observations and modification of insect behaviour by chemicals.

## 3. Simple behavioural observations for insect control

Simple behavioural observations can sometimes be used to great advantage for the management of forest insect pests. Some examples follow.

The caterpillar of the moth, *Hyblaea puera* is a serious pest of teak plantations throughout India. Experimental aerial application of insecticides has been tried in the past (see Sen-Sarma and Thapa 1981) to control this insect. A 4-year field study conducted in Kerala (Nair and Sudheendrakumar, unpublished) showed that in spite of the insect's potentiality to pass through at least one generation per month, serious

outbreaks occurred only once or twice a year in most plantations, usually in late April to August. Unpublished recent observations on the egg-laying behaviour of natural populations of *H. puera* moths, on the behaviour of mature larvae, and on the movement pattern of newly emerged moths have revolutionised our concepts of the population dynamics of this important pest. The current working hypothesis is that the greater proportion of the moth population, resulting from a larval build-up in one area, migrate to another area about 5 to 10 km away, to start a new infestation. *H. puera* moths lay eggs only on tender leaves. Although teak is a deciduous species, observations have shown that there exists enough phenological variation among individual trees within an area and among populations of trees in different areas within a larger geographical region to make tender leaves available continuously, though not at the same place, to sustain a residual population of larvae. With the onset of the general flushing season, the insect population builds up, step by step, colonising newer and newer areas, moving in the general direction of late flushing areas from early flushing areas. Later, populations decline when parasite populations build up or large quantities of tender leaves are no longer available. Existence of a residual population during the off-season (non-flushing period of teak) and gypsy-like migration of adult moths, are new concepts prompted by simple behavioural observations (unpublished), which if proved, can lead to simple methods to control the outbreak. In plantations, initial build-up of larval populations can be located by surveillance during the critical period. As mature larvae descend to the ground for pupation, application of a contact insecticide or appropriate microbial agent can prevent emergence of the moth population and their subsequent spread to other areas. This method is much simpler, practicable and environmentally less hazardous than aerial application of insecticides that had been experimented with in the past.

Increasing evidence is now accumulating to suggest that migration may be more common than hitherto suspected in many pests, particularly the noctuid moths (Barfield and Stimac 1981; Oku 1983; Stinner *et al* 1983; Riley *et al* 1983). Management strategies for such highly mobile pests must necessarily be based on observations on the behaviour of natural populations.

Several examples can be cited to show how careful observations on the behaviour of pests can lead to improvements in their control. Subterranean termites damage young transplants of eucalypts within a few months of planting out. Field observations revealed that characteristically the termites (mostly *Odontotermes* spp.) begin their attack in the root collar region a few centimeters below groundlevel (Nair and Varma 1982). It is therefore possible to control the attack by treating the soil core immediately surrounding the tap root, instead of the entire planting area. This can be accomplished by drenching the polythene bag containing the seedling with an insecticide before transplanting it in the field, thus effecting considerable reduction in environmental contamination as well as cost of treatment. In African countries, on the other hand, different species of termites, notably *Macrotermes* spp. cause most damage to eucalypts; they forage at the soil surface level and attack the stem of saplings at or above ground level. This behaviour of termites calls for a different approach to control.

Control of the sapling borer, *Sahyadrassus malabaricus* Moore (Hepialidae) by spot treatment of the tunnel mouth is another example. This borer attacks saplings of forest plantations and lives inside a large tunnel along the pith. The tunnel mouth is covered by a mat-work of wood particles and faecal pellets. Observations revealed that if the tunnel mouth cover is removed, the larva comes out at night and makes a new cover

with wood particles gathered from the vicinity of the tunnel mouth. Control of insects which live inside wood is generally difficult, but the above behavioural observation was made use of to control this insect by pulling off the cover and applying a contact insecticide at the tunnel mouth region (Nair 1982).

Behavioural observations also find application in the control of cerambycid wood borers. Observations have revealed that they lay eggs only on logs with bark. Debarking is therefore a standard practice for protection of stored logs from cerambycid borers.

## 4. Modification of insect behaviour by chemicals

Over the last 15–20 years there has been considerable excitement among entomologists on the possibility of using behaviour-modifying chemicals for control of pests. A large number of chemicals have been isolated from a variety of insects and other sources, including host plants or animals, and their exact chemical structures have been established; many have also been synthesized outside the animal or plant system. These are elegant achievements made possible by the active involvement of expert chemists and general advances in instrumental analysis. Many such chemicals have been tested in the laboratory for behavioural responses and found promising. Pilot-scale field tests have also been carried out for some of them. We shall consider them under two heads – food plant chemicals and pheromones.

### 4.1 *Food plant chemicals*

Since Fraenkel's (1959) classic paper on the '*raison d'être* of secondary plant substances', the role of plant chemicals in influencing the orientation of insects to food plants for feeding or oviposition has been well recognized. Since then we have learned much about the mechanisms of host plant selection of various phytophagous insects. Several excellent reviews of the topic are available (Kennedy 1965; Dethier 1970a, b; Schoonhoven 1972, etc.) and several national and international symposia have also been conducted on the topic (Ananthakrishnan 1977; Visser and Minks 1982; etc.). Except in a few instances, however, it has not been possible to use this new knowledge for developing control measures against pests. Nonspecific food lures like sugar solutions or protein hydrolysates mixed with toxic compounds have often been used to attract and kill insects like ants, cockroaches and flies. A toxic bait containing aldrin, soya bean oil and dried citrus meal applied aerially at the rate of 2·2 kg/ha was reported to be collected by leaf-cutting ants (*Atta* sp.), a pest of forest trees in the neotropics, in preference to fresh leaves and to kill about 91 % of the ants in some experiments (Lewis 1972). The best known example in Indian forestry is the use of trap logs to attract the sal borer, *Hoplocerambyx spinicornis*. This cerambycid borer is a serious pest of natural and man-made forests of *Shorea robusta* (sal), an important timber tree in the northern states of India (Beeson 1941) bad epidemics of which have occurred in the past in UP, MP, Bengal and Assam. Normally a borer of sickly trees, at times of epidemics, even healthy trees are attacked and killed. Adult beetles of both sexes are attracted to newly exposed inner bark and sapwood of trees over distances upto 400m. It is therefore standard practice to fell unhealthy or injured trees, cut them into 3m logs, beat up the cut ends to expose fresh sapwood and distribute them in small heaps in the affected area

to attract the beetles. Trap trees at the rate of 3 to 5 per hectare are used; at intervals, the logs are again cut into smaller billets to expose fresh sap. The beetles are collected and destroyed daily. Collections of upto 1000 beetles per trap tree per day have been reported (Beeson 1941). The nature of attractant has not been determined.

In general, although there has been initial optimism on the use of behaviour-modifying host plant chemicals, particularly the secondary plant substances, for insect control, this has not been borne out by experience. The main handicap is the involvement of several chemicals, each having some influence on the chain of behavioural responses of the insect leading to host plant recognition and acceptance. The problems of using them for control can be illustrated using the example of the cabbage root fly, *Delia radicum* (Anthomyiidae) a pest of cruciferous crops in Europe and North America which has been studied in some detail (Traynier 1965, 1967a, b; Coaker 1969; Coaker and Finch 1971; Nair *et al* 1974, 1976; Nair and McEwen 1976; Finch 1978; Städler 1978; Hawkes and Coaker 1979; Ellis *et al* 1982). In this insect, the first step in host selection is taken by the adult female when it lays eggs in soil close to the host plant. The newly hatched larvae bore into the roots where they feed and grow. Highly volatile mustard oils, break-down products of glucosinolates (mustard oil glucosides) present in cruciferous plants, stimulate the flies into greater activity and attract them to the plant. The parent glucosinolates then induce oviposition. Allyl isothiocyanate (AITC), one of the mustard oils tested in the laboratory, did not induce oviposition by itself, but in the presence of a glucosinolate, very low concentrations of it caused an increase in the number of eggs laid (Nair and McEwen 1976). Many of the nutrients tested did not influence oviposition, but a protein hydrolysate inhibited it. Flies also arrive and land on non-host plants by random movements. The gravid female which lands on a plant probes the leaves with the proboscis (other sense organs may also be involved) and if the plant is judged suitable, walks down to the bottom and lays eggs in soil, close to the plant stem. A large number of factors, chemical, visual and physical, apart from the physiological state of the insect, are involved in the sequence of behavioural events leading to oviposition. In the next major step, similar factors affect the establishment of the neonate larvae on the root. All crucifers are believed to contain one or more glucosinolates, usually a mixture of several, but some crucifers did not elicit oviposition (Nair *et al* 1974). Experiments (Nair and McEwen 1976) revealed that although glucosinolates appeared to be the only oviposition inducing substances present in crucifers, there was no correlation between the total glucosinolate content of crucifer leaves and the oviposition response of the fly and that some glucosinolates elicited more oviposition than others. It appeared that both the absence of glucosinolates and the presence of inhibitory chemicals could make a plant unacceptable for oviposition. With more than 30 glucosinolates known from different plants, and with several glucosinolates occurring together in a given species, Nair *et al* (1976) suggested that a total 'glucosinolate pattern' may be more important in deciding the oviposition response. In the light of Dethier's (1973) conclusions based on extensive electrophysiological studies on the gustatory receptors of lepidopteran larvae, Nair *et al* (1976) also discussed how host selection may depend not on the presence or absence of a single stimulant or deterrent, but upon the total sensory impression derived from an integration of sensory responses to several plant constituents. Dethier (1973) has discussed in detail how subtle differences in one or more plant constituents result in different patterns of sensory input, and consequently, different magnitudes of response of insects.

In general, since a chain of sequential behavioural responses to food plant chemicals is involved in food selection and since most of these responses can at least partially be modified by other factors, the chances of utilizing any of the food plant chemicals to control insects by radically modifying their behavioural response is meagre. Recent studies have also shown (Ellis *et al* 1982) that the egg laying behaviour of the cabbage root fly is also influenced by microbial activity in or around its host plants. To compete with natural food plants, we need chemicals that will elicit superoptimal responses from the insect, but we are beginning to understand that though the insect may act "instinctively" rather than intelligently, optimal responses have been instinctively built into their behavioural repertoire. For example, while low concentrations of AITC will attract the cabbage root fly and promote egg laying, high concentrations of it will either be ignored through sensory adaptation or may elicit the opposite reaction. For use in controlling the insect, we should expect that a high concentration of attractive chemicals will attract the insects more effectively than the optimal concentration released by the natural hosts. Such responses have seldom been observed under natural conditions, although the search must continue in the hope that it may turn out useful in some cases.

### 4.2 *Pheromones*

There is vast literature on pheromones of insects and their potential uses (see Jacobson 1972); we shall limit our discussion to two examples of forest pests and examine the usefulness of pheromones for their control.

The first example is the gypsy moth *Lymantria dispar* (= *Porthetria dispar*) (Lymantriidae). The caterpillar of this moth is a pest of oak, birches and many other hardwoods in Europe, USSR and some parts of USA, particularly eastern New York and New England. As early as 1925 it was suspected that the flightless virgin females of this moth produced a sex attractant (pheromone) which lured the males. A series of investigations over several years culminated in the identification of the active component of the sex pheromone in 1970, designated as 'disparlure' (Bierl *et al* 1970). In between, preparations called 'gyptol' and 'gyplure' were also isolated and/or synthesized. Early attempts (in 1961) by the US Department of agriculture to control gypsy moth populations by disruption of the mating communication systems (male confusion method) with gyplure distributed by air craft over a 160 ha island located near New Hampshire did not show any adverse effect on male mating activity, but this was attributed to weak attractiveness of the commercial 'gyplure' used in the experiment (Jacobson 1972). A subsequent field experiment in 1964 with preparations of 'gyplure' previously shown to be effective in both laboratory and field tests, also did not yield encouraging results. In later experiments using disparlure, traps baited with the chemical were air dropped over large infested areas to attract and kill the males or strips of paper impregnated with the chemical were dropped to confuse the males. Both approaches appeared to be successful in areas of light infestation although not in areas of heavy infestation (see Jacobson 1972).

While disparlure has not yet become a practical tool for control *per se*, it is widely used as a surveillance tool, particularly to monitor the spread of this introduced pest within the USA to undertake timely control measures. General conclusions on the usefulness of these chemicals for control may be drawn after considering the second example, the pine bark beetles.

Several species of the small scolytid beetles belonging to the genera *Dendroctonus*, *Ips*, *Scolytus*, *Orthotomicus*, *Xyleborus* etc., attack pines and other trees in different parts of the world. Most investigations have been made on the pine bark beetles, *Dendroctonus* spp. in the USA and USSR and the literature is vast (see Wood 1970, 1980; Stark 1973; etc). In general, infestation occurs in the following sequence. Depending on the species, adult beetles of one of the sexes make the initial attack, usually on weakened trees which apparently have a characteristic odour profile. Once established, the beetles produce a population aggregating pheromone, possibly using precursors ingested from the tree, which is passed out through the frass and serves to attract other beetles to cause mass attack. In severe infestations, healthy trees are also attacked and killed. Several pheromones *viz*, frontalin, brevicomin, etc., have been isolated and synthesised from different species of bark beetles. The results of field experiments for control using bark beetle pheromones have been discussed by Wood (1970) and Roelofs (1975). In general, pheromone mixtures have been found effective in trapping large numbers of bark beetles, but unequivocal evidence of their effectiveness in reducing tree mortality under field conditions is yet to come, although the results are reported to be promising.

We shall now consider the prospects of using pheromones for pest management in forestry. The most obvious and profitable use of such chemicals is to detect the presence of the insect, either to monitor its spread into an area or to time the application of control measures. In the case of pests which appear all of a sudden in large numbers, like the teak defoliator, pheromones will be of little value to detect the time of their appearance. It is, however, an important research tool to elucidate many aspects of the ecology of the insect; for example, to determine whether residual populations of the insect occur in plantations or natural forests during periods when no visible defoliation occurs.

Although high hopes have been raised on the possibility of using pheromones for control of insects, either through mass trapping or the male confusion method, and optimism still prevails, no significant practical achievement has so far been reported, in spite of intensive efforts over the past 15 to 20 years – a sufficiently long period for experimentation. An excellent and critical discussion, full of insight, of the problems and prospects has been given by Greenway *et al* (1977), which must be consulted by anyone who contemplates use of pheromones for insect control. Some of the difficulties in using pheromones for direct control are:

(1) Isolation, identification and synthesis of the pheromones have progressed very fast, but our understanding of the responses they induce in individuals, and especially of the responses they induce in populations has not progressed fast enough (Greenway *et al* 1977). Biological response studies are more cumbersome and time consuming and tall claims have often been made with preliminary data. As Greenway *et al* (1977) pointed out, variability of responses in behavioural assays has not always been reported in published studies.

(2) In many cases it has been found that the functional sex pheromone is not a single compound but a mixture of compounds which constitute a 'pheromone system' (Minks *et al* 1977). For example, four components are involved in the sex pheromone system of the sugarcane stalk borer *Chilo auricilius* and effectiveness depends on an optimum combination of these compounds (H. David personal communication). There are several examples in which only specific combinations of certain chemicals are active (Minks *et al* 1977). The greater problem, which has not been generally recognised, is the

likelihood that different individuals in the given population may respond (reference here is to optimum response) to different combinations of the chemicals.

(3) In general, many predictions based on laboratory results have not been realized under field conditions due to various interfering factors. The behavioural reaction of a living organism, more particularly of a population of organisms cannot be expected to follow rigid principles, or perhaps, if they do, we are still ignorant of the principles which govern them and unable to predict the outcome. In spite of such knowledge, in control trials using pheromones we often expect that behavioural responses of the insect in the field will be as predictable as the outcome of a chemical reaction.

(4) Because of the cost and effort involved, development and use of pheromones for direct control may prove economically feasible only in high-value, intensively managed crops, and not in extensive low-value plantings (Greenway *et al* 1977) like forest plantations, especially in developing countries.

(5) As noted in the few field trials on gypsy moths, artificially used pheromones were effective in controlling the insect only when the pest population was low. When enormous numbers of insects are involved, as in an epidemic of forest insects like the teak defoliator or bark beetles, pheromone traps cannot cope up effectively with the numbers. Theoretical calculations suggested (Roefols 1975) that an initial trap to female ratio of at least 5:1 would be needed to obtain 95% suppression of mating.

(6) The possibility of insects developing resistance to pheromones has generally been ignored in discussions on the use of pheromones for insect control. Even Greenway *et al's* (1977) otherwise elegant discussion is silent on this point. Green *et al* (1960), however, discussed this problem. Development of pheromone resistance appears to be as simple and imminent as development of insecticide resistance, if we resort to direct control of insects with pheromones. One of the arguments in favour of use of sex pheromones for control was the idea that an insect cannot develop resistance to a chemical on which it depends for mating communication. However, resistance can develop in the following manner. Most insects make use of a pheromone system involving more than one chemical. As discussed above, different individuals of a given population may show optimal response to different combinations or ratios of the pheromones. Widespread use of a particular pheromone combination for mass trapping or mating disruption will eliminate that part of the population which responds to it, leaving the small unresponsive part of the population to mate and multiply. With continued selection pressure, a new population will evolve which makes use of a new slightly altered pheromone communication system. Such a population will no longer be responsive to the standard pheromone system; in other words, resistance has developed. If the pheromones are used only for survey purposes, it will not exert much of selection pressure and therefore will not lead to development of resistance.

## 5. Conclusions

Behavioural observations are important in developing control measures against forest pests. Simple observations on the behaviour of natural populations can often be used to great advantage in developing strategies for management of pests. In the management of forest pest populations behaviour modifying chemicals such as food lures, sex pheromones and population aggregation pheromones are useful as research and survey tools, but not for direct control.

Most new ideas useful for management of pests are likely to come from behavioural studies of populations, particularly natural populations, rather than observations made on individual insects in the laboratory. Populations exhibit properties that cannot be understood by studying individual insects.

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# Behavioural strategies of emergence, swarming, mating and oviposition in mayflies

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**Abstract.** Behavioural strategies of emergence, swarming, mating and oviposition in mayflies are reviewed in the light of available literature.

**Keywords.** Behavioural strategies; emergence; swarming; mating; oviposition; mayflies.

## 1. Introduction

Mayflies are unique among the insects in having two winged adult stages, the subimago and the imago. The adults live from 1–2 hr to a few days and even up to 14 days in some ovoviviparous species. The brevity of the adult stage is possible because the sole function of the adult is to reproduce. In fact, the process of natural selection has resulted in insects whose every adaptation is directed towards the process of reproduction. Moreover, the abbreviated adult life of Ephemeroptera is an adaptation to minimise exposure time to predators (Edmunds and Edmunds 1980). An attempt is made to review the data on behavioural strategies of emergence, swarming, mating and oviposition in mayflies.

## 2. Emergence

Emergence, the transition from the aquatic nymph to the terrestrial subimago, is a critical period for mayflies. Shedding of the nymphal skin usually occurs at the water surface on some object such as a stone or macrophyte stem or in mid-water (Brittain 1982). The latter is more typical of the burrowing species which inhabit deeper waters, and of a number of river species. Genera such as *Siphlonurus*, *Isonychia* and *Baetisca* crawl completely out of the water before they moult (Edmunds *et al* 1976). The mechanism of emergence has been well documented in *Baetisca rogersi* (Pescador and Peters 1974). The emergence process begins with a medial split of the thoracic notal shield. The abdominal segments contract repeatedly in a peristalic fashion followed by the outward bulging of the thorax until the entire medial line of the mesothoracic notal shield opens. The split gradually progresses anteriorly and posteriorly. Anteriorly it reaches the vertex of the head, usually between the compound eyes along the obscured ecdysial line, but sometimes extend to the base of the frontal process of the head. Posteriorly the split terminates at the posterior margin of the median carina. As the split progresses, the subimago wriggles out from the old skin. The dorsum of the subimaginal thorax emerges first, followed by the compound eyes and then the head. At this point the emerging subimago assumes a slanted position with the head and anterior

half of the thorax completely exposed, and the abdomen still encased in the old cuticle. Quick jerky body movements and abdominal contractions complete the process with the release of the abdominal segments and caudal filaments. Sometimes the subimago spreads out its prothoracic legs immediately upon exposure and firmly anchors the claws on the supporting objects. This probably helps the emerging subimago pull itself from the nymphal skin. Normally the prothoracic legs and the mesothoracic legs remain firmly drawn under the venter of the thorax until the metathoracic legs appear and all three pairs spread out at the same time. At emergence the wings of the subimago are moist and are often curled at the apex. A newly emerged subimago remains motionless for a while, and then crawls up on the supporting object. This resting behaviour probably allows the subimago time to regain its strength and dry its wings (Pescador and Peters 1974).

Mayfly emergence patterns can be analysed under two categories:

## 2.1 *Diel patterns*

Edmunds and Edmunds (1980) makes an interesting comparison of emergence in specialized short life (< 2 hr) and longer adult life (> 6 hr) unspecialized species of Ephemeroptera in tropical and temperate regions (figure 1). Specialized genera have the potential of being similar in the two regions. For example, the emergence of the short-lived Caenidae invariably takes place either at dawn or dusk and appears to be

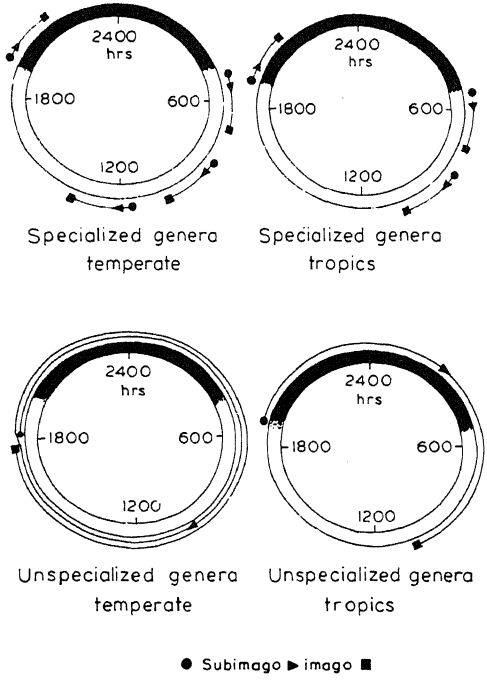


**Figure 1.** A generalized comparison of emergence and moulting to imago stages in specialized and unspecialized species of Ephemeroptera in tropical and temperate regions (after Edmunds and Edmunds 1980).

controlled by light intensity (Muller–Liebenau 1960). On the other hand, whereas in the unspecialized genera of the temperate region, emergence of subimagos is scattered, and length of subimaginal and imaginal life is variable, unspecialized genera in the tropical lowlands tend to be strongly restrained to a short daylight life, although exceptions are known (Edmunds and Edmunds 1980). Furthermore, nearly 100% of the vulnerable subimagos emerge in almost total darkness in tropical lowlands whereas emergence of the subimago during full daylight occurs in many temperate species. The most common time of emergence for most temperate species is from late afternoon through the first hour of darkness. In warm temperate regions with suitable night-time temperatures subimagos of various species may emerge during the night.

Edmunds and Edmunds (1980) point out that apparently many of the activity patterns and adaptations of adult mayflies (subimagos and imagos) have formed in response to selection pressure from predators. Mayfly subimagos are slow and clumsy fliers and are highly vulnerable to predation. Flying imagos are much less vulnerable but resting subimagos or imagos, being Paleoptera, are unable to fold the wings and hide in leaf litter, crevices or other protected areas. The brevity of their winged lives is itself an adaptation to reduce exposure to predation. Apparently the most significant daytime predators in tropics are Odonata. Birds are secondary. Most mayflies are killed also in spider webs. The only significant night time predators are bats. It is also clear that predation on mayfly subimagos and imagos is several times as great in the day as it is at night. Apparently the lowest predation rate of subimagos in the temperate regions also would be during hours of darkness. However, the selection pressure which seems to counteract selection for night-time emergence appears to be cool climate that slows transformation from the subimago to the imago. In the lowland tropics, nights are warm and most imagos which emerge in the first $1\frac{1}{2}$ hours of darkness transform to the imago stage before 0300 hr the next morning.

### 2.2 *Seasonal patterns*

Mayflies have distinct and finite emergence periods, especially in temperate and arctic areas. In cold temperate and arctic areas, mayfly emergence is more or less restricted to the summer months, owing to the physical barrier of ice cover and the low air temperatures during the rest of the year (Boerger and Clifford 1975; Brittain 1982; Ulfstrand 1969). Probably only a few species such as *Baetis macani* are able to emerge at water temperatures below 7°C (Brittain 1975). As one approaches the tropics, and also in more oceanic climates, there are fewer restrictions and emergence may occur throughout much of the year, although most emergence still occurs during the warmer months (Clifford 1981). In the tropics emergence is often non-seasonal, (Tjonneland 1960, 1970), although some species have clear emergence patterns. The lunar rhythm of emergence of the African species, *Povilla adusta*, is well known from a number of lakes (Hartland–Rowe 1958).

In habits with several mayfly species, peak emergence of the major species may be separated in time, especially in congeneric species (Brittain 1982). Such temporal separation over the emergence season may serve to reproductively separate species (Friesen *et al* 1980). Recent authors who have studied emergence suggest the following factors as possibly important in influencing or synchronizing emergence: temperature of air and water, light, moon phase, flow, humidity, wind, rainfall, photoperiod,

successive instars, hormones and endogenous rhythms (Corbet 1964; Humpesch 1971; Thibault 1971; Fremling 1973 a, b; Langford 1975; Peters and Peters 1977). In species with well defined emergence periods, males and females usually emerge synchronously, which may ensure survival of adequate numbers of organisms for successful reproduction (Friesen *et al* 1980). Emergence should be viewed as an integral part of the species' overall life cycle strategy (Brittain 1980).

## 3. Swarming and mating

Swarming is a male activity, apart from the Caenidae and Tricorythidae where both males and females may participate. The females fly into these swarms and mating occurs almost immediately and usually in flight. The flight of mayflies is a sort of wedding dance. Swarming may take place over the water itself, over the shore area, or even remote from the water. For instance, the swarms of *Baetis*, *Paraleptophlebia* and *Rhithrogena* have been observed up to several kilometers from the nymphal habitat (Edmunds and Edmunds 1980). Most swarms are oriented according to terrain markers such as areas of vegetation, the shoreline, and trees (Savolainen 1978). The time of swarming varies considerably, although dusk is the most common time of the day in temperate areas. Light intensity and temperature are major factors in determining the timing of swarming. The time of nuptial flight appears to be the result of selection to reduce the time of daylight. Table 1 is the estimate of Edmunds and Edmunds (1980) regarding the most to least frequent times of swarming in tropical and temperate species.

The behavioural adaptations for mating as evident in the shape and location of eyes, and foreleg modifications in males are really striking. The prominent turbinate eyes of males, especially well-developed in the Baetidae and some Leptophlebiidae, provide both high acuity and good sensitivity (Horridge 1976). This enables them to detect and capture single females in a swarm at low light intensities. The forelegs of male are unusually long for grasping and holding the female during mating. The other legs are reduced. The backward bent of tarsus necessary for the suspension during mating is made possible by a reversible joint at their bases.

Brinck (1956) observed mating in *Parameletus chelifer*. Males dominate during the early swarming period. But soon numerous females mixed with the swarms. The male pressed himself under the female abdomen and stretched the front legs forwards and upwards along the sides of her body, until they reached the prothorax. Then the tarsus

**Table 1.** Nuptial flight time (after Edmunds and Edmunds 1980).

| Decreasing frequency ↓ | Tropics | Temperate |
|---|---|---|
| | Morning | Dusk |
| | First dark hours | Afternoon |
| | Dawn | Afternoon/Morning |
| | Midday | Morning |
| | Dusk | Midday |
| | Afternoon | Dawn |
| | | First dark hours |

were bent so that each clasped round a wing-base. At the same time the abdomen was curved and the forceps grasped the 8th or 9th abdominal segment of the female. The female abdomen was usually held like an S so that the penis was easily pressed into the female genital opening. The male cerci were stretched forward, fixing the female abdomen at the same vertical plane as the male abdomen. The female cerci were directed obliquely hindward (figures 2 A,B). The copulation lasted about 20 sec and then the male took off, soon followed by the female.

The suspension of the male body in the anterior legs and the forceps is certainly very safe. It is most probable that the above type of male suspension in anterior tarsus and forceps is characteristic of this insect group. This is indicated by the presence of the forceps in all males investigated and lengthened male front legs in all species known.

## 4. Oviposition

Specialized structure like ovipostor found in other groups of insects, is lacking in mayflies. However some authors call the produced subgenital plate an ovipositor. We prefer to call it 'egg channel' (Sivaramakrishnan 1984). The problem of downward displacement of eggs and immature nymphs, can be compensated for by the adult mayflies flying upstream before they lay eggs.

Oviposition behaviour of mayflies can be categorized under five basic types (Elliott and Humpesch, Personal communication).

(i) *Female goes underwater and eggs laid on substratum:*
*Baetis rhodani* belongs to this category (Elliott 1972). The female lands on a partially submerged stone in rapidly flowing water, folds her wings along the abdomen, then walks under the water and searches for a suitable oviposition site, usually on the

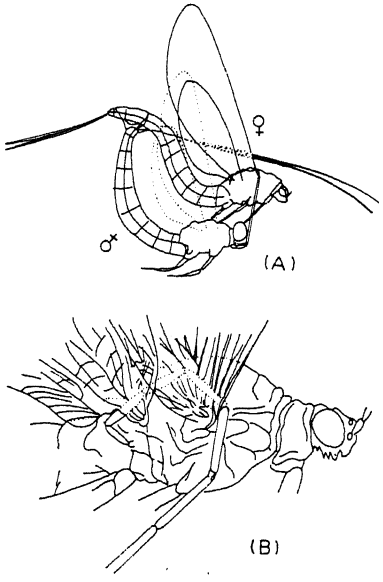


**Figure 2.** *Parameletus chelifer* Bengts (after Brinck 1957) **A.** Mating couple in flight. **B.** The backward bent of the tarsus of male clasped round the wingbase of female.

underside of the stone. This behaviour may permit assessment of water quality before oviposition (Sutcliffe and Carrick 1973). The female lays contiguous rows of eggs to form a flat semi-circular plate. When oviposition ceases, the female may walk out of the water and fly away, but she is usually swept away downstream.

(ii) *Female rests on a stone above water and eggs laid on substratum under water:* *Habroleptoides modesta* (Pleskot 1953) belongs to this category in which the female dips its abdomen in the water and lays eggs. The female is never totally submerged. The tails are usually broken off before oviposition starts.

(iii) *Female flies down to the water surface and eggs are released in a single mass:* In *Ephemerella ignita* (Elliott 1978) the egg mass forms a spherical greenish ball that is carried at the genital aperture with the posterior abdominal segments curved downwards and round the ball to hold it in position. The female flies upstream and descends to the water surface, releasing the egg-ball on contact with the water.

(iv) *Female flies down to the water surface and eggs are released in several batches:* In *Rhithrogena semicolorata* (Humpesch and Elliott 1980) the female flies upstream, descends to the surface of the water and releases a few eggs by dipping the tip of her abdomen at intervals. Most species belong to this category.

(v) *Ovoviviparous species:*

*Cloeon dipterum* is the only species in which the females rest for 10–14 days after copulation and then lay their eggs on the surface of the water. As soon as the eggs come into contact with the water, they hatch and the larvae swim away, (Degrange 1959).

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# Functional morphology of air-breathing fishes: A review

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**Abstract.** This review of the air-breathing fishes deals with morphology, histochemical analyses of respiratory membrane and muscles, morphometrics and development of respiratory organs, hematology and other blood parameters, oxygen uptake by gills and skin, physiological and behavioural aspects, ecology and effects of pollutants on the morphology and behaviour. The scientific significance and application of the studies of functional morphology and physiology in understanding the alteration caused by pollutants have also been elucidated.

**Keywords.** Air-breathing fishes; morphology; hematology; histochemistry; morphometrics; physiology; behaviour; pollutants.

## 1. Introduction

The air-breathing structures appeared in freshwater fish during the late Silurian or early Devonian period and are thought to have evolved as adaptation to hypoxic water conditions due to severe periodic droughts (Smith 1931; Johansen 1970). Recent studies on air-breathing fish have demonstrated that various types of morphological and physiological adaptations have made it possible for fish to utilize aquatic and aerial respiration. The morphological and physiological adaptations in these fishes are designed in a manner so that they derive maximum advantage of their surrounding environment. There is an intimate relationship among the physico-chemical characters of habitat, nature of biota, food chain and finally the morpho-physiological adaptation of animals.

## 2. Morphology

The structures of the different types of respiratory organs have been studied for many years by several scientists. Munshi (1961, 1962, 1962a, 1968), Hughes and Munshi (1973), Dutta (1968), Jordan (1976), Peters (1978), and Burggren (1979) found that certain air-breathing fishes such as *Clarias batrachus*, *Heteropneustes fossilis*, Blue gourami, *Trichogaster trichopterus*, *Anabas*, *Macropodus* and *Betta* possess labyrinthine air-breathing organs. These organs are derived from the epibranchial regions of the first and second branchial arches, and extend dorsally as plate-like organs to fill the suprabranchial chamber and the air-sacs which are the extensions of the opercular cavity or branchial chamber. The earlier hypothesis of Munshi (1962b) that the "respiratory islets" of *H. fossilis* and *C. batrachus* are modified lamellar structures has been confirmed later by electron microscopic studies of Hughes and Munshi (1973a).

According to them, the cells forming the vascular spaces in the dendritic organs and respiratory islets are typical pillar cells of the gills.

Although the suprabranchial chambers in the Anabantidae group are extensions of branchial chambers and the labyrinthine organs which have developed on the epibranchials, the "respiratory islets" of the accessory respiratory organs have evolved in different fashion. The hypothesis that the labyrinthine organs and the respiratory islets of the suprabranchial chambers represent modified gill structure (Munshi 1968) is now no longer tenable as the electron microscopic studies of Hughes and Munshi (1973a) revealed that in *Anabas testudineus* the pillar cells of the air-breathing organs do not have the same relationship as in gills, but are modified epithelial cells. Obviously we are dealing with analogous rather than homologous structures that are used to serve the same function, supporting the contiguous vascular units which make up these respiratory organs. Such a supporting function is clearly of importance. During evolution, different structural arrangements have been selected to provide surfaces with minimum diffusion barrier between blood and the respiratory medium (Hughes and Munshi 1968). Further, the blood capillaries of the respiratory islets of *Anabas* are unique structures, having a series of most characteristic type of unicellular valves ever discovered in any animal system so far (Hughes and Munshi 1973a). These valves control the movement of blood through the respiratory islets. In *Amphipnous cuchia* and *Channa* sp. the suprabranchial chambers are the extensions of the pharynx. The vascular mucosa of the air-sacs have evolved independently (Hughes and Munshi 1973a) and not from the gill lamellae as it appears in the histological preparations under light microscope (Munshi 1962a; Munshi and Singh 1968).

In *Amphipnous* (*Monopterus*) *cuchia*, the structure of the valve is very unique. It projects freely into the papilla lumen and appears to be involved in the regulation of blood flow through individual papilla (Hughes and Munshi 1973a). These structures were discovered for the first time and are not only new contributions to knowledge, but open up new lines of further research.

In *Periophthalmus vulgaris* and *Boleophthalmus boddaereti*, opercular chambers are modified for air-breathing purposes. The accessory respiratory organs of *P. vulgaris* provide an excellent example of adaptation by modification of opercular chambers (Singh and Munshi 1969). In this fish, the opercular chambers have enlarged and become vascularized for respiratory purposes. Intricate mechanisms for opening and closing of the inhalent and exhalent apertures have evolved and the branchiostegal apparatus has developed a special type of safety valve which is workable by stripes of muscles.

Further, the studies on ultrastructure of gills of these air-breathing fishes have made new contributions to our knowledge. The earlier hypothesis of Munshi and Singh (1968a) that the pillar cells are modified smooth muscle cells have been confirmed (Munshi 1976).

Some other forms of accessory gas exchange organs have evolved in numerous groups of fish in order to obtain oxygen from air. These air-breathing organs may be in the form of modified swimbladders, pharyngeal cavities, stomach and intestine (Johansen 1966; Munshi 1976; Singh 1976). Kramer and McClure (1980) found that similar tc other callichthyids, the posterior end of the intestine of *Corydoras aeneus* works as an accessory respiratory organ and its anterior end is provided with a muscular bulb. Generally, these organs are utilized for oxygen uptake and gills are used for the elimination of $CO_2$, and these processes seem to be similar to aquatic respiration (Randall *et al* 1978; Burggren 1979).

It has been observed by Jordan (1976) that some air-breathing fishes are facultative air-breathers and can survive indefinitely on dissolved oxygen, while some such as *Protopterus*, *Lepidosiren* and *Electrophorus* are obligate air-breathers that will drown when access to air is denied. She also found that *Clarias batrachus* is a bimodally breathing teleost. *C. batrachus* possesses an air-breathing organ with highly branched dendritic organs or respiratory "trees" that develop as outgrowths of the second and fourth gill arches, and are located in the suprabranchial chamber.

## 3. Histochemistry

### 3.1 *Histochemical studies of the respiratory membrane*

A complete knowledge of the cellular structure of the respiratory membrane of the air-breathing organ is essential to understand its physiology. The histochemical study of the respiratory membrane reveals five kinds of specialized cells: (a) mucous cells, (b) acidophil granular cells, (c) basophil mast cells, (d) large bi- or trinucleate glandular cells, and (e) mitochondria rich chloride cells in the gills and accessory respiratory organs of many air-breathing fishes (Munshi 1960).

The typical goblet type of mucous glands are present in large numbers in freshwater fishes such as, *Catla catla*, *Labeo rohita*, *Channa punctatus*, *Mastacembellus armatus*, *Clarias batrachus*, *Heteropneustes fossilis*. In *C. catla* these cells respond to the chloride test (Munshi 1964). This means that besides mucous secretion, they also play an important role in chloride regulation. In air-breathing fishes *viz C. punctatus*, *C. batrachus* and *H. fossilis* only few mucous cells give positive reaction with $AgNO_3/HNO_3$ test for chloride. The medullary hormones of adrenal have profound effect on the mucous cells of air-sacs and the gills of *H. fossilis* and *M. aculeatum* respectively (Guha *et al* 1967). The sulphated acid mucopolysaccharide component of the mucous keeps the air-sac moist and lubricated during gaseous exchange.

The acidophil granular cells of the gill epithelia are diastase resistant, PAS-positive. Those belonging to the connective tissue system are PAS-negative, however, almost all the granular cells are PAS-positive after extraction of lipids. The granules of these cells are composed of tyrosine-rich protein. The cells also appear to contain a large amount of RNA. The eosinophilic granular cells appear to contain carbohydrate, protein and lipid firmly bound with each other. Some of the cells give positive reactions for alkaline phosphatase. These cells do not respond to $AgNO_3/HNO_3$ test for chloride (Singh and Munshi 1968).

The basophilic mast cells are present in large numbers in the sub-epithelial connective tissues of the gill lamellae of *Hilsa ilisha*. They are closely associated with blood capillaries (Munshi 1960). It is quite meaningful that mast cells (which are reservoirs of heparin and histamine) are found in the gills of fishes.

The bi- and trinucleate glandular cells are found in the gills of siluroid fishes. They have been derived from the glands of skin of these fishes (Munshi 1960). The cytomorphosis of these glands has been studied for the first time and throw light on their origin (Mittal and Munshi 1970).

Chloride cells are found in good numbers in many of the air-breathing fishes *viz Anabas* and *Clarias* which live in brackish waters also. The endoplasmic reticulum is very well developed and a large number of mitochondria is found in these cells (Hughes and Munshi 1973a).

Large amounts of reserve fats have been discovered in true air-breathing organs of amphibious fishes (Singh *et al* 1973). The stored lipids lie in the well-developed fat cells of the connective tissue layer between the respiratory islets and the muscles of the air-sacs. A direct correlation exists between the vascularity of the organs and the concentration of the fat globules, and they contain both acidic and neutral fats. The pharmacological action of adrenalin and atropine was effective in bringing about complete mobilization of the fat deposits of the air-sacs of *Saccobranchus fossilis in vivo* condition.

### 3.2 *Enzyme histochemistry of the respiratory muscles*

The gill ventilation is under the influence of buccal pressure and opercular suction pumps. These pumps are operated by means of a series of respiratory muscles. The enzyme histochemistry of these respiratory muscles has opened up a new field of investigation and it also reveals that the muscles operating these respiratory pumps are composite in nature. Red, white and intermediate muscle fibers have been distinguished in the respiratory muscles depending on their intensity of reaction for succinic dehydrogenase (Munshi *et al* 1975). It has been noted that the muscles innervated by the facialis nerve are dominated by red fibers, whereas those innervated by trigeminal are dominated by white muscle fibers (Ojha and Munshi 1975). The cytochemical differentiation of the muscle fibers will reflect their metabolic activities during gill ventilation. The combined study of ultrastructure and enzyme activities of muscle fibers provides an indepth understanding of the muscle physiology which can be correlated with behavioural patterns of air-breathing fishes.

Both electron microscopy and enzyme determination techniques were used by Hochachka *et al* (1978) and Johnston (1979) to determine the ultrastructures and enzyme activities of white and red muscle fibers of *Aruana* and *Arapaima*, both obligate air breathers. Their study reveals that the white muscle fibers in both species possess a rather similar ultrastructure, characterized by large diameter, very few mitochondria, and few capillaries. They also found that white muscle fibers of *Aruana* displayed higher levels of enzyme activity, while enzymes in aerobic metabolism occurred at about one-half the levels in *Arapaima*. No red muscle was found in *Aruana*, but it was present in *Arapaima* and was fueled by glycogen and lipid droplets. Their studies led to a revealing conclusion that the surface skimmer sustained a higher oxidative capacity in its myotomal muscles than that of the facultative air-breather.

## 4. Morphometrics and development of respiratory organs

An earlier study (Das 1927) detailed only the morphometric aspect of the air-breathing organ of *Channa striatus* and *C. punctatus* during their ontogenic development. Whereas the more recent morphometric studies (Hughes *et al* 1973; Hakim *et al* 1978) explain the role of the gas exchange machinery of the amphibious fishes during their development and growth. Recently, Dube and Munshi (1974) have observed that *Anabas testudineus* of the lower weight group survived for a longer period than that of higher weight group, when prevented from surfacing. They reasoned that as the fish grow, the rate of increase in gill surface becomes less than that in the surface of the accessory respiratory (labyrinthine) organ.

Dube and Munshi (1974) also found out that the $O_2$ gas-diffusing capacity of the gill of *Anabas testudineus* decreases at faster rate with increasing body weight than that of *Clarias* and *Heteropneustes*. These findings explain why *Anabas* of higher weight group dies when not allowed to breathe atmospheric air whereas the gills and skin of *Clarias* and *Heteropneustes* are efficient enough to take care of the total metabolic demands of the fishes as they grow in size. The estimation of the diffusion capacity of the accessory respiratory organs of the air-sacs of *Amphipnous* are less suited for oxygen uptake than that of *Anabas*.

The morphometry of respiratory surface area can be used for gas transfer by using the following equation (Hughes and Morgan 1973)

$$VO_2 = K\ (A\ PO_2)/T.$$

where $VO_2 = O_2$ uptake in ml $O_2$/min; $A$ is the area in $cm^2$ for gaseous exchange; $PO_2$ is the mean difference between the oxygen tensions of water and blood; $K$ is the permeation coefficients (ml $O_2/m/cm^2$ mm Hg/min).

On the basis of morphometric deduction of the surface area, its physiological aspects can be inferred. These studies further have practical importance in rearing and transporting of these air-breathing fishes (Munshi and Ojha 1974; Munshi and Dube 1974; Munshi *et al* 1974). Other researchers (Lenfant and Johansen 1968; Farber and Rahn 1970; Hughes and Singh 1970a, b, 1971; Singh and Hughes 1971; Magid and Babiker 1975; Stevens and Holeton 1978; Magnuson *et al* 1982; Ischimatsu and Itazawa 1983a, b) have also shown that there are considerable variations among air-breathing fishes in the degree of dependence on aquatic or aerial respiration depending on degree of development and efficiency of the respiratory and related structures, environmental limitations and metabolic needs of the fish.

To correlate form with function, the morphometric study of these respiratory (both aerial and aquatic) structures during development is extremely important. A correlation exists between the surfacing behaviour and different stages of ontogenic development of *Channa striatus*. The surfacing frequency is most erratic in the fingerling and becomes regular in the fish of 750 mg. The individuals weighing more than 30 g also can survive on aquatic breathing for more than 16 hr, when prevented from surfacing. It seems that this fish size can accumulate sufficient energy-rich substances that they are able to switch over to anaerobic respiration (Vivekanandan 1977). Babiker (1979) has also found out that the dependence on aerial respiration appeared to develop gradually with age in *Clarias* but occurred over limited age range (200–300 g) in *Protopterus*.

It has also been observed by Natarajan (1977) that labyrinthine organs of certain air-breathing fishes such as *Anabas scandens* have the capacity of regeneration. It was observed that when the first branchial arch was amputated at the level of the origin it never regenerated, whereas regeneration was detected in those fishes which have a remnant of labyrinthine tissue at their base. This suggests that the base of the labyrinthine organ acts as a root for regeneration. Therefore, a knowledge of the process of regeneration will definitely promote the understanding of the developmental mystery of the respiratory organs.

## 5. Ecology, pollutants and air-breathing fishes

The extensive use of insecticides is continuously polluting fresh water. There are manifold effects of insecticides on living organisms including economically important

fishes. They are also responsible for a number of physiological and biochemical disturbances. Metasystox is known to be the most widely used insecticide against paddy sucking aphids, spiders, mites, saw flies etc. It has been proven by Natarajan (1981) that in the pesticide contaminated water, the air-breathing organs of *Channa striatus* play a very important role by extracting $O_2$ from air during their stay in such contaminated water. He also observed the existence of DDT and Dieldrin-induced anemia as shown by the low erythrocyte count, low Hb content, light MCH and colour index in *Channa punctatus*. The progressive decrease in erythrocyte count, Hb concentration and total leucocyte count were found in *C. punctatus* exposed to malathion and methyl parathion. It has also been observed by Natarajan (1978) that when a climbing perch, *Anabas scandens* is exposed to a lethal dose of sumithion, it used its air-breathing organs extensively to overcome the pollution stress for survival.

The effect of pollutants on air-breathing fishes has also been investigated by Munshi and Singh (1971). Different aspects of the biology of air-breathing fishes (reproduction, feeding habits, and body composition) have been correlated with the ecology of ponds and swamps (Bilgrami 1977).

The presence of bacteria in high concentration on the surface of supra-branchial chambers of *Anabas* has been recorded in many collections made by Munshi from several localities of W. Bengal and Bihar. This is an interesting association and practically nothing is known about the role of these bacteria. The possibility of their playing some decisive role connected with respiration or other physiological processes cannot be ruled out. It is also quite likely that some of these bacteria produce biotic substances that can protect these fishes against possible injury under stressed physiological environment. The bacteria in turn get their oxygen supply readily available in the suprabranchial chambers.

## 6. Hematology

### 6.1 *Hematology and other blood parameters*

A series of hematological studies on *Amphipnous*, *Anabas*, *Channa* and *Heteropneustes* indicate that the oxygen-carrying capacity of blood is related to body size of these amphibious fishes (Dube and Munshi 1973; Mishra *et al* 1977; Pandey *et al* 1977). Recently, a comparative study of the bloods of 45 species of Amazonian fishes (Powers *et al* 1979) pointed out that there is a significant difference between water and air-breathers $CO_2$ tension in the blood. Air-breathing fishes have much higher $CO_2$ tension in the blood and the arterial $CO_2$ tension of water breathers is generally below 5 torr (Rahn 1966). Whereas, in the air breathers $CO_2$ tension ranges from 15 to 43 torr (Rahn and Garey 1973).

Thus, it is apparent that with the evolution of air-breathing mechanism, adjustments occur at the molecular level of $CO_2$ which is the causative factor for efficient hemoglobin function to counteract the increased $CO_2$ load. Therefore, some of the differences in the hemoglobin of air and water breathers are related to the effect of carbon dioxide on the hemoglobins of the air-breathers (Farmer 1979). Further, while studying the effects of $CO_2$ on hemoglobin function of air-breathing fish, Farmer investigated three major points. First, to what extent are the oxygen binding properties of non-mammalian hemoglobins get influenced by $CO_2$ and whether these properties

are independent of pH or not. Second, whether or not the hemoglobins of water breathers differ from the hemoglobins of air-breathers with respect to the magnitude of the effect of carbamino $CO_2$ affinity. Third, whether or not there is a correlation between Bohr effect and effect of carbamino $CO_2$ on the $O_2$ affinity of hemoglobin. He also found that the blood $CO_2$ content of air-breathing fish and amphibians is much higher than that of water breathers, but hemoglobin showed no adaptation to an increased $CO_2$ load. But the drop in oxygen affinity of hemoglobin caused by $CO_2$ is increased by increasing pH for each hemoglobin examined.

Blood oxygen, erythrocyte nucleoside triphosphate (NTP) concentration and several other hematological parameters in facultative air-breathing fish from the Amazon acclimated in normoxic and hypoxic water were measured by Weber *et al* (1979). They found that the armored catfish *Hypostomus* sp and *Pterygoplichthys* sp in hypoxic water undertake surfacing intermittently and result in lower NTP level.

Benesch and Benesch (1967) found in eel, *Anguilla* that the decrease of NTP under hypoxic condition increases blood $O_2$ affinity, by reducing the direct allosteric interaction and also affecting the Donnan distribution of proteins across the red cell membranes. These changes help to increase the intraerythrocytic pH of eels in hypoxic water and thus increases the $O_2$ affinity of the hemoglobin via the Bohr effect (Wood and Johansen 1973).

Similar studies on the catfish indicated changes in acid-base balance and blood-buffering associated with hypoxic acclimation (Wood *et al* 1979).

### 6.2 *Determination of blood proteins*

Although some work has been done on serum and plasma proteins of freshwater fishes, little is known about the air-breathing fishes specially those exposed to the pollutants. Comparative electrophoretic studies of serum and plasma proteins of different fish have been made (Lepkovsky 1929; Deutsch and Goodloe 1945; Drilhan 1954; O'Rourke 1960; Nyman 1965; Badawi 1971 and Hattingh, 1972). Most of them deal with blood protein patterns of bass, carp, trout, catfish, perch, pike and whitefish. Badawi (1971) investigated blood protein patterns of four Tilapia species. Hattingh (1972) studied the plasma and serum samples by SDS-PAGE of five South African species. Hattingh's results indicated that plasma and serum of these fishes differ in electrophoretic pattern, and also in the number of fractions and the protein concentration of fractions. It appears that each fish has its own characteristic pattern of electrophoresis, which is fairly constant in mature and healthy fishes within a species (Hattingh 1972). Badawi (1971) and Lysak and Wojcik (1960) postulated that the variable amounts of protein found in blood samples might be related to diet and/or season.

The serum proteins of bluegills, *Lepomis macrochirus*, exposed to methyl mercuric chloride were electrophoretically investigated by Dutta *et al* (1983). They found significant changes in the qualitative and quantitative profiles at 24 and 48 hr. A variety of peptides, showing differential loci, staining intensity, mobility and molecular weight were seen at 48 hr compared to 24 hr. However, 72 hr samples showed a reduction in polypeptide bands approximating a profile similar to controls. A few other investigators have found changes in serum protein of fishes exposed to MeHg (Lagler *et al* 1977; Hilmy *et al* 1980).

## 7. Oxygen uptake by gills and skin in relation to body size of amphibious fishes

The exponent *b* values related to oxygen consumption and the body weight show a wide range of variation among the teleostean species. Winberg (1956) reported an average value of 0·81 for a number of species, whereas Paloheimo and Dickie (1965) suggested 0·80 to be the characteristic value of most teleosts. These exponent values are based on data available on water breathing forms.

The rate of oxygen consumption ($VO_2$) through gills and skin (Munshi *et al* 1982) was measured in air-breathing fishes of different body weights under two experimental conditions, *viz* (i) when access to air was allowed and (ii) when it was prevented. Characteristics of the regression lines relating the logarithm of oxygen consumption to log body weight were calculated by the method of least squares. These physiological studies revealed that in *Anabas testudineus*, *Channa punctatus* and *Clarias batrachus* the oxygen consumption through gills and gill and skin (*Clarias*) increases by a power of 0·67, 0·79 and 0·869 respectively when these fishes have free access to air. Further, lowering of the exponent values was indicated in *A. testudineus* ($b = 0{\cdot}53$), *C. punctatus* ($b = 0{\cdot}62$) and *C. batrachus* ($b = 0{\cdot}841$) when they were not allowed to breathe atmospheric air. The lowering of the exponent value was perhaps related to the stress condition of the air-breathing fishes when they were not allowed to breathe atmospheric air. The fall of "b" value due to stress condition in air-breathing fishes is a phenomenon newly discovered in the physiology of these fishes.

## 8. Other physiological and behavioural aspects of air-breathing fishes

During the last decade or so, a large number of physiologists have become interested in the study of various adaptive features of the amphibious vertebrates (Hughes and Munshi 1979; Johansen 1966, 1970; Rahn *et al* 1971; Lenfant and Johansen 1968; Liem 1980). Gas exchange and respiratory patterns of Indian air-breathing fishes such as *Anabas testudineus*, *Heteropneustes fossilis* and *Clarias batrachus* have been studied in detail by Hughes in his Research Unit for Comparative Animal Respiration, University of Bristol, U.K. These studies clearly indicate that there are large variations in the metabolic rates among the species of these fishes inhabiting more or less similar waters. They also indicate differences in the relative dependance on air and water for the oxygen and $CO_2$ exchange. The morphometric studies have been interpreted in relation to the physiology of respiratory system (Hakim *et al* 1978).

## 9. Acclimation of air-breathing fishes

The bimodal (aerial and aquatic) gas exchange capacity of hypoxia-acclimated *Ancistrus chagresi*, the armored catfish has been investigated by Graham (1983). He found that hypoxia-acclimated *Ancistrus* have higher blood $O_2$ affinity, more hemoglobin (Hb) and can maintain a higher aquatic oxygen consumption rate ($VO_2$) in hypoxic water. These fishes also have a 25% larger stomach which they use as an air-breathing organ. Johnston and Bernard (1983) have inquired in detail the aquatic and aerial respiration rates, muscle capillary supply and mitochondrial volume density in

air-breathing catfish (*Clarias mossambicus*) acclimated to either aerated or hypoxic water. They determined that hypoxia acclimation did not cause changes in either muscle mitochondrial density or capillary density. They ascertained that increased ventilation of the suprabranchial chambers and greater oxygen extraction across the gills inhibit the need for modifications in the above mentioned structures.

Pettit and Beitinger (1980) have searched into the respiration of the reedfish, *Calamoichthys calabaricus* which encounters hypoxic conditions over much of its range. Their experiments have confirmed that *Calamoichthys* can exist exclusively on aerial respiration when exposed to hypoxic aquatic environment. Burggren and Randall (1978) demonstrated that the sturgeon, *Acipenser transmontanus* can reduce its aerobic metabolism drastically in response to hypoxic water. Though Pettit and Beitinger (1980) have also found a remarkable reduction in the respiration rates with time in seven experiments (in hypoxic water), they attributed this reduction to the stress caused by handling.

Babiker (1979) discovered that when the fish was allowed to partition oxygen uptake in hypoxic water, the rate of pulmonary ventilation in *Protopterus* and oxygen consumption in *Clarias* also increased with enhanced hypoxia. However, this increase was very noticeable in *Clarias* and insignificant in *Protopterus*. This finding shows that the independence of pulmonary ventilation from the level of oxygen in the water in the case of dipnoans and dependence of *Clarias* on accessory respiratory organ.

Enhancement of the respiratory frequency in hypoxic water has been observed in many other air breathing teleosts (Gans 1970; Hughes and Singh 1970a, b, 1971; Singh and Hughes 1971; Stevens and Holeton 1978, 1978a; Gee and Graham 1978). Effects of hypoxia acclimation on the respiratory system of air-breathing fishes have also been studied by Weber *et al* (1979), Gee (1980), and Graham and Baird (1982).

It has been observed that all air-breathing fish utilize aquatic gas exchange to some extent, because air-breathing organs have a low gas exchange ratio. Since carbon dioxide is highly soluble in water, it is naturally eliminated mainly through gill or skin (Singh and Hughes 1971, 1973).

The oxygen uptake of air-breathing fishes varies in various environmental conditions. Jordan (1976) and Munshi *et al* (1982) have measured the rate of oxygen uptake ($VO_2$) of *Clarias batrachus* and *Heteropneustes fossilis* under various experimental situations, such as, in normoxic water, forcible submergence, and air exposure.

Most studies on oxygen uptake of fishes have been carried out under experimental conditions and controlled environment. In the future investigation it is proposed to measure the rate of $O_2$ uptake under natural environment with different types of waters.

Pandey and Chanchal (1977) demonstrated that if access to atmospheric air was not allowed *C. punctatus* and *A. testudineus* could not survive or asphyxiate if the oxygen content of the water fell below 2·79 and 2·93 ml/L respectively. Immediate death may occur due to brain anoxia.

There are some air-breathing fishes such as *Piabacina fistae*, a central American fish, which breathes air frequently even in air-saturated water, yet, it is not an obligate air-breather. Without an access to air this fish can maintain $VO_2$ by aquatic respiration down to a $PO_2$ of about 70 torr. Its vascularized respiratory compartments or cells located in the second chamber of the physostomus gas bladder function for aerial respiration (Graham *et al* 1977).

## 10. Behaviour

Behaviour such as way of aerial respiration and time required for the same can be accurately observed by cinematography. Some of the earlier investigators have recorded the time required for gas release and gas intake. Gradwell (1971) has recorded the time needed for entire cycle from breaking the surface to gas release (which requires about 0·2 sec) and inspiration (which requires less than 0·07 sec) for *Plecostomus* (*Hypostomus*) *punctatus*, the Loricariid catfish. Whereas, *Lepidosiren* needs about 3·3 sec for entire cycle (Bishop and Foxon 1968) and *Protopterus* 1·1 sec (McMahon 1969). Garfish *Lepisosteus* requires 0·9 sec. (Rahn *et al* 1971) and *Hopterythrinus* (a teleost) needs 0·86 sec (Kramer 1978). The shorter breath duration of *Corydoras* has been related to the continuous rather than tidal ventilation (Kramer and McClure 1980). Longer breath duration may be related to the size of the above mentioned species. Kramer and McClure (1980) have searched into the surfacing behaviour of the catfish *Corydoras aeneus* (Callichthyidae) by using cinematography, which made it possible to determine the sequences and timing of the ventilation movement of this fish. They also studied the effect of dissolved oxygen on surfacing and depth on surfacing rate and activity. They concluded that brief and infrequent surface visits are essential for survival when $PO_2$ falls below 15 torr and surfacing is not obligatory at high oxygen level. They confirmed Gee and Graham's (1978) findings which revealed that air-breathing was not obligatory in the Callichthyids. Compared to impressive research made in ultrastructure, physiology, and biochemistry of the respiratory organs of air-breathing fishes, knowledge of air ventilation mechanism has been largely neglected. Very few researchers have paid attention to neuromuscular and biomechanical aspects of intake and expulsion of air into the accessory respiratory organs. The aerial respiration and the movements of the related bony elements of *Anabas testudineus* were recorded cinematographically by Dutta (1968), establishing a network of functional correlation between the specific bones, muscles and the air-breathing mechanism of the fish. It has been noted that *Anabas* uses only upper and lower jaws, opercular bones, branchial chamber and the hyoid apparatus during normal respiration. The suspensorium is not involved and a small expansion of the buccal cavity seems to be conducted only by the ventral movement of the hyoid apparatus. The aerial respiration (gulping) is realized by the above mentioned elements of the head, but in this case a concurrent lateral movement of the suspensorium is also recorded. There is a remarkable similarity between the aerial respiration (gulping) and feeding mechanisms of *Anabas*. The same functional elements of the head are involved in carrying out both functions. To create a suction-force in the oral cavity, expansion of this cavity is essential which is performed by the lateral movement of the suspensorium, caused by the contraction of the musculus levator arcus palatini. Similar type of expansion of the oral cavity has been observed when *Anabas* engages itself to engulf fresh air.

In *Anabas*, the branchial chamber is expected to be much reduced, because of the large antero-dorsally situated labyrinthine organs. On the contrary, the branchial chamber is not small. The moderate sized branchial chamber seems to be maintained by the caudal displacement of the pectoral girdle, which is reflected by the long supracleithrum, supratemporal, and post-temporal bones. The caudal displacement of the pectoral girdle can be considered as a classic example of an interelemental dependence. It seems that in order to accommodate the labyrinthine organs, the size,

shape and even entire architecture of the head had to make certain compromise. Therefore, air-breathing organs of any fish should be analyzed in a functional-morphological perspective which emphasizes the mechanical and spatial arrangements of the structural features in accordance with their use or function. Dutta (1975) has also correlated the functional and structural aspects of the suspensorium of another air-breather, *Ctenopoma acutirostre* with those of *Anabas testudineus*. It has been cinematographically recorded that *Ctenopoma's* suspensorium is involved in aquatic and aerial respirations as well as in feeding, whereas, in *Anabas* the same bone functions only during air-breathing and feeding. Further, the suspensorium has been analyzed as a part of the architectonic structure of the entire head by using a diagrammatic model based on mutual influence, integration and couplings including other functional elements.

Dutta (1979) has traced the structural configurations and functional mechanics of jaws of *Macropodus opercularis*, *Ctenopoma acutirostre* and *Anabas testudineus* (air-breathing fishes) in feeding. The mechanics of air-breathing in all the three fishes are similar during feeding. In these fishes the protrusion of the upper jaw has been discerned during feeding and air-breathing. The ventral groove of the premaxillae and caudo-lateral articular process in *Macropodus* and *Ctenopoma*, the median condyle and the antero-lateral articular process on premaxillae in *Anabas* are vital structures for the protrusion of the upper-jaw. In *Ctenopoma* the dorsally inclined mouth seems to be correlated to surface feeding as well as air-breathing. Easy access to the surface may be a functional advantage obtained by this fish by having the afore-mentioned structural modification. In between the air-breathing and feeding behaviours, *Ctenopoma* rests on the bottom of the aquarium. The condylar articular process on the articular bones of the lower jaw in *Ctenopoma* is a structural modification, which is adapted to work as balancing wheel when fish is in resting stage.

The movement of the jaws is a vital mechanics which is involved in life-sustaining functions like respiration and feeding. The patterns of articulation between premaxilla and maxilla, maxilla and vomer, palatine and ethmoid, maxilla and palatine, the structural configuration of rostral cartilage and the surrounding ligaments have an integrally vital role in realization of upper-jaw protrusion (Dutta and Chen 1983). Any change in the structural and functional patterns of these elements will have a definite unfavourable effect on the function of the fish, whether the function is an aerial respiration or feeding, even though the fish might have a well-developed labyrinthine organ, swim-bladder or any other kind of air-breathing organs.

Jaw protrusion of a fish is not only influenced by its closely associated functional elements but a distantly located element, hyomandibula, also, has a greater impact on the protractibility of both lower and upper jaws. Dutta (1980) discussed the comparative structural and functional aspects of the hyomandibula in three air-breathing fishes, *Macropodus opercularis*, *Ctenopoma acutirostre*, and *Anabas testudineus* and concluded that the structural analysis of the hyomandibula and its relationship with the function like aquatic and aerial-respirations depict the inter-relation between form and function. Features such as the vertical ridge and articulations like the hyomandibulo-neurocranial, the hyomandibulo-opercular, and the hyomandibulo-interhyal have been specially analyzed in relation to the respiratory function in the above three air-breathers. Peters (1978) not only indicates that hyomandibula is one of the apparatus which form the lateral wall of the suprabranchial

chamber in Anabantoids, but "the air ventilation is regarded as being caused by the activity of a double pumping mechanism consisting of the buccal and opercular apparatus." Therefore, the hyomandibula being one of the functional elements of the buccal apparatus plays equally an important role in air ventilation in Anabantoids (Dutta 1980). Peters (1978) and Kramer (1978) have used cineradiography to analyze the mechanics of air ventilation of the air chamber and respiratory gas bladder in some teleosts and have achieved remarkable results.

The physiological origin of the mechanisms underlying air ventilation in air breathing fish of different phylogenetic lineages has also been researched by using high speed cinematography, cineradiography and electromyography (Liem 1980). With the help of these experiments Liem was able to disprove the hypotheses of Bader (1937) and Mishra and Munshi (1958). Munshi (1968) attributed a key functional role to the constrictor suprabranchialis during ventilation. With the electromyographic profiles and more intense kinematic analyses Liem (1980) revealed that the "monophasic" pattern of air breathing as indicated by Peters (1978) to be explicitly "triphasic" and the "diphasic" is actually "quadriphasic." Therefore, with the help of modern experimental tools and kinematic analyses Liem has accurately traced out the "monophasic", "diphasic", "triphasic" and "quadriphasic" air ventilations in Anabantoidei.

Based on kinematic and electromyographic profiles of *Helostoma* and *Anabas*, Liem (1980) interpreted the air ventilation mechanism as follows: as the fish rises to the surface it compresses its buccal cavity by the contraction of adductor arcus palatine, geniohyoideus and adductor mandibulae to minimize the volume of the buccopharyngeal cavity in order to expel as much water as possible from the buccal cavity as preparatory phase for air intake. Then the air is sucked in by the sudden expansion of the buccal cavity conducted by the contraction of the levator arcus palatini which then move the suspensorium laterally. Sternohyoideus muscle engages itself to lower the floor of the buccal cavity.

Therefore, cinematography provides the precise movements of the bony elements and the synchronous electromyography reveals the related muscles' potential which are involved in carrying out the air-ventilation in air-breathing fishes.

Not only the mechanisms, but the behavioural patterns of air-breathers can be studied elusively by high speed cinematography. Behavioural aspects will unfold the yet unknown nature of these fish at the time of their aerial respiration. A synchronous aerial respiration by the walking catfish in Florida has been studied by Loftus (1979). According to him, in turbid water the synchronous air-breathing amongst several catfishes is done by detecting the vibrations of respiring fish with their lateral line system. Whereas, Kramer and Graham (1976) stated that in low turbid water air-breathing fishes seem to maintain synchrony by visual means. They have also observed catfish *Hoplosternum* to breathe synchronously in very murky water, probably without any visual aid.

## 11. Scientific significance and application of functional morphological work

A fundamental knowledge of the morphology and physiology of the air-breathing organs of these fishes is indispensable in understanding the alterations caused by pollutants.

Development of normal fishes will reveal the sequential changes which incur in the

progressive stages of ontogeny of their air-breathing organs. The developmental studies will indeed unravel the interesting adaptations and many convergences of these fishes to the ancestral vertebrates. Development study will also give us the indepth understanding of the structural adaptations and will also reveal how they have helped the vertebrates to achieve their transition from water to land. For example such studies might include the morphometrics of the developing gills and the accessory respiratory organs by employing the modern stereological methods (Weibel 1973) and structural patterns of the bimodal gas exchange system.

The developmental studies of the pollutant-exposed fishes will give us the knowledge of the varied alterations in the structures and the functions in their different stages and pinpoint the stages which are most susceptible to the pollutants. The developmental study includes the fertilized eggs, larvae, juvenile and adult specimens.

Further, an indepth knowledge of their physiology, micro and macroecology, the development of the respiratory organs and general growth would be especially important in relation to any work envisaged in the culture of these fishes. Many of these species form an important food source in India. A knowledge of their mode of life and environmental conditions can improve methods for their successful culture and help to protect them from dangers of environmental pollution.

## 12. Conclusions

Being a warm water tropical fish the air-breathers are not only widespread in India, but the Indian biologists have made significant contributions to the understanding of their functional morphology. Gross morphology and the behavioural aspects of the air-breathing fishes have been researched more intensively than the applied aspects of physiology and effects of pollutants on morphology. With the use of more chemicals in our food and growing environmental pollution, the air-breathing fishes are likely to undergo varied changes in their functional morphology. Studies in this aspect, therefore, will have new openings of far reaching significance.

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# Bionomics of hill-stream cyprinids. I. Food, parasites and length-weight relationship of *Labeo dyocheilus* (McClell)*

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**Abstract.** Food habits, parasites and length-weight relationship in the hill-stream fish *Labeo dyocheilus* were examined. The frequency distribution of the pseudophyllidean cestode, *Bothriocephalus teleostei* in this fish has been reported to be log-normal.

**Keywords.** Parasites; riverine ecosystems; cyprinids; parasitization index; *Labeo dyocheilus*.

## 1. Introduction

Studies on the biological and statistical significance of length-weight relationship among ichthyofauna of Himalayan riverine ecosystems are available on only three fish species: *Barilius bola* (Ham) (Chauhan and Malhotra 1981). *Labeo dero* (Ham) (Malhotra and Chauhan 1984) and *Tor tor* (Ham) (Malhotra 1982). The present investigation is aimed at analysing the biology and length-weight relationship in *L. dyocheilus* (McClell) in high altitude ecosystems.

## 2. Materials and methods

Methods of collection of fish samples (N = 152) and their analyses were published earlier (Malhotra 1982). *L. dyocheilus* (9·5–49 cm length range) collected from River Nayar (650 meters ASL) were used in the present investigation. The length-weight relationship was estimated by the formula, $W = aL^n$ where $W$ is the weight, $L$ the body length, and $a$ and $n$ are constants. Logarithmic transformation of the equation may be written as $\log W = \log a + n \log L$ where, $\log W$ is the dependent variable ($y$), $\log L$ the independent variable ($x$), $n$ the regression coefficient or slope ($b$), and $\log a$ the $y$-intercept. Analysis of covariance and student's t-test were applied and the coefficient of determination (Croxton 1953), the values of least squares regression slopes and the proportion of correlated variance (Zeller and Carmines 1978) were computed.

## 3. Results

### 3.1 *Food*

Qualitative and quantitative (percentage by weight) analysis of gut contents including food and parasites showed 0·496 % worms, and 99·504 % *Cladophora* sp., *Spirogyra* sp., *Sphaerocystis* sp., *Volvox* colonies and plant debris.

### 3.2 *Parasites*

The frequency of parasites (mean worm burden, 2–3(2) in the alimentary canal of the examined fishes was 0·49 % cestodes (female, 0·248 %; male, 0·248 %). *Bothriocephalus teleostei* Malhotra, 1984a was the only cestode recorded. Interestingly, no trematode, nematode or acanthocephalan infection was found in *L. dyocheilus*. The parasitization index and prominence values of the cestode were 0·004–0·009 and 1·277–1·592 respectively. The optimum water temperature and humidity during infection period were 13·75 ± 0·25/0·18°C (13·5–14°C) and 85 ± 5/3·54 % (80–90 %) respectively.

### 3.3 *Length-weight relationship*

Various body measurements in the ratio of total and standard length of fish including body weight are given in table 1.

### 3.4 *Estimated regressions*

An initial assessment of the fishes of the length range 9·5–49 cm suggested that the same equation would not fit the data for the entire length range and that a break occurred around < 17 cm and > 17.1 cm groups. Separate parabolic equations and linear regressions were therefore computed for both the sexes and length classes as mentioned in table 2. The significance of the differences between the regression coefficients (*b*) was

**Table 1.** Mean values of body weight and ratios of total *vis-a-vis* standard length of *Labeo dyocheilus*.

| | | Mean ± S. E. | | | |
|---|---|---|---|---|---|
| Category | Sample size | Total length (cm) | Standard length (cm) | TL/SL ratio | Body weight (g) |
| Female | 86 | 22·6116 ± 0·9789 | 19·6209 ± 0·7323 | 1·1524 ± 0·0123 | 178·9419 ± 45·5443 |
| Male | 66 | 20·5955 ± 0·9744 | 17·2773 ± 0·7458 | 1·1921 ± 0·0039 | 132·2879 ± 29·0144 |
| < 17 cm | 76 | 14·956 ± 0·2062 | 14·4605 ± 0·2718 | 1·0343 ± 0·0110 | 49·84 ± 3·6949 |
| > 17·1 cm | 76 | 32·391 ± 0·6251 | 22·7461 ± 0·7849 | 1·4240 ± 0·0379 | 268·2368 ± 54·5471 |
| Pooled | 152 | 21·7362 ± 0·7016 | 18·6033 ± 0·5342 | 1·1684 ± 0·0631 | 159·0395 ± 28·7343 |

**Table 2.** Regression equations describing length-weight relationship in *Labeo dyocheilus*.

| Category | Logarithmic regression equations (log W + log L) | Parabolic equations (W =) |
|---|---|---|
| Female | = $\bar{2}$·946 + 2·2103 | $0{\cdot}0011324L^{2.2103}$ |
| Male | = $\bar{2}$·5998 + 2·1396 | $0{\cdot}00251304L^{2.1396}$ |
| Pooled | = $\bar{2}$·8199 + 2·0871 | $0{\cdot}00151391L^{2.0871}$ |
| < 17 cm | = $\bar{1}$·7670 + 1·7559 | $0{\cdot}00171002L^{1.7559}$ |
| > 17·1 cm | = $\bar{3}$·0889 + 2·2850 | $0{\cdot}000814892L^{2.2850}$ |

tested by the method of analysis of covariance. The relevant data has been presented in table 3. The test for heterogeneity of regressions revealed that the differences between the regression coefficients were significant at 1 % level (sum of squares 0·0239149, mean square 0·00797163, d*f* 3, $F = 8{\cdot}337$, $F_{1\%} = 3{\cdot}91$). The test of heterogeneity was again performed for the sexes (within and with each of the two length classes) and length classes (within). It was observed that the differences between the regression coefficients between male and female ($F = 0{\cdot}002$, $F_{5\%} = 3{\cdot}91$ d*f* 1; 151) and between < 17 cm and > 17·1 cm ($F = 0{\cdot}139$, $F_{5\%} = 3{\cdot}91$, d*f* = 1; 151) were not significant while those between sexes and < 17 cm ($F = 141{\cdot}521$, $F_{1\%} = 4{\cdot}71$, d*f* 2; 226) and between sexes and > 17.1 cm ($F = 10{\cdot}693$, $F_{1\%} = 4{\cdot}71$, d*f* 2; 226) were significant at 1 % level.

The application of *t*-test revealed that the departures of regression coefficients from the isometric growth value of 3 were significant at 1 % level in < 17 cm ($b - 3$, $-1{\cdot}1609$; *t*, $-19{\cdot}720$; d*f*, 74; $t_{1\%} = 2{\cdot}66$), > 17·1 cm ($b - 3$, $-0{\cdot}8842$; *t*, $-7{\cdot}392$; d*f*, 74; $t_{1\%} = 2{\cdot}66$) and sexes ($b - 3$, $-0{\cdot}9129$; *t*, $-31{\cdot}325$; d*f*, 150; $t_{1\%} = 2{\cdot}58$).

A comparison of the regression lines of the length-weight relationship of *L. dyocheilus* has been presented in table 4. According to the standardized least squares linear regression line, for each standard unit of length, the fish gained, 0·8703–0·8722; 0·7888–0·7918; 0·8192–0·8291; 0·5509–0·5584; and 0·88–0·9074 of a standard unit of weight for females, males and pooled, and < 17 cm and > 17.1 cm classes of fish, respectively. In both the sexes and weight classes *r* is significant.

**Table 3.** Analysis of covariance between the regression coefficients (*b*) for *Labeo dyocheilus*.

| | Female | Male | Pooled | < 17 cm | > 17.1 cm |
|---|---|---|---|---|---|
| *N* | 86 | 66 | 152 | 76 | 76 |
| $\Sigma(X-\bar{X})^2$ | 3·5984 | 3·3848 | 3·8244 | 2·6300 | 3·5445 |
| $\Sigma(Y-\bar{Y})^2$ | 7·1849 | 6·5631 | 7·2805 | 4·8910 | 7·2417 |
| $\Sigma(X-\bar{X})(Y-\bar{Y})$ | 5·3318 | 4·8717 | 5·4684 | 3·5046 | 5·3443 |
| $b\Sigma(X-\bar{X})(Y-\bar{Y})$ | 11·7849 | 10·4235 | 7·9819 | 6·1537 | 12·2117 |
| $\rho^2$ | 0·7590 | 0·6245 | 0·6792 | 0·3077 | 0·7986 |
| $r^2$ | 0·7572 | 0·6233 | 0·6874 | 0·3035 | 0·7979 |

*N* = numer of observations; $\rho^2$ = proportion of correlated variance; $r^2$ = coefficient of determination.

**Table 4.** Comparison of the regression lines of the length-weight relationship of *Labeo dyocheilus*.

| Category | Sample size | Variance | | Covariance | Standardized least squares regression slope predicting | | P |
|---|---|---|---|---|---|---|---|
| | | Length | Weight | | *X* from *Y* | *Y* from *X* | |
| Female | 86 | 1·6639 | 5·2504 | 3·3973 | 0·8703 | 0·8722 | P < 0·001 |
| Male | 66 | 1·5652 | 4·7435 | 3·0522 | 0·7888 | 0·7918 | P < 0·001 |
| Pooled | 152 | 1·6426 | 5·0987 | 3·2866 | 0·8192 | 0·8291 | P < 0·001 |
| < 17 cm | 76 | 0·7492 | 3·0102 | 1·6238 | 0·5509 | 0·5584 | P < 0·001 |
| > 17·1 cm | 76 | 1·6637 | 5·3609 | 3·4634 | 0·9074 | 0·8800 | P < 0·001 |

## 4. Discussion

### 4.1 *Food and parasites*

The analysis of food reveals that *L. dyocheilus* maintains a predominantly herbivorous feeding habit. The curves for frequency distribution of *B. teleostei* during infection period, fish age, size and weight classes have been reported to be a simple logarithmic function and revealed the familiar gaussian hump when plotted in octaves (Malhotra 1984b). The implications of this tendency have already been discussed by the author in the earlier study (Malhotra 1984b).

### 4.2 *Length-weight relationship*

To conform to the results based on analysis of samples of *L. dero* collected from natural habitats in the same locality (Malhotra 1984b) no major difference was noted in the ratio values of total *vis-a-vis* standard length of *L. dyocheilus*. The agreement between fish length and weight was good ($P < 0{\cdot}001$) in female, male and pooled fishes and by $< 17$ cm and $> 17{\cdot}1$ cm classes of fish (table 4). Based on coefficient of determination more than 75% of the variation in weight in females, 62% in males, 68% in pooled, 30% in $< 17$ cm length class and 79% in $> 17{\cdot}1$ cm length class was attributable to the variation in length of *L. dyocheilus*. Similarly the proportion of correlated variance ($\rho^2$) suggests that 75·9071% variance in length in female fishes, 62·4532% in males, 67·9212% in pooled, 30·7659% in fishes of $< 17$ cm length class, and 79·8611% in fishes of $> 17{\cdot}1$ cm length class was associated with weight.

The length-weight relationship for female, male, pooled, $< 17$ cm and $> 17{\cdot}1$ cm length classes of *L. dyocheilus* is defined and illustrated in this investigation. Similar to the earlier observations in *T. tor* (Malhotra 1982) and *L. dero* (Malhotra 1984b) in the Himalayan riverine ecosystem the larger fishes ($> 17{\cdot}1$ cm) showed higher value of regression coefficient ($b = 2{\cdot}2850$) than the smaller ones i.e. $< 17$ cm ($b = 1{\cdot}7559$). This conclusion illustrates a relatively rapid change in body outline of the fishes of $> 17{\cdot}1$ cm length class as they increase in length compared to those of the fishes of smaller length class ($< 17$ cm).

As $a$ depends upon the obesity of the fish (LeCren 1951), by comparing log $a$ values it becomes obvious that there is no significant difference ($F_{1;151} = 0{\cdot}002$) in the general fatness of the two sexes in the present study similar to the findings of Malhotra (1982). The regression for the pooled lot of fishes was calculated. The value of $n$ (2·0871) indicated that the growth rate is lesser than the cube length. Similar deviations have been discussed earlier by Malhotra (1984b) on another species of the genus *i.e. L. dero* from the same locality. In the present case too the departures of regression coefficients from 3 were found highly significant (1% level) for sexes and both length classes. As emphasized earlier (Malhotra 1984b) the suitability of the exponential formula $W = aL^n$ used in the present analysis to the cubic formula $W = CL^3$ ($C$, constant) has been justified by several authors (Sekharan 1968) in similar studies. Beverton and Holt (1957) discussed the merit of both allometric and cubic formula and reported that the former works much better as $a$ and $n$ of the allometric formula vary within a wide range for very similar data and are very sensitive to even quite unimportant variations in $n$. Hence the high value of coefficient of correlation indicates that the allometric relationship of length and weight is suitable for the fish.

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# pH and dissolution of crystalline style in some bivalve molluscs of Porto Novo coastal waters

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**Abstract.** A comparative study of the pH of crystalline style, digestive diverticula and stomach fluid in six bivalve species, *Anadara rhombea*, *Crassostrea madrasensis*, *Meretrix meretrix*, *M. casta*, *Katelysia opima* and *Donax cuneatus* revealed that the digestive diverticula are more acidic while crystalline style is either slightly acidic or nearly neutral. The significance of this finding is discussed. *In vitro* dissolution of crystalline style of all the six species in buffer solutions of varying pH from 3·6 to 9·0 was observed and the study indicated that the optimum pH for style dissolution is around 8·0 in all species excepting *Donax cuneatus* where some dissolution was recorded only at pH 9·0. Based on the physical changes observed in the style, a mechanism of style dissolution has been put forward.

**Keywords.** Bivalve molluscs; crystalline style; style dissolution; *Anadara*; *Crassostrea*; *Meretrix*; *Katelysia*; *Donax*.

## 1. Introduction

The crystalline style is a long, transparent rod of glycoprotein, lodged in the midgut of most bivalves and some gastropods aiding in the processes of feeding and digestion through its dissolution. Dissolution of the crystalline style had been shown to be pH dependent by Yonge (1925, 1926), Venugopalan (1955), Mathers (1974, 1976) Mathers *et al* (1979) and Alyakrinskaya (1977, 1979). Yonge (1925, 1926) proposed a theory that the style was the most acidic part of the gut in bivalves and that the dissolution of the style in the stomach was effected by the higher pH of the stomach content. On the other hand, Purchon (1971) and Morton (1973) found that the style was not the most acidic part of the gut and that the style pH was near neutral or slightly acidic. Further, the pH in different regions of the gut was reported to be variable (Mathers 1974; Owen 1974). Therefore, the present study was undertaken to clarify the actual situation in the bivalve species of Indian coastal waters.

## 2. Materials and methods

The bivalve species, *Anadara rhombea* (Born), *Crassostrea madrasensis* (Preston), *Meretrix meretrix* (Linnaeus), *M. casta* (Chemitz) and *Katelysia opima* (Gmelin) were collected from the mud flats of Vellar estuary joining Porto Novo sea shore (11°29′ N lat. and 79°46′ E long.) and *D. cuneatus* from the marine intertidal zone. The pH of aqueous solutions of the crystalline style, the digestive diverticula and the stomach

content was measured in a digital pH meter (Toshniwal Cat. No. CL 46) to the nearest 0·01 pH following the method of Mathers (1974). *In vitro* dissolution of the style was observed in buffer solutions with the pH range from 3·6 to 9·0 and in distilled water, sea water and estuarine water at room temperature (34° C). The time required for the complete dissolution was recorded following the method of Mathers (1974). Changes occurring in the style during dissolution was observed under a microscope. Three estimations were made at each pH and the mean dissolution time ($t$) and the rate of dissolution ($1/t$) were calculated.

## 3. Results

Table 1 presents the mean pH of the crystalline style and the digestive diverticula in six species and that of stomach fluid on four species. The mean pH was either slightly acidic or neutral. It is interesting to note that pH of any of these three regions of the gut was not stable but variable within a limited range (table 1) and the digestive diverticula was the most acidic part in all the species tested. The relationship between the pH of the medium and the rate of style dissolution ($1/t$) was studied on all the six species (figure 1). In general, at low pH (3·6 to 4·6) the styles were stable for a prolonged period with low rate of dissolution. The rate of style dissolution was rather slow at pH range of 5 to 7. Faster dissolution was observed at pH 8·0 in all species tested excepting *D. cuneatus*. At this optimum pH, a high rate of dissolution was recorded in *C. madrasensis* (3 min) and in *A. rhombea* (9 min). The pattern of style dissolution in *D. cuneatus* was quite distinct from that of other species tested in that the style of this species was highly resistant to dissolution in the pH range of 3·6 to 8·0. At pH 9·0 the style dissolved at a slow rate (175 min) which was close to the dissolution time in sea water (215 min).

The data on the style dissolution in distilled water (pH 7·0), estuarine water (pH 8·2) and sea water (pH 8·5) indicated that the style dissolved faster in natural media than in distilled water (table 2).

Observation on the mode of style of dissolution revealed two distinct patterns. In *D. cuneatus* the style underwent a process of lengthening before dissolution. In all other species dissolution was followed by the shortening of the style. In both cases the style had a central granular matrix around which concentric crystalline layers were found. During dissolution, the granular matrix was observed to be continuously flowing out largely through the anterior end and to a small extent through the posterior end.

## 4. Discussion

It is evident from the present study that the pH of the crystalline style of bivalve is either slightly acidic or neutral. Morton (1969, 1970, 1971) recorded that the pH of styles of *Dreissena* (pH 7·0) and *Ostrea* (pH 6·8) is approximately neutral whereas that of *Cardium* (pH 6·4) is slightly acidic. Similar results were also reported by Langton and Gabbott (1974) in *Ostrea*, Langton (1977) in *Mytilus* and Mathers (1974, 1976) in *Ostrea*, *Crassostrea* and *Pecten*. In a given individual, the digestive diverticula were always more acidic than its crystalline style. The pH of the style, digestive diverticula and stomach content were not stable but fluctuated within a range. Fluctuating pH in these three regions of the gut was also recorded by Mathers (1974, 1976) and Morton

**Table 1.** pH of style, digestive diverticula (DD) and stomach content of bivalves

| Species | Style pH | | DD pH | | Stomach content pH | |
|---|---|---|---|---|---|---|
| | Mean[a] | Range | Mean[a] | Range | Mean[b] | Range |
| *A. rhombea* | 6·39 | 5·99–6·98 | 5·83 | 5·63–6·06 | 6·2 | 5·75–6·65 |
| *C. madrasensis* | 7·17 | 6·88–7·52 | 6·43 | 6·31–6·79 | 6·4 | 6·41–7·10 |
| *M. meretrix* | 7·11 | 6·11–7·46 | 6·42 | 6·58–6·66 | 6·5 | 6·50–7·00 |
| *M. casta* | 7·37 | 7·08–7·57 | 6·56 | 6·76–7·32 | — | — |
| *K. opima* | 6·41 | 6·05–6·59 | 5·80 | 5·70–5·86 | 6·0 | 5·90–6·90 |
| *D. cuneatus* | 6·79 | 6·45–7·03 | 6·31 | 6·09–6·59 | — | — |

[a]Average of 10 estimates; [b]Average of 5 estimates;—not determined.

**Table 2.** Dissolution of crystalline style of bivalve species in natural media and distilled water

| Species | Mean dissolution time (Minutes) | | |
|---|---|---|---|
| | Estuarine water (pH 8·2) | Sea water (pH 8·5) | Distilled water (pH 7·0) |
| *Anadara rhombea* | 53 | — | 174 |
| *Crassostrea madrasensis* | 12 | — | 16 |
| *Meretrix meretrix* | 65 | — | 180 |
| *Meretrix casta* | 23 | — | 203 |
| *Katelysia opima* | 44 | — | 174 |
| *Donax cuneatus* | — | 215 | 2580 |

(1969, 1970). The present work confirms the current concept that in bivalves, the most acidic part of the gut is the digestive diverticula and not the crystalline style; the pH of the crystalline style, digestive diverticula and stomach content fluctuates considerably and that the stomach pH is not stable and not buffered by style dissolution (Purchon 1971; Morton 1973; Owen 1974).

Bivalve crystalline style had long been known to dissolve at a faster rate in alkaline medium and stable in acidic medium (Yonge 1925, 1926; Venugopalan 1955; Mathers 1974, 1976; Mathers *et al* 1979; Kristensen 1972; Alyakrinskaya 1977, 1979). On the basis of the rate of dissolution, the crystalline styles of the bivalve species examined in the present work could be classed into two types: (i) rapidly dissolving styles and (ii) slowly dissolving styles. The rapidly dissolving styles were found to be housed in the style sac conjoined with midgut as observed in *A. rhombea*, *C. madrasensis*, *K. opima*, *M. meretrix* and *M. casta*. These estuarine bivalves had a high rate of dissolution at pH 8·0 (figure 1) and the complete dissolution time ranged between 3 minutes in *C. madrasensis* and 25 minutes in *M. meretrix*. On the other hand, *D. cuneatus* possessed a slowly dissolving style and its style sac is entirely separated from the midgut. Even at the pH of 9·0 its dissolution rate is very slow (22 hr). Probably the rate of dissolution depends on other factors as well such as the organic content of the style (Alyakrinskaya 1979).

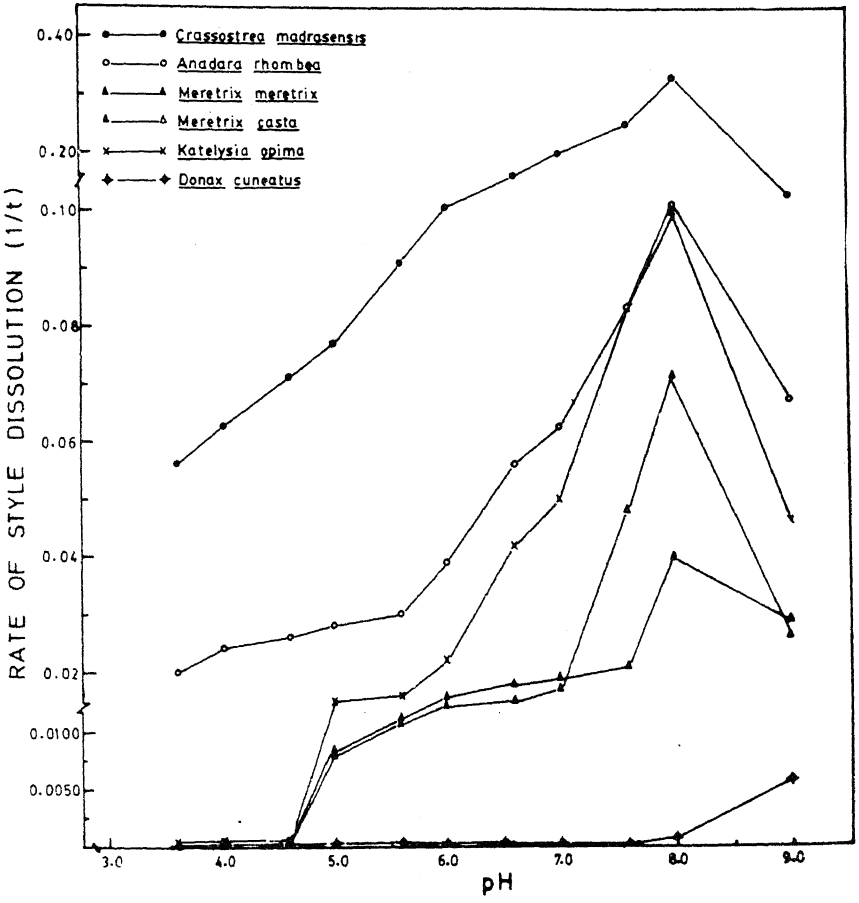


**Figure 1.** Relationship between the rate of style dissolution and pH.

The optimum pH for dissolution of the style coincided with the pH of the medium (table 2). It would seem that during feeding the entry of water into the stomach would raise the pH facilitating an increase in the rate of style dissolution and the consequent release of digestive enzymes. The inference is in conformity with the observations of Mathers (1974, 1976) in *Ostrea* and *Pecten.*

According to Yonge (1925, 1926, 1949) the dissolution of the style was thought to be restricted to its anterior end projecting into stomach lumen. But *in vitro* observations on the mode of style dissolution did not support this view. Dissolution of bivalve style is a physical phenomenon involving the entire style body. Kristensen (1972) reported that the outer crystalline layers of the style are relatively hard and more resistant to dissolution while the central core is hygroscopic. It is suggested that the dissolution of the style is brought about by the liquefaction of the crystalline layers into granular core through uptake of water. The actual transport of the dissolved materials was observed in the present study as an anteriorly directed streaming movement of the central granular matrix.

**Acknowledgement**

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# Studies on the induced spawning and larval rearing of a freshwater catfish, *Mystus punctatus* (Jerdon)

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**Abstract.** The freshwater catfish *Mystus punctatus* was successfully bred in the laboratory by injecting the pituitary extract of the marine catfish *Tachysurus maculatus*. The number of eggs released by a single female fish was 9050 ± 700, the relative fecundity was 43·1 and the fertilization success was 85 ± 2·8 per cent. The average diameter of the unfertilized eggs ranged from 1·27 to 1·35 mm and the fertilized eggs ranged from 1·45 to 1·50 mm. The eggs hatched within 18 to 24 hr at a water temperature of 28·5 ± 1·8°C, and the percentage of hatching was 78%. The larvae metamorphosed into juveniles within fifteen days of their hatching. A description of the egg and larval development to metamorphosis is given.

**Keywords.** *Mystus punctatus*; induced breeding; pituitary extract; hatching; metamorphosis; larval development.

## 1. Introduction

The freshwater catfish *Mystus punctatus* is one of the good table fishes in the inland regions of India and hence has good culture potential.

The effect of pituitary gland extract from the marine catfish *Tachysurus maculatus* to induce the breeding of freshwater catfish *Clarias batrachus* was studied by Devaraj *et al* (1972). In the present study the freshwater catfish *Mystus punctatus* was successfully induced to spawn in the laboratory by injecting marine catfish pituitary gland extract as adopted by Devaraj *et al* (1972). The larvae that hatched out from the fertilized eggs were reared in the laboratory and their development was studied.

## 2. Material and methods

The matured adult fish of both the sexes were collected from natural waters of Kalladaippu tank near Tuticorin of Tirunelveli district of Tamil Nadu (South India) during January to February 1981. The fish thus collected were maintained in fibreglass tanks of size 200 × 50 × 50 cm, and were fed with artificial pelleted feeds (Tilapia flesh, groundnut oil cake and rice bran at a ratio of 2 : 1 : 1) at a rate of 5% body weight of fish. The water temperature maintained was 28·5 ± 1·8°C. The pituitary glands were collected from the marine catfish *Tachysurus maculatus*. The breeding experiments were conducted in three fibreglass tanks of 200 × 50 × 50 cm size, in which a water depth of 25 cm was maintained.

The females were injected with the extract containing 6 mg of dry pituitary/kg of body weight in two split doses with an interval of 3 hr while the males were given the extract of 3 mg/kg of body weight in single dose at the time of second injection to the

female. Six males (68 to 73 g) and 3 females (197 to 223 g) were used for the experiment. Very clear water was maintained in the tank. The water temperature was 28·5 ± 1·8°C. The hatched larvae were reared in nursery tanks of size 5 × 5 m with a water depth of 75 cm. In these nursery tanks the water temperature was 28·5 ± 1·8°C. The larval development was studied and discussed (Mansueti and Hardy 1967).

## 3. Results and discussion

After 15 minutes of the second doze of the pituitary extract injection, the experimental fish started the courtship behaviour. The males started chasing the females and twisted around the body of the females with the tail. The females released the eggs eight hours after administering the pituitary injection.

The eggs were siphoned from the tank after removing the spent fish and placed in clear water in another tank of the same size, and the temperature of the water was 28·5 ± 1·8°C. The fertilization of the eggs was estimated as 85 %. The average number of eggs released was 9050 and the diameter of the fertilized egg was 1·45 to 1·50 mm.

The eggs hatched out within 18 to 24 hr after fertilization. The percentage of hatching was 78 %. The standard deviation ($\bar{x} \pm$ SD) for number of fish, number of eggs collected, relative fecundity, percentage of fertilization, egg diameter and the number of degrees-days for spawning and incubation are given in table 1. The hatchlings had a large yolk sac and the larvae were found attached to the sides and bottom of the tank. The yolk was completely absorbed on the third day of hatching and subsequently the larvae were fed with plankton obtained from the ponds. The larvae started feeding from the fourth day onwards. They were then transferred to a manured cement nursery tank of size 5 × 5 m with a water depth of 75 cm. The postlarvae metamorphosed into juveniles within 15 days from hatching.

A description of the eggs to the fully metamorphosed juvenile is given below in order to facilitate its identification at any stage of its larval development as it was not described earlier.

### 3.1 *Unfertilized ova*

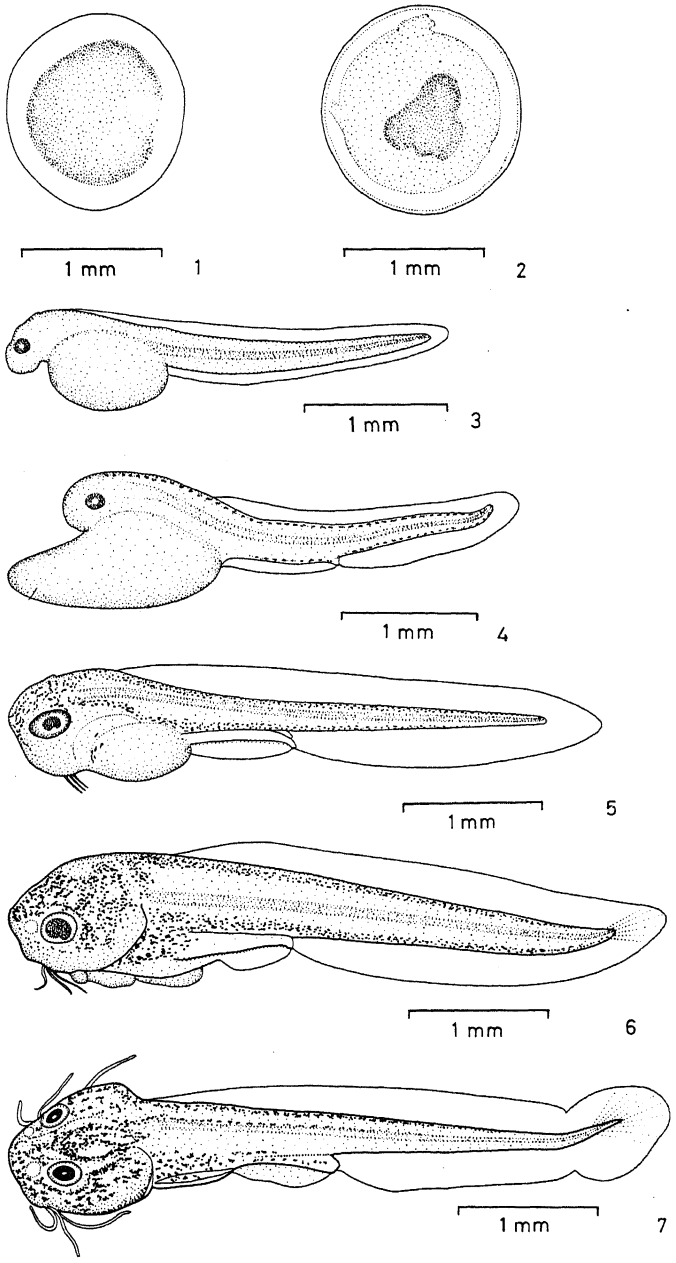
The ova (figure 1) are light yellowish in colour with a denser area in the centre. Their diameter ranges from 1·27 to 1·35 mm. The outer surface is smooth and concretions are visible.

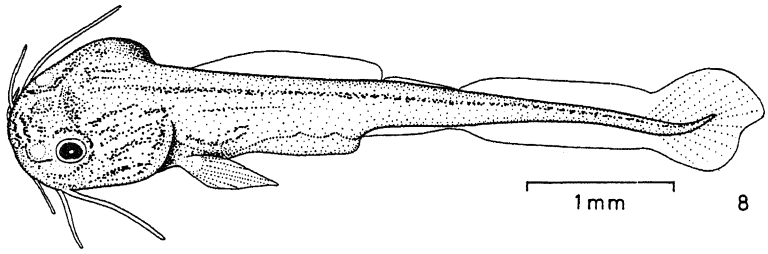
**Table 1.** Standard deviation (X ± SD), of the fecundity description

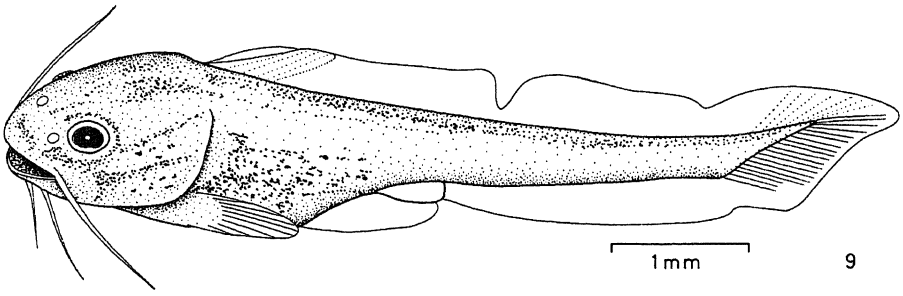
| | Experiment | Control |
|---|---|---|
| Number of fish | ♂ – 3; ♀ – 6 | ♂ – 2; ♀ – 1 |
| Fish size | ♂ 69 ± 4g; ♀ 210 ± 13g | ♂ 69g, 71g; ♀ 210g |
| No. of eggs released | 9057 ± 700 | — |
| Relative fecundity | 43·1 | — |
| Percentage of fertilization | 85 ± 2·8 | — |
| Egg diameter — Unfertilized eggs | 1·27–1·35 mm | — |
| Egg diameter — Fertilized eggs | 1·45–1·50 mm | — |
| Number of degrees for spawning and incubation | 8 ± 2 | — |
| Temperature | 28·5 ± 1·8°C | 28·5 ± 1·8°C |

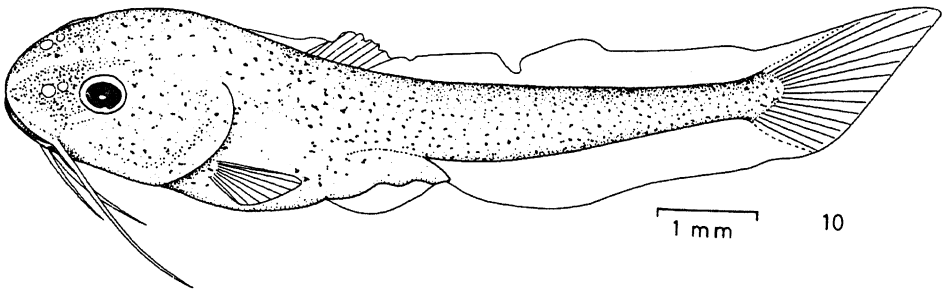
### 3.2 *Fertilized egg*

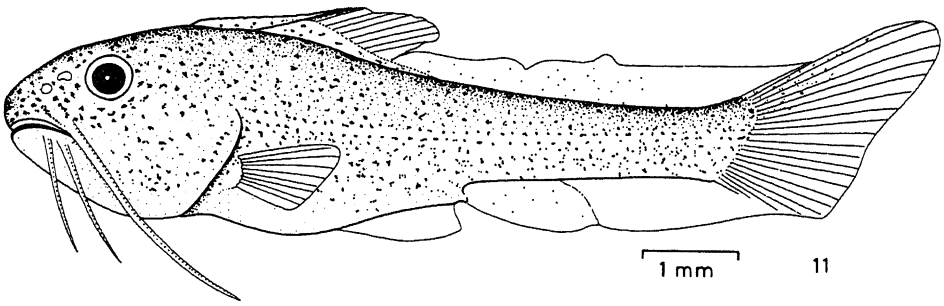
The fertilized eggs (figure 2) are also light yellowish in colour and the diameter ranges from 1·45 to 1·5 mm. The outer surface of the egg is somewhat thickened when compared to that of the unfertilized ova. The developing embryo is clearly visible under a microscope.



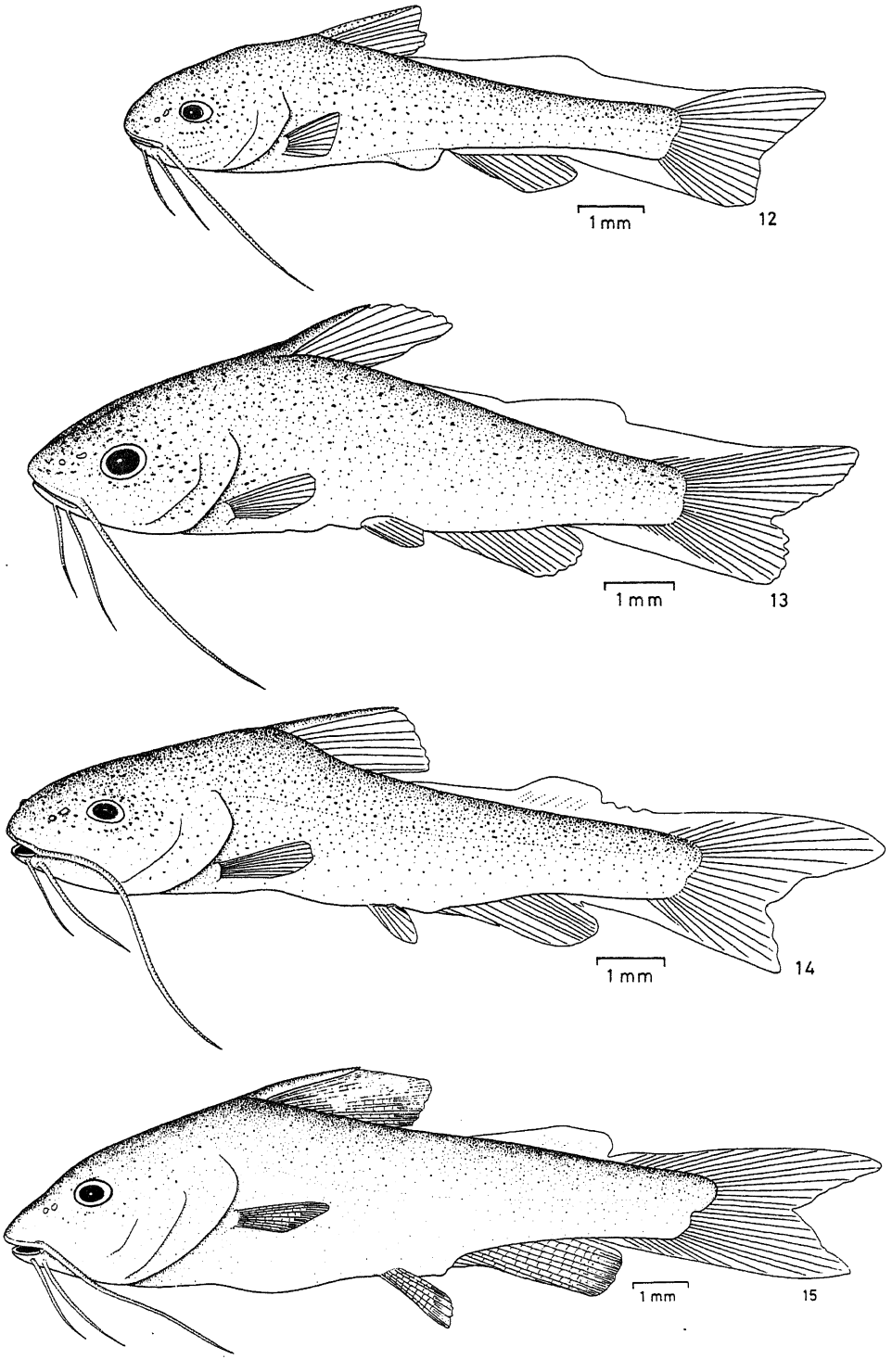


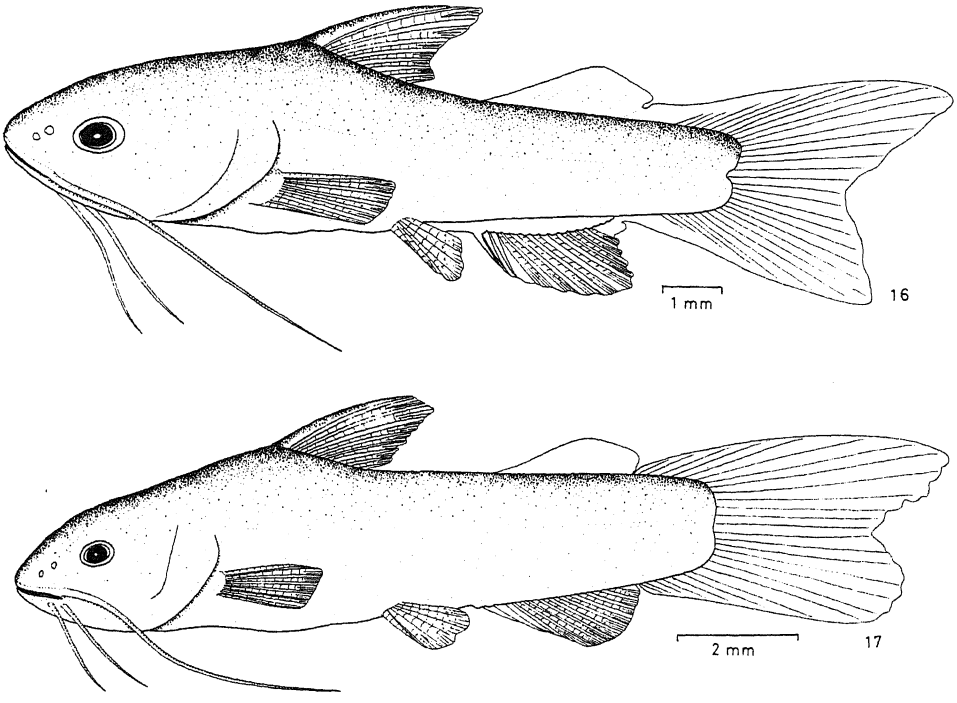




**Figures 8–11.**




**Figures 12–15.**




**Figures 16–17.**

### 3.3 *Just hatched out larva*

The just hatched out larva (figure 3) is laden with yolk sac. The larvae measure 3·1 to 3·3 mm in total length and the yolk sac 0·8 mm on an average. The mouth has not yet formed, while the optical sac has already developed.

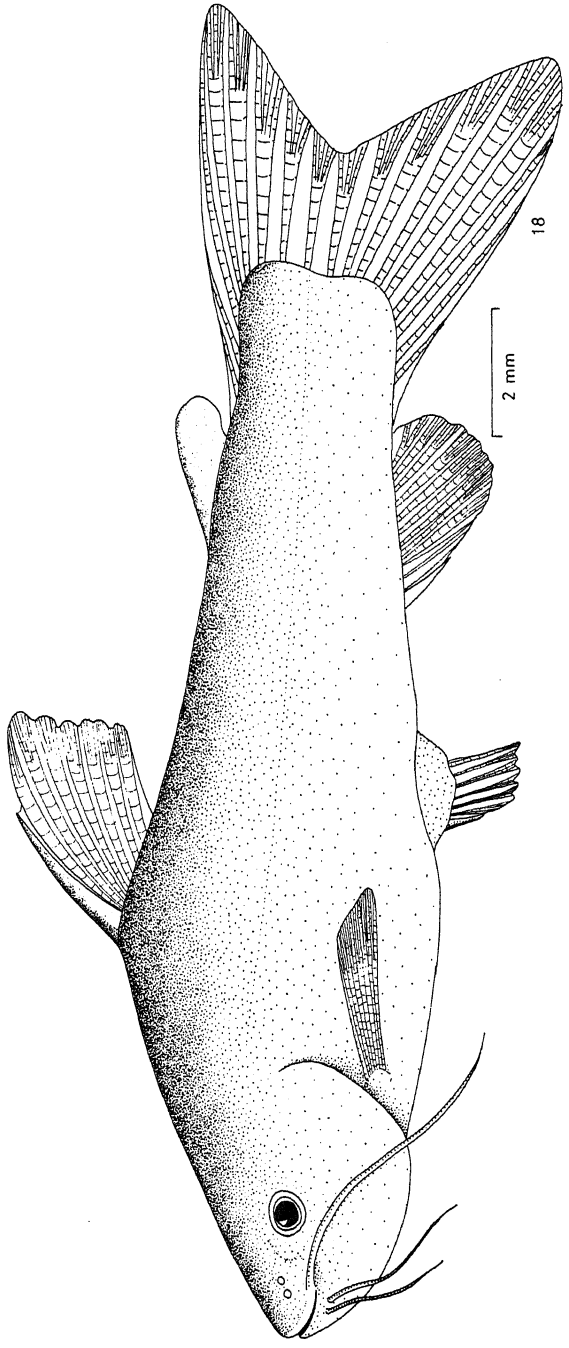
The larvae settle at the bottom and sides of the tank and their movements are restricted on account of the large yolk sac. The body is devoid of any chromatophores.

### 3.4 *One-day larva*

The one-day old larvae (figure 4) measure 3·4 to 3·6 mm in total length and the yolk still persists measuring 1·6 mm on an average. As in the just hatched out larva, the mouth has not yet developed. The anal opening has developed by the constriction of the ventral fin fold in the middle. The dorsal and ventral margins of the body have a row of simple chromatophores and the larvae do not swim except when disturbed.

### 3.5 *Two-day old larva*

The larvae (figure 5) measure 4·3 to 4·6 mm in total length. The yolk is almost absorbed. Three pairs of barbels have developed (one pair maxillary and two pairs mandibular).




**Figures 1–18.** Fertilized egg and larvae of *Mystus punctatus*. **1.** Unfertilized ova. **2.** Fertilized egg. **3.** Just hatchedout larva. **4.** One-day larva. **5.** Two-day old larva. **6.** Three-day old larva. **7.** Four-day old larva. **8.** Five-day old larva. **9.** Six-day old larva. **10.** Seven-day old larva. **11.** Eight-day old larva. **12.** Nine-day old larva. **13.** Ten-day old larva. **14.** Eleven-day old larva. **15.** Twelve-day old larva. **16.** Thirteen-day old larva. **17.** Fourteen-day old larva. **18.** Juvenile.

The mouth has just formed. The chromatophores are distributed over the head and along the dorsal and ventral margins of the body. Chromatophores are also seen scattered over the opercular region. The larvae are able to swim freely.

### 3.6 *Three-day old larva*

They (figure 6) measure 4·6 to 5·0 mm in total length. The nostril is formed and the mouth developed. The operculum is seen as a slit. The chromatophores are found scattered over the head, below the opercular slit and along the dorsal and ventral margins of the body. The caudal rays have begun to develop. The larvae are active and freely swimming at this stage.

### 3.7 *Four-day old larva*

They (figure 7) measure 4·7 to 5·1 mm in total length. The eyes and nostrils are well formed. The size of maxillary barbels at this stage is more than that of the mandibular barbels. The chromatophores are scattered over the head, dorsal and ventral margins of the body, except along the posterior half of the ventral margin. The caudal fin is slightly constricted from the dorsal and ventral fin folds.

### 3.8 *Five-day old larva*

They (figure 8) measure 5·2 to 5·4 mm in total length. The pectoral fins are developed. The dorsal and anal fins are separated from the fin folds. The chromatophores are scattered over the head, on the dorsal margin of body and above the pectoral fin. The body is dark compared to the four day old larva.

### 3.9 *Six-day old larva*

They (figure 9) measure 6·4 to 6·7 mm in total length. The rays of the pectoral fins and the lower lobe of the caudal fin are calcified. The dorsal fin rays have developed and so also the ventral and caudal fin lobes. The chromatophores are scattered over the head, around the orbit, along the dorsal margin of body and over the belly region.

### 3.10 *Seven-day old larva*

They (figure 10) measure 9·8 to 10·1 mm in total length. Nostrils have formed. The rays of dorsal and upper lobe of caudal fin are calcified. The chromatophores are scattered throughout the body. A spine is developing in the dorsal fin.

### 3.11 *Eight-day old larvae*

They (figure 11) measure 10·3 to 10·6 mm in total length. The first dorsal fin spine is well calcified and the chromatophores are star-shaped over the head.

3.12 *Nine-day old larva*

They (figure 12) measure 10·4 to 10·6 mm in total length. The anal fin spines and rays are calcified. The caudal fin is clearly forked. The chromatophores are scattered throughout the body, but are more concentrated over the dorsal region of the body.

3.13 *Ten-day old larva*

They (figure 13) measure 10·6 to 10·9 mm in total length. The ventral fin rays are calcified and the caudal fin is clearly forked as in the juveniles. The adipose second dorsal fin is still attached to the upper lobe of the caudal fin. The chromatophores are more concentrated on the dorsal surface of the body.

3.14 *Eleven-day old larva*

They (figure 14) measure 12·3 to 12·7 mm in total length. The adipose second dorsal fin has just started detaching itself from the caudal fin. The spines in the anal fin are fully calcified. The chromatophores are found only along the dorsal surface of the body.

3.15 *Twelve, Thirteen- and fourteen-day old larvae*

These larvae (figures 15–17) show only minute differences in their morphology. In the twelve-day old larva, the chromatophores are very few and started disappearing. The thirteen- and fourteen-day old larvae are devoid of any chromatophores. The adipose second dorsal fin has completely separated from the caudal lobe in the fourteen day old larva and appears almost similar to that of a juvenile catfish.

3.16 *Juvenile catfish*

The juveniles (figure 18) just metamorphosed from the post-larvae show all the morphological characters of the adult. The metamorphosis from postlarvae to juvenile stage takes place mostly in about fifteen days after hatching.

**Acknowledgements**

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# Wing microsculpturing in the Brazilian termite family Serritermitidae (*Serritermes serrifer*, Isoptera), and its bearing on phylogeny

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**Abstract.** The results of our studies on the Brazilian family Serritermitidae are presented here. Microsculpturing is simple and consists of a few rows of small, tongue-shaped papillae on the anterior and posterior wing margins and a few rows of angular arrowheads in the anterior one-third of the wings. Hairs are almost absent, a few small ones being scattered on some of the veins. The bearing of wing microsculpturing on the phylogeny of the Serritermitidae is discussed. It is concluded that the family arose as a lone sideline from the common ancient rhinotermitid stock. The other line from this stock gave rise to the Stylotermitidae on the one hand and the Rhinotermitidae on the other.

**Keywords.** Wing microsculpturing; Isoptera; Serritermitidae; *Serritermes serrifer*; phylogeny

## 1. Introduction

In a long series of papers, Roonwal and co-workers (1967–1983) have published the results of studies on wing microsculpturing in all the major families and subfamilies of termites except two tiny single-genus families, the Serritermitidae (neotropical, Brazil) and the Indotermitidae (oriental). The occurrence of a thick carpet of eight different types of microsculpturing elements on both wing surfaces has been demonstrated, with densities as high as over 12500/mm$^2$.

Imagoes of *Serritermes serrifer* (Bates in Hagen), the sole representative of this rare and unique neotropical (Brazilian) family, have, after years of effort, recently become available, and the results of its study are presented here.

## 2. Materials and methods

Imagoes collected from Coxipo de Ponte (about 5 km SE of Cuyaba, Mato Grosso Province, Brazil; lat. 15·35 S, long. 56·00 W) were studied. Glycerine mounts of wings gave excellent delineation of the microsculpturing elements.

## 3. Results

### 3.1 *Family SERRITERMITIDAE Holmgren*

Various authors have placed the subfamily Serritermitinae in either the family Rhinotermitidae or the Termitidae. Emerson (1965, p. 17) raised it to family rank

(characters later elaborated by Emerson and Krishna 1975), and we accept that status (also vide Krishna 1970; Weidner 1970).

Genus **Serritermes** Wasmann

*Serritermes serrifer* (Bates in Hagen)

*Wings* (figures 1–3)

Wings small (size without scale: forewing 5·1 × 1·2 mm; hindwing 4·0 × 1·3 mm), transparent, colourless, veins somewhat dark yellowish; almost hairless, a few small hairs (length 28–40 μm) scattered on some of the veins; margins smooth and totally hairless; at the distal end the margin is wavy and rugose (figure 3C). Venation variable. In the two pairs of wings examined by us the media is absent (according to Emerson and Krishna 1975, it is present in some wings and absent in others).

*Microsculpturing* (figures 2 and 3)

Consisting of papillae and arrowheads on both the dorsal and ventral wing surfaces. No micrasters or any other type present.

Papillae: Consisting of 2 or 3 rows of distally directed, tongue-shaped papillae on anterior margin of first vein (costal-subcosta), and 1 or 2 rows of similar but smaller papillae on the posterior margin. Size of anterior papillae 6–11 μm × 4–5 μm. Density (in the concerned region) *ca* 6400/mm$^2$.

Arrowheads: Consisting of 3 or 4 rows of distally directed, angular, pointed, arrowhead-like bodies on the second vein (radius) and 2 or 3 rows of similar but smaller

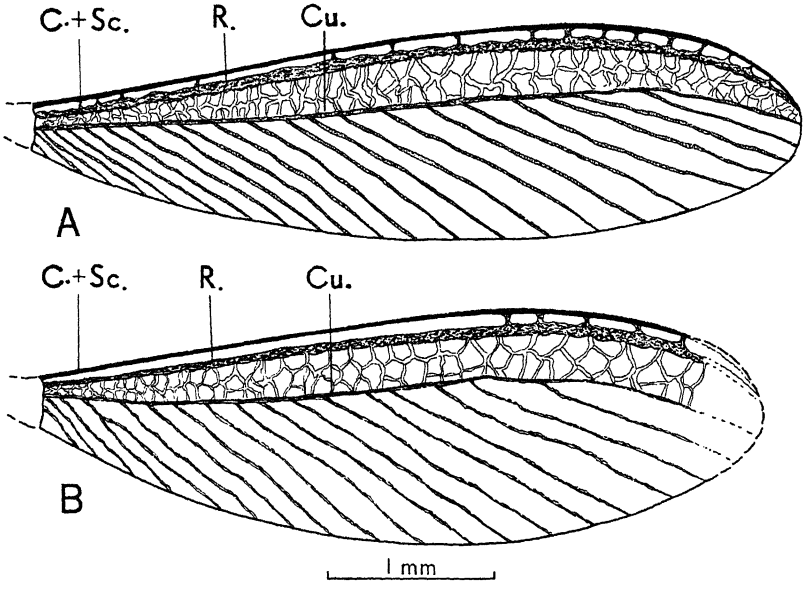


**Figure 1.** *Serritermes serrifer*. Right wings. **A.** Forewing. **B.** Hindwing. C + Sc, Costa-subcosta (costal margin of Emerson); R, radius (radial sector of Emerson); Cu, cubitus.

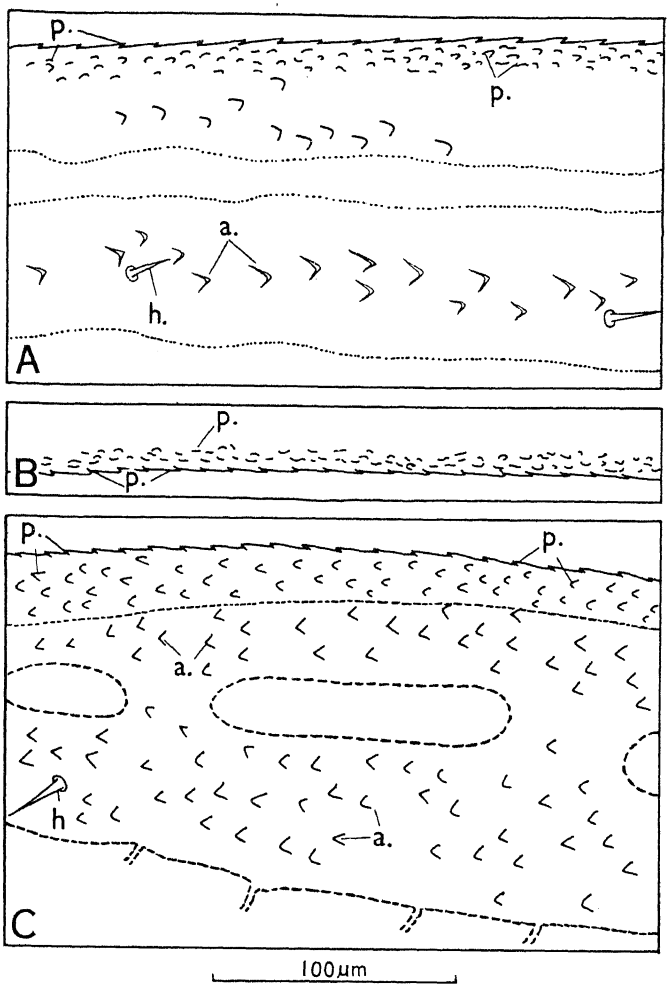


**Figure 2.** *Serritermes serrifer*. Portions of right wings, magnified to show microsculpturing. **A.** Middle of anterior margin of forewing, dorsal surface. Papillae (on anterior edge of first vein) and arrowheads (on first and second veins), **B.** Same, posterior margin (papillae), **C.** Middle of anterior margin of hindwing, ventral surface. a., arrowheads; h., hairs; p., papillae.

structures on the first vein below the papillae. Size (on the second vein) 8–11 μm × 6–9 μm. Density (on second vein) *ca* 3060/mm$^2$. Arrowheads mostly V-shaped with a sharp, narrow to broad angle, and with a thick base and pointed tips; those on the first vein tend to be smaller and somewhat subcresentic.

## 4. Discussion

### 4.1 *General*

No information was hitherto available on wing microsculpturing in *Serritermes serrifer*. Emerson and Krishna (1975) gave a preliminary and incomplete account as follows:

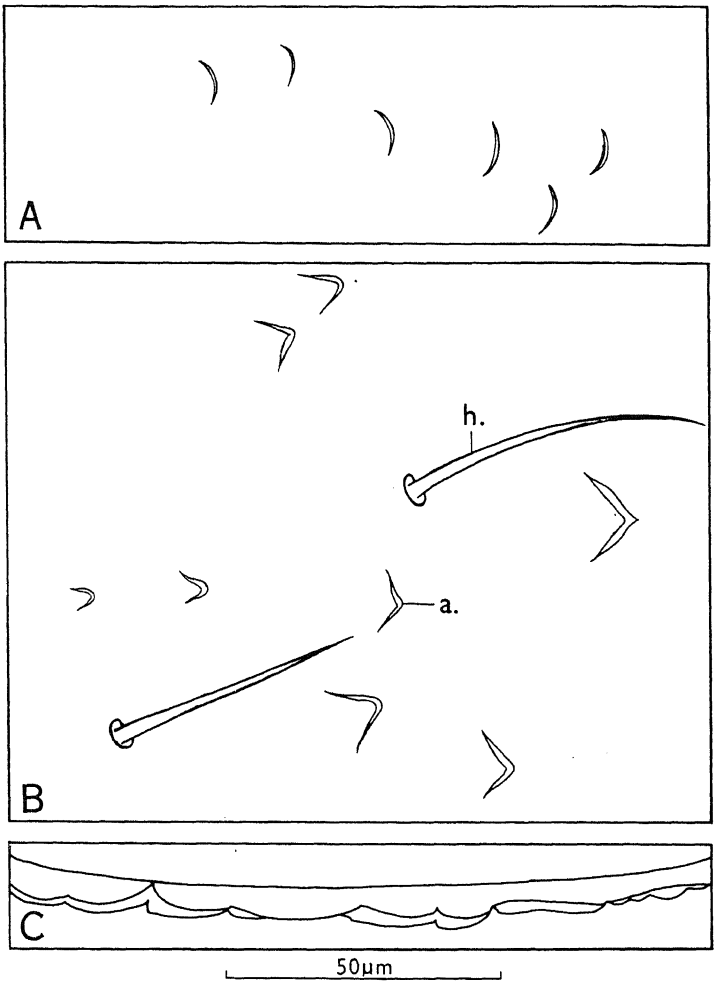


**Figure 3.** *Serritermes serrifer.* Portions of right forewing, dorsal surface, greatly magnified to show microsculpturing. **A.** On first vein (below anterior edge), **B.** On second vein. **C.** Distal margin to show rugosity of outer edge. a., arrowheads; h., hairs.

(i) Hairs are "absent on the costal margins, inner margins, membranes, and strong veins beyond the humeral suture . . ." (p. 12). The absence of hairs was regarded as a derivative (*i.e.* secondary) condition. (ii) Wing membranes "lack punctations" (p. 16). The precise meaning of punctation was, however, not made clear, and further down the same page they mentioned the presence of "rounded punctations or micrasters" in several genera of other families, *e.g. Coptotermes*, *Psammotermes*, *Heterotermes*, *Termitogeton*, *Stylotermes* and *Parastylotermes*. Some, but, not all, of these genera possess true, multi-armed, asteroid micrasters, and it is clear that these authors neither distinguished punctations from micrasters, nor gave their precise meaning.

The presence of weak papillae and simple arrowheads (and no other form of microsculpturing) provide valuable clues to the phylogenetic affinities of the Serritermitidae, as discussed below.

## 4.2 *Phylogeny*

*Serritermes serrifer* is a rare and peculiar species whose phylogenetic position has been the subject of much diversity of opinion. Hagen (1858) placed it with the Kalotermitidae (his *Calotermes serrifer* Bates). Wasmann (1897) erected for it a new subgenus *Serritermes* (of genus *Calotermes*), while Silvestri (1901, 1903) raised it to full generic rank. Holmgren (1911) placed it in a new subfamily, Serritermitinae (of his family Mesotermitidae = Rhinotermitidae). Subsequent authors placed the Serritermitinae either with the Rhinotermitidae (Grassé 1949) or the Termitidae (Snyder 1949). Emerson (1965) raised it to family rank. Emerson and Krishna (1975) regarded it as close to the rhinotermitid subfamily Psammotermitinae (genera *Psammotermes* and *Glossotermes*). They concluded (p. 28) that "the Serritermitidae can be traced backward to an origin from the base of the Psammotermitinae, and in sequence back to the primitive rhinotermid stem, to the hodotermitid-rhinotermitid stem, or to the primitive isopteran stem that arose from primitive blattoids possibly as early as Permian times."

We may now discuss briefly the significance of the more important characters, including wing microsculpturing, which have a bearing in determining the phylogenetic positions of the Serritermitidae.

The very small size of this termite, its characteristics imago-worker mandibles with a single marginal tooth (versus two or three in others), the peculiar, long, sword-like, serrated soldier mandibles (figure 4A) and the soldier pronotum (figure 4D) with a very deep, median indentation in both the anterior and posterior margins are characters which distinguish *Serritermes* from all other genera.

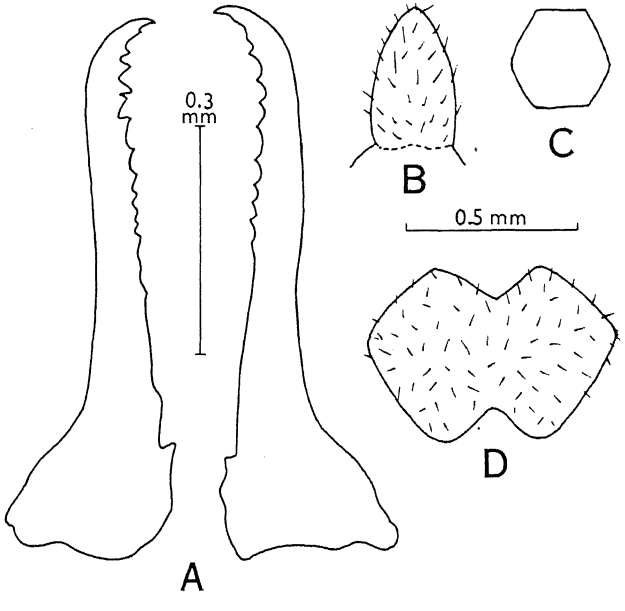


**Figure 4.** *Serritermes serrifer*. Soldier. **A.** Mandibles. **B.** Labrum. **C.** Postmentum. **D.** Pronotum.

Several characters suggest a rhinotermitid or a stylotermitid affinity. The simple, elongate, tongue-shaped soldier labrum (figure 4B) recalls the similar labrum of the primitive rhinotermitids (*Psammotermes*, *Coptotermes*), but lacks the pair of long apical bristles. The differences from the latter genera are also emphasised by the small hexagonal postmentum in *Serritermes* (figure 4C) and a long one in the other two.

Regarding wing microsculpturing, unlike the Psammotermitinae (Roonwal *et al* 1979b) where only pimpules are present in addition to the papillae, there are no pimpules in *Serritermes* but only arrowheads in addition to the universally occurring papillae. Arrowheads first appeared in the family Stylotermitidae (*Stylotermes*, Roonwal *et al* 1979a; Roonwal 1981, 1983b) and are present in considerable abundance in addition to pimpules and papillae. They are only occasionally present in a few Rhinotermitidae and Termitidae (Roonwal 1983b), but their main concentration seems to be in the Stylotermitidae. Their presence, in fair abundance, in the Serritermitidae would thus suggest its affinity with the Stylotermitidae, but this is offset by the important fact that the tarsi are 3-segmented in all the three legs of the Stylotermitidae (Roonwal 1975) and 4-segmented in all the legs of the Serritermitidae (figures 5 A–D).

Emerson and Krishna (1975) have suggested that the origins of the Serritermitidae can be traced back not only to the primitive rhinotermitid stem at the base of the Psammotermitinae, but even earlier, near the primitive blattoid stock. The two most primitive families which also arose from primitive blattoid stocks are the Mastotermitidae (*Mastotermes*) and the Termopsidae (*Archotermopsis*). But the Serritermitidae can be separated from these primitive genera by the fact that the apical tibial spurs are naked in *Serritermes* (Figure 5 E) and clothed with scaly papillae in the other two.

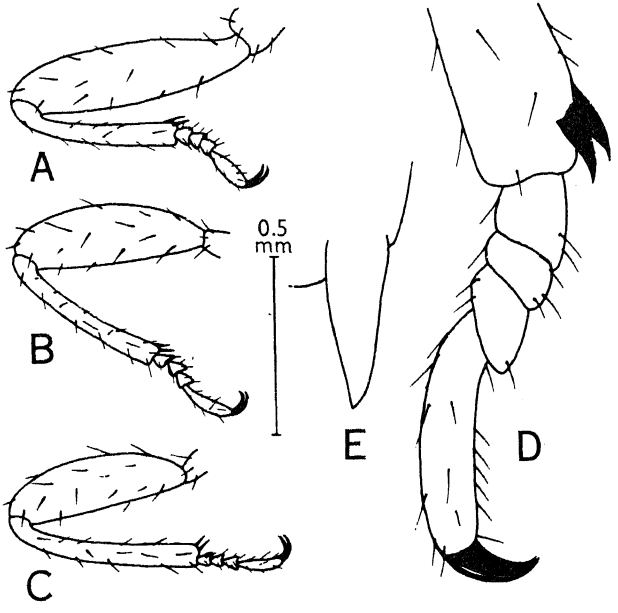


**Figure 5.** *Serritermes serrifer*. Soldier, legs of left side. **A.** Foreleg. **B.** Middle leg. **C.** Hindleg. **D.** Lower part of hindleg, more magnified. **E.** An apical tibial spur.

On the whole, it may be concluded that the Serritermitidae, while being a primitive and isolated family, differs from the most primitive ones and has close affinities with the Rhinotermitidae, especially the Psammotermitinae. It seems (also vide Roonwal 1983b, p. 367, chart) that the ancestral rhinotermitid stock (with 4-segmented tarsi and potentiality for varied sorts of wing microsculpturing) evolved into two main lines (figure 6), *viz* (i) a lone serritermitid line (with 4-segmented tarsi and simple wing microsculpturing consisting of only papillae and arrowheads); and (ii) a rhinotermitid-stylotermitid line with (initially) 4-segmented tarsi and varied types of microsculpturings. This latter line further evolved into two branches: (a) A small Stylotermitidae branch (with a simple wing microsculpturing consisting of only 3 kinds, *e.g.* papillae, pimpules and arrowheads, and with the number of tarsal segments reduced to 3); and (b) a large Rhinotermitidae branch with 4-segmented tarsi and wing microsculpturing consisting of 5 types of structures, *e.g.* papillae, arrowheads, pimpules, tubercles and micrasters, though all the five do not occur together in the same species.

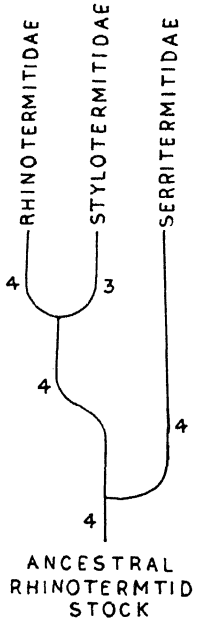


**Figure 6.** Diagram to illustrate the probable phylogeny of the Serritermitidae from the ancient rhinotermitid stock. Numerals indicate the number of tarsal segments (for details see text.)

**Acknowledgements**

We are indebted to the following for assistance: to Dr Eliana M Cancello (Zoology Museum, Sao Paolo University, Sao Paolo, Brazil) for kindly sending examples of *Serritermes serrifer*; to Dr R C Sharma (Zoological Survey of India, Jodhpur) for assistance with some of the drawings.

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Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94, No. 4, August 1985, pp. 407–411.


# Electrical stimulation—Effects on the protein in the ventral nerve cord of cockroach, *Periplaneta americana*

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MS received 15 June 1984; revised 8 September 1984

**Abstract.** Changes in protein concentration due to electrical stimulation have been investigated at different time intervals in cockroach ventral nerve cord. Significant increase in total protein concentration was observed at 15 min interval of stimulation. Increase in protein concentration was also observed in ventral nerve cords incubated with exogenous glucose. Microdisc polyacrylamide gel electrophoresis at 15 min interval has revealed an increase in low molecular weight protein fractions in nerve cords from stimulated group. The data clearly depict the changes in protein due to electrical stimulation stress.

**Keywords.** Cockroach; electrical stimulation; ventral nerve cord; protein; microdisc polyacrylamide gel electrophoresis.

## 1. Introduction

Studies on the correlation of the functional activity of the brain with metabolic parameters due to chemical, electrical and light stimuli are available on vertebrates (Chitre and Talwar 1963; Chitre *et al* 1964; Jones 1972; Jones and MacIlwain 1971; Kol's *et al* 1974; Luxuro 1960; Selvanayagam and Habibulla 1979; Talwar *et al* 1961, 1966). Review of literature shows absence of knowledge on the effects of electrical stimulation on the metabolic parameters of nervous system (NS) of invertebrates. Hence the present study was undertaken to gain information of electrical stimulation on the protein concentration and pattern in an invertebrate central nervous system.

## 2. Material and methods

In order to eliminate the effect of sex on protein concentration, only male animals were chosen for study. Male cockroaches, *Periplaneta americana* were taken in two groups. One served as control and the other as the experimental group. From each group, atleast ten animals were used for removal of ventral nerve cord. The dissection was carried out in insect ringer solution (Orchard and Finlayson 1977). One set of the nerve cords from the control group was incubated in ringer with glucose and another set in ringer free of glucose for 15 min (Edstrom and Mattison 1972). The nerve cords from the experimental group were incubated in two sets similar to control before stimulation. After the incubation period, all the sets of ventral nerve cords were hooked separately to a pair of silver-silver chloride electrodes, supported by a wax bath in a petridish. The preparation was prevented from drying by periodically adding insect ringer. The nerve cords of the experimental sets alone were given a repetitive stimuli of

0·1 m sec duration with a current strength of 3 V at a frequency of 1/sec using an electronic stimulator (Palmer, England Electronic square-wave stimulator) for 5, 10, 15, 30 and 60 min.

### 2.1 *Total protein estimation*

Both control and stimulated nerve cords were removed, washed with insect ringer and weighed separately after carefully blotting out the moisture. They were homogenised separately in prechilled glass-glass homogeniser with 80% ethanol and centrifuged at 3500 rpm for 5 min. The residue was dissolved in 1N NaOH solution and the proteins were estimated following the method of Lowry *et al* (1951).

The results were tested for significance using student's *t* test.

### 2.2 *Microdisc electrophoresis*

Protein pattern was analysed by homogenising the ventral nerve cord from both the sets of control and experimental groups in 0·01 M Tris-HCl buffer (pH 7·4) and subjecting the supernatent to microdisc polyacrylamide gel electrophoresis (Ganesan *et al* 1979). The gels were stained in 0·25% coomassie brilliant blue, destained and stored in 7% acetic acid (Smith 1968). Gels were photographed over an x-ray viewer as suggested by Oliver and Chalkley (1971) and scanned using Shimadzu-Dual length TLC scanner CS-910 with C-RIA chromotrophe recorder at 640 and 430 nm.

## 3. Results

### 3.1 *Total proteins*

The electrical stimulation of the cockroach ventral nerve cord (incubated in exogenous glucose) at 15 min interval has revealed highly significant ($p < 0.001$) increase in total protein concentration ($40.81 \pm 5.56$ mg/g). Though the protein concentration increases from 5 min up to 15 min, a decrease is observed after that period (table 1).

**Table 1.** Effect of electrical stimulation on the total protein concentration (mg/g) from the cockroach ventral nerve cord with exogenous glucose.

| Duration of stimulation | Total protein | | Absolute difference | *P* value |
|---|---|---|---|---|
| | Control | Experimental | | |
| 5 | $24.30 \pm 2.14$ | $29.54 \pm 2.54$ | + 5·24 | < 0·005 |
| 10 | $25.91 \pm 3.26$ | $35.86 \pm 1.89$ | + 9·95 | < 0·005 |
| 15 | $26.02 \pm 4.21$ | $40.81 \pm 5.56$ | + 14·79 | < 0·001 |
| 30 | $24.90 \pm 5.04$ | $20.79 \pm 4.15$ | − 4·11 | NS |
| 60 | $30.00 \pm 6.01$ | $19.10 \pm 5.76$ | − 10·90 | < 0·010 |

Mean ± S.D. of 6 observations; +: indicates increase; −: indicates decrease; NS: not significant.

**Table 2.** Effect of electrical stimulation on the total protein concentration (mg/g) from the cockroach ventral nerve cord without exogenous glucose.

| Duration of stimulation | Total protein | | Absolute difference | *P* value |
|---|---|---|---|---|
| | Control | Experimental | | |
| 5 | 21·05 ± 1·85 | 20·11 ± 1·02 | − 0·94 | NS |
| 10 | 22·23 ± 2·12 | 20·23 ± 2·03 | − 2·00 | NS |
| 15 | 30·72 ± 2·92 | 21·98 ± 1·65 | − 8·74 | < 0·001 |
| 30 | 20·56 ± 1·99 | 9·89 ± 0·10 | − 10·67 | < 0·001 |
| 60 | 21·81 ± 2·942 | 7·12 ± 0·59 | − 14·69 | < 0·001 |

Mean ± S.D. of 6 observations; −: indicates decrease; NS: not significant.

In nerve cords incubated free of exogenous glucose, stimulation showed a decreasing trend in the total protein concentration significantly ($p < 0·001$) (table 2). The decrease was consistently found at all intervals of stimulation.

### 3.2 *Protein pattern*

Densitometric scanning of the gels from unstimulated and stimulated ventral nerve cords are shown in figure 1. The number of fractions resolved from the unstimulated control group was eleven and from the stimulated group was nineteen. The control group thus depicted only six high molecular weight protein fractions. But the experimental group showed an increase by two fractions over the control group. Similarly the low molecular weight protein fractions were only five in control groups but the experimental group revealed an increase by six fractions. Thus the rise in total protein concentration in the stimulated nerve cord may be due to the increase in both the low and high molecular weight protein fractions.

## 4. Discussion

Electrophoretic and immunological studies have shown that brain contains several proteins which presumably involved in specific neural functions (Bock 1978). Hence, factors regulating protein synthesis are known to play an important role in the functioning of the NS. The changes observed in the total protein concentration with and without exogenous glucose in the present study suggests that glucose exhibits considerable role as an energy source to meet the altered condition. Selvanayagam and Habibulla (1979) report glucose as an energy source in the absence of adequate energy supply in frog sciatic nerve.

The significant increase in total protein concentration in electrically stimulated nerves with glucose suggests the increased rate of protein biosynthesis. The observed increase in total protein concentration up to 15 min is in accordance with the findings of Jones (1972) who has stated that the increase could be due to the electrical activity causing localized increases in concentration of leucine or neutral amino acids which are known to enhance the incorporation of amino acids into proteins. The decrease

observed after 15 min may be due to the induced hyperactivity for longer duration resulting in protein catabolism leading to fatigue and exhaustion as suggested by Selvanayagam and Habibulla (1979).

The polyacrylamide gel electrophoresis depicts a greater increase in the total number of low molecular weight protein fractions. Such a change suggests the dissociation of complex proteins into simple low molecular weight proteins to meet the stimulation stress. Luxuro (1960) has put forth the view that a small fraction of nerve proteins may split probably to the level of amino acids during the nerve activity. Maheswari (1983) has also reported similar observation of dissociation of proteins in the nervous system of *Scylla serrata* due to pesticide stress. The present investigation has clearly shown alteration in proteins and is probably a geared mechanism to functional demand. However, further investigation on protein change to stress condition is needed.

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# Studies on the silk gland of *Bombyx mori*: A comparative analysis during fifth instar development

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**Abstract.** Middle and posterior parts of the silk gland of fifth instar bivoltine and multivoltine races of *Bombyx mori* and their hybrid were analysed for the concentration of fibroin, DNA, RNA and total protein. Fibroin content of the silk gland increased rapidly from the beginning of fifth instar upto the spinning stage. Concentration of DNA in the middle silk gland was maximum at 24 hr and decreased thereafter. In the posterior silk gland, the concentration of DNA increased upto 72 hr and then decreased. RNA concentration was maximum at 72 hr and 120 hr, in the middle and posterior silk gland respectively. The total protein content increased gradually upto the spinning stage in the middle silk gland whereas it increased upto 120 hr and decreased sharply thereafter in the posterior silk gland. The difference in the concentration of these constituents in the silk gland was correlated with the differential silk output in both the pure races and their hybrid.

**Keywords.** *Bombyx mori*; bivoltine; multivoltine; hybrid; silk gland; fibroin; DNA; RNA; total protein.

## 1. Introduction

Structural and functional aspects of the silk gland of silkworm *Bombyx mori* have thoroughly been investigated (Dhavalikar 1962; Lucas 1966; Machida 1970; Tashiro and Otsuki 1970; Sasaki and Noda 1973 a, b; Prudhomme *et al* 1973; Tashiro *et al* 1976). Variations in the concentration of fibroin, DNA and RNA in the silk gland of several strains of bivoltine race and their hybrids have also been reported (Shigematsu and Takeshita 1968; Tashiro *et al* 1968; Shigematsu and Moriyama 1970; Moriuchi *et al* 1972; Shigematsu *et al* 1974). However, no information is available on the quantitative variations in the silk gland of multivoltine race. Bivoltines are shown to produce more quantity of silk compared to multivoltines (Tanaka 1964). It is therefore interesting to undertake a comparative study on the quantitative analysis of the silk gland of both bivoltine and multivoltine races of *Bombyx mori* and their hybrid to highlight the influence of these variations on the differential silk output by the silkworm varieties.

The silk gland grows enormously during the fifth instar development of silkworm (Sakaguchi 1978). The middle and posterior parts of the silk gland are known to synthesize sericin and fibroin respectively (Machida 1927; Oba 1957; Shibukawa 1959). Further it has been shown that fibroin is synthesized very rapidly during the fifth instar development (Shimura *et al* 1955; Noguchi *et al* 1974) being associated with an increase in DNA, RNA and total protein content of the posterior silk gland (Tashiro *et al* 1968).

The present communication deals with a comparative account of the middle and posterior silk gland of the fifth instar bivoltine and multivoltine races of *Bombyx mori* and their hybrid. A few important commercial characters are analysed in different silkworm varieties to correlate with the variations in the silk gland.

## 2. Materials and methods

### 2.1 *Silkworms*

Bivoltine ($NB_{18}$), multivoltine (Pure Mysore) races of *Bombyx mori* and their hybrid ($NB_{18}$ ♂ X PM ♀) were maintained under standard laboratory conditions at a temperature of 25–28°C and relative humidity of 75–90% with good quality mulberry leaves ($M_5$ variety). Fifth instar larvae of average body weight were used at different time intervals.

### 2.2 *Analyses of the silk gland*

The silk gland was dissected and washed with 0·9% NaCl. The intraglandular fibroin was extracted separately from the middle and posterior parts of the silk gland according to the procedure of Tashiro *et al* (1968). After complete extraction of fibroin, the extracts from both parts of the silk gland were pooled and weighed. The nucleic acid was extracted from the middle and posterior silk gland separately and estimated according to Schneider (1957). The concentration of DNA was determined by diphenylamine method using calf thymus DNA as standard and the concentration of RNA was determined by orcinol method using rat liver RNA as standard. The total protein from the middle and posterior silk gland was extracted by the reduction of disulphide bonds, according to the procedure of Gamo *et al* (1977) and estimated according to Lowry *et al* (1951). Usually 4–5 larvae were used for each determination and average value of four independent determinations was calculated.

### 2.3 *Analyses of cocoon characters*

Four to five lots of 10 cocoons each, were taken after harvesting from bivoltine, multivoltine and the hybrid silkworm varieties for analysis of cocoon characters. Important characters like cocoon weight, shell weight percentage, floss weight percentage and filament length were determined. The shell weight percentage was calculated as the ratio between the cocoon shell and the whole cocoon, while the floss weight percentage was the ratio between the total floss and the whole cocoon. Mean values of the lots with standard deviation are presented in table 1.

## 3. Results

As shown in figure 1, total fibroin content increased significantly ($P = <0{\cdot}01$) from 24 hr to reach the maximum level prior to spinning in both the pure races. In hybrid male, a significant increase ($P = <0{\cdot}01$) in fibroin content was observed between 72 hr and 120 hr which remained more or less at the same level upto the spinning stage. On the other hand, the fibroin content increased significantly upto 120 hr followed by a slight increase thereafter in hybrid female. However, the concentration of fibroin at the final stages of fifth instar was high in bivoltine and low in multivoltine, while hybrid showed a sex difference in fibroin content being high in male and low in female.

**Table 1.** Important cocoon characters of bivoltine, multivoltine and the hybrid silkworm varieties

| Silkworm variety | Av. Cocoon weight (g) Mean SD | Shell weight (%) Mean SD | Floss weight (%) Mean SD | Av. filament length (m) Mean SD |
|---|---|---|---|---|
| $NB_{18}$ (bivoltine) | 1·28 ± 0·06 | 14·35 ± 0·80 | 0·79 ± 0·08 | 780 ± 55 |
| Pure Mysore (multivoltine) | 0·88 ± 0·04 | 9·59 ± 1·02 | 2·25 ± 0·18 | 350 ± 25 |
| Hybrid male | 1·08 ± 0·06 | 12·02 ± 1·05 | 1·38 ± 0·09 | 620 ± 35 |
| Hybrid female | 0·94 ± 0·06 | 11·00 ± 0·90 | 1·40 ± 0·11 | 575 ± 50 |

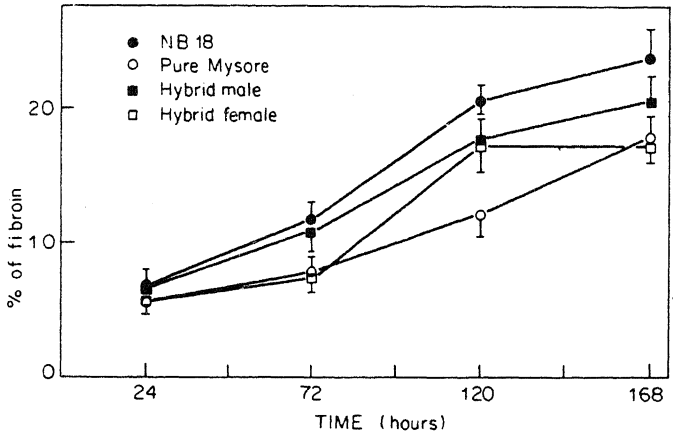


**Figure 1.** Increase in fibroin content of the silk gland (middle plus posterior) during fifth instar development.

The concentration of DNA in the middle silk gland was maximum at 24 hr which decreased gradually upto 120 hr and remained at a more or less constant level (figure 2A). The DNA concentration was high in bivoltine and both sexes of the hybrid while in multivoltine it was significantly low ($P = <0·005$). As shown in figure 2B, the concentration of DNA in the posterior silk gland increased to reach the maximum level at 72 hr and decreased thereafter. However, DNA concentration was different in different races being high in bivoltine and low in multivoltine. Further, at the final stages of fifth instar the DNA content remained constant between 0·4 and 0·5 mg/g wet wt in both the pure races and the hybrid.

RNA concentration increased upto 72 hr and then decreased to a more or less constant level by 120 hr in the middle silk gland of bivoltine and hybrid male, whereas it was maximum at the beginning of fifth instar (24 hr) and decreased thereafter in multivoltine and hybrid female (figure 3A). The level of RNA in the middle silk gland of bivoltine and both sexes of the hybrid was high compared to a significantly low ($P = <0·001$) level in multivoltine. The concentration of RNA in the posterior silk gland increased gradually to reach the maximum at 120 hr and decreased significantly

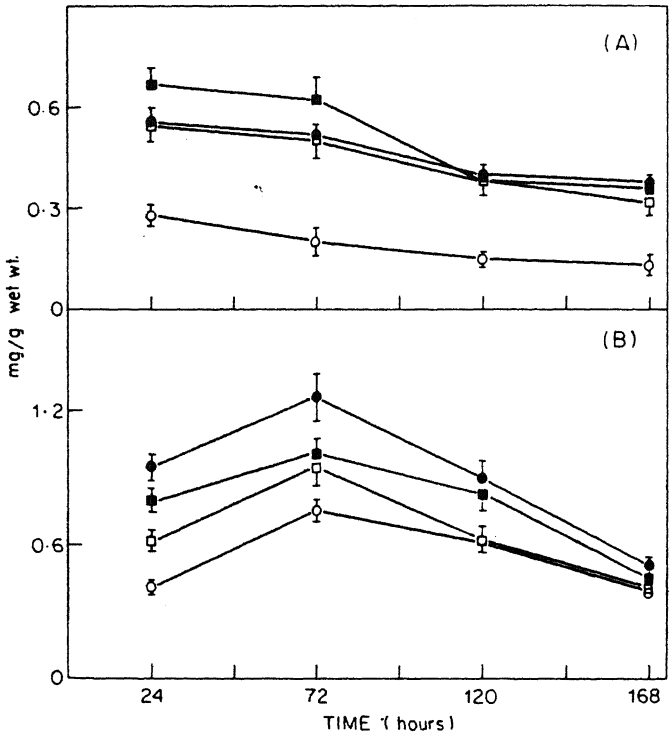


**Figure 2.** Change in DNA content of the middle (A) and posterior (B) silk gland during the fifth instar.

($P = <0{\cdot}001$) thereafter in bivoltine and hybrid male (figure 3B). But in multivoltine and hybrid female, the RNA content was maximum at 72 hr and then decreased gradually. The concentration of RNA in the posterior silk gland was significantly high ($P = <0{\cdot}01$) in bivoltine and hybrid male as compared to multivoltine and hybrid female.

There was a significant increase ($P = <0{\cdot}001$) in the amount of total protein in the middle silk gland from the beginning to the end of fifth instar (figure 4A). The final concentration was about five times that at the beginning of the fifth instar. Further, a significant difference in the concentration of total protein was observed in different races, being high in bivoltine (160 mg/g wet wt) and low in multivoltine (112 mg/g wet wt) prior to spinning. As shown in figure 4B, the concentration of total protein in the posterior silk gland increased upto 120 hr, reached the maximum level and then decreased significantly ($P = <0{\cdot}005$), unlike in the middle silk gland. Further, the concentration of total protein in the posterior silk gland at 120 hr was significantly different in the silkworm varieties studied being high in bivoltine and low in hybrid female.

Table 1 shows the difference in a few important commercial characters of the cocoon from different silkworm varieties. The average cocoon weight, shell weight percentage and the average filament length were significantly high in case of bivoltine as compared to multivoltine. But the hybrid showed an improvement of all these characters over the

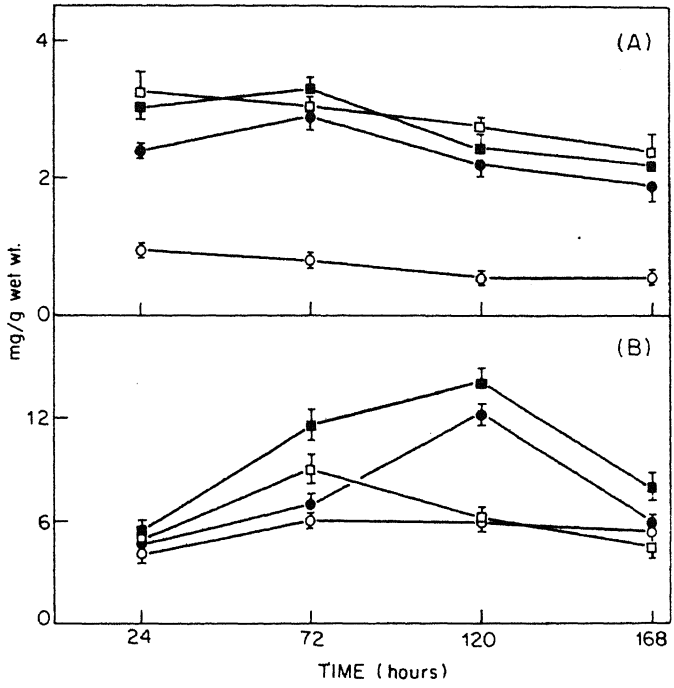


**Figure 3.** Change in RNA content of the middle (A) and posterior (B) silk gland during the fifth instar.

multivoltine parent. Further, a difference, though not significant, was observed between both sexes of the hybrid.

## 4. Discussion

The amount of fibroin in the silk gland increases significantly from the beginning to the end of fifth instar. This can be correlated with the high rate of fibroin synthesis in the silk gland during fifth instar development (Shigematsu and Takeshita 1968; Tashiro *et al* 1968; Noguchi *et al* 1974). A higher concentration of fibroin in the silk gland of bivoltine might account for more quantity of silk produced as compared to multivoltine. The concentration of fibroin in the silk gland of hybrid male is more concomitant with larger quantity of silk produced as compared to the female. However, no significant sex difference in the concentration of fibroin is observed in pure races.

High level of DNA at the beginning of fifth instar shows that the synthetic activity starts early in the middle silk gland. In contrast, there is a time-lag for the increase in DNA content of the posterior silk gland suggesting that the fibroin synthesis starts a little late during the fifth instar. Incorporation studies have also shown that the synthesis of fibroin is significant only from 96 hr of the fifth instar development (Fukuda and Florkin 1959). This result, however, contradicts the earlier reports of Tashiro *et al* (1968) who reported no time-lag for the increase in DNA content of the posterior silk gland. The difference in the DNA content of both middle and posterior silk gland accounts for the

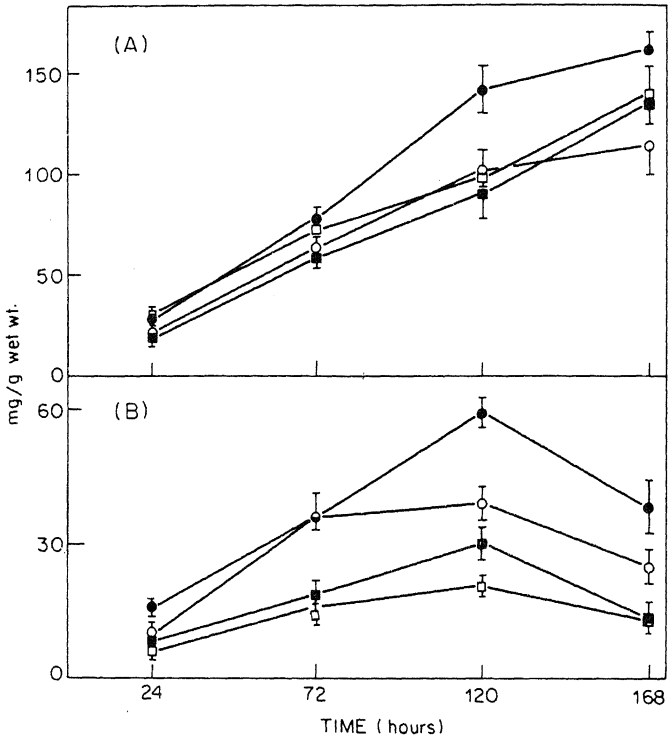


**Figure 4.** Change in total protein content of the middle (A) and posterior (B) silk gland during the fifth instar.

difference in the degree of their synthetic activity. Further, the DNA content of the silk gland of both the races is different showing that it is probably specific to the race.

The concentration of RNA in the middle silk gland of multivoltine is low concomitant with a low DNA concentration. This reflects the lower rate of synthetic activity in the middle silk gland of multivoltine compared to bivoltine and both sexes of the hybrid. Maximum level of RNA at 72 hr and 120 hr in the posterior silk gland of multivoltine and bivoltine respectively, suggests that the synthetic activity in the posterior silk gland starts later than in the middle silk gland.

The initial increase in the total protein content of the middle silk gland might be due to the synthesis and accumulation of sericin as suggested by an early increase in its DNA and RNA contents. The rapid increase in the amount of protein after 72 hr, supports the view that there is an inflow of fibroin to the middle silk gland for storage (Fukuda and Florkin 1959; Gamo *et al* 1977; Sakaguchi 1978). The significant decrease in total protein content of the posterior silk gland after 120 hr is in accordance with earlier reports (Tashiro *et al* 1968). This suggests that the outflow of fibroin from posterior silk gland is more rapid during the later part of the fifth instar.

Thus it is evident that the concentration of fibroin, DNA, RNA and total protein in the middle and posterior silk gland is different in both the pure races and that the hybrid shows an increase in the concentration of these constituents over the multivoltine parent. The final silk output, however, is directly correlated with the concentration of these constituents in the silk gland of the silkworm varieties studied.

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# Studies on mating, spawning and development of egg in *Macrobrachium nobilii* (Henderson and Mathai)

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**Abstract.** The chronomorphological events during the development of the egg of the freshwater caridean prawn, *Macrobrachium nobilii*, were followed from spawning to hatching. The female is receptive only for a 30-minute period after the premating moult. After mating, spawning ensued within $9 \pm 3$ hours and was completed within 15–20 seconds. Unmated females also spawned but the eggs did not survive. The rate of egg development increased 2·5x for a temperature rise of 10°C. Hatching was synchronised under *in vitro* conditions indicating uniform development of a clutch.

**Keywords.** *Macrobrachium nobilii*; mating; spawning; egg development.

## 1. Introduction

*Macrobrachium nobilii*, a caridean freshwater prawn moults and breeds once in 19 days. In carideans, there is a premating moult before spawning. A freshly moulted female remains receptive to a male for only a limited period (Ling 1964). If a male is not available during the receptive period, then fertilization of a brood will be missed and such a brood will be lost. Hence, it is important for culture purpose to know the receptive period. Therefore, studies were carried out on the sexual receptiveness and mating of *M. nobilii*.

Like most other decapods, *M. nobilii* incubates the developing eggs in the ovigerous setae of the pleopods till hatching. However, incubation by the mother is less productive in terms of larvae for culture needs (Balasundaram 1980). To circumvent the disadvantages associated with incubation, the eggs can be hatched under artificial conditions, simulating the ventilation technique of the mother (Balasundaram and Pandian 1981). An average female ($39 \pm 5$ mm : total length from tip of rostrum to telson) incubates about 2,200 eggs/clutch. For mass incubation, eggs from more than one female are needed. Incubation of egg masses of identical age will enable simultaneous hatching. The percentage of hatching is influenced by the age of egg mass at the time of relieving (Balasundaram and Pandian 1981).

Hence, it is advantageous to procure egg masses of identical age for mass incubation under *in vitro* conditions. Therefore, an attempt has been made in this study to know the age of eggs based on simple chronomorphological features of the developing egg from 0 hour to hatching.

In general, temperature is known to influence egg development of decapods (Wear 1974). This indicates that the time required for development can be accelerated by

controlling the temperature. Hence, the effect of temperature on development of egg and hatching also was studied.

## 2. Material and methods

Healthy individuals of *M. nobilii* were collected from the river Cauvery at Tiruchi (10°5′N; 78°43′E). They were maintained in 90 litre/laboratory tanks at a density of 1 male to 7 females. The water temperature was 28 ± 2°C (mean ± SD). The pH and dissolved oxygen content were 7·5 ± 1 and 6 ± 1 ml/l respectively. The photo-period was 14L : 10D. The prawns were fed once daily, *ad libitum*, with a mixture of beef, goat liver and boiled Bengal gram (*Cicer arietinum*). Water was changed once a day. The female about to moult and spawn was identified by its fully developed dark green ovaries beneath the transparent carapace extending upto the third rostral spine anteriorly and into the first abdominal somite posteriorly (Balasundaram 1980). Such females were transferred into circular troughs at a density of 1 male to 1 female and observed for moulting and spawning.

Sexual receptivity of the female was studied by introducing several freshly moulted individuals in separate troughs. Males were released individually into each one of these troughs at different time intervals (5, 10, 15, . . . minutes) after moult, till sexual attractiveness ceased to exist when the prawns ignored each other.

Two different techniques were tried to obtain eggs from the mother, for *in vitro* incubation. In the first technique, 6 of the freshly moulted and mated females were selected. Each one was gently stretched and enveloped with a flexible aluminium wire mesh (0·5 cm mesh) around the body. This prevented the animal from bending its abdomen to form the brood chamber. However, the enclosure was loose enough to allow appendage movements. This arrangement enabled the eggs, on release, to pass through the wire mesh to be collected at the bottom of the trough. The collected eggs were washed with sterile water and transferred to incubating chambers. The incubating chambers were 100 ml conical flasks with 50 ml water disinfected by boiling. Each incubating chamber contained 10 eggs. The flasks were then covered loosely with cotton plugs to avoid atmospheric contamination.

The second technique involved the removal after 3 hours of berrying of small egg masses (25 ± 5 eggs/mass) from a clutch. These small masses were then carefully teased manually with the help of needles into individual eggs and incubated as in the previous method.

A batch of 6 berried females were allowed to incubate their eggs normally. Eggs were removed from these females and examined under a light microscope once an hour. The morphological stages attained by these eggs were compared with those of similar age incubated *in vitro*.

To study the effect of temperature on egg development and hatching, samples of egg mass from freshly berried females (3 hour old), reared at room temperature of 28 ± 2°C, were teased individually and transferred into disinfected water in incubation chambers as described above. The chambers were kept at 19, 22, 25, 29, 32 and 34 ± 0·5°C at normal photoperiod (14L : 10D). Six replicates were maintained at each temperature till the eggs hatched. The water in the chambers was carefully decanted and fresh disinfected water at the same temperature was added everyday.

## 3. Results and discussion

### 3.1 *Mating and spawning*

When a male was introduced into a trough containing a freshly moulted female with ripe ovaries, the male quickly rushed to the female, turned it over and mated. In less than 5 minutes the female, lying between the maxillipeds of the male, started moving freely and kept away from the male darting backwards even on chance encounters. Spawning occurred within $9 \pm 3$ hr after mating and lasted for a period of 15–20 seconds. The eggs on release were fertilized externally and passed into the brood chamber. The pleopods secreted a glue-like substance into which the eggs were enveloped and finally kept attached to the ovigerous setae of the pleopods as observed by Ling (1969) in *M. rosenbergii.*

The female after undertaking the premating moult, remained receptive only for 30 minutes. After this period when a male was introduced, they ignored each other. In *M. rosenbergii* such attractiveness persisted for 3 hours (Ling 1964) and *Palaemonetes* was receptive only for 20 minutes (Burkenroad 1947).

Females which were not mated also spawned and got berried as usual but these unfertilized eggs turned golden yellow owing to cytolysis and were lost subsequently. Such females with golden egg mass have also been collected from the field (3 out of 456 females over a period of one year). These eggs when examined under a light microscope were similar to the unfertilized eggs obtained from unmated females in the laboratory. This clearly reveals the importance of the male availability at the right time for mating to ensure fertilisation of the eggs.

**Table 1.** *Macrobrachium nobilii*: Chronomorphological features of the developing egg from 0 hour to hatching

| Time: Hour | Identification features |
|---|---|
| 0 | Egg-oval in shape and measures 570 $\mu$ long and 437 $\mu$ wide. Uniformly granulated. |
| 7 | Cleavage commences as furrows at 4 equidistant points—8-celled stage. |
| 14 | Many hexagonal cells are seen—Egg pale green in colour. |
| 25 | Egg appears olive green in the centre due to the presence of large yolk cells and pale green ectodermal cells in the periphery (see also Anderson 1973) |
| 35 | White opaque blastoderm appears |
| 60 | Blastoderm extends anteriorly |
| 80 | Blastoderm occupies 8·3% of the egg's surface area |
| 120 | Blastoderm occupies 16·7% of the egg's surface area |
| 160 | Blastoderm occupies 36·7% of the egg's surface area |
| 170 | Heart beat begins—Eyes appear as crescentic streaks |
| 205 | Crescentic eye becomes oval in shape—Carapace appears |
| 220 | Length of egg increases from 570 to 608 $\mu$—Breadth from 437 to 497 $\mu$ |
| 240 | Cornea formed—Mouth parts twitch occasionally |
| 250 | Setae of exopodite extend beyond carapace posteriorly |
| 270 | The outer and inner flagellae of antennule, their aesthetes and setae are seen distinctly—larva twitches often and tries to stretch out abdomen—Hatching imminent. |

### 3.2 *Egg development*

The eggs on release were fertilized externally and passed into the brood chamber. The pleopods secreted a glue-like substance into which the eggs were enveloped and kept attached to the ovigerous setae. Ling (1969) made similar observations in *M. rosenbergii*. Eggs removed from the pleopods within the first 3 hours of berrying developed abnormally. This timing appears to vary in different species. For example in *Homarus* it takes at least 29 hours before the eggs can be removed for normal development to proceed (Templeman 1940). Patel and Crisp (1960) too observed that initial development stages are highly sensitive and develop abnormally when incubated *in vitro*.

The chronomorphological events occurring in the developing egg of *M. nobilii* incubated at 29 + 0·5°C is summarised in table 1. The segmentation of the egg was completed on the first day and the blastoderm appeared on the second day. On the seventh day the eyes appeared and heart beat began (figure 1b). The size of the egg

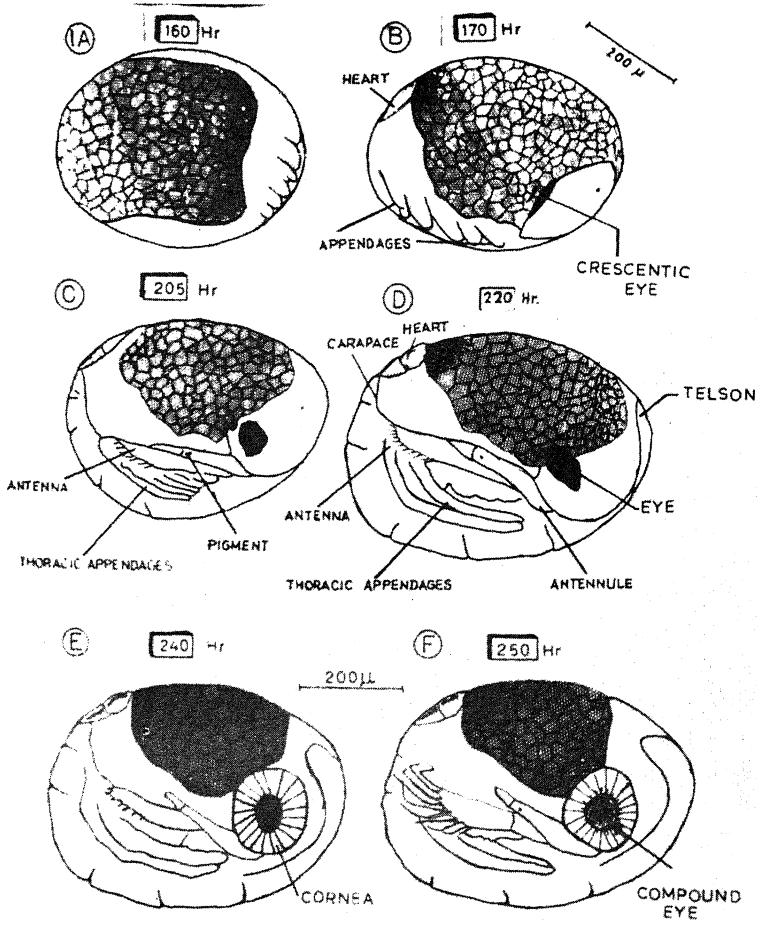


**Figure 1.** *Macrobrachium nobilii*: Chronomorphological features of the developing egg.

increased 1·6x on the tenth day (figure 1d). Such volumetric expansion of eggs due to imbibition of water has been reported by Pandian (1984) for several species of decapods and range from 1·2x in *Palirurus gammarus* (Berry 1971) to 5·4x in *Petrolisthes elongatus* (Greenwood 1965). On the 12th day the egg hatched as zoea (figure 1f).

### 3.3 *Effect of temperature*

The developmental stages attained by the eggs of *M. nobilii* at 22, 25, 29 and 32°C were observed. The eggs required 333, 300, 270 and 173 hours respectively to complete development to hatch (table 2). At any chosen temperature once the egg development is completed, hatching of simultaneously incubated eggs lasts for a period of 6 hours irrespective of the time of the day. The regression lines fitted by the method of least squares bear a linear relationship between the stages attained and time taken for development (figure 2). However, the percentage hatchability did not vary significantly and ranged from 73–78 % ($P < 0{\cdot}05$) (table 2). At 19 and 34°C the eggs failed to develop and suffered total mortality due to cytolysis within 9 ± 3 hours of incubation.

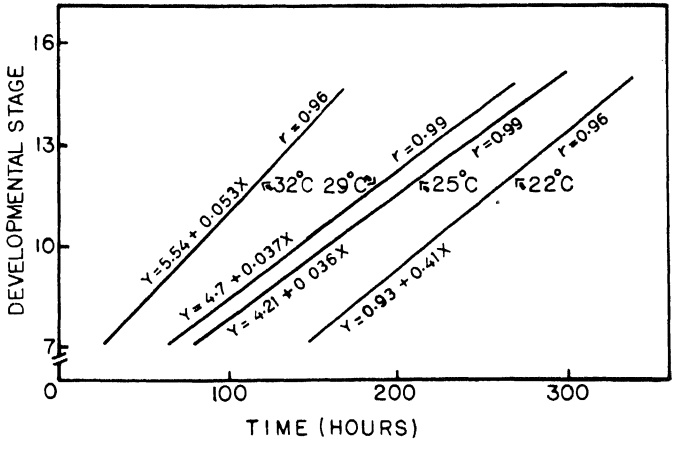


**Figure 2.** *Macrobrachium nobilii*: Effect of temperature on egg development.

**Table 2.** Effect of temperature (±0·5°C) on incubation period and hatchability of eggs of *Macrobrachium nobilii*.

| Temperature (°C) | Time Hour | Hatchability (%) |
|---|---|---|
| 22 | 333±6·6 | 73±9·1 |
| 25 | 300±7·1 | 77±5·2 |
| 29 | 270±6·9 | 78±6·4 |
| 32 | 173±6·6 | 75±8·3 |

Each value (mean ± SD) is based on 6 observations

The development of egg at the lowest temperature (22°C) was the least. The rate increased 2·5x times for a 10°C rise in temperature. This is comparable to the period required for the development of several species of barnacles (Patel and Crisp 1960).

Under *in vitro* conditions all the eggs of a single batch incubated at any particular temperature hatched simultaneously. This indicates the synchronous nature of the development of all the eggs. The rate of egg development when incubated by the mother (29 ± 1°C) was also uniform as in amphipods (Fish 1975), Cirrepeds (Patel and Crisp 1960) and decapods (Bensam and Kartha 1967).

The present study indicates that whether incubated by the mother *in situ* or under *in vitro* conditions, all the eggs of a brood commence development at the same time. They are at the same stage of development at any given time during incubation. However, when incubated by the mother the eggs are hatched in intermittent batches (Balasundaram and Pandian 1981).

Information on the chronomorphological events during the development will help to procure egg masses of identical age so that hatching can be synchronised. At room temperature (28 ± 2°C) the eggs take 12 days to hatch whereas at 32°C they need only 8 days. This indicates that hatching can be timed to suit the need for larvae.

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# Effect of castration and androgen treatment on the androgen dependent parameters in the accessory glands of the slender loris, *loris Tardigradus lydekkerianus* (Cabra).

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**Abstract.** The accessory glands of reproduction in slender loris exhibit reduction in secretory activity following castration for 15 days and 30 days. Administration of androgens (testosterone—propionate and 5α dihydrotestosterone) to castrated animals had a differential effect in maintaining the biochemical parameters like citric acid and fructose.

**Keywords.** Loris Tardigradus lydekkerianus; slender loris; castration; androgen treatment.

## 1. Introduction

Of the several secretions of the male sex accessory glands of mammals, fructose and citric acid are important and are found in large quantities in the semen. Their synthesis and secretion are entirely regulated by the androgens secreted by the testis (Price and William Ashman 1961; Prasad *et al* 1973a,b). Amongst the non-human primates, besides the monkey (Dinakar *et al* 1974a,b) not much information about the prosimian primates is available. The present investigation deals with the secretions and their androgen control in the accessory sex glands of the male slender loris, *Loris tardigradus lydekkerianus* (cabra).

## 2. Materials and Methods

Lorises, also known by the native names as ceylon sloths, sherminds, unhappen luma and thevangu, occur in some of the forested areas of Southern India and Ceylon from sea level to an elevation of about 1800 inches. Lorises are found mostly in casurina, tamarind and pongamia trees, quite close to rural habitation. They were collected from the forests around Bangalore, brought to the laboratory, maintained in cages and provided with food and water.

Adult male lorises used for the experimental groups were divided into nine groups of 3/5 animals in each (table 1). They were castrated by opening the inguinal passage under asceptic conditions using sodium pentobarbitone (Nembutol, Abbot laboratories) as anesthetic. Through a small incision in the inguinal passage, the testis were exposed, carefully freed from the epididymis after ligating the efferent ductules and testicular blood vessels without damaging the vascular supply to the epididymis and ductus deferens. The different doses of testosterone propionate (TP) used were 125 μg, 250 μg

**Table 1.** Changes in the weights (mg) of the accessory glands in the castrated, testosterone propionate (TP), and 5α-dihydrotestosterone (5α-DHT) treated lorises (Mean ± S.E.)

| Treatment | Body weight (g) | Seminal vesicles | Prostate gland | Cowper's glands |
|---|---|---|---|---|
| Intact control | 279(5) | 293·4 ± 6·3 | 75·4 ± 14·9 | 104·0 ± 3·2 |
| ♂ for 15 days | 252(3) | 195·4 ± 9·0$^c$ | 55·2 ± 7·0$^c$ | 85·4 ± 12·4$^c$ |
| ♂ for 30 days | 260(3) | 192·1 ± 4·8$^b$ | 53·3 ± 3·6$^b$ | 83·9 ± 11·1$^b$ |
| ♂ + 500 μg of TP/day | 241(5) | 315·0 ± 38·4$^b$ | 137·0 ± 9·0$^b$ | 122·7 ± 9·2$^b$ |
| ♂ + 250 μg of TP/day | 283(5) | 393·0 ± 48·0$^c$ | 71·7 ± 3·5$^a$ | 174·1 ± 3·1$^b$ |
| ♂ + 125 μg of TP/day | 259(5) | 292·2 ± 89·7$^a$ | 70·7 ± 19·3$^a$ | 114·5 ± 13·7$^a$ |
| ♂ + 250 μg of DHT/day | 285(5) | 299·1 ± 25·6$^a$ | 71·3 ± 10·5$^a$ | 117·0 ± 18·0$^a$ |
| ♂ + 50 μg of DHT/day | 284(5) | 286·0 ± 20·4$^a$ | 56·5 ± 6·4 | 116·0 ± 21·5$^a$ |
| ♂ + 5 μg of DHT/day | 272(5) | 216·6 ± 10·4 | 58·9 ± 3·8 | 74·3 ± 7·9 |

Figures in parentheses represent the number of animals used in each group. Levels of significance compared with intact control animals. $^aP < 0{\cdot}05$; $^bP < 0{\cdot}01$; $^cP < 0{\cdot}05$. ♂ = Castrated.

and 500 μg/day and of 5α- dihydrotestosterone (5α-DHT) were 5 μg, 50 μg and 250 μg/day. The androgens were administered subcutaneously in 0·2 ml of olive oil daily for 15 days from the next day of castration. Intact and castrated lorises of comparable body weights received the vehicle only and they served as controls.

The lorises were autopsied 24 hr after the last injection; one castrated group without androgen treatment was autopsied 30 days after castration. The accessory glands were removed, cleaned of fat and connective tissue and weighed to the nearest 0·1 mg in a torsion balance; fructose was estimated in the accessory glands by the method of Roe (1934) as modified by Linder and Mann (1960) and citric acid by the method of Ettinger *et al* (1952). The results were analysed statistically using student's *t* test.

## 3. Results

Response of the accessory glands of reproduction in the lorises to the administration of different dosages of TP/5α-DHT in terms of change in weight is shown in table 2. Changes in the content and concentration of fructose in the accessory glands resulting from castration and androgen replacement are shown in table 3. In loris, fructose is secreted by the prostate gland in maximum quantities. The seminal vesicles and Cowper's glands also secrete some quantities of the fructose. Castration for 15 days resulted in significant decrease of fructose concentration in the accessory glands and a further decrease was observed in 30-day castrated animals. More than 500 μg of TP or 5 μg of 5α-DHT/day maintained the fructose concentration in the seminal vesicles but there was a three-fold increase in the fructose concentration in the animals treated with 50 μg of 5α-DHT. Cowper's gland required 250 μg of TP or more than 50 μg of 5α-DHT for the maintenance of normal fructose concentration and in the prostate gland, it required 250 μg of TP/5α-DHT/day.

Changes in the levels of citric acid in the accessory glands of loris resulting from castration and androgen replacement is shown in table 3. Citric acid is mainly secreted

**Table 2.** Effect of administration of TP and DHT on the content and concentration of fructose in the accessory glands of the castrated slender loris (Mean ± S. E)

| | Seminal vesicles | | Prostate glands | | Cowper's glands | |
|---|---|---|---|---|---|---|
| | A | B | A | B | A | B |
| Intact control | 199·0 ± 2·8 | 69·4 ± 15·6 | 267·9 ± 99·2 | 140·3 ± 51·9 | 132·5 ± 11·7 | 79·2 ± 50·6 |
| ♂ for 15 days | 149·6 ± 20·3[d] | 42·5 ± 22·7[d] | 137·7 ± 1·3[d] | 84·0 ± 15·3[d] | 94·5 ± 6·6[d] | 54·8 ± 6·0[d] |
| ♂ for 30 days | 34·1 ± 4·4[d] | 16·3 ± 12·3[d] | 62·2 ± 9·4[d] | 18·1 ± 8·0[d] | 50·6 ± 19·5[d] | 30·8 ± 15·0[d] |
| ♂ + 500 μg of TP/day | 300·0 ± 21·4[b] | 131·8 ± 28·8[a] | 302·9 ± 151·9[a] | 126·2 ± 33·2 | 267·6 ± 121·3[a] | 66·2 ± 12·7[c] |
| ♂ + 250 μg of TP/day | 241·2 ± 137·1[d] | 64·1 ± 14·0[a] | 218·2 ± 94·8 | 87·5 ± 24·0 | 157·4 ± 51·6[a] | 63·0 ± 15·9 |
| ♂ + 125 μg of TP/day | 214·8 ± 74·3 | 52·9 ± 5·7 | 97·1 ± 43·7 | 85·3 ± 29·9 | 108·2 ± 6·6 | 52·0 ± 7·9 |
| ♂ + 250 μg of DHT/day | 267·2 ± 50·2[b] | 179·1 ± 95·0[a] | 690·4 ± 318·2[a] | 347·9 ± 88·0[a] | 282·0 ± 37·2[b] | 288·9 ± 68·0[a] |
| ♂ + 50 μg of DHT/day | 213·0 ± 82·4[a] | 162·2 ± 59·3[a] | 164·5 ± 45·7 | 68·5 ± 2·6 | 144·3 ± 25·7[a] | 69·9 ± 24·8[c] |
| ♂ + 5 μg of DHT/day | 203·0 ± 16·6[a] | 55·7 ± 29·3 | 131·3 ± 47·4 | 54·8 ± 12·9 | 51·6 ± 19.5 | 23·8 ± 5·4 |

Levels of significance compared with intact control animals. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P \leqslant 0.05$, $^{d}P < 0.05$ A = μg of Fructose/organ, B = μg of Fructose/100 mg of tissue.

**Table 3.** Effect of administration of TP and DHT on the content and concentration of citric acid in the accessory glands of the slender loris (mean ± S. E)

| | Seminal vesicles | | Prostate glands | | Cowper's glands | |
|---|---|---|---|---|---|---|
| | A | B | A | B | A | B |
| Intact control | 501·3 ± 282·3 | 430·1 ± 42·0 | 478·5 ± 136·6 | 375·8 ± 44·4 | 373·6 ± 223·1 | 195·0 ± 32·8 |
| ♂ for 15 days | 174·6 ± 115·9[d] | 129·5 ± 88·0[d] | 166·1 ± 20·7[d] | 134·9 ± 46·8[d] | 133·7 ± 22·7[d] | 65·0 ± 0·0[d] |
| ♂ for 30 days | Absent | Absent | Absent | Absent | Absent | Absent |
| ♂ + 500 μg of TP/day | 481·5 ± 39·4[c] | 481·9 ± 39·4[a] | 131·3 ± 21·9 | 157·8 ± 0·0 | 302·0 ± 58·6 | 135·8 ± 2·1 |
| ♂ + 250 μg of TP/day | 231·1 ± 105·1 | 129·8 ± 0·0 | 73·4 ± 25·0 | 126·2 ± 10·4 | 58·6 ± 12·1 | 126·3 ± 0·0 |
| ♂ + 125 μg of TP/day | 110·9 ± 51·4 | 118·0 ± 7·0 | 46·3 ± 12·5 | 92·9 ± 16·0 | 42·5 ± 20·2 | 15·1 ± 2·0 |
| ♂ + 250 μg of DHT/day | 799·6 ± 217·2[b] | 143·8 ± 38·0 | 378·1 ± 26·6 | 353·6 ± 23·0 | 225·9 ± 24·9 | 310·4 ± 87·6[a] |
| ♂ + 50 μg of DHT/day | 437·9 ± 264·0 | 134·6 ± 94·9 | 167·9 ± 1·9 | 191·5 ± 9·0 | 101·3 ± 32·1 | 232·4 ± 58·8[a] |
| ♂ + 5 μg of DHT/day | 305·8 ± 55·3 | 71·6 ± 43·1 | 166·7 ± 1·5 | 107·8 ± 32·8 | 41·8 ± 10·4 | 78·6 ± 3·8 |

Levels of significance compared with intact controls [a] $P < 0.05$, [b] $P < 0.01$, [c] $P \leqslant 0.05$, [d] $P < 0.05$ A = μg of citric acid/organ. B = μg of citric acid/100 mg of tissue.

by the seminal vesicles in maximum quantities. Like fructose, citric acid in the accessory glands also depends on the androgens. Prostate and Cowper's glands also contribute citric acid to the seminal plasma in the slender loris. In the 15-day castrated animals the concentration of citric acid had decreased significantly and it was completely absent from the glands of 30-day castrated animals. Requirement of androgens for the maintenance of citric acid concentration by the different glands varied. 500 $\mu$g of TP or 250 $\mu$g of 5$\alpha$-DHT maintained the citric acid concentration in the seminal vesicles while more than 500 $\mu$g of TP or more than 250 $\mu$g of 5$\alpha$-DHT was required for the maintenance of the citric acid concentration in the prostate and Cowper's glands.

## 4. Discussion

A decrease in the weights and secretory activity of the male reproductive system of slender loris has been observed following castration (Manjula and Kadam 1980) for 15 days. These tissues showed a further significant decrease in weight after 30 days of castration. A similar decrease has been observed in laboratory rodents (Price and William Ashman 1961; Mann 1964; Gupta *et al* 1974), hamster (Ortiz 1953; Karkun *et al* 1974), and rhesus monkey (Dinakar *et al* 1974a,b).

Fructose has been found in the semen of man, monkey, ram, guinea pig, rat and other mammals (Mann 1964). Androgen is necessary for the production of fructose (Mann 1964; Gassner and Hopwood 1952) in the male reproductive system. Fructose is secreted mainly by the prostate gland in the slender loris, but the seminal vesicles and Cowper's gland also contribute. In the castrated guinea pig and rats (Harold and Clara 1955a,b) a lower dosage of testosterone (0·5$\mu$g) was sufficient to restore the fructose content. In the accessory glands of mouse, fructose level had decreased significantly following castration for 3 days (Mawhinney *et al* 1970). In the castrated loris, a higher dose of androgens, 500 $\mu$g of TP and 250 $\mu$g of 5$\alpha$-DHT, was required by the prostate and Cowper's glands to restore the secretory activity in 15-day castrated animals. The seminal vesicles required 250 $\mu$g of TP and a lower dose of 5$\alpha$-DHT (5 $\mu$g). Thus 5$\alpha$-DHT appears to be more potent than TP in restoring and maintaining the secretory activity of the accessory glands. Similarly in the rhesus monkey, the seminal vesicles required four implants of 5$\alpha$-DHT and eight implants of testosterone (Dinakar *et al* 1974a,b). The content of fructose in dorsolateral prostate was maintained at control levels with 500 $\mu$g of TP or 250 $\mu$g of 5$\alpha$-DHT in rats (Gupta *et al* 1974). In loris, as in monkey and rat, 5$\alpha$-DHT is more potent, whereas in hamster (Karkun *et al* 1974) testosterone appears to be more potent. This shows that testosterone and 5$\alpha$-DHT were not equipotent in stimulating the production of fructose in the slender loris.

Citric acid is also an androgen-dependent parameter in the accessory glands. Most of the higher mammals (Barron and Huggins 1946a,b; Humphrey and Mann 1948, 1949; Schersten 1929, 1936), have a high concentration of citric acid in semen. A direct relationship exists between the plasma testosterone and citric acid in seminal plasma in man and it is a good index of androgen secreted by the gonads (Dondero *et al* 1972).

Decrease in the levels of citric acid in the seminal vesicles of rabbit following castration has been observed (Humphrey and Mann 1948) and it reappears following administration of testosterone. In the accessory glands of loris, castration for 15 days resulted in a significant decrease in the levels of citric acid and it was completely absent

in the seminal vesicles of 30-day castrated animals, prostate and Cowper's glands also showed significant reduction in the citric acid content. In the castrated loris, seminal vesicles required a low dosage of 5α-DHT (50 μg) and a high dosage of TP (500 μg) for the maintenance of citric acid content. The prostate and Cowper's glands required more than 500 μg of TP or 250 μg of 5α-DHT, of both the androgens, 5α-DHT is more potent in low doses than TP in maintaining citric acid content in the seminal vesicles thus indicating greater sensitiveness to low doses of 5α-DHT, than to testosterone itself.

Androstendione and 5β-androsten 3-01-17-one have been found in the seminal vesicles after administration of testosterone (Harding and Samules 1962; Kinson 1962; Evalkar *et al* 1964) and 5α-androstane 17-01-3-one is found bound to the macromolecular fraction of the homogenate of the seminal vesicles in the ventral prostate of the rat (Unjem and Tveter 1969). In the slender loris the results indicate that 5α-DHT is more potent than TP and hence it is possible that similar DHT binding sites to macromolecules are present in the accessory glands of loris also.

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# Community and succession of the round-head borers (Coleoptera: Cerambycidae) infesting the felled logs of White Dhup, *Canarium euphyllum* Kurz

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**Abstract.** Succession and assemblage of the round-head borers infesting *Canarium euphyllum* Kurz have been described. The borer pests have been categorized into two major groups. The first group includes the borers of standing trees and freshly felled logs, while the second constitutes the species infesting the dead logs after some seasoning. Even among the borers belonging to each of these groups, there is a clear sequence of succession of species infesting the logs depending upon the period lapse after felling and subsequent conditions of the host.

**Keywords.** *Canarium euphyllum* Kurz; Cerambycidae; community and ecological succession; overlapping of species

## 1. Introduction

It is well known that various stages of a gradually disintegrating log or tree harbour particular groups of insects, some in the living or dying parts, some in the recently dead material, others in the moderately dry wood and still others in the wood which has seasoned for several years (Shelford 1913; Adams 1915). Round-head borers are generally recognized as the 'second group of invaders', preceded by the bark- and ambrosia-beetles (Scolytidae and Platypodidae), infesting the host-material when it is still green (Howden and Vogt 1951; Sen-Sarma 1983; Maiti *et al* 1983).

However, the round-head borers themselves exhibit a clear succession of species in infesting the wood, depending upon its progressive drying and decaying due to many physico-chemical and biological factors (Khan 1984). The present communication summarizes the succession and assemblage of the round-head borers infesting the logs of different period lapse after felling of an economically important timber yielding plant, *Canarium euphyllum* Kurz.

## 2. Materials and methods

The work was carried out in several localities of Andaman and Nicobar islands (latitude 6°40′ to 13°41′ N and longitude 92°11′ to 94°10′ E) from 1978 to 1981. Observations were made in different forest areas, timber extraction and logging centres, timber depots, wood-based industries, etc. The frequency of infestation of different round-head borers attacking the standing trees and felled logs of *C. euphyllum* was regularly

recorded. The period lapse after the felling of the logs was also recorded, as could be ascertained from the authorities of the Forest Department. Population density of different stages of borers per log or tree was determined by random samplings from unit-area of 25 cm × 25 cm.

In all these localities, several batches of trees of *C. euphyllum* (girth varying from 1·38 m to 2·87 m) were felled and were cut into billets of uniform size. The number of adult beetles active on each of these logs and the number of females observed ovipositing thereon on the subsequent days were recorded. For each day, the egg-nitches made by females on each logs were also counted and were marked with flags. The infested logs were dissected every week till the entire development of the invader species was completed. The population density of the developmental stages was also determined. The number of emerged adults of different species was determined from the emergence-hole counts.

Insectary studies were conducted at Port Blair using portions of the infested logs collected from both the extensive and intensive study areas. They were kept in spacious galvanized iron cages (1 m × 50 cm × 50 cm) and were examined each day between 1000 and 1100 hours IST, prior to which the newly emerged adults were collected. Insectary studies permitted observation of the characteristic features of larval galleries, pupal chambers and exit-holes, duration of development and periods of adult emergence. These were finally correlated with those collected from the field.

The immature stages of different species were identified following Khan and Maiti (1983).

## 3. Results

### 3.1 *Categories of the round-head borers and their succession*

The round-head borers assembled in standing trees and felled logs of *C. euphyllum* are listed in table 1. It is worthy of mention that this tree is the most preferred host of round-head borers and harbours nearly 10% of the total cerambycid-fauna of these islands. About 36% of these borers attack unhealthy or dying trees in the forest stands. These species are *Plocaederus obesus* Gahan, *Pharsalia* (*Cycos*) *subgemmata* (Thomson), *Acalolepta rusticator* (Fabricius) and *Olenecamptus bilobus* (Fabricius).

**Table 1.** Grouping of round-head borers on the basis of their infestation to standing trees and logs of different period lapse after felling.

| Group-I | Group-II |
|---|---|
| Infesting living trees and freshly felled logs of 15 to 40 days after felling | Infesting recently dead trees and logs after 40 to 80 days of felling |
| *Plocaederus obesus* Gahan, *Epepeotes* sp., *Pharsalia* (*Cycos*) *subgemmata* (Thomson), *Acalolepta andamanica* (Breuning), *Acalolepta rusticator* (Fabricius), *Batocera rufomaculata* var. *andamana* Thomson, *Olenecamptus bilobus* (Fabricius) | *Clyzomedus annularis* Pascoe, *Coptops rufa* Thomson, *Macrochenus atkinsoni* Gahan and *Marmaroglypha nicobarica* Redtenbacher |

On the basis of the community composition on the living trees and logs of different period lapse after felling, Khan (1984) categorized the round-head borers of these islands into four major groups. In the present study, two of these groups have been recognized to infest the living trees and felled logs of *C. euphyllum* (table 1).

Figure 1 (a,b) presents the succession and assemblage of the round-head borers belonging to each of these two groups. It clearly shows that most of the borers belonging to group-I infest the logs within the period from about two weeks to two months of felling and their populations in the host attain a maximum density within this period. After that, the population declines gradually as a result of the completion of development and mortality due to parasites, predators, competitors, diseases and other environmental factors. The representatives of group-II, on the other hand, attack the logs after about five weeks of felling. Their infestation continues upto about $3\frac{1}{2}$ months of felling (figure 1a).

### 3.2 *Succession and assemblage of species*

Further observations suggest that even among the borers belonging to each of the two groups, there is a clear sequence of succession of species infesting the logs depending

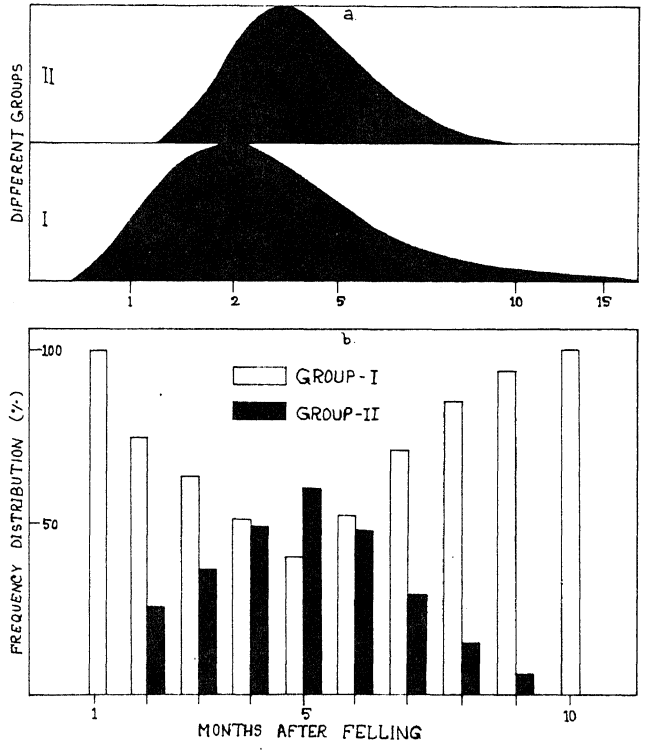


**Figure 1.** Succession and assemblage of round-head borers belonging to different groups in the logs after different period lapse of felling; **(a)** infestation and continuation of existence; **(b)** frequency distribution.

upon the period lapse after felling and subsequent conditions. A brief account of this phenomenon is furnished below.

3.2a *Group-I:* The data on succession and assemblage of the species constituting this group are presented in figure 2 (a,b). Figure 2a presents the cumulative number of eggs and early instar larvae harboured per unit-area of the bark and wood-surface of the logs. *Acalolepta rusticator* has been recognized as the first cerambycid to infest the logs within 15 to 30 days of felling, while *Plocaederus obesus* is the last one invading after 30 to 45 days. Other species, *viz Olenecamptus bilobus*, *Acalolepta andamanica*, *Epepeotes* sp., *Pharsalia* (*Cycos*) *subgemmata* and *Batocera rufomaculata* var *andamana* infest the logs in succession between the two extremities (figure 2a).

Almost all the species in general exhibit a definite period of infestation. Amongst them *Plocaederus obesus* has the longest period of existence inside the wood, while *Acalolepta rusticator* and *Epepeotes* sp. have the shortest life cycle. Other species continue their destructive activities inside the wood for a varying period from 5 to 8 months (figure 2b).

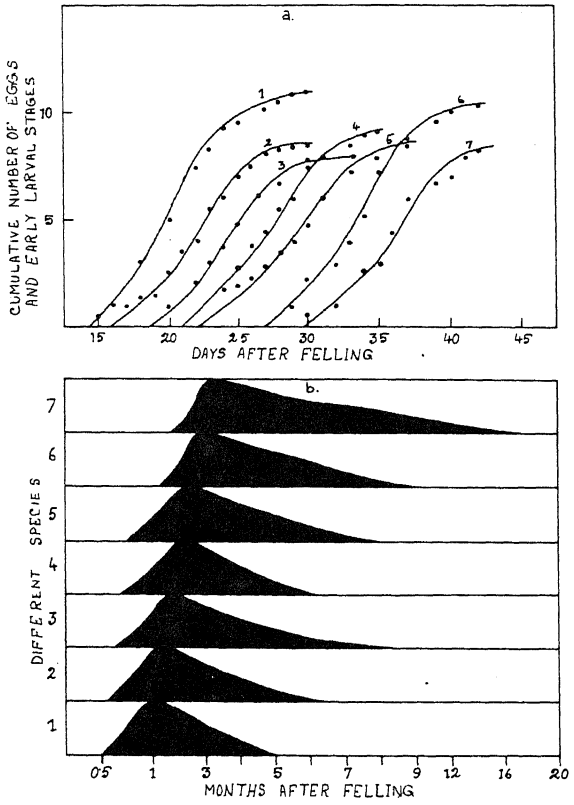


**Figure 2.** Successive infestation (**a**) and distribution and existence (**b**) of round-head borers belonging to group-I; 1. *Acalolepta rusticator*, 2. *Olenecamptus bilobus*, 3. *Acalolepta andamanica*, 4. *Epepeotes* sp, 5. *Pharsalia* (*Cycos*) *subgemmata*, 6. *Batocera rufomaculata* var *andamana* and 7. *Plocaederus obesus*.

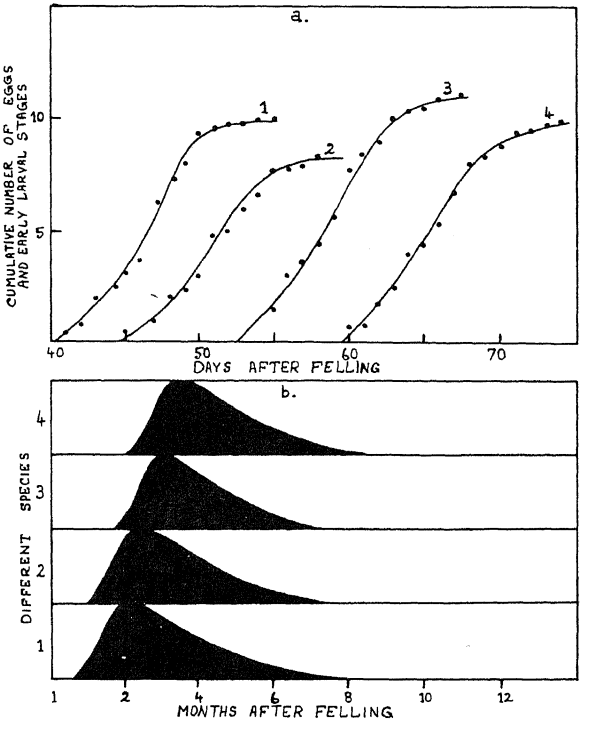


**Figure 3.** Successive infestation (**a**) and distribution and existence (**b**) of round-head borers belonging to group-II; 1. *Macrochenus atkinsoni* 2. *Marmaroglypha nicobarica* 3. *Clyzomedus annularis* and 4. *Coptops rufa.*

3.2b *Group-II:* Figure 3 (a,b) shows the succession and assemblage of the borers constituting Group-II. All these species exhibit a similar pattern of succession as shown by those of the group-I. However, in this group the sequence of succession of species is more pronounced. *Macrochenus atkinsoni* is the first invader of the group (infesting after 40 to 55 days of felling), followed by *Marmaroglypha nicobarica* (stepping in after 45 to 60 days). *Coptops rufa*, on the other hand, is the last invading the logs of more than two months old, being preceeded by *Clyzomedus annularis* (figure 3a).

Amongst these borers, *Macrochenus atkinsoni* and *Coptops rufa* exist inside the wood for a longer period of more than 9 months (figure 3b). *Clyzomedus annularis* is the least survivor (survival period 6 months).

## 4. Discussion

The results clearly reveal that the cerambycid-community colonizing in the felled logs of *C. euphyllum* is rather poor, and this is expected in islands which support depauperate faunal elements. Moreover, the period between felling, extraction and storage is too short to permit development of climax community. The logs situated inside forests, normally harbour a higher number of species with higher population densities than in those located in non-forested areas for the same duration.

On the other hand, the existence of different round-head borers almost simultaneously appears to be influenced by the microhabitat differences among themselves. Thus, *Rhaphipodus* (*s. str.*) *andamanicus* is dominant in and around the pith, *Batocera rufomaculata* var *andamana* inside comparatively upper layers of the heartwood, *Pharsalia* (*Cycos*) *subgemmata* inside deeper layers of the sapwood and outer heartwood, *Acalolepta andamanica* inside the sapwood, while *Coptops rufa* is restricted to inside the bark. Such co-existence is obviously due to availability of different food-material in different zones of the wood. Thus, a bark or superficial wood borer feeds mostly on the soluble sugars and simple carbohydrates, while a deep heartwood borer feeds on the cellulose, as pointed out by Beeson and Bhatia (1939).

With regard to the succession, *Acalolepta rusticator* is the first to invade, while *Coptops rufa* is the terminal one. The invasion of these two species is bridged up by succession of other invaders. However, an overlapping of their infestations is not very uncommon. Such overlapping is more pronounced among the species constituting the group-I than in those belonging to the group-II. The higher frequency of overlapping among the members of the group-I may, at least in part, be attributed to the higher degree of competition for suitable oviposition-sites as should normally be expected from a higher number of species.

Lastly, it is clear that round-head borers colonizing in the felled logs of *Canarium euphyllum* utilize dying or recently dead green host for oviposition, but complete their development at a period when the wood becomes comparatively drier. The advanced developmental stages are, thus, associated with the exploiting efficiencies of drier wood for their nourishment. It, therefore, appears that these borers requiring fresh tissues for their early immature stages, can continue their growth and development in the drier wood in the later phases of their life indicating an increase in their digestive power with the progressive age (Graham 1925).

## Acknowledgements

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# Behavioural responses in terms of feeding and reproduction in some grasshoppers (Orthoptera: Insecta)

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**Abstract.** Studies on the host preference in some acridids revealed sequentiality of feeding behaviour aided by various sensillae, the suitability of the host plant greatly depending upon the physical and chemical factors. Studies on acridids and pyrgomorphid species indicated that *Truxalis indica* feed exclusively on grasses, *Orthacris maindroni* only on dicot plants and *Atractomorpha crenulata* on both monocot and dicot plants. Significant variations in the quantitative food intake on diverse host plants during post-embryonic development were also evident. Distribution and density of diverse sensillae on the antennae, labrum, maxillary and labial palps as well as their role in food selection are documented. The impact of antennal and palpal ablation on host selection, the quantity of food intake as well as the influence of diverse host plants on fecundity are discussed.

**Keywords.** Quantitative food utilization; sensillae; degree of preference; post embryonic development; fecundity; grasshoppers.

## 1. Introduction

Food selection in polyphagous insects is known to involve the cumulative effects of physical, chemical and sensory factors which act independently or simultaneously. Short-term feeding behavioural effects on host selection appears to be influenced by the secondary plant chemicals acting as phagostimulants and/or deterrents and long-term physiological effects are known to involve nutrition and antibiosis (Chapman and Bernays 1977). The role of sensillae in the maxillary and labial palps in food selection was suggested by Blaney and Chapman (1969) and Bernays and Chapman (1970, 1973), their numerical variations among acridids feeding on graminaceous and broad-leaved plants reflecting their role in food selection (Chapman and Thomas 1978). Available information on feeding and food selection of essentially graminivorous acridids relates to specific aspects concerning short term behavioural effects pertaining to quantitative food intake and utilization. Long term physiological effects involving growth and reproduction on diverse host plants as well as the role of sensillae in food discrimination/avoidance, especially in dicot feeding grasshoppers, appear meagre. For a further understanding of the food selection of grasshoppers, an attempt has been made to study the feeding and behavioural dynamics involving feeding and reproduction of the polyphagous pest species, *Atractomorpha crenulata* (Fabricius), *Orthacris maindroni* Boliver (Pyrgomorphidae) and *Truxalis indica* Boliver (Acrididae).

## 2. Materials and methods

*A. crenulata*, *O. maindroni* and *T. indica* were collected from the fields in and around Madras, reared in cages and provided with their respective host plants for

feeding. Nymphal stages were isolated and reared individually in separate cages as well as in glass chimneys. In order to find out the exact day of moulting, the pterothorax of the nymphs was marked with cutex. Mating pairs were isolated from the cages and provided with transparent plastic vials (8 × 12 cm) filled with loose wet soil for oviposition. The number of egg pods laid in the vials could be easily counted through the transparent wall of the vials. The plastic vials containing egg pods were covered with thin muslin to prevent the escape of the emerging nymphs. The emerging nymphs were counted and reared individually in different host plants. Increase in the weight of the nymphal stages while feeding on different host plants was recorded through the use of a monopan balance.

To calculate the quantitative food intake and food utilization, fresh host plants were weighed initially and kept in a flask containing water and 15 adults/nymphs were allowed to feed for 24 hr, another set of host plants of similar weight were kept as control simultaneously to find out the quantitative food intake by the insects. After 24 hr, both the experimental as well as the control host plants were weighed. In order to assess the loss of weight in experimental leaves due to the feeding damage, both the control and experimental leaves were desiccated for 7 days. The difference in the dry weight between the control and experimental plants indicated the quantity of food intake/number of insects. Excreta of the experimental insects were collected in polyethylene papers during 24 hr of experimental period and weighed immediately as well as after complete desiccation for 7 days. The difference in the quantity of food intake and the quantity of excreta indicated the quantitative food utilization for specific host plants.

For light microscopic observations, the labrum, labium, maxillae and antennae of the grasshoppers were dissected in insect ringer, washed with distilled water, incubated in 1 % boiling KOH for 5 min, washed well in distilled water and mounted in glycerine. For obtaining scanning electron microscope pictures, the labrum, labium, maxilla and antenna were washed well in 80 % ethanol, dried and fixed on aluminium stubs using a double adhesive tape and coated with gold using an ion coater. The coated materials were examined in a Hitachi SEM and photographed using an attached Mamia camera.

For quantitative estimations of proteins, carbohydrates, total lipids and phenols, the standard methods of Lowry *et al* (1951), Dubois *et al* (1956), Kok (1971) and Bray and Thorpe (1954) respectively were followed.

## 3. Results

### 3.1 *Role of sensillae in feeding behaviour*

3.1a *Sensillae of the inner side of the labrum:* Studies by Chapman and Thomas (1978) on the distribution of sensillae on the inner side of labrum indicated the occurrence of diverse sensillae arranged in the 'alpha' and 'beta' tract in addition to the A-1 to A-3 sensillae. Further studies on the distribution of sensillae of *A. crenulata*, a dicot feeder and the *T. indica*, a monocot feeder indicated further variation in the distribution and the nature of sensillae. Hence as a convenient measure the sensillae of the innerside of the clypeo-labrum was further differentiated as S1 and S2 of the inner side of the distal margin of clypeus. In addition to the alpha and beta tracts of the labrum another tract was also distinguished, referred to here as gamma tract, with a

higher number of sensillae. All the sensillae of the gamma tract appeared to be of the campaniform type arranged in a 'V' shaped fashion. A-1 sensillae were not noticed both in light microscopic as well as in SEM studies. Further recognition of the sensillae of the alpha tract was made into the campaniform alpha-B sensillae (S-4a) and (S-4b) sensillae. In the beta tract all the sensillae appeared large, consisting of campaniform sensillae (S-5) and trichoid sensillae (S-6). In the free distal margins, another group of sensillae designated as S-7 was also recognised. In the inner side of the labrum, A-2 and A-3 sensillae alone were noticed (figure 8).

In *T. indica*, a similar distribution of sensillae was evident with the wide beta tract covering the entire distal margin of the labrum. The type S-7 sensillae were unrecognisable and numerical differences in the sensillae were also evident as compared to that of the other species studied (figure 8).

3.1b *Sensillae of maxillary and labial palps:* Studies by Haskell and Schoonhoven (1969) and Blaney (1974, 1975) indicated the presence of trichoid sensillae which are mechanical receptors as well as another group of chemosensory trichoid sensillae.

During the post-embryonic development of *A. crenulata* from the nymphs to the adults, a gradual increase in the number of sensillae at the palpal apices as well as on the surfaces of both maxillary and labial palps was observed (figures 1 and 2). A bottom collar is characteristic of the short trichoid apical sensillae, occurring at the apices of both maxillary and labial palps, the entire surface of the maxillary and labial palps bearing a number of trichoid and campaniform sensillae (figure 7).

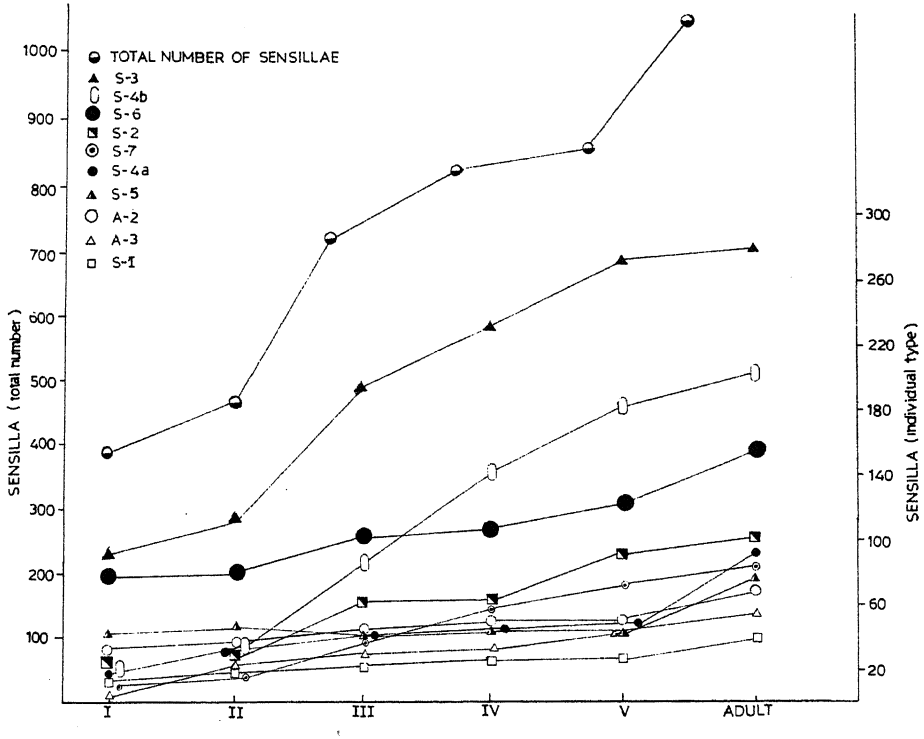


**Figure 1.** Numerical increase of sensillae on the inner side of the labrum of *A. crenulata* during post-embryonic development.

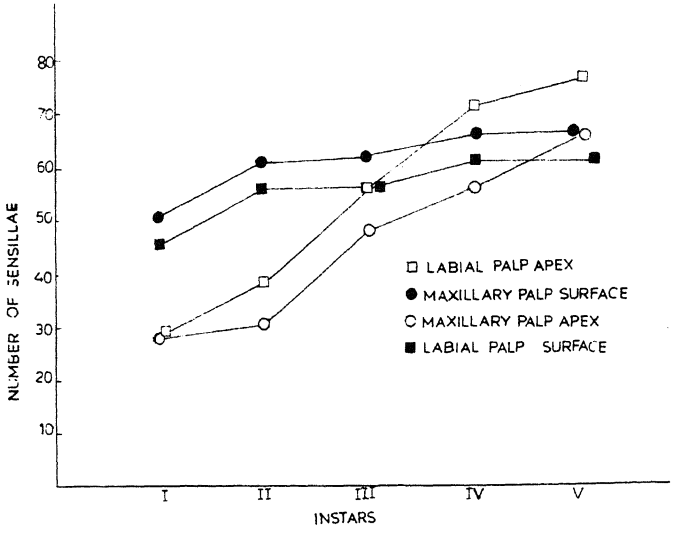


**Figure 2.** Numerical increase of sensillae on the maxillary and labial palps of *A. crenulata* during post-embryonic development.

3.1c *Numerical increase of sensillae during post-embryonic development:* A consistent increase in the total number of sensillae of the labrum was evident during post-embryonic development of *A. crenulata* (figure 2), the rate of increase of each type of sensillae varying considerably. A significant increase in the sensillae types S-4b, S-6 and S-3 to the extent of about 3–4 times was observed, while others like S-2, S-4a, S-7, S-5, A-2; A-3 and S-1 showed a gradual increase as they reached the adult stage. S-2 sensillae appeared to be numerically identical being constant during the first and second nymphal stages, increasing in numbers during the third, retaining the same number in the fourth and increasing again in the fifth stage nymphs.

Similarly the number of sensillae at the apices of the maxillary and labial palps also increased during post-embryonic development, with the trichoid sensillae on both palps being 22–27 and 24–30 in the first nymphs and 34 and 38 in the second nymphs respectively. Further increase in the number of sensillae was evident in the other stage also bearing 47 and 55, 57 and 72, 67 and 77 respectively in the maxillary and labial palps of 3rd, 4th and 5th instar nymphs (figure 2).

3.1d *Influence of sensory structures in the discrimination of host plants:* The antennae, maxillary and labial palps, labrum and hypopharynx are known to play a key role in food selection, aided by visual host location. To the control individuals, when nine host plants were offered simultaneously, the quantitative food intake in terms of dry weight consumed was found to be more in *Ricinus communis* L. and *Dolichos lablab* L. and, *Prosopis spicigera* L and *Calotropis gigantea* R. Br were not consumed. One side antennectomised individuals showed a similar feeding range, but with a substantial reduction in the quantity of food intake. Total antennectomy resulted in the quantitative food intake becoming very much reduced. Palpectomy involving labial and maxillary palps individually or together reduced the feeding range to three plants

when nine plants were offered simultaneously, with the quantity of food intake being reduced. Labrectomy had no influence on the feeding range, except for a substantial decrease in food intake.

### 3.2 *Host range*

*Atractomorpha crenulata* is a recognised cosmopolitan polyphagous pest species feeding on diverse crop plants. Both the adults and nymphs feed on a variety of crop as well as weed plants belonging to both dicot and monocot plants. Screening of diverse host plants in and around the habitat of *A. crenulata* showed their ability to feed on the members of family Euphorbiaceae, Solanaceae, Fabaceae, Poaceae, Amarantaceae and Asclepidaceae, their incidence being very high (more frequent) on the dicot plants especially by the nymphal stages. Screening of these host plants for various nymphal instars showed that they preferred Euphorbiaceae, Fabaceae, Amarantaceae and other dicot plants like *Clerodendrum* sp. However their incidence was low on monocots especially on the members of the family Poaceae. Though *A. crenulata* is on record as occurring on the members of the families Asclepiadaceae, Cucurbitaceae, Verbanaceae they avoided these plants for feeding. The present observations indicate that crop plants like *Ricinus communis* L., *Solanum melongena* L., *S. torvum* Sw., *Dolichos lablab* L., *Panicum maximum* Jacq., *Clerodendrum* sp., and *Achyranthes aspera* L., form the major food plants for the growth of *A. crenulata.* Though the nymphs have a wide host range, *R. communis, D. lablab* and *A. hypogaea* appear to be the preferred host plants.

The pyrgomorphid, *Orthacris maindroni*, in particular their nymphs fed exclusively on dicot plants like *R. communis*, *D. lablab*, *S. melongena*, *S. trilobatum*, *C. gigantea*, *Clerodendrum* sp. and *P. spicigera* exhibiting a preference within their range of host plants, to *R. communis* and weeds like *Clerodendrum* sp. They are polyphagous, feeding on the members of the families of Euphorbiaceae, Fabaceae, Solanaceae, Asclepidaceae, Vernoniaceae, Bignoniceae and Mimoseae and their occurrence on *C. gigantea* appeared only more casual.

*Truxalis indica* feeds exclusively on monocots especially on *Cynodon dactylon* Pers. (Poaceae), *Cyperus rotundus* L. (Cyperaceae), *Carex* sp.

### 3.3 *Host preference and food plant selection*

Host preference of *A. crenulata* on the basis of quantitative food intake indicated that when seven preferred host plants *viz R. communis, S. melongena, D. lablab, P. maximum, S. trilobatum* and *Clerodendrum* sp were provided, quantitative food intake was more in *R. communis, D. lablab* and *S. melongena.* When the same host plants were offered individually, quantitative food intake was high among *R. communis, S. melongena* and *D. lablab,* and comparatively low in the other food plants. Simultaneous offering of the three host plants showed increased preference to *R. communis* and *D. lablab,* the former being more preferred than the latter.

Similar variation in the quantitative food intake of different nymphs on either host plants was also evident, with the first instar nymphs tending to feed on six out of eight host plants offered, indicating a distinct preference towards *R. communis* than to *D. lablab.* But the second stage nymphs showed a preference for all the six hosts offered

with a higher quantitative food intake in respect of *R. communis* and *S. melongena*. The third instar nymphs showed an increase in the host range, feeding on seven of eight hosts offered with a higher, but equal amount of food utilization on both *S. melongena* and *R. communis*, but lesser in *D. lablab*. Both fourth and fifth instar nymphs fed on all the eight host plants provided, a higher quantity of food intake being evident on *R. communis*, and very much less on *S. trilobatum* and *Oryza sativa* L (figure 3).

Individuals of *Orthacris maindroni* feed on a wide spectrum of dicot plants, exhibiting specific preference towards *R. communis* and to a lesser extent on *D. lablab* than the other host plants. Though they feed only on dicot plants, under experimental conditions it was possible to make them feed on monocot plants like *P. maximum*. *T. indica*, a monocot feeder showed a specific preference to *C. dactylon* and to lesser extent to other grasses and never fed on dicot plants. Though the adults of *O. maindroni* preferred *R. communis*, all the nymphal stages preferred to feed more on *Clerodendrum* sp, the adult showing a reduced preference.

### 3.4 *Quantitative food utilization*

In addition to the amount of food intake, the quantity of food utilized and the quantity of water excreted varied considerably while feeding on different host plants. When eight host plants were offered to *A. crenulata* individually, the amount of food consumed by 15 adults, as expressed in the form of dry weight was very high in *R. communis* (1,240 mg/15 adults), comparatively lower in *D. lablab* (780 mg/15 adults) and very low

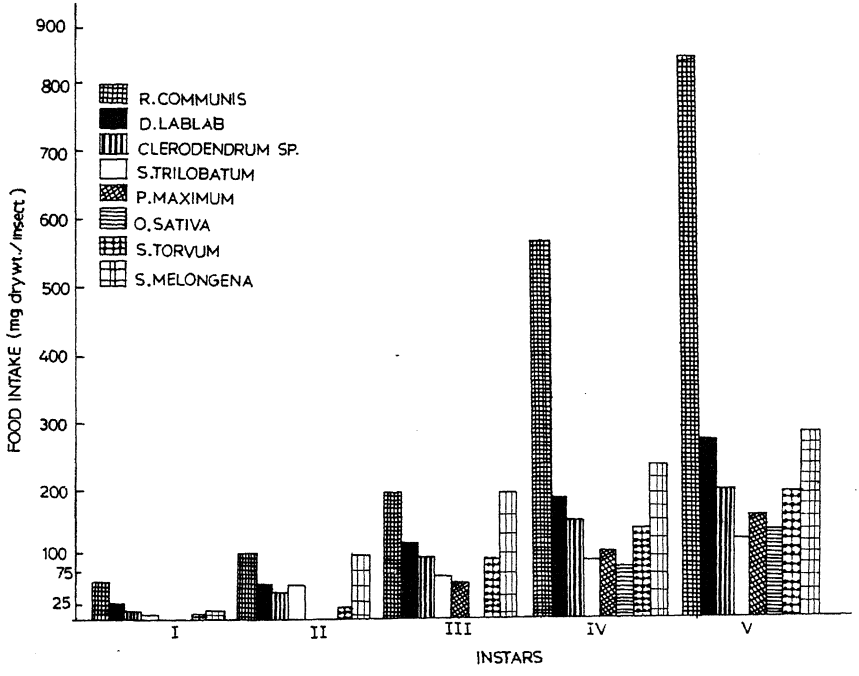


**Figure 3.** Quantitative food utilization by nymphal stages of *A. crenulata*.

in the case of *S. melongena*, *S. trilobatum*, *Clerodendurm* sp. and *P. maximum* (170–300 mg/15 adults). However the dry weight of food excreted was very high when fed on *R. communis* (430 mg/15 adults), as compared to (100–135 mg/15 adults) on other host plants. The amount of water consumed was also high (3,140 mg/15 adults) on *R. communis*, comparatively lower in *D. lablab* (1,040 mg/15 adults), and very low (286·1–664·6 mg/15 adults) in other host plants. Similarly water assimilation was also high for both *R. communis* (3·12 mg/15 adults) and *D. lablab* (1·01 mg/15 adults) than on other host plants (table 1).

Similar variations in the quantity of food utilization of diverse host plants were also noticed in *O. maindroni*, where the quantity of food consumed was high for *R. communis* (190 mg/15 adults) than on other host plants. When the same host plants were offered individually to *O. maindroni*, intake was very high in *D. lablab* (550 mg/15 adults) and comparatively lower (80–330 mg/15 adults) on other host plants like *S. melongena*, *S. trilobatum*, *P. maximum*, *C. gigantea*, *Clerodendrum* sp and *Bougainvilla* sp. The dry weight of excreta was very high (160–175 mg/15 adults) when fed on *D. lablab*, *P. maximum* and *C. gigantea*, and comparatively lower (60–135 mg/15 adults) on *S. melongena*, *S. trilobatum*, *R. communis*, *Clerodendrum* sp and *Bougainvilla* sp. Highest dry weight was assimilated when fed on *D. lablab* (375 mg/15 adults), comparatively lower on *Clerodendrum* sp and *R. communis* with 195 and 138 mg/15 adults respectively. In the other host plants it was considerably lower. Correspondingly the amount of water assimilated was equally high in *C. gigantea* (1,441 mg/15 adults), *D. lablab* (1,310 mg/15 adults) and *R. communis* (1,010 mg/15 adults) and in other plants it was considerably lower (table 2).

When a wide range of host plants were offered simultaneously to *Truxalis indica* there was a specific preference towards *Carex* sp (360 mg/5 adults), *Commelina* sp (200 mg/5 adults). However under continuous starvation they tend to feed on other dicot plants like *R. communis*.

### 3.5 *Life cycle*

*A. crenulata* being a polyphagous species, the different host plants seem to influence fecundity and the rate of post-embryonic development. Of the seven preferred host plants provided as nymphal diet, the development was quicker on both *R. communis* and *A. hypogaea*. On *Clerodendrum* sp, *S. torvum* and on *P. maximum* the duration of development was comparatively longer, whereas no development was observed when reared on *O. sativa* (Var. I.R. 50) (figure 4, table 3).

The rate of mortality of different nymphal instars as well as the number of nymphs attaining adult stage differ considerably on various host plants. Nymphal mortality in all host plants tested was very high in the first nymphal stage and was lower in the second with no mortality in the other stages. A very low nymphal mortality and a higher rate of adult emergence were evident in *R. communis* and *A. hypogaea*, whereas a very high nymphal mortality and low adult emergence were observed when reared on *Clerodendrum* sp and *P. maximum*. Interestingly mortality was 100% even in the first instar itself when reared on *O. sativa* (figure 5).

**Table 1.** Quantitative food utilization of *Atractomorpha crenulata* (adult)

| | Host Plants | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Ricinus communis* | *Solanum melongena* | *Dolichos lablab* | *Panicum maximum* | *Solanum trilobatum* | *Clerodendrum* sp. | *Calotropis gigantea* |
| Dry weight fed (mg) | 1240 | 300 | 780 | 170 | 200 | 208 | — |
| Dry weight excreted (mg) | 430 | 110 | 135 | 100 | 115 | 105 | — |
| Dry weight assimilated (mg) | 305 | 109 | 645 | 70 | 85 | 103 | — |
| Moisture content of leaf (%) | 71·51 | 68·90 | 57·07 | 63·95 | 58·37 | 67·34 | 79·4 |
| Amount of water consumed (mg) | 3140 | 664·6 | 1040 | 301.6 | 286·1 | 438·7 | — |
| Water excreted (mg) | 31·4 | 270 | 25·1 | 10·6 | 30·0 | 12·0 | — |
| Water assimilated (mg) | 3·12 | 637·6 | 1·01 | 290·9 | 256·1 | 426·7 | — |

**Table 2.** Quantitative food utilization of *Orthacris maindroni*

| | Host plants | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Ricinus communis* | *Dolichos lablab* | *Solanum melongena* | *Solanum trilobatum* | *Panicum maximum* | *Calotropis gigantea* | *Cleroden-drum* sp. | *Bougain-villea* sp. |
| Choice of 8 sp (dry wt. fed) (mg) | 190 | 110 | 28 | 24 | 59 | 72 | 75 | 5 |
| Dry wt. excreted when offered separately, dry wt. fed (mg) | 300 | 550 | 175 | 130 | 270 | 300 | 230 | 80 |
| Dry wt. excreted (mg) | 112 | 175 | 90 | 80 | 170 | 160 | 135 | 60 |
| Dry wt. assimilated (mg) | 138 | 375 | 85 | 50 | 100 | 140 | 95 | 20 |
| Moisutre content of the leaf (%) | 72·52 | 58·79 | 74·83 | 50·57 | 76·84 | 79·4 | 76·78 | 60·88 |
| Amount of water consumed (mg) | 1090 | 1330 | 695·2 | 262·9 | 1170 | 1460 | 990 | 205 |
| Amount of water excreted (mg) | 81·2 | 25 | 20 | 40·45 | 15 | 15 | 27 | 15 |
| Water assimilated (mg) | 1010 | 1310 | 675·2 | 222·5 | 1150 | 1,441 | 963 | 190 |

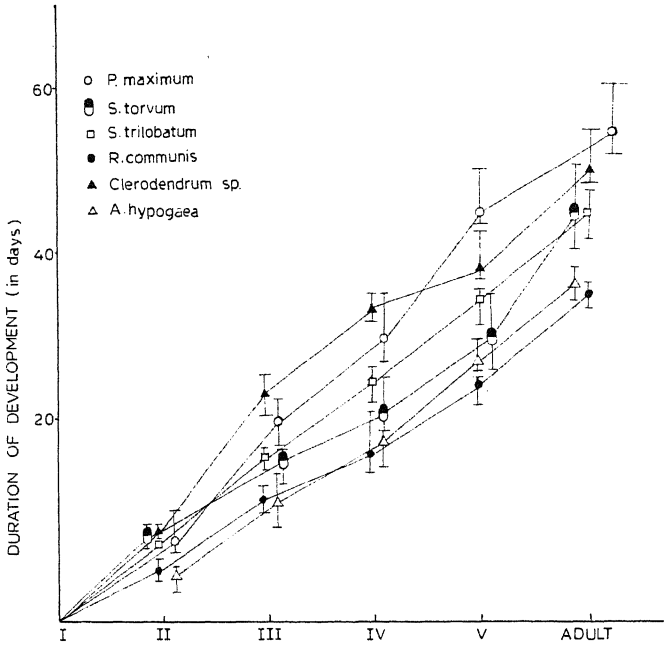


**Figure 4.** Developmental duration of *A. crenulata* when fed on different hosts.

### 3.6 *Weight increase during the post-embryonic development*

In addition to normal variations in the developmental rates as well as percentage of mortality of the nymphs when reared on different host plants there were considerable variation in the increase in the weight of the nymphs when reared on different hosts. The first instar nymphs when developed on *R. communis* showed a higher weight increase than on other hosts. From third instar nymphs onwards the weight increase became more pronounced in female nymphs than in male nymphs. Adults emerging from those nymphs fed on *R. communis* showed an increase in weight ranging from 75–90 mg in males and 220–250 mg in females, and a similar but comparatively lower weight increase was noticed on *A. hypogaea* with the weight increase of 168–260 mg in females and 65–70 mg in males respectively. Weight increase during the post-embryonic development was very low when reared on *P. maximum* where the emerging adults showed a very low weight, ranging from 60–65 mg and 110–115 mg in males and females respectively. On the other host plants the weight increase was moderate, ranging from 60–85 mg in males and 120–210 mg in the females (figure 6).

The first nymphal stage also differed considerably when developed on different host plants. Weight increase on both *R. communis* and *S. trilobatum* was higher (20–25 mg), and comparatively lower on *A. hypogaea* (15–20 mg). When developed on other host plants like *P. maximum*, *Clerodendrum* sp and *S. torvum* the weight increase was very low, ranging from 12–20 mg. Similarly the second stage nymphs also showed a higher weight increase when reared on *R. communis* and *A. hypogaea* (30–55 mg). A very low weight increase was observed on *S. torvum* (20–30 mg). In other host plants like *P. maximum*, *S. trilobatum* and *Clerodendrum* sp the weight increase was intermediate

**Table 3.** Impact of host plants on the life cycle and fecundity of *Atractomorpha crenulata* and *Orthacris maindroni*

| Host plants | Incubation period | No. of egg pods laid | No. of nymphs emerged from each pod | Total no. of nymphs emerged | Nymphal duration | | | | | Total duration |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | I | II | III | IV | V | |
| ***Atractomorpha crenulata*** | | | | | | | | | | |
| *Ricinus communis* | 35·4 ± 2·5* | 6·34 ± 0·6 | 56·7 ± 30·6 | 198·4 ± 12·6 | 5·7 ± 1·2 | 9·0 ± 1·0 | 6·7 ± 1·5 | 7·0 ± 1·0 | 8·0 ± 1·0 | 37·7 ± 5·9 |
| | (33–38) | (6–7) | (30–90) | (185–210) | (5–7) | (8–10) | (5–8) | (6–8) | (7–9) | (31–42) |
| *Panicum maximum* | 49·4 ± 1·5 | 2·70 ± 0·6 | 36·7 ± 15·3 | 94·0 ± 3·5 | 11·4 ± 2·0 | 12·7 ± 2·0 | 10·7 ± 1·5 | 14·7 ± 1·5 | 12·0 ± 2·0 | 63·4 ± 9·9 |
| | (48–51) | (2–3) | (20–50) | (90–96) | (9–13) | (11–15) | (9–12) | (13–16) | (10–14) | (52–70) |
| *Solanum trilobatum* | 43·4 ± 2·3 | 4·70 ± 0·6 | 40·4 ± 28·0 | 134·7 ± 4·0 | 8·7 ± 0·6 | 11·0 ± 1·0 | 8·7 ± 1·5 | 10·4 ± 1·5 | 10·0 ± 1·0 | 50·4 ± 6·4 |
| | (42–46) | (4–5) | (12–68) | (130–137) | (8–9) | (10–12) | (7–10) | (9–12) | (9–11) | (43–54) |
| *Solanum torvum* | 41·7 ± 9·7 | 3·70 ± 0·6 | 37·0 ± 22·5 | 120·4 ± 1·2 | 9·0 ± 1·7 | 9·0 ± 2·0 | 14·0 ± 2·7 | 11·0 ± 1·0 | 9·4 ± 2·5 | 99·4 ± 10·7 |
| | (31–50) | (3–4) | (14–59) | (119–121) | (7–10) | (7–11) | (11–16) | (10–12) | (7–12) | (37–56) |
| *Arachis hypogaea* | 38·7 ± 2·5 | 5·40 ± 0·6 | 53·0 ± 25·0 | 179·0 ± 3·6 | 7·0 ± 1·0 | 9·0 ± 2·0 | 7·7 ± 2·3 | 6·7 ± 1·4 | 9·4 ± 2·0 | 40·7 ± 9·3 |
| | (36–41) | (5–6) | (27–77) | (175–182) | (6–8) | (7–11) | (5–9) | (5–8) | (7–11) | (30–47) |
| ***Orthacris maindroni*** | | | | | | | | | | |
| *Clerodendrum* sp. | 39·7 ± 1·2 | 2·70 ± 0·6 | 22·7 ± 3·0 | 51·7 ± 2·0 | 9·4 ± 0·6 | 10·0 ± 2·0 | 4·0 ± 1·0 | 5·4 ± 1·5 | 11·4 ± 2·0 | 41·7 ± 7·6 |
| | (39–41) | (2–3) | (20–26) | (51–54) | (9–10) | (8–12) | (3–5) | (4–7) | (9–13) | (33–47) |
| *Ricinus communis* | 42·0 ± 1·7 | 2·00 ± 0·0 | 23·0 ± 4·0 | 41·4 ± 1·5 | 6·0 ± 1·0 | 9·0 ± 1·0 | 6·7 ± 0·6 | 7·0 ± 1·0 | 10·4 ± 2·5 | 40·1 ± 6·3 |
| | (40–43) | (2–2) | (19–27) | (40–43) | (5–7) | (8–10) | (6–7) | (6–8) | (8–13) | (33–45) |
| *Dolichos lablab* | 36·4 ± 1·5 | 1·00 ± 0·0 | 22·4 ± 1·2 | 40·7 ± 0·6 | 5·4 ± 1·2 | 7·4 ± 0·6 | 5·7 ± 0·6 | 6·0 ± 0·0 | 7·0 ± 2·0 | 26·7 ± 1·5 |
| | (35–37) | (1–1) | (21–23) | (40–41) | (4–6) | (7–8) | (5–6) | (6–6) | (5–9) | (25–28) |
| *Solanum trilobatum* | 40·0 ± 1·0 | 1·70 ± 0·6 | 25·4 ± 2·5 | 50·4 ± 1·2 | 6·4 ± 1·2 | 8·7 ± 1·2 | 6·0 ± 1·7 | 6·7 ± 2·0 | 9·7 ± 2·5 | 40·4 ± 9·0 |
| | (39–41) | (1–2) | (23–38) | (49–51) | (5–7) | (8–10) | (4–7) | (5–9) | (7–12) | (30–46) |

*Standard deviation; figures in parenthesis indicate range.

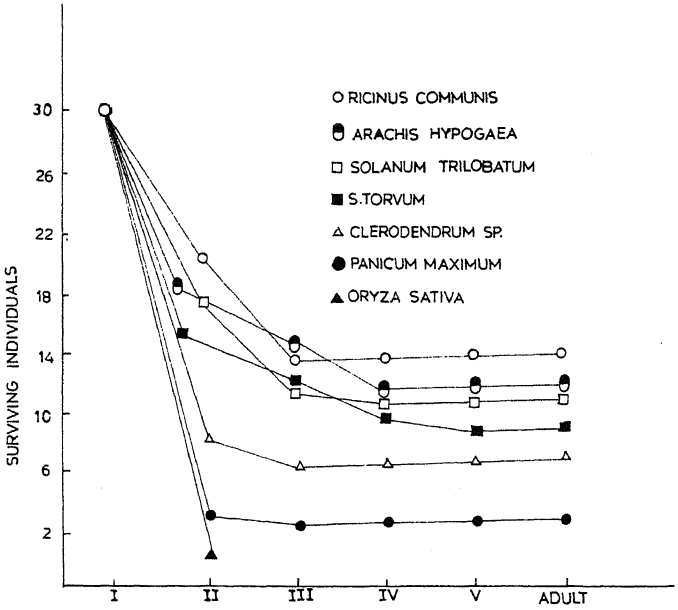


**Figure 5.** Survival rate of *A. crenulata* on different host plants.

ranging from 30–50 mg. The third instar nymphs reared on hosts like *R. communis* and *A. hypogaea* showed a higher rate of weight increase with 25–40 mg/nymphs (figure 6).

### 3.7 *Impact of diverse host plants on fecundity*

In many of the phytophagous insects, it is well known that the quality of food has a great impact on their fecundity and fertility. Many of the acridids, though polyphagous without showing any discrimination towards specific host plant, also indicated a significant variation in the number of egg pods laid as well as in the total number of nymphs that emerged. The present observations based on laboratory studies showed that the nature of host plants greatly influenced the fecundity and fertility. The number of pods laid was easily recognizable as they were laid close to the wall of the transparent plastic vial. Since it was not possible to count the number of eggs in each pod, the number of young ones emerging from each pod was taken into account. The highest number of egg pods (6/female) was laid when fed on *R. communis* and *A. hypogaea*, whereas the lowest number of egg pods (3/female) was laid when fed on *P. maximum*. Similarly the total number of nymphs emerging from all the pods was high (182–210/female) on *R. communis* and *A. hypogaea*, whereas it was low (96/female) on *P. maximum*. A moderate fecundity (120–137/female) was evident on *S. trilobatum* and *S. torvum*.

In *Orthacris maindroni*, no significant variation was apparent in the incubation period of eggs when fed on different host plants indicating that they did not influence much on it. However the number of egg pods laid by each female in its life time varied significantly. The highest number of pods (3/female) was laid on *Clerodendrum* sp and a

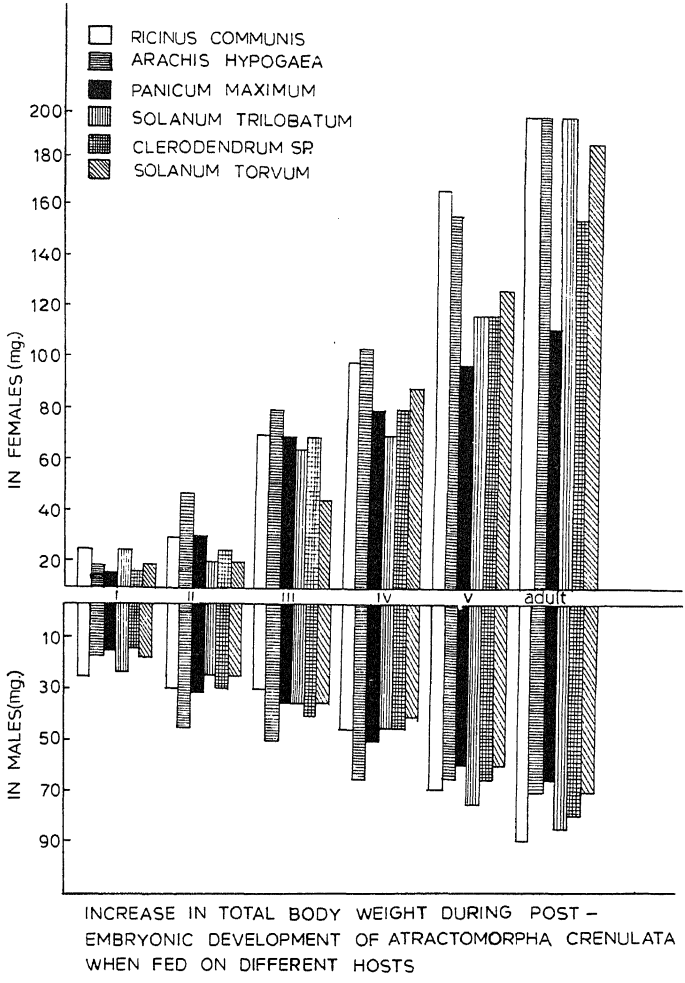


**Figure 6.** Increase in total body weight during post-embryonic development of *A. crenulata* when fed on different hosts.

lesser number of pods (2/female) on both *R. communis* and *S. trilobatum*, and only one pod was laid when fed on *D. lablab*. However no eggs were laid when fed with *P. maximum*. The total number of nymphs emerging was high and more or less equal (51–54/female) when fed on *Clerodendrum* sp and *S. trilobatum* and comparatively lesser (41–43/female) on *R. communis* and *D. lablab*.

### 3.8 *Biochemical parameters of host plants*

In order to assess the impact of various biochemical parameters of the host plants on the host preference of the grasshoppers, the following biochemical estimations were made (table 4).

Total phenols as expressed in mg/g dry weight (DW) of host tissue was very high (55 mg/g DW) in *D. lablab*, and comparatively low (12–3–40 mg/g DW) in other host plants. Total protein content was very high in *C. gigantea* (259.3 mg/g DW) and significantly lower in the rest of the host plants. *Clerodendrum* sp., showed a higher level of carbohydrates (425·9 mg/g DW), comparatively lower in *R. communis* and *S. trilobatum* (200–225 mg/g DW) and very low in the other host plants. A very high lipid content was recorded in *R. communis* (53 mg/g DW) and lower amounts in the other host plants ranging from 10·0–51·5 mg/g DW. Carbohydrate/protein ratio was greater in *C. gigantea* (1 : 20·75), comparatively lesser in *A. hypogaea*, *D. lablab*, *P. maximum*, *P. spicigera* and *S. torvum* (1 : 2·65–1 : 6·06) and very low in *Clerodendrum* sp., *R. communis* and *S. trilobatum* (1 : 0·19–1 : 0·57) (table 4).

## 4. Discussion

Studies on the distribution and abundance of diverse sensillae on the labrum, maxillary and labial palps, in *A. crenulata* (dicot-feeder), *O. maindroni* (feeding on both dicot and monocot plants) and *T. indica* (monocot feeder) indicated significant numerical and distributional variations, besides recognition of further types of sensillae in the inner side of the labrum. In all the three species discussed, A-1 sensillae were not evident, but with the 'beta' tract further divisible into the 'beta' and 'gamma' tracts. In each tract further sub-types were recognised. As many as seven types (S-1 to S-7) were evident in labrum itself. Such a recognition of sensillae was based only on their morphology, size and distribution and no functional significance is evident so far. Haskell and Schoonhoven (1969), Cook (1972), Chapman and Thomas (1978) and Chapman (1982) however showed the chemoreceptory nature of the A-1, A-2 and A-3 sensillae and suggested others probably as mechanoreceptors.

The recognition of two types of sensillae, type-A and type-B, on the surface of the maxillary and labial palps follows similar report by Abushama (1968) in *Poecilocerus hieroglyphicus* (Klug). But later studies by Blaney (1974, 1975) and Haskell and Schoonhoven (1969) suggested the mechanical and chemosensory nature of these sensillae, which are known to increase as they grow into adults. Chapman and Thomas (1978) noted that the total number of sensillae increased by 250 % for a 10-fold increase in the surface area of the labrum. In addition to S-1 to S-7 sensillae all the other A-2 and A-3 sensillae also increased in number during post-embryonic development, a fact not reported earlier. As no prior report exists regarding the increase of sensillae in the maxillary and labial palps, the present observation showed an increase in the number of sensillae during post-embryonic development varying for each type of sensillae. Trends of an increase in sensilla number were recognised only from 3rd nymphs onwards; such an increase can be correlated with the increase in the quantitative food intake as well as the range of preference for their host plants. However, the role of various sensillae located at various regions of the mouth parts in host plant selection still remains unclear. Though Blaney and Chapman (1969) and Bernays and Chapman (1970) indicated the role of various sensillae in food plant selection, the removal of labrum, maxillary palps and labial palps individually or in combination did not narrow down the host plant spectrum. However there appears to be a definite role by these organs on the quantitative food intake, since their removal significantly reduced the food intake. Such a reduction in the food intake is also possibly due to the stress caused

**Table 4.** Percentage water content, dry weight (DW) and quantitative profile of phenol, protein, carbohydrates and lipids in host plants of some acridids*

| | Water content (%) | % dry wt. of leaf over fresh wt. | Total phenol (mg/g DW) | Total protein (mg/g DW) | Total carbo-hydrate (mg/g DW) | Total lipids (mg/g DW) | Carbo-hydrate/ Protein ratio |
|---|---|---|---|---|---|---|---|
| *Arachis hypogaea* | 72 | 28 | 24 | 92·39 | 17 | 20 | 1:5·43 |
| *Calotropis gigantea* | 88 | 12 | 22 | 259·33 | 12·5 | 20 | 1:20·75 |
| *Clerodendrum* sp | 80 | 20 | 35 | 80·00 | 425·0 | 51·5 | 1:0·19 |
| *Dolichos lablab* | 83 | 17 | 55 | 198·53 | 75 | 20 | 1:2·65 |
| *Panicum maximum* | 85 | 15 | 40 | 66·66 | 11 | 47·5 | 1:6·06 |
| *Prosopis spicigera* | 73 | 27 | 40 | 109·70 | 40 | 25 | 1:2·74 |
| *Ricinus communis* | 75 | 25 | 38 | 68·00 | 200 | 53 | 1:0·34 |
| *Solanum trilobatum* | 82 | 18 | 12·3 | 129·17 | 225 | 15 | 1:0·57 |
| *Solanum torvum* | 77 | 23 | 20·5 | 163·04 | 44 | 10 | 1:3·70 |

* Mean of 3 replications

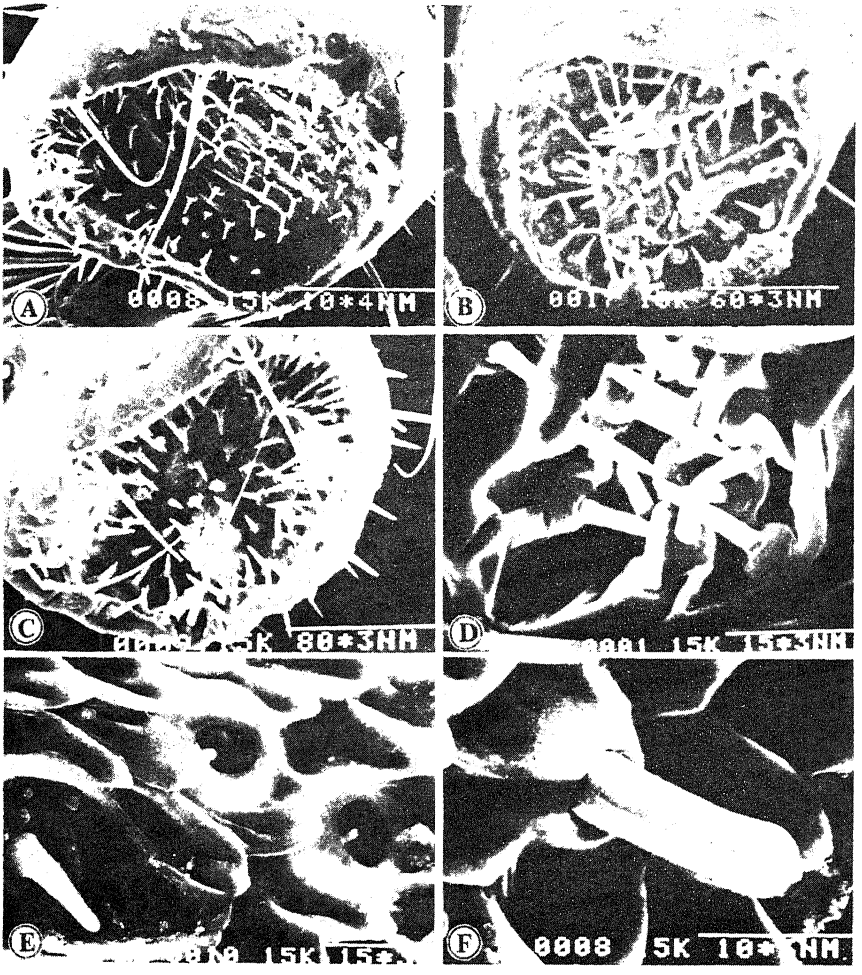

**Figure 7. A–D.** Sensillae at the palpal apices of late instars (D) portion of (B) enlarged; E, F. collared sensillae.

by their removal of various organs like maxillary and labial palps, labrum, antennae etc. To avoid such stress by mechanical injury, such ablated individuals were allowed for one to two days till they are normal in their feeding behaviour. Though in the present observations, ablations of sensillae in both maxillary and labial palps did not interfere with the host selection, earlier studies on *Locusta* (Blaney and Chapman 1969; Bernays and Chapman 1970) indicated the role of various sensillae at each palpal tip in food selection. The 'dome-shaped sensillae' alone appear to be chemosensory, functioning as feeding sensory structures. Though the host range or the host preference of acridids as in *Paulinea acuminata* does not depend upon the relative numbers of sensillae (Bennett 1970), the number of sensillae appears considerably small in acridids with a narrow host range than in those with a higher food range (Abushama 1968; Hummelen and Gillon

1968; Bennett 1970; Gillon 1972; Perkins 1973). Present investigations showed that the sensillae were larger in number in dicot feeders, lesser in mixed feeders and still lesser in monocot feeders supporting the observations by Chapman and Thomas (1978).

Detailed analysis of the host range of the three species of grasshoppers revealed their highly polyphagous nature, thereby exhibiting certain preference towards specific host plants. Both *A. crenulata* and *O. maindroni* preferred *R. communis* and *Clerodendrum* sp., respectively, whereas the monocot feeder *T. indica* preferred *C. dactylon.* Though the adults of both species can effectively survive on monocot plants, nymphal stages especially the first and second stage nymphs did not feed on the monocot plants like *P. maximum* and *O. sativa*, which is mainly due to the higher silica content of the leaves. Similar avoidance of nymphs of *Oxya nitidula* towards *P. maximum* due to the higher silica content was also reported by Meera (1982). In the present observations, food preferences were estimated on the following basis:

(i) Short term feeding behavioural effects including
  (a) frequent visit to specific host plants in the fields,
  (b) orientation towards the preferred plants in the cages,
  (c) higher quantitative food intake, and
  (d) higher food utilization ability.
(ii) Long term physiological effects involving
  (a) duration of post-embryonic development,
  (b) weight increase of the developing nymphs on diverse host plants, and
  (c) fecundity and fertility.

On the basis of the all the above parameters, *R. communis* appeared to be the best suited and preferred host plant for both *A. crenulata* and *O. maindroni* and *C. dactylon* for *T. indica.* In addition the range of host plants of the above grasshoppers can be successfully correlated with their relative number of sensillae at various mouthparts like labrum, maxillary and labial palps etc. For example, *O. maindroni* and *A. crenulata* showing higher number of sensillae, fed on plants representing as many as 10 families, whereas the *T. indica* with a comparatively lower number of sensillae fed exclusively on members of Poaceae.

In addition the preference towards the specific host plants also varied between nymphs and adults. The nymphs preferred leaves of *R. communis* as they are thin, succulant without any trichomes or any other physical barriers. But it is well known that the host preference of grasshoppers is influenced by physical and chemical characteristics like nutrition or secondary chemicals (Gangwere 1972; Otte and Joern 1975, 1977; Chapman and Bernays 1977; Bernays and Chapman 1977). The quantitative food intake and utilization were also equally high when fed on *R. communis* than on other hosts. The nymphal diet also had a significant influence on the adult preference. However the adults showed a wide spectrum of host plants than that of the nymphs, their restriction is based upon the physical characters of the host plants.

Though the nymphs of *A. crenulata* were found to feed and develop well on a wide range of plants, the development was quicker with a lesser percentage of mortality on *R. communis* and *A. hypogaea.* Simultaneously the nymphs developing on *R. communis* and *A. hypogaea* showed a higher weight increase than on other plants. In addition, the fecundity and fertility were also equally high on the above host plants indicating that they are the most preferred hosts. Though there appears to be no positive correlation

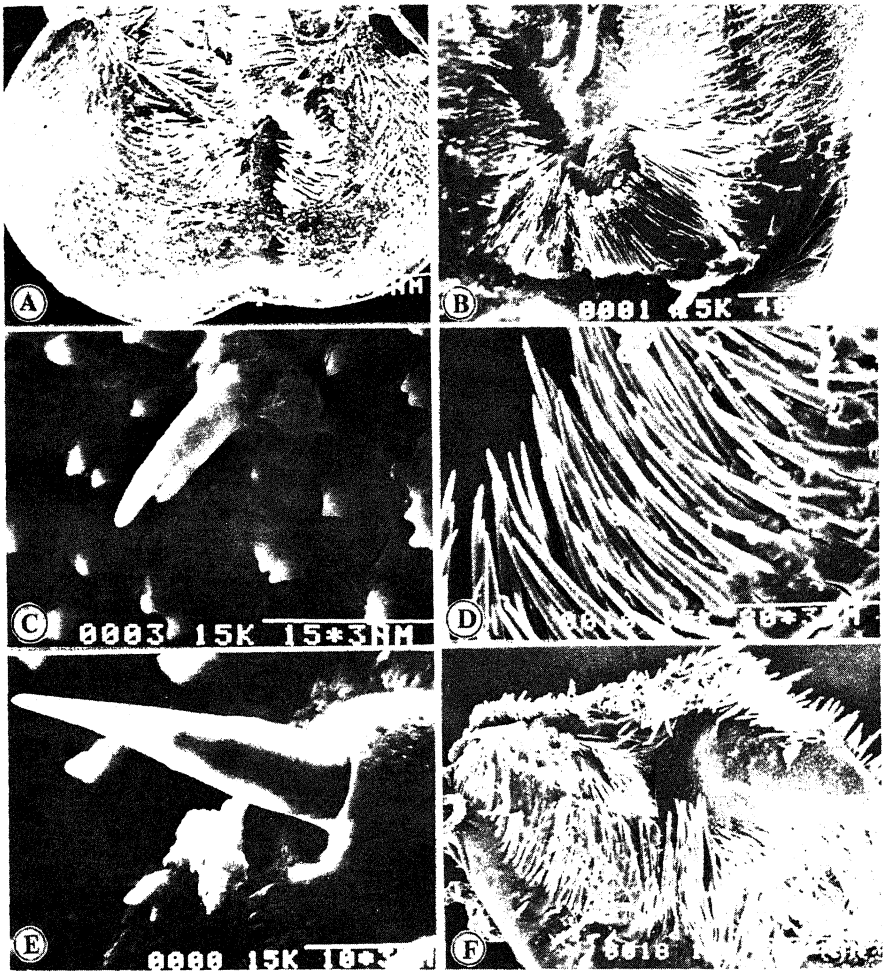


**Figure 8.** (**A**) Inner side of the labrum of *Atractomorpha crenulata*; (**B**) Inner side of the labrum of *Truxalis indica*; (**C**) Bifid sensilla in the labrum of *T. indica*; (**D**) Sensillae of the 'Beta' tract in the labrum of *T. indica*; (**E**) Sensillum with a bottom collar in the labrum of *T. indica*; (**F**) Hypopharynx of *A. crenulata*.

between biochemical parameters and host suitability or between incidence of higher lipid content and higher carbohydrate, protein ratios among these preferred hosts appear to be an effective factor for the higher developmental rates and increased fecunidty of grasshoppers. Though the protein content of the hosts was known to increase the fecundity, present observations indicate that other nutrients like lipids, carbohydrates and carbohydrate : protein ratio may also influence the fecundity.

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# Investigations on *Heliothis armigera* (Hubner) in Marathwada-XXVIII. Key mortality factors in regular and overlapping generations on pigeonpea

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**Abstract.** The parasitisation of *Heliothis armigera* (Hubner) larvae by *Campoletis chlorideae* Uchida and *Enicospilus biconatus* Townes, Townes & Gupta (= neorufus Rao & Nikam) was 8·70 and 13·79% respectively during the first generation on pigeonpea during 1983–84 at Parbhani. The parasitisation of *H. armigera* by these parasites was low in second overlapping generation. Thus, *C. chlorideae* and *E. biconatus* were identified as key mortality factors for *H. armigera* on pigeonpea.

**Keywords.** Key mortality factors; *Heliothis armigera* (Hubner); pigeonpea.

## 1. Introduction

*Heliothis armigera* (Hubner) is the most destructive pest of pigeonpea in Marathwada. Naturally-occurring predators and parasites are more important in regulating populations of *H. armigera*. Explicit instructions in insect control guides, developed through qualitative and quantitative evaluation of natural enemies, are needed so that the natural enemy numbers can be used more directly in decision-making (King *et al* 1981). Because of the high value of pigeonpea and the importance of *Heliothis*, there is a need for more ecological research approach for its management.

## 2. Materials and methods

The sampling techniques described by Harcourt (1961) were followed with slight modifications as it was felt desirable to sample larval stage instead of egg and pupa.

### 2.1 *Time of sampling*

Egg stage was sampled when the activity of adult moths was noticed in the field. The first incidence of *H. armigera* was observed on 24 October 1983.

### 2.2 *Laboratory culture*

The known number of eggs, deposited for the first time in field were collected on the

above date for further rearing in laboratory. This laboratory culture was reared on pigeonpea pods till the pest disappeared from the field.

### 2.3 *Sampling of larvae*

The sampling for the larvae (2 × 2 m quadrate) for regular generation in field was based on the developmental stages of laboratory culture. When the larvae in plastic boxes (5 × 5 cm) reached in I–II, III–IV sampling for the larvae was done in the field. Similarly when the larvae in the plastic boxes passed in V–VI instars of laboratory culture, the respective instar group was sampled in the field. In each generation 50 quadrates of 2 × 2 m were sampled randomly and the larvae were brought to the laboratory for further rearing. The infested plants in the quadrates were carefully examined for recording pest population in larval stage and thus the population was computed on hectare basis.

### 2.4 *Study of generations*

To determine the distinct regular generations, an interval of five days was provided before sampling of larvae of next generation, after the mean adult emerged from previous generation in laboratory. This period was considered for oviposition by the moths of previous generation. The start of overlapping generation was considered after 15 days of the start of regular generation.

### 2.5 *Mode of observations*

The larvae collected from the field were brought to the laboratory and reared on pigeonpea pods till the adults emerged. Larval and pupal parasitism as well as, because of unknown causes mortality were observed.

### 2.6 *Life table*

The column headings used in the life tables of the present study were similar to those proposed by Morris and Miller (1954). The data were analysed according to the method suggested by Harcourt (1963, 1969) and Atwal and Bains (1974). Separate budgets were prepared to find out the key factors that influence the population trend in different generations. The method of Varley and Gradwell (1963, 1965) was chosen for key factor analysis.

## 3. Results and discussion

The results on the key mortality factors for regular generations of *H. armigera* are presented in table 1. The larval mortality in I–II instar group due to *Campoletis chlorideae* Uchida was 8·70 % in the first generation. The parasitisation of the larvae by

**Table 1.** Key mortality factors for three regular generations of *H. armigera* on pigeonpea during 1983–84 (population/ha)

| Age interval<br>*x* | Numbers alive at beginning of *x*<br>lx | Factors responsible for *dx*<br>*dxF* | Numbers dying during *x*<br>*dx* | dx as a per cent of lx<br>100 *qx* | Survival rate<br>*Sx* | Log (lx) | *k* |
|---|---|---|---|---|---|---|---|
| I Generation(24–10–83 to 24–11) | | | | | | | |
| Expected eggs | 3,632 | Sterility/dead | 182 | 5·01 | 0·95 | 3·5601 | |
| Viable eggs | 3,450 | | | | | 3·5378 | 0·0223 |
| *Larval instars*: | | | | | | | |
| I–II ($N_1$) | 3,450 | *C. chlorideae* | 300 | 8·70 | 0·84 | 3·5378 | |
| | | Unknown | 250 | 7·25 | | | |
| III–IV | 2,900 | *E. biconatus* | 400 | 13·79 | 0·81 | 3·4624 | 0·0754 |
| | | Unknown | 150 | 5·17 | | | |
| V–VI | 2,350 | Virus | 100 | 4·25 | 0·87 | 3·3711 | 0·0913 |
| | | Unknown | 200 | 8·51 | | | |
| Pupae | 2,050 | *G. halli* | 50 | 2·44 | 0·93 | 3·3117 | 0·0594 |
| | | Unknown | 100 | 4·88 | | | |
| Moths | 1,900 | Sex 50% females | | | 1·00 | 3·2787 | 0·0330 |
| Females × 2 ($N_3$) | 1,900 | (Reproducing females = 950) | | | | 2·9777 | 0·3010 |
| Trend Index ($N_2/N_1$) | | | 7·78 | | | | |
| Generation survival ($N_3/N_1$) | | | 0·55 | | | | |
| II Generation (30–11–83 to 2–1–84) | | | | | | | |
| Expected eggs | 27,680 | Sterility/dead | 830 | 3·00 | 0·97 | 4·4422 | |
| Viable eggs | 26,850 | | | | | 4·4289 | 0·0133 |
| *Larval instars*: | | | | | | | |
| I–II ($N_1$) | 26,850 | *C. chlorideae* | 150 | 0·56 | 0·98 | 4·4298 | |
| | | Unknown | 450 | 1·67 | | | |
| III–IV | 26,250 | *E. biconatus* | 100 | 0·38 | 0·95 | 4·4191 | 0·0098 |
| | | *Carcelia* sp. | 50 | 0.19 | | | |
| | | Unknown | 1,050 | 4·00 | | | |
| V–VI | 25,050 | Unknown | 650 | 2·59 | 0·97 | 4·3988 | 0·0203 |

*(Contd.)*

**Table 1.** (*Contd.*)

| Age interval *x* | Numbers alive at beginning of *x* lx | Factors responsible for *dx* *dxF* | Numbers dying during *x* *dx* | *dx* as a per cent of lx 100 *qx* | Survival rate *Sx* | Log (lx) | *k* |
|---|---|---|---|---|---|---|---|
| Prepupa | 24,400 | Unknown | 950 | 3·89 | 0·96 | 4·3874 | 0·0114 |
| Pupa | 23,450 | *G. halli* | 250 | 1·07 | 0·92 | 4·3701 | 0·0173 |
| | | Unknown | 1,550 | 6·61 | | | |
| Moths | 21,650 | Sex 50% females | | | 1·00 | 4·3354 | 0·0347 |
| Females × 2 ($N_3$) | 21,650 | (Reproducing females = 10,825) | | | | 4·0344 | 0·3010 |
| Trend index | | | 0·29 | | | | |
| Generation survival | | | 0·81 | | | | |
| III Generation: (8–1–84 to 9–2–84) | | | | | | | |
| Expected eggs | 8,611 | Sterility/dead | 861 | 10·00 | 0·90 | 3·9350 | |
| Viable eggs | 7,750 | | | | | 3·8893 | 0·0450 |
| *Larval instars*: | | | | | | | |
| I–II ($N_1$) | 7,750 | *C. chlorideae* | 100 | 1·29 | 0·98 | 3·8893 | |
| | | Unknown | 50 | 0·64 | | | |
| III–IV | 7,600 | *Carcelia* sp. | 150 | 1·97 | 0·97 | 3·8808 | 0·0085 |
| | | Unknown | 50 | 0·65 | | | |
| V–VI | 7,400 | Unknown | 100 | 1·35 | 0·99 | 3·8692 | 0·0116 |
| Prepupa | 7,300 | Unknown | 200 | 2·74 | 0·97 | 3·8633 | 0·0059 |
| Pupa | 7,100 | *G. halli* | 250 | 3·52 | 0·95 | 3·8512 | 0·0121 |
| | | Unknown | 100 | 1·41 | | | |
| Moths | 6,750 | Sex 50% females | | | 1·00 | 3·8293 | 0·0219 |
| Females × 2 ($N_3$) | 6,750 | (Reproducing females = 3,375) | | | | 3·5283 | 0·3010 |
| Trend index | | | Nil | | | | |
| Generation survival | | | 0·87 | | | | |

*Enicospilus biconatus* Townes, Townes & Gupta (= neorufus Rao & Nikam) was 13·79%. The pupal parasitism was 2·44% by *Goniophthalmus halli* Mesnil. The positive trend index (7·78) indicated that the mortality factors were ineffective in checking further multiplication of population in the second generation. The high value of $k$ (0·0913) indicated that the mortality parameters in III–IV instars resulted in highest mortality of larvae in the first regular generation. The parasitisation by *C. chlorideae*, *E. biconatus* and *Carcelia* sp in the larval stage and *G. halli* in the pupal stage was very low in the second generation. The total larval mortality due to *C. chlorideae* and *Carcelia* sp was 3·26% during the third generation. The results on overlapping generations are presented in table 2. The mortality of *H. armigera* larvae because of *C.*

**Table 2.** Key mortality factors for two overlapping generations of *H. armigera* on pigeonpea during 1983–84 (Population/ha)

| $x$ | $lx$ | $dxF$ | $dx$ | 100 $qx$ | $Sx$ | Log ($lx$) | $k$ |
|---|---|---|---|---|---|---|---|
| I Overlapping generation (8–11–83 to 10–12–83) | | | | | | | |
| Expected eggs | 20,990 | Sterility/dead | 840 | 4·00 | 0·96 | 4·3220 | |
| Viable eggs | 20,150 | | | | | 4·3043 | 0·0177 |
| *Larval instars*: | | | | | | | |
| I–II ($N_1$) | 20,150 | *C. chlorideae* | 1,750 | 8·68 | 0·86 | 4·3043 | |
| | | Unknown | 1,000 | 4·96 | | | |
| III–IV | 17,400 | *E. biconatus* | 2,900 | 16·67 | 0·79 | 4·2405 | 0·0638 |
| | | Virus | 150 | 0·86 | | | |
| | | Unknown | 650 | 3·73 | | | |
| V–VI | 13,700 | Virus | 250 | 1·82 | 0·92 | 4·1367 | 0·1038 |
| | | Unknown | 800 | 5·84 | | | |
| Prepupa | 12,650 | Unknown | 300 | 2·37 | 0·98 | 4·1021 | 0·0346 |
| Pupa | 12,350 | *G. halli* | 900 | 7·29 | 0·82 | 4·0917 | 0·0104 |
| | | Unknown | 1,250 | 10·12 | | | |
| Moths | 10,200 | Sex 50% females | | | 1·00 | 4·0086 | 0·0831 |
| Females × 2 ($N_3$) | 10,200 | (Reproducing females = 5,100) | | | | 3·7076 | 0·3010 |
| Trend index | | | 1·19 | | | | |
| Generation survival | | | 0·51 | | | | |
| II Overlapping generation (16–12–83 to 20–1–84) | | | | | | | |
| Expected eggs | 24,639 | Sterility/dead | 739 | 3·00 | 0·97 | 4·3916 | |
| Viable eggs | 23,900 | | | | | 4·3784 | 0·0132 |
| *Larval instars*: | | | | | | | |
| I–II ($N_1$) | 23,900 | *C. chlorideae* | 150 | 0·63 | 0·98 | 4·3784 | |
| | | Unknown | 250 | 1·05 | | | |
| III–IV | 23,500 | *E. biconatus* | 50 | 0·21 | 0·97 | 4·3711 | 0·0073 |
| | | Unknown | 600 | 2·55 | | | |
| V–VI | 22,850 | Unknown | 500 | 2·19 | 0·98 | 4·3589 | 0·0122 |
| Prepupa | 22,350 | Unknown | 800 | 3·58 | 0·96 | 4·3493 | 0·0096 |
| Pupa | 21,550 | *G. halli* | 100 | 0·46 | 0·92 | 4·3334 | 0·0159 |
| | | Unknown | 1,500 | 6·96 | | | |
| Moths | 19,950 | Sex 50% females | | | 1·00 | 4·2999 | 0·0335 |
| Females × 2 ($N_3$) | 19,950 | (Reproducing females = 9,975) | | | | 3·9989 | 0·3010 |
| Trend index | | | Nil | | | | |
| Generation survival | | | 0·83 | | | | |

*chlorideae*, *E. biconatus* and nuclear polyhedrosis virus was 8·68, 16·67 and 1·82% respectively. The nil trend index in the second overlapping generation indicated that pest population was not present in the field and only two overlapping generations were recorded in pigeonpea. The *k* value of 0·0638 indicate highest contribution of mortality in III–IV instar group in the second overlapping generation.

The parasitisation of *H. armigera* larvae (26·74%) and pupae by different parasites was comparatively greater in the first regular generation when compared to second (1·13%) and third (3·26%) generations. Similar trend regarding the role of larval parasites was observed in the first overlapping (28·03%) generation while the parasitisation of *H. armigera* larvae was very low in the second overlapping generation. Irrespective of high parasitisation, the population increased in second regular and overlapping generations. The little showers of rain at the end of the first regular and at the start of second overlapping generation could be the reason for increasing pest population on pigeonpea. Thus, it could be concluded that *C. chlorideae* and *E. biconatus* acted as key mortality factors in first regular generation only. Similarly these parasites were also identified as key factor in reducing the population of larvae to the extent of 25·35% in the first overlapping generation. *C. chlorideae* alone parasitised the *H. armigera* larvae to the extent of 24·92% in chickpea (Bilapate *et al* 1979). The importance of this parasite in regulating the population of *H. armigera* has been indicated by many researchers in India (Achan *et al* 1968; Bilapate 1984; Gangrade 1964; Raich 1973; Rao 1968; Raodeo *et al* 1974).

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# Transpiration rates and acclimation to water and temperature of the tropical woodlice, *Porcellionides pruinosus* Brandt and *Porcellio laevis* Latreille

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**Abstract.** The transpiration rates and acclimation to water and temperature of *Porcellionides pruinosus* Brandt and *Porcellio laevis* Latreille, the common terrestrial isopods occurring in and around Trivandrum, India, were studied. Transpiration rates in different temperatures (24 to 50°C) were higher in both the species during the first 15 minutes' exposure when compared to 1-hour duration. Previous acclimation to different humidity and temperature conditions affected the transpiration rates in both the species and lower rates of transpiration occurred in isopods acclimated to dry conditions and higher temperatures. Survival rates improved at lethal temperatures in specimens acclimated to 34°C.

**Keywords.** Transpiration; temperature; acclimation; isopods; *Porcellionides pruinosus*; *Porcellio laevis*.

## 1. Introduction

Although terrestrial isopods are widespread in the tropical south-west coast of India, very little is known concerning their physiological adaptations. In the present study, *Porcellionides pruinosus* Brandt and *Porcellio laevis* Latreille, commonly found in and around Trivandrum, were selected and their rates of transpiration under different temperatures and the nature of temperature and humidity relations have been examined.

Attempts were made to study the physiological adaptations of terrestrial isopods to varying temperature and humidity conditions, the notable contributors of such studies among others being those of Bursell (1955), Paris (1963), Warburg (1965), Edney (1968), Cloudsley-Thompson (1969), Sutton (1969) and Dubinsky and Steinberger (1979).

## 2. Materials and methods

### 2.1 *Topography*

Trivandrum lies at 8°20'N lat. and 76°55'E long. in Kerala State, S.W. India. The state is bounded on the west by the Arabian Sea and on the east by the Western Ghats. The area has a tropical climate and the year may be divided into the hot dry pre-monsoon (February to May), the rainy season (June to November) and the comparatively cool

post-monsoon (December and January) periods. The relative humidity is generally above 70% and the maximum goes upto 90% during the monsoon. The atmospheric temperature also varies from a mean maximum of 27·5°C during August to 33·5°C in April and a mean minimum of 21·6°C during January to 25·5°C in April. Rainfall varies from month to month with the maximum during July–November and the minimum during February–April. The region is covered with green bushes, coconut palms and trees and the heavy litter covering the ground provide ideal conditions for the life and propagation of isopods.

### 2.2 *Material*

*Porcellionides pruinosus* Brandt and *Porcellio laevis* Latreille, are common terrestrial isopods found in and around Trivandrum. The former is smaller than the latter and the mean weights of specimens used in the experiments were 4·0 mg and 14·7 mg respectively. For transpiration studies, the largest specimens available, mostly adult females, were selected.

### 2.3 *Sites of collection and methods*

The isopods were collected from 2 localities, one from the Aquarium campus near the seashore, in sandy soils under bricks and stones in humid and shady places, and the other from the city proper about 12 km away from the Aquarium, in loamy soil with rich humus content and also from beneath bricks and stones in cool and shady places. A marked difference was noticeable in the distribution of these isopods in these localities. Over 95% of woodlice collected from the Aquarium area were *P. pruinosus* while an even higher proportion of those taken from the city proper was *P. laevis*. Overlapping of these two populations was not observed in both the localities. Soil pH in the two localities was measured with an analytical pH meter. It averaged 6·8 at the Aquarium campus while in the city it averaged 7·4.

The isopods were maintained in large petri dishes containing damp filter paper with rotting leaves lying on it at room temperature (average about $31 \pm 4$°C) in natural day light and darkness.

To study the transpiration rates, the effect of acclimation in different temperature and humidity conditions on the rates of transpiration and the effect of acclimation in different temperatures on the lethal temperature, the methods described by Edney (1951, 1954) and Cloudsley-Thompson (1969) were adopted. For transpiration studies the specimens (10 each in number) which were weighed individually were exposed separately for 15 minutes and 1 hour over phosphorous pentoxide at 24, 29, 34, 39, 45 and 50°C before the water-loss through evaporation was estimated by reweighing. The relative humidity was kept constant at 22% in all the temperatures tested. To calculate the surface area of the animal, a value of $K = 12$ was adopted as used by Cloudsley–Thompson (1956, 1969) for many African woodlice and it is the mean of the figures calculated by Edney (1951) for various British woodlice.

## 3. Results

### 3.1 *Transpiration*

Water-loss by transpiration is one of the most important physiological factors affecting the distribution of woodlice (Edney 1954, 1968; Cloudsley–Thompson 1969). The results on the transpiration for exposures of *P. pruinosus* and *P. laevis* for 15 minutes and 1 hour in various temperatures are given in table 1. The transpiration rate was higher during the first 15 minutes' exposure for both the species than for 1 hour duration. Thus the mean water-loss for 15 minutes' exposure for *P. pruinosus* at 24°C was 3·80 mg/cm$^2$/hr which increased to 8·76 mg/cm$^2$ /hr at 34°C and 18·89 mg/cm$^2$/hr at 50°C whereas the same for 1 hour exposure at 24°C was 1·94 mg/cm$^2$/hr which further increased to 3·99 mg/cm$^2$/hr at 34°C and 9·45 mg/cm$^2$/hr at 50°C. In the case of *P. laevis* the water-loss was lower for both 15 minutes and 1 hour exposures at different temperatures when compared to that of *P. pruinosus*. Thus the mean water-loss for this species for 15 minutes' exposure at 24°C was 2·01 mg/cm$^2$/hr which gradually increased to 3·68 mg/cm$^2$/hr at 34°C and 8·21 mg/cm$^2$/hr at 50°C whereas the values for 1 hour exposure of these animals for the same temperatures were 1·00 mg/cm$^2$/hr, 1·06 mg/cm$^2$/hr and 4·26 mg/cm$^2$/hr respectively (table 1).

### 3.2 *Effect of acclimation to humidity and temperature on transpiration*

The mean rates of transpiration from both the species previously acclimated in moist and dry conditions and also at two different temperatures of 24°C and 34°C are given in table 2. The transpiration is lower in those acclimated to dry conditions (relative humidity 48%) as compared to those acclimated to moist conditions (relative humidity 68%) and the difference in rates of transpiration between these two conditions is quite significant (at 5% level) in the case of *P. pruinosus*. Such a significant difference, however, was not evident in the case of *P. laevis* (table 2).

Studies on the effect of acclimation to temperature (24 and 34°C) on transpiration rates for both the species show that the transpiration rates are higher in both the species

**Table 1.** Rate of transpiration in *P. pruinosus* and in *P. laevis*.

| Temperature (°C) | Water loss (mg/cm$^2$/hr) | | | |
|---|---|---|---|---|
| | 15 min exposure | | 1 hr exposure | |
| | *P. pruinosus* | *P. laevis* | *P. pruinosus* | *P. laevis* |
| 24 | 3·80 ± 0·07 | 2·01 ± 0·04 | 1·94 ± 0·03 | 1·00 ± 0·02 |
| 29 | 4·15 ± 0·04 | 2·94 ± 0·06 | 2·91 ± 0·03 | 1·04 ± 0·03 |
| 34 | 8·76 ± 0·05 | 3·68 ± 0·05 | 3·99 ± 0·05 | 1·06 ± 0·03 |
| 39 | 10·20 ± 0·05 | 4·34 ± 0·02 | 5·82 ± 0·07 | 2·44 ± 0·04 |
| 45 | 14·78 ± 0·05 | 6·48 ± 0·03 | 7·87 ± 0·04 | 3·57 ± 0·05 |
| 50 | 18·89 ± 0·17 | 8·21 ± 0·02 | 9·45 ± 0·05 | 4·26 ± 0·03 |

The relative humidity was kept constant at 22%.

**Table 2.** Mean water loss (mg/cm²/hr) in < 10% relative humidity at room temperatures (34 ± 1°C) for exposure of 1 hr, in woodlice previously conditioned in various ways ($N = 16$).

| Species | Conditioning | | $t$ | Inference |
|---|---|---|---|---|
| | *moist* | *dry* | | |
| *P. pruinosus* | 1·76 ± 0·06 | 1·43 ± 0·09 | 2·81 | Significant at 5% level $P < 0·05$ |
| *P. laevis* | 1·36 ± 0·19 | 1·15 ± 0·09 | 0·87 | N.S. |
| | 24°C | 34°C | | |
| *P. pruinosus* | 1·65 ± 0·20 | 3·45 ± 0·51 | 2·98 | Significant at 5% level $P < 0·05$ |
| *P. laevis* | 0·96 ± 0·18 | 1·31 ± 0·02 | 1·71 | N.S. |

N.S.—not significant.

acclimated at 34°C when compared to those acclimated to 24°C. Here also a significant difference (at the 5% level) between the transpiration rates at 24°C and 34°C is discernible in the case of *P. pruinosus* whereas such a difference was not noticed in the case of *P. laevis*.

### 3.3 *Effect of acclimation on the lethal temperatures*

In preliminary tests conducted at room temperature, most individuals of *P. pruinosus* survived at 41·5°C for 30 min but very few survived at 42·5°C. The lethal temperature of *P. pruinosus* for an exposure of 30 minutes was, therefore, assumed to be around 42°C. In the case of *P. laevis*, however, the lethal temperature was around 44°C although a slight variation occurred on account of the dampness of the filter paper. Studies on the survival rates in their respective lethal temperatures for 2 hr for both the species which were previously acclimatized at two different temperatures of 24°C and 34°C showed that survival was better for both the species acclimated at 34°C as compared to 24°C. The results are presented in table 3. From the data it is apparent that during the initial 30 min exposure, no mortality of *P. pruinosus* and *P. laevis* acclimated at 34°C occurred whereas a gradual reduction in the percentage survival took place in both the species acclimated at 24°C. After 90 min of exposure, however, a sudden increase in the mortality rates for both the species acclimated at 34°C was evident and after 120 min, the percentage of animals survived equalled, irrespective of the temperatures in which they were acclimated (table 3).

## 4. Discussion

A higher rate of water-loss from the body surfaces of *P. pruinosus* and *P. laevis* during the first 15 min of exposure to different temperatures was noted as compared to 1 hr exposure. The cuticle of land isopods does not possess the water-proofing mechanism,

**Table 3.** Percentage survival of *P. pruinosus* and *P. laevis* (previously acclimated at 24°C and 34°C) in their lethal temperatures of 42°C and 44°C respectively.

| Time of exposure (min) | Percentage survival | | | |
|---|---|---|---|---|
| | *P. pruinosus* Acclimated at | | *P. laevis* Acclimated at | |
| | 24°C | 34°C | 24°C | 34°C |
| 0 | 100·00 | 100·00 | 100·00 | 100·00 |
| 15 | 90·91 | 100·00 | 91·67 | 100·00 |
| 30 | 72·73 | 100·00 | 83·33 | 100·00 |
| 45 | 72·73 | 90·91 | 41·67 | 100·00 |
| 60 | 63·64 | 90·91 | 33·33 | 83·33 |
| 75 | 45·45 | 81·83 | 16·67 | 66·67 |
| 90 | 36·36 | 81·83 | 16·67 | 41·67 |
| 105 | 36·36 | 45·45 | 8·33 | 8·33 |
| 120 | 36·36 | 36·36 | 8·33 | 8·33 |

an oriental layer of lipid molecules in the epicuticle so characteristic of insects and arachnids (Lees 1947; Baement 1961). Thus the higher initial rate may be due mainly to loss of water from layers of the cuticle external to lipid barrier, which is later followed by shrinkage of the cuticle thus leading to a closer packing of the lipid molecules and, therefore, to decreased permeability (Bursell 1955). This is clearly advantageous to the animal since it acts as a regulating mechanism that reduces the rate of water-loss at a time when conservation is most needed (Cloudsley-Thompson 1969).

Observations on the transpiration rates of *P. pruinosus* and *P. laevis* previously acclimated to moist and dry conditions and also at two different temperatures of 24°C and 34°C, show a significant difference in their transpiration rates in different acclimated conditions and that the transpiration rates are higher in those conditioned under moist conditions and at a higher temperature of 34°C. Higher cuticular permeability at higher temperatures is a typical character of cryptozoic animals (Lawrence 1953). The ability to loose water rapidly by transpiration certainly enables the woodlice to withstand high temperatures for brief periods (Edney 1954). Edney (1951) also found significantly different rates of evaporation from specimens of *Armadillidium vulgare* obtained from different localities of England and concluded that the same species showed considerable difference in their rates of evaporation resulting from selection over a long period or an effect of acclimation within the lifetime of the individual, or both. This seems to be true in the present study also where these animals showed marked differences in transpiration in varying temperature and humidity conditions.

Regarding lethal temperatures, the highest ambient temperature that land isopods tolerate is a reflection of their genetic constitution, the period of exposure, the rate of rise, the ambient humidity and the temperature history (acclimation) (Edney 1968). Thus factors such as size, duration of exposure, permeability of the cuticle and humidity, seem to play significant roles in determining the effects of high ambient temperatures. Studies on the survival rates of *P. pruinosus* and *P. laevis* in their respective lethal temperatures showed that the survival was better for both the species acclimated at higher temperature. Edney (1964) also made similar observations on

*Armadillidium vulgare* and *Porcellio laevis* in California and he found that neither size nor mortality affected the lethal temperature but that acclimation for some days in different temperatures had a marked effect. This is true in the case of African woodlice also (Cloudsley-Thompson 1969).

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# Changes in some biochemical constituents of the fiddler crabs *Uca annulipes* Latreille and *U. triangularis* (Milne Edwards) in response to eyestalk removal

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**Abstract.** Metabolic effects of eyestalk removal in the fiddler crabs *Uca annulipes* and *U. triangularis* were studied. Bilateral extirpation of eyestalks results in hypoglycemia and a fall and rise in the glycogen content of hepatopancreas and muscle respectively. Eyestalk removal also caused a fall in protein and fat content of hepatopancreas and muscle respectively. Injection of eyestalk extract restored the level of blood sugar, glycogen, protein and fat to normal level.

**Keywords.** Hypoglycemia; metabolic effects; eyestalk ablation; fiddler crabs; biochemical constituents.

## 1. Introduction

Among crustaceans, the presence of hyperglycemic condition was noticed after eyestalk ablation in *Callinectes* by Abramowitz *et al* (1944), in *Astacus* by Kleinholz *et al* (1950), in *Ocypode platytarsis* by Parvathy (1972), in *Cambarus robustus* by Telford (1975) and in *Parapenaeopsis hardwickii* by Nagabhushanam and Kulkarni (1980). But interestingly, the extirpation of eyestalks did not produce hypoglycemia in the spiny lobsters *Panulirus japonicus* and *P. penicellatus* (Scheer and Scheer 1951) or in the prawns *Metapenaeus monoceros* (Rangneker and Madhyastha 1971). In addition, no change in the blood sugar levels was observed in the crabs *Libinia emarginata* and *Callinectes sapidus* after eyestalk removal. The eyestalks of crustaceans also contain some other factors that can regulate the levels of fats and proteins (Rangneker and Madhyastha 1971; Madhyastha and Rangneker 1976; Raghavaiah *et al* 1980). Recently Sedlmeier and Keller (1981) investigated the effect of crustacean hyperglycemic neurohormone (CHH) on cyclic nucleotide levels of different tissues of crayfish *Orconectes limosus* and found elevation in the levels of cAMP and cGMP in all the tissues after hormone administration.

It is evident from literature that the ablation of eyestalks causes either an increase or decrease in any of the biochemical constituents and this is also sometimes different in different parts of the body. Till now, there has been no convincing evidence in general of the metabolic effects of eyestalk extracts in crustaceans. Moreover, it is apparent that less attention has been paid to this aspect in crabs in particular when compared to other crustaceans. Therefore, an attempt has been made to give a comparative account of the effect of eyestalks on the levels of different biochemical constituents like carbohydrates, fats and proteins in two species of fiddler crabs, *Uca annulipes* and *U. triangularis*.

## 2. Material and methods

The fiddler crabs collected from a marshy area near Visakhapatnam harbour, were maintained in the laboratory in aquaria containing sea water. The crabs were not fed during the experimental period. After 24 hr, twenty animals of the same size (male, intermoult) were selected for the experiment and divided into four groups (A,B,C and D) of five each. Bilateral eyestalk ablation was performed by cutting the eyestalks at their bases and stubs were quickly cauterised to avoid bleeding. The animals of group A served as intact controls, while those in group D were used for sham-operation.

A single injection of eyestalk extract was given to each animal in group C. Eyestalk extract was prepared by triturating a pair of eyestalks in 0·2 ml of sterilised sea water ($32°/_{oo}$). The extract was centrifuged and the supernatant was used for injection. Each animal received a dose of 0·03 ml which was injected into the ventral sinus by piercing the needle of the syringe in the region between the bases of the third and fourth walking legs. Sham-operation was performed by removing the chitinous exoskeleton around the bases of eyestalks. Before and after eyestalk removal, animals were given cold treatment for 30 min to avoid shocks. After eyestalk ablation and injection of eyestalk extract, all the four groups of animals were maintained separately in glass troughs containing sea water ($32°/_{oo}$) for 24 hr.

After 24 hr, blood samples from the animals of all the four groups were withdrawn by piercing a hypodermic needle through the carapace in the pericardial region. Blood sugar was estimated by the Folin-Wu method (1920).

At the same time hepatopancreas and muscle tissues were separated from all the animals of the four groups and were dried separately at 110°C till constant weights were obtained. Then, the dried tissue material was used for the estimation of glycogen (Carrol *et al* 1956), protein (Lowry *et al* 1951) and fats (Folch *et al* 1957). Student's *t* test was used to compare the values (Snedecor and Cochran 1967).

## 3. Results

Changes in the blood sugar level and variation in glycogen, fat and protein level of hepatopancreas and muscle under different experimental regimens of *U. annulipes* and *U. triangularis* are given in figures 1 to 4.

The eyestalk ablation results in a significant ($P < 0·001$) decline in the average glycemic level (group B) in both the species of crabs (figure 1). Further, when the eyestalk extract is injected into the destalked animals (group C) the average blood sugar level is restored to its normal level. No marked alteration ($P > 0·001$) in the concentration of blood sugar is noted after sham-operation (group D) (figure 1).

Bilateral eyestalk ablation also causes a significant ($P < 0·001$) fall in the average value of hepatopancreatic glycogen (figure 2). Moreover, injection of eyestalk extract into destalked animals restores the amount of glycogen to normal level and this restored value compared well with those of the control and sham-operation groups (figure 2).

Bilateral ablation of eyestalks causes a significant ($P < 0·001$) increase in the concentration of muscle glycogen (group B) (figure 2). Injection of eyestalk extract in the operated animals (group C) produces a partial restoration of the raised glycogen level (figure 2).

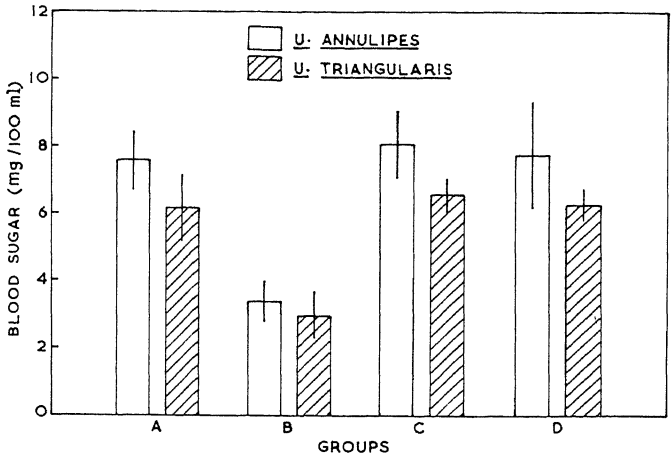


**Figure 1.** Changes in the blood sugar levels of *U. annulipes* and *U. triangularis* during different experimental conditions (for groups A, B, C and D, see text).

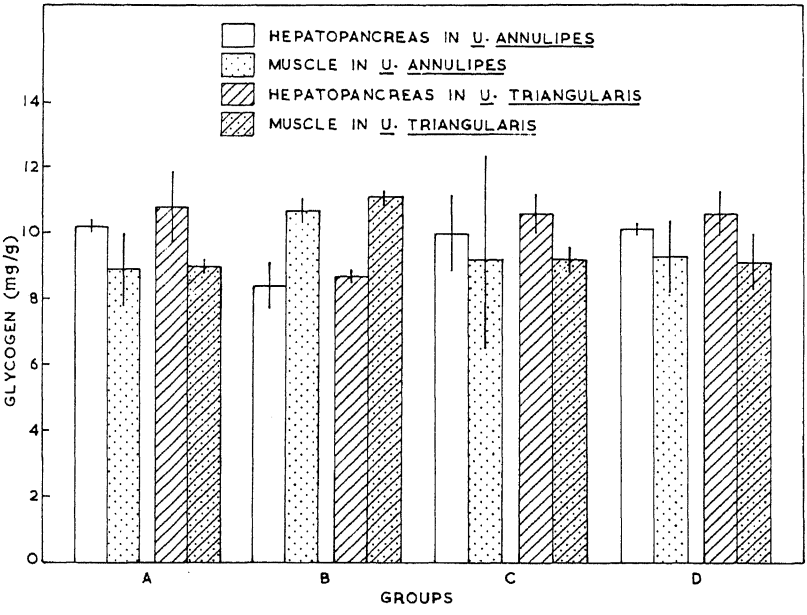


**Figure 2.** Changes in the amounts of glycogen in the hepatopancreas and muscle of *U. annulipes* and *U. triangularis* during different experimental conditions.

Eyestalk removal also causes (group B) a significant ($P < 0{\cdot}001$) decline in the concentration of lipids and proteins of the hepatopancreas (figures 3 and 4). Though there is a decline in the lipids and proteins of muscle tissue of the destalked animals (group B), it is not statistically significant ($P > 0{\cdot}001$). However, injection of eyestalk extract into the destalked animals (group C) brings about a re-establishment of the lipid and the protein levels within the normal range (figures 3 and 4).

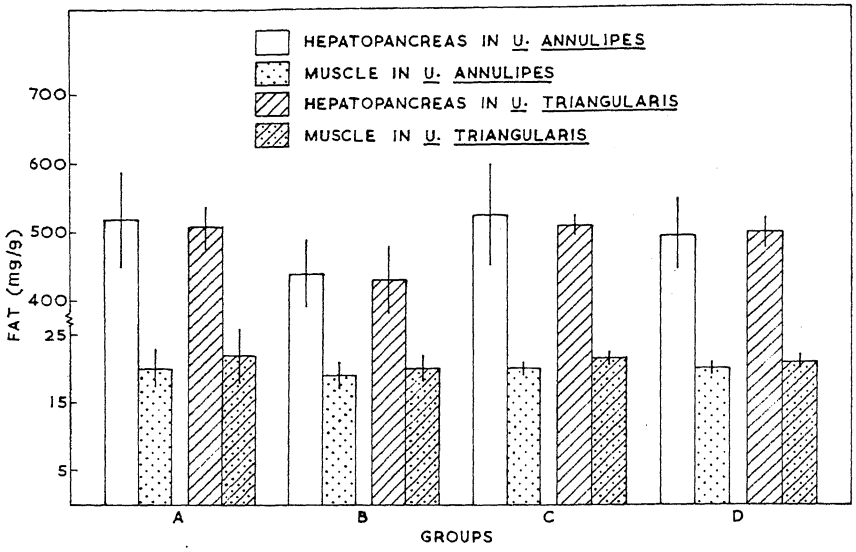


**Figure 3.** Changes in the amounts of fat in the hepatopancreas and muscle of *U. annulipes* and *U. triangularis* during different experimental conditions.

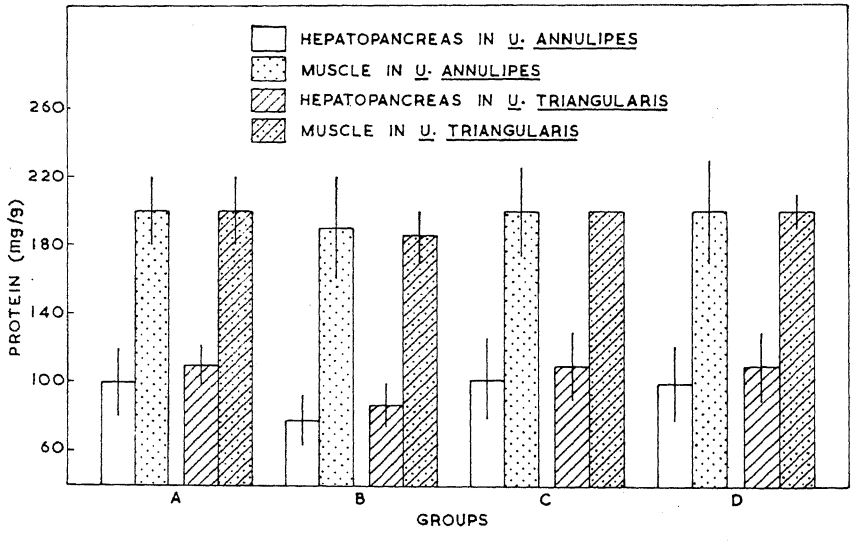


**Figure 4.** Changes in the amounts of protein in the hepatopancreas and muscle of *U. annulipes* and *U. triangularis* during different experimental conditions.

## 4. Discussion

The observation of hypoglycemia in both the crabs after surgical removal, differ from those on other crabs, *Callinectes sapidus* (Kleinholz *et al* 1950), *Scylla serrata* (Deshmukh 1968) and *Paratelphusa jacquamontii* (Rangneker *et al* 1961) where the eyestalk ablation resulted in hyperglycemia. But it is interesting to note that such a

condition of hypoglycemia after eyestalk removal was reported earlier in only one crab *Varuna litterata* by Madhyastha and Rangneker (1976). However, similar observations were recorded in other crustaceans, *Metapenaeus* sp. (Dall 1965), *Panulirus japonicus* and *P. penicillatus* (Scheer and Scheer 1951) and *Metapenaeus monoceros* (Rangneker and Madhyastha 1971).

It may also be said from the results that carbohydrate metabolism in the fiddler crabs, *Uca annulipes* and *U. triangularis* is controlled by the interaction of two separate factors originating from eyestalks: one might be maintaining the normal levels of blood sugar and glycogen concentration of hepatopancreas in the intact crabs and another might be inhibiting the synthesis of glycogen in the muscle. Therefore, eyestalk ablation results in decrease of blood sugar and glycogen concentration in hepatopancreas and increase in muscle glycogen concentration. Injection of eyestalk extracts supplemented both the factors restoring to normal range.

The reason for the depletion of glycogen leading to rapid glycogenolysis in the hepatopancreas of destalked *U. annulipes* and *U. triangularis* may be due to the inhibitory capacity of the enzyme phosphorylase as suggested by Fingerman (1974) and Nagabhushanam and Kulkarni (1980). Wang and Scheer (1963) observed that the enzyme uredinediphosphate glucoseglycogen transglucosylase (UDPG-GT) present in muscles of crabs is under the control of eyestalk hormones. The increase of glycogen in the muscles of *U. annulipes* and *U. triangularis* may be ascribed to the lack of inhibition to this enzyme.

A post operative decline in the lipids and proteins of hepatopancreas and muscle may be due to the absence of the factors that regulate the incorporation of amino acids into proteins and partly due to conversion of lipids and proteins to carbohydrates to maintain a steady supply of glycogen. Similar observations were made by Raghavaiah (1977) in *Paratelphusa hydrodromus*, Rangneker and Madhyastha (1971) in *Metapenaeus monoceros* and Skinner (1966) in *Gecarcinus lateralis*.

Thus the pattern of increase or decrease in different constituents is the same in both the animals but the differences in their amounts may be attributed to the species specificity.

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# Fecundity of the allochthonous feeder, *Rasbora daniconius* (Ham.) and of the autochthonous feeder, *Puntius amphibius* (Val.)

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**Abstract.** The fecundity of an allochthonous feeder, *Rasbora daniconius* and an autochthonous feeder, *Puntius amphibius* in a perennial tropical pond was assessed and found to be higher in the latter. The fecundity of each of the species was correlated with parameters like standard length, weight of fish, length and weight of ovary. A comparison of the regression coefficients in the relations statistically confirmed that as the length or weight of the fish and the length of the ovary increased the rate of increase in the number of eggs was greater in *P. amphibius* suggesting a better assimilation of the autochthonous food by *P. amphibius* than of the allochthonous food by *R. daniconius*.

**Keywords.** Allochthonous feeder; *Rasbora daniconius*; fecundity; *Puntius amphibius;* autochthonous feeder.

## 1. Introduction

Knowledge about the number of eggs produced by fishes is of great importance for aquaculture. Qasim and Qayyum (1963) discussed the different ways in which knowledge about the fecundity would be useful to the fishery biologist. Fecundity studies have been carried out on a large number of freshwater fishes of India (Alikunhi and Chaudhuri 1954; Qasim and Qayyum 1963; Bhatnagar 1964, 1972; Das 1964; Parameswaran *et al* 1971; Saxena 1972; Selvaraj *et al* 1972; Varghese 1973; Sinha 1975; Bhatt *et al* 1977; Jhingran 1977; Pathak and Jhingran 1977; Bisht and Upadhay 1979; Pathani 1981). In the present paper the fecundity of an allochthonous feeder, *Rasbora daniconius* and an autochthonous feeder, *Puntius amphibius* is compared in relation to the type of food they consume.

## 2. Material and methods

The fishes were collected wild from a perennial pond during their breeding season (June to August). Various body measurements were taken before cutting open the abdomen and removing the ovary. On the whole 55 ripe ovaries of *R. daniconius* (47 and 88 mm standard length) and 47 ovaries of *P. amphibius* (65 and 105 mm standard length) were used for the study. For calculating the fecundity, the gravimetric method by which the number of eggs in accurately weighed subsamples being multiplied by the total weights of the ovaries was adopted. The final figure of fecundity was arrived at based on the average of the weights and number of eggs in three subsamples of each ovary.

In both the species the correlation between the fecundity and parameters like standard length, weight of fish, length and weight of ovary was calculated. These relationships were studied by the method of least squares according to which either the linear equation, $Y = a + b\ X$ or $\log Y = \log a + b \log X$, a linear transformation of $Y = a x^b$, was fitted, where $Y$ stood for fecundity and $a$ and $b$ were constants; $X$ stood for body measurements such as standard length (*Sl*); total body weight (*Bw*); length of ovary (*Ol*) and ovary weight (*Ow*). The constants $a$ and $b$ were calculated in each case.

## 3. Results and discussion

The fecundity varied between 929 and 7398 in *R. daniconius* and from 3379 to 24485 in *P. amphibius*. The higher fecundity of the latter clearly highlights a higher rate of egg production in *P. amphibius* than in *R. daniconius*.

According to table 1, the relationship between the fecundity ($F$) and the total body weight, the length and weight of ovary was linear (figures 2, 3 and 4) and that between fecundity and standard length of the fish, was curvilinear (figure 1) in *R. daniconius*. In *P. amphibius*, the relationship between fecundity and total body weight of the fish and length of ovary was linear (figures 6 and 7 respectively) and that between fecundity and standard length of the fish and weight of ovary was curvilinear (figures 5 and 8 respectively) suggesting that the pattern of relationship between fecundity and other parameters, except the weight of ovary was similar in both *R. daniconius* and *P. amphibius*.

The correlation coefficient worked out between the fecundity and other parameters were significant in both the species ($P < 0{\cdot}01$).

Therefore, as the length or weight of fish and the length of ovary increased, the rate of increase in the number of eggs was greater in the autochthonous feeder, *P. amphibius* than in the allochthonous feeder, *R. daniconius*. This suggests that the autochthonous food in the pond, though according to the chemical analysis was less nutritive than the allochthonous food is better made use of by *P. amphibius* which may result in higher egg production also. This is natural because *P. amphibius* was found to have better

**Table 1.** The regression and correlation coefficients between the fecundity of *R. daniconius* and *P. amphibius* and other variables of each species with equations used in each case.

| Species | Variable | Equation | Regression coefficient | Correlation coefficient |
|---|---|---|---|---|
| *R. daniconius* | *Sl* | Log F = −0·2693 + 2·0441 *log Sl* | 2·0441 | 0·7138* |
| | *Bw* | 482·0670 + 0·4315 *Bw* | 0·4315 | 0·7350* |
| | *Ol* | −2537·8400 + 18·2253 *Ol* | 18·2253 | 0·6260* |
| | *Ow* | 166·2626 + 3·0051 *Ow* | 3·0051 | 0·8866* |
| *P. amphibius* | *Sl* | Log F = −1·2835 + 2·6633 log *Sl* | 2·6633 | 0·6904* |
| | *Bw* | −1989·5533 + 0·6023 *Bw* | 0·6023 | 0·7893* |
| | *Ol* | 12085·8130 + 518·6034 *Ol* | 518·6034 | 0·7200* |
| | *Ow* | Log F = 9·9793 + 0·8765 log *Ow* | 0·8765 | 0·9581* |

* Significant ($P < 0{\cdot}01$)

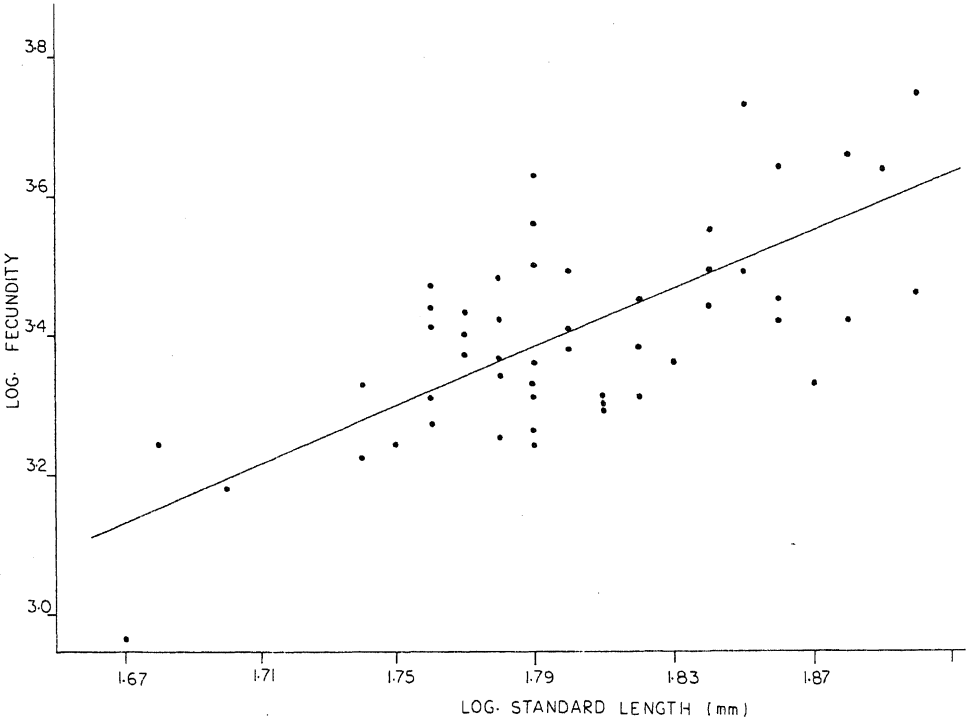


**Figure 1.** Standard length-fecundity relationship in *R. daniconius*.

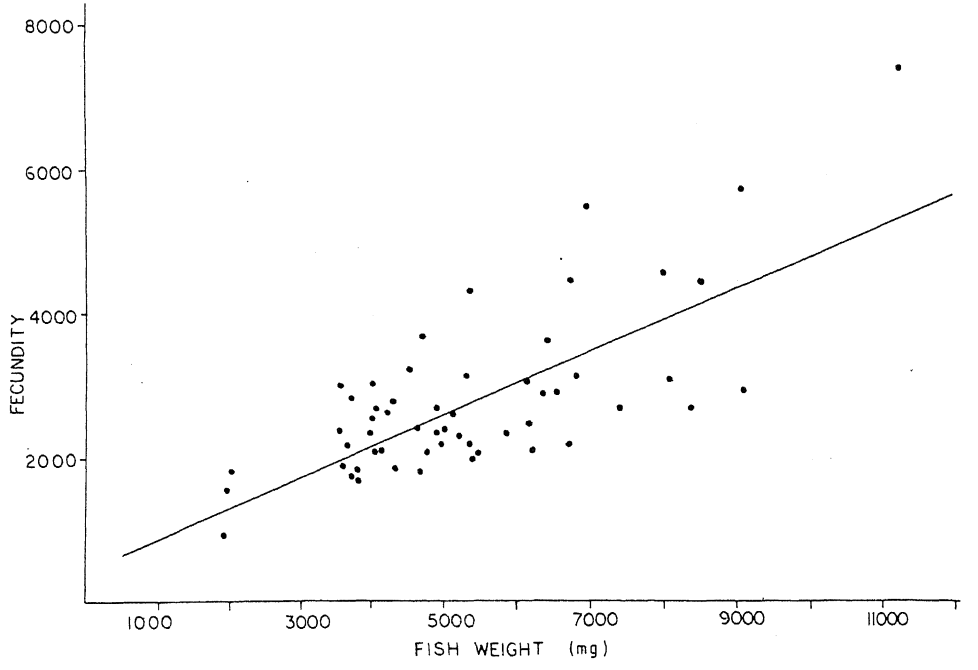


**Figure 2.** Body weight-fecundity relationship in *R. daniconius*.

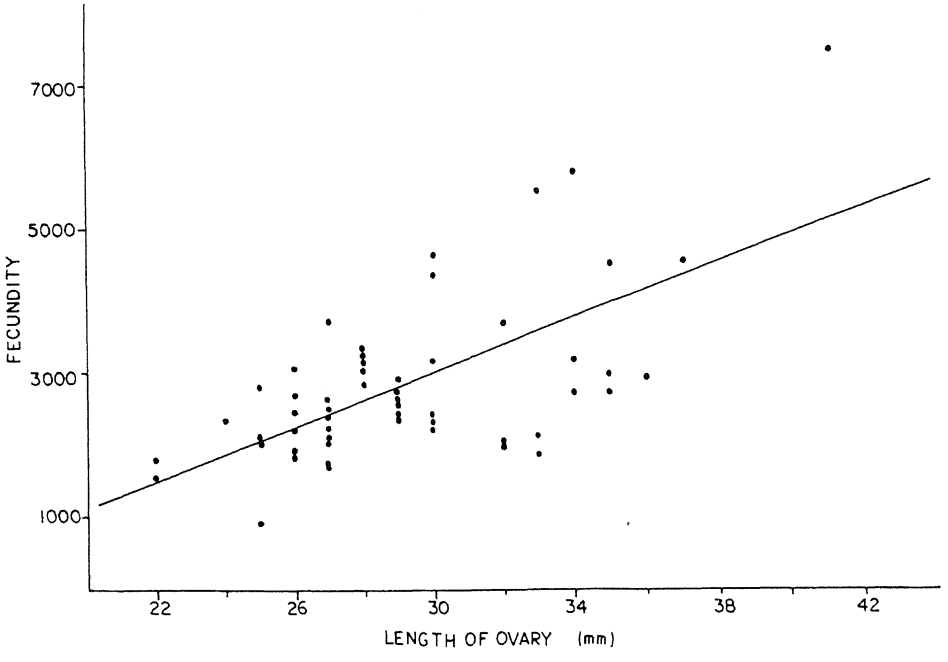


**Figure 3.** Length of ovary-fecundity relationship in *R. daniconius*.

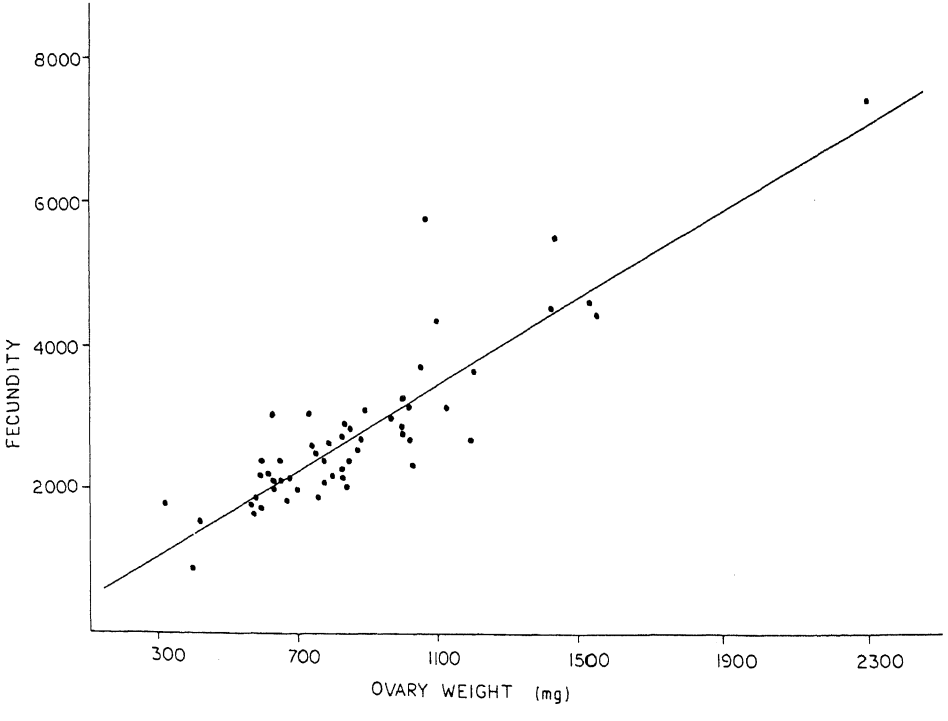


**Figure 4.** Ovary weight-fecundity relationship in *R. daniconius*.

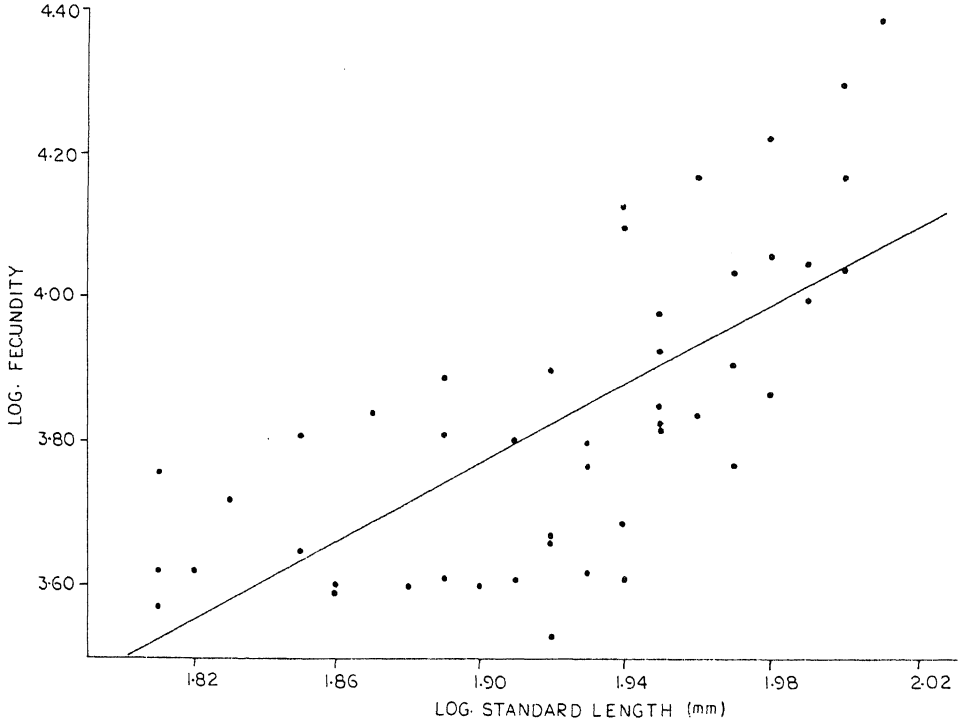


**Figure 5.** Standard length-fecundity relationship in *P. amphibius*.

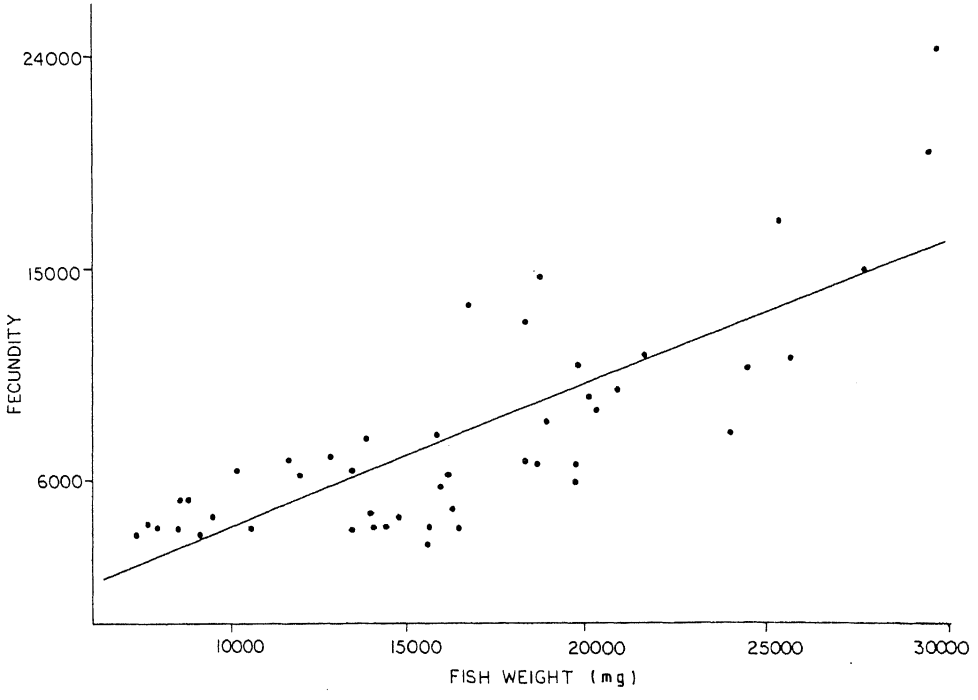


**Figure 6.** Body weight-fecundity relationship in *P. amphibius*.

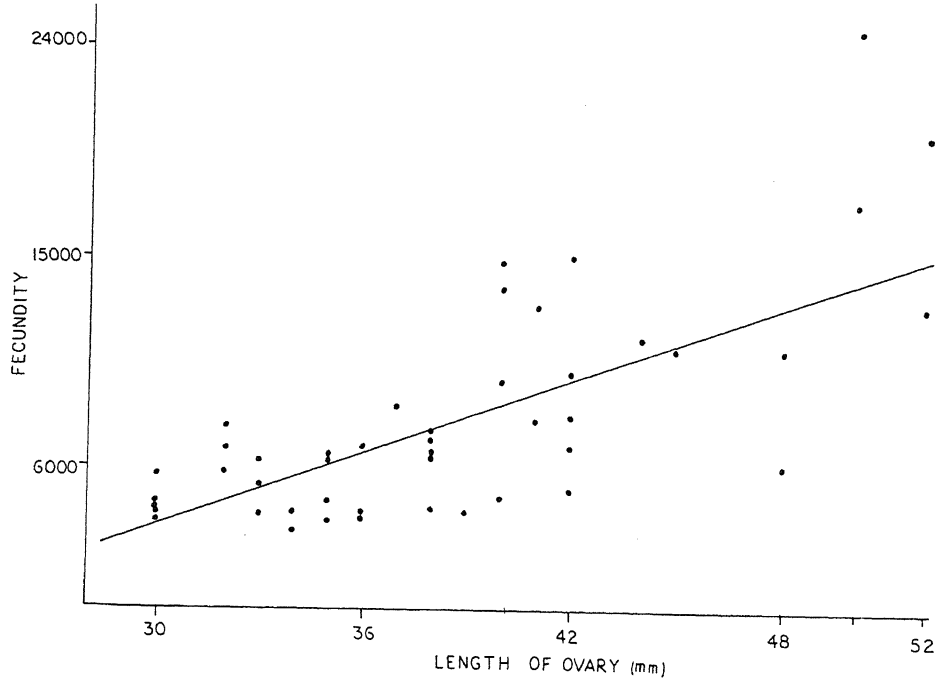


**Figure 7.** Length of ovary-fecundity relationship in *P. amphibius*.

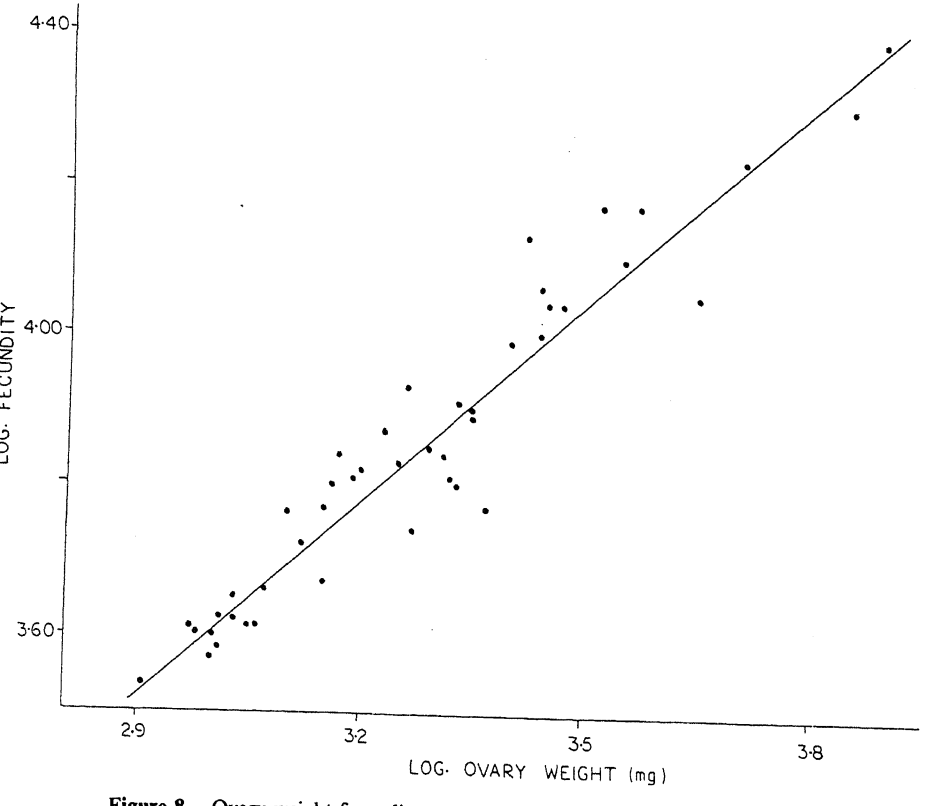


**Figure 8.** Ovary weight-fecundity relationship in *P. amphibius*.

conversion efficiency than *R. daniconius* (Prem Kumar and John 1984). It may also be more easy for the fish to absorb the nutrients from the organisms living in the same medium than from the terrestrial organisms.

The curvilinear relationship between the fecundity and standard length as seen in *R. daniconius* and *P. amphibius* agrees with the findings of Gupta (1968) and Sinha (1975). But, according to Qasim and Qayyum (1963) in fishes which seldom grow more than a few inches in length there exists a linear relationship between body length and fecundity.

A linear relationship between fecundity and fish weight similar to that in *R. daniconius* and *P. amphibius* has been reported in *Labeo fimbriatus* by Bhatnagar (1972), in *P. sarana* by Sinha (1975) and in *R. daniconius* by Nagendran *et al* (1981). Yuen (1955), however, has reported a curvilinear relationship between fecundity and fish weight in the big eye tuna.

The linear relationship between the ovary weight and fecundity as seen in *R. daniconius* is a common feature which has been reported in several fishes (Pantalu 1963; Qasim and Qayyum 1963; Bhargava 1970; Bhatt *et al* 1977; Nagendran *et al* 1981). On the other hand, the curvilinear relationship which was found to exist between the fecundity and ovary weight in *P. amphibius* has also been reported in other freshwater fishes (Rita Kumari 1977; Babu 1981).

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# Development, endocrine organs and moulting in the embryos of *Dysdercus cingulatus* Fabr (Heteroptera: Pyrrhocoridae)

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**Abstract.** Embryonic development of *Dysdercus cingulatus* is briefly described. In the embryos, neurosecretory cells become evident in the median, lateral and ventral aspects of protocerebrum 84 hr after egg laying. The corpora cardiaca, the corpora allata and the prothoracic glands arise more or less simultaneously at 78 hr from the dorsolateral wall of the stomodaeum, from the mandibular segments and from the labial segments respectively. Secretory material appears in the brain neurosecretory cells and in the intrinsic cells of the corpus cardiacum at 84 hr and in the nervi corpori cardiaci and aorta at 90 hr. The cells of the prothoracic glands show signs of secretory activity at 90 hr, reaching maximum activity around 96 hr. The corpus allatum appears to be inactive in the embryo. Two embryonic moults appear between 96 hr and 110 hr. Consequence of events suggests that the neurosecretory material stimulates the prothoracic glands including embryonic moulting.

**Keywords.** Neurosecretory cells; corpora cardiaca; corpora allata; prothoracic glands; secretory activity; embryonic moulting; *Dysdercus cingulatus*

## 1. Introduction

Endocrine activity during insect embryogenesis appears to have been first reported by Jones (1956a,b) in *Locusta pardalina* and *Locusta migratoria*. Ontogeny of the neurosecretory cells was studied by Khan and Fraser (1962) in *Periplaneta americana*, and by Dorn (1972, 1975b) in *Oncopeltus fasciatus*. Development of corpora cardiaca was traced in the embryos of *Melanoplus differentialis* (Baden 1936), *Rhodnius prolixus* (Mellanby 1936), *Drosophila* (Poulson 1937) and in *Oncopeltus fasciatus* (Dorn 1972, 1975c). Development of the corpus allatum has been worked out in *Oncopeltus fasciatus* (Dorn 1972). Earlier work on the allatum includes that of Nussbaum (1889), Wheeler (1893), Mellanby (1936) and Roonwal (1937). Development of prothoracic glands has been studied by Toyama (1902) in the silkworm, and Darquenne (1978) in *Leucophaea maderae*. Sharan and Sahni (1960) reported the presence of neurosecretory cells in the embryos of *Dysdercus cingulatus* and Wells (1954) studied the development of its prothoracic glands. The purpose of the present investigation has been to study in detail the development of the endocrine glands in the red cotton bug *Dysdercus cingulatus*, and to find out if there is any interrelationship between the endocrine activity and embryonic moulting in this animal.

## 2. Materials and methods

Stock colony of *Dysdercus cingulatus* was reared in the laboratory as described by Jalaja and Prabhu (1976). Pairs were kept isolated in glass chimneys on soaked cotton

seeds, and the eggs laid in clutches of 100–150 during the first gonotrophic cycle were collected immediately after laying. Thus eggs of known age were available for the study.

### 2.1 *Development of the embryo and the endocrines*

Development of the embryo, the neurosecretory cells, the corpora cardiaca, the corpora allata and the prothoracic glands was studied after fixing the eggs in warm Smith's fluid. After processing for general embryology, sections were stained in Heidenhain's iron haematoxylin and eosin; paraffin sections were stained in Gomori's chrom alum haematoxylin phloxin (Gomori 1941) for the study of the cerebral neuroendocrine complex. The brain was also dissected out from four-day and five-day old embryos and fixed in formol saline, and stained using Humberstone's performic acid victoria blue technique (Dogra and Tandon 1964), for whole mounts.

### 2.2 *Measurements*

All measurements were taken using a calibrated ocular micrometer. In the embryo, there were spherical, oval and pear-shaped neurosecretory cells. The diameter of the spherical cells, the mean of the length and the breadth of the oval and pear-shaped cells were taken and the volume of the cells calculated (see below). In the embryonic prothoracic glands, there were spherical and oval cells. The diameter of the spherical cells and mean of the length and the breadth of the oval cells were found. The diameter of nuclei was also measured. The volume of spherical cells was calculated using the formula $4/3\,\pi r^3$ where $r$ was the radius, while that of oval cells was calculated using the formula $4/3\,\pi\ ab^2$ where $a$ and $b$ represented half the length and breadth respectively (Penzlin 1971). The volume of the nuclei was also calculated using the first formula. The length and the breadth of the corpus cardiacum were noted and the approximate number of cells in this organ was estimated from the number of nuclei counted. The volume of the corpus allatum was calculated according to the method of Scharrer and Von Harnack (1960). The number of their nuclei was counted from serial sections and the volume of the allatum per nucleus was calculated. The diameter of the corpus allatum nuclei was also measured and the nuclear volume calculated.

## 3. Observation

### 3.1 *Development of the egg*

The main events during embryonic development are summarised in table 1. The eggs which are centrolecithal, undergo first cleavage at 9 hr. The divisions are initially synchronous, but by 16 hr become asynchronous. By 24 hr, cleavage is complete, most of the nuclei move to the periphery, leaving behind a few remaining nuclei scattered among yolk granules. The nuclei at the periphery undergo rapid proliferation leading to the formation of a thin layer of blastoderm. The whole yolk is enclosed in a cellular blastoderm by the end of 35 hr. Between 36 hr and 38 hr the cells of the blastoderm begin to aggregate at the posterior pole towards the ventral side. The cells of the

**Table 1.** Table showing stages of embryonic development in *Dysdercus cingulatus*

| Number | Stages | Hours of development |
|---|---|---|
| 1 | Oviposition and Maturation | 0–8 |
| 2 | Cleavage | 8–24 |
| 3 | Blastoderm | 24–36 |
| 4 | Germ Band | 36–49 |
| 5 | Gastrulation | 49–51 |
| 6 | Segmentation | 51–74 |
| | (a) Differentiation of mesoderm | 52–53 |
| | (b) Differentiation of neuroblast | 52–55 |
| | (c) Fusion of amniotic folds | 55–70 |
| | (d) Differentiation of stomodaeum and proctodaeum | 60–74 |
| 7 | Blastokinesis | 74–84 |
| | (a) Anatrepsis | 74–78 |
| | (b) Katatrepsis | 78–84 |
| 8 | Definitive dorsal closure and absorption of dorsal organ | 84–90 |
| 9 | Consolidation of nervous system and Pigmentation of eye and abdomen become apparent | 90–96 |
| 10 | Embryo-larval transition | 96–120 |
| | (a) I moult | 96–98 |
| | (b) Thoracic legs develop claws | 98–108 |
| | (c) II moult | 108–110 |
| | (d) Pigmentation deepened | 110–120 |
| 11 | Hatching | 120–124 |

remaining portion of the blastoderm get flattened and form the extra-embryonic blastoderm. The embryonic primordium or the germ band is formed by tubular invagination into the yolk. Rarely two lateral, faintly marked and irregular thickenings develop, which later fuse together from which a tubular invagination arise. By 49 hr the germ band reaches maximum size, the blunt end extending half way along the dorsal side, rather superficially. The thinner, inner wall of the germ band is the amnion; the future embryo develops from the thicker outer wall; the anterior end of germ band is comparatively broader representing the head region of the future embryo.

Gastrulation takes place between 49 and 51 hr by migration of the cells proliferating from the longitudinal groove along the germ band. These cells form a single layer below the original layer by 55 hr, the latter being now ectoderm. By 60 hr the germ band gets segmented, with appendage rudiments on the head and thorax. By 65–70 hr the serosa covering the entire egg, and the amnion covering the embryo are established.

Segmentation is distinguishable first in the thoracic region; then the cephalic region and finally the abdominal region get segmented. In 51–52 hr old embryos, four transverse grooves appear at the thoracic region giving rise to the three thoracic segments, the cephalic region and the abdominal region. In close connection with the head lobe appear three pairs of appendage rudiments: the small labral, the larger antennal and smaller mandibular rudiments, one behind the other. Below these are the

maxillary and the labial segments with their appendage rudiments. Rudiments of the thoracic legs also develop on the thoracic segments. In the abdomen appear eleven segments demarcated by transverse grooves. The head lobes are pushed in from the surface between 65–70 hr and are covered by amnion. A fully segmented germ band with rudiments of appendages on head and thorax was visible by 72 hr (figure 1).

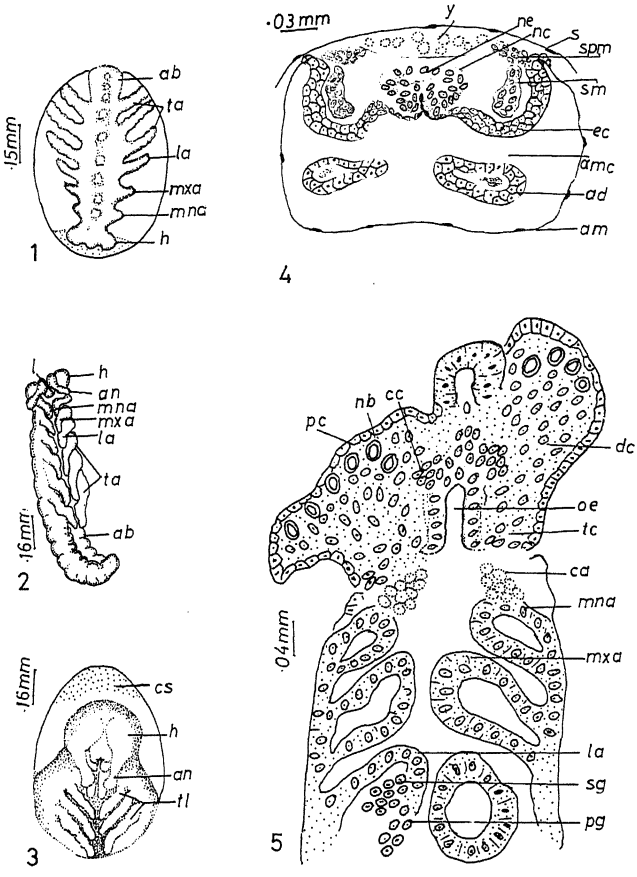
The stomodaeum is in the form of a shallow depression at 60 hr, but becomes a conspicuous tube by 74 hr. The proctodaeal invagination is also visible now; subsequently, the cells at the tip of the proctodaeal invagination proliferate to form the posterior enteron rudiment. The cells at the tip of the developing stomodaeum spread over the already formed inner layer to form the anterior enteron rudiment from which cells grow gradually to form the wall of the midgut. The inner layer which gets separated during gastrulation constitutes the mesoderm, and this layer at anatrepsis differentiates to somatic and splanchnic mesoderm (figure 4).

A continuous neural groove from the anterior to the posterior region is seen by 65 hr, and neuroblasts develop from the neural ridges situated on either side of the groove. Between 65 and 70 hr segmentally arranged ganglionic masses differentiate and by 78 hr the ventral nerve cord is completely separated from the ectoderm (figure 4). However, the rows of neuroblasts in the mandibular segment diverge on either side of the stomodaeum and are hence further apart in the head lobe. At 72 hr neuroblasts are peripherally arranged; neurons are subsequently set apart from neuroblasts in the head region, thus forming the three ganglionic masses: the proto, deuto, and the tritocerebrum. A well-developed neuropile becomes evident only after rotation of the embryo. The nervous system of the embryo just after blastokinesis consists of the brain, the three thoracic ganglia and 11 abdominal ganglia. By 90 hr the abdominal ganglia get consolidated to form a single ganglionic mass attached to the last thoracic ganglion.

Anatrepsis, the folding of the appendages to the ventral side, and shortening of the germ band as a whole, constituting the first phase of blastokinesis is over by 78 hr (figure 2). The second phase or rotation of the embryo, constituting katatrepsis (figure 3) is over by 84 hr; the provisional dorsal closure is replaced by permanent embryonic ectoderm by 90 hr. The dorsal organ is now absorbed by the yolk.

### 3.2 *Endocrine organs*

3.2a *Neurosecretory cells:* After blastokinesis, at 84 hr of development when the brain develops neuropile, the neurosecretory cells become evident, when many of them already contain quite a lot of neurosecretory material (table 2) stainable blue with Gomori's haematoxylin. The cells may be either oval, spherical or pear (figure 8) shaped. They are distributed in the median, lateral and ventral aspects of the protocerebrum (figure 9). The data on these cells during embryonic development are shown in table 2. The secretory material gradually decreases in quantity from around 94 hr. Cell size as well as nuclear size of the neurosecretory cells, which may be taken as an index of their activity, also decreases from 96 hr onwards or slightly earlier. Later on fifth day towards hatching, the number of recognizable neurosecretory cells are very few as many of them have emptied their secretory content; the cells as well as the nuclei also dwindle substantially in size, indicating very low activity.

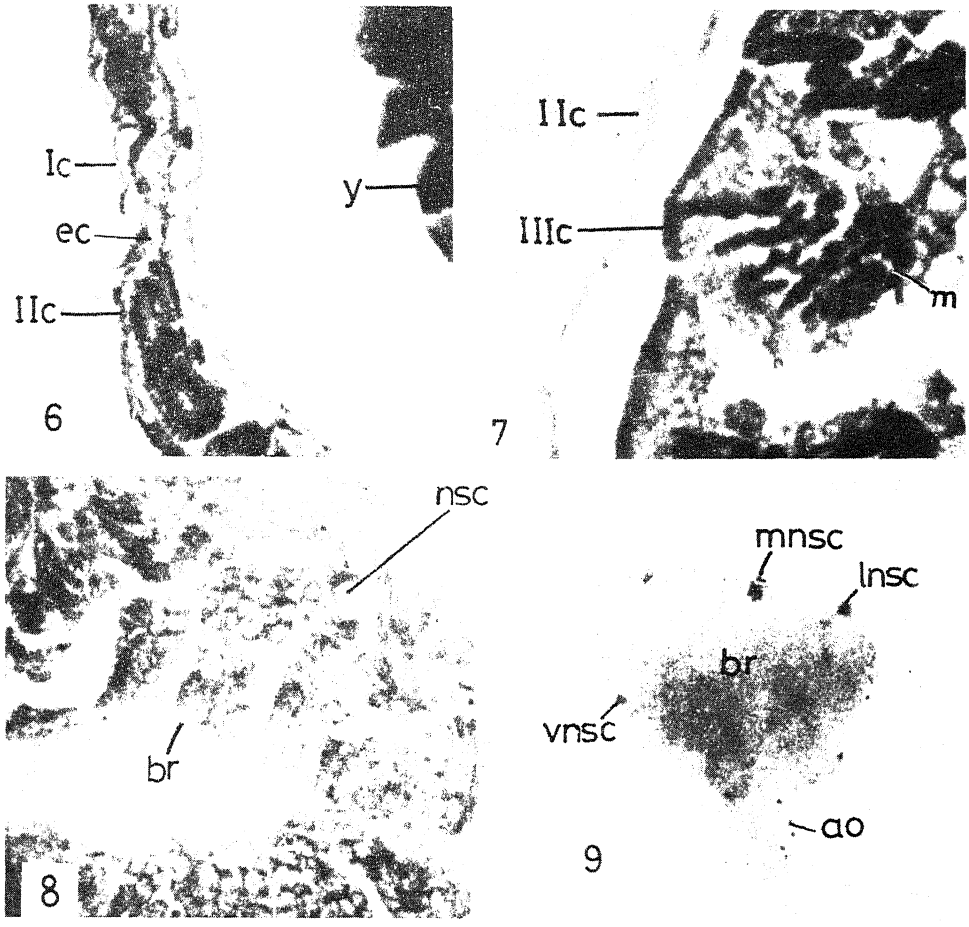



**Figures 1–5.** Camera lucida sketches. (**1**) 72 hr old embryo showing fully segmented germ band with developed appendages, dorsal view. (**2**) 78 hr old embryo showing anatrepsis. Note that the appendages are withdrawn from the surface of the yolk and folded on the ventral surface of the embryo, ventral view. (**3**) 82 hr old embryo showing advanced katatrepsis, where the head reached almost at the anterior pole of the egg, ventral view. (**4**) Cross-section through germ band showing differentiation of mesoderm and nerve cord. (**5**) Frontal section through embryo at 78 hr. Origin of corpora cardiaca, corpora allata, and prothoracic glands have been shown (**Abbreviations:** ab—abdomen; ad—appendage; am—amnion; amc—amniotic cavity; an—antenna; ca—corpus allatum; cc—corpus cardiacum; cs—contracted serosa; dc—deutocerebrum; ec—ectoderm; h—head; la—labial appendage; mna—mandibular appendage; mxa—maxillary appendage; nb—neuroblast; nc—nerve cord; ne—neuron; oe—oesophagus; pc—protocerebrum; pg—prothoracic gland; s—serosa; sg—salivary gland; sm—somatic mesoderm; spm—splanchnic mesoderm; ta—thoracic appendage; tc—tritocerebrum; tl—thoracic legs; y—yolk).

3.2b *The corpora cardiaca:* Rudiments of the corpora cardiaca make their appearance at 78 hr during anatrepsis (figure 5). The corpora cardiaca originate as a string of cells on either side from the dorsolateral walls of the stomodaeum (figure 10). After blastokinesis they appear triangular, placed behind the brain on either side of oesophagus, below the aorta. The cells of the cardiaca are confined to the posterior portion of the organs whereas the anterior portion is composed of nerve fibres. The

**Table 2.** Changes in size of neurosecretory cells of *Dysdercus cingulatus* during embryonic development (data represent mean values of cells from six embryos at each hour)

| Development (Hours) | Type of cell and size | | Cell volume $\mu m^3$ | Number of cells on − to +++ scale | | | | Neuro-secretory index | | Nuclear size $\mu m$ | Nuclear volume $\mu m^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type | Size | | − | + | ++ | +++ | | | | |
| 84 | Spherical | dia = 12 | 904·31 | 0 | 1 | 2 | 2 | 11 | 32 | 9 | 381·50 |
| | Oval | l = 13, b = 8 | 435·41 | 0 | 1 | 2 | 3 | 14 | | 7·5 | 220·78 |
| | Pear shaped | l = 10, b = 7 | 256·43 | 0 | 0 | 2 | 1 | 7 | | 5 | 65·41 |
| 96 | Spherical | dia = 12 | 904·31 | 0 | 1 | 3 | 1 | 10 | 25 | 9 | 381·50 |
| | Oval | l = 12, b = 8 | 401·91 | 1 | 2 | 2 | 1 | 9 | | 7 | 179·50 |
| | Pear shaped | l = 11, b = 6 | 207·43 | 0 | 1 | 1 | 1 | 6 | | 5 | 65.41 |
| 120 | Spherical | dia = 9 | 381·5 | 1 | 2 | 0 | 0 | 2 | 5 | 5 | 65·41 |
| | Oval | l = 10, b = 6 | 188·39 | 1 | 3 | 0 | 0 | 3 | | 4 | 33·49 |




**Figures 6–9.** Microphotographs. (**6**) Sagittal section through 98 hr old embryo showing first embryonic moult. See the separating first cuticle and the depositing second cuticle × 700. (**7**) Sagittal section through 108 hr old embryo showing second moult. Original cuticle has already been removed along with chorion and hence not visible in section. Separation of second cuticle and deposition of third cuticle are shown × 700. (**8**) Frontal section through brain of four-day-old embryo showing lateral pear shaped neurosecretory cell × 700. (**9**) Whole mount of brain of four-day-old embryo showing median, lateral and ventral neurosecretory cells × 400 (ao—aorta; br—brain; Ic—first cuticle; IIc—second cuticle, IIIc—third cuticle; ec—ectoderm; lnsc—lateral neurosecretory cells; m—muscle; mnsc—median neurosecretory cells; nsc—neurosecretory cells; vnsc—ventral neurosecretory cells; y—yolk)

corpus cardiacum is about 30–35 $\mu$m long, 20–25 $\mu$m broad and 20 $\mu$m thick. No change in size is observed in the cardiaca during further embryonic development. There are altogether 25–30 cells in a cardiacum. Of these only five to six cells, which occupy the posterior portion, are stainable blue by Gomori's method and these constitute the intrinsic secretory cells of the cardiacum. The remaining cells, the chromophobe cells, which do not contain any stainable material, surround the nerve fibres; the nerve fibres hence occupy the central region of the cardiacum.

3.2c *Stainability of the brain-cardiacum tract:* As already reported above, at 84 hr, the brain neurosecretory cells and five to six cells of the corpus cardiacum are stainable blue by Gomori's method. At this time the tract from the brain or the nerve fibres of the cardiacum is not stainable. Evidently, transportation of secretory material to cardiacum has not yet started. However at 90 hr both the tracts become stainable indicating initiation of transportation. At 102 hr, though in the brain neurosecretory cells there is decrease in their neurosecretory content and many of these cells are scarcely recognizable, the connecting nerve tracts are stainable due to transportation of material. At 120 hr only a few neurosecretory cells in the brain are distinguishable and they show very little neurosecretory material. The secretory material decreases in the connecting tract and the nerve fibres in the cardiacum. By now the stainability of the cardiacum also decreases. Apparently, most of the material from the nerve tract and cardiacum has now been released.

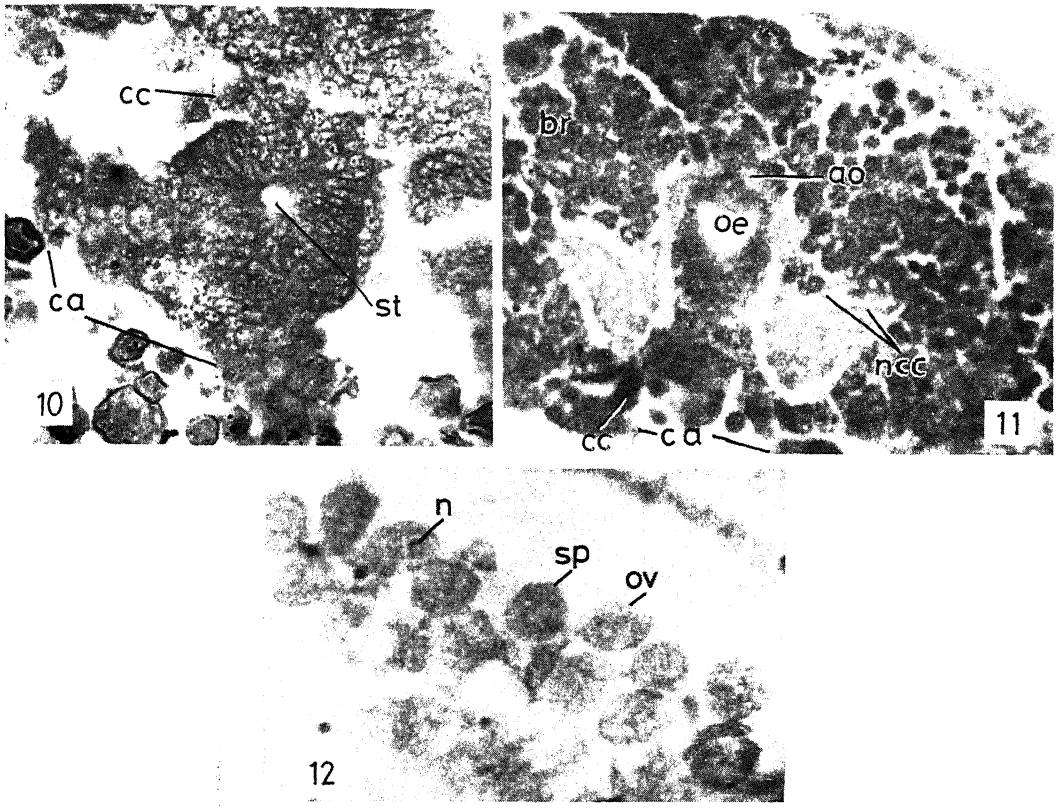
3.2d *Neurosecretory material and the aorta:* Till 84 hr, there is very little evidence for the presence of neurosecretory material in the aorta wall. But there is gradual accumulation of secretory material in the aorta thereafter and by 96 hr, the aorta contained as much secretory material as in the nervi corpori cardiaci (figure 11). However, by 120 hr all the secretory material in the aorta appears to have been released as there is hardly any secretory product in the aorta now.

3.2e *Corpora allata:* The corpora allata develop simultaneously with corpora cardiaca as paired invaginations from the mandibular segments at 78 hr during anatrepsis (figure 5) and migrate in the anterodorsal direction. They finally come to be situated behind the corpora cardiaca attached to the oesophagus below the aorta (figure 10). Each corpus allatum has now about 35 cells which are closely packed. The corpora cardiaca and the corpora allata are seen in close association after blastokinesis. The corpora allata do not appear to undergo any change with regard to size during embryonic development (table 3). Their nuclei however undergo a further increase to over 50 in number.

3.2f *Prothoracic glands:* The primordia of the prothoracic glands make their appearance at the time of anatrepsis (figure 5). They originate as a small pair of invaginations from the labial segments along with a larger pair of invaginations (the future salivary glands). After blastokinesis the salivary gland rudiments extend backwards into the thorax and the prothoracic gland rudiments are also drawn along with it. There are 90–100 cells in the prothoracic glands of a four-day-old embryo. Prothoracic gland is a ribbon of cells which are loosly embedded in their connective tissue. During 78–84 hr, the glands consist of oval as well as spherical cells, the majority being oval. The changes undergone by the cells are shown in table 4 (figure 12). Around 96 hr the cell size and nuclear size have increased to about 8 times. By 120 hr both the cells as well as the nuclei have again become smaller, shrinking to their original size, indicating an apparant cessation of activity.

3.3 *Embryonic moults*

By 96–98 hr the first cuticle appears to separate from epidermis and a new one is




**Figures 10–12.** Microphotographs. (**10**) Frontal section through 78 hr old embryo showing the development of corpora cardiaca and corpora allata × 700. (**11**) Frontal section through brain of four-day-old embryo showing close association of corpora allata and corpora cardiaca and the presence of secretory material in the aorta and nervi corpori cardiaci × 700. (**12**) Sagittal section through 84 hr old embryo showing prothoracic gland cells × 1000 (ao—aorta; br—brain; ca—corpora allata; cc—corpora cardiaca; n—nucleus; ncc—nervi corpori cardiaci; oe—oesophagus; ov—oval cells; sp—spherical cells; st—stomodaeum).

**Table 3.** Changes in corpus allatum of *Dysdercus cingulatus* during embryonic development (data represent mean values of cells from six embryos at each hour)

| Development hour | Volume of Ca $\mu m^3$ | Number of nuclei | Mean cell volume | Nuclear size | Nuclear volume |
|---|---|---|---|---|---|
| 84 | 3401 | 53 | 64·16 | 2·33 | 6·61 |
| 96 | 3924 | 60 | 65·40 | 2·33 | 6·61 |
| 120 | 3640 | 58 | 62·75 | 2·33 | 6·61 |

observed attached to the epidermis, constituting the first embryonic moult (figure 6). Similarly, at 108–110 hr a thicker cuticle is observed close to the epidermis, the second cuticle having separated from it, constituting the second moult (figure 7). During the first moult muscle fibers are not well differentiated whereas during the second moult thick muscle fibers are seen.

**Table 4.** Changes in cell size and nuclear size of prothoracic glands in the embryos of *Dysdercus cingulatus* during development (data represent mean values of cells from six embryos at each hour)

| Development (hours) | Cell diameter (μm) | | Cell volume μm³ | Nuclear diameter μm | Nuclear volume μm³ |
|---|---|---|---|---|---|
| | Spherical cells | Oval cells | | | |
| 84 | 4·67 | — | 53·32 | 2·33 | 6·61 |
| | | l = 5·8<br>b = 4·28 | 55·60 | 2·11 | 4·91 |
| 96 | 9·32 | — | 423·88 | 4·61 | 51·29 |
| 120 | 5·16 | — | 71·93 | 2·16 | 5·27 |
| | | l = 5·61<br>b = 4·80 | 67·64 | 2·20 | 5·57 |

## 4. Discussion

The pattern of embryonic development in *Dysdercus cingulatus* follows the general heteropteran pattern (Anderson 1972). The blastoderm in this animal forms a median thickening, though very rarely two lateral thickenings are also observed, when they are rather irregular and faintly marked on the surface of the embryo, as in *Oncopeltus fasciatus* (Butt 1949) but unlike *Blissus leucopterus hirtus* (Choban and Gupta 1972), *Rhodnius prolixus* (Mellanby 1935) and *Pyrrhocoris apterus* (Seidel 1924). The developing germ band lay close to the ventral surface of the eggs of *Dysdercus cingulatus* as in *Pyrrhocoris apterus* (Seidel 1924) and in *Oncopeltus fasciatus* (Butt 1949). However, the germ band of *Rhodnius prolixus* lay superficially on the dorsal side of the egg (Mellanby 1935) whereas in *Blissus leucopterus hirtus* (Choban and Gupta 1972) it is neither on the dorsal side nor on the ventral side, but it took a position in between.

In *Dysdercus cingulatus*, the present studies revealed that masses of cells proliferated from the embryonic layer, migrating into the yolk. Subsequently, they arranged themselves as a layer inner to the original layer, forming the mesoderm as in *Blissus* (Choban and Gupta 1972). In *Locusta*, Roonwal (1936) proposed a multiphased gastrulation. In *Pyrrhocoris* gastrulation was by sinking of a ridge of cells without a lumen into the yolk (Seidel 1924), and consisted not only of invagination but also delamination of the inner layer corresponding to the entomesoblast whose cells differentiated from the ectoblast (Matolin 1973). In *Rhodnius prolixus* however the central part of the embryonic rudiment invaginated, which overgrew to the lateral portions (Mellanby 1935).

In the head region, six segments got differentiated during embryonic development in *Dysdercus cingulatus*, as in *Oncopeltus fasciatus* (Butt 1949) and in *Blissus* (Choban and Gupta 1972), with five pairs of appendage rudiments viz. labral, antennal, mandibular, maxillary and labial. No appendage rudiment was observed on the first abdominal segment in *Dysdercus cingulatus* as in *Blissus* (Choban and Gupta 1972) or in *Oncopeltus* (Butt 1949) though Dorn (1972) and Matolin (1973) observed a pleuropodium in *Oncopeltus fasciatus* and *Pyrrhocoris apterus* respectively. A prominent pleuropodium was also present in *Belostoma* and *Ranatra* (Hussey 1926).

In *Dysdercus cingulatus*, the anterior and posterior enteron rudiments developed independently of the inner layer by proliferation of ectoderm cells from the tip of the stomodaeal and proctodaeal invaginations, unlike in *Pyrrhocoris* (Seidel 1924) and in *Rhodnius prolixus* (Mellanby 1935) in which the enteron arose from masses of cells from the anterior and posterior ends of the inner layer.

Neurosecretory cells have been reported earlier also in the embryos of many insects (Khan and Fraser 1962; Dorn 1972; Jones 1956a), including *Dysdercus cingulatus* (Sharan and Sahni 1960; Mariamma Jacob and Prabhu 1979), but the present studies give details of the secretory activity of the cells and their relationship with other endocrine glands during development, as well as moulting. The corpora cardiaca in the embryos of *Dysdercus* arose from the stomodaeum as in other insects (Mellanby 1936; Baden 1936; Pflugfelder 1937; Poulson 1937; Roonwal 1937; Dorn 1972). The allatum in the embryo of *Dysdercus cingulatus* arose from mandibular segments as ectodermal invaginations, though they appeared to arise from mandibular and maxillary segments in *Carausius morosus* (Leuzinger *et al* 1926), and in *Locusta migratoria* (Roonwal 1937) and from maxillary segments in *Oncopeltus fasciatus* (Dorn 1972), *Rhodnius prolixus* (Mellanby 1936). The present studies in *Dysdercus cingulatus* showed that the allatum is apparently inactive during embryonic development of the animal, as it did not show any cyclic changes in the embryo. This is in step with the findings of Jones (1956a) and Sharan and Sahni (1960) though in *Periplaneta americana* and *Oncopeltus fasciatus* the corpus allatum showed fluctuation in size suggesting secretory activity (Khan and Fraser 1962; Dorn 1972, 1975a). The present studies show that in *Dysdercus cingulatus* also prothoracic glands arose from labial segments as already reported in insects (Wells 1954; Dorn 1972; Darquenne 1978).

From the present studies, it is clear that there is secretory cycle in the brain neurosecretory cells in the embryos of *Dysdercus cingulatus*. There were about 14 neurosecretory cells in the brain; their activity decreased from 84 hr to 120 hr while the size of the cell and of nuclei and the secretory content were taken as the criteria. When stainability of the cells, the nerve tract and the cardiacum are considered, there was clear accumulation by 84 hr followed by a release of material which is complete by 120 hr when the cells, the tract and the aorta, all become devoid of secretory material, indicating that the release has already been taken place by 120 hr. It may be noted that though in the adult *Dysdercus cingulatus* the neurosecretory tract from the A-cells does not enter the corpus cardiacum, but bypasses it to enter the aorta (Jalaja and Prabhu 1977), in the embryo the tract passes through the cardiacum. This is comparable to the condition in *Oncopeltus fasciatus* (Dorn 1975b, 1977). The prothoracic gland cells tremendously increase in size from 84 hr to 96 hr when their nuclei also reach maximum size indicating very high activity around this period, followed by gradual inactivity. It would appear that the release of neurosecretory material stimulates prothoracic gland activity which release its products around 96 hr and thereafter. Two embryonic moults in *Dysdercus cingulatus* observed during 96–98 hr and 108–110 hr in the present study are apparently connected with the release of moulting hormone by the prothoracic glands during their secretory cycle reported above. The corpus allatum however does not appear to play any role in the embryo. Possibly the maternal juvenile hormone found in embryos (Gilbert and Schneiderman 1961) serves the purpose, whereas the embryo appears to be capable of producing its own ecdysone required for embryonic moults.

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# Seasonal variations and the role of neurosecretory hormones on the androgenic gland of the prawn *Macrobrachium lamerrii*

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**Abstract.** The androgenic gland of *M. lamerrii* is situated near the seminal vesicle and along the vas deferens of the male prawns. Histologically it is made up of cords of cells which are arranged loosely. Histochemical analysis of the androgenic gland cells showed the presence of cystine/cysteins, protein bound amino acid groups. Androgenic gland of *M. lamerrii* shows signs of increased secretory activity during the sexually active phase of the male. In adult prawns, eyestalk ablation results in the hypertrophy of androgenic gland. Brain and thoracic ganglion extracts also showed enhanced secretory activity of androgenic gland and corresponding gonadal activity in the male prawns.

**Keywords.** Androgenic gland; eyestalk ablation; *Macrobrachium lamerrii*; eyestalk extract; brain extract; thoracic ganglion.

## 1. Introduction

The androgenic gland which secretes androgenic hormones has been established as the endocrine gland which is responsible for the differentiation of the primary, secondary sexual characters in the malacostracan crustaceans (Charniaux-Cotton 1960, 1964). Seasonal variations in the androgenic gland activity has been reported in the crustaceans (Hoffman 1968, 1969). The neuroendocrine control of androgenic gland by eyestalk ablation and eyestalk extract injections was experimented in crabs (Demeusy and Veillet 1958; Rangnekar *et al* 1971). The above literature shows that the freshwater prawns were neglected in this aspect which prompted us to note the seasonal cyclicity of androgenic gland and the role of neurosecretory hormones on the androgenic gland of the freshwater prawn, *Macrobrachium lamerrii.*

## 2. Material and methods

The androgenic glands (AG) used in the present study were obtained from intermoult (C) prawns. The AG were dissected out and kept in crustacean saline for morphological observations. For histological preparations, the AG were fixed in Bouins fluid and sectioned at 8 $\mu$m, stained in Gomori's chrome alum hematoxylin phloxine (CHP). The eyestalk ablation, eyestalk extract and brain, and thoracic ganglion extract injections were done as described by Diwan and Nagabhushanam (1974).

Histochemical nature of the androgenic gland was studied by treating the tissues in Susa, carnoy and alcoholic Bouins fixative for 24 hr. The tissues were dehydrated, paraffin embedded and sections (7–8 $\mu$ in thickness) were treated for histochemical tests

**Table 1.** Results of histochemical tests on the androgenic gland of *M. lamerrii*.

| Tests | Cytoplasm | Nucleus |
|---|---|---|
| For proteins | | |
| Mercuric bromophenol blue | ++ | + |
| Millons reaction (Bensley and Gersh modification) | − | − |
| Millons reaction (Baker modification) | − | − |
| Ninhydrin-Schiff method | + | + |
| Ferric cyanide method for -SH groups | − | − |
| Aldehyde fuchsin | + | ± |
| For carbohydrates | | |
| Best's carmine | − | − |
| Performic acid (Schiff method (PFAS)) | − | − |
| Periodic acid-Schiff method (PAS) | ± | ± |
| For lipids | | |
| Sudan black B | ± | ± |

− = negative; ± = doubtful; + = positive; ++ = intensity moderate.

as given in table 1. The histology of the testis was done by fixing the tissue in Bouin's fluid and staining the sections with Harri's haematoxylin eosin. The number of testicular follicles were counted.

## 3. Observations and results

### 3.1 *Histology and histochemistry of* AG

In the male *M. lamerrii* the AG was located near the terminal part of the seminal vesicle and extended over the vas deferens to which it is superficially attached (figure 1). The diameter of the AG measured at the seminal vesicle was 75 $\mu$m and near the vas deferens it is in the form of a diffused structure.

In whole mounts the AG looked transparent and the size varied with the reproductive stage of the animal. (21·8–90·5 $\mu$m). In histological preparations, the cords of the cells

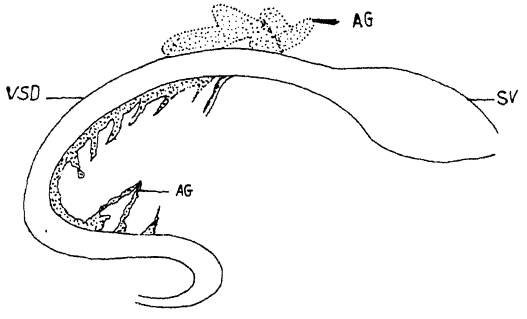


**Figure 1.** Gross morphology of androgenic gland. (AG-androgenic gland, SV-seminal vesicle, VSD-vas deferens.)

are loosely arranged, the cytoplasm in the cells scanty and the nuclei stained dark blue-black.

The histochemical tests revealed that the AG cells are positive to bromophenol blue and ferric ferricyanide and Millon's reaction failed to detect SH groups and tyrosine, but aldehyde fuchsin and ninhydrin tests gave positive results. The performic acid Schiff and Best's carmine tests gave negative reports.

In routine histological preparations, the gland cells appear almost identical and the cell boundaries can be hardly made out in light microscopy when the gland is active, the cells contain large amounts of CH positive granules. During inactive stages, signs of nuclear pycnosis becomes distinct.

### 3.2 *Seasonal variations in the AG*

The AG remained in a regressed state all through, May, June and July (table 1). Pycnotic and shrunken nuclei were observed throughout the gland. The gland cells very rarely showed the presence of fine basophilic granules. The AG started hypertrophy by August and September. The nuclei were large and the cells were multinucleolated and fine basophilic granules were observed scattered evenly in the cytoplasm. Nuclear pycnosis was rare in the cells. By October, November and December the hypertrophy of the AG increased steadily and during February the hypertrophied AG showed regional differences in appearance and histology also. Some areas in the gland showed basophilic granules in the cytoplasm and enlarged nuclei. The AG of March animals was largely comparable to those of February except that in some areas, the cells showed accumulations of granules into masses. By April the cytoplasmic granular bodies increased in size to form larger and larger units and nuclear pycnosis was increasingly evident. By May and June the AG as a whole atrophied significantly and the cytoplasmic granules became scarce and the cells started degenerating.

### 3.3 *Seasonal variations in the testicular follicles*

The testis showed cyclic changes and this was evident by the variation in the number of testicular follicles. The number of testicular follicles were highest during February. No testicular follicles appeared in May and June. From November onwards these showed a steep incline reaching maximum in February. By end of April the testis did not show any activity.

### 3.4 *Effect of neurosecretory hormones on AG*

Eyestalk ablation caused hypertrophy after 15 days of operation. This was evident by the increase in the cell diameters and also the nuclear diameters. In the eyestalk ablated individuals, the cell and nuclear diameters showed an enhancement from $0{\cdot}98 \pm 0{\cdot}29\,\mu$ to $2{\cdot}16 \pm 0{\cdot}10\,\mu$ and $0{\cdot}47 \pm 0{\cdot}02\,\mu$ to $1{\cdot}53 \pm 0{\cdot}07\,\mu$ respectively. When the eyestalk extract injection was given to the eyestalk ablated animals, there was a significant decrease in the cell and nuclear diameters (table 2). Central nervous tissue extracts when injected into normal and eyestalk ablated individuals brought about enhancement in

**Table 2.** Seasonal variation of androgenic gland activity.

| Month | Size of AG ± SD ($\mu$) | Secretory activity of AG | No. of testicular follicles ± SD |
|---|---|---|---|
| May | 5·2±1·2 | No activity | No follicles |
| June | 4·9±0·9 | No activity | No follicles |
| July | 6·4±0·7 | No activity | 3·2±0·1 |
| August | 7·3±0·2 | Cells active | 3·4±0·2 |
| September | 10·4±0·6 | No. of cells increased | 5·0±0·4 |
| October | *15·3±0·8 | Cells compactly arranged | 7·6±0·5* |
| November | *20·0±0·5 | Cytoplasm developed | 12·8±0·7* |
| December | *25·0±0·9 | Hypertrophy | 19·8±0·7* |
| January | *29·6±0·1 | Hypertrophy | 21·2±0·4* |
| February | *45·9±0·6 | Hypertrophy | 39·2±0·4* |
| March | *34·2±0·7 | Regression started | 16·4±0·2* |
| April | *30·1±0·1 | Regression started | 14·2±0·6* |

* $P < 0{\cdot}05$.

the above parameters. However, this enhancement was more in the individuals who had eyestalk ablation than normal ones.

## 4. Discussion

The location and gross morphology of the AG of *M. lamerrii* is comparable to that of other decapod crustaceans (Charniaux-Cotton *et al* 1966). The AG showed its peak activity during February to March and it remained inactive from May to July. Gain in size of the gland, abundance of basophilic granules in the cytoplasm, increase in the size of nuclei and multinucleolated nature are some of the parameters taken into account to consider signs of increased secretory activity of the AG. All these cytological events in the gland cells occur when the male is in the sexually active phase. Seasonal changes in AG activity have been reported earlier in the crayfish, *Orconectes nais* (Carpenter and Deroos 1970). In the present study when the secretory activity of AG is correlated with the number of testicular follicles there appears to be a direct relationship (table 1).

Secretory activity of the AG in crustaceans is maintained by the influence of neurosecretory hormones (Charniaux-Cotton 1960, for review; Payen *et al* 1971). Eyestalk ablation brought about hypertrophy of AG in the marine crab, *Scylla serrata* (Rangnekar *et al* 1971). Inhibitory gonadotropins are produced in the neurosecretory complex of the eyestalks which inhibit testis development and AG. From the available literature it can be said that inhibitory action of gonadotropins is at first at AG level and further changes occur in the spermatogenic activity of crustaceans. Brain and thoracic ganglion extracts enhanced the spermatogenic activity and corresponding increase in androgenic gland activity. A stimulatory gonadotropin released from the photo-cerebrum was reported in *Orchestia gammarella* (Bounenfant 1967) and *Paratelphusa hydrodromous* (Adiyodi and Adiyodi 1974). Amato and Payen (1978) have suggested two types of specific neurohormones, one controlling the growth of the AG and the other

**Table 3.** Effect of eyestalk ablation and injection of central nervous tissue extracts on the androgenic gland activity.

| Treatment | Cell diameter ± SD ($\mu$) | Nuclear diameter ± SD ($\mu$) |
|---|---|---|
| Normal (control) | 5·98 ± 0·21 | 2·51 ± 0·50 |
| Normal + eyestalk extract | 5·71 ± 0·10 | 2·50 ± 0·10 |
| Normal + brain extract | *12·07 ± 0·3 | 4·37 ± 0·30 |
| Normal + thoracic ganglion extract | *12·87 ± 0·1 | 4·23 ± 0·06 |
| Eyestalk ablated | *12·16 ± 0·1 | 4·53 ± 0·70 |
| Eyestalk ablated + eyestalk extract | 7·01 ± 0·5 | 2·52 ± 0·60 |
| Eyestalk ablated + brain extract | *13·56 ± 0·4 | 1·57 ± 0·22 |
| Eyestalk ablated + thoracic ganglion extract. | *13·69 ± 0·2 | 1·73 ± 0·10 |

*$P < 0·05$.

synthesis of male hormone. Further work on these lines is needed to establish the above facts in the freshwater prawn, *M. lamerrii.*

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# Mermithid nematodes as parasites of *Heliothis* spp. and other crop pests in Andhra Pradesh, India

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**Abstract.** Insect pests were collected from cultivated and wild plant species to study their parasites in Andhra Pradesh, India. Besides insects, nematodes emerged as parasites. While *Hexamermis* spp. were common in most lepidoptera, *Ovomermis albicans* (Siebold) was recovered from *Heliothis* spp. The nematodes were active, even more than insect parasites, during early monsoon. They were more active on light-soils than on heavy-soils. Against *Heliothis armigera* (Hubner) in particular, their incidence was more on "low-growing" crops like *Arachis hypogaea* (L.), and *Lycopersicon esculentum* (L.), and weeds. The nematode *Pentatomimermis* sp. was recorded from the bug *Nezara viridula* L.

**Keywords.** Mermithids; *Ovomermis albicans*; *Hexamermis* spp; *Pentatomimermis* sp.; *Heliothis* spp.

## 1. Introduction

The mermithid nematodes, in general, are known to infect a wide range of insects in 15 different orders (Nickle 1972). Ramakrishnan and Kumar (1976) reported the association of species of *Mermis*, *Agamermis*, *Hexamermis*, and *Geomermis* with 40 insect species in India. In this paper, observations on mermithids as parasites of some important insect pests on dry-land crops and their role in regulating the pests populations are described.

## 2. Material and methods

Insects were collected (1975–83) in their available stages from Medak, Rangareddy and Mahaboobnagar districts of Andhra Pradesh, India and reared in glass vials (9 × 2·5 cm) in the laboratory on the same natural hosts to study critically for nematode and insect parasites. The nematodes, when emerged, were preserved by the method suggested by IA Rubtsov (personal communication). The rates of nematode parasitism recorded over years in different months on different crops were calculated on the basis of total larvae in the samples which showed the nematodes.

## 3. Results and discussion

### 3.1 *Nematode species and insect hosts*

The mermithid nematode species identified from different insect hosts were as follows

| | |
|---|---|
| *Ovomermis albicans* (Siebold) | : *Heliothis armigera* (Hubner) |
| | : *H. assulta* Guenee |
| | : *H. peltigera* Schiff |
| *Hexamermis* spp | : *Achaea janata* L. |
| | : *Chilo partellus* Swinhoe |
| | : *Cydia critica* Meyr. |
| | : *C. ptychora* Meyr. |
| | : *Lampides boeticus* L. |
| | : *Marasmia suspicalis* Walker |
| | : *Menochilus sexmaculatus* F. |
| | : *Mythimna separata* Walker |
| | : *Scirpophaga incertulas* Walker |
| | : *Spodoptera exigua* Hubner |
| | : *S. litura* F. |
| *Pentatomimermis* sp. | : *Nezara viridula* L. |

*O. albicans* was recovered from the larvae of all the three *Heliothis* species found in India. *Hexamermis* spp. were recovered from many insects including a coleopteran *M. sexmaculatus* which predates on eggs and larvae of some insect pests. *Pentatomimermis* sp. emerged from *N. viridula*. The nematodes also emerged from the adults of *H. armigera*, *N. viridula* and *S. incertulas*.

The nematodes recorded on *Heliothis* and *N. viridula* are new records for these are not listed by Poinar (1975, 1979) in his reviews on entomophagus nematodes.

### 3.2 *Nematode parasitism in relation to season and host crops*

The rates of nematode parasitism recorded in different insects on cultivated and uncultivated host plants in different months are given in table 1. Although the collection of insects was from almost all months of year, the nematode parasitism was seen only between June to December with peak activity generally during July–September.

Nematodes, in general, were more active on light-soils (alfisols) than on heavy-soils (vertisols). This is, however, comparable in our data only for *S. bicolor* (L.) Moench, *Zea mays* L., and *C. cajan* (L.) Millsp which are grown on both types of soils. *A. hypogaea* (L.) and *Lycopersicon esculentum* (L.) are normally grown on light-soils, and weeds are also most common on these soils.

Amongst weeds, *Heliothis* was greatly parasitised on *Acanthospermum hispidum* DC., *Gomphrena celosioides* Mart., and *Cleome gynandra* (L.) Briq., and relatively less on *Datura metel* L. It should be noted here that *H. peltigera* is more predominant on *A. hispidum* and *H. assulta* on *D. metel* (Bhatnagar and Davies 1978).

**Table 1.** Occurrence of nematode parasitism (%) in insect pests on cultivated and wild host plants in Andhra Pradesh, India (1975–83).

| Insect | Plant species | Month | Light soils (Alfisols) | Heavy soils (Vertisols) |
|---|---|---|---|---|
| | *Ovomermis albicans* (Siebold) | | | |
| *Heliothis armigera* | *Arachis hypogaea* (L.)., Groundnut | Jun. | 3·8 (424)[2] | — |
| | | Jul. | 20·5 (677)[4] | — |
| | | Aug. | 30·0 (1035)[7] | — |
| | | Sep. | 39·4 (279)[3] | — |
| | *Cajanus cajan* (L.) Millsp., | Jul. | — | 0·2 (444)[1] |
| | Pigeonpea | Sep. | — | 1·4 (418)[1] |
| | | Oct. | 2·4 (817)[3] | — |
| | | Nov. | 1·0 (960)[3] | — |
| | *Cicer arietinum* (L.)., Chickpea | Aug. | 1·3 (382)[1] | — |
| | | Oct. | — | 0·2 (429)[1] |
| | *Helianthus annus* L., Sunflower | Aug. | — | 0·4 (237)[1] |
| | *Ipomoea batatas* (L.) Lam., Sweet potato | Sep. | — | 16·6 (6)[1] |
| | *Lycopersicon esculentum* (L.)., | Jul. | 52·0 (50)[1] | — |
| | Tomato | Aug. | 42·5 (153)[1] | — |
| | *Sorghum bicolor* (L.) Moench., | Aug. | 0·7 (400)[1] | 2·9 (35)[1] |
| | Sorghum | Sep. | 1·0 (798)[3] | 0·5 (206)[1] |
| | *Vigna aureus* (Roxb.) Hepper., Mungbean | Aug. | 18·2 (11)[1] | — |
| | *V. radiata* (L.) Wilczek., Black gram | Aug. | — | 3·3 (150)[1] |
| | *Zea mays* L., Maize | Aug. | 31·2 (16)[1] | 1·0 (1122)[2] |
| | | Sep. | 8·3 (132)[1] | — |
| | *Acalypha indica* L.* | Aug. | 5·9 (17)[1] | — |
| | *Cleome gynandra* (L.) Briq.* | Jun. | 1·1 (94)[1] | — |
| | | Jul. | 57·8 (1404)[2] | — |
| | | Aug. | 33·5 (176)[1] | — |
| | *Gomphrena celosioides* Mart.* | Jun. | 4·3 (138)[1] | — |
| | | Jul. | 61·9 (698)[1] | — |
| | | Aug. | 18·0 (1626)[2] | — |
| | | Sep. | 1·0 (400)[1] | — |
| | *Leucas aspera* L.* | Aug. | 6·3 (16)[1] | — |
| | *Tridax procumbens* L.* | Jul. | 50·0 (2)[1] | — |
| *H. assulta*** | *Datura metal* L.* | Jul. | 4·0 (297)[2] | — |
| | | Aug. | 0·4 (526)[2] | — |
| *H. peltigera*** | *Acanthospermum hispidum* DC.* | Jun. | 1·3 (80)[1] | — |
| | | Jul. | 43·1 (788)[4] | — |
| | | Aug. | 46·1 (475)[4] | — |
| | | Sep. | 19·0 (79)[1] | — |
| | | Nov. | 4·2 (24)[1] | — |
| | *Hexamermis* Spp. | | | |
| *Achaea janata* | *Ricinus communis* L., Castor | Aug. | 25·7 (140)[2] | — |
| *Chilo partellus* | *Sorghum bicolor* (L.) Moench., Sorghum | Jul. | 20·6 (475)[5] | — |
| | | Aug. | 9·0 (575)[4] | 2·3 (220)[2] |
| | | Dec. | 15·4 (13)[1] | — |
| *Cydia critica* | *Cajanus cajan* (L.) Millsp., Pigeonpea | Aug. | — | 3·0 (162)[2] |
| | | Sep. | 3·3 (180)[1] | 1·2 (240)[1] |
| *C. ptychora* | „ „ | Aug. | 50·0 (6)[1] | — |

**Table 1.** (*Contd.*)

| Insect | Plant species | Month | Light soils (Alfisols) | Heavy soils (Vertisols) |
|---|---|---|---|---|
| *Lampides boeticus* | *Vigna radiata* (L.) Wilczek., Black gram | Jul. | — | 20·0 (45)[1] |
| | | Aug. | — | 6·0 (50)[1] |
| *Marasmia suspicalis* | *Sorghum bicolor* (L.) Moench., Sorghum | Oct. | 3·4 (89)[1] | — |
| *Menochilus sexmaculatus* | „ „ | Aug. | 6·7 (330)[2] | 5·3 (342)[3] |
| *Mythimna separata* | „ „ | Jul. | 50·0 (100)[1] | — |
| | | Aug. | 24·5 (233)[2] | 25·9 (309)[2] |
| *Spodoptera exigua* | *Cicer arietinum* (L.)., Chickpea | Jul. | 59·0 (52)[1] | — |
| | | Oct. | — | 1·0 (100)[1] |
| *Spodoptera litura* | *Arachis hypogaea* (L.)., Groundnut | Aug. | 2·0 (50)[1] | — |
| | | Sep. | 12·5 (24)[1] | — |
| | *Pentatomimermis* sp. | | | |
| *Nazara viridula* | *Pennisetum americanum* (L.) Leeke., Millet | Oct. | — | 3·1 (96)[1] |

* : Weeds.

** : 90–95% in total *Heliothis* larvae on the weed.

Figures in parentheses give the total collection in samples showing parasitism over years. Superscripts 1–7 refer to the number of years of data available.

### 3.3 *Nematode and insect parasitism*

The overall level of nematode parasitism recorded in *Heliothis* spp. irrespective of host plants on alfisols at ICRISAT Center are shown in figure 1. It was higher than insect parasitism in the early part of the season and ceased subsequently to almost nil by October. Amongst *Heliothis* spp., *H. peltigera* was most vulnerable, and *H. assulta* the least. In August 1979, *Heliothis* collection from one of the light-soil grazing areas, about 45 km from ICRISAT Center and uncultivated for at least 15 years, showed as high as 92·4% ($n = 305$) nematode parasitism on *A. hispidum*, but no parasitism due to insects. The weed was growing as 18 plants/m$^2$ and had as many as 74 *Heliothis* larvae/100 plants.

The multiple parasitism, involving the nematodes and insects, was rare. Nematodes were recorded only twice in association with hymenopterans *Campoletis chlorideae* Uchida and *Microchelonus curvimaculatus* Cameron in *Heliothis* larvae collected in August on *A. hispidum*.

### 3.4 *Nematode activity and distribution in the soil*

Nematodes were common on lepidoptera than on other insects. One to four nematodes emerged from a majority of *Heliothis* larvae, but as many as 63 nematodes were recorded from a larva of *H. peltigera* collected on *A. hispidum* (table 2). Usually one nematode emerged from a larva of *H. assulta*. The nematodes that emerged from

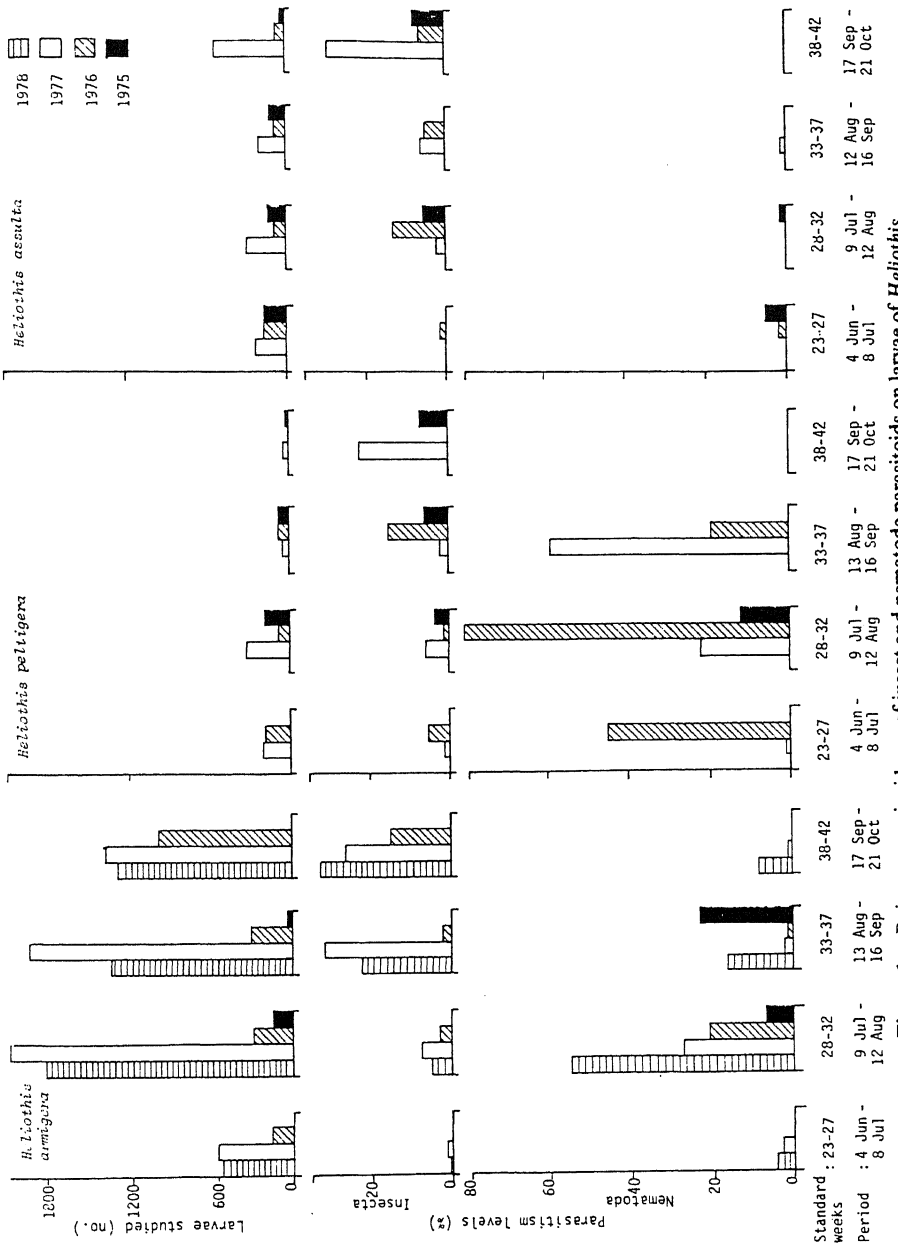


**Figure 1.** Rainy season incidence of insect and nematode parasitoids on larvae of *Heliothis* spp obtained from crop and weed hosts in alfisol, ICRISAT Centre (1975–78).

**Table 2.** Number of nematodes emerged from *Heliothis* larvae (1976–77)

| No. of Nematodes | Number of larvae | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Heliothis armigera* | | | *Heliothis peltigera* | | | *Heliothis assulta* | | |
| | Jul | Aug. | Sep. | Jul. | Aug. | Sep. | Jul. | Aug. | Sep. |
| 01-05 | 36 | 15 | 3 | 53 | 143 | 14 | 2 | 0 | 1 |
| 06-10 | 3 | 3 | 0 | 64 | 22 | 1 | 0 | 0 | 0 |
| 11-15 | 3 | 1 | 0 | 62 | 4 | 0 | 0 | 0 | 0 |
| 16-20 | 0 | 0 | 0 | 60 | 1 | 0 | 0 | 0 | 0 |
| 21-25 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 |
| 26-30 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| 31-35 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 50-60 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| > 60 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cumulative average of nematodes/ larva | 3·6 | 3·5 | 2·3 | 13·9 | 3·4 | 2·0 | 1·5 | 0·0 | 1·0 |

*Heliothis*, measured 3–22 cm in length. The nematode number declined from July to September.

Nematode activity appeared to be stimulated by the arrival of the premonsoon showers in June, and varied seasonally remaining often localised. In soil samples from 1–30 cm depth, collected only in alfisols, more nematodes were recovered from 20–30 cm, and more so in July when the monsoon is normally well set. Nematodes frequently had a patchy distribution and varied in population.

*Heliothis* larvae parasitised by nematode were creamy yellow and sluggish and ate little. They survived for one to two days when juvenile nematodes emerged, but died soon with the emergence of adults. Emergence was observed both during the day and night. The nematodes emerged usually from the abdominal region of insects.

Glaser *et al* (1942) recorded *Neoaplectana chresima* Steiner as a natural endoparasite of *Heliothis* spp. including *H. armigera* from the USA. Poinar (1979) considered *N. chresima* as a strain of *N. carpocapsae* Weiser. It should be noted here that *H. armigera* is present only in the old world and the report from USA could be because of the taxonomic confusion during that time (Hardwick 1965; Nye 1982). In India, Achan *et al* (1968) identified the nematode parasitic on *H. armigera* as *Hexamermis* sp., and specific identification was considered difficult for want of adult nematodes. However, in the present study it was possible because of recovery of sufficient number of adult nematodes. The nematode has been identified as *Ovomermis albicans* (Siebold).

Achan *et al* (1968) considered the nematode on *H. armigera* to be specific on *L. esculentum*. However, the present investigation shows that the nematode is parasitic on all the three *Heliothis* species in India, and is associated with many host plants. Achan *et al* (1968) recorded a maximum of six juvenile nematodes from a larva of *H. armigera*, but our record is of 14 juveniles from a larva of *H. armigera*, and of as many as 63 juveniles from a larva of *H. peltigera*.

Laumond *et al* (1979) reported the infectivity of *N. carpocapsae* to *N. viridula* in the

laboratory, but there is no record of its natural occurrence on this bug, at least from India. *Hexamermis* sp. has been identified in general from pentatomid bugs (Gokulpure 1970). The nematode *Pentatomimermis* sp. is a new record on *N. viridula.*

## 4. Conclusions

The occurrence of nematode parasites early in the season and their higher incidence on alfisols, and on "low growing" crops like groundnut, tomato and weeds are important findings of this study. This should help plant protectionists to plan effective utilisation of the total parasitic fauna of the pest in nature, and particularly in integrated management of pests like *H. armigera* which attack many crops in succession. The soil application of insecticides to control a few insect pests in some crops needs to be investigated for their effects on entomophagus nematodes.

In view of the importance of mermithid nematodes as parasites on insect pests, a clearcut information is required on individual species in relation to a given insect host to consider their utility in pest management. Further a gathering of base data with large samples from farmers' fields is essential to know how beneficial these mermithids could be in suppressing the pest in nature.

## Acknowledgements

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# Wing microsculpturing in the termite genus *Amitermes* (Termitidae, Amitermitinae)

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**Abstract.** Wing microsculpturing has been described in the genus *Amitermes*. It occurs on the upper and lower wing surfaces and is composed of a single row of small (3–5 μm long), thorny papillae at the anterior margin and numerous micrasters all over the wing membrane (size 6–8 μm × 5–7 μm; density 9200–9600/mm$^2$). The micrasters are with 5–8 arms and of the complex type (types V–X). No arrowheads are present. The position of *Amitermes* is discussed in the general scheme of termite microsculpturing. Comparison is also made with the condition in the Zoraptera, Embioptera and the Blattoidea.

**Keywords.** Wing microsculpturing; *Amitermes belli*; termites; isoptera; Amitermitinae.

## 1. Introduction

The occurrence of an elaborate and dense pattern of cuticular microstructures of various shapes and sizes has been established in recent years in a long series of studies (1967–1983) by Roonwal and co-workers; for a summary see Roonwal (1983a, b).

Over 80 genera and 250 species belonging to all the major families and subfamilies of termites (Isoptera) have been studied in this respect, including the subfamily Amitermitinae (Roonwal 1981; Roonwal and Rathore 1977, 1982; Roonwal and Verma 1980, 1983; Roonwal *et al* 1974). The genus *Amitermes* Silvestri (Amitermitinae, Termitidae), however, remained unstudied due to dearth of material. Recently, we were able to procure winged examples of a member of this genus (*A. belli*), and the present account fills the lacuna in our knowledge of wing microsculpturing in this subfamily.

## 2. Material and methods

Winged forms, along with soldiers and workers, of *Amitermes belli* were collected from Sariska Forest, 18 km south-east of Alwar (Alwar District, Rajasthan, India, *ca* 27·30 N lat., 76·30 E long.), ex rotten date palm trunk; N. S. Rathore Coll., 18 June 1983. It is a soil dwelling, wet and semi-arid zone species occurring in Western India and Pakistan. Its ecology and distribution are described by Roonwal (1976) and Roonwal and Bose (1964). *Sex ratios* (in colony): Alates, 45 males (57%), 34 females (43%).

Wings were mounted in glycerine as well as the quick-drying D.P.X. mounting medium (BDH/Glaxo); both gave good results.

## 3. Results

### 3.1 *Genus Amitermes Silvestri*

This is a large, widespread genus of which the winged forms of a single species, *A. belli*, were alone available for study.

### 3.2 *Amitermes belli* (Desneux)

(*Termes belli Desneux*, 1906, *Annales Soc. Ent. Belge*, Brussels, **49** (12); p. 352.)

Wings (figure 1) small; with scale, forewings 8·5 × 2·0 mm, hindwings 8·2 × 2·1 mm. Membrane transparent, pale smoky; anterior veins brown, rest paler. Hairs (length 50–110 μm) fairly numerous on anterior margin and a little below it; similar but smaller ones on posterior margin; on membrane a few small ones here and there, somewhat longer (45–135 μm) and more numerous on basal scales.

### 3.3 *Microsculpturing* (figures 2 and 3)

Microsculpturing consisting of papillae and micrasters; is found on both upper and lower surfaces of the wing.

3.3a *Papillae:* A single row of small (length 3–5 μm), pointed, thorny papillae on anterior margin; none on posterior margin and membrane.

3.3b *Micrasters:* Present in considerable density all over membrane right up to margins; lumpy and distorted on basal scales and on adjacent portions. Generally of the complex, thick, many-armed, type (types V–X of Roonwal *et al* 1974), with 5–8 arms, mostly 6–7; with asteroid and odd shapes. Sizes 6–8 μm × 5–7 μm. Density (per mm$^2$) in the middle of wing is fairly uniform and is as follows: Dorsal surface: Forewing 9600, hindwing 9280. Ventral surface: forewing 9200, hindwing 9600.

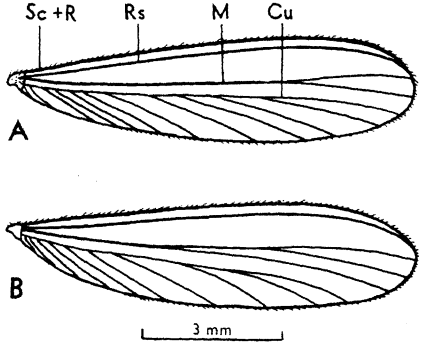


**Figure 1.** *Amitermes belli* (Sariska, Rajasthan). Right wings, to show venation. **A.** Forewing. **B.** Hindwing. Cu., cubitus; M, media; Rs., radial sector; Sc. + R., subcosta + radius.

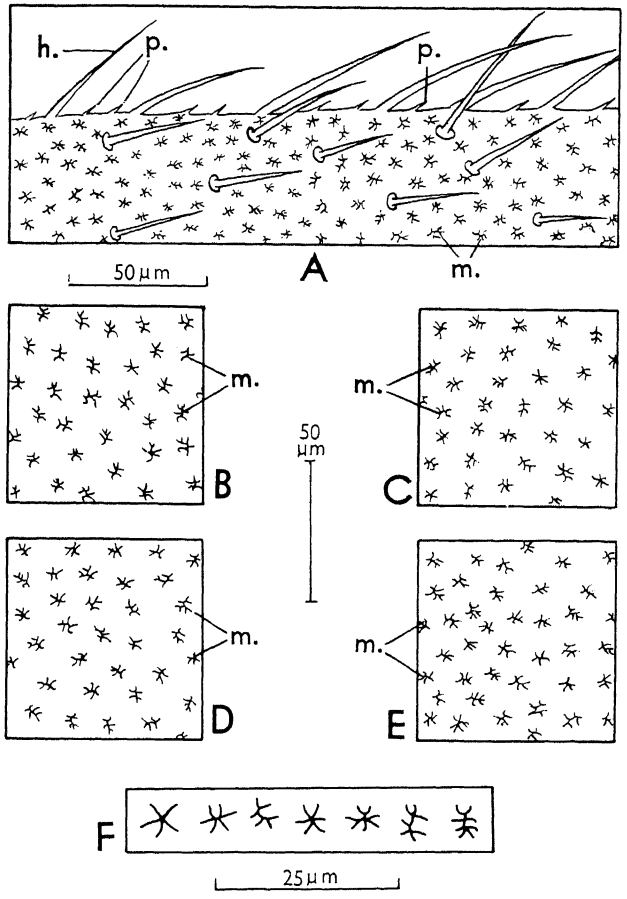


**Figure 2.** *Amitermes belli* (Sariska, Rajasthan). Portions of wing surfaces to show microsculpturing. **A.** Portion of anterior margin of right forewing, in dorsal view, to show micrasters. **B.** Part of middle of wing membrane of right forewing, in dorsal view. **C.** Same, of hindwing. **D.** Same, of ventral view of forewing. **E.** Same of hindwing. **F.** Micrasters from dorsal surface of forewing, enlarged and rearranged. h., hairs; m., micrasters; p., papillae.

## 4. Discussion

*Amitermes* conforms to the amitermitine pattern as discussed by Roonwal (1983b, chart 1) and falls in major group B, *i.e.* "Higher Group I (with micrasters but without rods)", and in the second amitermitine category, of advanced genera, *e.g.*, *Eremotermes*, *Microcerotermes*, etc., which have complex, many-armed micrasters. The papillae too are pointed and thorny as is characteristic of the Amitermitinae. Similarly, hairs are common on the wing margins, but rare on the membrane. All these similarities emphasise the relative uniformity of the subfamily Amitermitinae.

Termite-like wing microsculpturing is absent in the allied orders Zoraptera (Roonwal 1983b) and Embioptera (Roonwal and Rathore 1984) where only hairs or hair-like structures are present. In the cockroaches (Blattoidea), however, microsculpturing is present but is less elaborate (Roonwal and Rathore 1983).

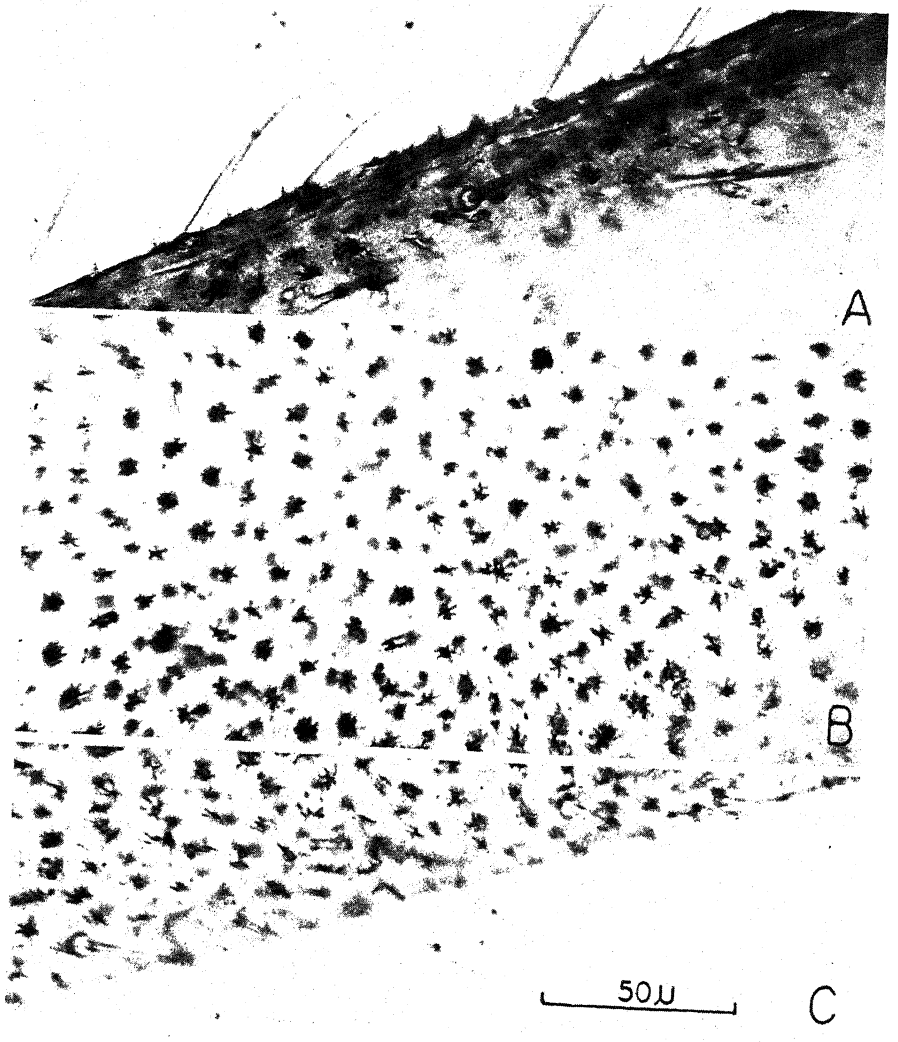


**Figure 3.** *Amitermes belli*. Photomicrographs of dorsal surface of right forewing, to show microsculpturing. **A.** Anterior margin. Note small, pointed papillae on margin; also long hairs. **B.** Middle of wing membrane. Note the micrasters, often arranged in curves and circles. The clear ones, in focus, are those on the dorsal surface; those out of focus, showing as dark masses with no detail, belong to the ventral surface. **C.** Posterior margin.

## Acknowledgements

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# Effect of two graded doses of x-irradiation on the rat adrenal gland and its protection by S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride

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**Abstract.** In the present study the amount of protection offered by the use of S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride to adrenal gland was investigated on albino rats against two graded doses of x-rays (1500R each). Total body x-irradiation brought about the hypertrophy and degranulation of adrenal cortical and medullary cells. The extent of hypertrophy and degranulation increased after the 2nd exposure to x-rays. Treatment with S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride prior to each dose of irradiation precluded the radiation changes caused in the adrenal cortex and medulla of the rats.

**Keywords.** X-rays; S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride; adrenal gland.

## 1. Introduction

Earlier studies have demonstrated that ionizing radiation decreases production of corticoids from the adrenal gland (Nabors *et al* 1974; Nabors 1962; Berliner *et al* 1962; Stevens *et al* 1963). It is known that adrenal glands respond appreciably to irradiation, and the response is generally dose-dependent (Dougherty and White 1946; French *et al* 1955). Hasan *et al* (1977) reported degranulation and hypertrophy of adrenal cortex and medullary cells at various intervals of post-irradiation. Studies conducted hitherto on the adrenal gland in relation to radiation dealt with a single dose of irradiation. Similarly there seems to be paucity of information on the chemical protection of adrenal cortex and medulla against two graded doses of x-irradiation (Bacq *et al* 1955; Sarkar *et al* 1978). Thus in the present study the protection offered to the adrenal by S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride—a known chemical radio protector (Hasan *et al* 1983) against two graded doses of x-irradiation was studied on the albino rats.

## 2. Materials and methods

One hundred albino rats of porton strain weighing $100 \pm 10$ g were used in this study. Before the commencement of the experiment rats were acclimatized to laboratory conditions for about a fortnight. During acclimatization and experimentation, rats were maintained on balanced laboratory diet procured from the Hindustan Levers Limited (Bombay) and water *ad libitum*. After acclimatization, the rats were divided into four groups each containing equal number of animals.

Group I: rats receiving physiological saline and serving as control for groups II and IV.

Group II: rats were exposed to two graded doses of x-rays (3000R), 1500R at one week interval.

Group III: rats receiving twice 30 mg/kg of the antiradiation compound and exposed to two graded doses of x-rays (3000R), 1500R at one-week interval.

Group IV: rats receiving twice 30 mg/kg of antiradiation compound at the time when group III rats were administered the drug to serve as control for group III.

S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride (kindly supplied by Dr S N Pandey, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi) was used as antiradiation compound (Hasan *et al* 1983).

Before preparing the injectable solution the compound was activised with the use of acetone. The injectable solution was prepared in 0·9 % physiological saline and the pH was adjusted to 9·0. Half ml of the injectable solution containing 3 mg of the compound was administered to rats intraperitoneally 30 minutes before each exposure to x-ray

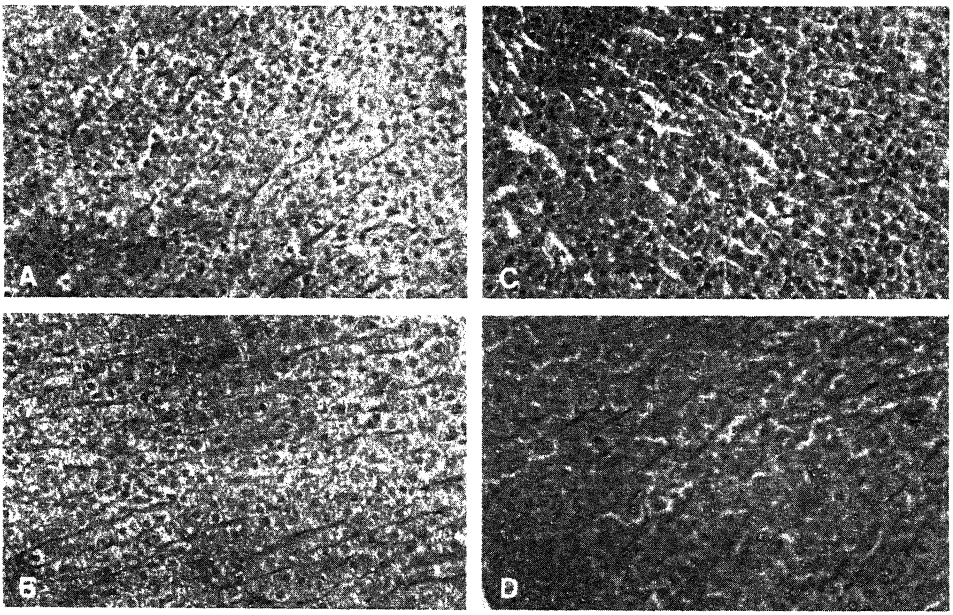


**Figure 1.** Sections of adrenal cortex of normal control and experimental rats, haematoxylin and eosin ( × 160) **A.** Normal control showing sparsely granulated cells in all the three layers and cortex with rich supply of blood vessels. **B.** 7 days after second exposure to x-ray treatment showing granulated cells, nuclei with granular nucleoplasm and cortex with rich supply of blood vessels. **C.** 7 days after second dose of drug treatment showing granulated cells and nuclei with agranular nucleoplasm, cortical cells separated by sinusoids in some places. **D.** 7 days after second injection of the drug/second exposure to x-rays showing degranulated cells in zona glomerulosa and granulated cells in zona fasciculata and zona reticularis, nuclei with granular nucleoplasm, cortex with rich supply of blood vessels.

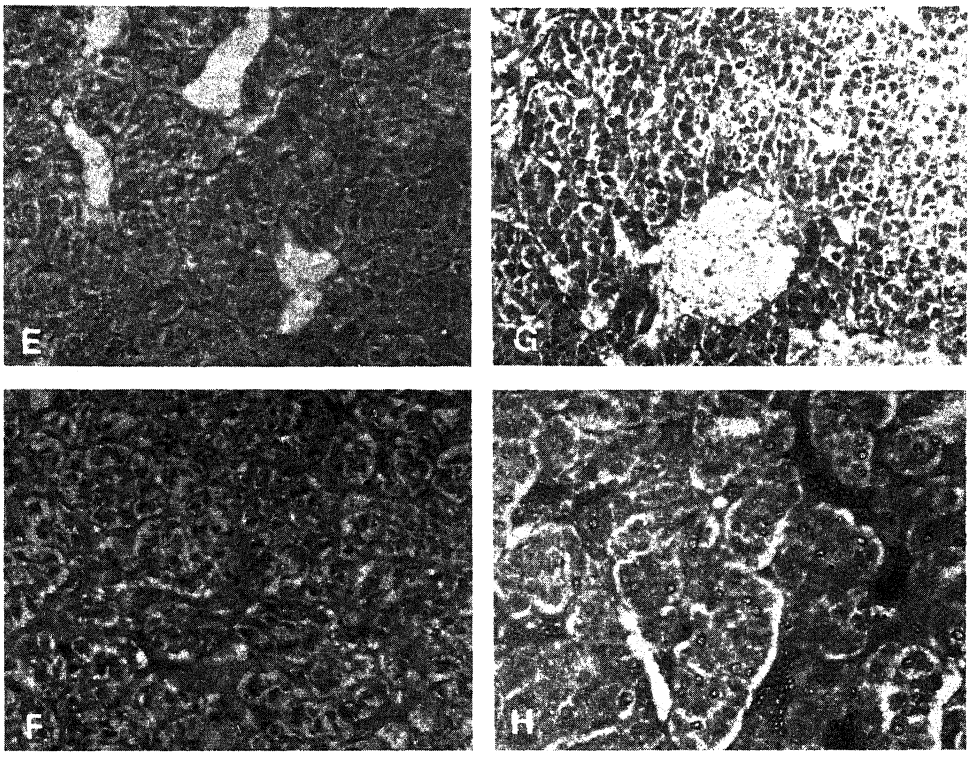


**Figure 2.** Sections of adrenal medulla of normal control and experimental rats, haematoxylin and eosin ( × 160). **E.** Normal control showing granulated medullary cells. **F.** 7 days after second exposure to x-ray treatment showing chromaffin granules bordering the nuclei, nuclei with granular nucleoplasm, dilated blood vessels. **G.** 7 days after second dose of drug treatment showing chromaffin granules filled medullary cells and nuclei with agranular nucleoplasm. **H.** 7 days after second dose of drug/second exposure to x-rays showing scantly granulated chromaffin cells and rich supply of blood vessels.

source. The vehicle carrier (0·9 % NaCl solution) of the drug was injected likewise to rats of group I to serve as control. The whole body of each rat of groups (II) and (III) was exposed twice to an x-ray source at one-week interval each time for a dose of 1500R (80 kV; 200 MAS; time 1 sec; distance 80 cm). The rats of the control as well as the experimental groups were housed under identical animal husbandry conditions.

The $LD_{50}$ of the drug was found to exceed 0·5 g/kg body weight.

The first sacrifice from each group was made at the end of the 7th day after the first exposure to 1500R and the later sacrifices were performed at intervals of 7,14 and 28 days after the second exposure to 1500R. At the time of sacrifice adrenal of each rat was dissected out and fixed in Bouin's fluid for histological examination. Thick paraffin sections (5$\mu$) were cut and stained with haematoxylin and eosin.

## 3. Results and discussion

In the present study the animals exposed to x-rays showed hypertrophy and degranulation of cortical and medullary cells and the extent of degranulation increased

after the second dose of irradiation (figures 1B and 2F). The hypertrophy and depletion of granular contents from the cells indicate an increase in the cellular activity of cortex and medulla in comparison with normal control animals whose adreno-cortical and medullary cells were granulated; besides, there was little mobilization of granular contents in the cells (figures 1A and 2E). In the cortex of the irradiated animals pretreated with the drug S-phenetyl formamidino 4(N-ethyl isothioamide) morpholine dihydrochloride, the cells are arranged in whorls and have a densely staining cytoplasm. Throughout the cortex and the medulla runs a rich vascular bed of sinusoids (figures 1D and 2H), whereas cortical and medullary cells of the rats treated with the antiradiation compound alone appeared to show the tightly packed cells particularly in the cortex were separated by wide vacant spaces which were devoid of vascular bed of sinusoids (figures 1C and 2G). Similarly there was no mobilization of granular mass in the wide medullary spaces of the animals treated with the antiradiation compound alone (figure 2G). This suggested the low profile of cellular activity in the cortex and medulla of drug-treated animals. Bacq (1965) opines that transformation of DNA from a metabolically active into resting state plays a major role in the radiation protection. Thus this study infers that the radiation injury induced in this resting state of nuclei of adreno-cortical and medullary cells could be repaired with the treatment of the drug prior to each dose of radiation than in the non-treated irradiated animals.

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# Effects of DDT and malathion on tissue succinic dehydrogenase activity (SDH) and lactic dehydrogenase isoenzymes (LDH) of *Sarotherodon mossambicus* (Peters)

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**Abstract.** Liver and muscle succinic dehydrogenase enzyme activity in *Sarotherodon mossambicus* subjected to sublethal concentrations of DDT and malathion declined significantly when compared to the control. The isoenzyme patterns of serum, liver and muscle in the fishes exposed to toxicants showed marked variations from that of the control. The variations in LDH isoenzyme patterns attribute alteration in the oxidative capacity of the tissues. The histological changes in the liver of the experimental group also revealed the harmful effects of DDT and malathion. The results suggest an alteration in the tissue metabolism towards an anaerobic type.

**Keywords.** Organochlorine; organophosphorous; isoenzymes; oxidative metabolism.

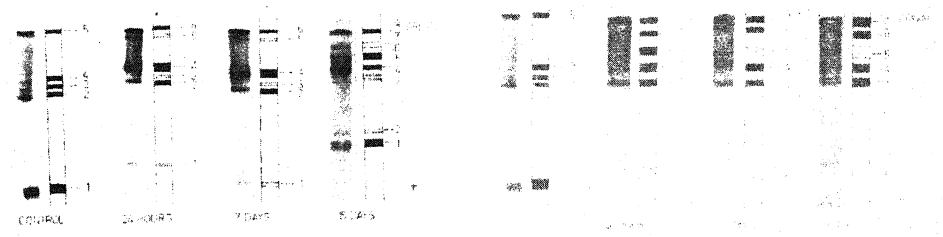
## 1. Introduction

The discernible effects of toxicants on the histological profile of specific tissues in fish as demonstrated by several investigators include necrobiotic changes in the liver cells, tubular damage of the kidneys and lamellar abnormalities of gills (Baker 1969; Gardner and Yevish 1970; Skidmore 1970). Taking a cue from the above studies and also of their own on *Salmo gairdneri*, Bilinski and Jonas (1972) inferred that such changes in tissues are the resultant effects of oxygen deficiency in them. As the activity of oxidative enzymes is known to imply the tissue oxygen levels, studies on enzyme systems in fishes exposed to toxicants would be of interest. However investigations on such enzymes are meagre. In the present study succinic dehydrogenase activity in liver and muscle, lactic dehydrogenase isoenzymes of serum, liver and muscle and the liver histology were determined in *Sarotherodon mossambicus* exposed to a sublethal dose of two pesticides.

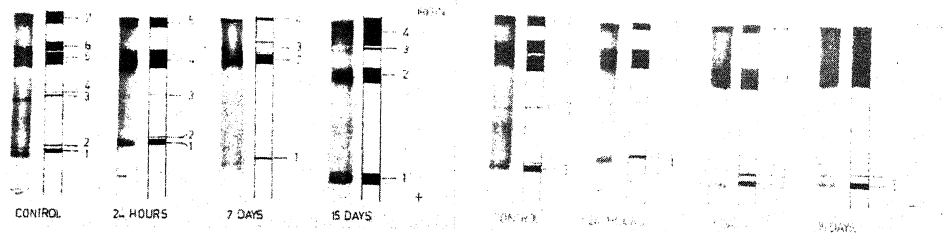
## 2. Material and methods

Specimens of *S. mossambicus* (15–20 g) were obtained from the local ponds maintained by the State Fisheries, Tamil Nadu and acclimated to laboratory conditions for 15 days as suggested by Chavin and Young (1970). The fishes were fed daily with cooked rice mixed with dried prawn powder *ad libitum*. A set of 10 fishes each was exposed to 100 l of dechlorinated water containing DDT (chlorinated compound) and malathion (organophosphorous compound) at concentrations of 0·01 and 0·95 ppm respectively. The above sublethal doses were derived as reported previously (Ramalingam and Ramalingam 1982). A time course study at intervals of 24 hr, 7 days and 15 days was

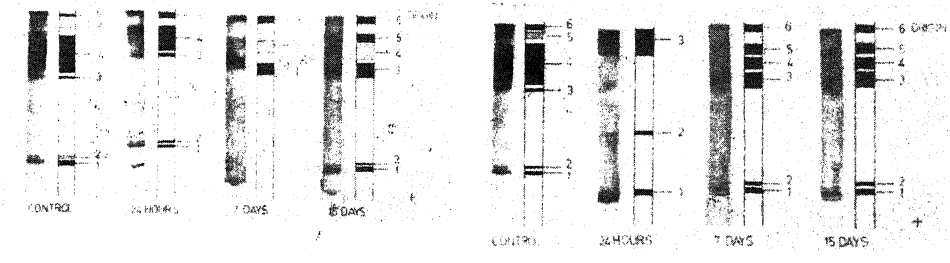
conducted with controls run simultaneously. Every 24 hr, water was changed in both control and experiment. The toxicants were also added to water along with renewal and thus exposure to DDT and malathion was repeated. At the end of each interval, fishes were dissected and the tissues, liver and muscle were removed for analyses. Before taking the tissues, blood collected by severing the tail was allowed to clot and the serum formed after the clot retraction was used for the LDH isoenzyme analysis. The time of sampling was confined to 9–10 hr. The tissue SDH activity was estimated by the method of Kun and Abood (1949). For LDH isoenzymes in serum, liver and muscle, disc electrophoresis as detailed by Smith (1968) was followed. The LDH fractions in the polyacrylamide gel were numbered according to their electrophoretic mobility, considering the fast moving fraction which is discernible next to the bromophenol blue marker end as No.1. The patterns of LDH fractions of control *vs* experiment are indicated in figures 1–6. Analysis of variance was applied to study the significance of SDH values within the groups at the three intervals. Student *t*-test was made to determine the significance of values between control group and experiment.



**Figures 1 and 2.** Serum LDH isoenzymes pattern. Control *vs* experiment. (1. DDT, 2. Malathion).




**Figures 3 and 4.** Liver LDH isoenzymes pattern. Control *vs* experiment. (3. DDT, 4. Malathion).




**Figures 5 and 6.** Muscle LDH isoenzymes pattern. Control *vs* experiment. (5. DDT, 6. Malathion).

## 3. Results and discussion

The SDH activity of the tissues of control and experiment is illustrated in table 1. The mean values of SDH activity range from 51·91 ± 1·49 to 86·10 ± 7·63 μg TTC reduced/100 mg wet wt/hr in the liver and 8·05 ± 0·85 to 12·00 ± 0·98 μg TTC reduced/100 mg wet wt/hr in the muscle of the control. The above variations in the control could be due to the fluctuations present in the blood and tissue total carbohydrate and its other intermediary metabolites of Kreb's cycle in this species (Ramalingam 1982). Similar variations in the metabolites of the Kreb's cycle enzymes have also been reported in several species of normal unstressed fishes (Chavin and Young 1970).

The SDH values in DDT and malathion exposed fish tissues are significantly lower compared to control at all the intervals ($P = 0{\cdot}05$). This suggests an impairment in the aerobic capacity of the tissues. Similar to the present study lower activity of oxidative enzymes by chlorinated compounds (Janicki and Kinter 1971; Timothy *et al* 1974; Sreeramulu Chetty *et al* 1978) as well as by organophosphorous compounds (Sivaprasada Rao and Ramana Rao 1979; Ranganatha and Ramamurthi 1978) has been observed in other fishes and vertebrates also. They also indicated the depression of cellular oxidation. The accumulation of lactic acid in the liver and muscle of *S. mossambicus* and also the dimunition of whole animal respiration at the corresponding intervals when the SDH activity was lowered (Ramalingam 1980) also supports the suggestion that DDT and malathion cause impairment in the aerobic capacity of the tissues. The results also reveal variations in the level of the activity of SDH within the test groups namely DDT and malathion exposed fishes (ANOVA). Such variations may be due to the action of detoxifying mechanisms by the tissue microsomal enzymes (Janardhan *et al* 1972), which also occur after exposure to the toxicants.

Analysis of lactic dehydrogenase isoenzymes in the present study reveal that samples from individual fish as well as the pooled sample show identical isoenzyme pattern in the control. This suggests that there is no genetic variability in the species. However, the LDH isoenzyme pattern was altered, as a sequel to the changes in the SDH activity in the test groups. In the serum of DDT and malathion exposed fishes, an increase in the slow moving fractions was noticed (figures 1 and 2). Bostrom and Johansson (1972) reported that the fast moving aerobic fraction no. 1 of LDH is most affected by PCP, a chlorinated compound. Quantitative studies using organophosphate also revealed an anaerobic mode of LDH activity in *T. mossambica* (Ranganatha and Ramamurthy 1978) corroborating the appearance of slow moving fractions in the present study. In mammals treated with pesticides, the slow moving LDH fractions were attributed to the

**Table 1.** SDH activity (mean ± SD in μg reduced TTC/100 mg wet wt/hr) in liver and muscle.

| | 24 hr | | 7 days | | 15 days | |
|---|---|---|---|---|---|---|
| | Liver | Muscle | Liver | Muscle | Liver | Muscle |
| Control | 51·91 ± 1·49 | 8·05 ± 0·85 | 58·11 ± 3·78 | 8·07 ± 0·80 | 86·10 ± 7·63 | 12·00 ± 0·98 |
| DDT | 40·71 ± 3·25* | 5·11 ± 0·32* | 20·25 ± 3·57* | 3·79 ± 0·52* | 31·12 ± 1·50* | 5·95 ± 0·20* |
| Malathion | 42·30 ± 3·96* | 4·24 ± 0·11* | 14·86 ± 3·85* | 4·40 ± 0·35* | 29·03 ± 1·76* | 3·97 ± 0·20* |

*Significant ($P = 0{\cdot}05$).

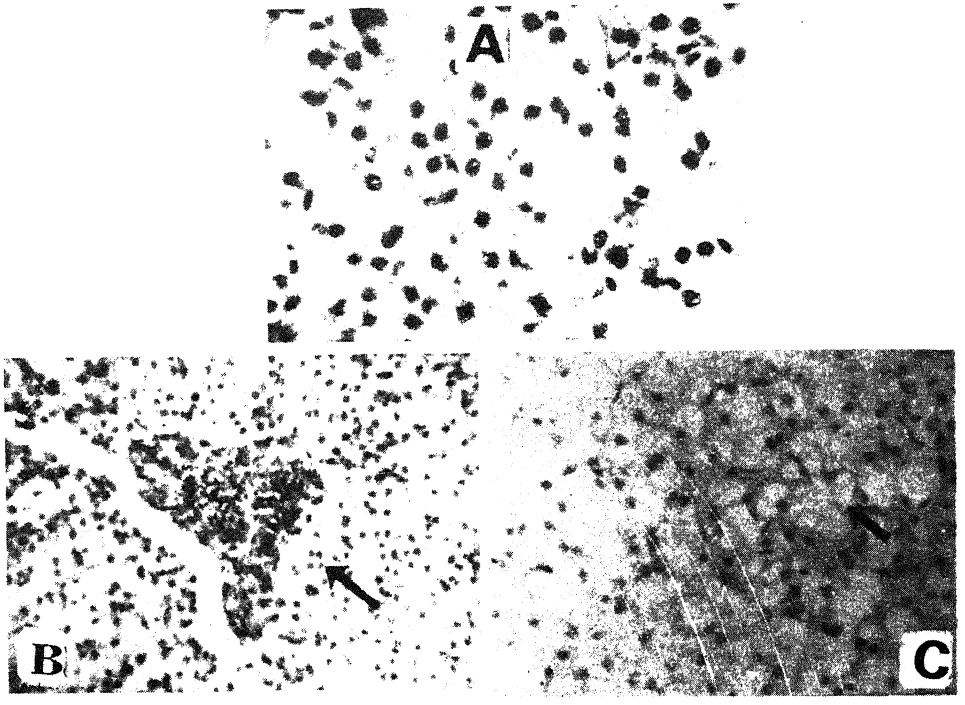



**Figure 7.** **A.** Section of the liver of normal fish (control). **B.** DDT treated fish liver section (arrow indicates periportal necrosis). **C.** Malathion treated fish liver section (arrow indicates vacuolation and fatty degeneration).

necrotic changes in the hepatic cells and the consequent release of isoenzymes into circulation (Weime and Van Maercke 1961; Zimmerman *et al* 1971; Truhaut *et al* 1973). The liver histology in the present study also reveal vacuolation, fatty changes and degeneration in the fishes exposed to DDT and malathion (figures 7A,B and C). Concurrent to the increase in the LDH fractions in serum, a decrease in the total number of fractions was noticed in liver and muscle. Other changes in these tissues are the appearance of additional new slow moving anaerobic fractions, change in the intensity to staining of the above fractions and the disappearance of some of the fast moving fractions. Similar changes have been noticed in the tissues of rats and rabbits administered with other toxicants (Zimmerman *et al* 1971; Truhaut *et al* 1973) and also in *Carassius auratus* subjected to thermal stress (Smit *et al* 1974). In the light of the above studies and also of the changes noticed in the metabolites of this species, the changes observed in SDH activity and LDH isoenzymes in the present study are suggestive of alteration of the metabolic pathway more towards the anaerobic side during DDT and malathion intoxication.

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# Ultrastructure of the eggs of Reduviidae: I. Eggs of Piratinae (Insecta—Heteroptera)

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**Abstract.** Eggs of Piratinae are unique among reduviids in possessing stellate chorionic filaments that remain exposed even after the insertion of the egg into the ground. These eggs have developed many structures for supplying ambient oxygen to the developing embryo inside them. The ultrastructure of the chorion, the operculum and the aeromicropylar system of the eggs of seven species of piratinae bugs are reported.

**Keywords.** Reduviidae; Piratinae, egg; chorion; operculum.

## 1. Introduction

Reduviid bugs are known to colonise varied habitats, depositing their eggs in a variety of situations, scattering them loosely, inserting or burying them in the soil, or attaching them to barks or twigs or leaves of trees and shrubs or even to the under surfaces of rocks and stones. These peculiar eggs have attracted the attention of several workers including Readio (1926), Southwood (1956), Miller (1953, 1971), Cobben (1968) and Hinton (1969, 1981). Though the eggs of a number of subfamilies are studied in detail, those of Piratinae, considered unique among Reduviidae, have not been given sufficient emphasis and hence an attempt has been made to study the ultrastructure of seven species collected from southern India.

## 2. Materials and methods

Eggs of the following species were studied.

(a) *Pirates affinis* Serville
(b) *Pirates mundulus* Stal
(c) *Ectomocoris tibialis* Distant
(d) *Ectomocoris ochropterus* Stal
(e) *Ectomocoris cordiger* Stal
(f) *Sirthenia flavipes* Stal
(g) *Catamiarus brevipennis* Serville

Eggs were collected from laboratory cultures after oviposition in the soil and also from dead gravid females with swollen abdomen that were treated with 5% KOH for few hours. Due to the hard nature of the egg shells, embedding with paraffin wax was not successful, hence hand sections of liquid nitrogen-frozen eggs were made using sharp razor blade. Such sections obtained from ovarian and oviposited eggs, as well as from empty shells were dehydrated in alcohol, cleared in clove oil or xylene and mounted in canada balsam or euparol. Longitudinally split entire egg shells were similarly mounted for counting aeropyles and micropyles. Ultratome (LKB Nova) was used to cut epoxy

resin-embedded eggs for ultrathin sections for observations of chorionic details. The surface areas and volumes of the eggs (the shape of which can be approximated reasonably to that of a cylinder mounted on a semi ellipsoid of revolution, the bottom one fourth of the total height being the height of the ellipsoidal part) were calculated

$$\left(\text{Surface area: II ab}\left(1-\frac{e^2}{24}-\frac{e^4}{160}-\frac{e^6}{468}\right)+\frac{\text{II}b^2}{2}\right.$$

$$\text{where } e^2 = 1-\frac{4^b}{a^2};\ \text{volume:}\left.\frac{11\,\text{II}ab^2}{48}\right).$$

The approximate number of the follicular pits was counted using an occular grid under a microscope for a given square area and these values were used to arrive at the total follicular number of the chorion of different eggs. Alcohol preserved eggs, both ovarian and oviposited, were sonic cleaned, gold coated (EIKO I B.2 ion coater) for 2–4 minutes and scanned with an electron microscope (Hitachi—450 A) using 10 and 15 kV emission currents.

## 3. Observation

### 3.1 *Oviposition*

All piratine bugs examined insert their eggs in loose soil. After suitable site selection, the gravid female assumes a slanting posture with raised head and thorax and with the abdominal apex making a twisting side to side, as well as downward, thrusting movements. The plate-like ovipositors guide the eggs for vertical insertion into the soil. The posterior pole of the egg is always directed away from the head of the female and after oviposition the apical parts of the egg alone are exposed outside the soil. The female covers up the egg with sand grains and particles of debris using her hind legs. The entire oviposition of a single egg lasts 3–4 minutes and in a day a maximum of 9 eggs are laid and females exhibit 3–4 oviposition cycles. The eggs are abandoned, there being no parental care in any of the species observed and all of them displayed a similar kind of oviposition behaviour.

### 3.2 *Structure of the egg*

3.2a *Structure, colour and size:* Eggs of piratine species are more or less of an uniform shape, cylindrical apically, the basal fourth of the total height being ellipsoidal with a cap-like point at the centre of the basal end. Freshly laid eggs have distinct concave and convex surfaces, corresponding to the ventral and dorsal sides of the fully developed embryo inside the egg. After the commencement of embryogenesis the eggs increase in size, altering their dorsal and ventral curvatures, tending to make them more spherical. The anterior end of the egg is characterised by an operculum surrounded by chorionic filaments. Generally the eggs are light yellowish or cream-coloured, more due to the large quantity of yolk inside. Eggs of *Pirates mundulus*, however, are reddish brown. The chorionic filaments resemble the eggs in colouration, except in *Pirates affinis* and *Ectomocoris cordiger* where these are brownish black. The sizes of the

**Table 1.** Eggs of some Piratinae

| Species | Number of Aeropyle | Number of Micropyle | Egg* Length (in mm) (a) | Egg* Width (in mm) (b) | Chorionic filament Length (in $\mu$) | Chorionic filament Width (in $\mu$) | Surface area (in sq. mm) Volume** (in c. mm) |
|---|---|---|---|---|---|---|---|
| *Pirates affinis* | 270 | 26·4 | 2·6 | 1·29 | 1280·4 | 37·14 | 12·54<br>3·12 |
| *Pirates mundulus* | 243 | 18·0 | 1·34 | 0·62 | 341·44 | 13·92 | 3·49<br>0·38 |
| *Sirthenia flavipes* | 169 | 19·3 | 1·96 | 0·85 | 437·47 | 32·48 | 6·34<br>1·02 |
| *Catamiarus brevipennis* | 369 | 15·6 | 2·73 | 1·34 | 320·1 | 9·28 | 13·55<br>3·64 |
| *Ectomocoris tibialis* | 240 | 16·7 | 1·98 | 0·95 | 160·05 | 11·6 | 7·32<br>1·29 |
| *Ectomocoris ochropterus* | 246 | 13·3 | 2·12 | 0·92 | 192·06 | 6·96 | 7·31<br>1·31 |
| *Ectomocoris cordiger* | 175 | 25·0 | 1·37 | 0·73 | 288·09 | 13·92 | 4·34<br>0·53 |

*Values are for the average of 10 eggs. The shape of the egg can be approximated to that of a cylinder mounted on a semi-ellipsoid of revolution, the bottom one fourth of the total height being the height of the ellipsoid part.

++Surface area $= \pi ab\left(1-\frac{e^2}{24}-\frac{e^4}{160}-\frac{e^6}{468}\right)+\frac{\pi b^2}{a}$, where $e = 1-\frac{4b^2}{a^2}$ **Volume $= 11\frac{\pi ab^2}{48}$

different eggs, along with the various measurements of their parts are tabulated (table 1).

3.2b *Body of the egg:* The main body of piratine eggs is always inserted into the soil and the surfaces of these are characterised by numerous polygonal follicular areas. In *Ectomocoris tibialis, E. ochropterus*, such areas have deep central pits (figure 1, E,F; 4, G,H; 5G). In *Pirates mundulus, Catamiarus brevipennis, Sirthenia flavipes* and *Ectomocoris cordiger* the polygonal areas are flat with rounded or elongated tubercles marking their boundaries (figure 1 B–D,G; 4A, C,E and F). In *Pirates affinis* the follicular pits are prominent, being shallow anteriorly, but increasing in depth progressively towards the posterior, giving the appearance of overlapping tiles (figure 1; 3B). The posterior-most part of all eggs examined, has a central cap-like projection, the surface of which is always beset with deep pits extending well into the interior (figure 3D,H).

The chorion of the eggs shows only two layers, the exo- and endochorion. Very close to the collar, the exochorion is thinner than the endochorion, but towards the posterior part of the egg, the former is thicker than the latter (figure 2A, D). The boundaries of the prominent follicular pits and tubercle-bounded follicular areas arise as extensions from the exochorion possessing thicker exochorion than the central areas. The innermost part of the endochorion shows an aerostatic layer; the net-work of air spaces of this is separated from the lumen of the egg by a very thin layer with numerous rounded, blunt

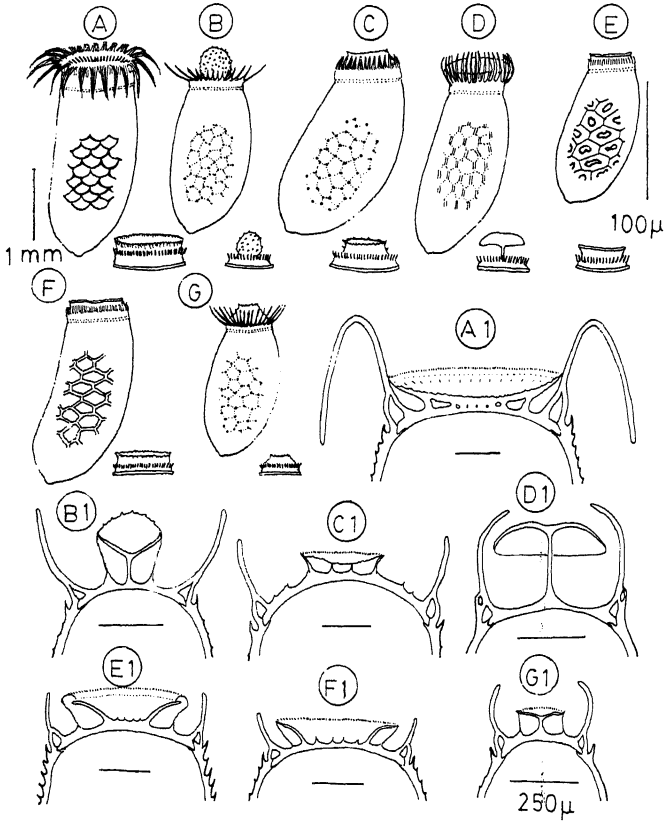


**Figure 1.** Eggs of Piratinae. **A**: Entire egg, operculum and follicular pits of *Pirates affinis*. **A1**: L.S. of the anterior half of the egg. **B**: Entire egg, operculum and follicular pits of *Pirates mundulus*. **B1**: L.S. of the anterior half of the egg. **C**: Entire egg, operculum and follicular pits of *Catamiarus brevipennis*. **C1**: L.S. of the anterior half of the egg. **D**: Entire egg, operculum and follicular pits of *Sirthenia flavipes*. **D1**: L.S. of the anterior half of the egg. **E**: Entire egg, operculum and follicular pits of *Ectomocoris tibialis*. **E1**: L.S. of the anterior half of the egg. **F**: Entire egg, operculum and follicular pits of *E. ochropterus*. **F1**: L.S. of the anterior half of the egg. **G**: Entire egg, Operculum and Follicular pits of *E. cordiger*. Bar scale: 1000 $\mu$ for entire egg and operculum; 250 $\mu$ for L.S. of the egg; 100 $\mu$ for follicular pits.

tubercles on the outer surfaces abutting the rest of the endochorion (figure 3G; 4J). The basal specialised prominence of the egg with deep follicular pits shows numerous pore canals extending into the endochorion and opening finally into the aerostatic layer (figure 2D; 3H).

3.2c *Collar:* The anterior rim of the cylindrical egg has a prominent collar consisting of two distinct regions: (i) an outer collar rim, composed mostly of exochorion, projecting away from the central axis and enclosing a ring-like spermatic groove on its inner side; (ii) an inner 'J' shaped sealing bar, formed wholly of endochorion, the basal-free edge of it is slightly turned upwards and projecting into the lumen of the egg like a ring (figure 1 A1–G1; 2A; 3C, E). This wedge of sealing bar encloses on its upper side a circular, shallow, but wide groove with numerous vertical columns of alternating ridges

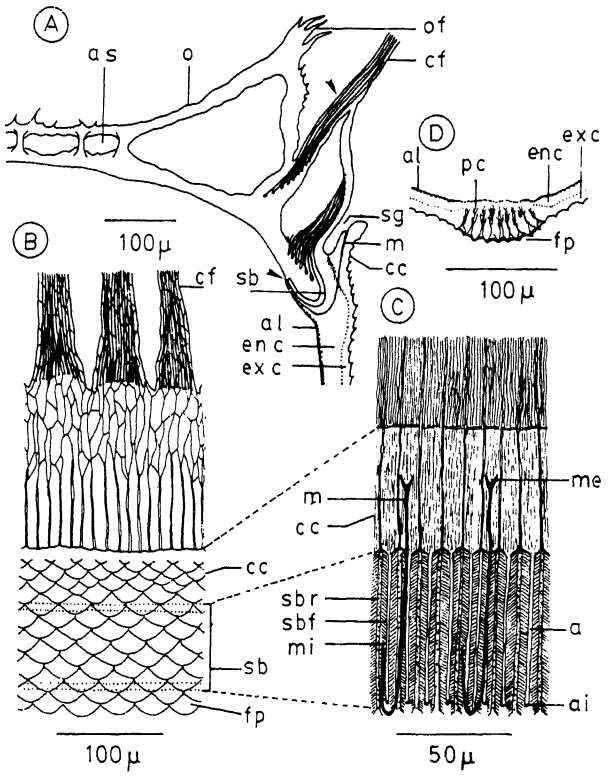


**Figure 2.** Egg of *Pirates affinis*. **A**: L.S. of the anterior lateral part of the operculum and collar region of the chorion. Arrows indicate points of breakage at the time of eclosion. **B**: Outer surface view of the collar region of chorion. **C**: Inner surface view of the collar region of chorion. **D**: L.S. of the basal part of chorion.

**Abbreviations.** a, aeropyle, ai, aeropylar inner opening, al, aerostatic layer, as, air space, cc, chorionic collar, cf, chorionic filament, enc, endochorion, exc, exochorion, fp, follicular pit, m, micropyle, me, micropylar external opening, mi, micropylar inner opening, o, operculum, of, opercular filament, pc, pore canal, sb, sealing bar, sbr, sealing bar ridge, sbf, sealing bar furrow, sg, spermatic groove.

and furrows on its inner wall (figure 3F). The blunt circular basal margin of the operculum with similar vertical columns of ridges and furrows (figure 2C; 5A–D & H), flush with the ridges and furrows of the sealing bar, enabling the operculum to fit exactly into the groove of the sealing bar (figure 2A; 3C, E). The aerostatic inner layer of the chorion takes its origin from the distal parts of the wedge of sealing bar, from below the groove which receives the margins of the operculum (figure 2A).

The anterior apices of the collar side of the sealing bar, inner to the spermatic groove, give rise to numerous upwardly projecting endochorionic extensions, each of which corresponds to one furrow and two half ridges of the sealing bar. At about the middle level of the operculum, between 2 and 5 of these extensios merge into one and immediately above this fusion they join again with similar extensions arising from the outer walls of opercular rim. The chorionic filaments thus formed are contributed by

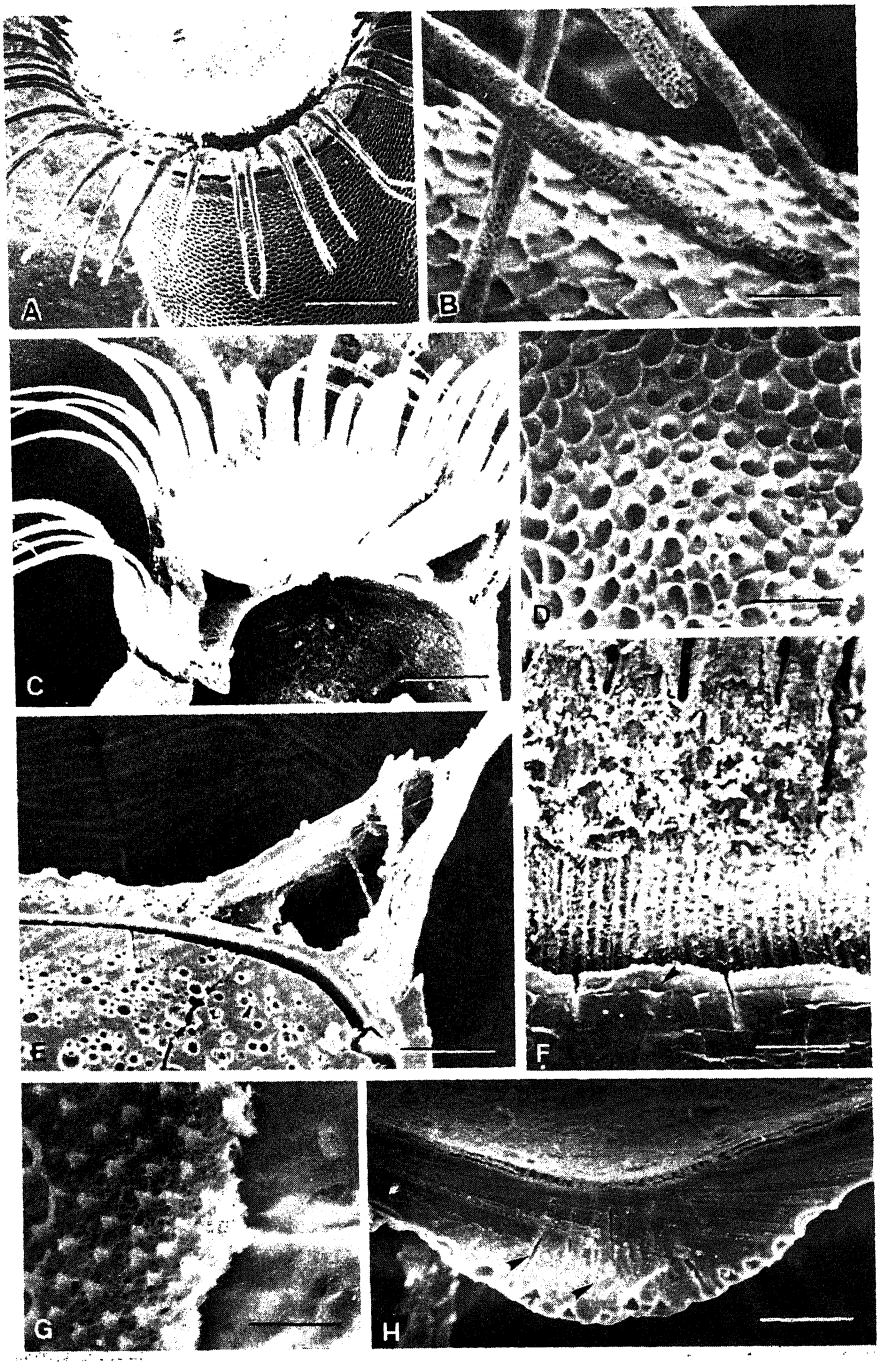


**Figure 3.** Scanning electron micrographs of the egg of *Pirates affinis*. **A**: Anterior and opercular surface view of the egg (scale: 250 $\mu$). **B**: External aeropylar openings on the chorionic filaments (scale: 38 $\mu$). **C**: L.S. through anterior part of the collar region and operculum (scale: 300 $\mu$). **D**: Follicular pits at the basal part of the egg (scale: 60 $\mu$). **E**: L.S. through the anterior part of the collar region and operculum of an ovarian egg (scale: 130 $\mu$). **F**: Ridges and furrows of the sealing bar on the inner surfaces of the chorionic collar. Arrow points to the outer micropylar opening at the free edge of the sealing bar (scale: 72 $\mu$). **G**: Outer view of the aerostatic inner layer after separation from the endochorion (scale: 5 $\mu$). **H**: Pore canals (arrows) from the basal follicular pits (scale: 42 $\mu$).

both operculum and collar. These filaments finally project further up and radiate away from the central egg-axis (figure 1; 2A; 3A, C). While the opercular extensions of the chorionic filaments may or may not be coloured, those from the sealing bar are always colourless. During eclosion the opercular connections with chorionic filaments get snapped like rings at two places, one at the innermost part of the wedge of the sealing bar below the operculum, from where the aerostatic layer originates and the other at the junction where the opercular extensions join with those of the collar (figures 5 A–D & H). Hence after the escape of young nymphs, the chorionic filaments of empty eggs appear to arise only from the inner aspects of the collar rim, inner to the spermatic groove.

In freshly laid eggs the chorionic filaments converge over the operculum and project towards the central axis of the eggs, but 20–30 minutes after oviposition, they open out and project away giving the eggs a star-like appearance characteristic of piratinae (figure 3A; 4E & F).

There is considerable variation among the different species examined in so far as the length and structure of the chorionic filaments are concerned. While these filaments in *E. tibialis*, *E. ochropterus* and *C. brevipennis* are short and extend only upto the level of the opercular tip (figure 1C, E & F; 4C, G, & H), those of *E. cordiger*, *S. flavipes*, and *P. mundulus* are longer and extend well beyond the operculum (figure 1B,D & G; 4A,E and F). The maximum development of the filaments is seen in *P. affinis*, the blackish-brown filaments projecting around the periphery of the collar (figure 1A; 3A, C). In *S. flavipes* the collar region is peculiar in that the collar rim is united at regular intervals with the outer basal parts of the chorionic filaments all around, enclosing the spermatic groove completely, except for regularly placed openings at the apical outer surfaces (figure 2E; 5E).

3.2d *Aeropyles and micropyles:* The apical parts of the chorionic filaments, extending to about a fourth to one half of their length from the apex, show numerous irregularly shaped and linearly arranged external aeropylar openings. These openings in most of the species become progressively reduced in size and fused towards the bases of the filaments (figure 3B; 4B). In *S. flavipes* however, these external openings are distinct and extend to about three fourths of the filaments (figure 5E). The external aeropyle openings lead to many irregular air spaces that extend towards the collar and in the endochorion of the sealing bar, the air conduits join into regularly arranged vertical channels, the aeropyles. The aeropyles are placed, in the centre of the vertical furrows of the sealing bar, bounded on either side by its ridges, ultimately opening into the inner aerostatic layer of the endochorion through distinct apertures, the inner aeropylar openings (figure 2C). These openings, placed between the bases of the ridges of the sealing bar, demarcate the lower limits of the collar region. The inner aerostatic layer distributed throughout the interior of the body, commences from the lower wedge of the sealing bar where the inner aeropylar openings finally open (figure 2A).

The micropyles, easily identified by their funnel-shaped external openings, originate from the spermatic groove, in the inner wall of the collar rim (figure 2A; 5I). They extend down along the chorion, running almost parallel to the sealing bar and at the level of the inner aeropylar openings, make a 'U' turn and run through the wedge of sealing bar to open finally into the lumen of the egg prior to the line of weakness between the collar and the opercular base. When viewed from the apical side of the egg,

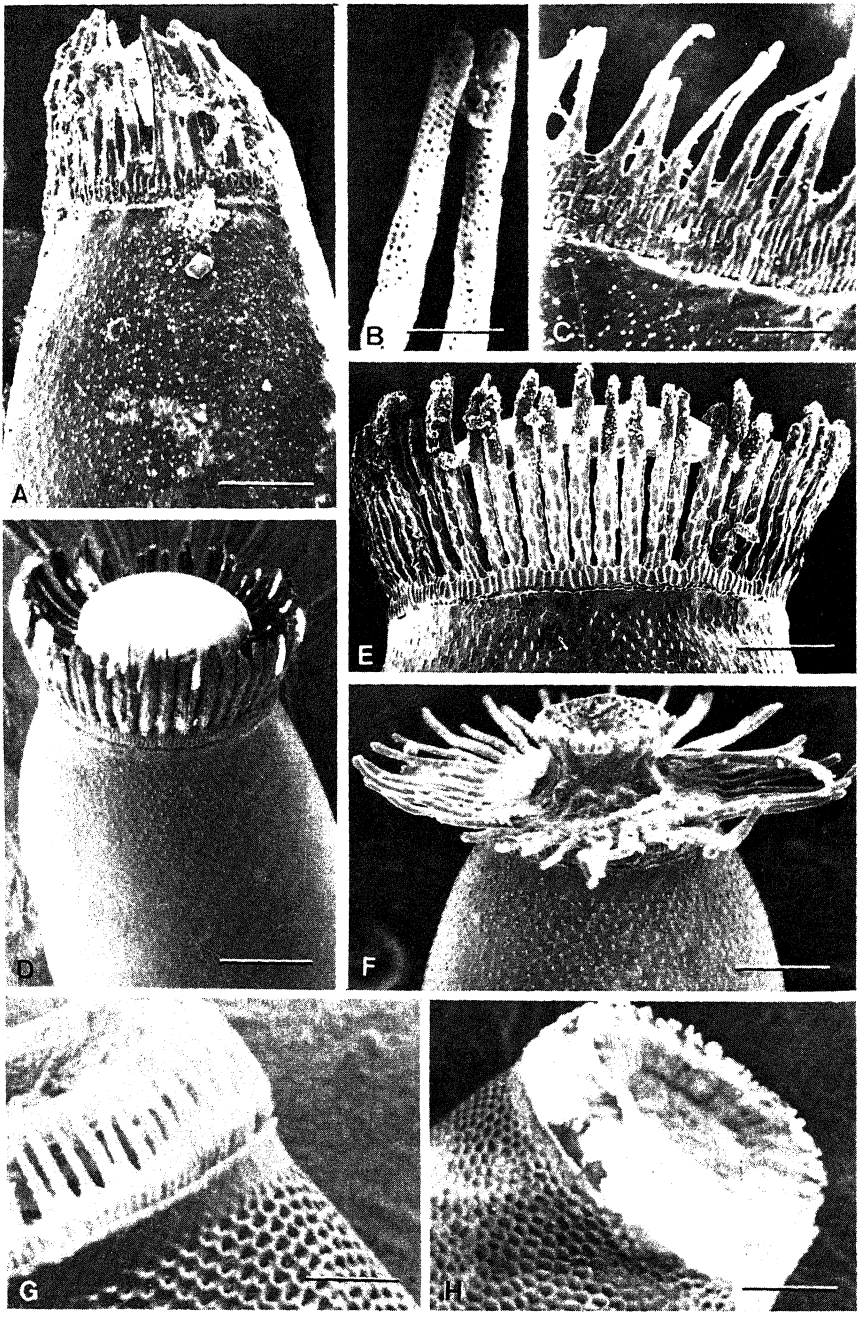


**Figure 4.** Scanning electron micrographs of the eggs of Piratinae. **A**: *Pirates mundulus*: Anterior half of the egg with operculum (scale: 125 $\mu$). **B**: Chorionic filaments with external aeropylar openings of the same (scale: 26 $\mu$). **C**: *Catamiarus brevipennis*: Collar rim and chorionic filaments (scale: 97 $\mu$). **D**: *Sirthenia flavipes*: Anterior half of the egg with operculum (scale: 250 $\mu$). **E**: Enlarged view of the same (scale: 172 $\mu$). **F**: *Ectomocoris cordiger*: Anterior half of the egg with operculum (scale: 162 $\mu$). **G**: *Ectomocoris tibialis*: Anterior half of the egg with operculum (scale: 95 $\mu$). **H**: *Ectomocoris ochropterus*: Anterior half of the egg with operculum (scale: 215 $\mu$).

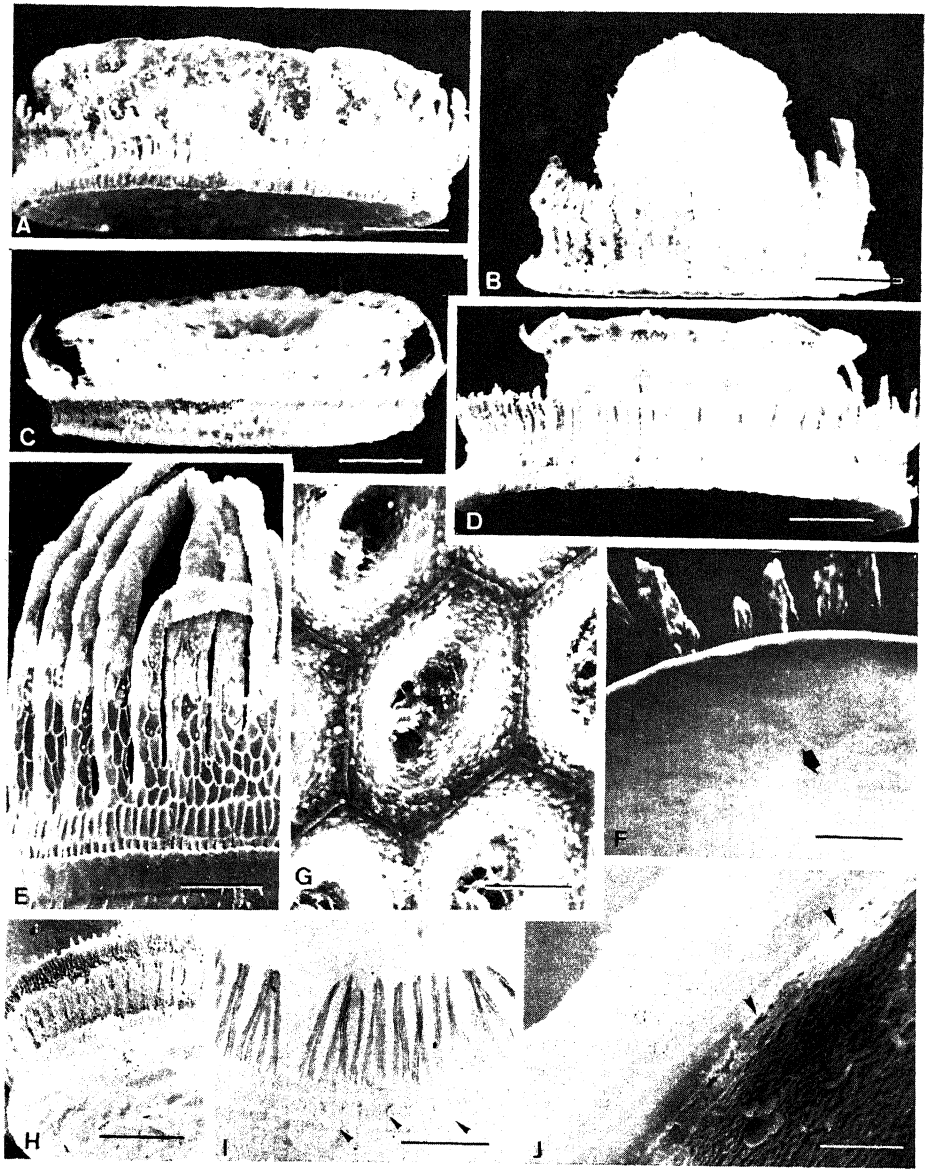


**Figure 5.** Scanning electron micrographs of the eggs of Piratinae. **A**: Operculum of *Ectomocoris tibialis* (scale: 118 $\mu$). **B**: Operculum of *Pirates mundulus* (scale: 86 $\mu$). **C**: Operculum of *Ectomocoris ochropterus* (scale: 154 $\mu$). **D**: Operculum of *Catamiarus brevipennis* (scale: 137 $\mu$). **E**: *Sirthenia flavipes*: Collar and chorionic filaments (scale: 75 $\mu$). **F**: Spongy area at the operculur disc of the same (scale: 57 $\mu$). **G**: Enlarged view of the follicular pits of *Ectomocoris tibialis* (scale: 17 $\mu$). **H**: Operculum of *Pirates affinis* (scale: 267 $\mu$). **I**: Micropyles of *Pirates mundulus* at the collar region (Photomicrograph: scale: 376 $\mu$). **J**: L.S. of chorion of *Pirates affinis* showing the aerostatic layer (arrows; scale: 14 $\mu$).

the bent portion of the micropyles are arranged in a clockwise direction. The number of micropyles and aeropyles, along with their dimensions is tabulated (table 1).

3.2e *Operculum:* The operculum of piratine eggs is disc-like and fits in the collar through a very distinct sealing bar. The exo-endochorionic differentiation, seen clearly in the body of the egg, is absent in the operculum and it is wholly composed of endochorion. While the posterior part of the operculum is simple and dome-shaped, the anterior surface shows variations in different species. In *E. tibialis*, *E. ochropterus*, *E. cordiger*, *C. brevipennis* and *P. affinis* (figures 1, 2, 4 & 5), the upper surface of the operculum has a central concave disc of shallow area consisting of air containing spongy network, the height of which gradually increases towards the opercular periphery and ultimately project as small, but numerous filaments. The spongy network is supported by slanting rings of endochorionic extensions. In addition, the centre of the disc may show additional smaller supports. In *E. tibialis* and *E. ochropterus* a thin highly porous veil-like extension connects the extremities of the extensions to the upper middle of the operculum (figure 1, E1, F1; 4G, H; 5A, C). In *E. cordiger*, instead of the shallow central depression, the operculum has the central area elevated like a button with the spongy network restricted to this elevated part (figure 1G; 4F). In *P. mundulus* the entire upper surface is in the form of an upturned air-filled cup having tubercular projections (figure 1B 1, 5B). In the last two species the central part of the opercular disc has a vertical endochorionic column with the apex flared giving an 'X' or 'Y' shape in cross-section. The most peculiar operculum is that of *S. flavipes* where the central disc of the upper surface is produced into a vertical pillar supporting in its extreme an inverted air-filled saucer-shaped disc with spongy area confined to the centre (figure 1, D1; 5F). The pillar-like support has numerous pore canals running through it.

The periphery of the operculum is nearly 4–5 times as thick as its central area and shows large air-filled spaces supported by struts or pillars. The height of such struts of pillars, as well as the sizes of the air spaces progressively get reduced towards the central area of the operculum (figure 2). Through the basal wall of the air-filled cavities, especially from the central shallow area, run numerous pore canals through the endochorion and open into the lumen of the egg. The aerostatic inner layer visible throughout the body is distinctly absent in the endochorion of the operculum. During eclosion the operculum breaks free from the sealing bar of the collar at two places (figure 2A) and is removed like a lid from the body of the egg without causing any damage to the latter.

## 4. Discussion

A detailed study of the eggs of several terrestrial heteroptera enabled Southwood (1956) to distinguish Cimicomorpha from Pentatomomorpha on the basis of operculum, sealing bar and aero-micropylar systems. In spite of the general similarities with other families, the characters of the reduviid eggs were recognised as unique in several aspects (Readio 1926; Miller 1953; Southwood 1956). Among the reduviids the eggs of Piratinae and Harpactorinae stand out as most characteristic. Though there are several descriptions pertaining to the eggs of Harpactorinae, not much attention has been given

to the eggs of Piratinae. Readio (1926) was the first to mention the chorionic filaments in Piratinae. While describing the biology of many southern Rhodesian reduviids, Miller (1953) provided information on the eggs of some Piratinae. Even in the classical work of Cobben (1968) and Hinton (1981) there is hardly any mention about this subfamily.

The cylindrical shape of the piratine eggs with a pointed and semi-ellipsoidal basal region enables their easy insertion into the ground. This type of vertical deposition of eggs in the soil, leaving only the apical parts exposed, is unique to Piratinae (Haridass 1974) and such a method does not fit into the ovipositional schemes of insects provided by Southwood (1956).

The structure of the shell or chorion of insect eggs is a product of the follicular cells and this has been a subject of controversy for long. The architectural as well as the chemical diversity of the chorion have been shown to be highly variable and there has been neither strict correspondence between layers of eggs of different insects nor any homology with the terms exo- and endochorion (Cobben 1968; Hinton 1969). In fact, Pantel (1913), Hinton (1963), and Hartley (1964) suggested that these layers be merely called outer and inner chorionic layers. Generally the most accepted terminology for the outer and inner layers of chorion are exochorion and endochorion, respectively. All other layers secreted outside the eggs, either by the oviduct or by the female accessory glands, are called extrachorion (Hartley 1961) or as suprachorion (Cobben 1968), as against the layer/s secreted inner to the chorion by the developing embryo as subchorion (Beament 1949). Eggs of all the piratine species examined revealed only two distinct layers, the exo- and endochorion, throughout their body, but varying in thickness in those eggs with marked follicular pits or with tubercle-bounded follicular areas. The seven different proteinaceous layers reported for *Rhodnius* eggs (Beament 1946) have not been observed presently and the existence of such layers has also been doubted (Cobben 1968; Hinton 1969). Unlike the body, the operculum of these eggs is made up of only the endochorion. The true operculum common in Cimicomorpha, is defined as one which is formed only of endochorion, with sealing bar and micropyles (Southwood 1956). The innermost layer of the shells in all piratine species examined is a continuous aerostatic layer separated from the rest of the endochorion by blunt tubercular struts enclosing air spaces. This layer appears to be common in many reduviids (Southwood 1956; Cobben and Henstra 1968; Cobben 1968; Salkeld 1972; Hinton 1981) and it is also believed to have evolved independently along different lines among various groups of insects (Cobben 1978).

Like other terrestrial insects, Piratinae lay eggs in relatively dry environment and hence face the requirement of extensive surface area for obtaining oxygen from the ambient air, as well as the danger of losing considerable water through such surfaces. In spite of all these adverse factors, the piratine eggs have evolved efficient respiratory system that has satisfied their oxygen needs without losing too much water. The surface area/volume ratios of the eggs being high (table 1) the contrasting problems of oxygen uptake and water loss are very great for the small-sized eggs and so the existence of complicated respiratory system in them appears very relevant.

The air containing aerostatic inner layer in piratine eggs is connected with the ambient air. The numerous external aeropylar openings of the chorionic filaments establish a pathway for air passage between ambient oxygen and the inner aerostatic layer through a network of air channels in the filaments and in the collar region. In addition, the oxygen available inside the ground is also taken to the aerostatic layer

from the deepest part of the egg lying buried, through a system of pore canals of the specialised basal part. The absence of a distinct aerostatic layer in the operculum is compensated by the development of network of spongy areas and large air-filled spaces in this part of the egg and with a system of well-developed pore canals, the air spaces are connected to the lumen of the egg enabling the apical parts of the developing embryos to get their share of the ambient oxygen.

Terrestrial habitats of Piratinae are often liable to sudden flooding, particularly during rainy seasons, and to get submerged in water for periods longer than the embryonic development is a natural and regular problem for these eggs. Hence the adaptations of these terrestrial eggs for respiration water are as complex as those of aquatic eggs. Eggs which can submerge in water always support a thin layer of air all around them and this physical gill has been termed as plastron (Brocher 1912 a,b). Such plastrons can be formed by an aggregation and/or enlargement of aeropyles as well as by a close network of air containing areas of the chorion. The importance of plastron is well documented for Coleoptera (Thrope and Crisp 1949) and the instances of plastron respiration among terrestrial insects are believed to outnumber those of aquatic insects (Hinton 1981). Plastron enables the egg to remain submerged indefinitely and to obtain oxygen from ambient water as well as to provide direct air routes in dry conditions. Terrestrial insects, having a small ratio of the total aeropylar area per mg of egg to effect an efficient plastron respiration, are known to lay eggs in habitats that are usually wetted by rain and in many such insects, like the mirids, the aeropyles are restricted to the anterior end of the eggs on elevated tubercles or stalks (Hinton 1962; Cobben 1968). The elevation of aeropyles on such respiratory horns helps the eggs to utilize atmospheric air when the egg is covered by a thicker layer of water. In piratine eggs the operculum is provided with a complicated system of air-containing network and the general surface of the chorion is never smooth; they always have prominent follicular pits or tubercle bounded follicular areas. These structures of the chorion and the operculum, the irregular prominences of the collar and the external aeropylar openings of the chorionic filaments, all act as hydrofuge. When dipped in water these areas resist wetting and always hold a thin layer of air all around them. Though not so well developed as in other terrestrial eggs reported so far, such an air layer still serves as efficient plastron for those eggs.

As in other Cimicomorpha, the eggs of Reduviidae are fully formed before fertilization and it is essential that the eggs are provided with openings in the chorion for the entry of sperms for fertilization. Leuckart (1855) was the first to describe these in insects eggs and such canals are termed as micropyles. Complete forms of true aeropyles and micropyles are found only in Reduviidae (Southwood 1956) and in piratine eggs they are typical like those described in many other Reduviidae (Beament 1947; Southwood 1956; Cobben 1968; Salkeld 1972; Haridas 1974; Hinton 1981) arising from the inner aspects of the spermatic groove in the collar and opening into the lumen of the egg through the endochorion near the sealing bar. When viewed from the apical side, the inner bent portions of these sperm canals are arranged in a clock-wise direction, as has been reported for other reduviid eggs (Cobben 1968). Though the maximum of micropyles reported for Reduviidae is only 15 (Cobben 1968) the species presently examined possessed a higher number, the maximum for a single egg being 31 (table 1). This number is reduced in eggs laid at the fag end of the ovipositional cycle and as Beament (1947) suggested, this reduced number of micropyles could be one of the reasons for the higher number of unfertilized eggs laid by older females.

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# Host selection and food utilization of the red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas) (Chrysomelidae: Coleoptera)

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**Abstract.** An analysis of the host plant relationships with respect to the red pumpkin beetle, *Raphidopalpa foveicollis* Lucas is presented based on the role of receptors involved in host selection, the quantitative food utilization on different cucurbitaceous host plants and the biochemical parameters involved in food plant selection. Orientation of the beetles towards the host plants appeared to be profoundly affected when the receptors present on the antennae and mouthparts were ablated or coated. Though significant differences were observed with regard to the quantity of food ingested among different host plants, ingestion of food was higher for mature leaves and flowers compared to young and senescent leaves. Accordingly, mature leaves and flowers showed high nitrogen and proteins, low sugars, moderately high phenols and narrow $C/N$ ratio compared to other plant parts. The chemosensory receptors present on the antennae and mouthparts were also studied using scanning electron microscope.

**Keywords.** Host selection; food utilization; *Raphidopalpa foveicollis*.

## 1. Introduction

An individual plant is heterogenous to any phytophagous insect and since the plant parts vary in their nutritional value, the food utilization on different parts of the same plant appears to be important in order to determine the pest status of the concerned species. The red pumpkin beetle, *Raphidopalpa foveicollis* Lucas feeds on a variety of cucurbitaceous crops causing economic losses. Observations by Sihna and Krishna (1971) and Grewal and Sandhu (1982) have shown the relative preference of the beetle towards different parts of the cucurbits, *Lagenaria vulgaris* Ser and *Cucumis melo* L. In view of the fact that the information on the comparative utilization of the different plant parts *viz* flowers, young, mature and senescent leaves is meagre, an attempt has been made to study the relative preference and quantitative food utilization of some economically important cucurbits and a weed host by *R. foveicollis* in terms of some important chemical parameters. In addition, the role of sensory structures in host selection by *R. foveicollis* was also investigated using scanning electron microscope (SEM).

## 2. Material and methods

Adult individuals of mixed age and sex groups, collected from the college campus were used in this investigation. The different cucurbitaceous plants *viz Luffa cylindrica*

Roem, *Luffa acutangula* Roxb, *Trichosanthes anguina* L, *Cucurbita maxima* Duch, *Benincasa cerifera* Savi, *Momordica charantia* L and *Mukia scabrella* Arn were collected from the college farm.

### 2.1 *Role of receptors in host selection*

The role of receptors in host selection was studied by ablation of antennae, painting the eyes with nail polish, and coating the mouthparts with nail polish. In each of these tests ten adults of *R. foveicollis* were subjected to various treatments and another ten adults were used as control. The beetles were released into a glass trough containing the preferred host plant at the centre and covered by a glass plate. The number of beetles (control and experimental) orienting towards the host plant was monitored for 15 min. Each set of experiment was repeated three times.

### 2.2 *SEM studies*

To get a detailed picture of the sensilla on the antennae and mouthparts SEM studies were made. The mouthparts and antennae were detached from etherized specimens, dried in a dust-free desiccator for 1–2 days, fixed to aluminium stubs using double adhesive tapes and then coated with gold for 2–3 min in a standard ion-coater. Micrographs were made using a Hitachi scanning electron microscope, table top model S 415 A under 15 kV emission current.

### 2.3 *Quantitative food utilization*

To study the quantitative food utilization by *R. foveicollis*, leaf discs (7·5 cm dia) or an entire leaf were placed inside a petri dish over a moist filter paper of the same dimension. The initial weight of the leaf disc and the filter paper was determined. The test insects were fed with water for 2–3 hr by providing a moist cotton to clear their guts before the start of the experiment. A pair of beetles (male and female) were released into the dish and covered by another petri plate. A control was also maintained without the test insects. Twentyfour hours after the release of the insect, the leaf discs and filter paper were removed and reweighed. Before reweighing the filter paper, the sides of the petri dishes were carefully wiped with it to collect any excrement left on the dishes. After correcting for the weight loss due to transpiration by the leaf, the comparative food utilization on different host plants was assessed by determining the quantity of food ingested/24 hr and the coefficient of digestibility (Waldbauer 1968). Each set of experiment was repeated three times.

### 2.4 *Biochemical analysis*

The different cucurbitaceous host plant parts *viz* flowers (1-day old), young (3–5 days old), mature (5–10 days old), and senescent leaves (more than 10 days old) were analysed for various biochemical components. The total nitrogen was estimated (Humphries

1956) and multiplied by 6·25 to estimate the total protein content. In addition, the total carbohydrates (Dubois *et al* 1956) and phenols (Bray and Thorpe 1954) were also estimated. The $C/N$ ratio was computed for the different host plants.

## 3. Results

### 3.1 *Role of receptors in host selection*

It was observed that orientation towards the host plant by *R. foveicollis* was profoundly affected when subjected to various tests such as antennal ablation, blinding of eyes and coating of mouthparts. Antennectomized and blinded beetles took a longer time in moving near the host plant. The mean numbers of antennectomized beetles that located the host plant and involved in active feeding were 2·0 and 0·67 respectively. When eyes were blinded, the mean number of beetles which located the host plant was only 0·33 and they did not involve in active feeding. However, in control, the number of beetles involved in host location and active feeding was 7·00 and 5·00 respectively. The beetles, whose mouthparts have been suitably coated, did not show any positive response to initiate feeding even after locating the host plant (table 1).

### 3.2 *SEM studies*

Figure 1 depicts the sensory structures involved in perceiving the odours/stimulus emanating from the host plants and are located on the antennae and mouthparts of *R. foveicollis*, involving (1) sensilla trichoidea: four long sensory hairs at the apex of each antennae, functioning as olfactory chemoreceptors, (2) sensilla basiconica: short sensillae distributed all along the antenna and designated as trichoid sensillae involved in olfaction, and (3) sensilla chaetica: bristles with blunt tips or sometimes with sharp pointed ends serve as mechanoreceptors. Ablation of antennae in *R. foveicollis* deprived the insect of the ability to perceive stimuli and interfered with the normal orientation towards the host plant. The outer and inner face of the labrum bears sparsely distributed trichoid sensillae. While the mandibles did not bear any sensory hairs, on

**Table 1.** Effect of antennectomy, blinding of eyes and coating of maxillae on the host plant location and feeding by *R. foveicollis*.

| | Mean number of beetles orienting towards the host plant | | | |
|---|---|---|---|---|
| Treatments | Host location | *t* value | Active feeding | *t* value |
| Antennectomized | 2·00 | 6·10* | 0·67 | 6·69* |
| Eyes blinded | 0·33 | 10·30** | 0·00 | 12·20** |
| Maxilla coated | 0·33 | 10·30** | 0·00 | 12·20** |
| Control | 7·00 | | 5·00 | |

Data represent mean of three replicates. *Significant at 5 % level, **significant at 1 % level.

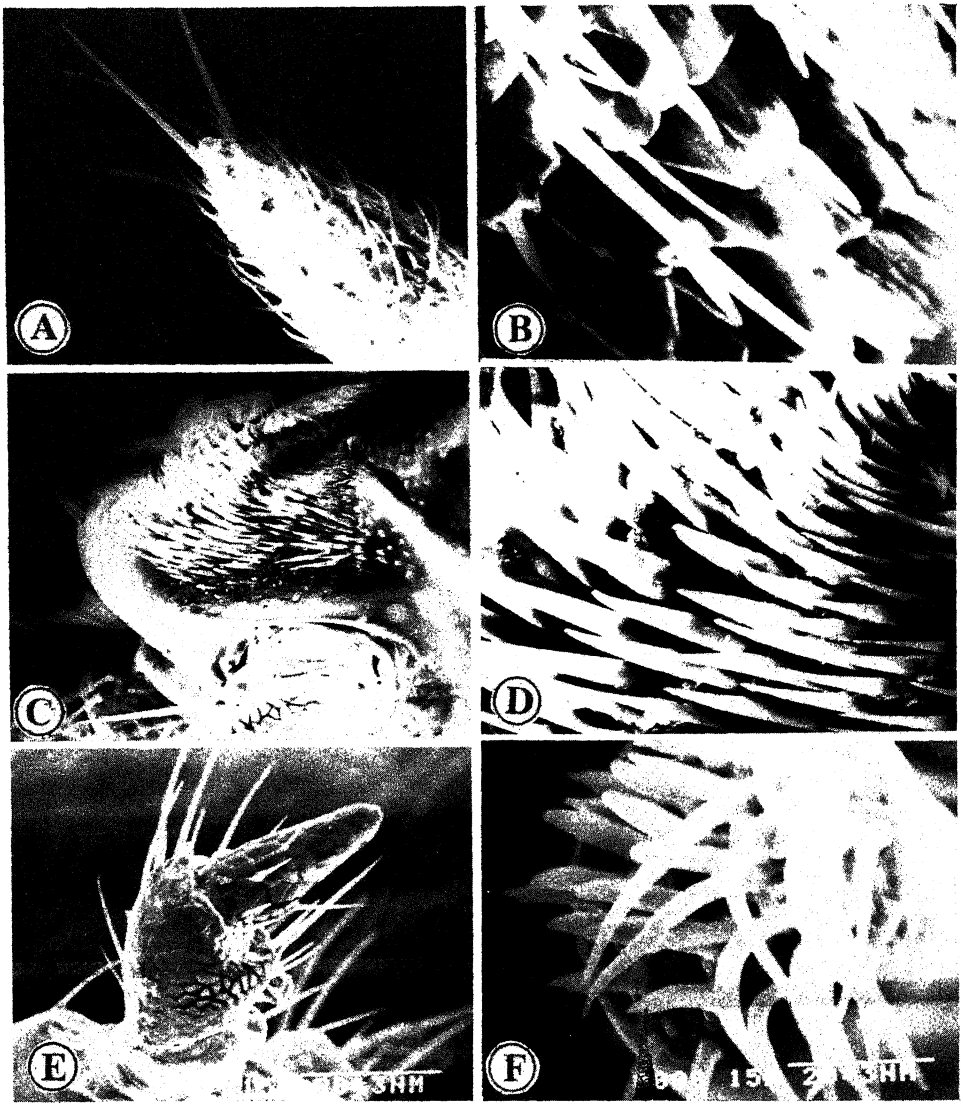


**Figure 1.** **A.** Antenna showing four long *Sensilla trichoidea* at the tip. **B.** Distribution of *sensilla basiconica* (short sensillae) and *Sensilla chaetica* (bristles with blunt tips) on the antenna. **C.** Stipes of maxilla showing the campaniform sensilla. **D.** Enlarged view of the campaniform sensilla on the stipes. **E.** Labial palp. **F.** Labial palp showing the presence of two types of sensillae at the tip.

the maxilla, the stipes carried large number of sensory hairs (figure 1). The maxillary palp did not carry any sensilla but the labial tips showed the presence of two types of sensillae, one group with sharp and pointed ends and the other with blunt ends. These sensillae appear to be the campaniform sensillum and act as gustatory chemoreceptors.

### 3.3 *Quantitative food utilization*

The quantity of food ingested by *R. foveicollis* varied considerably among different cucurbitaceous host plant parts (table 2). On young leaves, maximum consumption was observed on *B. cerifera* (0·367 mg) followed by *T. anguina* (0·362 mg), *L. cylindrica* (0·342 mg), *L. acutangula* (0·259 mg) and *C. maxima* (0·256 mg). The food ingested was the least on the weed host *M. scabrella* (0·199 mg). However, the quantity of food ingested on mature and senescent leaves was the highest for *T. anguina* (0·510 and 0·427 mg) as against 0·469, 0·335 mg; 0·385, 0·339 mg; 0·350, 0·307 mg; 0·295, 0·272 mg and 0·188, 0·178 mg on *L. cylindrica*, *B. cerifera*, *L. acutangula*, *C. maxima* and *M. scabrella* respectively. With regard to the flowers, the highest consumption was observed for *C. maxima* (0·395 mg) compared to other host plants. Thus an overall assessment of the pattern of food ingestion by *R. foveicollis* showed that apart from the

**Table 2.** Quantitative food utilization by *R. foveicollis* on certain cucurbitaceous hosts

| Plant parts | Host plants | Quantity of food ingested (mg) | Coefficient of digestability (%) |
|---|---|---|---|
| Young leaves | *L. cylindrica* | 0·342 ± 0·000* | 92·950 ± 0·562 |
| | *T. anguina* | 0·362 ± 0·038 | 93·770 ± 1·490 |
| | *C. maxima* | 0·256 ± 0·033 | 89·456 ± 2·919 |
| | *L. acutangula* | 0·259 ± 0·008 | 89·575 ± 1·219 |
| | *B. cerifera* | 0·367 ± 0·027 | 94·005 ± 1·041 |
| | *M. charantia* | — — — | — — — |
| | *M. scabrella* | 0·199 ± 0·001 | 85·929 ± 1·489 |
| Mature leaves | *L. cylindrica* | 0·469 ± 0·005 | 94·570 ± 0·271 |
| | *T. anguina* | 0·510 ± 0·001 | 96·275 ± 0·873 |
| | *C. maxima* | 0·295 ± 0·027 | 91·985 ± 1·983 |
| | *L. acutangula* | 0·350 ± 0·039 | 94·000 ± 0·481 |
| | *B. cerifera* | 0·385 ± 0·007 | 94·805 ± 1·292 |
| | *M. charantia* | — — — | — — — |
| | *M. scabrella* | 0·188 ± 0·036 | 86·705 ± 0·708 |
| Senescent leaves | *L. cylindrica* | 0·335 ± 0·004 | 90·290 ± 0·572 |
| | *T. anguina* | 0·427 ± 0·065 | 95·316 ± 0·053 |
| | *C. maxima* | 0·272 ± 0·065 | 88·971 ± 1·021 |
| | *L. acutangula* | 0·307 ± 0·053 | 90·228 ± 0·553 |
| | *B. cerifera* | 0·339 ± 0·022 | 93·805 ± 0·512 |
| | *M. charantia* | — — — | — — — |
| | *M. scabrella* | 0·178 ± 0·016 | 86·516 ± 0·038 |
| Flowers | *L. cylindrica* | 0·347 ± 0·049 | 84·725 ± 0·802 |
| | *T. anguina* | 0·355 ± 0·010 | 85·070 ± 0·140 |
| | *C. maxima* | 0·395 ± 0·066 | 87·848 ± 0·539 |
| | *L. acutangula* | 0·349 ± 0·022 | 85·673 ± 0·138 |
| | *B. cerifera* | 0·372 ± 0·071 | 85·022 ± 0·412 |
| | *M. charantia* | — — — | — — — |
| | *M. scabrella* | 0·165 ± 0·040 | 65·455 ± 0·412 |

*Indicate mean ± S.E.

preference among different cucurbitaceous host plants, the preference was more for mature leaves and flowers compared to young and senescent leaves. Observations on the feeding behaviour of the beetle under field conditions also revealed a similar picture. The non-utilization of the cucurbitaceous host, *M. charantia* is of interest since the beetles were not found feeding on this plant in the field as well as under laboratory conditions. The computation of the coefficient of digestibility of various plant parts also showed a direct proportionality with the quantity of food ingested. Moreover, the digestibility was comparatively higher when *R. foveicollis* was fed on mature leaves as against other host plant parts.

### 3.4 *Biochemical analysis*

Data on the biochemical analysis of different cucurbitaceous host plants are furnished in table 3. With regard to water content, it was found to be higher in young leaves and flowers compared to mature and senescent leaves, but significant differences were not observed among different host plants, excepting in the case of senescent leaves. The estimation of nitrogen and protein content showed significant differences between different parts of the plant as well as among different host plants. While the nitrogen and protein content was appreciably high for *B. cerifera* and *L. cylindrica*, on other host plants they were comparatively lower. It was maximum in mature leaves compared to young and senescent leaves and moderately high in flowers.

Estimation of carbohydrates showed that it was lower in *L. acutangula*, *T. anguina* and *M. scabrella* when compared to *C. maxima*. Variation was evident between the different parts of the plant such as mature and senescent leaves exhibiting lower carbohydrates as compared to young leaves and flowers. Similarly, the phenol content in *M. scabrella* and *T. anguina* was less when compared to other host plants and young leaves showed higher phenolic contents compared to other parts. Computation of C/N ratio also indicated considerable differences among different cucurbitaceous host plants as well as between plant parts. The ratio was narrow for mature leaves followed by senescent leaves, flowers and young leaves. However, it was high in *M. charantia* compared to other host plants.

### 3.5 *Host plant preference in terms of food utilization and biochemical parameters*

An assessment of the host plant preference of *R. foveicollis* in terms of the quantity of food ingested and various biochemical components also revealed some interesting results. With regard to the utilization of flowers, the preference among different plants is as follows: *C. maxima* > *B. cerifera* > *T. anguina* > *L. acutangula* > *L. cylindrica* > *M. scabrella*. Although the percentage of nitrogen and protein was maximum in *L. cylindrica*, the quantity of food ingested was comparatively lower when compared to *C. maxima*, probably due to the increased carbohydrates and phenols. A similar trend was also evident for *L. acutangula*. Moreover, the low nitrogen and protein in *C. maxima* have been compensated by high food intake by the beetles.

The presence of increased amounts of nitrogen and protein, low phenols and moderate carbohydrate in the young leaves of *B. cerifera* resulted in an increased quantity of food intake by *R. foveicollis*. On the contrary, the high concentration of

**Table 3.** Chemical composition of certain cucurbitaceous host plants

| Host plants | Plant parts | Moisture (%) | Nitrogen (%) | Proteins (%) | Carbohydrates (mg/g fresh wt) | Phenols (mg/g fresh wt) | C/N ratio |
|---|---|---|---|---|---|---|---|
| *L. cylindrica* | Y | 83·28 | 5·32 | 33·25 | 2·38 | 2·60 | 0·45 |
| | M | 77·76 | 6·44 | 40·25 | 1·25 | 2·53 | 0·19 |
| | S | 62·73 | 4·79 | 29·94 | 1·08 | 2·19 | 0·23 |
| | F | 80·43 | 5·95 | 37·19 | 2·13 | 2·60 | 0·36 |
| *T. anguina* | Y | 84·39 | 3·64 | 22·75 | 1·13 | 1·85 | 0·31 |
| | M | 84·00 | 5·18 | 32·38 | 1·00 | 1·63 | 0·19 |
| | S | 76·84 | 3·15 | 19·69 | 0·93 | 1·80 | 0·29 |
| | F | 83·10 | 3·41 | 21·81 | 2·00 | 2·10 | 0·59 |
| *C. maxima* | Y | 82·24 | 1·61 | 10·06 | 2·60 | 2·00 | 1·61 |
| | M | 72·94 | 3·15 | 19·69 | 1·48 | 1·83 | 0·47 |
| | S | 68·32 | 2·10 | 16·63 | 1·13 | 1·35 | 0·54 |
| | F | 80·10 | 2·66 | 16·63 | 1·18 | 1·35 | 0·44 |
| *L. acutangula* | Y | 78·14 | 2·17 | 13·56 | 1·63 | 2·60 | 0·75 |
| | M | 73·75 | 2·94 | 18·38 | 1·13 | 2·53 | 0·38 |
| | S | 66·00 | 2·01 | 18·81 | 0·85 | 2·30 | 0·28 |
| | F | 79·75 | 4·69 | 29·31 | 1·73 | 1·56 | 0·37 |
| *B. cerifera* | Y | 84·00 | 6·09 | 38·06 | 2·23 | 3·03 | 0·37 |
| | M | 79·26 | 6·58 | 41·13 | 2·13 | 2·60 | 0·32 |
| | S | 77·62 | 4·34 | 27·13 | 2·23 | 2·18 | 0·51 |
| | F | 80·00 | 2·38 | 14·88 | 1·75 | 2·18 | 0·74 |
| *M. charantia* | Y | 82·34 | 1·96 | 12·25 | 2·39 | 2·61 | 1·22 |
| | M | 78·84 | 2·17 | 13·56 | 1·64 | 2·00 | 0·75 |
| | S | 73.92 | 1·26 | 7·88 | 1·08 | 1·08 | 0·85 |
| | F | 81·42 | 1·61 | 10·06 | 2·28 | 1·75 | 1·42 |
| *M. scabrella** | Y | 84·00 | 1·68 | 10·50 | 1·25 | 1·50 | 0·74 |
| | M | 78·77 | 1·75 | 10·94 | 0·95 | 1·55 | 0·54 |
| | S | 76·63 | 1·96 | 12·25 | 0·93 | 1·55 | 0·47 |
| | F | 82·50 | 1·12 | 7·00 | 1·10 | 2·15 | 0·98 |

*Indicates cucurbitaceous weed host. Y: Young leaves; M: Mature leaves; S: Senescent leaves; F: Flowers

phenols and carbohydrates in *L. cylindrica* reduced the food consumption. The food intake in *L. acutangula*, *C. maxima* and *M. scabrella* was sufficiently low presumably due to low nitrogen and protein. Thus the preference for young leaves indicate the following sequence: *B. cerifera* > *T. anguina* > *L. cylindrica* > *L. acutangula* > *C. maxima* > *M. scabrella*.

The pattern of food consumption on mature and senescent leaves also indicate a striking relationship between leaf nitrogen, protein, carbohydrate and total phenolic contents. High food intake was evident in the presence of increased nitrogen and protein, low carbohydrate and phenols, as in the case of mature leaves of *T. anguina* followed by *L. cylindrica* and *B. cerifera* respectively. In the case of low nitrogen and low phenols, compensation was achieved by a relatively high food intake as seen in the senescent leaves of *T. anguina*.

## 4. Discussion

Chemoreceptors of insects form a complicated and subtle sensory system that enables them to differentiate between many natural stimuli of fairly great diversity (Schoonhoven 1977). In the present study three types of sensory hairs were observed on the antennae of *R. foveicollis viz* sensilla trichoidea, sensilla basiconica and sensilla chaetica. In addition, the sensillum present on the stipes of the maxillae and tip of the labial palps appears to be gustatory receptors. Schneider (1964) opined that the sensilla present on the antennae acts as olfactory and mechanoreceptory chemoreceptors. In acridids, the campaniform and trichoid sensilla located on the maxilla and labium were found to act as gustatory chemoreceptors (Chapman and Thomas 1978). Thus the differences in sensitivity of chemoreceptors to different host plant odours enable the insect to orient towards its food plant and the delay in location of host plants and the failure to initiate feeding by *R. foveicollis* could be attributed to the inability to perceive the plant odours as a result of antennal ablation and coating of the sensory areas located on the mouthparts.

It is well known that both primary and secondary plant chemicals are indispensable in the regulation of feeding behaviour of phytophagous insects. While primary plant chemicals serve as essential phagostimulants to elicit initial feeding response and to maintain continuous feeding on the host plant, many secondary plant chemicals exhibit repellent, deterrant and toxic effects, and their presence provides the basis of insect resistance in the majority of plants (Hsiao 1974). The greater preference of *R. foveicollis* towards leaves and flowers was attributed to some chemical stimuli present in these regions (Sinha and Krishna 1971). In the present investigation, the differences in the relative preference and quantitative food utilization of *R. foveicollis* among different cucurbitaceous host plants as well as between different plant parts appear to be due to variations in their nutritive qualities, *M. charantia* being an exception. Mature leaves and flowers showed high nitrogen and proteins, low carbohydrates, moderately high phenols and narrow C/N ratio compared to other plant parts. The importance of organic nitrogen (McNeill and Southwood 1978), sugars and proteins (Beck 1956), and phenols (Hartfield *et al* 1982), in the host plant selection by phytophagous insects is very well established. Moreover, a lower C/N ratio is also associated with greater susceptibility of the host plant to insect attack (Jayaraj 1967). With increasing plant age, the total soluble nitrogen decreased appreciably thereby interfering with the development of the aphid, *Myzus persicae* Sulz on Brussels sprout plants (van Emden and Bashford 1971). Marian and Pandian (1980) showed that the consumption, assimilation and net-conversion of leaf proteins by the *Danaus chrysippus* L larvae fed on senescent leaf of *Calotropis gigantea* R. Br. were significantly less than those fed on normal leaf. However, in the present study it was observed that a definite relationship existed between the quantity of food ingested and the different biochemical parameters such as the nitrogen, protein, carbohydrate and phenolic contents of different plant parts. The presence of increased nitrogen and protein, low carbohydrate and phenol in the host plant resulted in increased food consumption by *R. foveicollis*. Similarly, the food intake was also relatively high in the case of host plants with low nitrogen and low phenols. This emphasises the fact that *R. foveicollis* exhibited a compensatory behavioural and physiological response i.e. altering the food consumption and utilization in response to variation in the nutritional suitability of their food (Slansky 1982).

Chandaravadana and Pal (1983) reported the presence of a triterpenoid feeding eterrant from *M. charantia* which could also be the reason for the non-utilization of his plant by *R. foveicollis*. In addition, Sinha and Krishna (1970) showed that for an ptimum level of the alkaloid, cucurbitacin E (a feeding incitant widely distributed in ucurbitaceous plants) was essential to stimulate feeding in *R. foveicollis*. While a lower oncentration of this compound will not initiate the beetle feeding, higher concentra- ions can also act as a feeding deterrant. Thus the differential feeding behaviour of *. foveicollis* on different parts of the cucurbitaceous plants could also be due to the istribution and varied concentrations of such secondary substances. It could therefore e concluded that the qualitative and quantitative differences in the primary and econdary chemicals coupled with the discriminating powers of the various chemosen- ory receptors (Visşer 1983) located in the antennae and mouthparts determine the election of food plants/plant parts by *R. foveicollis*.

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# Distribution of earthworms in Madras

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**Abstract.** Distribution of earthworms has been studied in four locations comprising of two sandy loam and two clay loam soils of Madras, and correlated with the physical and chemical parameters of the soil. *L. mauritii* and *O. serrata* are the dominant earthworm species in the sandy loams and clay loams respectively. Each location has limiting factors like temperature, moisture and/or soil texture which strongly govern the density and diversity of earthworm populations in that region.

**Keywords.** *L. mauritii*; *O. serrata*; distribution; density.

## 1. Introduction

The ecology and the manner of life of the oligochaeta, especially the earthworms has been extensively investigated since Darwin's contribution to the study of earthworms. Earthworms prefer well aerated and moist habitats. They are frequently found in medium textured upland soils where the moisture capacity is high, rather than in droughty sands or poorly drained lowlands (Brady 1974). Distribution of earthworms is usually irregular (Guild 1952; Satchell 1955; Svendsen 1957), the numbers varying in relation to the type of soil (Evans and Guild 1947), with edaphic factors playing a vital role in their distribution.

An attempt is made in the present study to investigate the distribution and abundance of earthworms in the soils of Madras correlating their distribution to physical and chemical parameters of the soils they inhabit. Atmospheric temperature, soil temperature, moisture, pore space, maximum water holding capacity, organic matter and pH have been investigated in the four locations studied for earthworm distribution, including two sandy loams and two clay loams from May 1981 to May 1982.

## 2. Material and methods

The four locations studied in Madras (13°5′N, 80°18′E), have been categorically designated as locations *A*, *B*, *C* and *D*. While locations *A* and *B* are located in Ameer Mahal (a stretch of private land in Madras city), *C* and *D* are located in the Deer Sanctuary which is a protected reserve forest at Guindy, on the outskirts of the city. Soil is light brown sandy loam at *A* and *B*, brown clay loam at *C* and light brown clay loam at *D*.

Location *A* is situated at the periphery of a heap of dung, which is cleared during December–January. Field grass (Graminae) are the only floral associates of the community.

Location *B* lies adjacent to an open type domestic sewage about 0·5 km from *A*. Field grass along with *Coccinia indica* W & A, *Amaranthus spinosus* Linn., and *Euphorbia hirta* Linn. are the plant species.

Location *C* is richly inhabited by plants, the canopy of *Cassia* sp providing ample shade and litter to this region.

Location *D* lies adjacent to the banks of the lake inside the Deer Sanctuary about 4 km from *C*. Organic matter to the location is contributed by leaf litter of *Syzygium jambolanum* DC and droppings left behind by spotted deer and black buck which frequent this lake.

Monthly samples were collected from three units in each location, each unit covering a soil volume of 0·1 $m^3$ (60 × 60 × 30 cm) from May 1981 to May 1982. Earthworms were hand sorted and identified. Population density (no/0·1 $m^3$) was recorded for each of the monthly samples analysed.

Atmospheric temperature (45 cm above soil level) and soil temperature (10 cm below soil level) were recorded. Moisture content (Misra 1968), porosity and maximum water holding capacity (Keen and Rackzkowski 1921), oxidizable organic matter (Walkey-Black 1947) and pH (Hanna 1968) of the soil samples were assessed. Total monthly precipitation was obtained from the Meteorological Department, Madras. Data were subjected to appropriate statistical analyses for correlation and significance (Rao 1965).

## 3. Results

Soils of locations *A* and *B* are organically rich whereas at *C* and *D* only the surface horizons can be distinguished as organically rich zones. The soil in location *D* is coarse and gravel below 16 cm of the surface thus not being able to support earthworm fauna.

Mean annual values for atmospheric temperature, soil temperature, moisture, pore space, water holding capacity, organic matter and pH are illustrated in figure 1.

Atmospheric temperature is negatively correlated to the distribution of earthworms in all the four locations especially at *A* where it is significant ($P < 0{\cdot}05$). Soil temperature is also negatively correlated to earthworm distribution, being significantly negative ($P < 0{\cdot}05$) at the non-shaded locations *A* and *B*.

Moisture functions as a limiting factor at *A* and *C*, but not at *B* and *D*, which are close to water sources. Correlation to earthworm distribution is positive in all locations, being significant at *A* and *C*. Earthworm distribution in these areas is therefore co-ordinated with the amount of rainfall. Significant amount of rainfall occurred between 16 July 1981 and 15 December 1981 precipitating 1042·5 mm of rain scattered during the five months with a maximum of 305·5 mm during October–November 1981.

Pore space and the water holding capacity distinguish the sandy loams at *A* and *B* from the clay loams at *C* and *D*, the latter soils having lesser pore space and greater water holding capacity.

Organic matter of soils is generally positively correlated to earthworm distribution.

A piece of land of about 0·4 ha is reserved and protected for cultivation of fodder within the Deer Sanctuary about 0·5 km from *D*. The soil is sandy here when compared to *C* and *D*. This location, for convenience, called *E* supports earthworm fauna even during summer due to its moisture content. Species of earthworms here include *Lampito mauritii* Kinberg, *Octochaetona pattoni* (Michaelsen) and *Octochaetona thurstoni* (Michaelsen).

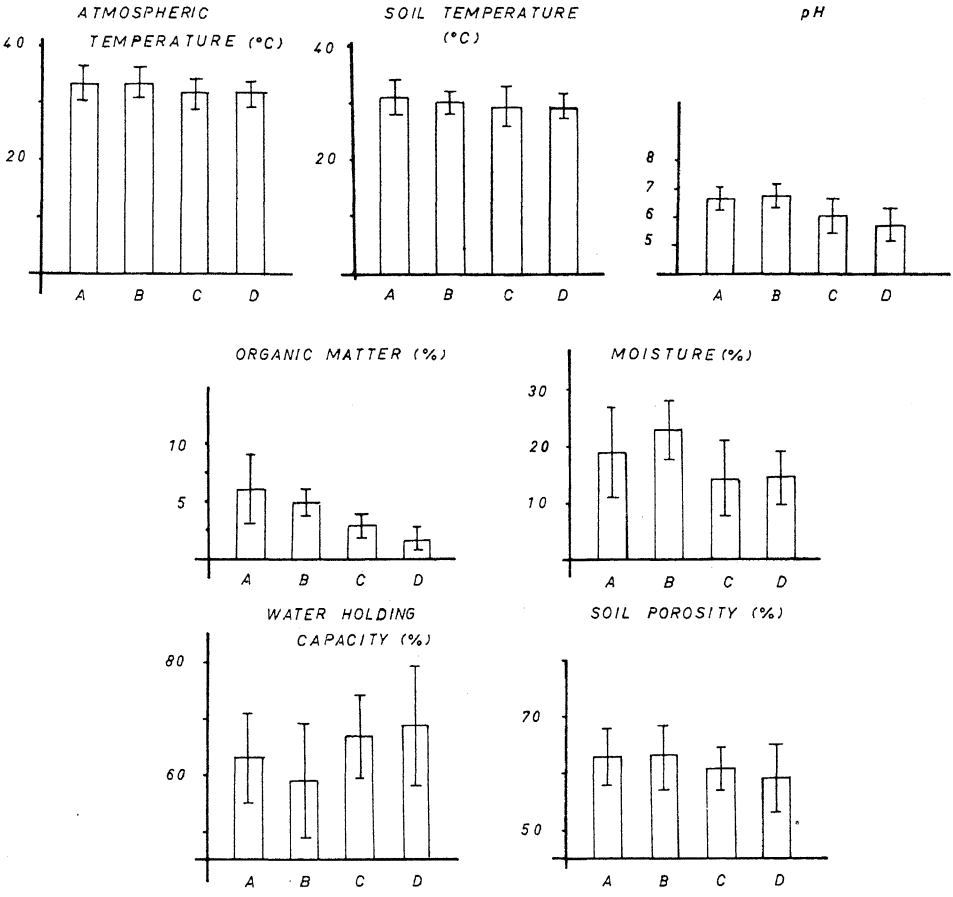



**Figure 1.** Annual mean with SD for atmospheric temperature (°C), soil temperature (°C), pH, organic matter (%), moisture (%), water holding capacity (%) and soil porosity (%) for locations *A*, *B*, *C* and *D* (May 1981 to May 1982).

*Lampito mauritii* Kinberg is the dominant species at *A* and *B* distributed in association with *Drawida modesta* Rao and *Ramiella pachpaharensis* Stephenson. *Octochaetona pattoni* (Michaelsen) and *Octochaetona thurstoni* (Michaelsen), have also been observed in these locations, but not frequently.

*Octochaetona serrata* (Gates) occurs as the dominant and the only species at *C* while *L. mauritii* and *O. thurstoni* occasionally trespass the domain of *O. serrata* at *D*, probably from *E*.

Population density of earthworms at *A*, *B*, *C* and *D* are compared to the respective mean monthly values for atmospheric temperature, soil temperature, pH, organic matter and moisture in three dimensional models, the parameters being marked with respect to the peak distribution of earthworms (figures 2 a–d).

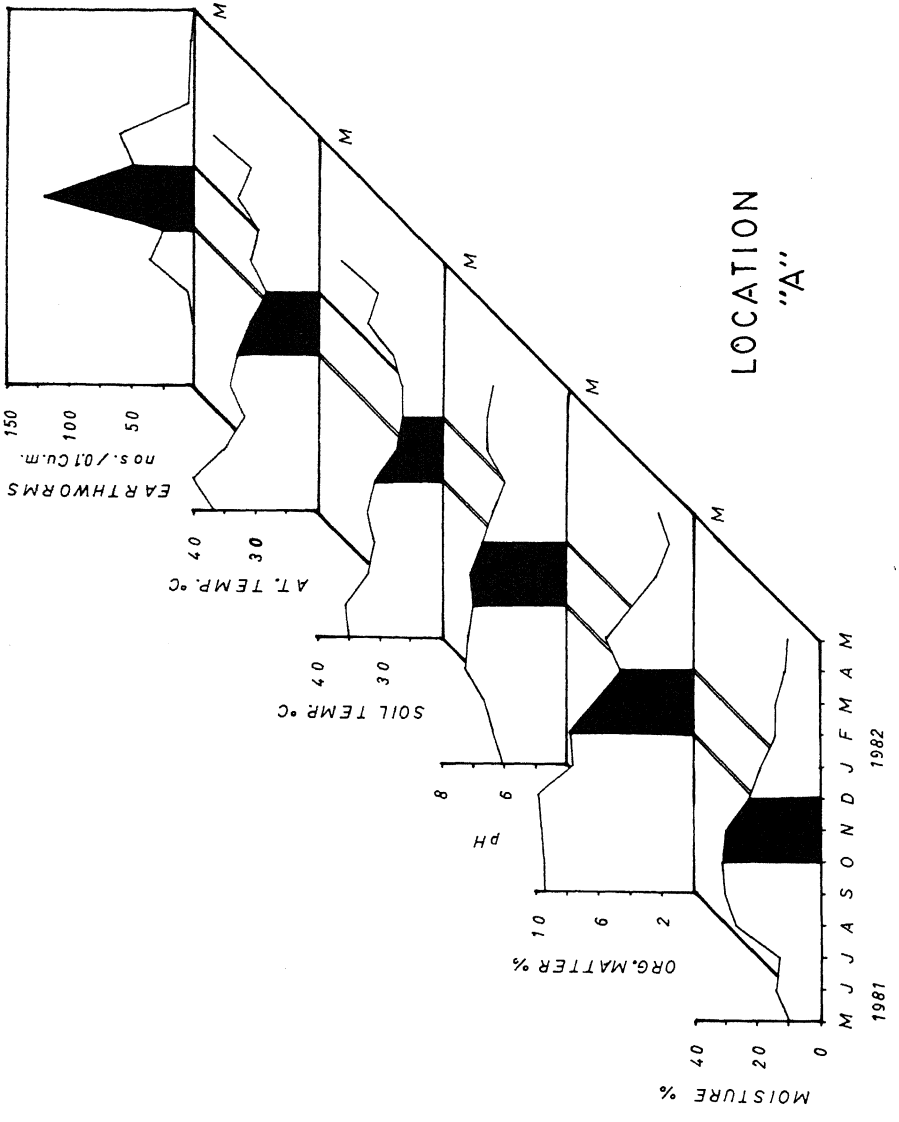
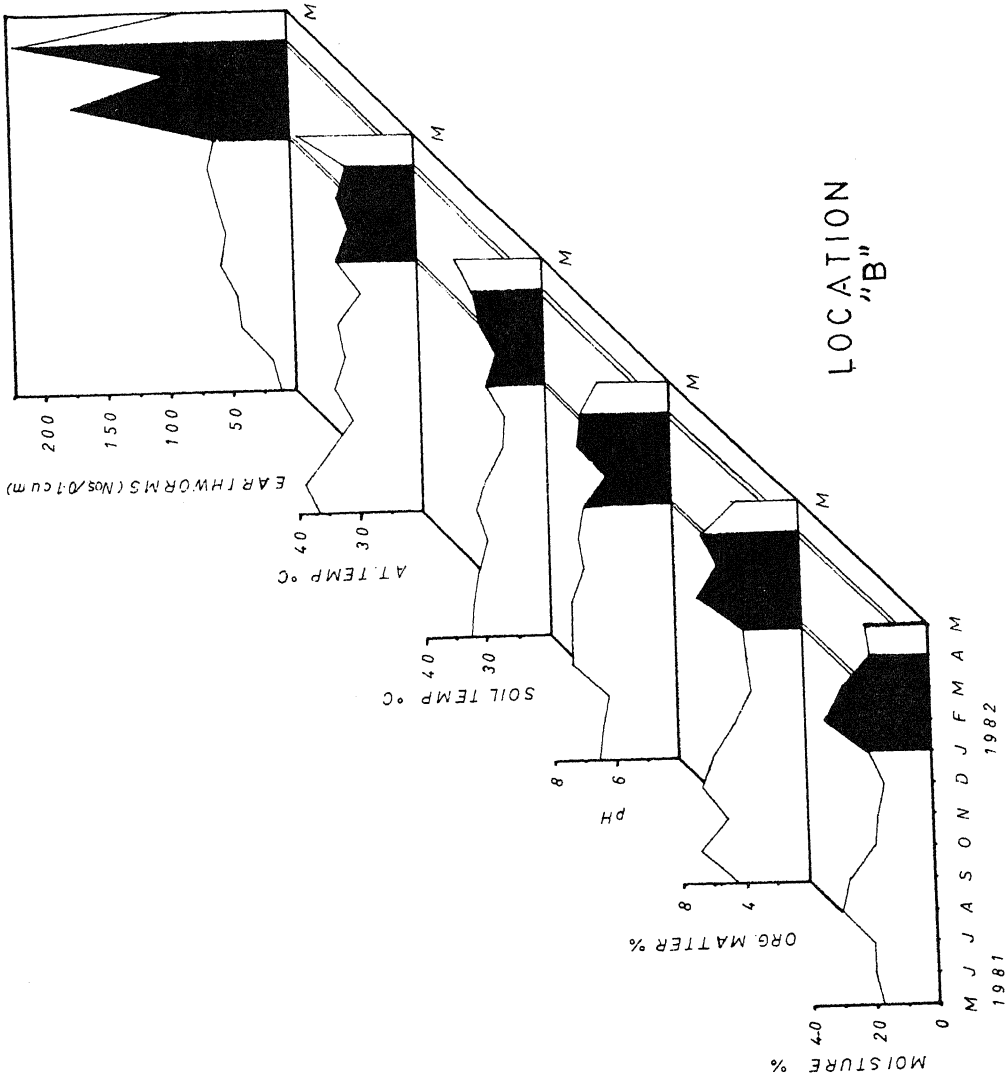

LOCATION
"A"
MOISTURE %
0
20
40
ORG. MATTER %
2
6
10
pH
6
8
SOIL TEMP °C
30
40
AT. TEMP. °C
30
40
EARTHWORMS
no s. / 0.1 Cu.m.
50
100
150
M J J A S O N D J F M A M
1981
1982

**Figure 2.**


LOCATION "B"
M J J A S O N D J F M A M
1981 1982
MOISTURE %
0 20 40
ORG. MATTER %
4 8
pH
6 8
SOIL TEMP °C
30 40
AT. TEMP °C
30 40
EARTHWORMS (Nos/0.1cu m)
50 100 150 200

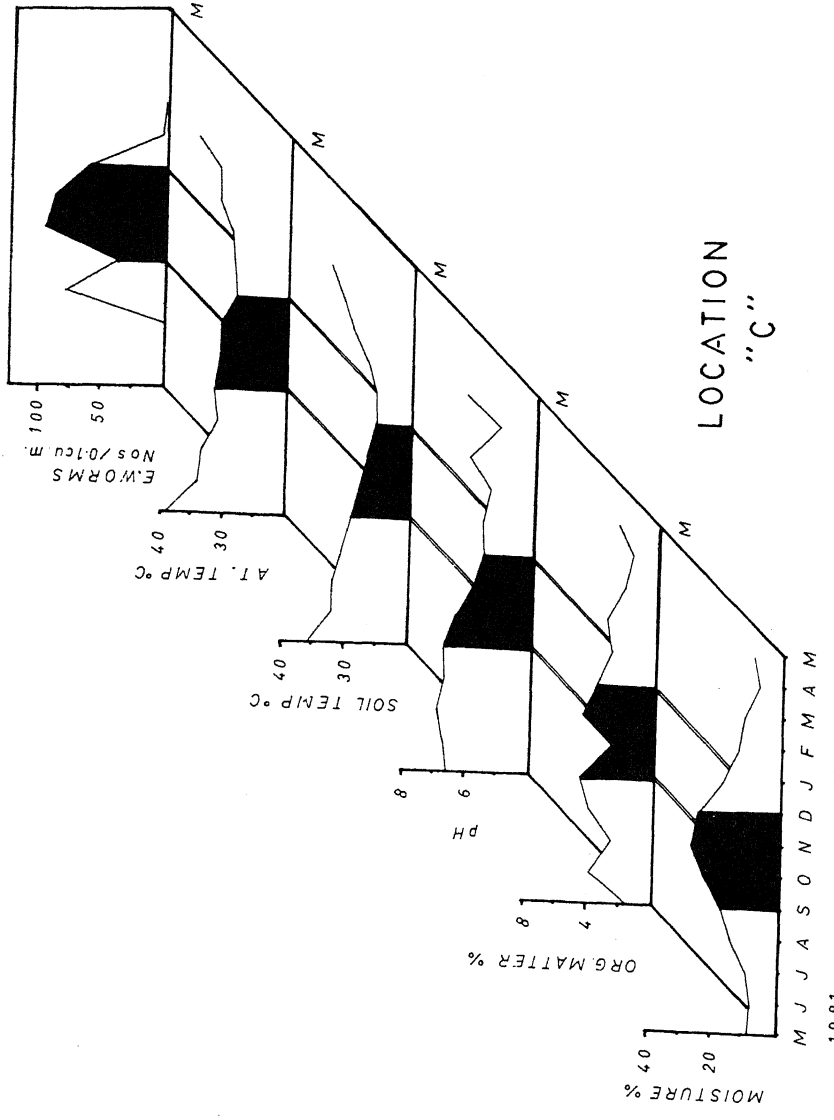

LOCATION
"C"
MOISTURE %
ORG. MATTER %
pH
SOIL TEMP °C
AT. TEMP °C
E.WORMS
Nos /0.1cu m.
M J J A S O N D J F M A M
1981
1982

**Figure 2.**

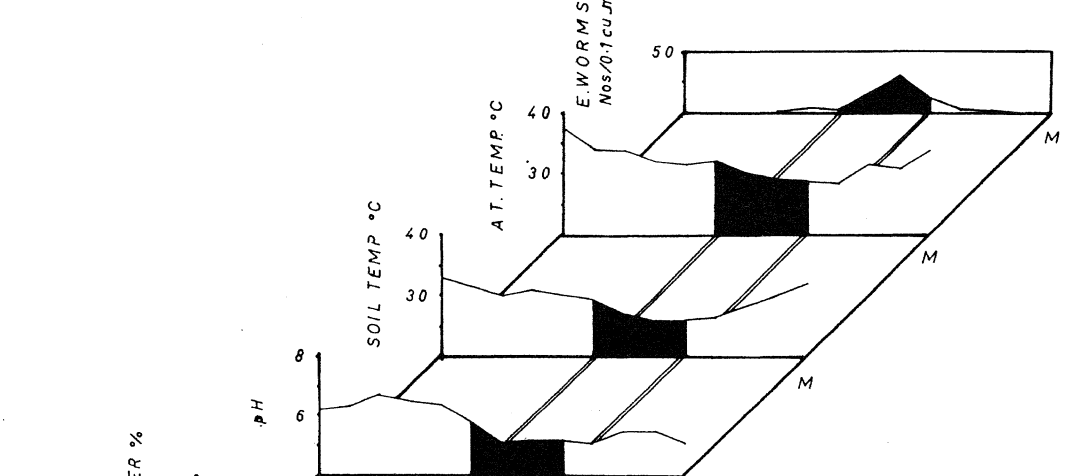



**Figure 2.** Three dimensional model for the distribution of earthworms in relation to atmospheric temperature, soil temperature, pH, organic matter, and moisture during May 1981–May 1982, for locations *A*, *B*, *C* and *D*. Parameters shaded to the peak period of earthworm distribution.

## 4. Discussion

"Earthworms, though in appearance a small and despicable link in the chain of nature, yet, if lost, would make a lamentable chasm . . . worms seem to be the great promoter of vegetation which would proceed but lamely without them, . . . " (Gilbert White of Selbourne, 1770; cf Satchell in Edwards and Lofty 1972). Much attention has been paid in recent years to the importance of the earthworms in increasing farm yields and as a source of protein for other organisms especially for fish, poultry, piggery, cattle and man.

A number of ecological parameters play a vital role in the distribution and abundance of earthworms, which may be due to the adaptability of that particular species to the soil sample studied. While *L. mauritii* inhabits the sandy loams of Ameer Mahal and neighbouring areas, *O. serrata* occurs in the clay loams of Guindy. That *L. mauritii* prefers sandy loams is evident by its presence at *E*, along with populations of *O. serrata* and *O. thurstoni*. *O. thurstoni* prefers the sandy loams of Ameer Mahal and Guindy.

Close tree canopy at *C* and *D* prevents heating of the soil surface. Soil temperature on an annual average is 31·04°C at *A*, 30·04°C at *B* and about 29°C at *C* and *D*. Location *A* being exposed to sun absorbs heat significantly faster, while at *B* vegetational ground cover as well as moisture from sewage check the effect of solar radiation. Soils at *C* and *D* are not only protected by the close tree canopy but the layer of litter also insulates the soil beneath it from getting heated up.

Temperature largely effects earthworm activity. Compared to temperate conditions tropical species can withstand higher temperatures. *L. mauritii* is available at *B* throughout the year where the annual temperature is $30 \pm 2$°C. At *C* population of *O. serrata* were available between 27 and 28°C. Occurrence of the tropical species of earthworms at higher temperatures has been reported for *Hyperiodrilus africanus* (Madge 1969).

Moisture is another limiting factor for earthworm distribution as water constitutes a major portion of the body weight of an earthworm (Grant 1955). Soil moisture *vs* population estimates are positively correlated at *A*, *B*, *C* and *D* being significant ($P < 0{\cdot}05$) at *A* and *C*. Locations *B* and *D* being closer to water bodies (*B* near sewage and *D* near lake) provide habitat to earthworms where water is not a limiting factor. *A* and *C* depend on precipitation for their content of moisture which makes the soils of these locations significantly correlated to moisture, for moisture controls earthworm activity (Barley 1959; Dash and Senapati 1980). Earthworms avoid much adverse conditions by either moving away to moist soils or by aestivating. Locations *A* and *C* are exposed to such droughty conditions annually, when their population of earthworms react suitably. *L. mauritii* and *O. serrata* are both surface dwellers. *L. mauritii* migrates either horizontally to moist soils or vertically to greater depths, while *O. serrata* undergoes diapause by encircling itself. *L. mauritii* during dry seasons were traced at *A* from depths of 80 to 105 cm, when their population within the quadrat was zero. The most interesting recording is of *O. serrata* which had zero population within the quadrat during July showed itself during August (*ca.* 77/0·1 $m^3$) in a quiescent state. September showed an activity of these worms which resulted in their dispersal. However, the population of *O. serrata* at *C* decreased by January in the field.

Occurrence of active *O. serrata* clitellate and non-clitellate at *E* during January–February, when the worms at *C* get prepared for the summer diapause, explains diapause to be facultative.

During August 1982 a large number of *O. serrata* in a quiescent stage were again observed. Each cell or "house" that harboured quiescent earthworms contained only one clitellate form, or two clitellates, or one clitellate and two non-clitellates, or just two or more non-clitellates. This stage which is the commencing point for dispersal can be termed as the quiescent stage and the stage during January–February when dry conditions commence as the diapause. Diapause in *O. serrata* commences when the temperature is about 28°C and soil moisture is 12–14 %, these values being almost similar to those reported by Dash and Senapati (1980) for *Octochaetona surensis.*

Though the earthworm has no significant correlation with soil porosity and water holding capacity of soils, they to a large extent alter these factors by their tunnelling activity (Guild 1955). This is reflected at *C* where soil porosity is high compared to *D*, where earthworm activity is negligible.

Organic matter in soils greatly influences the distribution of earthworms; soils with low organic matter do not support earthworm populations. Organic matter is comparatively high at *A* followed by *B* and *C*, and *D* having the least content of organic matter (1·79 %). Location *A* gets its high percentage of organic matter from the closely deposited dung heaps, while *B* gets it from the sewage. Location *C* draws organic matter through the litter of *Cassia* sp. while *D* through the litter of *S. jambolanum.* Location *D* shows a mat of undisturbed organic matter on the soil surface which is also an indication of a poor population of earthworms in this location. Such mats of undisturbed organic matter occur in both woodlands (Richardson 1938) and grassland (Raw 1962) where earthworms are scanty.

Location *A* which is enriched with organic manure, at an optimal condition of temperature and moisture supports a high density and diversity of earthworm populations (120/0·1 cu m). Species recorded here include *L. mauritii, D. modesta, R. pachpaharensis, O. pattoni* and *O. thurstoni.* Plots with organic manure are known to support higher populations of earthworms (Satchell 1955) as food supply appears to be the major factor for earthworm distribution (Abbott and Parker 1980).

Hydrogen ion concentration of soils is neutral at *A* and *B* and medium acidic at *C* and *D*. *L. mauritii* occurs in large numbers at *A* and *B*, where the soil is a neutral sandy loam. *O. serrata* dominates the medium acidic clay loams. Of *C* and *D*, the pH at *D* tends to be more towards the acidic range, which also probably limits the distribution of earthworms in this location. Though species of earthworms may be acid tolerant (Guild 1951; Satchell 1955; Leger and Millette 1979) earthworms normally prefer a neutral pH (Arrhenius 1921; Salisbury 1925; Bodenheimer 1935; Petrov 1946; Magdoom 1984).

Peak of earthworm distribution is during November at *A* (120/0·1 $m^3$; *L. mauritii* in November: 40/0·1 $m^3$), April at *B* (221/0·1 $m^3$; *L. mauritii* in February: 156/0·1 $m^3$), November at *C* (*O. serrata*—86/0·1 $m^3$), December at *D* (30/0·1 $m^3$, *O. serrata* in November: 12/0·1 $m^3$) (figure 2). Availability of earthworms at *A*, *C* and *D* coincides with the peak rainfall of 305·5 mm during October–November 1981.

A review of the four locations *A*, *B*, *C* and *D* investigated show variations with reference to physical and chemical parameters, each location having limiting factors which strongly govern the density and diversity of earthworm populations in that region; they being temperature at *A* and *B*, moisture at *A* and *C*, and soil texture at *D*.

## Acknowledgements

The authors thank the Prince of Arcot and the Wild Life Warden, Madras for permission to conduct ecological studies at Ameer Mahal and the Deer Sanctuary respectively. Thanks are due to Mr E G Easton, British Museum Natural History, London, for identifying/confirming earthworm species.

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Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94, No. 5, October 1985, pp. 567–572.


# Alkaline protease in the midgut of the silkworm *Bombyx mori* L: changes during metamorphosis

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**Abstract.** The protease activity in the midgut of bivoltine and multivoltine races of the silkworm, *Bombyx mori*, was studied. The enzyme activity increased during fifth instar, reached a peak, and decreased significantly through pre-pupal stage to the lowest level at the pupal stage. During pharate adult period, the protease activity increased to reach another peak just before emergence of the moth, and decreased thereafter. The enzyme activity in bivoltines was about 2–3 times higher than multivoltines at the peak levels. Bivoltines showed a sex difference in midgut protease activity while no significant difference was observed in multivoltines. Larvae and pharate adults showed a difference in the pH optima for the enzyme activity. From the results the possible role of midgut protease during the process of metamorphosis is discussed.

**Keywords.** *Bombyx mori*; bivoltine; multivoltine; midgut protease; metamorphosis.

## 1. Introduction

The most common proteolytic enzymes in the alimentary canal of insects have long been recognised as trypsin-like enzymes (Day and Waterhouse 1953; House 1974; Law *et al* 1977; Jany *et al* 1978). Eguchi and Iwamoto (1982) have also established that midgut protease, the activity of which has been earlier demonstrated in the alimentary canal of the larva of the silkworm, *Bombyx mori* (Horie *et al* 1963; Eguchi and Yoshitake 1967; Hamano and Mukaiyama 1970; Eguchi and Iwamoto 1976) is a trypsin-like enzyme possessing certain properties (Eguchi and Arai 1983). However, most of the studies on the midgut protease of silkworms are restricted to the larval stage mainly because of its important role in protein digestion. Eventhough Eguchi *et al* (1972) have studied the proteolytic enzymes in pharate adults, very little information is available on the pattern of change in total protease activity during larval-pupal-adult transformations in silkworms. It has already been reported that the trypsin-like enzyme in mosquitoes changes significantly during metamorphosis (Chen 1978). The present communication deals with the pattern of change in total protease activity of the midgut, a comparison of the enzyme activity in different races and sexes and the pH optima for midgut protease at different stages of metamorphosis.

## 2. Materials and methods

### 2.1 *Animals*

Bivoltine ($NB_{18}$ and $NB_4D_2$) and multivoltine (Pure Mysore) silkworm races were maintained under standard laboratory conditions at 25–28°C and a relative humidity

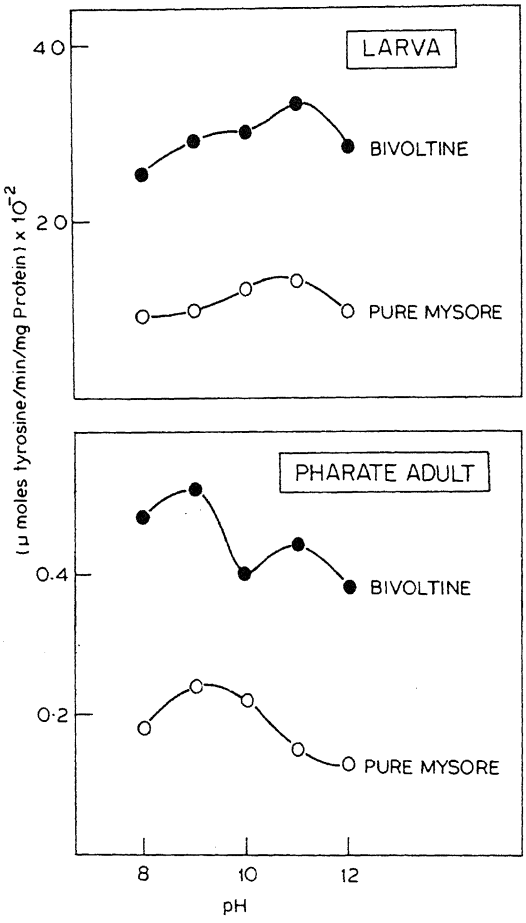


**Figure 3.** Effect of pH on the alkaline protease of the midgut.

bivoltines was about 2·5 times higher than that of multivoltine. During pharate adult stage, the bivoltines interestingly showed two peaks of enzyme activity at pH 9 and pH 11. But in case of pure Mysore only one peak was observed at pH 9. The enzyme activity in bivoltines was about 2·5 times higher than multivoltines during pharate adult stage.

## 4. Discussion

Midgut enzymes in insects are synthesized in the midgut epithelium and secreted into the gut lumen (Engelmann 1969; Engelmann and Geraerts 1980; Eguchi and Arai 1983). Thus changes in total protease activity of the midgut reflect changes in the overall production of the enzyme during metamorphosis. A significantly high activity of midgut protease during fifth instar larval development of silkworms shows a higher rate of enzyme synthesis corresponding to enhanced food intake (Waldbauer 1968). This might facilitate a greater utilization of proteins for larval growth and silk

production as well. Higher protease activity in bivoltines might result in better conversion of exogenous proteins which ultimately lead to production of more silk compared to multivoltines (Tanaka 1964). The sharp decrease in midgut protease activity from larval to pupal stage shows the difference between active feeding and non-feeding stages during metamorphosis. Thus it can be presumed that the rate of synthesis of midgut protease is dependent upon the rate of food intake. Furthermore, traces of protease activity during pupal stage might be the residual larval enzymes. In pharate adults, protease activity increases and reaches another peak just before emergence. This is in accordance with earlier reports (Eguchi *et al* 1972) and supports their view that midgut protease in pharate adult might contribute either in part or full towards the formation of cocoonase.

The present study shows that the optimum pH for total protease activity of the midgut is around pH 11·0 in the larval stage. Eguchi and Iwamoto (1976) have shown that the pH optima for protease from midgut tissue and digestive fluid are the same. Interestingly a shift in pH optima for protease activity is observed during metamorphosis from pH 11·0 at larval stage to pH 9·0 at pharate adult stage. It cannot be confirmed at present whether the larval protease is different from adult protease or whether they are two isoenzymes of protease active at different stages of metamorphosis. The presence of two peaks, each at pH 9·0 and pH 11·0, in midgut protease activity of bivoltine pharate adult, likewise, again provides a basis to speculate the existence of two isoenzymes of protease having different pH optima characteristics. However, the single peak at pH 9·0, in the enzyme activity of multivoltine pharate adult requires further studies to draw any conclusion.

## Acknowledgement

Grateful acknowledgements are due to the World Bank for financial assistance and to the co-ordinator, R & D Programme in Sericulture, Bangalore University for facilities.

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**Foreword**

This issue brings together contributions of five speakers who made presentations at a special symposium on "Animal communication" held on the occasion of the Golden Jubilee celebrations of the Indian Academy of Sciences on 6–8 February 1985. We are grateful to the speakers who readily agreed to have the papers based on their presentations published together in a special issue of the Proceedings of the Indian Academy of Sciences (Animal Sciences) and to the many organizations which so generously supported this meeting.

Madhav Gadgil
Convener, Symposium on
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# On the communication of well-being

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**Abstract.** The form that any communicatory exchange takes would depend on the extent to which the interests of the signaller and the recipient are at variance. Where such interests coincide, i.e. in cases of mutualism, the signals may be conspicuous when an immediate response is favoured, but rather subtle and variable otherwise. Over 80% of the events of tactile communication that we have noted in our study of the social behaviour of free ranging groups of tame elephants appear to belong to this latter category. On Smith's standard classification, they can only be classified as 'associative', related to remaining in the company of another individual. However, such signals are commoner by a factor of 20–100 amongst elephant calves and their mothers and allomothers when compared to exchanges between adult cows. We suggest that the function of these signals is mutual monitoring of the state of well being amongst related individuals. The considerable degree of altruistic behaviour displayed in social groups, such as those of elephants is now believed to subserve the function of enhancing the inclusive fitness of the individuals concerned. We explore a mathematical model of exchange of social aid which suggests that animals in social groups may enhance their inclusive fitness further by adjusting the amount of social aid exchanged in relation to the state of well being of the donor as well as the recipient. Our model further suggests that optimal social aid depends on the state of well being in a complex fashion making it difficult for the recipient to deceive the donor so as to extract more aid. We therefore expect that by and large honest communication of the state of well being would be characteristic of the higher social animals. Such communication would be based on normal physiological changes consequent on a change in well being. Thus animals with a superior degree of well being would take postures conducive to greater activity, would be more receptive to sensory inputs and may also shift the balance of production of various metabolites. This monitoring of the well being has greatly advanced in the human species and may be at the base of the elaborate health care amongst human societies.

**Keywords.** Communication; well-being; kin selection; elephants.

## 1. Introduction

Animals, whether they be lowly soil amoebae or highly evolved elephants, are creatures on the move. For them success depends on being alert to what is happening around them. They have therefore evolved a variety of sense organs to receive signals of relevance to their own welfare from the environment. Such signals may be picked up opportunistically, regardless of the interests of the signaller. Thus many species of soil amoebae possess on their body surface receptors for derivatives of folic acid. These chemical compounds are involved in the biosynthesis of purines and pyrimidines, the basic building blocks of nucleic acids that are essential for every living organism. It turns out that they leak out of the bodies of bacteria grazed on by soil amoebae. Soil amoebae then employ the folic acid derivatives as signals to locate their prey, moving up the gradient of concentration. This is thus an example of interception of a signal against

the interest of the signaller. In another paper in this issue Stanley Rand will add another, that of predatory bats homing on to male frogs calling to keep out the other males and to attract the females.

## 2. Eavesdropping

In general, we can classify signals into 4 categories depending on whether the signaller benefits or suffers from the recipient receiving it, and on whether the recipient benefits or suffers from acting on it (Wiley 1983).

| | | Signaller | |
|---|---|---|---|
| Recipient | | Benefits | Suffers |
| | Benefits | Mutualism | Eavesdropping |
| | Suffers | Deceit | Spite |

The genetic interests of the predatory soil amoebae and their bacterial prey are obviously divergent, making the use of folic acid derivatives to locate them an example of eavesdropping. But two of the species of soil amoebae *Dictyolstelium lacteum* and *D. minutum* use folic acid derivatives as communicatory signals in quite another context—to attract each other. These soil amoebae multiply by binary fission, feeding on bacteria in their vegetative phase. When the food supply runs out, members of a clone come together to form a many celled creature called the slug. Some cells of this slug forego reproduction to form a stalk, while other cells turn into spores which disperse and may enter again into the vegetative phase if they encounter a favourable environment.

In terms of our classification above, some of the soil amoebae that are thus attracted by the signal suffer, since they forego their chances of reproduction to form the stalk, while others benefit, since they form spores which retain the possibility of future reproduction. In the process of aggregation of soil amoebae, all cells produce the signal and all cells receive it. Is this communicatory episode then an example of deceit by future spore cells signalling the future stalk cells, or eavesdropping on the signal of future stalk cells by the future spore cells?

## 3. Altruism

A closer look suggests that in fact it is neither. The soil amoebae attracting each other are all members of a clone, and therefore genetically identical. The assumption of the role of stalk cells by some of the amoebae is an example of altruistic behaviour through kin selection. As Hamilton (1964) has shown, natural selection will favour such behaviour so long as:

$$\text{Cost to the altruist} < \text{Benefit to the recipient } x \text{ kinship coefficient}$$

where the kinship coefficient is the proportion of genes shared by virtue of common ancestry. Members of a soil amoebae clone will have a kinship coefficient of 1. Suppose now that a proportion $x$ of them sacrifices a chance to reproduce by forming a stalk; leaving $(1-x)$ to reproduce. Then this behaviour would be favoured if raising on a stalk improves the chance of survival of a spore by a factor greater than $\frac{1}{1-x}$.

There are good reasons to believe that this is in fact so. In that case the genetic interests of all the soil amoebae that are attracted to each other by signals employing folic acid derivatives are served by producing and responding to the signal. This is then an example of mutualism in our classification of communicatory systems. Vidyanand Nanjundiah discusses these fascinating organisms in greater detail in another paper in this issue.

## 4. Information and manipulation

We have thus far looked at two extremes—total divergence of genetic interests as with bacteria and their predators, and total congruence of genetic interests as with members of a clone of soil amoebae. There would however be many examples of only a partial congruence of genetic interests, resulting in a rich structure of animal communication (Dawkins and Krebs 1978; Krebs and Dawkins 1984).

Consider for a moment the call of male frogs. It is in the interest of female frogs to locate a mate, preferably a mate that will contribute qualities that will help the offspring succeed in life. It is likely that the biggest available male of their own species will be their best choice. It will therefore be in the interest of the females to be able to derive information on the size of the calling male from its call. On the contrary, it will be in the genetic interest of every male to misinform the females, conveying that the signaller is much bigger than in fact it is. We could then consider the information being transmitted during any communicatory exchange under 3 components:

| | Signaller | |
|---|---|---|
| True information sought to be transmitted in mutual interest | True information sought to be suppressed | False information sought to be transmitted |
| $\alpha$ | $\beta$ | $\gamma$ |
| True information sought to be received in mutual interest | True information sought to be received against the interest of signaller | False information sought to be discounted in interest of the recipient |
| | Recipient | |

Any communicatory exchange could be characterized usefully by the relative values of $\alpha, \beta$ and $\gamma$. In the case of bacteria and amoebae $\beta$ dominates, the bacteria would rather produce no signal if it were metabolically easily possible; while $\alpha$ dominates in case of members of a clone of soil amoebae attracting each other. With male frogs calling to attract females, $\gamma$ must assume significance with males evolutionarily favoured to convey an exaggerated impression of their size and females favoured to discount any

such bluff. As Wiley (1983) and Krebs and Dawkins (1984) have argued this should lead to the evolution of rather stereotyped, repetitive and unbluffable signals. These are the conspicuous displays that have so attracted students of animal behaviour.

## 5. Kinship discrimination

It appears reasonable to conclude that signals would tend to be conspicuous because of their stereotype and repetition when $\gamma$ is significant, and rather inconspicuous when $\beta$ dominates. In the context of mutualism, when $\alpha$ dominates, they may be conspicuous when immediate response is favoured, as with soil amoebae attracting each other. In other mutualistic contexts, however, the signals are expected to be rather subtle and variable and therefore escape the attention of the ethologist (Krebs and Dawkins 1984). One such context is that of discrimination of kinship level. This could be important in social animals, for as J B S Haldane is supposed to have said—he would be evolutionarily favoured to lay down his life to save two full brothers from drowning; but if it were only first cousins he must save at least 8 of them for this to make genetic sense. This is because while full brothers have a kinship coefficient of 1/2, first cousins share only 1/8 of their genes by virtue of common ancestry.

Discrimination of levels of kinship should therefore be important in social animals. But it may be based on very subtle and variable cues and it is only now that we are finding out that in some social insects individuals can discriminate kinship levels based on chemical signals, even in the absence of individual recognition. This is a topic that will be reviewed in detail in another paper in this issue by Raghavendra Gadagkar.

Amongst higher animals such as mammals on the other hand, sociality is based on individual recognition. Many mammals use chemical signals in the form of ratios of several compounds for this purpose. This individual recognition coupled with ties established from birth onwards between the mother and the young must permit a fine tuning of social behaviour in higher mammals such as elephants.

## 6. Elephant societies

Along with Dr P Vijayakumaran Nair of Kerala Forest Research Institute, we have investigated the structure of social behaviour in the Asiatic Elephant (*Elephas maximus*). For this purpose we used tame elephants maintained in elephant camps in South Indian forests. All the adults were caught from the wild in the same or nearby forests and the tame elephants were left for grazing in their natural habitat. These tame elephants often mingled with wild herds when thus left free. In fact all the calves born in captivity were sired by wild tuskers. We observed these elephants over a period of 23 months between February 1975 to March 1976 and January 1978 to August 1979. The total amount of time spent in the field recording behavioural details spanned 645 hr. Our parallel, but less detailed observations on the wild elephants confirmed that the behaviour patterns within the artificially constituted social groups of tame elephants closely resembled those of wild elephants. In particular, adult females reacted to calves of other females as did wild females to calves of other females in their herd. Under natural conditions, of course, the several adult females in a single herd are related to each other as mother-daughter-half-sister-aunts-cousins (Douglas Hamilton 1972).

The glue that holds the elephant society together is evidently the protection and nurture of calves. These herbivores would only reduce their feeding efficiency by being together; and the adults can resist any predation by themselves. The calves, however, are still susceptible to predation, and in fact an elephant calf fell prey to a tiger in Bandipur during our study period. Adult female elephants therefore stay together to help protect each other's calves, which under natural conditions have kinship coefficients of 1/2–1/16 with the adult females. The adult males wander from herd to herd and can have little clue as to which calves they have sired. As expected, they play no role in helping the calves.

The social group of elephants therefore comprises related adult females with their young sons, daughters, cousins, nephews and nieces. The calves station themselves in between adult females who run to them on slightest alarm. The adult females stand guard over the calves when the latter sleep, and also suckle them if they have no suckling calves of their own. Elephants are thus model aunts or allomothers. The role of calves in holding these females together was strikingly brought out when the only calf with 3 adult females of Bandipur Tiger Reserve was removed for weaning. As soon as the calf was gone, the cows started grazing separately by themselves (Gadgil and Nair 1984).

## 7. Communication amongst elephants

Elephants have poor vision and do not seem to base much of their social communication on visual signals. We have little information on the extent to which they depend on chemical signals except that they seem to base individual recognition on it. They do use vocal signals, especially to communicate alarm and aggression. Tactile communication however seems to be the dominant mode of communication, especially where the calves are involved. These tactile contacts are largely initiated by the calf towards mother or allomother. Thus in a group of 3 adult females and a calf of less than 6 months of age in Bandipur Tiger Reserve the calf touched the mother and one of the allomothers at a rate of about 4 times in 10 min while it touched a second allomother at a lower rate of once in 10 min. The mother touched the calf at the rate of once in 7 min, the first allomother did so at the rate of once in 20 min, while the second allomother touched the calf at the rate of once in 50 min. The adults touched each other at low rates ranging from once in 50 to once in 300 min.

A question of considerable significance is the function of the different acts of communication. In some cases the function is evident as when a calf follows a contact by suckling or rushes to the mother when the latter sounds an alarm call. A remarkable result however is that in the vast majority of cases, of the order of 80 %, no such clear cut immediate function can be attached to a communicatory exchange. The intriguing question therefore is this: what are the adult elephant, cows and calves conveying to each other most of the time?

I believe that our failure to assign any obvious function to the bulk of these communicatory exchanges is due to the fact that it does not subserve any immediate need. Consequently, in this mutualistic system the signals, as expected, are inconspicuous and variable. On Smith's (1977) standard classification they can be only classified as 'associative', related to remaining in the company of another individual. But then elephant females remain in company with each other often for their whole lives.

Nevertheless the adult females have a far lower frequency of tactile communication amongst themselves – by a factor of 20–100 than females and calves.

## 8. Monitoring well-being

We therefore suggest that there is an additional function of communication in case of higher social animals like the elephants that has not so far been clearly identified; this is the monitoring of the state of well-being of the young by related adults.

To understand why such communication of well-being could be favoured during the course of evolution, we have to go back to Hamilton's inequality:
An altruistic act will be favoured if:

Cost to altruistic donor <
Benefit to recipient $x$ kinship coefficient between donor and recipient.

A superficial examination of this statement suggests the following paradox: the kinship coefficient between a mother and her off-spring and between two full sisters is the same, namely 1/2. Nevertheless, a female mammal displays much greater level of altruistic behaviour towards her offsprings than towards her sisters, or towards her mother. The resolution of this paradox lies in recognising that the costs and benefits of a given altruistic act will differ substantially depending on the identity of the actors involved. These benefits and costs must be measured as marginal changes in the reproductive value of an individual as a result of a given social act. Since social aid will often make greater difference to the chances of survival and future reproduction of a young offspring than that of a grown sister or an old mother, a female mammal will be generally favoured by natural selection to behave far more altruistically towards her offspring rather than a sister or a mother. This need not always hold, of course, and one needs a better defined model to explore this proposition further. Such a model cannot of course reflect all the complexities of the real world; that would be too cumbersome to handle. Rather, the model we develop should be rich enough to reflect the essentials but simple enough to handle and be interpretable.

## 9. Modelling social interactions

In such a model the physiological status of the interacting individuals could be specified by 3 parameters: the ability to convert resources into somatic or reproductive growth, $\alpha$, the cost of maintenance, $\beta$ and the extent to which physical growth has been completed and independent abilities to gather resources achieved $S$. The extent of favourability of environment is reflected in a parameter $F$. Let us assume that each individual $i$ has at its disposal some resources taken to be proportional to $S_iF$ for maintenance and growth. Of these it retains a fraction $\phi_{ii}$ for its own use and donates a fraction $\phi_{ij}$ to the $j$th individual. Then the total amount of resources available to it, $\theta_i$, is given by

$$\theta_i = \sum_j S_j F \phi_{ji}$$

The marginal change in fitness or the reproductive value of the $i$th individual, $\Delta W_i$

depends on the change in $S_i$, i.e. $\Delta S_i$. We take:

$$\Delta S_i = S_i(\alpha(1-S_i)(1-e^{-\theta_i/S_i\beta_i}) - \beta_i e^{-\theta_i/\beta_i S_i})$$

and $W_i$ is given by:

$$W_i = 1 - e^{-3(S_i+\Delta S_i)}.$$

Hamilton's (1964) extension of the genetical theory of natural selection tells us that each individual will be so programmed as to maximise its inclusive fitness

$$H_i = \sum_j W_j \gamma_{ij}$$

where $\gamma_{ij}$ is the kinship coefficient between $i$ and $j$.

We can therefore determine for any given $\alpha_i, \beta_i, S_i, F$ values the $\phi_{ij}$ that will tend to maximize the inclusive fitness of each individual involved. The problem is made complex by the fact that the optimal allocation of resources for an individual $i$ depends on the allocation strategies adopted by other individuals. In such an interactive population, evidently the allocation strategy $\phi_{ij}$ of the $i$th individual will be such that the inclusive fitness of this individual is maximum for the existing strategies $\phi_{kj}(k \neq i)$ of other individuals. We know that such a point exists, and have a working algorithm for determining this matrix for the case of two interacting individuals, say the mother(1) and the offspring(2).

In this simple case of two individuals, it can be shown that $\phi_{ii}$ is always 1 for atleast one of the two individuals; i.e., one of the individuals may be a donor and the other the recipient keeping all of its own resources to itself, or both may keep all their resources to themselves. We naturally identify the donor in our model with the mother(1), and the recipient with the offspring(2). Our problem then is to determine the optimal level of maternal investment, i.e., social aid $\phi_{12}$ from the mother to the offspring.

## 10. Optimal maternal investment

There are two immediately interesting results of our model. The first is that optimal maternal investment is much more sensitive in variation to the offspring's cost of maintenance $\beta_2$, extent of development completed $S_2$, and the extent of favourability of the environment $F$, than it is to efficiency of growth $\alpha_2$ (figure 1). This result is probably related to the fact that the first 3 parameters appear in the exponential term of our specific model. Secondly, we find that the optimal level of maternal investment need not vary monotonically with the value of variables specifying offspring's state. That is to say the mother will not necessarily provide more and more maternal care as offspring's apparent needs increase. This is because the optimal level of maternal investment depends on the extent to which an offspring can enhance its reproductive value by receiving such help. An offspring not in much need can make little use of aid; at the same time, an offspring too much in need may also be a bad investment. We are reminded of Kafka's story 'Metamorphosis' in which the son of the family is turned into a cockroach. Initially, while there is hope that he will turn back into man much help is lavished on him. But as time goes on and he remains an insect, he is neglected and ultimately allowed to die. There will thus be an intermediate level of need by offspring

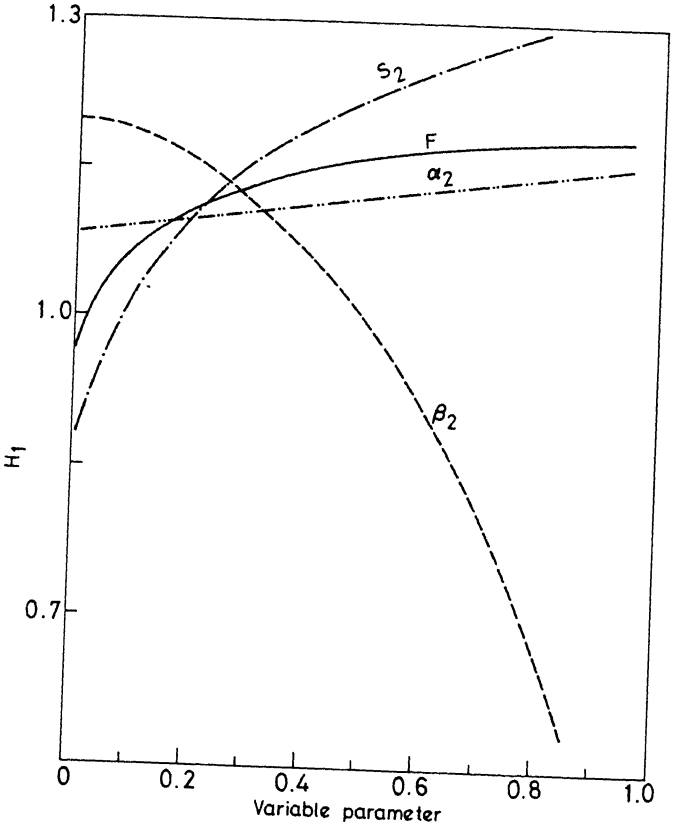


**Figure 1.** Inclusive fitness of mother ($H_1$) as a function of variation in $\alpha_2$, $\beta_2$ $S_2$ or $F$ varying one parameter at a time. The fixed values of other parameters are taken to be $\alpha_1 = 0{\cdot}6, \alpha_2 = 0{\cdot}1$, $\beta_1 = 0{\cdot}4$, $\beta_2 = 0{\cdot}4$, $S_1 = 1$, $S_2 = 0{\cdot}15$, $F = 0{\cdot}1$.

which will attract maximal investment. This level will also depend on mother's condition (figures 2 and 3).

What are the implications of these results for our central theme, the value of communication of well-being? A mother can be programmed to fix the level of maternal investment at the value which will maximize her inclusive fitness, if information on the offspring's state is available to her. In the absence of such information, she will either make too much or too little maternal investment and thereby suffer a decline in her inclusive fitness. Our model shows that the availability of information relating to the offspring's well-being can indeed make a significant difference in mother's inclusive fitness. Mothers of highly social species and with a nervous system developed enough to make possible fine adjustments in maternal care should therefore be favoured evolutionarily to look for information on the well-being of her offspring.

## 11. Mother-infant conflict

Trivers (1974) points to a very intriguing complication that must arise in this context due to the fact that since an offspring shares only 1/2 of its genes with its mother, its

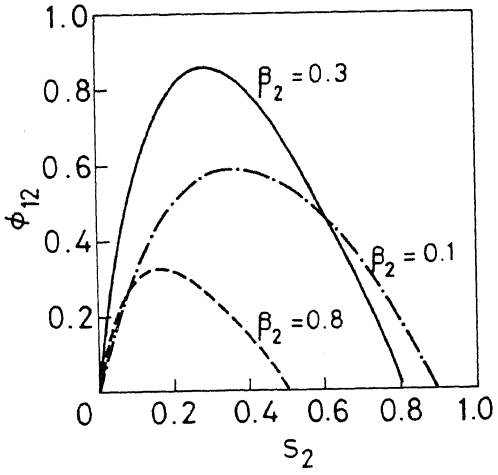


**Figure 2.** Optimal maternal investment, $\phi_{12}$ as a function of $S_2$ for different values of $\beta_2$. The values of other parameters are $\alpha_1 = 0{\cdot}6$, $\alpha_2 = 0{\cdot}6$, $\beta_1 = 0{\cdot}4$, $S_1 = 1$, $F = 0.1$.

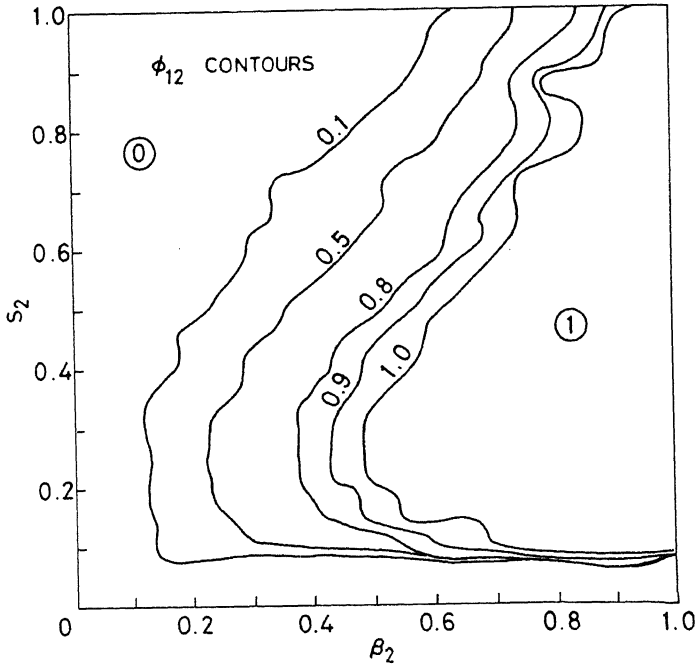


**Figure 3.** Contours of optimal maternal investment $\phi_{12}$ as a function of $S_2$ and $\beta_2$. The values of other parameters are $\alpha_1 = 0{\cdot}6$, $\alpha_2 = 0{\cdot}6$, $\beta_1 = 0{\cdot}4$, $S_1 = 1$ and $F = 0{\cdot}1$.

genetic interests will diverge, though within limits, from those of its mother. It would then be expected to try to extract a higher level of maternal investment $\phi_{12}$, then the mother would be selected to offer. Hence the weaning conflict in mammals with the

mother attempting to stop suckling and the offspring attempting to continue doing so at a certain age. In context of the problem of our interest, the offspring can gain in its inclusive fitness by miscommunicating to the mother the value of its well-being so as to extract a higher level of $\phi_{12}$. Our model has a very interesting result bearing on this issue, namely that optimal $\phi_{12}$ that the mother would be programmed to adopt does not change in a simple fashion with parameters specifying the offspring's condition (figures 2 and 3). That is, a mother will not always increase her level of maternal investment if the offspring's condition is better or worse. Under certain conditions she will increase it, under others decrease it. There is therefore no simple strategy available to the offspring of misinforming its mother of its own level of well-being so as to prompt her to enhance the level of maternal investment. Hence natural selection would tend to disfavour any manipulation of information relating to its own state from the offspring to its mother (figure 4).

Our enquiry thus suggests that atleast in higher animals with a well developed nervous system and high levels of maternal care such as elephants and human beings, communication of well-being must be a significant component of social communication.

## 12. Communication of well-being

We began by noting that soil amoebae use folic acid derivatives which are normal products of bacterial metabolism as signals to locate their prey, and further that these have been elaborated to serve as signals for aggregation in some species of soil amoebae. Evolution has thus opportunistically siezed upon normal physiology of animals from which to elaborate communicatory signals. Signals communicating well-being must

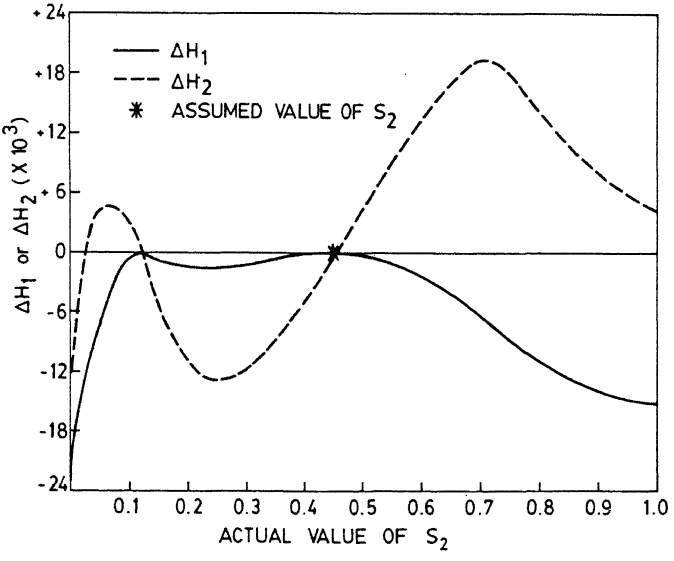


**Figure 4.** Change in inclusive fitness of mother ($\Delta H_1$) or offspring ($\Delta H_2$) when optimal maternal investment is based assuming $S_2$ to be 0·45, but is in fact different. The values of other parameters are $\alpha_1 = 0{\cdot}6$, $\alpha_2 = 0{\cdot}6$, $\beta_1 = 0{\cdot}4$, $S_1 = 1$, $F = 0{\cdot}1$.

similarly relate to normal physiological changes in an offspring brought about by a change in its state of well being. An animal must adjust its metabolism, activity level and behavioural patterns to its level of well being, though this problem does not appear to have been specifically investigated with this view point. Coming back to the elephants, Dr V Krishnamurthy of Tamilnadu Forest Department who has handled elephants for 30 years as a Veterinarian, tells us that a male elephant will come into musth only if he is given light work and fed well and is in excellent physical condition. A male elephant's physiology thus shifts into this mode only when its state of well-being is high. There must be other chemical substances that an elephant calf, say, will start producing in large quantities only when it is in good health; shifting to others as its health declines. An elephant mother may continually monitor the state of well being of its offsprings through monitoring such chemical signals.

The state of well being of an individual will also be reflected in its activity level. Andrew (1972) talks of an exertion/immobility continuum along which an individual mammal may be placed. We expect it to move towards greater exertion with an improvement in its state of well being. This will be reflected in its posture; thus in horses being more active leads to a high postural tonus with a raising of the tail. An active mammal also tends to adopt postures which would lead to a loss of heat, an inactive one curled up postures designed to conserve heat. We may also expect more active animals to be much more receptive to sensory cues from their environment; this could be reflected in their sense organs, for instance, cocked ears and dilated pupils.

## 13. The human species

We close with some less formal observations. In the human family the mother is continually monitoring the state of well being of her offspring based on general activity level, changes in sense organs such as 'sparkle' in the eyes, and what goes under the broad title of 'moods'. Further clues are picked up if necessary by monitoring body temperature, sensations of pain, excretions and so on. Beginning with this ancient heritage, human societies with their complex social ties going beyond kinship level have erected a whole system of monitoring of health status through increasingly specialized professionals detecting increasingly subtle cues. Communication of well-being has indeed been tremendously elaborated in human societies.

## Acknowledgement

We have benefitted greatly from discussions with P Vijayakumaran Nair.

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# Kin recognition in social insects and other animals—A review of recent findings and a consideration of their relevance for the theory of kin selection

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**Abstract.** Kin selection is a widely invoked mechanism to explain the origin and evolution of social behaviour in animals. Proponents of the theory of kin selection place great emphasis on the correlation between asymmetries in genetic relatedness created by haplodiploidy and the multiple origins of eusociality in the order Hymenoptera. The fact that a female is more closely related genetically to her full sister than to her daughters makes it more profitable for a Hymenopteran female, in terms of inclusive fitness, to raise full sisters rather than daughters or full siblings with a female biased sex ratio rather than offspring. This is sometimes referred to as the haplodiploidy hypothesis. In reality however, genetic relatedness between workers in social insect colonies and the reproductive brood they rear is far below 0·75, the value expected for full sisters, often below 0·5 the value expected between mother and daughter and, not uncommonly, approaching zero. Such values are on account of queen turnover, multiple mating by queens or polygyny. This situation raises doubts regarding the haplodiploidy hypothesis unless workers can discriminate between full and half sisters and preferentially direct their altruism towards their full sisters only. This would still mean an effective coefficient of genetic relatedness of 0·75 between altruist and recipient. For this to be possible however, workers should be able to recognise their full sisters inspite of growing up with and being habituated to an assortment of full sisters, half sisters and perhaps other even less related individuals. Even outside the Hymenoptera, social animals may find themselves growing up together in the company of individuals of varying degrees of relatedness. An ability to tell apart the more and less related individuals under such circumstances should favour kin selection.

Much effort is now going into assessing the abilities of animals to discriminate between kin and non kin. In every case studied carefully so far, animals appear to be capable of recognising their kin. Ants, wasps, sweat bees, honey bees, frogs, toads, mice, rats, voles, squirrels, monkeys and even humans appear to be able to recognise their kin in one circumstance or another. An ability to recognize true genetic relatedness requires genetically specified recognition labels and these must therefore be present. Recent findings of the role of the histocompatibility system provides some clues to the possible nature of recognition labels. An ability to recognise full sisters for example, inspite of being habituated to full and half sisters requires not merely genetically specified labels but also recognition templates which are based on the characteristics of the individual animals making the recognition and not templates based on all animals one grows up with. Some animals such as honey bees, tadpoles and ground squirrels appear to have such templates but others such as sweat bees and some mice appear not to. It is entirely possible that our inability to devise natural enough assays for recognition prevents us from understanding the full potential of the kin recognition abilities of many animal species. In any case, genetically specified labels and self based templates should greatly facilitate the evolution of social behaviour by kin selection.

**Keywords.** Genetic relatedness; kin recognition; kin selection; hymenoptera; haplodiploidy; evolution of social behaviour.

## 1. Introduction

There are two main grounds for expecting that animals must be capable of distinguishing between close genetic relatives and non or distant relatives. The first has

to do with inbreeding avoidance (or for achieving an optimum balance between inbreeding and outbreeding; see Bateson 1980). On theoretical grounds it can be shown that inbreeding leads to homozygosity of recessive lethal genes resulting in inviable offspring. In conformity with this expectation inbreeding avoidance is widely observed in most animal groups. The second has to do with models for the spread of 'altruistic' alleles by natural selection. The basic idea of current models is that animals must behave altruistically towards close genetic relatives and selfishly towards non relatives. Such 'nepotistic' behaviour has again been observed in a wide variety of animals. In spite of such strong theoretical and empirical grounds, efforts to unravel animals' abilities to recognise kin (other than parent-offspring recognition) began just over 5 years ago. In this paper I will review experimental evidence of kin recognition from different animal groups, both among insects and vertebrates. Given an ability to recognise kin, any animal can potentially use it both for mate selection and for structuring altruistic and selfish interactions. I will not specifically allude to the function of kin recognition in each case.

Whether animals can discriminate between close and distant relatives inspite of being habituated to both classes of relatives is of great theoretical interest. Such an ability is essential for the tenability of a widely discussed form of kin selection theory (the haplodiploidy hypothesis) that purports to explain the evolution of insect sociality. Even outside the Hymenoptera, an ability to discriminate between close and not so close relatives within a mixed cohort or family group will greatly facilitate the operation of kin selection. This is because such an ability can raise the effective coefficients of relatedness between donor and recipient in altruistic interactions. My intention here is not so much to exhaustively review the literature on kin selection or kin recognition but to examine the consequences of our present understanding of kin recognition and its possible mechanisms to the theory of kin selection.

## 2. The theory of kin selection

The concept of 'inclusive fitness' first put forward by Hamilton (1964a, b) has promised to provide a plausible mechanism for the evolution by natural selection of altruistic behaviour in general and sterile castes and 'worker behaviour' in Hymenoptera in particular. The basic idea is a very simple one and has now come to be known as the theory of kin selection. Since organisms are ephemeral combinations of genes it is the individual alleles that form the connecting link from one generation to the next. This being the case one must be concerned with the changes in frequency of alleles *per se* in a population and not merely with the numbers of offspring produced by the bearers of the alleles in question. An allele can increase in frequency not only by programming its bearers to produce more offspring (who are likely to carry the same alleles) but also by programming them to aid genetic relatives (who too are likely to carry the same alleles) and the latter could well be at the cost of offspring production. If an individual aids $n_i$ relatives (other than offspring) who are related to it by $r_i$ at the cost of producing $n_o$ offspring who are related to it by $r_o$ then, as long as

$$n_i r_i > n_o r_o, \tag{1}$$

even sterility could evolve by natural selection (this form of eq. is from Craig 1979). (Notice that this argument rests on the assumption that the offspring given up and

relatives reared are of equal reproductive value). Eq. (1) can be rewritten as

$$\frac{n_i}{n_o} > \frac{r_o}{r_i}. \tag{2}$$

This inequality is possible either if $n_i > n_o$ (i.e., the individual is able to rear more relatives than it is capable of rearing offspring) or if $r_i > r_o$ (i.e., if the individual is more closely related to the relatives in question than to its own offspring). The possibility of rearing more relatives in a social group compared to offspring in a solitary mode of living is conceivable for any group of animals under certain severe ecological conditions. One cannot say the same thing for the other alternative, namely, closer genetic ties with relatives compared to offspring because, no other genetic relatives can be more closely related to oneself than one's own offspring who bear a coefficient of relatedness of 0·5 with their parents in any diplo-diploid system. The insect order Hymenoptera is unusual in this regard because it is not diplo-diploid.

Two facts make Hamilton's arguments particularly attractive. First, haplodiploidy, which is nearly universal in Hymenoptera but rare outside that order makes a female more closely related (coefficient of genetic relatedness, $r = 0{\cdot}75$) to her fullsister than to her daughter ($r = 0{\cdot}5$) (table 1) so that $r_i$ can potentially be greater than $r_o$. Second, eusociality, a condition characterised by overlap of generations, cooperative brood care and reproductive division of labour, has arisen at least eleven times independently in the order Hymenoptera (Wilson 1971). On the other hand eusociality has only arisen twice in the rest of the animal kingdom [namely termites and the naked mole-rat; see Jarvis (1981) for evidence of eusociality in the naked mole-rat]. In other words, haplodiploidy, which makes possible for $r_i$ to be greater than $r_o$ has been an important factor in the multiple origins of eusociality in Hymenoptera. We shall henceforth refer to this as the "haplodiploidy hypothesis". It is true of course that if Hymenopteran workers rear sisters and brothers in equal numbers in place of sons and daughters, they gain nothing as the low relatedness to brothers ($r = 0{\cdot}25$) exactly cancels out the advantage due to high relatedness to sisters. In other words average relatedness to fill-sibs $\left(r \text{ siblings} = \frac{0{\cdot}75 + 0{\cdot}25}{2} = 0{\cdot}5\right)$ is the same as relatedness to offspring ($r = 0{\cdot}5$). It has now been pointed out however that if Hymenopteran workers (who are always females) skew their investment in favour of sisters, then they would capitalise on the asymmetries in genetic relatedness created by haplodiploidy. In fact at equilibrium workers would be expected to invest in their full sisters and brothers in the ratio 3 : 1 (being the ratio of their genetic relatedness to full sisters and brothers) (Trivers and Hare 1976; for a recent review see

**Table 1.** Co-efficients of relatedness under haplodiploidy assuming complete outbreeding.

| | Daughter | Son | Sister | Brother | Mother | Father |
|---|---|---|---|---|---|---|
| Female | 0·5 | 0·5 | 0·75 / 0·25 (Av = 0·5) | | 0·5 | 0·5 |
| Male | 1·0 / 0·0 (Av = 0·5) | | 0·5 | 0·5 | 1·0 / 0·0 (Av = 0·5) | |

Joshi and Gadagkar 1985). If workers who give up the production of a certain number of offspring in fact invest in an equivalent number of siblings skewing investment in favour of sisters, average $r_i$ would be greater than $r_o$, satisfying the condition for the evolution of sterile or other altruistic behaviour by the haplodiploidy hypothesis. The current status of theory and data on evolution of social behaviour has been extensively reviewed (Hamilton 1972; West-Eberhard 1975; Wilson 1975; Starr 1979; Gadagkar 1985).

## 3. Lower than expected levels of relatedness—the evidence

The legitimacy of the haplodiploidy hypothesis outlined above is crucially linked to the demonstration that values of $r_i$ greater than $r_o$ in fact occur. Isozyme patterns revealed by electrophoresis are now routinely used to determine genotypes of individual organisms (see Lewontin 1974 for a comprehensive as well as historical introduction to this subject). In recent years a number of methods with increasing levels of sophistication have been developed to estimate levels of genetic relatedness within subgroups of a population, using electrophoretic data (Metcalf and Whitt 1977a; Lester and Selander 1981; Craig and Crozier 1979; Pamilo and Varvio-Aho 1979; Pamilo and Crozier 1982; Pamilo 1984). Many social insect species have now been subjected to such an analysis and a sample of the results (not an exhaustive list) available in the literature are presented in table 2. With few exceptions most estimates of genetic relatedness among workers or between workers and the female reproductive brood they rear are very low; almost always less than 0·75, the value expected for full sisters, often below 0·5, the value for mother and daughter and, not uncommonly the values are not significantly different from zero. Most species listed in table 2, however, are ants which are all highly eusocial. In the context of the evolution of social behaviour by kin selection our focus should naturally be on the primitively eusocial species but there have been surprisingly few attempts to estimate genetic relatedness in such species. And the few attempts that have been made are not very encouraging (table 2).

Determining the frequencies of alleles in 5 polymorphic esterase loci Metcalf and Whitt (1977a) showed that in the primitively eusocial wasp *Polistes metricus*:

(i) foundresses mate at least twice using sperm from the two males in the ratio 9 : 1;
(ii) $\alpha$ foundresses share reproduction with their subordinate $\beta$ foundresses, the former contributing 78 percent of the females and 87 percent of the males;
(iii) workers lay male eggs if foundresses die and even here one worker produces 19 times as many eggs as another. Intra-nest genetic relatedness can vary drastically depending on the fate of the foundresses.

On these criteria six different types of nests were defined and intra-nest relatedness was calculated for each nest type: (a) solitary foundress alive, (b) solitary foundress dead, (c) $\alpha$ and $\beta$ foundresses alive, (d) $\alpha$ foundress alive, $\beta$ dead, (e) $\alpha$ foundress dead, $\beta$ alive and (f) $\alpha$ and $\beta$ foundresses both dead. Using the data provided by Metcalf and Whitt (1977a, b), Lester and Selander (1981) have calculated an average relatedness of 0·63 between a worker and her female reproductive siblings. Of the three studies pertaining to primitively eusocial wasps listed in table 2, this is in fact the only case where $r_i$ is greater than $r_o$. In *P. exclamans* and *P. apachus-bellicosus*, respectively, similar electrophoretic techniques revealed an average genetic relatedness of 0·390 and 0·429 between workers and their reproductive sisters. This value is not only far below

**Table 2.** Genetic relatedness in colonies of social insects.

| Species | Average genetic relatedness between workers and the reproductive female brood they rear or among workers | Reference |
|---|---|---|
| *Wasps* | | |
| *Polistes metricus* | 0·63[a] | Metcalf and Whitt 1977a, b; Lester and Selander 1981 |
| *Polistes exclamans* | 0·390[a] | Lester and Selander 1981 |
| *Polistes apachus-bellicosus* | 0·429[a] | Lester and Selander 1981 |
| *Bees* | | |
| *Apis mellifera* | Approaching 0·25[b] | Page and Metcalf 1982 |
| *Ants* | | |
| *Aphaenogaster rudis* | 0·75[c] | Crozier 1973 |
| *Myrmecia pilosula* | 0·172 ± 0·053[b] | Craig and Crozier 1979 |
| *Formica sanguinea* | 0·378 ± 0·173[a] | Pamilo and Varvio-Aho 1979 |
| *Formica sanguinea* | 0·19[b] | Pamilo 1981 |
| *Formica transkaucasica* | 0·33 ± 0·07[b] | Pamilo 1982 |
| *Formica aquilonia* (Espoo[d]) | 0·09 ± 0·09[b] | Pamilo 1982 |
| *Formica aquilonia* (Vantaa[d]) | − 0·02 ± 0·14[b, e] | Pamilo 1982 |
| *Formica polyctena* (Siuntio[d]) | 0·19 ± 0·34[b] | Pamilo 1982 |
| *Formica polyctena* (Kauniainen[d]) | 0·30 ± 0·23[b] | Pamilo 1982 |
| *Myrmica rubra* Site A-1975 | 0·1056[b, e] | Pearson 1983 |
| *Myrmica rubra* Site A 1977 | 0·0218[b, e] | Pearson 1983 |
| *Myrmica rubra* Site A 1978 | 0.0828[b, e] | Pearson 1983 |
| *Myrmica rubra* Site B 1977 | 0·5428[b] | Pearson 1983 |
| *Formica exsecta* (Espoo[d]) | 0·04 ± 0·07[b, e] | Pamilo and Rosengren 1984 |
| *Formica exsecta* (Tuusula[d]) | 0·09 ± 0·08[b, e] | Pamilo and Rosengren 1984 |
| *Formica exsecta* (Joskar[d]) | 0·62 ± 0·13[b] | Pamilo and Rosengren 1984 |
| *Formica exsecta* (Kalvholm[d]) | 0·78 ± 0·13[b] | Pamilo and Rosengren 1984 |
| *Formica pressilabris* (Espoo[d]) | 0·29 ± 0·13[b] | Pamilo and Rosengren 1984 |
| *Formica pressilabris* (Tuusula[d]) | 0·07 ± 0·08[b, e] | Pamilo and Rosengren 1984 |
| *Rhytidoponera mayri* | 0·158 ± 0·037[b] | Crozier *et al* 1984 |

[a] Genetic relatedness between workers and the female reproductive brood they rear.
[b] Genetic relatedness among workers.
[c] Inferred because monogyny and monoandry were demonstrated.
[d] Localities from where the populations were sampled.
[e] Not significantly different from zero.

0·75, the value expected for full sisters in a haplodiploid system, but even lower than 0·5, the value expected between a female and her offspring.

In the honey bee *Apis mellifera*, which of course is highly eusocial, Page and Metcalf (1982) again used isozyme polymorphism and set out explicitly to study multiple mating and patterns of sperm usage by queens. Their results showed that honey bee queens used sperm from at least 3 males at any given time and mixing of sperm in the spermatheca resulted in the average relatedness amongst her daughters approaching 0·25.

## 4. The causes and consequences of low levels of relatedness

The main reasons attributed to such low levels of relatedness are polygyny, queen turnover and multiple mating followed by sperm mixing. Queen turnover can reduce the average relatedness between workers and the reproductives they rear in the kinds of *Polistes* nests studied by Metcalf and Whitt (1977a, b) and Lester and Selander (1981). If the $\alpha$ foundress lays worker eggs and dies paving the way for the $\beta$ foundress to lay the reproductive eggs, then workers are not rearing their full sisters ($r = 0{\cdot}75$) as future reproductives, but their cousins ($\alpha$ and $\beta$ are assumed to have been full sisters) [$r = 0{\cdot}1875$; relatedness of workers to their mother $\alpha$ (0·5) × relatedness of $\alpha$ to $\beta$ (0·75) × relatedness of $\beta$ to her daughters who are the future reproductives (0·5) = 0·1875]. In tropical wasps the queen's daughters often replace the queens (see for eg. Jeanne 1972). Here the workers who are sisters of the new queens now raise nieces ($r = 0{\cdot}375$) rather than full sisters ($r = 0{\cdot}75$). Notice that this value would be even lower if the original foundresses $\alpha$ and $\beta$ were not full but half sisters in the temperate species and if the workers in the tropical species were not full but half sisters of their new queens. Polygyny or the simultaneous presence of more than one egg layer (a condition known among many social insects) would also similarly lower the levels of relatedness between workers and the reproductive brood they rear. For a discussion of the role of polygyny see West-Eberhard (1978). Yet another factor contributing to low levels of relatedness between workers and the reproductive brood would be usurpation of nests by unrelated conspecifics (Gamboa 1978).

When the queen mates with more than one unrelated male, her daughters would not all be full sisters of each other if she used sperm from more than one male at any given time. Any two randomly picked daughters would be full sisters with a certain probability $p$ and half sister with a probability $1-p$. Suppose a female mated with $n$ males who are respectively responsible for proportions $f_1, f_2, f_3, \ldots, f_n$ of her female progeny where, $\sum_{i=1}^{n} f_i = 1$. The average coefficient of relatedness ($\bar{r}$) between daughters is then

$$\frac{1}{2}\left(\frac{1}{2} + \sum_{i=1}^{n} f_i^2\right),$$

and if all males contribute equally, we have

$$\bar{r} = \frac{1}{2}\left(\frac{1}{2} + \frac{1}{n}\right) \text{ (Hamilton 1964b).}$$

The larger the number of males she has mated with the closer the relatedness between two average daughters approaches 0·25, the relatedness between two half sisters. This perhaps partly accounts for the low relatedness in the wasp studies referred to above and is in fact the reason behind the results in *Apis mellifera* (Page and Metcalf 1982). Although Page and Metcalf (1982) first conclusively demonstrated multiple mating and sperm mixing, these ideas have a long history. Indeed social insects have long been known to be notoriously polyandrous (Wilson 1971; Page and Metcalf 1982; Cole 1983). In fact this was well known at the time Hamilton first proposed the ideas of kin selection and the role of haplodiploidy. Hamilton's reaction to this was first (1964b) that 'multiple insemination will greatly weaken the tendency to evolve worker-like altruism

and $n > 2$ . . . should prevent its incipience altogether' and later (1972) that 'the occurrence of this special relatedness to sisters must not be over emphasized. Male haploidy is certainly not the only prerequisite for evolving a sterile caste'.

Queen turnover might be of unpredictable accidental occurrence. But polygyny and multiple mating by the queens, widespread as they are, are clearly evolved traits that might have some adaptive significance. From the point of view of social evolution one might wonder why polyandry has evolved at all in social hymenopterans. By mating with only one male a queen ensures the highest possible relatedness amongst her daughters and thereby might be expected to maximise the chances of their cooperating and helping each other. It is possible that in ancestral hymenopterans natural selection placed a higher premium on other factors correlated with multiple mating such as greater brood viability (Page 1980; Page and Metcalf 1982; Woyke 1963) and larger colony size (Cole 1983) rather than the evolution of sociality. In the few cases where it has been studied, sex determination in Hymenoptera appears to be determined by one or a few polymorphic loci. Diploid heterozygotes (heterozygous in at least one locus in multi-locus systems) are females; haploids (hemizygotes) are males while diploid homozygotes (homozygous at all loci in multi-locus systems) are inviable or sterile males (see Wilson 1971 for a lucid treatment of sex determination in Hymenoptera). In such a system (let us consider the single locus system for simplicity) a queen who mates with a male carrying one of her own sex determining alleles is destined to produce 50% inviable offspring. It thus pays for the queen to mate with several males and thereby reduce the proportion of inviable offspring (Page 1980; Page and Metcalf 1982). On the other hand Cole (1983) has shown that multiple mating is strongly correlated with large colony sizes in ants, and argues that multiple mating ensures sufficient sperm in the queen to make possible the maintenance of larger colonies (see Starr 1984 for a more comprehensive account of the consequences of multiple mating). Similarly polygyny has been considered as an adaptation against extinction in small or rare populations (Wilson 1963).

## 5. Rendering the low levels of relatedness consistent with the haplodiploidy hypothesis

There are several ways in which attempts have been made in the literature to explain away the difficulties rendered to the haplodiploidy hypothesis by the low levels of genetic relatedness especially when they result from multiple mating.

(i) If the two or more males that mate with a queen are very closely related to each other then their sperm will be nearly identical thus negating the effects of multiple mating (Wilson 1971).

(ii) If multiple insemination is restricted to the more highly advanced social groups and absent in the primitive ones, it can be thought of as secondarily evolved after sterile castes had already evolved by kin selection and had gone so far as to be now irreversible. Such irreversibility could arise because workers may no longer have any immediate reproductive options in response to low levels of relatedness to the brood on their parental nests. Neither of the above ideas is however supported by any strong empirical observations (Wilson 1971; Starr 1984).

(iii) Low levels of relatedness in general and multiple insemination in particular can be thought of as posing no special problems for the haplodiploidy hypothesis as long

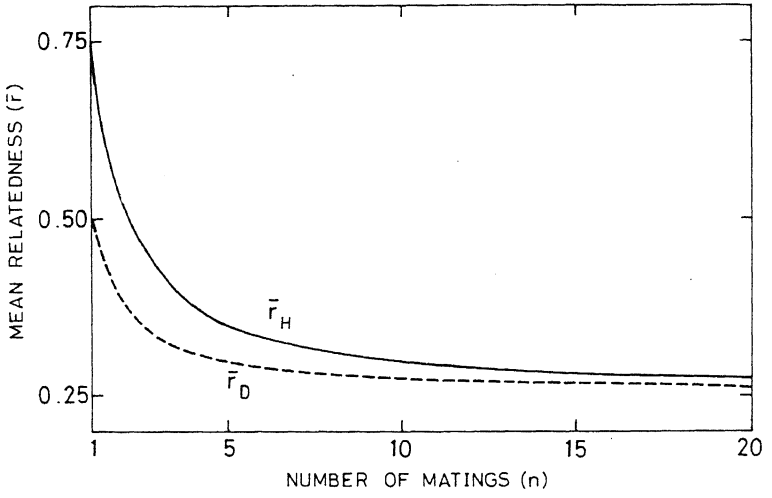


**Figure 1.** Mean relatedness among daughters as a function of the number of matings by the mother in haplodiploid ($\bar{r}_H$) and diploid ($\bar{r}_D$) genetic systems. This graph is drawn using the following equations provided by Page and Metcalf (1982): Assuming that each mate contributes equal amount of sperm $\bar{r}_H = 1/2(1/2 + 1/n)$ and $\bar{r}_D = 1/4(1 + 1/n)$.

as one does not find these phenomena more often in haplodiploid than in diploid groups. At any level of multiple mating haplodiploid groups are always at least slightly more predisposed towards sociality than their diploid counterparts (Page and Metcalf 1982). Note from figure 1 that if the queen mates with two males, then any two of her daughters on the average are related to each other by 1/2, which is the same as that between mother and daughter. The asymmetry in genetic relatedness caused by haplodiploidy is thus completely lost. This is probably why Hamilton (1964b) believed that more than 2 matings should prevent altruism altogether. It is true however that at any given number of matings two sisters in a haplodiploid populations are more closely related than two sisters in a diploid population. Ecological factors being identical haplodiploid populations are therefore more likely to develop female altruism than diploid populations (Page and Metcalf 1982).

(iv) A very popular way of getting out of the difficulty created by multiple mating has been to assume that although queens mate with more than one male the sperms from different males do not mix appreciably in the spermatheca. The queen is therefore effectively monoandrous using sperm from only one male for long stretches of time (Trivers and Hare 1976; Orlove 1975; Charnov 1978; Cole 1983). Taber (1955) conducted perhaps the first detailed investigation of sperm usage patterns using naturally and artificially inseminated queens. His results indicated a non-random usage of sperm but clearly sperm from different males was at least partially mixing in his experiments (Page and Metcalf 1982; Crozier and Brückner 1981). Other studies on honey bees (Alber *et al* 1955; Kerr *et al* 1962) strongly suggest sperm mixing. Comparable experiments have also been performed with solitary wasps (Wilkes 1966; Holmes 1974) and the conclusion here is that while sperm from different mates is not used in a perfectly random fashion there is no evidence of perfect sperm precedence either. Evidence for multiple mating and

patterns of sperm usage had until recently to depend entirely upon dissections or the use of genetic markers. In modern times however the use of isozyme markers has begun to yield far more reliable data. Some 9 species of Hymenopterans have been investigated using this technique (review in Page and Metcalf 1982; Starr 1984) of which 3 are polyandrous with a certain degree of sperm precedence in two species. The varying degrees of sperm precedence or biased sperm usage demonstrated in various species reduce the effective number of matings but the resultant relatedness between daughters would nevertheless be less than 0·75, the value expected for full sisters.

(v) Finally an ingeneous way of getting out of the difficulty caused by lower levels of relatedness between the workers and the reproductive brood is to argue that workers are capable of discriminating their full sisters apart from half sisters and that workers selectively rear their full sisters. This would still make worker behaviour advantageous by virtue of closer genetic ties between workers and their full sisters compared to that with daughters (Getz *et al* 1982; Page and Metcalf 1982).

## 6. Kin recognition—the evidence

### 6.1 *Sweat bees*

*Lasioglossum zephyrum* is a primitively social sweat bee that lives in a system of burrows under the soil. One of the bees usually assumes the role of a guard and, sitting at the entrance to the burrow, prevents both parasites as well as non-nest mate conspecifics from entering the burrow. Breeding these bees in artificial nests in the laboratory, Greenberg (1979) presented guard bees with intruders who are of known genetic relatedness to the guards (known to Greenberg!). Testing bees of 14 different geneological relationships against one another in this fashion a highly significant positive correlation between probability of acceptance into the nest and the genetic relatedness between intruder and guard bee was demonstrated (figure 2). This clearly implies a capacity to recognize different levels of genetic relatedness and there are reasons to believe that such recognition is based on odours (Barrows *et al* 1975). Using artificial laboratory colonies constituted by unrelated bees Buckle and Greenberg (1981) concluded that the bees do not recognise genetic relatedness to themselves. The guard bees appear to learn the odours of their nestmates and then, using these learnt odours as a guide, they accept or reject intruders depending on the similarity of the intruders' odour to those of the guard's nest mates (table 3). (see Getz 1982 for a refutation of this conclusion). Previous work had suggested that both genetic homogeneity as well as adult learning opportunities enhance nestmate recognition abilities (Kukuk *et al* 1977). Since environmentally acquired difference in odours are eliminated in these experiments it is presumed that similarity in odours reflect genetic relatedness. In this species males are also capable of assessing the genetic relatedness between successive female partners through a process of learning or becoming habituated to female pheromones (Smith 1983). Notice that such a system of recognition involving a template based on learning from individuals other than oneself is unlikely to permit workers to distinguish between full and half sisters in the same colony. This type of kin recognition will therefore not help get us out of the difficulties

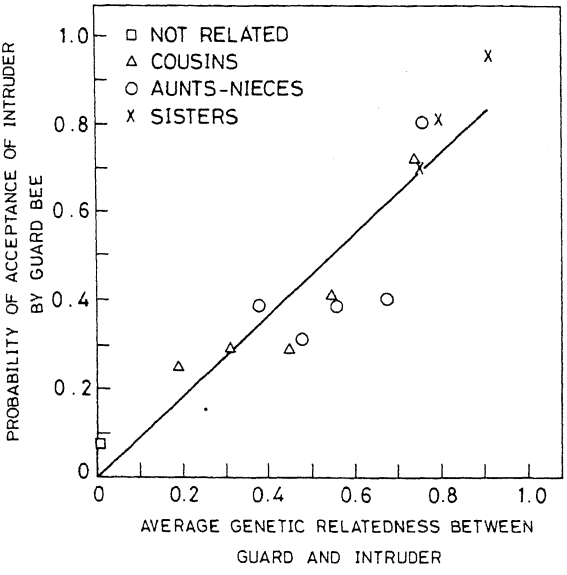


**Figure 2.** Individuals of the primitively eusocial bee *Lasioglossum zephyrum* were raised in the laboratory. In artificially constituted laboratory colonies guard bees were presented with intruder bees whom they had never encountered before. But these intruders were infact related to the guard bees as sisters, aunts, nieces, cousins or were unrelated to them. The probability of acceptance into the nest of the intruder bee by the guard bee was significantly positively correlated with the average genetic relatedness between guard and intruder bees. After Greenberg (1979). Copyright AAAS.

for the haplodiploidy hypothesis created by the low levels of genetic relatedness between workers and their reproductive siblings.

### 6.2 *Honey bees*

While colony odours and the recognition of hive mates is well known in honey bees, Breed (1981) first demonstrated a genetic basis in recognition of queens by workers. Replacing existing queens by new queens and observing the response of workers, Breed showed that the acceptance of new queens by the workers depends on genetic relatedness of the replacement queens to former queens (table 4). Thirty five per cent of the new queens were accepted if they were inbred sisters ($r \simeq 1$) of the previous queen, 12% if they were outbred sisters ($r \simeq 0{\cdot}75$) and 0% if unrelated. As in the case of sweat bees recognition by the workers is a learned phenomenon. Carbon dioxide narcosis abolished the memory of the recognition cue so that strange queens were now accepted. With time, of course, the identity of the new queen was learned as shown by rejection of subsequently introduced queens unrelated to that whose identity has been learned. Similar results were obtained when workers were transferred from one hive to another in the field or from a box containing one group of workers to one containing a different group of workers (Breed 1983). Although environmentally acquirable cues were held constant including the entire duration of larval development, genetically unrelated workers were attacked more often than genetically closely related ones. There

**Table 3.** Sweat bees don't smell themselves[a].

| Colony | Guard | Intruder | Accepted/ rejected |
|---|---|---|---|
| 3X 3Y[b] | X | Sister of X | Accepted |
| 3X 3Y | X | Sister of Y | Accepted |
| 3X 3Y | Y | Sister of X | Accepted |
| 3X 3Y | Y | Sister of Y | Accepted |
| 3X 1Y | X | Sister of X | Accepted |
| 3X 1Y | X | Sister of Y | Accepted |
| 3X 1Y | Y | Sister of X | Accepted |
| 3X 1Y | Y | Sister of Y | Rejected |

[a]Data from Buckle and Greenberg (1981).
[b]This means a colony consisting of 3 sisters from one inbred genetic line X and 3 bees from another inbred genetic line Y.

**Table 4.** Acceptance of foreign queens by honey bee workers[a].

| Queen type transferred | Sample size | Percentage transfers accepted |
|---|---|---|
| Inbred sister of old queen | 23 | 35 |
| Outbred sister of old queen | 26 | 10 |
| Non sister of old queen from same genetic line | 20 | 10 |
| Non sister of old queen from unrelated genetic line | 39 | 0 |
| Disturbance control | 10 | 90 |
| Non sister of old queen from unrelated genetic line transferred after $CO_2$ narcosis of workers | 20 | 90 |

[a]Modified from Breed (1981). Reprinted with permission.

is a strong suggestion of genetically determined odours and these appear to be already present at 5 days after post emergence. Getz and Smith (1983) performed similar experiments but with genetic relatedness more precisely defined. They set up experimental hives using queens of known genotypes which were artificially inseminated with sperms from males of known genotypes. In such hives full and half sisters among workers were obvious to the experimenter because the genetic markers used led to different colour morphs. Groups of worker bees were then removed from their parental hives little before their expected emergence and maintained as small groups of full sisters for 5–6 days. Now, when bees were transferred from one group to another, they were found to be significantly more likely to bite half sisters than full sisters. This result clearly indicates a genetic basis for the cue which is recognised because both full and half sisters were raised in the same hive and must therefore be almost identical in any environmentally acquired odours. The ability to recognize cues could however be based on learning the odours of one's nestmates because each test bee

had been allowed to habituate to its full sister for 5–6 days prior to testing. It is therefore possible that the test bee had learned the odour of its full sisters during this period.

In an earlier paper on the other hand, Getz *et al* (1982) suggest that distinction between half and full sisters could be occurring even when both are present in the same hive and therefore habituated to each other. The basis for this conclusion is the result that in hives with two different genetic lines of workers, the patrilineal worker groups segregate non-randomly during swarming. One colony had 36,700 bees with 74% of the cordovan mutant and 26% wild type. After swarming the bees remaining in the hives were 64% cordovan and 36% wild type while those in the swarm were 79% cordovan and 21% wild type. Another colony had 30,000 bees with 54.5% cordovan and 45·5% wild type. The swarm contained 58% cordovan and 42% wild type while those staying back in the parent hive were 43% cordovan and 57% wild type. The conclusion (Getz *et al* 1982) that, of the two patrilineal lines of workers, one line of full sisters leave while another line stay home is obviously very weak because of the very slight differences in composition. The high statistical significance of the data appear to be due to the inordinately large sample sizes (> 30,000 bees). Besides, as the authors themselves suggest, their data could simply 'reflect a propensity for cordovans to swarm more readily than normal workers'. Significantly, the same author states in a subsequent paper (Getz and Smith 1983) that 'at this stage there is no evidence that bees discriminate between full and half sisters in the hive once they are habituated to both sets of workers'. Unless this is shown we are still left with the difficulties posed by multiple mating for the haplodiploidy hypothesis.

In more recent experiments (Breed *et al* 1985) honey bee workers were allowed to mature (in cardboard boxes!) from day 1–5 after emergence either with other bees from the same hive (i.e. with full or half sisters) or with unrelated bees. When such bees were introduced into boxes holding other bees, an introduced bee is attacked depending only on its genetic relationship to the recipient bees. There is no effect of mixed rearing (from day 1–5) so that no odours appear to be transferred from one bee to another. Bees, however, appear to learn the odours of their nestmates (or boxmates!). If recipient bees are housed together in mixtures of two different genetic lines, bees of both genetic lines are equally likely to attack on introduced bee of either genetic line. This suggests that discrimination of heterogeneity within a hive is not possible. On the other hand, there is a tantalizing suggestion that bees learn the odour of their own genetic line as well as any other genetic line in their association and store these two memories separately. When feeding behaviour was studied, bees kept in mixed groups nevertheless interacted more often with unfamiliar kin than with the other genotype, some individuals of which they were also habituated to. In other words, as far as feeding behaviour goes, different genetic lines appear to be distinguished. As a matter of fact, if kin recognition is to counteract the effects of multiple mating and thereby rescue the haplodiploidy hypothesis, differential feeding of different genetic lines is probably more important than differential aggression. In any case, one cannot overemphasize the need for caution in interpreting results based on assays of kin recognition which, almost always bear unknown relationships to behaviour under natural conditions. Be that as it may, here is the first indication of what we have been looking for—ability of workers in a social insect colony to potentially discriminate between full and half sisters.

A conserted attack on the honey bee as a model system to unravel the possibility of discrimination of different genetic lines within a hive appears to have been conspired. Since the preparation of the first draft of this essay, I have received 4 unpublished

manuscripts, each taking us a step closer to a decisive answer to this question. Briefly, Getz W M, Bruckner D and Smith K B (unpublished results) asked if there is sufficient variability in the odours of full and half sisters to permit their differential recognition in the first place. The answer seems to be in the affirmative because they succeeded in conditioning bees to extend their probosides to only one of the two choice odours derived from their full and half sisters respectively. Getz W M and Smith K B (unpublished results) have now eliminated a lacuna in their previous experiments by showing that bees reared in complete isolation can still discriminate between their full and half sisters. Their results also corraborate those of Breed *et al* (1985) that, while odours from more than one genetic line can be learned, the respective templates used in recognition are not confounded. There is however a suggestion of possible transfer of labels from one bee to another unlike in the case of the experiments of Breed *et al* (1985). Noonan K C (unpublished results) has now demonstrated that worker honey bees in colonies of mixed patrilines show preferential care to queen and worker brood of their own patrilines. It thus appears that at least in the honey bee effective genetic relatedness between workers and the female reproductive brood they rear can be as high as 0·75. One hopes that further experimentation will reveal similar phenomena in primitively eusocial insects which is the critical focus for the haplodiploidy hypothesis.

### 6.3 *Ants*

Ants are a group where colony odours that help discrimination of nestmates from non-nestmates have been suggested almost a 100 years ago. There has been much discussion in the literature regarding the genetic versus environmental origins of such odour. It was not until a series of simple experiments by Jutsum *et al* (1979) however that it became clear that both exogenous (from diet) and endogenous (probably but not necessarily genetic) components exist and act synergistically. These experiments also indicated that even in the complete absence of any exogenous differences there exist sufficient differences in the endogenous component to permit distinction between nestmates and non-nestmates (table 5). The experiments just described used colonies of the leaf cutter ant *Acromyrmex octospinosus* maintained in the laboratory and where the endogenous and exogenous source of odour difference could be carefully controlled. Field experiments on aggression between workers drawn from local versus widely separated

**Table 5.** Endogenous and exogenous components of colony odour in ants[a].

| Colony (endogenous factor) | Forage (exogenous factor) | Mean time spent in investigating (minutes)[b] | Sample size |
|---|---|---|---|
| Different | Different | 12·4 | 60 |
| Same | Different | 7·0 | 15 |
| Different | Same | 4·4 | 55 |
| Same | Same | 2·6 | 23 |

[a] All means are significantly different from each other ($P < 0{\cdot}05$)

[b] Modified from Jutsum (1979). Copyright Baillière Tindall.

colonies confirmed these laboratory findings. More recently, experiments have been performed with interspecific mixed laboratory colonies using 5 species of monogynous carpenter ants belonging to the genus *Camponotus* (Carlin and Hölldobler 1983). When worker larvae from an alien species were introduced into a queen-right colony of a different species the larvae were accepted, groomed and fed to grow into adults. In several such colonies studied no pattern of preference for kin or rejection of heterospecific nestmates was observed. Moreover when interaction between an adoptee from a mixed colony was tested with its non nestmate genetic sisters taken from a stock laboratory colony, there was always intense aggression showing lack of recognition of genetic relatedness (figure 3). In other words the alien adoptee workers had acquired as

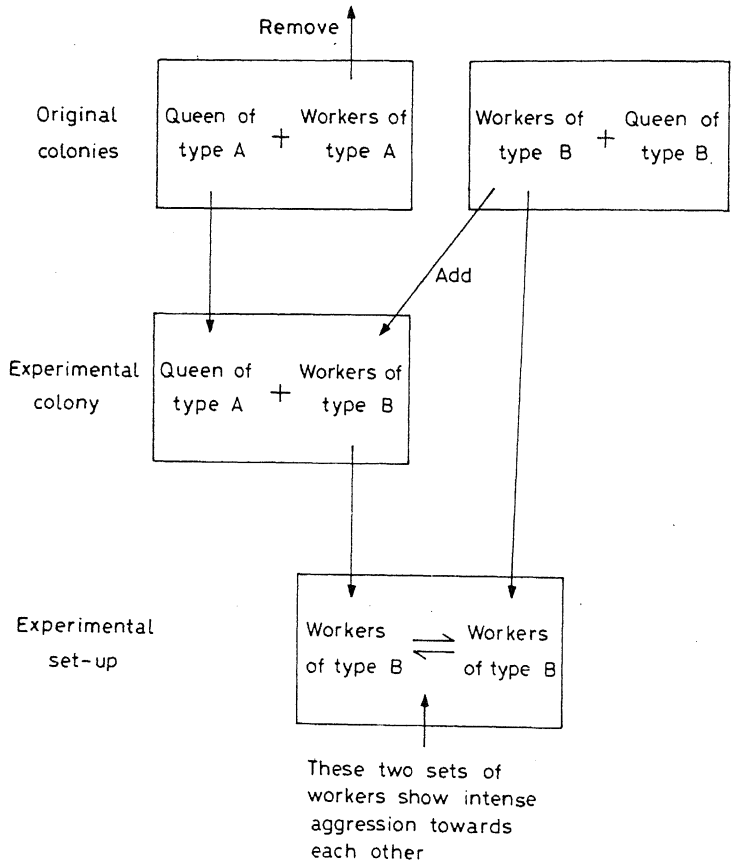


**Figure 3.** In laboratory colonies of the carpenter ants belonging to the genus *Camponotus* one colony (species A) was deprived of its workers and alien workers from a different laboratory colony of another species (species B) were introduced. The experimental colony thus consisted of a queen of one species (species A) while the workers were of a different genetic line (species B). These workers of species B in association with the queen of species A showed evidence of having learned as well as acquired the properties (odours) of their foster Queen. This is inferred from the observation of intense aggression between these workers of species B and other workers of species B drawn from the original species B colony. Data from Carlin and Hölldobler (1983). More recent experiments suggest that colony-specific odours and not species-specific odours are involved in these phenomena (Carlin and Hölldobler unpublished results).

well as learned an odour from the queen in its new nest. The adoptee workers must have acquired the queen's odour because they are discriminated against by their non nestmates genetic sisters and they must have learned the queen's odour because they now discriminate against their non nestmate genetic sisters. It is also clear that it is the queen that is the source of the discriminator odour because the same results were obtained with all-adoptee colonies where all the queen's brood had been removed. These results suggest that the queen's odour is learned by all workers in a colony and later used for discrimination of different individuals. Although interspecific colonies were used in these experiments, more recent studies have confirmed that indeed, colony-specific and not species-specific cues are being transferred from the queens to the workers. It also appears that in the absence of the queen, worker-derived as well as food-derived cues begin to exert their influence on recognition (Carlin N F and Hölldobler B, unpublished results). In any case, since the queen-derived cues are dominant there is little scope for different genetic lines of workers in the colonies of multiply mated queens to preferentially aid full sisters and discriminate against half sisters. On the contrary, nestmate recognition in the Acacia-ant *Pseudomyrmex ferruginea* appears to be of a rather different kind. The queen is not the source of recognition pheromone in this system because groups of worker brood separated from a stock colony and reared separately by different foster reproductives failed to show antagonistic interactions; although workers from different colonies are normally incompatible. Recognition must therefore be based on genetically specified cues although, whether the ability to discriminate nestmates is learned or not is unclear (Mintzer 1982). A follow-up study using colonies initiated by inbred lines suggests a multiple locus model for the production of recognition pheromones (Mintzer and Vinson 1985).

### 6.4 *Social wasps*

Comparable experiments have been performed using the primitively eusocial wasp genus *Polistes*. These wasps have not been bred in the laboratory and it has therefore not been possible to control genetic relatedness between experimental animals as precisely as in the case of sweat bees or honey bees. The discussion of the wasp data will therefore be in terms of nestmate discrimination. When nests are drawn from reasonably well separated localities, nestmates (wasps emerging in the same nest) must be significantly more closely related to each other than to non nest mates (animals emerging in different nests). The exact degree of genetic relatedness may however vary in an undefined fashion between different experiments. Wasps from the same nest will certainly be related and the values of relatedness may even fall within narrow limits. Wasps from nests drawn from distant localities will certainly bear low relatedness to each other. But pairs of wasps drawn from different pairs of distant nests may have very different values of relatedness. This can contribute to increase in variation in the results from experiment to experiment. Nestmate discrimination has been studied in *Polistes* in 4 situations: association of overwintered foundresses in spring, artificial associations of nest mates and non nestmates under experimental conditions, recognition of brood and mate preferences. For a review of the wasp work, see Gamboa *et al* (1985).

6.4.1 *Foundress associations:* *Polistes* wasps in the temperate regions terminate

their nest cycle during the fall season and newly emerged females overwinter by hibernating in places away from the nest sites. At the beginning of the following spring season the overwintered females return preferentially to the natal nesting sites and initiate new colonies, often by several females cooperating in what are termed multiple foundress colonies. Because females preferentially return to their natal nesting sites it has been possible to mark emerging females in the fall and observe association patterns in the following spring. This has been done repeatedly in several species of *Polistes* and two species of *Mischocyttarus* (another genus belonging to the sub-family Polistinae). The general result has been that cofoundresses are usually females emerging from the same nest (see West-Eberhard 1969; Noonan 1981 and references therein). Klahn (1979) working with *Polistes fuscatus* and Pratte (1982) working with *P. gallicus* have argued that preferential association of nest mates as cofoundresses is not based on recognition of nest mates *per se* but because of *philopatry*—the phenomenon of returning to the site of emergence. Because nests can be located very close to each other, however, philopatry is unlikely to be sufficient to ensure relatedness of cofoundresses. Besides, other workers have since provided strong evidence of recognition of nest mates *per se*. Ross and Gamboa (1981) collected nests from different localities and allowed the gynes (females emerging in fall which are potential foundresses for the next spring) from each nest to overwinter in the laboratory along with their nests and nest mates but separated from other gynes and other nests. After thus overwintering in the laboratory for 6·5 months the wasps were exposed to spring conditions. At this stage nest mates and non nest mates were introduced into enclosures where nest mates preferentially associated with each other to initiate nests. Behavioural interactions among such overwintered females who were isolated from all other conspecifics for 74–99 days showed that they still retained the ability to recognise the nestmates with whom they had hibernated. Similar results have been obtained with *P. fuscatus* where sample sizes were larger and observations blind (Bornais *et al* 1983). Using *P. fuscatus*, Post and Jeanne (1982) went a step further and showed that females overwintered in the laboratory preferentially associated with former nest mates even if they had not hibernated along with them and that they do not associate with non nest mates even if they had been forced to hibernate with them. The characteristics of nest mates must thus have either been learnt during the fall season soon after emergence and remembered or animals must be able to recognise nest mates without the need to have to learn anything from them. In other words, they must be selfsufficient in producing a template in their brain with which to compare other animals and assess relatedness to themselves. That the ability to distinguish nest mates from non nest mates does not depend on having to arrive at the same nesting sites is further strengthened by the observation of clumping patterns during overwintering in the laboratory by *P. exclamans* females. Artificial hibernating boxes containing only nestmates show few and large clumps during hibernation while those containing a mixture of nestmates and non nestmates contain many small clumps throughout the period of hibernation (Allen *et al* 1982).

6.4.2 *Associations of females emerging in the laboratory:* Shellman and Gamboa (1982) collected natural nests of *P. fuscatus* and kept them in the laboratory to allow emergence of adults from the puparia. Upon emergence females were either (i) isolated from the nest and nestmates within minutes of emergence, (ii) isolated from the nest but kept along with other newly emerged nestmates or (iii) exposed both to their natal nest and nestmates. After such treatment for 15–120 days each female was isolated into

individual boxes for 14–20 days. Now two nest mates and one unrelated female were introduced into a test box and the 3 females were observed for discrimination of nest mates from non nest mates. Using time spent in close proximity (less than 5 cm apart) as an assay of discrimination, Shellman and Gamboa (1982) showed that only females exposed to their natal nests and nest mates are capable of discriminating between nest mates and non nest mates. From more recent studies using newly emerged workers exposed to unrelated nest fragments and to unrelated conspecifics (Pfennig *et al* 1983a, b) it is quite clear that nest mate discrimination depends on learning of chemical cues from the natal nest or its brood by newly emerged adult wasps. Similar experiments have recently been performed with the bald-faced Hornet, *Dolichovespula maculata* (Hymenoptera: Vespidae) (Ryan *et al* 1985). These results are somewhat difficult to interpret because there appear to be discrepancies between different measures of recognition. Wasps isolated from their nests and nestmates also probably are capable of nestmate discrimination (unlike *Polistes*, see above). However, the authors conclude that the nest is somehow involved in the ontogeny of nestmate recognition ability because there is much more variability in the responses of the isolated wasps compared to those allowed to learn the characteristics of their nests and nestmates.

6.4.3 *Recognition of brood:* *Polistes fuscatus* wasps destroy brood or desert a nest significantly more often if their own nest is replaced by the nest of an unrelated female than if their nest is replaced by those of their sisters (former nest mates). Sisters normally nest in close proximity of each other and may therefore share common food, nesting material and other environmental odours. But the involvement of such environmentally originated odour is ruled out because brood destruction is based only on genetic relatedness even when sisters nesting far apart and non sisters nesting in close proximity were tested. A nest and its brood appear to be recognised as a unit and there is no evidence of discrimination of differently related brood within the same nest (Klahn and Gamboa 1983).

6.4.4 *Mate preferences:* In contrast to the female's demonstrable ability to discriminate nestmates from non nestmates, males of *P. fuscatus* appear to lack the ability of discriminating between nestmate and non nestmate females but, appear to recognize nestmate males at least under certain experimental conditions. When paired with nestmate and non nestmate females, males seem to choose their mates without regard to relatedness. This has been shown both by visual observation of mating behaviour (Larch and Gamboa 1981) as well as by actual assessment of insemination (Post and Jeanne 1982). The males also do not discriminate between nestmate and non nestmate males, as shown by Ryan *et al* (1984) in experiments where, spatial associations were observed in artificial associations of males in the lab. The techniques used were similar to those used for female-female recognition (e.g. Shellman and Gamboa 1982). Using slightly modified procedures, however, Shellman-Reeve and Gamboa (1985) conclude that mates can recognize their male nestmates. Here we must perhaps distinguish between ability to discriminate and actual discrimination. As suggested by Post and Jeanne (1982) these species are probably not selected to inbreed and therefore males, while still being able to distinguish between sisters and non sisters perhaps indiscriminately inseminate them. Similarly if the best strategy for males in natural situations is to ignore all other males, they are not likely to pair more (or less) often with nestmates in the laboratory.

In summary, studies on social wasps have shown that discrimination of nestmates from non nestmates depends on learning of recognition cues from their natal nests. Discriminations of different levels of genetic relatedness among nestmates has not so far been investigated. What we do know of kin recognition therefore does not help overcome the problems of low levels of relatedness caused by multiple mating for the haplodiploidy hypothesis.

## 6.5 *Vertebrates*

In recent years recognition of kin, other than offspring, in the absence of locational and other indirect cues has been demonstrated in several species of vertebrates. These studies also appear to have begun approximately around the year 1979 when the kin recognition abilities of ants and bees were first demonstrated (Greenberg 1979; Jutsum *et al* 1979). In many species of vertebrates there is evidence that totally naive individuals reared in isolation from all conspecifics also appear to recognize siblings. Although we are no longer concerned with the haplodiploidy hypothesis, our interest in kin recognition in vertebrates stems from a very similar logic. Given similar ecological conditions, altruistic behaviour can evolve more easily by kin selection if there is a high degree of genetic relatedness between the interacting individuals. Animals may however grow up with atleast some individuals who are not their full siblings (due to multiple mating for example). If kin recognition depends on learning the characteristics of all the individuals one grows up with coupled with an inability to distinguish different levels of genetic relatedness among them, the average coefficients of genetic relatedness between participants in social interactions will be relatively low. On the other hand, if kin recognition depends on matching encountered animals with oneself (either through an innate or learned knowledge about oneself) different levels of genetic relatedness can be recognised. Thus altruism can be so distributed that the effective coefficients of genetic relatedness between donor and recipient is relatively high. We will therefore once again be concerned with the possibility of discrimination between levels of genetic relatedness within a family unit.

6.5.1 *Toads and frogs:* Tadpoles of the toad *Bufo americanus* associate preferentially with siblings in the laboratory. Waldman and Adler (1979) released marked tadpoles of two different genetic lines in an indoor test pool and measured the positions of all tadpoles in repeated trials. The experiment was repeated 6 times with different sets of tadpoles and in each experiment the mean nearest neighbour distance between siblings was significantly less than that between non siblings, indicating a preferential association of siblings. In 37% of the trials the mean coordinates of the two groups were different indicating that the sibling groups separated out into different regions of the pool. But it was not as if the two groups of tadpoles preferred different regions of the pool due to any possible environmental gradients. They simply preferred to stay away from non siblings and closer to siblings. This is inferred because the position of the tadpoles kept changing from time to time. These experiments were subsequently repeated by releasing the tadpoles in outdoor ponds which are the natural habitats of the tadpoles. Here the sibship composition of 64% of all schools sampled were significantly biased in favour of one siblings or the other, once again demonstrating the ability of tadpoles to preferentially associate with siblings. There was again no evidence

of preferences for any specific habitats and the preference was clearly for siblings *per se* (Waldman 1982). Some light has been thrown on the mechanism of sibling recognition by rearing groups of tadpoles either in isolation or along with non siblings. Tadpoles reared together with siblings and non siblings together throughout their development failed to distinguish between familiar siblings and familiar non siblings in laboratory tests (Waldman 1981) although they appeared to be capable of doing so in field trials (Waldman 1982). It is now clear that the failure to distinguish siblings from non-siblings upon being reared together is not because of any convergence in recognition characteristics (labels) but because of learning of the characteristics of the non siblings by the experimental animals (Waldman 1985). On the other hand, tadpoles reared only with siblings for the first 18 days of their development and later exposed to non siblings successfully discriminated between familiar siblings and familiar non siblings in laboratory tests. Besides, tadpoles reared in total isolation from all conspecifics beginning prior to neural plate formation distinguished between unfamiliar siblings and unfamiliar non siblings. They also distinguished full siblings from paternal half siblings but not from maternal half siblings (Waldman 1981). It can be concluded from these experiments that tadpoles are capable of sibling recognition even without the aid of post-embryonic experience with conspecifics. However learning of the cues from conspecifics during early development not only reinforces recognition but also probably over-rides any ability innately present, acquired environmentally but pre-embryonically or acquired by learning from self (we must say 'probably' because here there is a discrepancy between laboratory and field experiments).

A number of very similar experiments have been conducted with tadpoles of the frog *Rana cascadae*. Here the testing procedure involved recording the time spent by test tadpoles in two different halves of a tank each holding different kinds of stimulus individuals. The basic result is similar to that with the toad study; tadpoles prefer to associate with siblings over non siblings (O'Hara and Blaustein 1981). With many controls and different kinds of rearing regimes it has been shown that tadpoles (a) reared with siblings, (b) reared with siblings and non siblings, (c) reared in isolation with their egg jelly mass, (d) reared in isolation without their egg jelly mass and (e) reared in isolation with egg jelly mass of non siblings all prefer full sibling over maternal half siblings, maternal half siblings over paternal half siblings and the latter are preferred over non siblings (Blaustein and O'Hara 1981; 1982; O'Hara and Blaustein 1981). These results reinforce our conclusion that there may be more than one mechanism of sibling recognition but the *Rana cascadae* study argues very strongly in favour of either an innate ability to recognise siblings or an ability dependent only on learning characteristics of oneself. More recent studies have shown that even adult *Rana cascadae* frogs prefer to associate with siblings over non siblings (Blaustein *et al* 1984).

6.5.2 *Bank swallows:* Bank swallows (*Riparia riparia*) breed in large dense colonies where errors in recognition of one's own burrow by young birds is not uncommon. Under such situations parent swallows recognise and evict their neighbour's chicks by means of what has been termed a 'signature' call given by the chick (Beecher *et al* 1981a, b). Siblings also appear to recognize each other by means of a similar call. Beecher and Beecher (1983) have recently shown that chicks recognise siblings by giving more calls in response to the recorded calls of their own sibling groups than they did to the calls of unrelated groups. Chicks hand reared in isolated from all their conspecifics but who had heard calls of unrelated chicks responded however to the familiar calls of

the unrelated groups rather than to the unfamiliar calls of their own siblings. Here again is an example of a learned recognition system but the critical data do not exist which might tell us if a residual innate recognition capacity persists in the absence of learning stimuli.

6.5.3 *Mammals:* Among mammals most work on kin recognition has involved rodents: mice, rats, squirrels and voles. Kin recognition can clearly take place in the absence of prior contact in white-footed deermice (*Peromyscus leucopus*) (Grau 1982). These mice were tested in pairs for frequency and duration of behavioural interactions. The mice investigated related but completely unfamiliar individuals (non litter mate siblings who were non cage mates) significantly more often than they did unrelated strangers (non siblings, non cage mates) showing evidence of kin recognition without prior contact with the very individuals tested with. All mice however had considerable social experience with their siblings both before and after weaning which must have provided them sufficient opportunity to learn the odours of at least some of their siblings. It is possible that they could later have used this information to discriminate between related and unrelated individuals as in the case of sweat bees (Buckle and Greenberg 1981). Similar results have been presented by Hayashi and Kimura (1983). In contrast Hepper (1983) ensured that at least some of his rats had no post natal experience with any related individuals. Preferences of the pups were tested in a *T* maze. When presented with an unrelated cage mate and an unrelated non cage mate, the pups preferred to associate with the unrelated cage mate suggesting that the characteristics of the cage mate had been learnt postnatally. On the other hand when pups were given a choice between genetically related but unfamiliar individuals and unrelated unfamiliar individuals, they now preferred genetically related strangers over unrelated strangers. In this case the discrimination had obviously been made without an opportunity for postnatal learning of sibling odours. Since the pups were not given a choice between familiar but unrelated individuals and unfamiliar relatives it is not clear whether any one mode of acquisition of information regarding the characteristics of relatives (self based or non self based) can be dominant over the other mode.

Gavish *et al* (1984) investigated sibling recognition in Prairie voles in the context of incest avoidance. They showed that individuals, whether related or not, but reared together, did not mate with each other while those not reared together mated whether or not they were genetically related. In nature this species has been shown to exhibit incest avoidance and the capability to do so is obviously due to postnatal learning abilities.

In two studies the importance of innate (or self based learning) and non self based learning were specifically investigated. In laboratory mice full sibs, half sibs and non sibs differed significantly from each other in aggressive interactions, but all such differences disappeared completely when the tested partners were familiar to each other (Kareem and Bernard 1982). The authors conclude that the mice use familiarity as a 'rule of thumb' during interactions. Porter *et al* (1983) applied artificial odours like musk oil, oil of clove, lemon lime and cherry to spiny mice pups. Pups who had a particular odour applied to themselves alone or to themselves and their littermates housed with them later reacted preferentially to unfamiliar animals treated with the identical artificial odour, indicating both the importance of postnatal learning in the recognition process as well as the possibility of using one's own odour as a standard of comparison. These results, however, are in contrast to earlier results where untreated

littermates isolated for a comparable period of time displayed no evidence of recognition (Porter and Wyrick 1979). It is somewhat difficult however to compare these results with the other mammalian studies because according to Porter and Wyrick (1979) spiny mice appear to be incapable of recognising unfamiliar siblings, quite in contrast to all the other studies described above.

Ground squirrels have been subjected to an impressive array of laboratory and field experimentation by Holmes and Sherman (1982). Each baby squirrel was marked for identification within about 3 hr of birth and from then was raised by either its biological mother or its foster mother along with some siblings and some non siblings. When later tested for aggressive interactions, animals reared together are much less aggressive than those reared apart. Among animals reared apart, biological sisters were more tolerant of each other than non kin. These results suggest that both genetic relatedness as well as rearing conditions affect recognition. In field studies there was clear evidence that animals were more tolerant to and cooperative with their full sisters than with their half sisters. Here full and half-sisters were reared together and genetic relatedness was assessed electrophoretically. As the authors have noted, there is an apparent contradiction between lab and field studies in whether different levels of genetic relatedness can be distinguished within a group being reared together, and this is the crucial question we have been interested in throughout this survey of the literature. Holmes and Sherman (1982) believe that their field tests are more sensitive and therefore that full and half sisters can be distinguished inspite of being reared together.

The study most cited as an illustration of kin recognition, not only in the absence of an opportunity to learn the characteristics of siblings or other relatives, but one which persists even if the experimental animal grows up entirely with unrelated conspecifics is that of Wu *et al* (1980). Sixteen infant pig tail monkeys (*Macaca nemestrina*) were separated from their dams within 5 min of birth and reared separately while allowing for social interaction with unrelated conspecifics for several hours a day. When tested subsequently these monkeys preferred to associate with their paternal half siblings over unrelated individuals. These results suggest that the monkeys were capable of distinguishing between related and unrelated animals without any prior experience with relatives and indeed inspite of prior experience with unrelated individuals. Fredrickson and Sackett (1984), however, appear not to have been able to reproduce these findings. Notice that the results of Wu *et al* (1980) in contrast to Buckle and Greenbergs' (1981) sweat bees, Breed's (1981) honey bees, *Polistes* wasps (Gamboa and colleagues, referenced above), Beecher and Beecher's (1983) bank swallows or Kareem and Bernard's mice squirrels where subjects preferred relatives of those individuals whose characteristics they had learned or those individuals emerging from nests whose characteristics they had learnt.

MacKenzie *et al* (1985) have studied the effects of companionship, kinship and rearing in social preferences of stump-tailed macaques (*Macaca arctoides*) using a qualitatively different set of techniques. A whole range of social interactions were observed as they occurred naturally in a heterogeneous group of 26 monkeys occupying a large enclosure. The monkeys varied widely in age, kinship and rearing conditions. Data on social interactions were subjected to partial correlational analysis. The results suggest that familiarity was the most important variable affecting social preferences. This was followed by correlation with kinship and very interestingly, kinship through the father was important but not through the mother. These results are in broad agreement with studies of other vertebrates suggesting an ability to recognise

kinship *per se*, but a strong masking influence of familiarity. As the authors note familiarity is probably sufficient to recognise matrilineal kinship (as the offspring of a female will grow up together) but natural selection appears to have favoured a special mechanism (not based on familiarity) to recognise patrilineal kinship. This is reminiscent of the ground squirrel study (Holmes and Shermann 1982; Holmes 1984) where, recognition abilities appear only at about the time that offspring are old enough to emerge from their natal burrows, move about and get mixed up. This is true both for the mother's ability to recognise her offspring as well as sibling recognition by the offspring itself.

There is some evidence that humans too are capable of assessing degrees of genetic relatedness amongst themselves. Dizygotic or fraternal twins are genetically no different from any pair of siblings but are likely to have shared a very similar environment during embryonic development. While the average genetic relatedness between co-twins would be 0.5, any pair of same sex twins could share from 1 to 46 chromosomes in common. The exact degree of genetic relatedness between a given pair of twins can be determined by the analysis of a large number of blood group factors. In a couple of rather fascinating studies it was found that the degree of genetic similarity as revealed by blood group analysis was significantly positively correlated with similarity in physical appearance as rated by the twins themselves, their mothers or other observers (Pakstis *et al* 1972; Carter-Saltzman and Scarr-Salapatek 1975). In retrospect this result is not so surprising after all. Some human siblings appear so similar to the casual observer that it is impossible not to guess their relationship. On the other hand, we all have remarked at one time or another that it would have been impossible to guess that certain pairs of individuals were siblings unless we were told of the fact. That same sex siblings can vary in their genetic relatedness by as much as from 1 to 46 shared chromosomes appears to be manifested in the widely varying degrees of similarity in physical appearance apparent even to the casual observer. We do not however know if this relationship between genetic relatedness and physical similarity is actually used by humans in recognition of unfamiliar relatives. Besides, our logic would be of course be in some trouble if all the genes controlling physical appearance (especially of facial features) were clustered on a single or a very small number of chromosomes but there is no evidence for or against this.

While sight may be more important than smell in the lives of humans we may be using smell in subtle ways not obvious without carefully controlled experiments. Such an idea is reinforced by a recent study showing that human mothers are capable of telling their infants apart from other infants by means of smell alone if they have been allowed to interact with their infants only for half an hour immediately after birth. Clearly here is an imprinting like phenomenon. Interestingly enough, fathers were unable to show any such capacity (Russell *et al* 1983).

## 7. The mechanism of recognition

There has been an explosion of studies on kin recognition in the last 5 years. Different studies use different methods to assess animals' abilities to discriminate kin from non kin and use a variety of different conditions for rearing experimental animals. Wilson (1986) provides a glossary that helps to face up to a concomitant explosion of terminology used by researchers in this field. There is also much discussion of

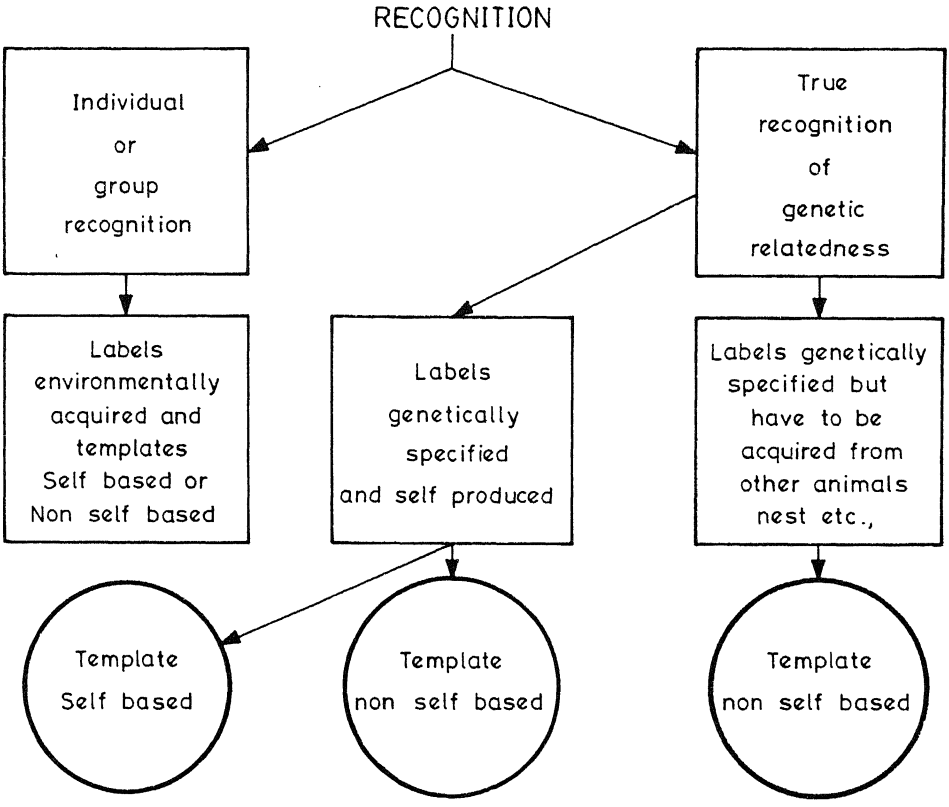


**Figure 4.** Facets of kin recognition.

In order for kin recognition to take place every animal must carry a label on its body and a template in its brain. Animals must examine the labels of any individual encountered and compare them with the template in their brains to determine the extent of relatedness between themselves and the individual encountered. The labels may be genetically specified or environmentally acquired. Genetically specified labels may be produced by each animal or some animals may acquire them from other animals (eg. from queens in social insect colonies), nests etc. Templates may be self based (either innate or dependent on learning from oneself) or non self based (dependent on learning from individuals other than oneself).

genetically determined versus learned or acquired abilities to recognise kin. For a discussion of quantitative genetic models for kin recognition see Crozier (1986). To facilitate comparison between different studies I suggest that we view the phenomenon of kin recognition in the following general frame work (figure 4).

### 7.1 *Group or individual recognition versus recognition of genetic relatedness per se*

This is an important distinction which is not always made. Group recognition by means of colony specific odour has long been well known in social insects such as ants and bees (Wilson 1971; Michener 1974). It is only in recent times however that the capabilities of ants and bees to assess actual genetic relatedness have become evident (Greenberg 1979; Jutsum *et al* 1979; Breed 1981).

### 7.2 *Distinction between labels and templates*

All that we know about kin recognition abilities of animals is consistent with the following scheme. In order to make kin recognition possible every animal should carry a label (or set of labels) on its body and a template in its brain. It should then compare any animal encountered to the template in order to decide if the label is similar to the template and therefore if the encountered animal is related to it. Although this scheme and terminology are used by some (Lacy and Sherman 1983; Holmes and Sherman 1982; Sherman and Holmes 1985; Waldman 1985; Getz 1982; Breed and Bennett 1986) the distinction between labels and templates has not always been made in discussing kin recognition abilities of animals.

### 7.3 *Labels*

Since most experimental results suggest olfaction as the sensory modality involved in recognition we shall refer to the labels as odours although a label could just as well be a visual label as it probably is in the human twin studies (Pakstis *et al* 1972; Carter-Saltzman and Scarr-Salapatek 1975) or an auditory label as in the case of bank swallows (Beecher and Beecher 1983). Unless labels used in recognition are genetically specified true recognition of genetic relatedness cannot occur. Entirely environmentally acquired labels can only be used in group or individual recognition. It is conceivable however that insects living in large colonies may use environmentally acquired labels for group recognition and thereby maintain colony integrity without having the ability to assess genetic relatedness. This is why much effort should go into discerning between group recognition and recognition of genetic relatedness (eg. Kalmus and Ribbands 1952; Boch and Morse 1979). Labels can be produced directly by the metabolic machinery of an animal or it can be acquired either from other animals (who have produced it by their metabolic machinery) or from the products of other animals (fecal matter, nests built by other animals etc.). These two possibilities were explicitly contrasted by Crozier and Dix (1979) who considered two kinds of models. In the 'individualistic' model each colony member is expected to 'retain its pheromonal integrity with no significant transfer of colony odour pheromones between colony members'. This clearly appears to be the case in the acasia ant studied by Mintzer (1982), Mintzer and Vinson (1985) as well as in Waldman's (1985) tadpoles. In contrast the 'gestalt' model supposes transfer of colony odour pheromones between different members of a colony by grooming and trophallaxis so that each individual responds to a common gestalt odour. A case intermediate between the gestalt and individualistic models is that where the queen is the source of the label which the workers acquire. This certainly seems to be the case in some ants (Carlin and Hölldobler 1983; Carlin N F and Hölldobler B, unpublished results). In most studies one cannot really distinguish between each animal producing its own label and labels being acquired from others because of the lack of distinction between labels and templates. Taking the case of the *Polistes* wasps, for example, in the experiments described so far, we do not know whether the wasps that were not exposed to their natal nests lacked the labels or templates or both. If they lacked labels then we may conclude that the labels must be acquired from the nest.

Getz (1981, 1982) has considered genetic models for the production of a sufficient diversity in labels to provide for recognition of genetic relatedness. Applying these

models to existing data on *Lasioglossum zephyrum* they suggest a genetic labelling system of 4 or 5 loci with 2 to 3 alleles at each locus. Only if labels are individualistic (self produced, figure 4) and not 'gestalt' or acquired from a common source such as the queen or nest (figure 4) will it be possible for animals to recognise different levels of relatedness within a hive or family unit. From the point of view of the haplodiploidy hypothesis or kin selection this distinction is thus essential.

### 7.4 *Templates*

Are templates the products of learning or are they somehow innately specified? This seems to be a question of great interest (Holmes and Sherman 1982, 1983; Sherman and Holmes 1985). The idea of innately specified templates by means of recognition alleles was first suggested by Hamilton (1964b). Dubbed as the 'green beard' effect by Dawkins (1976) in his inimitable style, the idea simply is that we need to postulate a gene that makes its bearer not only have a 'green beard' but also program it to aid all individuals in the population possessing 'green beards'. Such genes have been repeatedly considered highly improbable (Hamilton 1964b; Alexander and Borgia 1978; Dawkins 1976; Holmes and Sherman 1982). In contrast Alexander and Borgia (1978) and Lacy and Sherman (1983) have suggested 'phenotype comparison or matching' mechanisms with a learned component. Lacy and Sherman (1983) have modelled situations where an 'observer' assesses its genetic relationship to the 'observee' by means of 'templates' determined by 'referants' where the referants could be known relatives such as a parent or the observer itself. Given this definition of phenotype matching it cannot really be disproved (Blaustein 1983). The results of even the most carefully controlled experiments where learning of cues from relatives is ruled out (e.g. Blaustein and O'Hara 1981, 1982) can be construed as phenotype matching where individuals use themselves as 'referants' (see Holmes and Sherman 1982).

Holmes and Sherman (1982, 1983) and Sherman and Holmes (1985) distinguish 4 mechanisms of kin recognition namely, spatial distribution, association, phenotype matching and recognition alleles. Kin recognition based on predictable spatial distribution or predictable patterns of association are widespread and well-known (the relevant literature is reviewed in the just mentioned three papers). It is only in recent years that kin recognition in the absence of spatial and associational cues has become apparent. All known cases of such recognition are lumped under phenotype matching by these authors because recognition alleles have hitherto been defined in a way that precludes their search and possibly their very existence! I will argue here that it is useful to distinguish between two types of templates: (i) Self based templates where the templates do not have to be learned or they can be learned from oneself without the intervention of any other individual and (ii) non self based templates where the templates are learned from individuals other than oneself or even from some structure such as the nest as in some social insects. Once an individual produces a chemical label by means of its own metabolic machinery then, whether it releases this substance to the surface of its body, smells itself and then produces a template or whether a template is produced without it having to smell the surface of its body postnatally is perhaps not a terribly important distinction at the moment. Whether an animal acquires a template by smelling other individuals in the population (non self based) or whether the template is produced without the intervention of any other individuals (self based) is perhaps the

more important distinction. These two different mechanisms could drastically affect the abilities of animals to recognise levels of genetic relatedness. Consider for example the different lines of daughters in a honey bee hive with a multiply mated queen. The workers could get habituated to both their full and half sisters and acquire a template that prevents them from discriminating between full and half sisters as seems to happen in the case of sweat bees (Buckle and Greenberg 1981). If on the other hand each worker acquires a template by the action of its own alleles then even within a hive with a multiply mated queen, workers can selectively aid their full sisters. The effective genetic relatedness between a worker and the beneficiary of her altruism could thus be as high as 0·75, thereby drastically altering the conditions for the evolution of sociality by kin selection.

In this framework of the two kinds of templates there is evidence of self-based templates in honey bees (Breed *et al* 1985; Getz and Smith unpublished results; Noonan K C unpublished results) tadpoles of frogs and toads (Waldman 1981; Blaustein and O'Hara 1981, 1982; O'Hara and Blaustein 1981), rats (Hepper 1983), pig tailed monkeys (Wu *et al* 1980) as well as ground squirrels (Holmes and Sherman 1982). An example of the absence of self based templates and the need for non self based templates is the study of sweat bees by Buckle and Greenberg (1981). Several studies suggest a combination of self based and non self based templates. Indeed, most studies cited above as examples of self based templates are in fact instances of a combination of both kinds of templates. Notice that familiar individuals are almost always treated as kin. Given this fact what we need to be concerned about is how the self based and non self based templates are weighted. If self based templates are dominant over non self based ones then recognition of different genetic lines is possible within a mixed hive or family. This is what appears to be happening in the cases of ground squirrels (Holmes and Sherman 1982) and *Rana cascadae* tadpoles (Blaustein and O'Hara 1981, 1982; O'Hara and Blaustein 1981). On the contrary non self based templates appear to override any self based templates in sweat bees (Buckle and Greenberg 1981), *Bufo americanus* tadpoles (Waldman 1981), and laboratory mice (Kareem and Bernard 1982). In summary, what future experimental work should focus on is whether templates are self-based or non self based and if a mixture of the two kinds, whether the two templates are stored separately (as appears to be the case in honey bees, Breed 1985; Getz W M and Smith K B, unpublished results; Noonan K C unpublished results) and also whether the two templates can be differentially weighted during interaction. I suggest that we need not concern ourselves with whether true recognition allels exist and whether true genotypic comparison occurs simply because, these questions are not experimentally tractable. It has been hard enough to understand whether animals come with fully specified, hard wired knowledge of some features of the external world or they need some experience for complete specification, without getting lost in an irresolvable nature-nurture controversy. Understanding whether features of the animal itself can be hard wired can only be much worse.

### 7.5 *An experimental approach to distinguish between labels and templates*

Let us now consider a specific example to illustrate an experimental approach to discriminate between labels and templates. For the purposes of illustration let us use the experiments of Shellman and Gamboa (1982) with *Polistes* as our paradigm [although

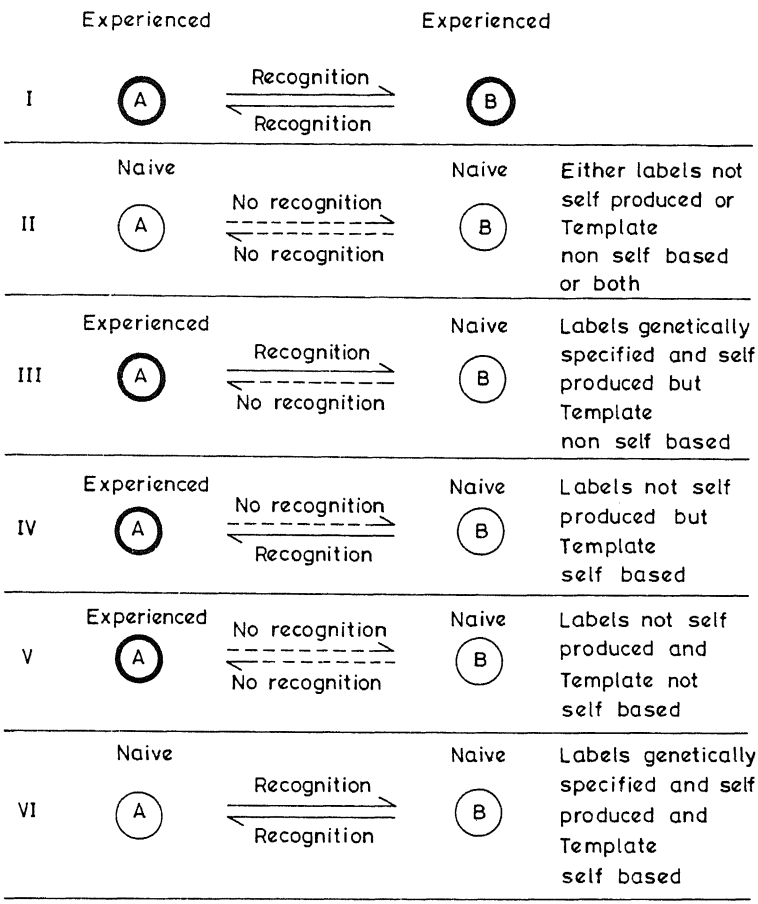


**Figure 5.** An experimental approach to distinguish between the roles of labels and templates in kin recognition.

A and B are two animals (say, wasps) who may or may not recognise each other as close genetic relatives depending on their rearing conditions. Based on this one can infer the ontogeny of the labels and the templates. See text for details.

Gamboa *et al* (1985) have recently used a different approach to address these questions and provided some additional information—see below]. Consider two wasp nestmates A and B (figure 5). We shall refer to the animals exposed to their natal nests during early life as 'experienced' and those animals isolated immediately after emergence from their natal nests as 'naive'. Let both animals A and B be able to recognise each other when both are experienced (figure 5, panel I). Let neither animal however be able to recognise the other when both are naive (panel II). This suggests that either the label is acquired after eclosion from the nest or the template is not self based or both. But notice that only one of these may be true. Consider now an extension of the experiment where animal A alone is experienced while animal B is naive. Now if animal A recognises B but B does not recognise A (panel III) we can conclude that A must have a normal template and B must have had a normal label. Since B did not recognise A this must mean that B did not have a normal template because A, being an experienced animal, must

necessarily have had a normal label. In other words it is the template that must be non self based while the label must have been innately specified and produced by B itself. This is because B is a naive animal. On the contrary consider a situation where A, the experienced animal, fails to recognise B but B, the naive animal, recognises A (panel IV). In this case we must conclude that A, being an experienced animal, must have had a normal template and therefore B lacked a normal label. B however must have had a normal template since it recognised A. In other words it is the label that must be acquired from the nest while the template is self based. If neither A nor B can recognise each other although one of them is experienced (panel V) then we must conclude that the label has to be acquired from the nest and that the template is not self-based. Finally, it is possible that even when both A and B are naive they may be able to recognise each other (panel VI) in which case labels must be self produced and templates must be self based. It should be possible to devise such experiments and understand the distinction between the possible ontogenies of labels and templates. Lack of distinction between labels and templates can potentially lead to erroneous conclusions. For instance Shellman and Gamboa (1982) showed that wasps exposed to their nestmates alone did not acquire the capacity to recognise nestmates. They therefore concluded that exposure to nestmates is neither a sufficient nor a necessary condition for the development of kin recognition abilities (Pfennig *et al* 1983a). But this conclusion will depend on the history of the animals that were used for exposure. Consider a situation where labels have to be acquired after emergence, which is certainly possible in the *Polistes* studied by them. Now if the animals used for exposure were themselves naive ones then they would not have acquired the required labels. On the other hand, if experienced animals were used for the exposure, then exposure to such experienced animals may be sufficient for other animals to acquire recognition abilities.

Recently Gamboa *et al* (1985) have used a different approach to address the same questions. Assuming that the templates are non self based (which is suggested by their earlier experiments) they set out to ask if the labels are self-produced or acquired from the nest. Taking wasps from two unrelated nests they exposed the animals not to their own nests but to each others nests. Now if labels are acquired from the nest (and templates are any way assumed to be learnt from nests) the animals should have labels and templates that match with each other (although corresponding to the unrelated nests). Two animals coming from a nest when exposed to the same alien nest should treat each other tolerantly. On the other hand, if labels are genetically specified and self produced then each animal should have mismatched labels and templates and be intolerant of each other. Since the results happen to be intermediate between these two possibilities the authors assumed that both endogenous odours as well as odours acquired from the nest are involved.

### 7.6 *A possible genetic basis for recognition labels*

True recognition of genetic relatedness must involve genetically specified labels that vary in a quantitative fashion between animals of different levels of genetic relatedness. This suggests a highly polymorphic multi locus system. A search for such a genetic system no longer appears like looking for a needle in a haystack with the recent demonstration of the role of the histocompatibility system in kin recognition. All multicellular animals, especially vertebrates, have a well developed immunological

system to prevent the invasion of their bodies by foreign cells. It is this histocompatibility system that frustrates transplantation of organs from one individual to another. The body's immune system unfailingly distinguishes between self and non self tissue by means of a set of antigenic molecules commonly referred to as transplantation antigens which are present on the surface of all cells. As this implies, the exact nature of the transplantation antigens present on the cells of any two individuals are different from each other unless of course the individuals are identical twins. There is considerable information on the genetic basis and Mendelian inheritance of the genes coding for the transplantation antigens. A large number of transplantation antigens coded for by an equally large number of genetic loci have been identified. Of the many gene complexes, the one known as the major histocompatibility complex (MHC) (designated as the H-2 in the mouse and the HLA in man) dominates the body's reaction to a graft. The MHC is a rather complex and highly polymorphic set of loci (see Roitt 1980 for an overview).

Recent work has unravelled another profound and rather surprising function for the MHC. The H-2 locus in the mouse appears to produce genotypically variable odour components on the basis of which mice can potentially assess their genotypic similarity with other conspecifics (see Jones and Partridge 1983 for an interesting commentary and Beauchamp *et al* 1985 for a non technical account). We know this from two kinds of experiments. Firstly, by various tricks strains of mice have been bred which differ from each other almost exclusively in the H-2 locus. Using such strains it has been demonstrated that mice can be trained to distinguish specific H-2 types by scenting the arms of a Y maze with the urine of an appropriate mouse (Yamazaki *et al* 1982). The idea that the H-2 loci produce distinctive odours which enable mice to distinguish one another is also supported by the observation that males of a certain H-2 type largely prefer to mate with females of alternative H-2 types (Yamazaki *et al* 1976). The second kind of experiment involves the use of the well known Bruce effect or 'pregnancy block'. If pregnant mice are exposed to 'strange' (males different from the one they have mated with) or even the urine or bedding of 'strange' males within the first 6 days of pregnancy, a neuroendocrine imbalance results leading to abortion of the embryo. This experimental situation has been utilized to show that the frequency of pregnancy block is higher if the 'strange' male is of a different H-2 type compared to the stud male (the original male with which the female was mated). Similar results were obtained with 'strange' females differing in H-2 type from the stud male although to a lesser extent (Yamazaki *et al* 1983). This once again suggests a role for the MHC in chemosensory recognition.

Another rather spectacular result that we should mention here is the recently discovered role of the *t* locus in determining mating preference in mice. The *t* locus is a highly polymorphic locus closely linked to the H-2. Most of the alleles at this locus are recessive lethals in spite of which a considerable amount of polymorphism is maintained in natural populations. It has been established that due to segregation distortion heterozygous males produce about 95% *t*-bearing sperm. There has been considerable interest in the *t* locus because the frequency of *t* alleles in natural populations is higher than would be expected on the basis of their lethality but less than would be expected after taking segregation distortion into account. It is now known that female mice given a choice between wild type males and males heterozygous for the *t* locus preferred to mate with the wild type males. (Levine *et al* 1980; Lenington 1983). The adaptive significance of this behaviour is easy to see because a wild type female

mating with a heterozygous male would produce 50% heterozygous offspring and could therefore potentially have some inviable grandchildren. On this argument a female who is herself heterozygous would be much worse off mating with a heterozygous male because she would then have 50% heterozygous offspring and 25% inviable offspring (homozygous for the lethal allele).

Females who are themselves heterozygous show a much stronger avoidance of heterozygous males when given a choice of mating with wild type and heterozygous males (Lenington 1983). Similarly males also prefer to mate with wild type rather than heterozygous females (Lenington 1983). These results suggest that the mice are capable of assessing their own as well as their potential mate's genotype at a single locus. There is evidence that this assessment is also on the basis of chemosensory perception of odours in the urine (Lenington 1983). It is unlikely that animals would be able to make such assessment at every genetic locus and these results were obtained probably because of the close linkage of the *t* locus to the H-2 locus. The H-2 is a highly polymorphic locus which is well known to produce sufficient genetic diversity in cell surface glycoproteins (the histocompatibility antigens) to permit recognition of self versus non self at the cellular level. Perhaps the H-2 locus also produces a similar diversity of odourous molecules that permits recognition of self versus non self at the behavioural level.

## 8. The physical basis of recognition

Today we understand rather little regarding the physical basis of kin recognition. That olfaction must be involved in most cases had however been suggested quite early. Most recent studies have confirmed this (except possibly in the case of birds where recognition could be acoustic and humans where recognition may be visual). Olfaction also appears to be the most suitable sensory modality to combine metabolically produced and environmentally derived cues in recognition, as many insects appear to do. The most precise statements regarding the basis of kin recognition have been made by Hölldobler and Michener (1980) who have coined the terms 'discriminators' or 'recognition pheromones' for 'the odour signals that differ among individuals in a population' but 'not of extrinsic origin'. It has also been hypothesised as discussed by Hölldobler and Michener (1980) that the recognition pheromones consist of several active components. What is believed to make a particular pheromone unique is not only its qualitative composition but also the concentrations of its different constituents (Barrows *et al* 1975). The resultant economy in producing and detecting pheromones under such a scheme is obvious. The properties of several known pheromones are clearly consistent with this idea (see for instance Cammaerts *et al* 1981).

There is one more aspect of the recognition system that we already know and that is that an imprinting like phenomenon is involved. Notice that while this strongly suggests a role for learning in kin recognition it does not rule out 'recognition alleles'. Animals might have to smell themselves and get imprinted on their own odour before acquiring the capacity to recognise kin. A recent neurophysiological study with Norway rat pups reinforces the idea of odour imprinting. Experimental Norway rat pups were exposed to peppermint odour during early postnatal development while control pups were exposed to clean air. The experimental pups showed an enhanced olfactory bulb response to peppermint odour as measured by radioactive glucose uptake, compared to

the control pups (Coopersmith and Leon 1984). It seems likely that a similar phenomenon may be involved in kin recognition. The idea that a developing animal forms olfactory representations in the nervous system which serve as templates to which incoming odours are later matched has already been suggested in the context of olfactory preferences in animals (Freeman 1981).

Apart from the involvement of olfaction and an imprinting like phenomenon we know scarcely little else. It should however be possible to begin to identify the recognition pheromones. Given that several complex pheromone systems have been identified it is well within the present technological capabilities to chemically identify recognition pheromones. One expects this to be a particularly exciting area of research in the coming years. Our knowledge of the basis of kin recognition thus seems to be poised for a quantum leap.

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# Tradeoffs in the evolution of frog calls

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**Abstract.** All biological characteristics are subject to conflicting selection pressures. This is particularly true of those characteristics that are subject to sexual selection. The classic example is the peacock's tail. Others are the calls used by male frogs and toads to attract their mates. The forces which have acted in the evolution of these calls are varied and the calls that we hear made by these animals are diverse.

Two kinds of factors can be recognized: constraints and forces. Constraints on the kind of a call that a frog might evolve include its phylogeny, the energy required to produce different kinds of calls, the risks incurred from attracting predators. Also important is the morphology of the frog: both the structures used by the males to make the calls and the apparatus with which the females hear the calls. For example, frog size has an important influence both on the frequencies of the sounds that a frog produces and the acuity with which they are heard.

Both passive and active selective forces can be identified. Passive forces include the distances that environments transmit sounds of different frequencies and the interference from other sounds that calls encounter. Active forces include the reactions of conspecific males and females to the calls. Males interact acoustically in a variety of ways to organize their choruses in both space and time. They position themselves, and time their calls so that they minimize the interference from other males while maximizing their chances of securing a mate. Female choice has been studied in test arenas. Females choose louder calls, calls that are most easily located, and the calls of their own rather than other species. In choosing among males of their own species, females have been shown to pick the males controlling the best resources, sometimes using calls to do so. They should also be expected to choose those males who can contribute the best genes to their offspring. The extent to which they do this and the role of calls in choosing is actively being argued.

Sexual selection, both interactions between males and female choice, have undoubtedly been important in the evolution of frog calls but only within the constraints imposed by a variety of other factors.

**Keywords.** Amphibian; anuran; frog; communication; vocalization; call; sexual selection; evolution.

## 1. Introduction

All biological characters are subject to conflicting selection pressures and the states that one sees represent points where opposing pressures balance. This is particularly evident in those characters in which sexual selection is involved; the classic example is the peacock's tail—frog calls are another.

Here, I will consider the various forces that shape the signals used in the long distance vocal communication of frogs and toads. A frog call is as we hear it not because there is no selection for it to change but because a change that might improve it in one way would make it less effective overall.

Standard English makes a distinction between frogs and toads. In this paper I will use frog as a general term for the Anura, including in this category the toads, the true frogs, the treefrogs and all of their varied relatives.

## 2. Vocalizations

Here I will be considering only advertisement or mating calls, one of the several kinds of vocalizations that frogs use. These are the calls used to communicate at long distances. They are given by males and serve to advertise their location, to attract receptive females and commonly, to organize the chorus of males. Females are not known to give advertisement calls.

There have been several review papers on frog communication, among them are: Arak (1983a), Bogert (1960), Blair (1964, 1968), Littlejohn (1977), Straughan (1973) and Wells (1977a, b).

Frog calls are diverse; no two species have exactly the same call, but they are not nearly as diverse as are bird calls nor so complex as some of them are. A few examples will illustrate the diversity of frog calls. Most calls are between 400 and 4000 Hz. Some are high in frequency, others are low. All have a relatively simple frequency structure; some are tonal, others are noisy. Some calls are frequency modulated usually with a simple sweep up or down. Calls also differ in their timing. Some are long, others short. Calls may be amplitude modulated with a simple change in loudness, modulated into regular pulses, or more complexly patterned. Some calls have a single note, others several identical notes, still others a long introductory note followed by one or more shorter notes. Among the most complex frog calls are those that combine notes that differ in several aspects as do the notes of the *Physalaemus pustulosus* advertisement call (Rand 1983; Ryan 1985).

Frog calls seem to be genetically determined. Males of two closely related species of *Hyla*, raised in isolation from tadpoles to adults, gave the appropriate species specific call regardless of whether they were exposed to the calls of their own or of the other species (Burger 1980, cited in Gerhardt 1982). Evidence that female call preference is also inherited comes from experimentally produced hybrids between two species of *Hyla* (Doherty and Gerhardt 1983). The hybrid males produced intermediate calls. Female hybrids preferred the synthetic calls with the pulse-repetition rate of the hybrids to synthetic calls with pulse-repetition rates of either parental species.

## 3. Constraints and selective forces

One can recognize two sets of factors that have been important in the evolution of frog calls. One set includes the forces that select for calls that are more effective in their social/sexual function, for example, the selection that would exist for low frequency calls in a population in which females always preferred the lowest frequency call that they heard. The other set are the constraints that seem to limit the effectiveness of the first set, for example the fact that larger structures vibrate at lower frequencies. Call evolution involves compromises and tradeoffs between and within both sets of factors.

### 3.1 *Constraints*

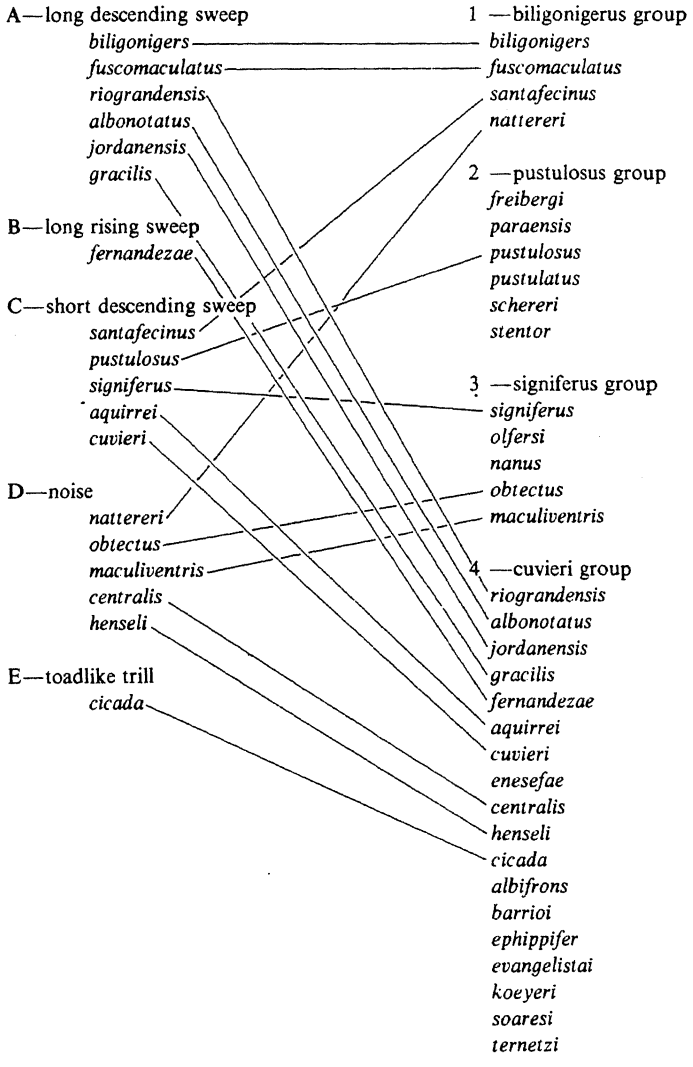
Constraints are imposed on frog calls by such things as: phylogenetic relationships, energetic costs of calling, risks of predation, and morphology, particularly the size and physical properties of the sound producing apparatus of the male, and the receptor

system of the female. These constraints do not specify what the call of a specific frog will be, but they do specify that certain things are more difficult and unlikely to evolve than are others.

### 3.2 *Phylogenetic constraints*

After reviewing the influence of phylogeny on frog calls, Schiøtz (1967, 1973) concluded that between subspecies within a species there are sometimes differences; between most

**Table 1.** Groups within the genus *Physalaemus*.




*Barrio (1965, 1967)
**Lynch (1970)

species within a genus there are clear differences, and in most cases there are similarities between the species in one genus that separate them from species of other genera; on the family level it is impossible to find characters common to the genera in one family, that are not shared by genera in other families.

One way to evaluate the phylogenetic constraints on the evolution of calls is to compare the calls of frogs within the same lineage with those from differing lineages.

As an example, table 1 shows the five types of calls that Barrio (1965, 1967) defined within the genus *Physalaemus* and the four groups into which (Lynch 1970), in his revision of the genus, arranged the species, on morphological grounds. The lack of correspondence between the two groupings is striking. Phylogeny does not seem to constrain this system in a simple way. However, the same call types do recur in related species. There do seem to be substantial phylogenetic constraints and major changes in call do not occur often.

### 3.3 *Energetic constraints*

A single frog call takes only seconds, at most, and a small amount of energy. Some frogs like the Panamanian forest species, *Eleutherodactylus fitzingeri*, may give only one or two calls in a night, but others calling nearby, like *Physalaemus pustulosus* may give 5,000 calls on the same night.

*Physalaemus pustulosus* males can be persuaded to call inside a respirometer (Bucher *et al* 1982). The energetic cost of a single call is small; an average of 0·024 joules. They will also nest in a respirometer and using these data energetic costs for a whole season can be estimated. Though a male calls many times in a night for a number of nights, he does not use as much energy in reproduction in a season as does a female (Ryan *et al* 1983). But he may be using all the energy available to him. Calling not only uses energy but it takes time that a male would have to spend feeding. In two species of Australian leptodactylids in the genus *Ranidella*, males have been shown to loose about 35% of their dry body mass in the course of a breeding season; between seasons, males gather enough energy to make up this loss but not enough to grow much (MacNally 1981). Thus, in at least some frogs, there is a tradeoff between expending energy in calling now, or using it for growth. Bigger males, as we shall see, are generally better at securing mates but a male that postpones calling in favor of growth risks dying without trying to breed at all.

### 3.4 *Risk from predators*

A frog, when it gives an advertisement call, takes a risk of attracting a predator. During the 7 nights that Ryan *et al* (1981) watched a *Physalaemus pustulosus* chorus they saw predation by crabs, opossums, bigger frogs and bats. These bats, *Trachops cirrhosus*, Tuttle and Ryan (1981) showed, were using the frog's calls to locate them.

Ryan (1985) lists several ways that a male frog might increase its probability of mating: call more intensely; more frequently; use call types that are more attractive to females; call longer; and remain at the calling site during disturbances. All of these probably also increase the risk of predation, so that bat predation is a selective force opposing the action of sexual selection. This tradeoff between probability of success

and risk of predation is an important one and we are just beginning to recognize the kinds of behavior that frogs seem to have evolved as antipredator tactics. One such is seen in the neotropical hylid, *Smilisca sila*, that does most of its calling on moonlight nights when the frog-eating bats are least active (Tuttle and Ryan 1982).

### 3.5 *Morphological constraints*

The morphology of the apparatus that a male uses to produce his advertisement call, and the auditory apparatus with which conspecifics hear it, both may influence the nature of the call.

### 3.6 *Emitter*

3.6.1 *Size and frequency:* A call is produced when air from the lungs is forced through the larynx into the vocal sac, causing the vocal cords to vibrate. The frequency of the vibrations is determined by the size of the vocal cords, their structure, and their tension (Martin 1972). The vibrations of the vocal cords seem to excite the air filled vocal sac to resonate so that some frequencies are emphasized. The frequencies at which a system resonates would be expected to decrease as its size increased.

Many groups of frogs, such as the neotropical hylids (Duellman and Pyles 1983), show an inverse correlation between size and call frequency. Slopes differ in different groups and there is substantial scatter around the line. The correlation shows that size influences call frequency but the scatter suggest that other factors are able to modify this.

Size also may have another effect. Acoustics textbooks say that, for a surface of a given area, frequencies above a threshold are radiated more or less equally, while frequencies below it are radiated less intensely. For a *Physalaemus pustulosus* male, Ryan (1985) calculated that threshold frequency should be about 3,500 Hz. Since most of the sound energy in a *P. pustulosus* call is below this, the call apparently is being radiated less effectively than it would be if the frequencies were higher. Presumably the advantages to having a call that would radiate better are outweighed by the advantages of having a lower frequency call. We will return later to what some of these advantages may be.

As we saw among species, within a species individuals in a population frequently show an inverse relationship between body size and the dominant frequency of their call. But this correlation is not nearly as ubiquitous nor always as tight as one might expect. In eastern North America, Zweifel (1968) found a significant relationship in *Bufo americanus*, but not in *Bufo woodhousei fowleri*. In a widespread North American hylid (Nevo 1969, 1973) size differences between populations have been interpreted as adaptations to differing environmental aridity and differences in dominant frequency as a direct consequence of these differences in size. There are also situations where closely related species, or isolated populations differ in frequency in ways that can not be explained by size alone. For example, there are populations of small toads that give calls of lower frequencies than those of their larger relatives (Porter 1966, 1968). Again, within, as among species, body size influences call frequency but does not determine it.

3.6.2 *Size and loudness:* Frog calls are surprisingly loud. Most species measured have calls with sound pressure levels between 90 and 120 dB at 50 cm. The loudest calls are almost painful when heard at close range.

Big frogs do not always make more intense noises than small ones. There have been three studies comparing the sound energy in calls of different species: one in Australia (Loftus-Hills and Littlejohn 1971), one in North America (Gerhardt 1975), and one in Africa (Passmore 1981). In Australia bigger frogs call more loudly than do smaller ones. Loftus-Hills and Littlejohn (1971) suggest that the size of the vocal sac may be correlated with the efficiency of coupling between the frog's acoustic generator and the atmosphere, giving an advantage to larger frogs. Likewise, the larger animals with larger larynxes and more powerful muscles should be able to produce more energy. Despite this very plausible argument, the 21 North American species (Gerhardt 1975) and the African species (Passmore 1981) show no consistent relationship between size and loudness.

Intraspecifically, Gerhardt (1975) reported a correlation between body size of male *Bufo americanus* and the loudness of their calls. However, Passmore (1981) did not find any such relationship within the African species that he studied. This seems anomolous. If small frogs can make such loud calls, why don't big frogs make even louder ones. One possible explanation lies in the very peculiar hearing of frogs.

3.6.3 *Receiver:* Frogs have very specialized, highly peculiar ears (Wilczynski and Capranica 1984). They have two areas sensitive to sound, one is homologus to that in other vertebrates; the other is unique to amphibians. Frogs have peaks of sensitivity at two, or three, different frequency bands (Capranica 1976). Advertisement calls commonly have two bands of emphasized frequencies and these coincide with peaks in hearing sensitivity. In at least some species conspecifics respond best to artificial calls that contain sound energy in both frequency bands (e.g. *Rana catesbeiana*, Capranica 1965, and *Hyla cinerea*, Gerhardt 1974, 1976, 1981).

The advantages that a male might gain from giving a call of a different frequency might be more than offset if the female's hearing was less sensitive at the new frequency.

In all species studied, the lower frequency peak in an individual is more sensitive than are the peaks at higher frequencies. When different species are compared the frequencies at which maximum sensitivity occurs goes up as size decreases. Further, Loftus-Hills (1973) showed that large species are more sensitive at their peak sensitivity than are smaller species. Thus size influences hearing so that big frogs hear better at lower frequencies than do small frogs and when compared at their most sensitive frequencies bigger frogs are able to hear fainter sounds. We saw that big frogs didn't generally call more loudly than small frogs. Is this possibly because they can hear better?

3.6.4 *What influences size:* Body size is, of course, strongly influenced by a variety of ecological factors. Among these are food, predators, competitors, mortality and fecundity schedules, and physical factors like climate. In addition, success in social interactions, particularly competition both for mates and for resources, may also influence size.

A frog's ecology influences its size and in turn its size influences sounds that it makes and hears. The reverse is at least conceivable, that selection for making or hearing certain sounds may influence the evolution of a frog's size and thus in turn its ecology.

## 4. Selective forces, passive and active

Two sorts of factors increase the effectiveness of an advertisement call. The first, Parker (1983) called passive attraction and the second, active discrimination. Factors that increase passive attraction are those that maximize transmission through the physical environment and minimize interference from ambient noises, including calls of conspecifics. Active discrimination involves differential responses to different calls even though they are equally audible.

### 4.1 *Passive forces*

The first of the passive forces to consider is transmission.

4.1.1 *Transmission:* In general, lower frequencies transmit through air better than do higher frequencies and so are audible from greater distances. Environments may, because of their structure, absorb, reflect and transmit different frequencies in more complex ways. An environment may transmit an intermediate band of frequencies better than those either higher or lower (Morton 1975).

In their review of bird vocalizations Wiley and Richards (1982) conclude that: "For maximum efficiency, long-range acoustic communication in any habitat should employ the lowest frequencies possible" p. 148.

Calls of monkeys (Waser and Waser 1977) and birds (Brenowitz 1982; Gish and Morton 1981) may be adapted to the transmission characteristics of their environments. But, when Zimmerman (1983) looked for similar adaptations in frogs in the Amazon, by comparing the calls of species from different habitats, she concluded that any differences among them that could be attributed to habitat were small and overshadowed by differences associated with differences in size and phylogenetic relationship.

Transmission experiments with *P. pustulosus* calls in the environments where it occurs showed that the call component which has more energy at lower frequencies, does in fact transmit further than does the component with more energy at higher frequencies (Ryan 1985).

Thus, in an otherwise silent environment, for maximum transmission a call should concentrate its energy in frequencies that are low, but not too low.

4.1.2 *Acoustic interference:* Frog calls aren't given in isolation. They compete with the sounds of a variety of other animals and with noises produced by wind and by water. The problem a frog confronts is akin to trying to shout instructions across a busy street. In Panama there are two bands of potential interference (Ryan and Brenowitz 1985). One from about 100–200 Hz, is produced by wind noise and a second, from about 4–7 kHz by insects, particularly cicadas and orthoptera. In the window between these two bands there may be interference from other kinds of frogs and, in some ways the most difficult to deal with, interference from the calls of conspecifics.

That a frog's ear is tuned so that it is more sensitive to the frequencies in its advertisement call reduces the interference from noise at other frequencies but the tuning of the ear is broad enough that noise interference can still be important (Narins 1982). There is direct evidence that this is true. Female *Hyla cinerea* will respond to

conspecific calls if the noise level is more than about 22 dB below that of the call, but not if the noise is relatively louder than this (Ehret and Gerhardt 1980). This is equivalent to the levels found for the few other animals that have been tested.

Wiley and Richards (1982) assert that in communities of birds there are no clear allocations of the frequencies and that, most passerine birds use approximately the same frequency band for their long-range acoustic signals. In contrast, frogs that call together, tend to use different frequency bands (Littlejohn 1977; Drewry and Rand 1983). Often there is at least some overlap between the frequency bands used, and species calling together usually also differ greatly in temporal characteristics. Usually there are differences among these species in addition to those in the calls. The species may call at different times, from different habitats, or like these same Australian frogs, from different kinds of perches. Species calling together may interact so that they partition signalling time and so reduce acoustic interference as has been reported for birds (Cody and Brown 1969; Moynihan 1963) and primates (MacKinnon 1974). Alternation between calling bouts is illustrated by the two neotropical hylids *Hyla phleboides* and *Hyla ebraccata* (Schwartz and Wells 1985).

Noise is usually a problem for a calling frog but perhaps not always. Tuttle and Ryan (1982) have argued that the neotropical hylid *Smilisca sila* uses the sounds of waterfalls to hide its calls from predators.

### 4.2 *Active discrimination—social/sexual selection*

4.2.1 *Responses of males:* Breeding male frogs may react to the advertisement calls of other conspecific males in a variety of ways.

(i) In some species, males moving to breeding sites are attracted by the calls of other males (e.g. *Ptychadena taenioscelis* in South Africa, Passmore 1976), in other species, breeding aggregations form with little or no calling (e.g. *Rana temporaria* in England, Savage 1962).

(ii) Males tend to space themselves out at calling stations in one small North American treefrog, *Hyla crucifer*, Wilczynski *et al* (1984) report that males position themselves so that each male can just hear the calls of its neighbors.

(iii) In some species at least, non-calling males may sit near calling males and attempt to intercept females attracted by the calls (e.g. *Hyla cinerea* Perril *et al* 1978).

(iv) Males are often stimulated to call by hearing the calls of other males, or even rather crude imitations or the calls of other species (e.g. *Hyla cinerea* Gerhardt 1974a).

(v) In many species, such as *Hyla microcephala* (Wells and Schwartz 1984), males react to calls of nearby conspecific males by giving aggressive calls that are responded to by males but ignored by females. *Eleutherodactylus coqui* has a two note call, the first note is responded to primarily by males as an aggressive call, the second, higher frequency note is responded to primarily by females as a mating call. There are corresponding differences in maximum sensitivity of hearing in the two sexes (Narins and Capranica 1976).

(vi) Males might by synchronizing their calls produce a louder sound than would be heard by females from further away. Certainly some frogs do synchronize their calls (e.g. *Bufo marinus*) but it is not clear that the resultant chorus is enough louder than an individual call to provide a selective advantage.

(vii) Males may time their calls so that they minimize overlap and presumably reduce

interference. A *Hyla microcephala* male responds to the call of a conspecific so that the notes of his call fall in the periods of silence between the notes of the other frog (Schwartz and Wells 1985). That the alternation of calls is due to active adjustment of timing, not the coincidental result of similar call rates as has been shown by measuring the responses to synthetic stimuli (e.g. *Eleutherodactylus cogui* Zelik and Narins 1982, *Hyla regilla* Awbrey 1978).

(viii) The calls may be structured to reduce interference. The whines of *Physlaemus pustulosus* and similar frequency modulated calls of other leptodactylids allows the calls to overlap in time and still be recognizable.

Thus males interact acoustically in ways that are more complex than the classic model of a shouting match for the attention of females.

4.2.2 *Preferences of females:* Still, attracting female attention is what the male is trying to do, and one of the advantages of studying frogs is that, in at least some species, female preferences for different mating calls can be tested directly. One captures a mating pair, separates them and introduces the female into an arena where she hears calls or other acoustic signals played through one or more loudspeakers. Many females will make a clear choice, approaching a speaker with a series of slow and deliberate hops.

There are several possible reasons why a female might select one call instead of another if both were equally audible. These include selecting a call that could be more easily located; a call of her own species; or a call that signalled a male that controlled more desirable resources, or had a better genotype.

## 4.3 *Locatability*

For a call to attract a mate, she must be able to find the sound source, as they clearly do. Konishi (1970) listed three ways in which animals could localize the direction of a sound source: sound shadows, time differences, and phase relationships. The mechanism by which frogs locate calls is not completely understood. At the relatively low frequencies that frogs use, their heads are too small to produce strong sound shadows, and the difference in times when a sound reaches the two ears is very short. A comparison of phase relationships between the two ears seems to require lower frequencies or bigger frogs than we observe. Gerhardt and Rheinlander (1980) suggested that anurans use some form of sound pressure gradient system, where directional sensitivity is caused by the interaction of two sound waves on the tympanic membrane, one acting on the external surface and the other travelling via an indirect route to the internal surface. Other neurophysiologists suggest that the situation is more complicated (Wilczynski and Capranica 1984). Speculation about how selection should act to maximize locatability will be on firmer ground when this issue is resolved.

Still, a female should select the mate who is easiest to find because by so doing, she minimizes the time and the energy she must invest. Evidence suggests that *P. pustulosus* females do this (Rand and Ryan 1981). Complex calls are preferred by females. Complex calls contain a wider range of frequencies and more transients and therefore should be more easily located. The parallel between the two components in the *P. pustulosus* complex call and with bird alarm calls evolved to be easy or hard to localize (Marler 1955) is striking. However, that female *P. pustulosus* really can locate

complex calls more easily than simple calls has not yet been demonstrated.

Increasing locatability, like increasing sound intensity, may bring gains in terms of females attracted but it also may bring increased attention from predators. The complex *P. pustulosus* calls that females find most attractive are also most attractive to the bats that hunt frogs by call (Ryan *et al* 1982).

### 4.4 *Species isolating mechanisms*

Since advertisement calls have as a primary function the attraction of a mate, one would think it important to both sexes that females be attracted to the call of their own species. That species calling together always differ in call, even when they are virtually identical morphologically (e.g. *Crinia* Littlejohn 1959), supports this idea. Cases of character displacement in advertisement calls have been interpreted as the results of selection for species isolating mechanisms in the sympatric populations. There are at least two well documented cases of character displacement in frog calls: between *Litoria ewingi* and *L. verreauxi* in Australia (Littlejohn 1965; Littlejohn and Loftus-Hills 1968) and in the *Pseudacris nigrita* complex in the southeastern United States (Fouquette 1975). However, such well documented cases are quite few. In most cases studied, the species are as distinctive in allopatry as in sympatry (Blair 1974). Paterson (1978, 1981) argues that mate recognition systems diverge only in allopatry. As Passmore (1981) restates this: "Sympatric species of frogs do not signal differently because they coexist, they are capable of coexisting because they signal differently," p. 95.

On the other hand, Gerhardt (1982) argues strongly that interactions between species are important. One of the cases he discusses is of two very similar hylids in the eastern United States. Their calls differ most distinctively in pulse repetition rate. This rate is positively correlated with the temperature of the calling male. In choice tests, females of both species prefer calls with rates appropriate to their species at the test temperature and discriminate more strongly against calls that resemble those of the other species than against those that differ by the same amount but in the other direction. This asymmetry in discrimination seems strong evidence of interaction between the two species. Selection has favored stronger discrimination where confusion with the other species is possible.

It appears that in frogs, species recognition may be an important force in the evolution of the advertisement calls, but it is clearly not the only one.

### 4.5 *Better resources*

Territoriality is much less common in frogs than in many other vertebrates, but it does occur and, in some species, larger males control better resources than do smaller ones. For example, Howard (1978a, b) has shown that territories of larger *Rana catesbyiana* have better oviposition sites, eggs laid in them have lower predation from leeches; and that these larger males are more successful at securing mates. Unfortunately, the territorial species are not easy ones with which to do female choice experiments, so we don't know if females are choosing males or choosing territories.

In a very real sense, effective fertilization is a resource that males provide for females. In at least two cases, *Bufo bufo*, (Davies and Halliday 1977) and *P. pustulosus* (Ryan

1983), certain combinations of sizes of male and female are optimal for maximum fertilization, and size-biased mating is observed. In the case of the *Bufo*, physical combat between males for females seems the important factor. In *Physalaemus* female preference is involved. Ryan (1980, 1983) tested a number of females and was able to show that they preferentially approached calls in which the chucks had a lower fundamental frequency. Further, Ryan (1983) showed that such a preference resulted in females mating with males whose size was such that the maximum number of eggs were fertilized.

Licht (1976) reported assortative mating by size in one *Bufo americanus* population and suggested that it was due to an advantage gained by the female from having a male big enough to achieve a secure axillary grip, but small enough to ensure cloacal alignment. He suggested that female choice based on dominant call frequency was involved, but he had no experimental evidence.

### 4.6 *Better genes*

Wilber *et al* (1978) reported that in several populations of *Bufo guercicus*, larger males mated relatively more frequently than did smaller ones. They advance the hypothesis "that females choose the largest males available because there is a selective advantage in large body size per se. . . . Large males may be more attractive to females because they are either older than the average male in the chorus or have had faster growth rates. Both are a sign of vigor and, perhaps, of a good genotype." p. 267. They suggest, as Licht did, that the females may be selecting mates on the basis of aspects of their calls that correlate with male size. However, Arak (1983a) reviewed the cases where size dependent and size assortative mating have been reported. He argues that "Non-random mating is not sufficient evidence for female choice since it may come about through incidental effects, such as size-related patterns of arrival at breeding ponds . . . or more likely, through male-male competition" p. 202.

The evidence of female preference for calls with lower frequencies is convincing for *P. pustulosus* (Ryan 1980); but it does not seem to occur in all species of frogs. Gerhardt (1982) argues that a species may be constrained by interactions with others and shows that in *Hyla cinerea* female choice of low frequency calls is constrained by the coexistence of another species with a similar but lower call. *Bufo calamita* in Britain does not breed in choruses with any other species of toad that uses an advertisement call. Females do not show any frequency preferences but males do (Arak 1983b).

Shine (1979) provided indirect evidence on the mating advantage of larger males. He showed that there is a correlation between those species in which males are larger than females and those in which fighting between males has been observed and/or in which the males have morphological structures that appear to have evolved for fighting.

All this argues that in many species, male-male competition is probably more important than female choice in giving large males mating advantages.

Frequency is not the only characteristic on which a female might select among calling conspecifics. There is evidence that females select the males that call more often, e.g. *Bufo woodhousei australis* (Sullivan 1982). The argument that by selecting the most vigorous males females are selecting for the best genes could plausibly be suggested here, but that it is true remains to be demonstrated.

In summary, the argument that females should be selecting males whose calls in some

way signal their greater fitness (in terms of natural selection) is persuasive; the evidence that they actually are doing so is still scant and some of it open to other interpretations.

4.6.1 *Runaway sexual selection:* Fisher (1930) suggested that selection would favor females who selected males that were attractive to other females, because in so doing they were increasing the probability that their sons would be attractive. This in turn would increase selection for the traits that made the males attractive. The positive feedback loop would continue with increasingly strong female preference and increasingly exaggerated male characteristics until strong counter-selection prevented further change.

The ways in which frog calls are subject to both active and passive selection have been discussed at length. Despite the constraints of phylogeny, morphology and the physics of sound, there is little question of the importance of female choice. There are instances of geographical variation in frog call characteristics, such as pulse rate, that can not easily be explained by differences in the sonic environment or frog size. The European treefrogs *Hyla arborea* show this kind of variation (Schneider 1977) and Gerhardt and Schneider (1980) showed that in the isolated population on the Canary Islands (*H. meridionalis*) most, but not all, females preferred calls of local males to those of males recorded in Germany. This kind of geographical variation is strong evidence for the importance of female preference in the evolution of frog calls as West–Eberhardt (1983) has argued for other organisms.

Curiously, since the evidence seems to implicate female choice as important, runaway sexual selection has not often been invoked to explain any particular frog call (an exception is Ryan 1985 for *P. pustulosus*). Indeed, do frog calls, diverse as we have seen them to be, include any cases of extreme elaboration? Is there a frog call equivalent of the peacock's tail? I would argue that selection for such extremes exists; that it is generated by competition among males, directly for mates and indirectly for resources, and by female preferences for calls that are maximally audible, minimally interfered with by other noises, locatable, and, where appropriate indicative of males in control of critical resources and perhaps with a good genotype. However, this selection is limited in its expression by the wide variety of constraints and counterselections; including the morphology of both the vocal apparatus and the hearing mechanism, the physics of sound production and environmental transmission, the presence of other sounds, and the activities of predators hunting by sound. However, the elaborate vocal apparatus and the loud and persistent calling of many frogs is itself an extreme elaboration that can only be interpreted as the result of sexual/social selection.

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# The evolution of communication and social behaviour in *Dictyostelium discoideum*

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**Abstract.** Exceptionally for a developing system, the pathways of intercellular communication are fairly well characterised in the cellular slime molds. This paper attempts to provide adaptive explanations for the origin of the following features and consequences of communication between cellular slime mold cells: the tendency to congregate, chemotaxis to a released signal, signal relay from cell to cell, oscillatory signal release and an invariant ratio of the terminally differentiated cell types. For the sake of specificity attention is directed at the species *Dictyostelium discoideum*. Central to the entire analysis is the assumption that contiguous groups of feeding cells are, and in the past were, genetically identical. It is suggested that, in respect of most of the features listed above, the critical event which started things off must have been the acquisition by the cell membrane of permeability for a substance normally produced intracellularly as a response to the stress of starvation. An argument is presented for treating social behaviour in these organisms, and in particular the suicide by cells which differentiate into stalk, as an example of group selection.

**Keywords.** *Dictyostelium*; slime mold; evolution; group selection.

## 1. Introduction

The aim of this paper is to advance adaptive explanations for some manifestations of organised behaviour in a primitive microorganism. A property will be said to be "adaptive" in the sense of being more useful for the survival and reproduction of the organism than the absence of the property and, sometimes, than other possible alternatives.

Almost by definition, communication or mutual signalling is a prerequisite for social behaviour (Wilson 1975). Excepting cases wherein sociality is only apparent and due to spatial proximity caused by other factors, the observation of social or cooperative behaviour indicates the existence of an underlying system of communication. Specifically, some form or forms of communication must be responsible for the integrated multicellular behaviour displayed by a developing organism. This leads one to the question of why any particular mode of communication, either identifiable directly or inferred from its effect on social behaviour, exists in development. In general there are two sorts of answer possible to such a question. One can think of a 'developmental' explanation, meaning basically an explanation in terms of whatever is known of the embryological process at a more basic level, ideally of its physics and chemistry. On the other hand one can aim for an 'evolutionary' explanation; that is, one can try to invoke natural selection. Seen in this light, the interesting questions which need to be answered are, in what manner is a particular form of communication adaptive? And how could it have evolved? Hardly anything is known about the details of

intercellular communication in most developing systems for these questions to be considered seriously. The cellular slime molds, especially their best studied member, *Dictyostelium discoideum*, provide an exception to this general rule (Bonner 1967; Loomis 1975).

In *D. discoideum* multicellularity arises on account of aggregation of single amoebae because of the emission of, and attraction to, a known chemical signal (Bonner 1967). During aggregation the signal is released periodically and also relayed from cell to cell (Gerisch and Wick 1975: Shaffer 1975; Roos *et al* 1975). Strong circumstantial evidence indicates that the cells continue to communicate—albeit by other, not yet understood means—in the multicellular aggregate (Raper 1940; Lokeshwar and Nanjundiah 1983). Eventually, after a series of morphogenetic movements, essentially all the cells in the aggregate differentiate into one of two types whose relative numbers are constant (Bonner 1967).

The present work is the result of an effort to look at the following 4 features of communication from an evolutionary point of view: aggregation by chemotaxis, periodicity of signal release and signal relay, patterning within the multicellular aggregate and cell number proportioning in the terminally differentiated structure. In order to fix our frame of reference we restrict our attention to *D. discoideum*. A word about methodology: the approach—not necessarily in the same order—will be to (i) describe an existing property; (ii) suggest an adaptive value for it; (iii) conjecture an ancestral state (by which is meant, somewhat loosely, a state in which the property is missing); and (iv) point out that starting with the ancestral condition, the presently existing property could be acquired via plausible mutational steps.

In what follows I first sketch the development of *D. discoideum* in just sufficient detail for our purpose and then go on to suggest specific evolutionary hypotheses for the 4 features mentioned above. General issues related to the hypotheses are discussed at the end. Comparative development in the cellular slime molds has been recently discussed in a paper by Bonner (1982) which has strongly influenced this one.

## 2. The life cycle of *Dictyostelium discoideum*

As this description is highly condensed, the literature (Bonner 1967; Loomis 1982 and references therein) should be consulted for a fuller picture. *D. discoideum* is a free living soil amoeba. Controlled experiments performed in the laboratory enable one to build the following plausible account of its life in nature (figure 1). In their vegetative phase, individual cells feed on bacteria, grow and divide by mitosis. Under extreme environmental conditions cellular slime mold cells are capable of following one of two protective strategies, that of forming microcysts or macrocysts. Both are resistant structures. Microcysts are not known as yet in *D. discoideum*, and macrocysts are the first step in the sexual pathway. Since they are not formed under standard experimental conditions, we will not refer to them any further (Bonner 1982 has considered their possible significance). In the laboratory, amoebae starved of food go through a seemingly quiescent phase for a few hours prior to aggregating. Aggregation is by means of chemotaxis, usually accompanied by relay of the chemoattractant. The attractant is a chemical, cyclic AMP (cAMP), which is produced and periodically released by the cells. A cell which senses external cAMP moves towards its source and in turn itself releases a burst of cAMP. Consequently local variations in cell density get amplified. Ultimately

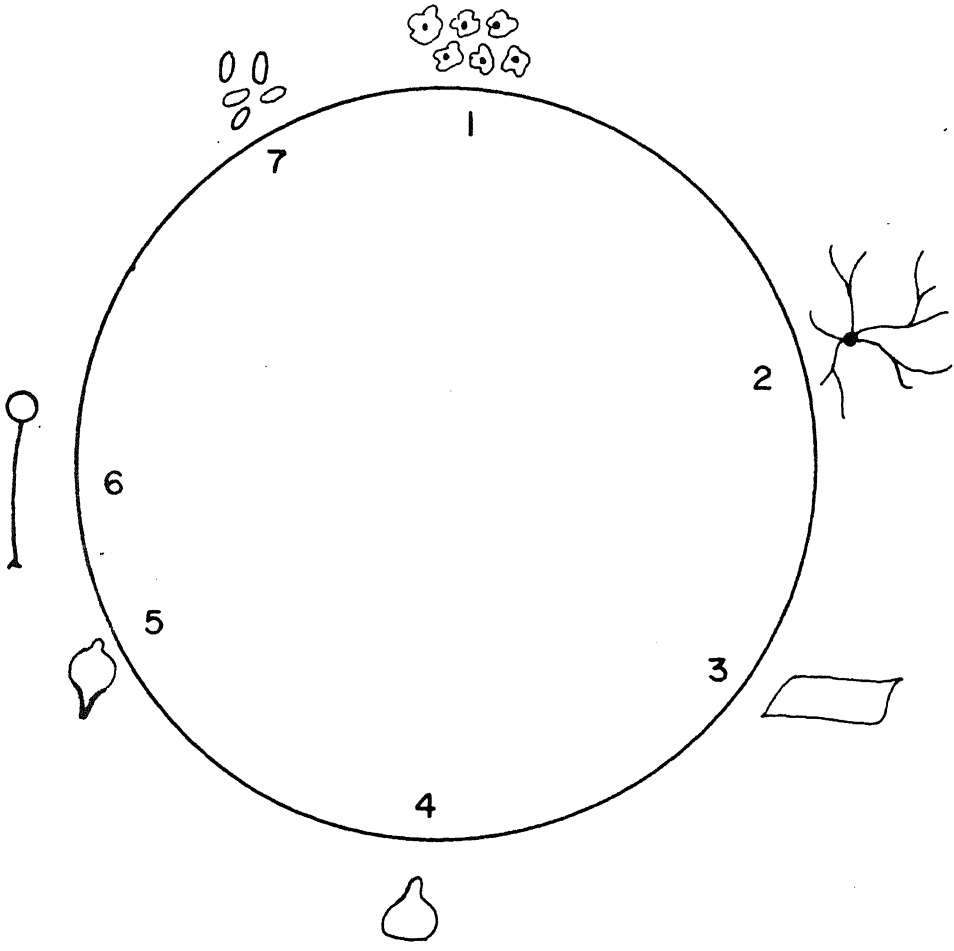


**Figure 1.** Schematic representation of the life cycle of *D. discoideum* (not to scale). (1), Free-living amoebae; (2), aggregation; (3), migrating slug, anterior to the right; (4), early culmination; (5), advanced culmination; (6), fruiting body with spore mass held aloft stalk; (7), spores.

all the cells in a neighbourhood end up piled on top of each other in a mound which is held together by specific adhesion molecules. The aggregate—now called the slug—often falls down and adopts a cylindrical form with a nipple-like protrusion, the tip, at its anterior end. After moving for a length of time which depends strongly on external conditions as well as the genotype, the slug stops, reverts to a mound-like shape, and the cells in it move in the manner of water in a reverse fountain and the entire mass rises upwards (the words 'up' and 'down' mean away from the two-dimensional substratum on which aggregation has occurred, or towards it). Irreversible differentiation sets in concurrently, most of the cells either turning into spores or, alternatively, dying and turning into the units of a cellulose-sheathed stalk. The final structure, called the fruiting body, has a disc-shaped base (also made of dead cells), an erect stalk and, above the stalk,

a spheroidal mass of spores. Under suitable conditions each spore can germinate and give rise to an amoeba, setting off the life cycle anew.

## 3. Aggregation

If not the earliest, aggregation is certainly the most striking example of social behaviour in the cellular slime molds [mutual repulsion, observed in the vegetative phase of some slime molds, is conceivably a device for maximising the efficiency of feeding (Keating and Bonner 1977)]. Since starvation is the primary trigger, it seems intuitively reasonable to suppose that aggregation, like the coming together of individuals in more evolved social groups, must be a behavioural trait which has evolved to improve the chances of survival of the individual itself or of its genetic relatives. Therefore one must make it plausible that because of aggregation there is an increase, either in the probability that an amoeba turns into a viable spore or in the probability that a spore disperses to a favourable environment. A large mass of spores probably stands a better chance of dispersal than many isolated spores (Bonner 1982). In a fruiting body, just the fact that the spores are on an elevation should also help in dispersal, and we will take this up later. That apart, the assumption that aggregation might enhance sporulation seems justified, since cell-cell contact is normally a requirement for development, and so for sporulation (Gregg 1971; Newell *et al* 1971; Takeuchi and Sakai 1971). Precisely how contact aids development is not known; one possibility is that the binding or recognition of sets of cell-surface molecules is necessary. Alternatively, from observations on mutants which can sporulate in the absence of contact if simulated by exogenous cAMP (Kay and Trevan 1981), we might conjecture that 'contact' really means a degree of proximity sufficient for some substance emanating from one cell to reach a level at the site of a second cell high enough to induce the latter to turn into a spore (extracellular cAMP by itself is a non-specific differentiation signal in present-day *D. discoideum*; in conjunction with other substances it can influence either stalk or spore formation (Morrissey 1982)). The suggestion is, then, that in the ancestor of *D. discoideum* a single cell could sporulate in isolation but that the process could be helped along by a stimulus emanating from another cell in the same condition. What sort of substance would be appropriate to provide such a stimulus? A natural candidate would seem to be a chemical which was already being synthesised in the (ancestral) isolated cell during sporulation. Such a substance would reinforce a cell's response to starvation if, in addition to being produced intracellularly, it moved from one cell to another through a 'leaky' membrane. The leak itself would be selected for when it first arose by mutation. This would be so since sufficiently small clusters of post-feeding amoebae very likely consist—and consisted—of clones; the mutation would be simultaneously expressed in an entire group of cells. The possibility that the leak could be disadvantageous to a cell on account of its losing some of the signal substance must have been compensated by the benefit it conferred when it entered and stimulated other, genetically identical, cells. To sum up, a group of amoebae which stayed together after feeding was over would hold a selective advantage over one whose members continued to move randomly and so drifted apart. Thus the mechanism of 'aggregation', when it first arose, may have been nothing more than a decrease in cell motility once the density of food became too low to be reliably sensed; the effect may have been further strengthened by the acquisition of the ability by cells to adhere to one another.

## 4. Chemotactic attraction

Continuing with the line of argument we have used so far, it is not difficult to see how mutual attraction, by decreasing the spatial spread of post-vegetative cells, would also have been selected for. The agent of attraction could either be the same one that enhanced sporulation or, perhaps, a substance which worked inside the cell and guided cell movement by acting on contractile filaments, thereby causing local psuedopodial extrusion (the observed rapid accumulation of cytoskeletal actin in *D. discoideum* following an external cAMP stimulus (McRobbie and Newell 1984) could be a pointer to the original situation). The same substance entering from outside would have the same effect, but this time in a definite direction. A mutation which made the cell membrane porous for this substance would polarise cell movement centripetally towards wherever the cell density was slightly higher than in the neighbourhood, or wherever—if development were not perfectly synchronised in all cells—the first cell to leak out the substance was situated.

Until now I have tried to suggest how non-directed aggregation could be adaptive in itself, and how—by improving the efficiency of aggregation—a further beneficial adaptation could be conferred by either the simultaneous and mutual attraction of all cells in a neighbourhood or the attraction of most of the cells towards a center.

## 5. Relay

Consider now the possibility that a primary chemotactic stimulus might excite a sensitive cell to act as a secondary source of the stimulus. The advantage of having an attractant relayed outward from a centre, rather than having it released at about the same time by all cells, would seem to be the prospect of increasing the size of an aggregate. Since the signal should have the same range whether it is released by a cell on its own or whether a cell releases it in response to an external stimulus, it appears that the ability to relay would not have been favoured if all cells had—in the course of their development—started to signal simultaneously. In the absence of a means for synchronising development, simultaneous signalling does seem unlikely; the special care needed to achieve developmental synchrony in the laboratory suggests this. Therefore, in order to associate a selective advantage with the ability to relay, it needs to be assumed that under natural conditions some cells in a group are developmentally more advanced than others and, specifically, are capable—purely on statistical grounds—of releasing the chemoattractant earlier than most others. Given that relaying the chemotactic signal causes aggregates to increase in size, how might this be beneficial? It could be that large aggregates, with large volume-to-surface ratios, imply a reduction in the amount of the hypothetical substance which leaks to the outside world and is lost. For the same energetic investment, the yield in terms of effective signal strength would be higher. Another possibility, alluded to earlier (Bonner 1982), is that a large aggregate might improve the prospects for spore dispersal. Significantly, large slugs of *D. discoideum* move faster than small ones (Bonner *et al* 1953). Consequently if the time available for dispersal (before sporulation begins) is limited, the distance of dispersal would be greater for cells contained in the larger of two slugs. If this is to be used as a justification for increased aggregate size *via* relay, one will have to make the further supposition that

aggregate motility existed earlier, probably having being selected as an aid to dispersal by itself.

There is another way of looking at the phenomenon of relay (Nanjundiah 1978). So far we have assumed that chemotaxis-mediated aggregation came first and that relay was a later refinement. Suppose things had been the other way round: consider a stage in evolution at which a clone of cells, lying close to one another, were faced with starvation. Just as we made a case for the original aggregation signal being a pre-existing component of the cell's internal response, we can argue that the cell-to-cell transfer of a substance, until then produced inside the cell in response to starvation and remaining there, would have enabled an entire group of cells to cope with a deteriorating environment faster than they would have in the absence of relay. Both clustering and chemotactic attraction could have arisen, in this way of looking at things, as later adaptations. Note that the first hypothesis (relay as a device for increasing the range of the chemotactic signal) makes it essential that the relay substance and the chemoattractant be the same (as is indeed the case in *D. discoideum*); on the second hypothesis, though it would probably be of advantage to have them the same, they could well be different.

An interesting point regarding the origin of relay is that on the second hypothesis the relay substance must have been able to activate its own synthesis. In other words, the reaction pathway responsible for forming the substance must have had in it an autocatalytic (positive feedback) step. The critical mutational event would then have been, as already suggested, a specific membrane 'leak'. If, on the other hand, chemotactic aggregation had arisen as an earlier adaptation (the first hypothesis), the important mutation would have been the one which made chemoattractant synthesis autocatalytic: that is, an early step in the synthetic pathway would need to be activated by a later step.

## 6. Oscillations

Given relay, and therefore an autocatalytic step; given that the living cell is thermodynamically an open system; and given that most biochemical reactions *in vivo* operate far from equilibrium, oscillatory reaction fluxes are almost a "natural" consequence (Higgins 1967), meaning that no special adaptive explanations are called for. Goldbeter and Segel (1977) have in fact shown that the observed cAMP oscillations in *D. discoideum* can be successfully modelled by assuming that the condition for relay exists, that is that an extracellular cAMP stimulus activates the intracellular synthesis of cAMP. Oscillations are on the other hand ubiquitous in biological systems (Winfree 1980); in particular, morphogenetic oscillations are found in myxobacteria (Kaiser *et al* 1979) as well as in other cellular slime molds, possibly with the involvement of cAMP (Schaap and Wang 1984). Even temporal patterning in chemical communication, though rare, is not unknown (Conner *et al* 1980). So it is worth considering whether the relatively rapid (period ca. 8 min) oscillations of cAMP in *D. discoideum* might be adaptive after all.

Under certain conditions, the course of glycolysis in yeast is oscillatory, also with a periodicity of some minutes (Hess and Boiteaux 1971). Since these oscillations involve the adenine nucleotide pool and therefore the adenylate charge (Atkinson 1968) of the system, Goldbeter (1974) has conjectured that the cell benefits by partitioning each oscillatory cycle into distinct energy-utilising and energy-yielding phases, something which is supposed to make for an improved metabolic efficiency. How far this argument can be carried over to cAMP oscillations in *D. discoideum*, where ATP levels do not vary

significantly within a period (Gerisch *et al* 1977), is not certain. Richter and Ross (1981) have offered yet another adaptive explanation for the glycolytic oscillation. Using a quantitative model they have calculated that energy dissipation in the latter part of glycolysis (the pyruvate kinase step) is minimal precisely when an earlier step (the phosphofructokinase reaction) is periodic with a frequency within a defined range. Again, one does not know how good this explanation, depending as it does on the "tuning" of one reaction step by another, would be for the slime mold oscillations. The oscillations in this case occur—as far as is known, unlike glycolysis in yeast—both extracellularly and inside the cell, and it is easier to speculate on a possible adaptive role for extracellular oscillations. It has been shown (Nanjundiah 1973) that when the relevant parameters are assumed to have reasonable values, a pulsatile source of cAMP has a spatial range which is about an order of magnitude more than the range of an equivalent steady source which releases cAMP at the same average rate. The reasoning is based on the facts that (a) (in three dimensions) the concentration of a signal diffusing from a steady source falls off inversely with the distance, whereas the peak concentration due to an impulsive source decreases as the cube of the distance, and (b) the lower the frequency of successive pulses, the weaker the effect of the equivalent steady source. Then, given a plausible threshold for the sensitivity of a cell, either for the concentration of cAMP or for its spatial gradient, the threshold is reached at a farther distance from the source when the signal is released in a series of brief pulses than when it is released at a uniform rate. The conclusion remains valid even when the released signal profile is no longer a sharp pulse but is somewhat sinusoidal. However, in the latter case the range of the signal is enhanced by roughly a factor of two rather than by one order of magnitude. A periodic signal has two other advantages. One is that the receiver would not get adapted to it as readily as it might to a steady source. It is to be expected that a train of stimuli reiterated at an appropriately intermediate frequency would be much better at eliciting a response than the same train of stimuli applied either at a very low frequency (in which case each stimulus would—so to speak—be like the first stimulus, there being no reinforcement) or at a very high frequency (in which case there would effectively be one constant stimulus, to which the system could adapt). Just this has been observed in the case of *D. discoideum* cells with folic acid, a chemoattractant for feeding cells (Wurster and Schubiger 1977). Also, periodic pulses of cAMP are efficient at eliciting cell differentiation whereas steady levels are not (Gerisch *et al* 1975; Darmon *et al* 1975). Unfortunately, none of these experiments tested the effect of irregular pulses or different waveforms; one is still unsure as to precisely which stimuli constitute signals and which ones do not. The other advantage of a periodic signal is that at close distances there would be a lesser degree of ambiguity in locating a periodic source than a steady source (the amplitude of oscillations will, in any case, get damped with increasing distance from the source, the higher frequencies dropping out first).

## 7. Pattern formation and tissue proportioning

### 7.1 *Pattern in the slug*

The slug is a facultative migratory structure formed by an aggregate of *D. discoideum* before it differentiates into a fruiting body. It is in some ways a preparatory phase; the

future stalk and spore cells are identifiable in its front and rear portions (Raper 1940). Depending on the type of experiment carried out, this separation into pre-stalk and pre-spore can be seen either as an expression of autonomous cellular predispositions (Takeuchi *et al* 1977) or as a consequence of the relative positioning of cells along the long axis of the slug (Raper 1940). Probably both factors are important and reinforce each other. Whatever be the cause, a spatial separation of presumptive cell types occurs within a few hours after aggregation is completed. It is difficult to avoid the conclusion that this spatial segregation must have co-evolved either with, or following, the evolution of the process of fruiting body formation and the geometry of the fruiting body. The chain of events could have been first, the transition of an aggregate into a fruiting body of a certain structure (a spore mass held aloft on erect stalk) and later, the appearance of an intermediate migratory phase in which the form of the fruiting body was anticipated. Migration would most likely be on account of an extension in time of the mechanism of amoeboid movement. However, morphogenesis in two other cellular slime molds displays features which indicate that this cannot be the whole story. In *Dictyostelium mucoroides*, stalk cells are formed and continuously released from a common pool consisting of all the cells in the migrating slug; in *Acytostelium* the stalk, which is acellular, is made up of material extruded from cells. Bonner (1982) treats this in some detail and goes on to speculate that patterning might be related to some developmental constraint imposed by the use of cAMP in chemoattraction.

It must be mentioned that the reasons for believing that cells in the slug communicate with one another go beyond the facts of integration (in a general sense) and pre-stalk—pre-spore patterning (in particular). The oldest and most convincing reason is that slugs belong to the class of regulative embryos; a fragmented slug gives rise to a diminutive but normally proportioned fruiting body (Raper 1940). Regulation was probably an adaptation in response to the selective pressure provided by physical fragmentation (Bonner 1982). Quantitative studies (Lokeshwar and Nanjundiah 1983) directed at a very early event in regulation—the regeneration of a new tip at the anterior margin of tipless fragments—indicate the involvement of long-range communication within the slug. How communication actually occurs is not known; the evidence from experiments on the rate of tip regeneration in genetically mosaic slugs is in agreement with, but does not prove, cell-to-cell relay (Lokeshwar and Nanjundiah 1985). If this result is confirmed, and if the same signal is responsible for both the rate of tip regeneration and long-range communication in the slug, one might conjecture that cell-to-cell relay during aggregation was a preadaptation which facilitated patterning in the slug.

### 7.2 *Tissue proportioning*

Under constant environmental conditions the number of spore cells in a fruiting body is in a more or less constant ratio to the number of stalk cells. The data of Stenhouse and Williams (1977), as analysed by Lokeshwar (1983), shows that $87{\cdot}6\,\% \pm 4{\cdot}5\,\%$ of the cells in small fruiting bodies (630–3901 cells) form spores; the corresponding figure for large fruiting bodies (8404–18301 cells) is $87{\cdot}1\,\% \pm 4{\cdot}8\,\%$. Lokeshwar also makes a case for neglecting, in comparison with stalk and spore, the number of cells which form the basal disc (less than $1{\cdot}5\,\%$ of the total); his own studies reveal no undifferentiated amoebae in the fruiting body. Two questions follow: (i) Why is the ratio of spores to stalk constant? (ii) Why do a certain fraction of cells—those that form the stalk—die and so sacrifice

their genetic potential? Wilson (1975) and Bonner (1980) cite stalk formation in *Dictyostelium* as an example of altruistic behaviour and invoke kin selection in order to explain it. The following simple argument (V Nanjundiah, unpublished results) makes this explicit. However, I suggest (see later) that 'group selection' is a more appropriate term than 'kin selection' to describe the phenomenon.

The critical assumption will be that, at least under the conditions obtaining when the present form of the fruiting body first evolved, an aggregate consisted of genetically identical cells. The problem facing this aggregate would have been to transfer all or part of itself elsewhere because food was finished at the site of aggregation. Migration of the entire aggregate towards food (not known to occur in *D. discoideum* slugs), might have been a possibility. The alternative, given that some of the cells had committed themselves to form spores, would have been to ensure that as many spores as possible dispersed and helped to give rise to viable amoebae. With an erect stalk and a spore mass on top, the distance of dispersal would depend on the elevation of the fruiting body and so on the number of stalk cells, but too high a stalk would mean that there would not be many spores left to propagate. An optimal balance would be reached at some intermediate value for the proportion of cells turning into spores. The actual ratio of spore to stalk at this optimal balance will depend on the mode of spore propagation—which could be by means of water, insects or other small animals, or wind. Purely as an illustrative exercise let us imagine that the spore mass is detached by a gust of wind and free-falls on account of its own weight (turbulence being neglected). The distance of dispersal will be the horizontal distance covered by the mass in the time it takes to fall to the ground. For a fruiting body consisting of $N$ cells of which $N_1$ are spores and $N_2 = N - N_1$ make up the stalk, the height of the structure will be proportional to $N_2$. Then the time of fall, and so the distance covered, will be proportional to $\sqrt{N_2}$ or $\sqrt{N - N_1}$. Now, suppose that the availability of food, and so the probability of a spore germinating, is proportional to the distance of dispersion. A single spore, $N$ of whose clonal descendants formed a fruiting body, would give rise to $N_1 . \sqrt{N - N_1}$ (times some constant) spores. It is easily seen that the product $N_1 . \sqrt{N - N_1}$ is a maximum when $N_1 = \frac{2}{3} N$, that is, at a fixed ratio of $N_1$ to $N$. Therefore according to this highly simplified model, $\frac{2}{3}$ of the cells in an aggregate should differentiate into spores and the rest into stalk if reproductive fitness is to be maximised. This conclusion still leaves us with the difficult problem of speculating on the possible mutational steps leading to proportioning. An intriguing but attractive possibility would be for proportioning to have evolved in parallel with patterning and spatial segregation of presumptive cell types, either in the late aggregate or in the early fruiting body.

## 8. Discussion

I have tried to provide adaptive explanations for some observed and inferred examples of communication between cells of *D. discoideum*. The sequence of evolutionary stages is suggested to have been as follows (table 1): sporulation of single spores → non-specific aggregation and sporulation in a group → aggregation by chemotaxis → relay of chemoattractant and oscillations → formation of the fruiting body with division of labour into stalk and spore → motility and patterning in the aggregate. Relay could have preceded chemotaxis instead of following it. The types of mutational events which could

**Table 1.** A hypothetical sequence in which the later mutational events enhance, but do not otherwise interfere with, the effects of the earlier ones. The initial situation is supposed to be one in which cells sporulate individually when starved; 'X' is a substance synthesised intracellularly in the course of sporulation. For simplicity X is assumed to be the same in each step; this would correspond to the roles played by cAMP in *D. discoideum*.

| Effect of mutation | Consequence | Possible reason(s) for increase in fitness |
|---|---|---|
| Starvation-induced leak of substance ('X') through cell membrane | X can enter a cell from outside | (a) Improved ability to sporulate;<br>(b) Cell responds to deteriorating environment more rapidly than it would have |
| Tendency of cells to stick to each other | Cells form clumps | Improved prospects for (post-feeding) dispersal, but probably also a decrease in feeding efficiency |
| Stickiness inducible by X | Cells clump only after feeding is over | As above, but no decrease in feeding efficiency |
| Chemotaxis to X | Cells actively congregate | Eventually, better dispersal |
| X relayed from cell to cell | Aggregate size increases | As above |

have led to each new stage have been conjectured. In this discussion I confine myself to making a few general points regarding these explanations.

Two questions which occur at the outset are, how reasonable are adaptive explanations in this context? And how reasonable are the arguments that have been advanced suggesting a specific form of selective force for the appearance of a particular phenotypic trait? The answers to both of these questions will necessarily be indirect and partial, and so not conclusive.

Cellular slime molds differ quite a bit in their life cycles, but aggregation and the formation of fruiting bodies consisting of spore masses held aloft stalks are common to all species. The reason why the details of development differ from one species to another could have to do either with differing adaptations to different selection pressures or to the same selective pressures leading to more than one adaptive peak. The possibility that the more "primitive" species are in the process of evolving towards the *D. discoideum* state cannot be ruled out, but appears to be unlikely. More relevant, the adaptive explanation itself might be incorrect or, at any rate, no less likely than a non-adaptive one (Gould and Lewontin 1979). It has already been mentioned that the phenomenon of oscillations is a possible candidate for a selectively neutral trait, even though a plausible adaptive explanation exists. The extracellular release of a cyclic AMP phosphodiesterase might be a second such candidate; arguments for and against the viewpoint that this is a nonadaptive (or maladaptive) trait will be found in the literature (Nanjundiah and Malchow 1976; Gerisch 1976; Darmon *et al* 1978). So also the widespread occurrence of regulation—the constancy in relative proportions of adult cell types—

might well have a purely developmental, as opposed to evolutionary, explanation.

All the same, in the absence of a specific rival hypothesis—based on selective neutrality, developmental correlations, even maladaptation—presenting itself, experience indicates that it is a useful exercise to see whether an adaptive explanation can be constructed at all and if it can, whether the construction is natural or forced.

A necessary (though not sufficient) condition for a trait to have evolved by selection amongst differentially adapted phenotypes is that it be subject to genetic control; and a "natural" construction of an adaptive explanation is one that makes use of plausible mutational steps. In the case of intercellular communication in *D. discoideum* both these requirements are satisfied. Consider the following properties of known mutations in *D. discoideum*: (i) an ability to differentiate without cell-to-cell contact (Kay and Trevan 1981) or normal aggregation (Ishida 1980); (ii) a failure to produce cAMP (Bonner *et al* 1969); (iii) a failure to respond to cAMP by chemotaxis (Bonner *et al* 1969); (iv) an inability to amplify and so relay an external cAMP signal, with the consequent absence of oscillations (Wurster and Bumann 1981); (v) a failure of normal stalk-to-spore proportioning (Morrissey *et al* 1981); and (vi) the absence of a correlation between the spatial patterning of the presumptive cell types in the slug, which can be normal, and proportioning in the fruiting body, which can be highly aberrant (Morrissey *et al* 1981; Amagai *et al* 1983). The intention is not to suggest that these mutations must represent a reversion to an evolutionarily primitive situation, but rather to indicate the plausibility of the origin and evolution of (present-day) wild-type genes with the properties demanded. It must be admitted that in one respect this could be a misleading argument: because selection acts on the genotype and not on single genes, or in other words because the relation between genes and phenotypic traits is normally not one-to-one, the life cycle must have evolved as a whole—and not, as in a sense we have imagined, in bits and pieces. It might appear that caution is all the more warranted when discussing *D. discoideum*, given the manifold effects that extracellular cAMP has on its development (Loomis 1982). Nevertheless, if the effect of a mutation is such as to influence one aspect of the phenotype more than any other, and if the mutation arises early enough in evolution for phenotypic effects to be significant, one can assume, as a good first approximation, that the components of (in this case) the communication pathway have been independently selected (table 1).

Even if we accept that adaptive explanations for the various features of intercellular communication we have considered might be valid, could the same end result have been achieved by other selective forces? The forces we have taken into account have been (a) selection for an improved efficiency of sporulation and (b) selection for dispersal. The assumption which runs through our entire analysis is that spatially contiguous, genetically identical amoebae form the units on which selection can act. Bonner (1967) starts with the opposite assumption—that in nature cells of diverse genotypes commonly come together in an aggregate—and comes to quite different conclusions regarding the function of aggregation. One is that aggregation serves as a partial substitute for sexuality, because the nuclei of the many genotypes gathered together in one spore mass have the potential to be redistributed as new combinations. He also suggests that genetically diverse cells living in close proximity might have developed mutual deficiencies which could have been overcome by aggregation. There are hints from the literature that two other selective agencies, predation and the occurrence of symbiotic or commensal organisms, might favour aggregation and fruiting. Waddell (1982) has reported the isolation, from bat guano, of a species of predatory cellular slime

mold. When tested in combination with a number of other species, the predator is able to delay the morphogenesis and (very probably) consume the amoebae of the prey species. Ellison and Buss (1983) have found a case of synergism between field specimens of *D. mucoroides* (a species similar in many respects to *D. discoideum*) and the fungus *Mucor hiemalis*: the presence of the fungus induces stalk formation in *D. mucoroides* and fungal extracts advance rate of fruiting in this and other species (though not in a laboratory strain of *D. discoideum*, which conceivably had already been selected for very rapid development). The stalkless variant of *D. mucoroides*, which forms in the absence of the fungus, aggregates normally but neither forms a slug nor migrates. The aggregating mass rounds up and differentiates straightaway into a mixture of spores and dead amoebae. While it is not possible at present to seriously consider which features of communication in *D. discoideum* might have been selected as adaptations to predation (to discuss commensal living is even harder), we can imagine that anything which lowered the risk of predation must have been strongly favoured. So, for instance, both the tendency to form fruiting bodies as well as an increase in the overall rate of development would have helped (rate, because in a communication network the same set of events can have different effects on fitness if the temporal relationships of the events differ (Bonner 1982)). Both environmental conditions (Schindler and Sussman 1977) and the genotype (Kessin 1977) can influence the life cycle of *D. discoideum* by affecting the speed of development. Similarly, using a substance which was harmful to a predator as an agent of communication or using a misleading signal (imagine that cAMP is an alarm pheromone for some predator) would clearly improve the prospects of survival.

It is important to note that the speculations in this paper refer only to the first steps in the evolution of pathways of communication in *D. discoideum*; what one finds today must be the result of many further steps of refinement. The most important refinement was probably the origin of receptors (Newell 1977). Receptors would enable signals to work their effect without having to enter a cell, allowing for increased efficiency and specificity. Also, the same substance used as a signal could then serve different ends, depending on which subset of receptors it happened to interact with at any given time. Other refinements could result in the signal becoming ritualised as has happened in higher animals (particularly in the case of courtship signals; see Wilson 1975). The signalling pathway would pick up successively arbitrary modifications and develop into a link between source and receiver which is highly reliable and specific but displays few clues as to how it could have evolved. Whatever be the extent to which the primitive signal has become ritualised, if the view adopted here is correct it must follow that all the conjectured "signal substances" must be components of the cell's internal response to a deteriorating environment. Haldane (1955) has asserted as a general principle that a signal is always a sign of a physiological or psychological condition within the sender; what I am suggesting is that in the present context the signal is so to speak the condition itself. To take a specific example, the choice of cAMP as a chemoattractant would not be totally arbitrary (in the sense that human language is said to be), but would be related to a role played by cAMP in the internal metabolism of a starved cell, even if the role were that of a waste product to be discarded. This is a testable statement.

Cellular slime molds are social organisms, and the origin and continued evolution of sociality all the way to the extreme altruism displayed by stalk cells must surely be a consequence of, in part, the high degree of genetic relatedness, or even genetic identity, of the amoebae in an aggregate (Wilson 1975; Bonner 1982). Nevertheless, I believe the

term 'group selection' is better suited to describe the situation than 'kin selection'. Kin selection is usually invoked when an individual sacrifices part of its potential reproductive fitness for the benefit of another individual related to it by common descent (Hamilton 1964). In the slime mold case, leaving aside the suicide by presumptive stalk cells for the moment, each of the adaptations we have considered has the characteristic that it is adopted by every member of the group; therefore its cost or benefit is the same for every member. It is the group which must succeed or fail, in comparison with other groups, with these adaptations. Even if this is not true at any one time because an aggregate consists of more than one genotype, it must be a factor which comes into play once the less fit genotypes have been eliminated (we take it of course that the genotypes differ in respect of the adaptations referred to in this paper). An implicit assumption is that all pre-fruiting cells are functionally identical: if this were not so, 'kin selection' might well be an appropriate description. An important example of functional non-identity would be if, for example, only the future stalk cells were to release the chemoattractant.

What if a cell belonging to a cheater genotype fortuitously gets into an aggregate, a genotype which responds to external signals with the rest of the group but does not produce the signal itself? Armstrong (1984), in analysing the survival of "cheaters" which never form stalk cells, suggests that division of labour would not be an evolutionary stable strategy under conditions which favour such cheaters. I feel this side-steps the basic problem, which is to explain why division of labour is observed even though a "cheater" (of whichever sort) is always at an advantage whenever it arises. The correct answer, according to me, is that the frequency of the cheater genotype will increase from one generation to the next until an aggregate consists of so many cheaters that the strategy does not pay any more. If the cheater only responds to signals released by the wildtype but does not produce them itself, at some stage the signal strength will be so weak essentially everywhere except at the locations of the signalling cells that viable aggregation will simply not occur. If the cheater always differentiates into spores the final state in evolution will be an aggregate consisting only of mutant spores; in line with the assumption under which we have operated the spores will be unable to disperse and therefore to survive. The cheater genotype increases in fitness but eventually drives the group to extinction. Therefore the normal division of labour is best described as a case of group selection.

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# Communication and synchronization of circadian rhythms in insectivorous bats

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**Abstract.** There is communication and social synchronization of the circadian rhythm in the flight activity of the microchiropteran cave-dwelling bat *Hipposideros speoris*. Thus captive bats surrounded by free-flying conspecifics synchronize their activity to the colony activity. The circadian rhythm of a solitary bat in a solitary cave freeruns. Even the rhythm of an 'alien' bat (*Taphozous nudiventris kachhensis*) held captive in the hipposiderid bat cave freeruns. But the rhythms of a closely-related species, *Hipposideros fulvus* partially entrain to social cues from *Hipposideros speoris*. Social synchronization of circadian rhythms in bats may be species-specific. This synchronization is abolished when continuous light of 10–20 lux is shone inside the natural cave.

**Keywords.** Circadian rhythms; bats; ultrasonics; communication; synchronization.

## 1. Introduction

Circadian rhythms, in nature, entrain to the light-dark cycles (LD cycles) generated by sunrise/sunset. If the organisms displaying such entrained 24 hr rhythms are brought into constant conditions of continuous light (LL) or constant darkness (DD) and invariant temperature of laboratories they 'freerun' (Moore-Ede *et al* 1982). Freerunning circadian rhythms have also occasionally been observed to be induced by the LL of the arctic summer and DD of the arctic winter (Swade and Pittendrigh 1967; Mueller 1968; Erkinaro 1969). LD cycles are clearly the most universal and dominant of all zeitgebers (synchronizers). It is now becoming clear, however, that there may be zeitgebers other than LD cycles. Cyclic variations in temperature (Hoffmann 1968), cyclic availability of food (Aschoff 1981), noise-silence cycles (Sulzman *et al* 1977), the state of the tides in the oceans and even the phases of the moon may act as zeitgebers (Neumann 1981). Apart from these abiotic fluctuating factors there are also biotic factors such as bird songs (Gwinner 1966; Menaker and Eskin 1966), mother-pup interaction (Davis 1981; Viswanathan and Chandrashekaran 1984) and such other social stimuli that can synchronize circadian rhythms. In humans social zeitgebers are more effective than physical zeitgebers (Conroy and Mills 1970; Aschoff 1981; Minors and Waterhouse 1981). We have been investigating (Marimuthu *et al* 1978) how the members of a colony of microchiropteran bats inhabiting a true cave under DD and constant temperature and constant humidity conditions still time their activity. Such conditions normally as already explained release circadian rhythms into freeruns.

*Abbreviations used*: LD, Light-dark cycles; LL, Continuous light; DD, Constant darkness; $\tau$, Period length (time between onset of activity from one day to the next); CF, constant frequency; FM, frequency modulation.

Experiments with trapped bats and flight activity monitoring inside a few such caves indicate that there is clear-cut evidence for social synchronization of the circadian rhythm in the bat *Hipposideros speoris* (Marimuthu *et al* 1978, 1981). Bats held captive some 40 m inside a cave in DD still began their nightly activity to coincide with the onset of the foraging activity of the colony.

We now have information that the circadian rhythm of *Hipposideros speoris* is indeed being synchronized by social communication and that such social communication is inexplicably operant in DD but not in LL.

## 2. Materials and methods

### 2.1 *Study site*

The site where the observations and experiments were carried out is a 'true cave' (cave 1), i.e. a cave with just one opening (Twente 1955), situated in a rock complex close to the Madurai Kamaraj University campus (lat. 9°58′ N, long. 78°10′ E). The cave has several labyrinthine ramifications 15–50 m from the cave mouth, which opens on the northern flank of the rock complex. The bats use several of these pockets as their daytime roosting place. We chose a site, ca. 40 m inside the cave, that showed great constancy of temperature (27°C ± 0·5°) and relative humidity of 95% (Lambrecht-Goettingen thermohygrographs) and was absolutely dark (no light measurable over periods of 1,000s even on the energy scala log scale of a United Detector Technology Optometer). A second cave (cave 2) was situated in the same rock complex but with the cave mouth on the southern side. The conditions obtained in cave 2 were very similar to the conditions in cave 1; temperature was constant at 30°C, relative humidity was 85% and darkness complete. Since the depth of cave 2 was only approximately 5 m an artificial mud wall had to be constructed and a black cloth curtain erected to ensure absolute darkness.

### 2.2 *Recording techniques*

Our behavioural observations and feeding of captive experimental bats were made using a noctovision apparatus with a far-red source of light projection and a viewing scope-screen. The bats did not respond to the switching on of the noctovision by either turning their heads towards the light source or flying away. We also used battery-powered torch lights with a combination of filters transmitting red light of > 610 nm, which seemed not to disturb the animals as much as white light.

Bats were captured on their return flight to the cave in the early hours of the morning before sunrise and placed in light aluminium-framed activity cages wrapped on all sides with synthetic gauze material. The dimension of the cubical cages (30 × 30 × 30 cm) permitted flapping flight. Bouts of flight jiggled the cages, which were suspended from the arms of a metal column held by retort stands. The movements of the cages were transferred directly to mechanically wound thermohygrograph drums with the aid of bamboo strip stylets fitted with felt writing tips. Observations and experiments were made with a minimum of disturbance to the bat population within this rather restrictive cave, in which the ceiling in its roomiest region was 1–1·5 m from the floor. The captive

bats were hand-fed at irregular hours of day and night with minced cockroaches.

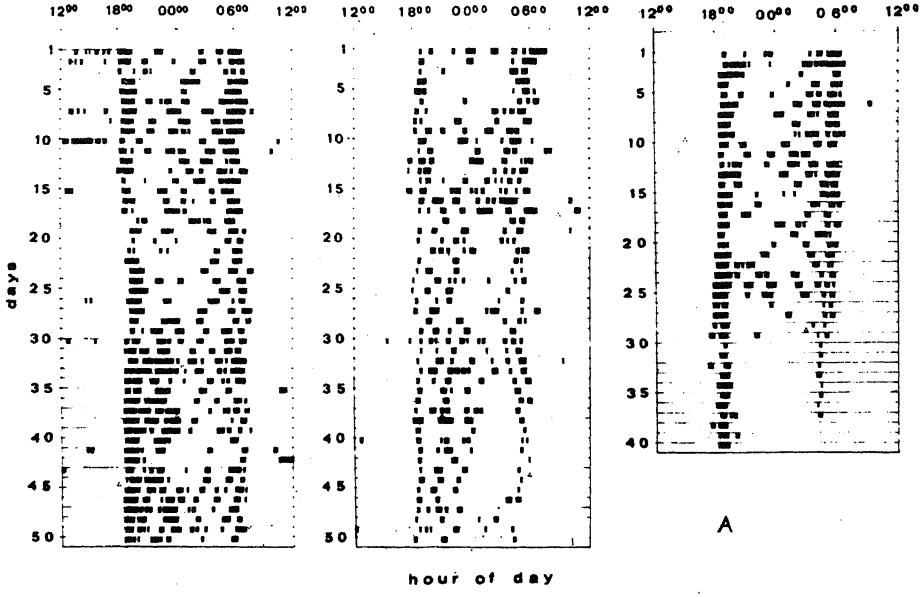
LL conditions were created by using incandescent bulbs and an automobile lead-acid battery. The incident light at the cage level varied between 1 and 20 lux. The battery was changed after 36–60 hr at random intervals for recharging, by replacing a second freshly charged battery. The caged bats could obviously hear orientation and/or communicative sounds from their unrestrained conspecifics.

## 3. Results

The hipposiderid bats awaken well before sunset. They then stretch, preen themselves and undertake short flights within the dark recesses. The bats then fly to a chamber proximal to the cave mouth. The bats fly around in this chamber 'sampling light' as other bats have been reported to do (Twente 1955; Voûte *et al* 1974). When it gets sufficiently dark outside after sunset they begin flying out to forage. Figure 1A, setting out the results of a 50-day experiment inside cave 1, indicates that 3 captive bats placed and experimented upon 40 m deep into the cave and surrounded by free-flying conspecifics still 'knew time'. They timed their flight within their confining cages, to the flight pattern of the colony. In further confirmation of the need for conspecific-communication, the results of another 50-day experiment with a solitary bat (in cave 2) with no conspecifics (figure 1B) reveal an impressive 'freerun'. No free-flying conspecifics, no social communication and no synchronization.

Interestingly even the rhythm of an 'alien' bat (*Taphozous nudiventris kachhensis*, an emballonurid species) held captive in the hipposiderid bat cave (cave 1) freeran. Figure 2 shows that even though the social cues emitted by the members of the colony of *H. speoris*, during their outflight and return before sunrise, were available to the captive emballonurid bat *T. n. kachhensis* the locomotor (flight) activity of the latter exhibited a spectacular freerunning rhythm with a $\tau$ shorter than 24 hr. But the rhythms of a closely related species *H. fulvus* partially entrain to social cues of *H. speoris*. Figure 3 illustrates how the flight activity rhythm of only one of three members of captive *H. fulvus* showed, synchronization to the colony activity of its sympatric species *H. speoris*.

To find out the effect of constant illumination on the social synchronization of the circadian rhythms we have recorded the flight activity of *H. speoris* inside cave 1 in LL. The circadian rhythms of *H. speoris* freeruns in LL in the laboratory. LL would tend in itself to induce a freerun and the social input would tend to entrain it. Figure 4 shows that under LL conditions of 1 lux two of the three captive bats still entrained to the social cues of their free-flying conspecifics, but the rhythm of the third bat freeran with a $\tau$ more than 24 hr. Since we suspected that the LL intensities reaching the bats might have been sub-threshold, the experiment was repeated with an increased light intensity of 10–20 lux. Figure 5 sets forth data for one bat. During the initial period of DD its daily activity rhythm coincided precisely with that of the free-flying conspecifics thus undergoing social entrainment. However, the same bat (and 3 other bats whose activity data are presented in figure 6) freeran in LL of 10–20 lux in spite of the social cues still available to them. The $\tau$ is longer than 24 hr in LL. LL seems to have abolished the social synchronization of the circadian rhythm in this bat. Bats in figures 6A, B exhibited in addition to freerunning rhythms, brief activity bouts for 28 days and 13 days, respectively, corresponding to onset of activity of the conspecifics. The bats flew inside activity cages for about 11–100 min just as the conspecifics flew out of the cave to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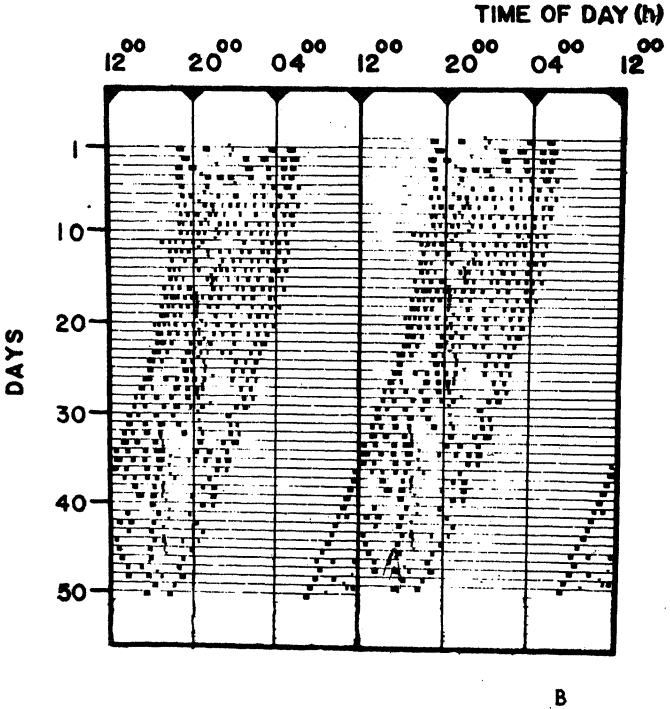
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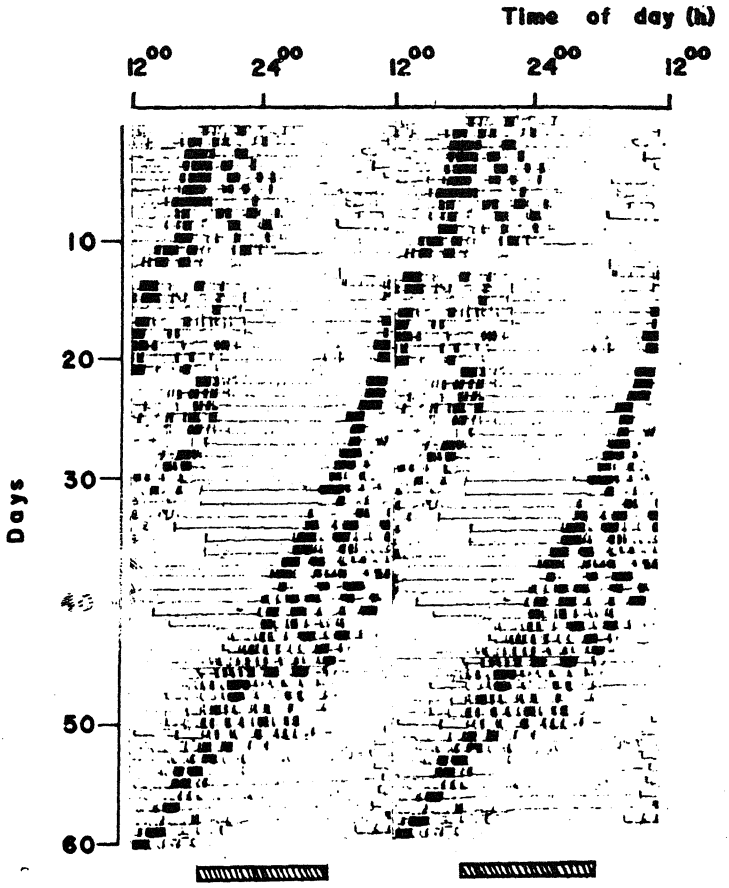


**Figure 2.** A typical example of the freerunning of the flight activity pattern of an emballonurid bat confined in a hipposiderid cave for 60 days. The original felt pen tracings are double-plotted. The hatched area at the bottom of the figure indicates the time over which the members of the hipposiderid colony would be active, leaving the cave in a mass exodus during early night and returning individually for the rest of the night. Other details are as in figure 1 (after Marimuthu and Chandrashekaran 1983b).

**Figure 1.** **A.** The flight activity patterns of 3 captive bats of *Hipposideros speoris* for 40 days in one case and 50 days in the other two cases recorded 40 m inside a narrow 'true cave' in Madurai. The bats could fly within the flight activity cages and the movements of the cages were directly recorded. Activity bouts are indicated by vertical patches and the horizontal lines indicate rest. The activity/rest data are schematized from original data and presented one below the other for successive days (after Marimuthu *et al* 1981). **B.** A double plot of activity/rest pattern of a solitary male *H. speoris* recorded in a cave without any conspecifics over a period of 50 days. The activity data for day 1 leading horizontally to data for day 2, data for day 2 to data for day 3 etc to facilitate visual evaluation. Triangles indicate feeding time. The details are schematized from original felt pen tracings. Other details are as in "A" (after Marimuthu *et al* 1981).

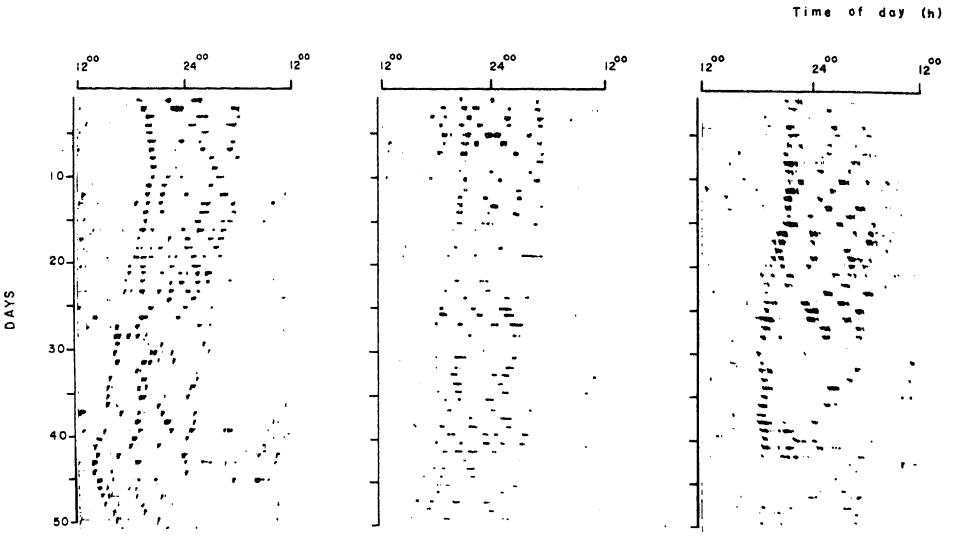


**Figure 3.** The flight activity patterns of three captive bats of *Hipposideros fulvus* confined in *H. speoris* cave for 50 days. Activity bouts indicate the original felt pen tracings. Other details are as in figure 1.

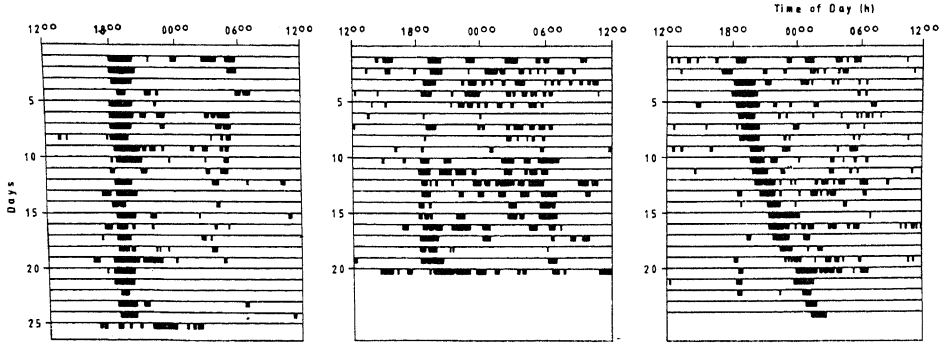


**Figure 4.** The flight activity patterns of 3 captive bats of *H. speoris* in LL of 1 lux. The activity/rest data are schematized from original felt pen tracings. Other details are as in figure 1.

forage. Such flights of these bats coinciding with the onset of the colony activity account for an exogenous component which expresses itself regardless of the phase of the freerunning rhythms. These exogenous components merge into the activity bouts when the rhythm crosses them during the freerun. The onset of the freerunning oscillatory component for bat in figure 6A crosses the exogenous component around days 37 and 38 without any sign of even a temporary synchronization, i.e. there is no 'relative coordination' (Holst 1939) during the whole run. The freerunning rhythm re-entrained to the social cues as seen in figure 5 when DD was restored. The light was turned off at phase which was 180° off course relative to colony activity. Re-entrainment set in after a few 'transient cycles' with the onset component of the re-entrained rhythm coinciding

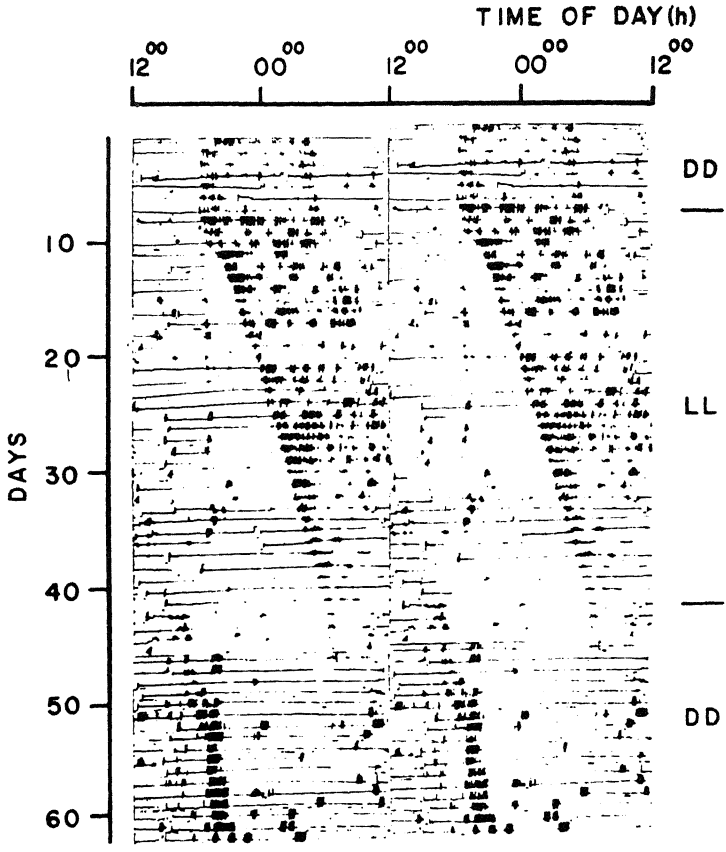


**Figure 5.** The flight activity patterns of a captive bat for 62 days. Days 1–7, DD; days 8–41, LL of 10–20 lux; days 42–62, DD. The activity/rest data containing the original felt pen tracings are double-plotted and other details are as in figure 1. (after Marimuthu and Chandrashekaran 1983a).

with the onset of colony activity. Another possibility that cannot be ruled out, however, is that the freerunning rhythm persists even after turning LL off and what is seen in figure 5 is some form of 'masking' (Aschoff 1965).

## 4. Discussion

Literature on communication and synchronization of circadian rhythms is altogether sparse. Even those of the reports that impute social synchronization are only rarely based on rigorous experimentation. One of the earliest reports to impute social synchronization among conspecifics was that of Johnson (1926) for the mice of the genus *Peromyscus*. Subsequent reports described similar effects for blinded mice *Mus musculus* (Halberg *et al* 1954), male chevrotain antelopes (Dubost 1975), wolf-coyote hybrids (Roper and Ryan 1977), beaver colonies of *Castor canadensis* (Potvin and Bovet 1975), deer mice (Crowley and Bovet 1980), macaque monkeys (Rohles and

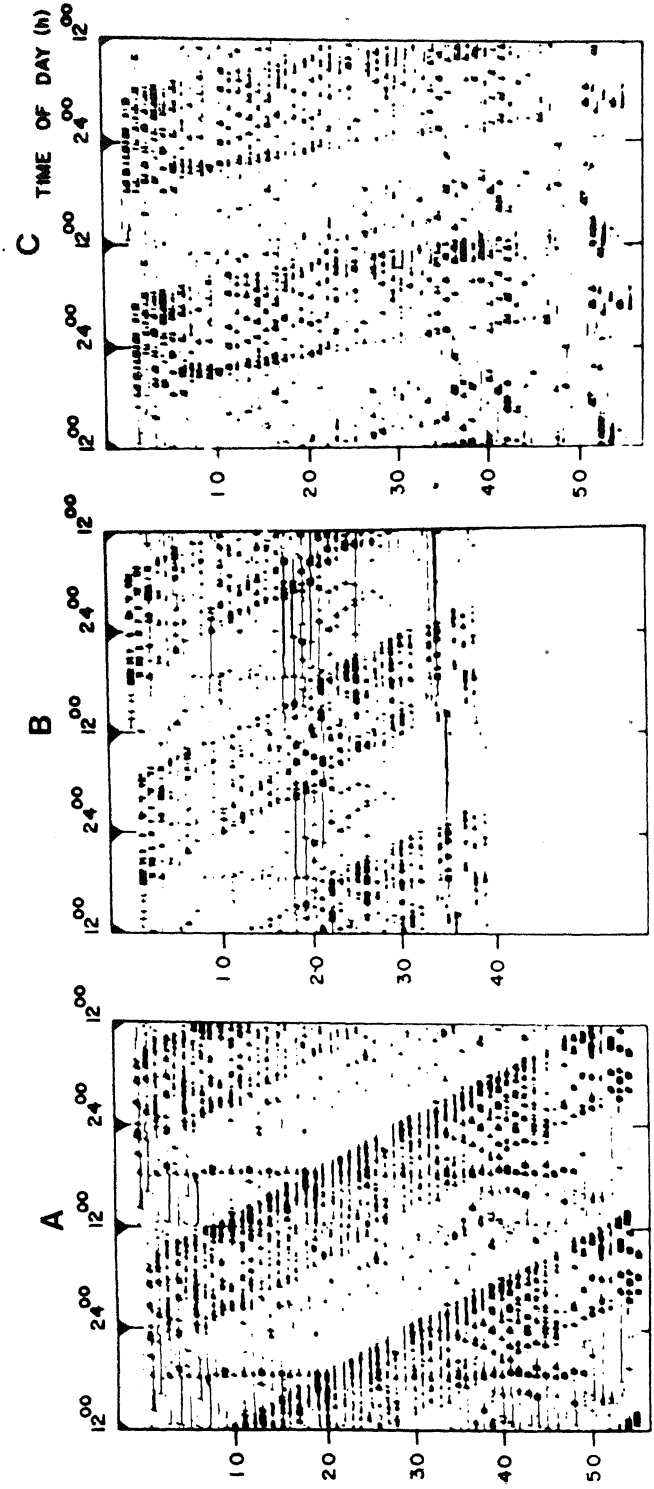


**Figure 6.** The flight activity patterns of 3 captive bats for 39 days (B) and 55 days (A and C) in LL of 10–20 lux. The activity/rest data containing the original felt pen tracings are double-plotted and other details are as in figure 1 (after Marimuthu and Chandrashekaran 1983a).

Osbaldiston 1969), sexual cyclicity of female mammals (Rusak and Zucker 1979) and so on. Several other reports seeking to causally connect social cues with circadian rhythms are anecdotal (Rusak 1981; Chandrashekaran 1982). The case we present here for synchronization of the circadian rhythm by social communication for the cave-dwelling microchiropteran bat *H. speoris* incorporates the outcome of our researches on this subject since 1977. We have applied the methodologies of the field ethologist and experimental chronobiologist. To this extent we may be justified in believing that our results are relatively free of experimental/laboratory artifacts.

The synchronization of the activity rhythm of all three captive bats illustrated in figure 1A, is absolute and stable. We conclude that the synchronization arises through communication. The communication could be in the form of (i) flight noise, (ii) pheromones and (iii) acoustics. Owing to the close intermingling of these factors in the cave environment, laboratory studies alone can resolve which of these factors plays a dominant role. It is also possible that all these factors have to be simultaneously available. Another feature (figure 1A) re-inforces the case for social synchronization. The captive bats while entraining show an unequivocal bimodality in their activity bouts. The first peak coincides with outflight of the colony and the second peak coincides with the return of the colony.

Figure 2 illustrates that an 'alien' bat (*T. n. kachhensis*) in a hipposiderid cave fails to synchronize. We postulate that the freerunning results from a 'communication gap' between the hipposiderid bat (*H. speoris*) which emits CF ultrasonics of 135 kHz and the emballonurid alien (*T. n. kachhensis*) which emits pulses consisting of a family of harmonics up to 80 kHz. Interestingly audiogram studies reveal that the bat *T. n. kachhensis* cannot indeed 'hear' (white) noise of 135 kHz which is the species specific emission frequency of *H. speoris* (Neuweiler *et al* 1984). Threshold of hearing may not be the only criterion for even the wing-beat noise of hipposiderid bats, well within the hearing range of the emballonurid bat, was apparently unable to synchronize.

A further ethological observation must be described in this context. In the experiment in cave 2 using a solitary bat we would often hear crows and mynas at a nearby waterhole during day and stridulation of crickets during night. These non-specific cues did not synchronize the activity rhythm. This finding contrasts with those of Lohmann and Enright (1967) on birds, and Wever (1979) on humans; bird and human circadian rhythms also entrain to buzzers and other non-specific noise.

It is tempting to assume that communication and synchronization of the circadian rhythm in bats may even be species specific. Thus evolutionarily closely related species, *H. speoris* and *H. fulvus* seem to be able to communicate even if only partially, in the context of synchronization of their rhythms. *H. fulvus* emits CF-FM ultrasonic pulses of 154 kHz with the downward sweeps touching 115 kHz.

Figures 4–6 represent the responses of the bat circadian system when exposed to a conflicting zeitgeber situation. This, however, almost never occurs in nature. Circadian rhythms of the captive bats freerun in LL of 10–20 lux in spite of the social cues available to them. LL thus apparently abolished the social synchronization of the circadian rhythm. The exogenous components exhibited by two individuals (figures 6A, B) represent a stimulus-response situation and may not merit oscillator status. This may be the phenomenon of response that was termed 'positive masking' (Aschoff 1965, 1981; Daan and Aschoff 1975). Figure 4 obviously mirrors a situation of subthreshold LL influence. This explains why two bats synchronized and the third did not.

The artificial light that shone inside the cave scared away a few bats that roosted

otherwise in and around the area inside the cave where the recording was carried out. To that extent the 'social cues' might indeed have attenuated in the immediate vicinity. However, within a week or so the bats returned to their original roosting sites, closer to the captive bats. The exogenous component seen so clearly in bats in figures 6A, B is further evidence that the onset of the activity of the bat colony had made itself felt, even though it could not synchronize the rhythm. The freeruns indicate that whatever social cues that prevail are not 'reaching the clock'. In LL the circadian rhythm apparently uncouples from social 'zeitgebers'. The reentrainment of the rhythm by the social cues after the light was turned off (figure 5) further confirmed that it is LL that uncoupled the circadian rhythm from the social cues. Sulzman *et al* (1977) reported that the locomotory activity of squirrel monkeys in noise/silence cycles of 24 hr periods did not synchronize the rhythm in LL of 600 lux, but the food cycles did synchronize it. It would be of great interest in the context of our findings to know whether squirrel monkeys would have entrained to the noise cycles in DD.

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