VOL. 17 1978

MALACOLOGIA

International Journal of Malacology

Revista Internacional de Malacologia

Journal International de Malacologie

Международный Журнал Малакологии

Internationale Malakologische Zeitschrift

Publication dates

Vol. 16, No. 2—17 September 1977
Vol. 17, No. 1—17 February 1978

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MALACOLOGIA

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The Academy of Natural Sciences of Philadelphia
Nineteenth Street and the Parkway
Philadelphia, Pennsylvania 19103, U.S.A.

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1978

MALACOLOGIA, 1978, 17(1):1-56

ON THE SYSTEMATICS OF *TRICHIA* S. LAT. (PULMONATA: HELICOIDEA: HYGROMIIDAE)

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ABSTRACT

Systematic revision of Asiatic, Caucasian and some European species of the *Trichia* s. lat. group of the Trichiinae has shown that it is not a united genus but a complex of genera. All known representatives (8) of the Central Asian group, all species but 1 occurring in the Caucasus (14), and 9 representatives of the main European taxa are treated in the Systematic Part (31 species). The anatomical investigation comprises 29 species, including 25 of the 27 species known in the USSR. Of the 9 genera recognized, 3 are new: *Hygrohelicopsis, Teberdinia, Plicuteria,* and also 4 species: *Leucozonella caria, Hygrohelicopsis darevskii, Kokotschashvilia tanta, K. eberhardi.*

Apart from shell and traditional anatomical characters, great attention has been paid to peculiarities of the inner structure of the genitalia. It was thus found that *Hygrohelicopsis darevskii* does indeed possess 2 pairs of dart sacs, though the inner pair is not visible from the outside. In this species the right ocular retractor does not pass between penis and vagina, as in *Trichia*, but only near them, which is considered to be characteristic for *Helicopsis* in the "Helicellinae" auct.; but from the totality of other characters *Hygrohelicopsis* is nevertheless regarded as belonging in the Trichiinae. *Teberdinia* was found to differ from other Caucasian forms by having a deep longitudinal groove on the surface of the penis papilla that separated off a lobe and from all other forms by having one of the intrapapillar cavities reaching into this lobe. In the genus *Plicuteria* the longitudinal vaginal plicae are subdivided by regular transverse prismatic folds forming a unique dense pattern on the internal wall of the vagina. *Kokotschashvilia tanta* and *K. eberhardi* can be distinguished, among other features, by the structure of the inner wall of the penis papilla, which in the latter is not smooth but plicate.

The Asiatic group is distinguished from the European-Caucasian group by the mode of formation of the intrapapillar cavity or cavity system. In the former group it arises from the closing of a groove on the surface of the verge, the main phases of the process being evident in the material studied. In the latter group the cavities arise as paired structures in the thickness of the papillar wall and then assume more complex forms. The proposed new systematics of the *Trichia* s. lat. group are based on various characters of a different nature, taking into consideration as far as possible the organization of the animal as a whole, as only such an analysis can reflect on the evolution of the various groups and the true relations among them.

INTRODUCTION

The use of gross genital morphology in stylommatophoran pulmonates has helped our understanding of systematics at the family level. Findings reflect relationships as they have evolved in nature. In practice, however, this approach has sometimes resulted in the creation of large genera comprising the anatomical characters of the many species included. One result is that the conchological features of such genera have become so hazy as to be quite useless.

The genus *Trichia* s. lat. (Trichiinae)[2] is very interesting in this respect. A conchological diagnosis of the group is not practi-

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[2]The author, replacing a classification that recognizes 2 subfamilies, the Hygromiinae and Helicellinae, subdivides (Shileyko, 1972) the family Hygromiidae Tryon, 1866, into 7 subfamilies:

Trichiinae Zilch & Jaeckel, 1962
Hygromiinae Tryon, 1866
Metafruticicolinae Shileyko, 1972
Monachinae Zilch, 1960
Cochlicellinae Shileyko, 1972
Ciliellinae (?) Shileyko, 1972
Geomitrinae Wenz, 1923

The "Helicellinae" auct. are not considered to form a natural group but to consist of various hygromiid "vital forms" (Shileyko, 1972: 28-41).

cable. The only diagnostic feature is the presence of 2 pairs of symmetrically situated dart sacs (stylophores). Other anatomical characters such as the shape and relative length of the flagellum, epiphallus, penis and spermathecal duct; the shape of the receptaculum seminis (spermatheca) and of the vagina; the number, location and nature of mucous glands and of their branches; and the presence of bends in the free oviduct are rather variable, and none of them is constant within the whole group. But one anatomical feature allows us to separate the present group from species of the genus *Helicopsis*, which belongs in the "Helicellinae" auct.: the right ocular retractor passes between the penis and vagina, not merely close to them, as in *Helicopsis*. This feature and its taxonomic value will be discussed in more detail below.

The present paper shows that the genus *Trichia* is more complex than hitherto imagined. The probable phylogenetic relationships in the genus complex will be discussed after a systematic review of the species studied.

This study includes the majority of species known from the USSR and also some eastern and central European forms allied to those from the European part of the Soviet Union. A total of 31 species that fall into 9 genera were considered as follows; 2 of these, marked by asterisks, were not examined.

Genus *Odontotrema* Lindholm, 1927
- *O. diplodon* Lindholm, 1927

Genus *Leucozonella* Lindholm, 1927
- *L. ferghanica* Lindholm, 1927
- *L. caryodes* (Westerlund, 1896)
- *L. rubens* (Martens, 1874)
- *L. mesoleuca* (Martens, 1882)*
- *L. rufispira* (Martens, 1874)
- *L. retteri* (Rosen, 1897)
- *L. caria* Shileyko, sp. nov.

Genus *Hygrohelicopsis* Shileyko, gen. nov.
- *H. darevskii* Shileyko, sp. nov.

Genus *Teberdinia* Shileyko, gen. nov.
- *T. zolotarevi* (Lindholm, 1913)

Genus *Kokotschashvilia* Hudec & Lezhawa, 1969
- *K. makvalae* (Hudec & Lezhawa, 1969)
- *K. tanta* Shileyko, sp. nov.
- *K. holotricha* (Boettger, 1884)
- *K. eberhardi* Shileyko, sp. nov.
- *K. phaeolaema* (Boettger, 1886)

Genus *Caucasigena* Lindholm, 1927
- Subgenus *Caucasigena* s. str.
  - *C.* (*C.*) *armeniaca* (L. Pfeiffer, 1846)
  - *C.* (*C.*) *tschetschenica* (Retowski, 1914)
  - *C.* (*C.*) *rengarteni* (Lindholm, 1913)
  - *C.* (*C.*) *eichwaldi* (L. Pfeiffer, 1846)
  - *C.* (*C.*) *abchasica* (Lindholm, 1927)*
- Subgenus *Anoplitella* Lindholm, 1929
  - *C.* (*A.*) *schaposchnikovi* (Rosen, 1911)
- Subgenus *Dioscuria* Lindholm, 1927
  - *C.* (*D.*) *thalestris* (Lindholm, 1927)

Genus *Plicuteria* Shileyko, gen. nov.
- *P. lubomirskii* (Slóssarski, 1881)

Genus *Trichia* Hartmann, 1840
- Subgenus *Petasina* Beck, 1847
  - *T.* (*P.*) *unidentata* (Draparnaud, 1805)
- Subgenus *Trichia* s. str.
  - *T.* (*T.*) *plebeia* (Draparnaud, 1805)
  - *T.* (*T.*) *concinna* (Jeffreys, 1862)
  - *T.* (*T.*) *hispida* (Linné, 1758)
  - *T.* (*T.*) *villosula* (Rossmaessler, 1838)
  - *T.* (*T.*) *striolata* (C. Pfeiffer, 1828)
  - *T.* (*T.*) *danubialis* (Clessin, 1874)

Genus *Edentiella* Polínski, 1929
- *E. bakowskii* (Polínski, 1924)

This material was collected from the following geographical areas (Fig. 1): *Odontotrema* and *Leucozonella* from Central Asia west of the great Tian-Shan Mountains (Fig. 2); *Hygrohelicopsis, Teberdinia, Kokotschasvilia* and *Caucasigena* from the Caucasus (Fig. 3); *Plicuteria, Trichia* and *Edentiella* from various European countries (Fig. 4).

SYSTEMATIC PART

In the following text, shell descriptions are given only for the little-known, rare or new species. The details of internal anatomy are mostly those relevant to taxonomic analysis. The characters of proximal reproductive structures do not distinguish genera and species; therefore the ovotestis, spermoviduct and albumen gland are not described.

The terminology of the features of the reproductive tract is not uniform in the literature, and some features have not been previously used in taxonomic distinction;

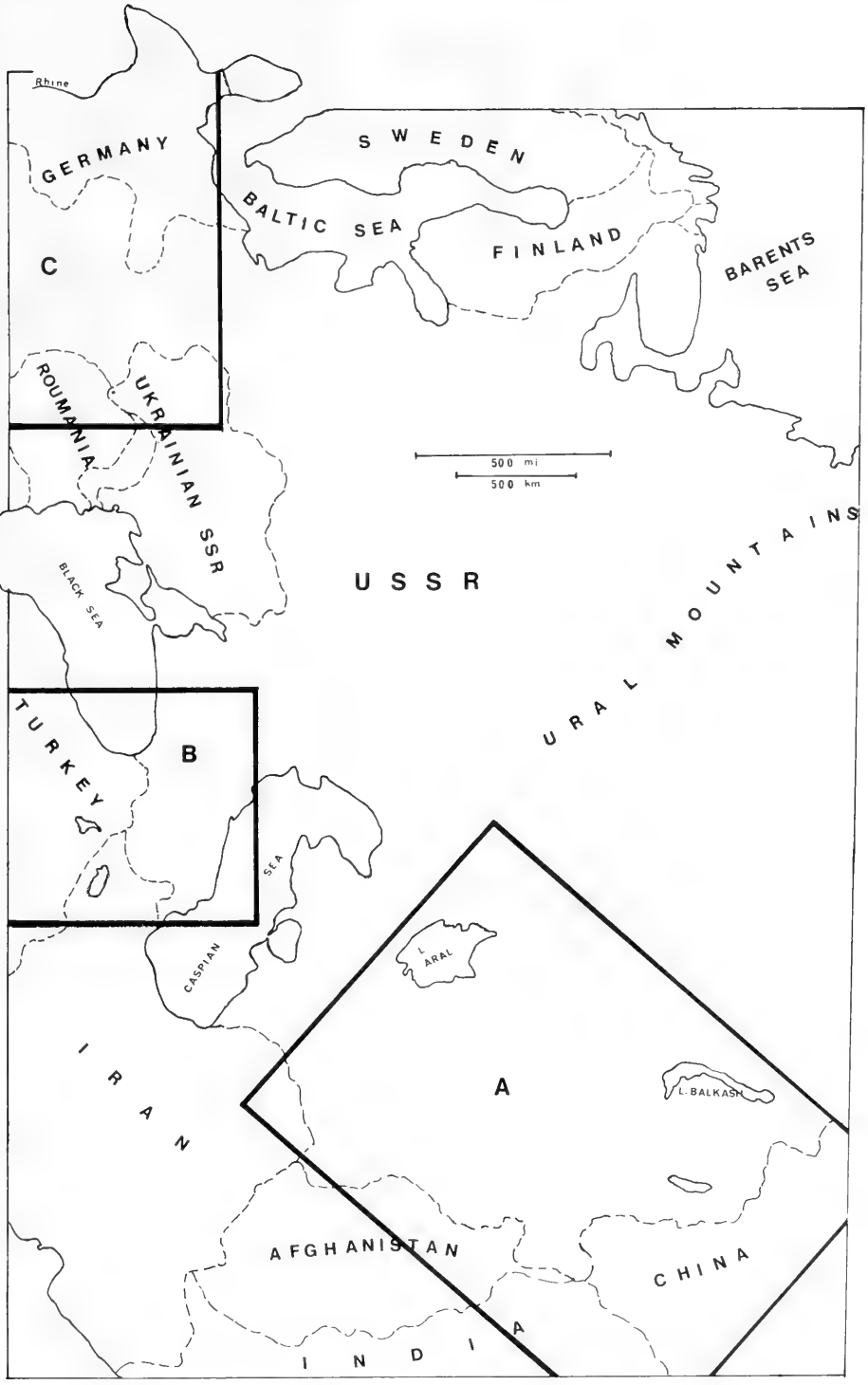


FIG. 1. Map showing the 3 general areas in which the species discussed were collected.

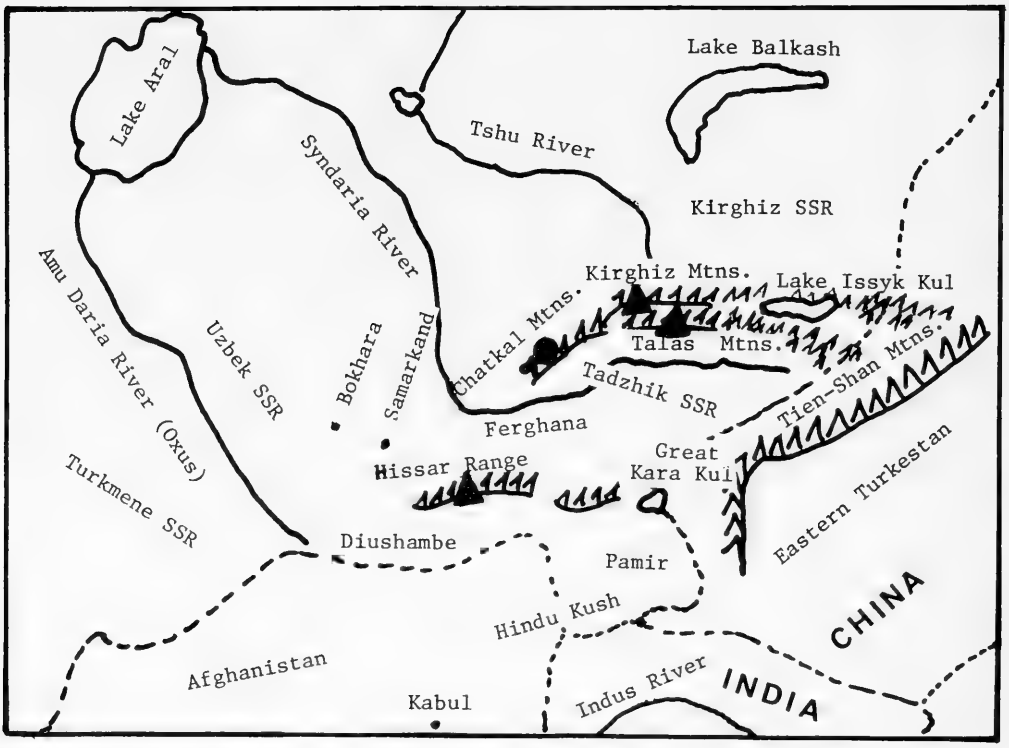


FIG. 2. Inset A of Fig. 1, the source area of central Asian specimens. This map shows the locations of Central Asian *Trichia* s. l. collected in the Soviet Union (in the Kirghiz, Chatkal, Talas, and Hissar Mountains, i.e., in the western spurs of the Tien-Shan Mountains. Black circles = *Odontotrema;* black triangles = *Leucozonella* spp. Note: kul = lake; Uzbek SSR = Uzbekistan; Tadzhik SSR = Tadzhikistan, etc.

therefore, certain terms used in this paper are defined and attention is drawn to particular features.

The free oviduct, i.e., that part of the female tract that continues the oviduct or uterine part of the spermoviduct, which runs from the point of departure of the vas deferens to that of the spermathecal duct, is here called "oviduct"; "bend of the oviduct" is used in specific differentiation. The following part of the tract, the vagina, is subdivided into an upper and lower portion: the upper lies in the mucous gland region above the dart sacs, and the lower included the dart sacs and continues to the genital atrium. The "dart sacs," which are accessory organs, are either rudimentary or contain lime shafts; "stylophore" is perhaps a shorter and more exact term for dart sac.

In the distal part of the male genital tract several features have been accorded special attention. The penis consists of the "penis sheath" and "papilla" ("verge"). Inside the walls of the inner verge surrounding the seminal canal we find one or more "intrapapillar cavities," i.e., a system of cavities, some of which may be separated by longitudinal tissue bands or by longitudinal septa. These communicate with the cavity of the penis sheath by a foramen, the "papillar lacuna" (or by several lacunae). The "papillar plicae," or folds, are ridges on the inner surface of the penis sheath. A "connective tissue penial membrane," i.e., a thin membrane that may contain muscle fibers, may be stretched among the vas deferens, epiphallus and penis. Between the epiphallus and the distal parts of the penis sheath there may be groups of muscle fibers, here termed "penial muscle bands," either alone or associated with bands of connective tissue, the "connective tissue penial bands."

The term "perspective" applied to the umbilicus or perforation of the shell denotes that the whorls can be seen in widely or narrowly umbilicate or even perforate shells.

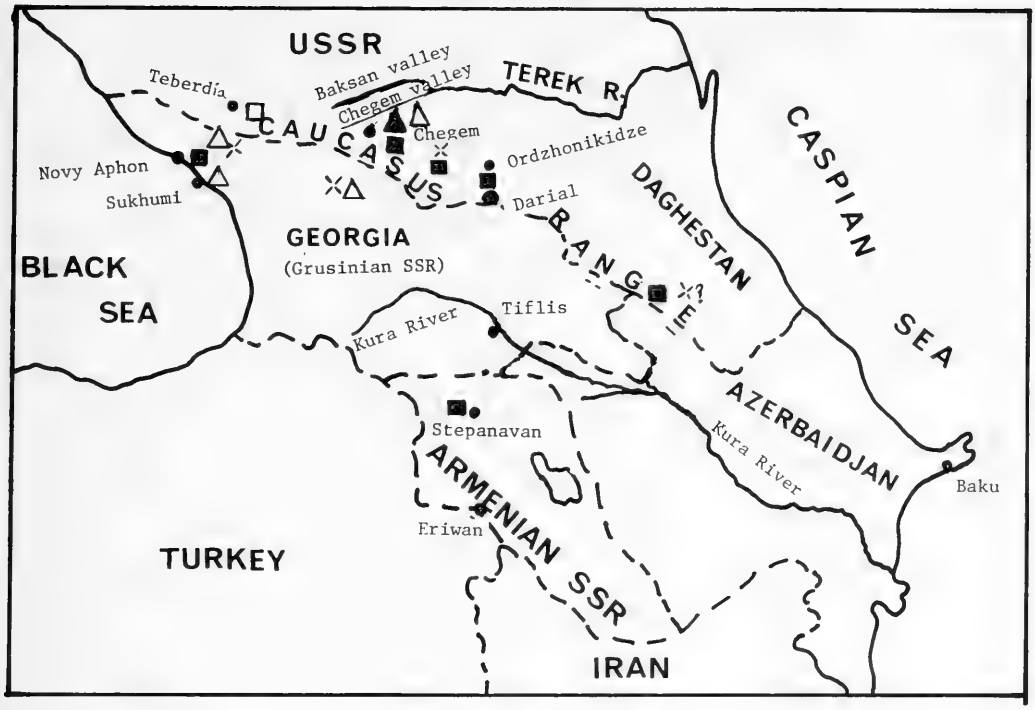


FIG. 3. Inset B of Fig. 1, the source area of specimens from the Caucasus. The locations of Caucasian *Trichia* s. l. collected in the Soviet Union. Open square = *Teberdinia;* black square = *Caucasigena* spp; open triangle = *Kokotschashvilia* spp; black triangle = *Hygrohelicopsis.* The crosses indicate that exact localities are not known but are located by district only.

LIST OF ABBREVIATIONS

AIG	albumen gland
BO	bend of oviduct
CML*	central mantle lobe
CPB	connective tissue penial band
CPM	connective tissue penial membrane
D	dart (stylet)
DS	dart sac (stylophore)
EDS	outer (external) dart sac
Ep	epiphallus
F	flagellum
GA	genital atrium
HD	hermaphrodite duct
IA	intrapenial appendix
IC	intrapapillar cavity
IDS	inner (upper) dart sac
LML*	left mantle lobe
MFE	main folds of epiphallus
MG	mucous glands
Ov	oviduct
P	penis
PF	penial folds (plicae)
PGr	papillar groove (intrapapillar cavity that still remains open)
PL	papillar lacuna
PMB	penial muscle band
PP	penis papilla (verge)
Pr	prostate
RML*	right mantle lobe
ROT	right ocular retractor
RP	retractor (muscle) of penis
SC	seminal canal (duct)
SD	spermathecal duct
SIC	septa in intrapapillar cavity
SOD	spermoviduct
Sp	spermatheca
V	vagina (lower portion)
VD	vas deferens
VF	vaginal folds (plicae)

*The mantle collar and its lobes have been figured for some species, for what it is worth, but the feature is not further discussed as the material is not sufficient for any conclusions.

FIG. 4. Inset C of Fig. 1, the source area of European specimens. Black square = *Edentiella;* open triangles = *Trichia* spp; black triangle = *Plicuteria.* CSSR = Czechoslovakia; GDR = German Democratic Republic; GFR = German Federal Republic.

Genus **Odontotrema** Lindholm, 1927

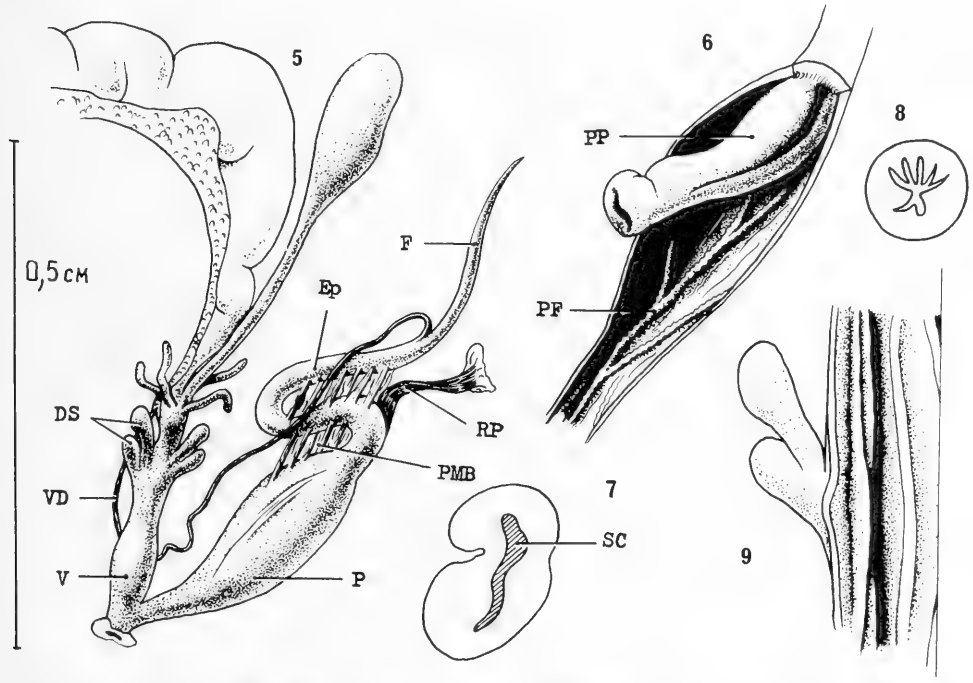
The shell is lowly conical with a smooth angle on the periphery; it is finely hirsute and brown. The umbilicus is relatively wide and perspective. There is a fine lip in the aperture on which there are 2 teeth: a larger basopalatal tooth and a basocolumellar one. The dart sacs are club-shaped and weakly fused together. There are 4 unbranched mucous glands. The inner surface of the penis sheath is covered with clear-cut, fluted ridges; the surface of the verge bears a longitudinal groove. There are no cavities in the verge walls. The flagellum is a little shorter than the epiphallus.

Genus monotypic.

Odontotrema diplodon Lindholm, 1927
Figs. 5-9; Pl. I, 1

Two specimens were examined. These were collected from the scree at the base of the Chatkal Range, NW Tian-Shan Mountains, Kirghiz SSR, central Asia, in May 1972, and identified by me.

The characteristic features of the internal anatomy are as follows. The oviduct (uterus) part of the spermoviduct passes straight into the free oviduct without any curve. The 4 mucous glands are simple, nonbranching and radially arranged. The dart sacs are thin and long, the inner dart sacs fused with the outer at their base; the inner dart sacs are almost free of the vagina. The lower vagina is long, fusiform; slight longitudinal plicae run within the whole of the inner vagina, the "vaginal plicae." At the outlet of the dart sacs these plicae form in distinct lobes. The flagellum is shorter than the epiphallus; the latter curves twice and is held in this position by connective tissue bands containing muscle fibers. The penis is relatively very massive, fusiform; its inner surface bears branching, slightly crimped, long ridges. The verge is generally cylindrical, with a long, deep groove which disappears distally. The distal part of the verge is constricted by an incomplete circular groove. Except for the vas deferens (seminal canal), the verge does not contain any cavities. There are some long, weak connective tissue bands on the surface of the penis sheath. The spermathecal duct (truncus receptaculi) has no abrupt curves and passes smoothly to the elon-



FIGS. 5-9. *Odontotrema diplodon* Lindholm, Chatkal range, NW Tian-Shan Mountains, Kirghiz SSR, May 1972. **5**, reproductive tract; **6**, penis, penis sheath partly removed; **7**, cross-section of verge; **8**, cross-section of epiphallus; **9**, inner structure of vagina in dart sac region.

gate-oval spermatheca (receptaculum seminis), which nearly reaches the lower edge of the albumen gland.

Genus **Leucozonella** Lindholm, 1927

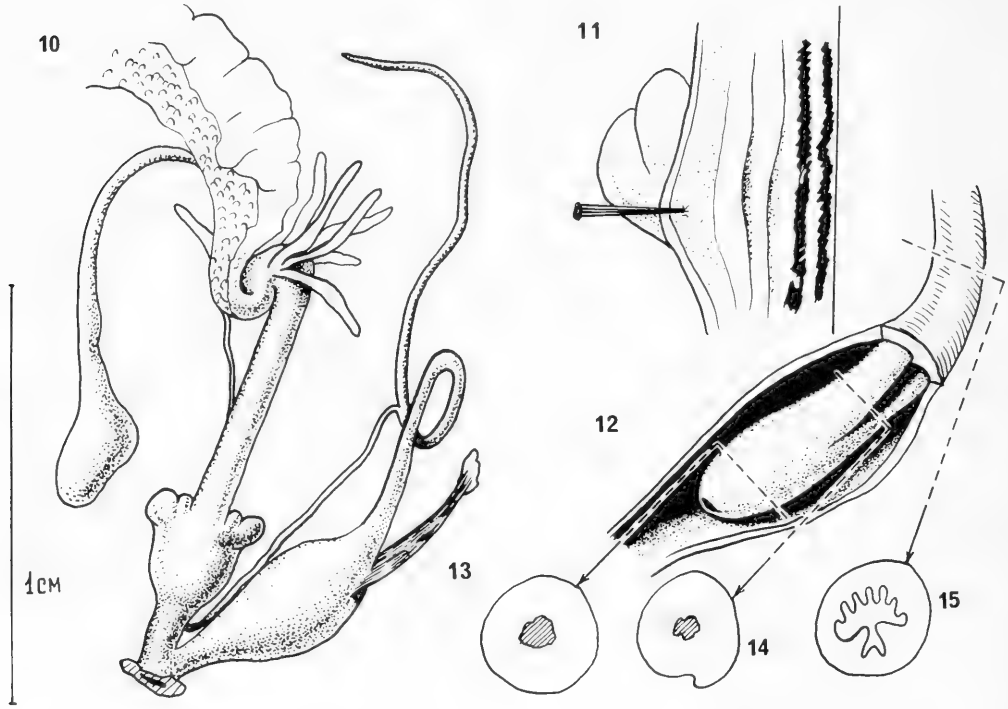
The shell is globose to lowly conical; in the latter case it may be angular at the periphery. Its color varies from light gray, yellowish, reddish to brown; on the periphery there is a light line, which is sometimes very faint. The umbilicus may vary from dot-like to relatively wide. Sometimes it is half covered by the reflected columellar edge of the aperture. The dart sacs are globose or elongate. There are 3 or 4 mucous glands, having 2-3 branches. The inner surface of the penis sheath is smooth. The verge has a long groove, developed to various degrees, or it is closed, forming an intrapapillar cavity.

Type-species: *Helix rubens* Martens, 1874.

Leucozonella ferghanica Lindholm, 1927
Figs. 10-15; Pl. I, 2

Two specimens were examined. They were collected in the Sary-Chileck Nature Reserve near Lake Kula-Kul, Chatkal Range, NW Tian-Shan Mountains, Kirghiz SSR, on 6 July 1966, by A. J. Jankowskaja and identified by I. M. Likharev. A description of the shell is given by Likharev & Rammelmeyer (1952).

The oviduct forms 2 sharp bends, and the walls of the tube are tightly pressed together (Fig. 10). The length of the straight part of the tube, the upper vagina, from the bend to the dart sacs, is 6-7 times its width. The dart sacs are globose; the sac region is separated from the lower vagina by a slight narrowing. The flagellum is thin, slightly longer than the epiphallus. The penis is very bulbous, fusiform. The verge bears a groove on its basal part (Figs. 12-14). The spermathecal duct is almost



FIGS. 10-15. *Leucozonella ferghanica* Lindholm, Sary-Chileck Nature Reserve, Chatkal range, NW Tian-Shan Mountains, Kirghiz SSR, 6 July 1966. **10**, reproductive tract; **11**, inner structure of vagina in dart sac region; **12**, penis, penis sheath partly removed; **13, 14**, cross-sections of verge at different levels; **15**, cross-section of epiphallus.

straight and ends in a small spermatheca nearly spherical in form, which almost reaches the albumen gland.

Leucozonella caryodes (Westerlund, 1896)
Figs. 16-20; Pl. I, 3

Four specimens were examined. I collected them from the Talas Range, NW Tian-Shan Mountains, Kirghiz SSR, on 4 June 1972, and identified them.

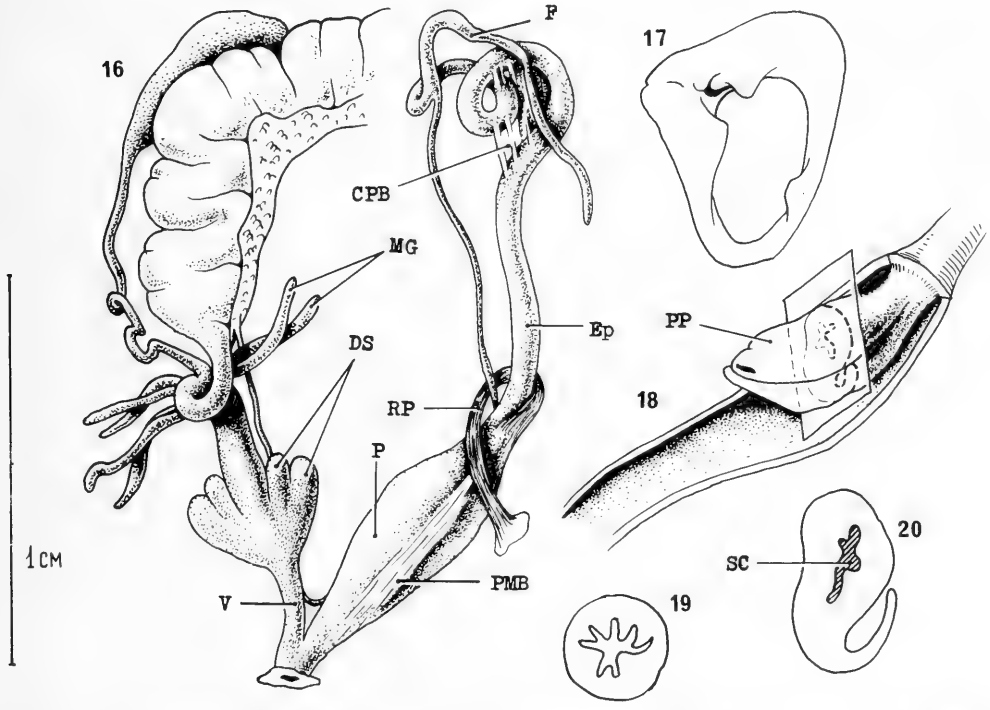
This species differs from *L. rubens* by its shell, which has considerably thicker walls and a narrower umbilicus (see Pl. I, 3b and 4b).

The oviduct forms a loop-like bend (Fig. 6). Usually there are 3 mucous glands, each with 2 branches. The distance between the basal part of the mucous glands and the upper limit of the dart sacs is approximately equal to the length of the lower vagina. The dart sacs are not globose but elongate, almost club-shaped. The lower vagina is thin; it is 2.5-4 times longer than it is wide. Vaginal plicae are very massive. The lobes at the outlet of the dart sac ducts are not well expressed. The flagellum is long, longer than the fine, curved, cylindrical epiphallus. The different portions of the epiphallus are connected by short connective tissue bands (CPB, Fig. 16); there are also longitudinal muscle bands (PMB) on the surface of the fusiform penis. The inner surface of the penis sheath is smooth. The verge bears a sharp, long groove, the plane of which forms an acute angle with the sagittal papilla plane (Figs. 18, 20). The penis retractor loops around the epiphallus. The spermathecal duct is thin and slightly curved and merges indistinctly with the elongate-oval receptaculum seminis. The latter does not quite reach the albumen gland.

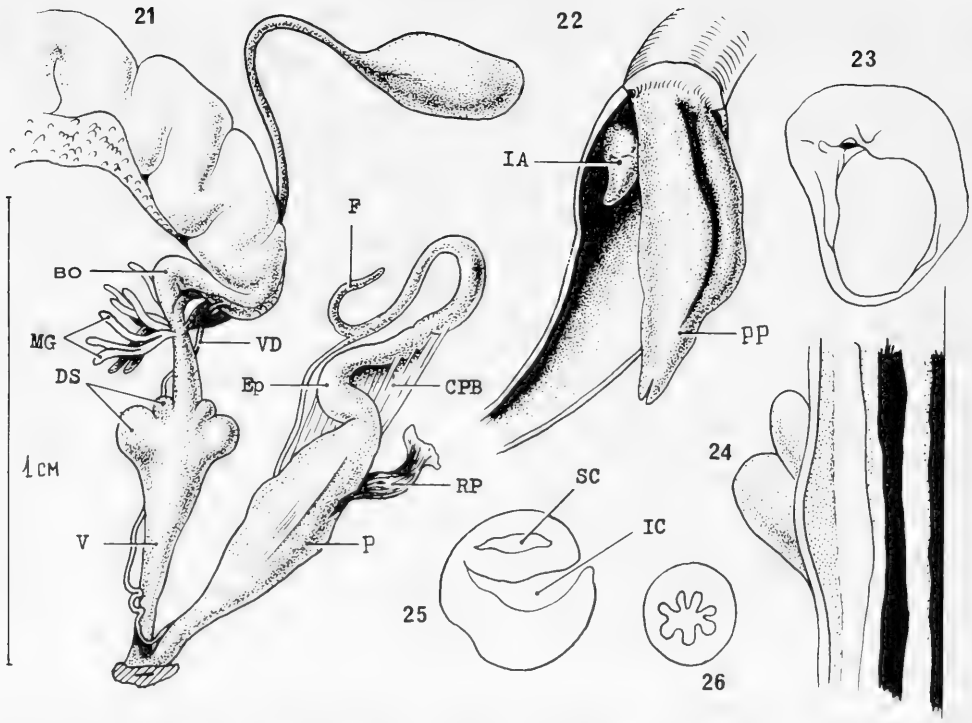
Leucozonella rubens (Martens, 1874)
Figs. 21-26; Pl. I, 4

Five specimens from the foothills of the Kirghiz Range (formerly Alexander Mountains), NW Tian-Shan Mountains, Kirghiz SSR, were examined; collection (15 June 1972) and identification are mine.

This shell is very much like that of *"Euomphalia" regeliana* (Martens, 1882)



FIGS. 16-20. *Leucozonella caryodes* (Westerlund), Talas range, NW Tian-Shan Mountains, Kirghiz SSR, 4 June 1972. **16,** reproductive tract; **17,** mantle collar with 3 lobes; **18,** penis, penis sheath partly removed; **19,** cross-section of epiphallus; **20,** cross-section of verge.



FIGS. 21-26. *Leucozonella rubens* (Martens), foothills of the Kirghiz range, NW Tian-Shan Mountains, Kirghiz SSR, 15 June 1972. **21**, reproductive tract; **22**, penis, penis sheath partly removed; **23**, mantle collar; **24**, inner structure of vagina in dart sac region; **25**, cross-section of verge; **26**, cross-section of epiphallus.

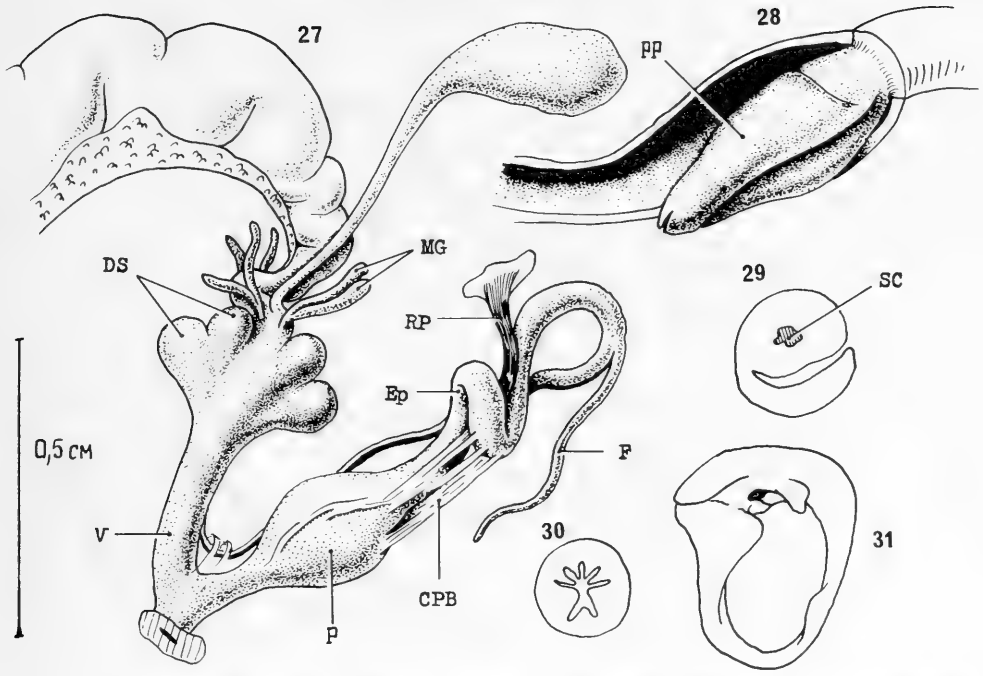
(cf. Pl. I, 4a, b, c and Pl. II, 5a, b, c); the shell of the latter species has a narrower umbilicus and in this respect it resembles *L. caryodes*, but it differs from it by being thinner. Nevertheless, despite these differences among the 3 species, positive identification is possible only after dissection.

The oviduct is rather narrow and long and bends suddenly (Fig. 21). There are 3-4 mucous glands, situated considerably higher than the dart sacs; each gland has 2-5 branches. The outer dart sacs are considerably larger than the inner ones; all are nearly spherical. The dart sac region tapers toward the vagina without any sudden narrowing. The vaginal plicae as well as the lamellae on them are indistinct. The flagellum is 2/5 to 1/4 the length of the epiphallus. The penis is fusiform to globose; the inside of the penis sheath is smooth, without any folds or lamellae. The verge bears a narrow, sharp, deeply incised groove on its surface. At the base of the papilla there is an appendix (IA, Fig. 22), developed to various degrees, in the form of a protuberance, of a conical callus or (when maximally developed) of a spongy lamina, partly superimposed upon the papilla. There is a connective tissue membrane between the penis and the epiphallus in this species. The spermathecal duct is thin and rather long; it ends in an oval spermatheca that reaches the lower edge of the albumen gland.

Leucozonella rufispira (Martens, 1874)
Figs. 27-31; Pl. II, 6

Four specimens were examined. They were collected from the Anzob Pass, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, on 28 July 1968, by Z. Izzatulaev and identified by him.

The oviduct forms a sudden bend, with the inner walls touching. As a rule there are 3-4 mucous glands sited around the upper vagina, all usually having 2 branches. The dart sacs are very massive and spherical, and the inner ones are not as short as the outer ones. The length of the lower vagina exceeds its width 3-4 times. The vaginal plicae are rather clear and show



FIGS. 27-31. *Leucozonella rufispira* (Martens), Anzob pass, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 28 July 1968. **27,** reproductive tract; **28,** penis, penis sheath partly removed; **28,** cross-section of verge; **30,** cross-section of epiphallus; **31,** mantle collar.

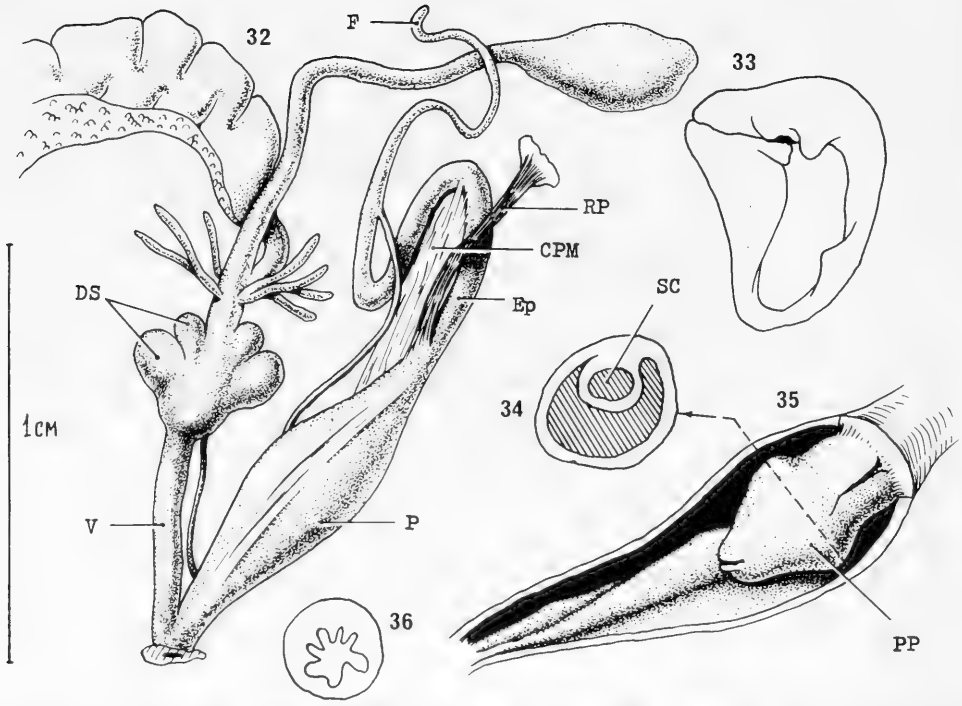
distinct laminae. The flagellum is a little shorter than the sharply curving and slightly coiling epiphallus. The loop of the epiphallus is drawn to the penis sheath by connective tissue bands (CPB, Fig. 27). The penis is globose, and the inner surface of its sheath is smooth. The verge is fusiform and has a very deep, long, longitudinal groove. In addition, there is a transverse groove not wholly encircling the proximal part of the papilla. The papilla wall has no cavities. The spermathecal duct is thin and almost straight, ending in an oval receptaculum seminis and stopping considerably short of the lower edge of the albumen gland.

Our observations differ somewhat from those of Likharev & Starobogatov (1967). According to these authors, there are 2 mucous glands on the very bend of the oviduct and the flagellum is 2/5 the length of the epiphallus.

Leucozonella retteri (Rosen, 1897)
Figs. 32-36; Pl. II, 7

Four specimens were examined; they were collected in Kandara Valley, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, on 2 July 1967, by Z. Izzatulaev and identified by him.

The oviduct forms only a slight bend. There are 4 mucous glands; they usually have 2 branches (1 specimen had 1 simple gland, Fig. 32). The dart sacs are massive, inflated, globose. The lower vagina is set off from the region of the dart sacs by a marked narrowing; it is long, narrow and cylindrical; its length is 5-6 times its width. The vaginal plicae are not distinct and form clear-cut lobes only at the entrance of the dart sacs. The flagellum is about as long as the epiphallus; the latter curves twice. There is a connective membrane between penis and epiphallus (CPM, Fig. 32); connective tissue bands also run along the penis sheath surface. The penis is massive, fusiform. The verge has a characteristic shape: its proximal part is cylindrical; it then suddenly widens out and ends in a conical distal part (Fig. 35). The seminal duct is fused to one side of the inner papilla wall, and it is embraced on all other sides by a vast intrapapillar cavity. A heavy crest-shaped plica runs on the inner surface of the penis. The spermathecal duct is



FIGS. 32-36. *Leucozonella retteri* (Rosen), Kandara Valley, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 2 July 1967. **32**, reproductive tract: **33**, mantle collar; **34**, transverse section of papilla showing single intrapapillar cavity with crescent-shaped cross-section; **35**, penis, penis sheath partly removed; **36**, cross-section of epiphallus.

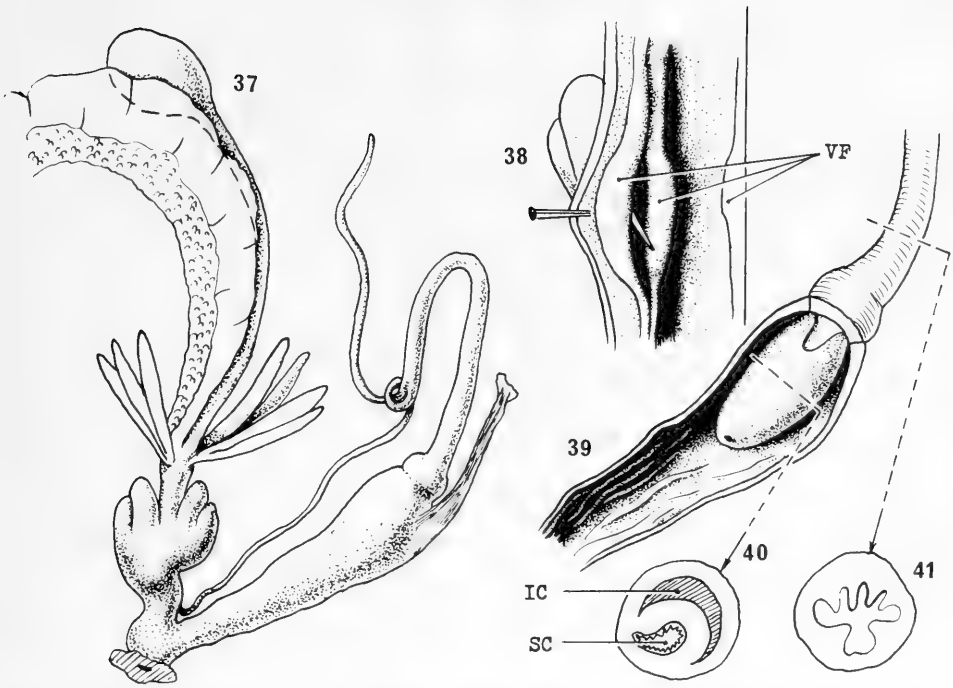
slightly curved; an oval spermatheca reaches the lower edge of the albumen gland.

Leucozonella caria Shileyko, sp. nov.
Figs. 37-41; Pl. II, 8

Three specimens from the Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, at the outskirts of the Khodzcha-Obi-Garm Rest Home, were examined. They were collected on 28 May 1968 by Z. Izzatulaev and identified by me. The holotype is at the Zoological Institute, USSR Academy of Sciences, Leningrad.

The shell is small (8-9 mm) and very much like that of the European *Trichia* s. str., but it has a constant distinction: it has no more than 4.5-5 whorls, whereas in fully adult *Trichia* s. str. there usually are 6 whorls. The shell may vary in aspect from lowly conical to nearly conical; it is brownish-horny with a washed-out light line at the periphery. It is sculptured with fine and rather light radial lines. The body whorl is 1.5 times wider than the penultimate whorl. The shell is covered with long periostracal hairs, curved at the ends as in *Trichia plebeia*. When the hairs are lost, marks are left in their place that look like short radial wrinkles. Spiral sculpture is absent. The nuclear whorls are glossy and not clearly limited from the adult whorls, and they have the same color. The whorls increase rather slowly in size, though more rapidly than European *Trichia*. The aperture is rounded inside; slightly away from the edge there is a low but wide, light-colored lip, which occupies the whole edge of the aperture in adults and is not limited to the basal part only. The umbilicus is narrow but perspective; though the columellar edge of the aperture is slightly reflected, it does not cover the umbilicus. The aperture is not deflected, moderately oblique. Measurements are as follows:

	Holotype (mm)	Paratype (mm)	
Shell height	4.7	6.0	4.2
Shell width	7.8	8.8	7.7



FIGS. 37-41. *Leucozonella caria* Shileyko, sp. nov., holotype, Khodzcha-Obi-Garm Rest Home, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 28 May 1968. **37,** reproductive tract; **38,** inner structure of vagina in dart sac region; **39,** penis, penis sheath partly removed; **40,** cross-section of verge; **41,** cross-section of epiphallus.

The shell of *L. caria* can be distinguished from that of *L. retteri* not only by its small size but also by its color and texture: *L. retteri* is light brown to reddish and has no hairs. In the structure of the verge, *L. caria* is characteristic for the Asiatic group and sharply differentiated from the European *Trichia* (cf. Fig. 160, IV, V).

The oviduct is short and does not form a bend; there are 3 mucous glands, each with 2 or 3 branches. These are long, 1.5-2 times longer than the upper vagina. The dart sacs are relatively very massive, elongate; the outer sacs are closely pressed to the inner ones. The lower vagina is straight or curved. The flagellum is fine, not as short as the epiphallus, which forms a curve. The penis is elongate; between epiphallus and penis there is a ring-like swelling (bulla). The verge is small and oval and does not measure more than half the penis length. The intrapapillar cavity is as in *L. retteri*: it embraces the seminal duct, which adheres to the inner papilla wall on one side. The interior of the penis sheath is covered by numerous small longitudinal folds. There are only 2 pairs of vaginal plicae. At the base of the dart sac ducts they form well-developed lobes. The spermathecal duct is almost straight, ending in a small oval spermatheca that falls considerably short of the lower edge of the albumen gland.

Genus **Hygrohelicopsis** Shileyko, gen. nov.

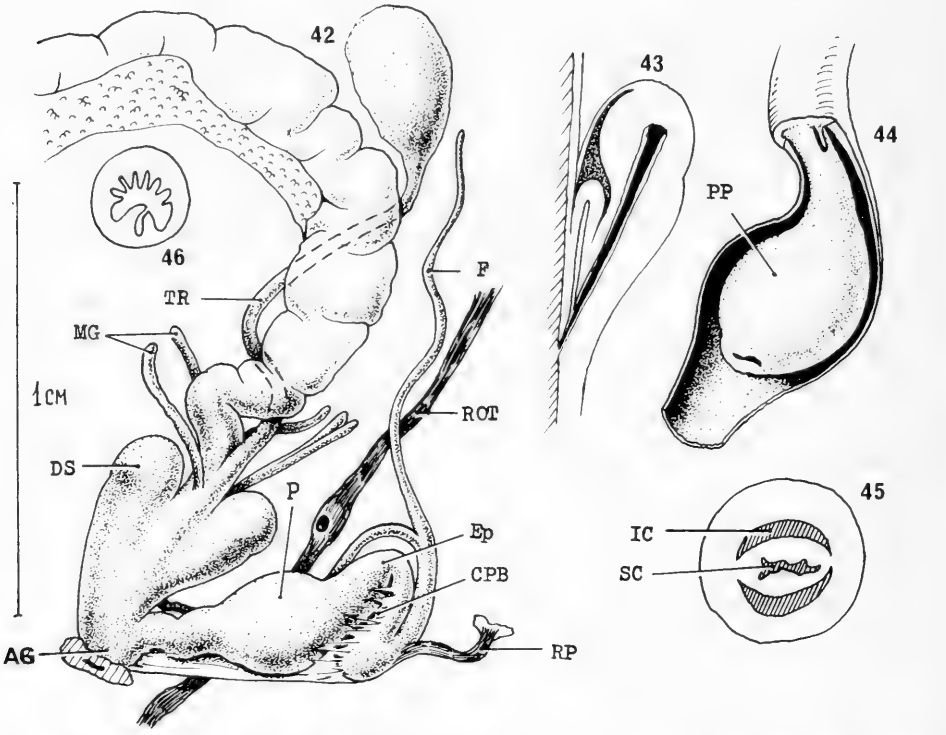
The shell is very flattened, lilac-chestnut-colored, with pale transverse spots. The aperture is rather large, with the body whorl rapidly increasing in height. The umbilicus is narrow but deep, penetrating well into the shell. Unlike all other Trichiinae, in this genus, the right-hand ocular retractor does not cross the distal part of the genitalia, passing between the penis and vagina, but only runs beside them. A further distinguishing mark is the seeming absence of the inner pair of dart sacs, which, however, are present internally. The flagellum is about as long as the penis and the epiphallus. Inside the verge the seminal duct is surrounded by a pair of intrapapillar cavities embracing it from 2 sides.

Hygrohelicopsis darevskii Shileyko, sp. nov.
Figs. 42-46; Pl. III, 9

Two specimens were examined. They were collected in Chegem Valley, N slope of the central Caucasus, USSR, at 200-2500 m above sea level, on 9-10 August 1965, by I. S. Darevski and identified by me. Holotype and paratype are in the Zoological Institute, USSR Academy of Sciences, Leningrad.

The shell is very lowly conical, of a lilac-chestnut color, with pale, wide transverse spots and a silky gloss. It is sculptured with irregular, smooth, transverse wrinkles, particularly on the upper part of the body whorl; the basal part is smoother. In places there are weak spiral lines. The aperture is rather large, oblique and somewhat deflected. The aperture edges are simple, but the basal edge is slightly reflected. At the rim there is a heavy, snow-white lip which is seen through the body whorl wall as a wide, white line with indistinct limits. During development not 1 lip alone but 2-3 lips are formed; earlier lips are also seen as wide, light lines. There are 5 whorls of moderately rapid growth. The body whorl increases rapidly in height so that the parietal wall of the aperture makes a rather acute angle with the periphery of the body whorl. This character easily distinguishes this species from *Caucasigena schaposchnikovi*, in which the lower part of the palatal aperture wall is almost parallel to the periphery of the body whorl (see Pl. III, 9a and Pl. V, 19a and 20a). The umbilicus is narrow but deep. The nuclear whorls are smooth, lightly horn-colored; they are vaguely separated from the definitive whorls. The dimensions are

	Holotype (mm)	Paratype (mm)
Shell height	6.0	6.0
Shell width	10.5	10.4



FIGS. 42-46. *Hygrohelicopsis darevskii* Shileyko, sp. nov., holotype, Chegem Valley, N slope of central Caucasus, USSR, 10 August 1965. **42**, reproductive tract; **43**, longitudinal section of dart sac region; **44**, penis, penis sheath partly removed; **45**, transverse section of verge showing paired intrapapillar cavities of crescent-shaped cross-section on either side of seminal duct, which is fused with the papillar wall at 2 opposite points; **46**, cross-section of epiphallus.

Anatomically this species stands apart from other representatives of the *Trichia* s. lat. group. The oviduct makes a sudden bend. There are 4 mucous glands; 1 or 2 are 2-branched. The upper and lower vagina in the dart sac region is bulbous; the dart sacs are very large. Externally only 1 pair of dart sacs is seen (Fig. 42) but upon dissection one can see small rudimentary inner sacs fully covered by a common connective tissue sheath (Fig. 43). The flagellum is thin, about twice as long as the epiphallus. The epiphallus sharply curves twice and is held in this position by connective tissue bands. The penis is globose, inflated. The verge is cylindrical proximally and bulbous distally. The seminal duct is surrounded by a pair of intrapapillar cavities (Fig. 45). The penis sheath is smooth inside. The spermathecal duct is long, slightly curved, with a bulky rounded spermatheca almost reaching the albumen gland.

It is emphasized that the right-hand ocular retractor (ROT, Fig. 42) is situated as is characteristic for the "Helicellinae" auct.: i.e., it passes near the distal genitalia but not between them. We shall return to this point in the discussion.

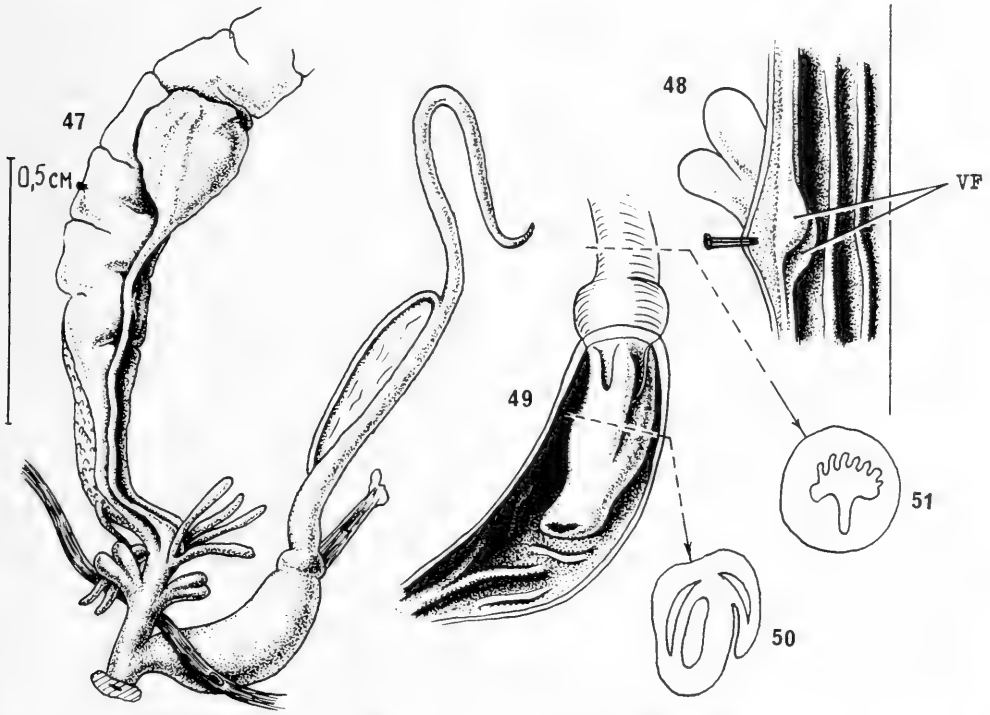
Genus **Teberdinia** Shileyko, gen. nov.

The shell is brownish-yellow in color with a slightly inflated basal part; it is lowly conical with an umbilicus of medium size. The outer and inner dart sacs are closely apposed and fused along the greater part of their length, but the inner sacs are distinctly separate from the upper vagina. The seminal duct is surrounded by a pair of intrapapillar cavities. It is the only Caucasian form with a deep longitudinal groove on the surface of the penis papilla that separates off a lobe, and the only form so far observed in which one of the intrapapillar cavities reaches into the lobe.

Genus monotypic.

Teberdinia zolotarevi (Lindholm, 1913)
Figs. 47-51; Pl. III, 10

One specimen was examined anatomically. It was collected from the Teberdia



FIGS. 47-51. *Teberdinia zolotarevi* (Lindholm), Teberdia Nature Reserve, NW Caucasus, USSR, 24 July 1958. **47,** reproductive tract; **48,** inner structure of vagina in dart sac region; note lobes on vaginal folds; **49,** penis, penis sheath partly removed; **50,** cross-section of verge; **51,** cross-section of epiphallus.

Nature Reserve, NW Caucasus, USSR, by L. Arens on 24 July 1958; I identified it. Holotype and paratype (not fully adult) are at the Zoological Institute, USSR Academy of Sciences.

The shell is lowly conical with a markedly bulbous basal part and flattened whorls. The color is brownish-yellow. It is sculptured with smooth radial wrinkles and spiral lines. The aperture is oblique, and in the basal (palatal) part it is twisted slightly to the right. The aperture edges are simple, slightly reflected. The columellar edge is more reflected. Near the edge there is a heavy white lip, visible through the shell as a wide, light line. The shell is perforate, and the aperture is slightly covered by the columellar edge. There are 5.75 whorls. The holotype shell height is 6.7 mm; width, 11.3 mm.

The oviduct forms a smooth bend. There are 3 mucous glands, each with 2 branches. The dart sacs are small, closely pressed together in pairs and directed away from the upper vagina. The lower vagina is short, and cylindrical, with narrow but rather high plicae. At the base of the dart sac duct they form rather round lobes. The flagellum is longer than the straight cylindrical epiphallus. Between the flagellum and the penis there is a clear-cut ring (bulla). Internally the penis sheath bears thin long plicae. The verge is short, bag-like, blunt at the edge. A deep longitudinal groove occupies about half the length of the verge. Of the 2 intrapapillar cavities, 1 is adjacent to the seminal duct and the other characteristically extends into the longitudinal lobe separated by the above-mentioned groove (Fig. 50). The spermathecal duct is very thin and closely pressed to the spermoviduct; it ends in a bulky, bag-like receptaculum seminis that does not reach the albumen gland.

Genus **Kokotschashvilia**
Hudec & Lezhawa, 1969

The shell is lowly conical to top-shaped, white or horny, perforate to umbilicate and perspective. It is sculptured with radial lines, spiral lines or granules. The flagellum is about half as long as the epiphallus. There are 4 mucous glands, each with 2-4 branches. The dart sacs are massive and rounded. The receptaculum seminis is very bulky; when it is not full its walls are collapsed, so in this condition it looks atypical. The seminal duct is surrounded either by a pair of intrapapillar cavities or, when a longitudinal partition between the cavities is absent, by a single intrapapillar cavity with a crescent-shaped cross-section that embraces the seminal duct from 3 sides. The seminal duct may either adhere closely to the inner papilla wall on one side or on a thin, long band on that same side or also on the opposite side.

Type-species: *Helix holotricha* Boettger, 1884

Kokotschashvilia makvalae
(Hudec & Lezhawa, 1969)
Figs. 52-56

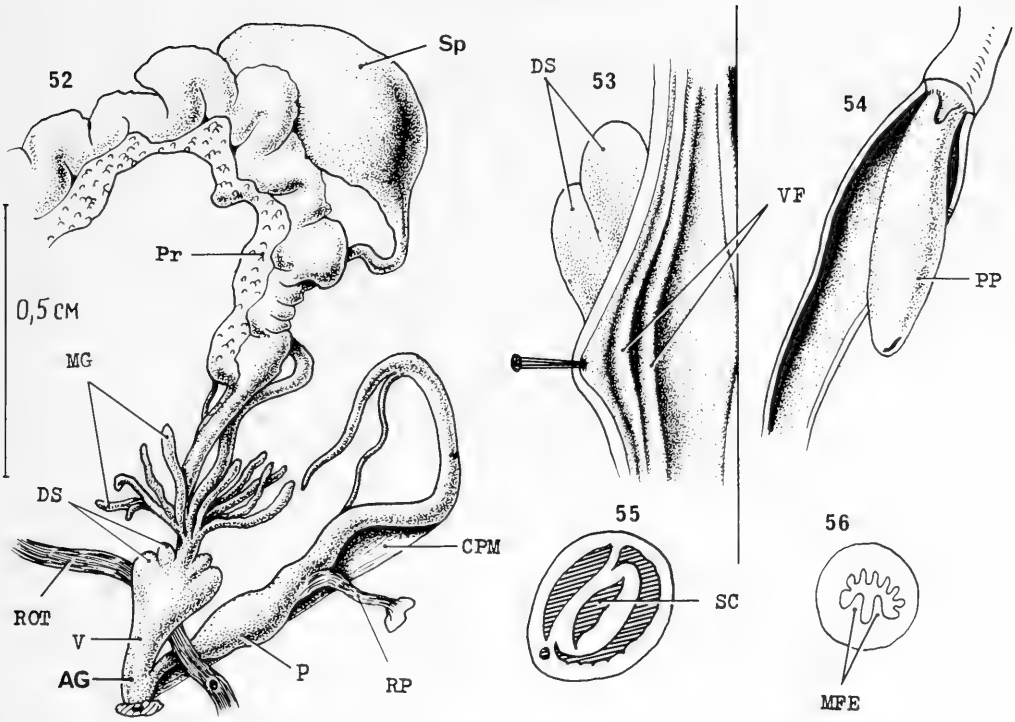
The anatomy of 1 specimen was studied. It was collected at Balda village, Gegechkor region, NW Georgia, Grusinian SSR, on 3 May 1967 by G. Lezhawa and identified by V. Hudec. A description of the shell is given by Hudec & Lezhawa (1969a, b).

The spermoviduct passes straight and without curving into the oviduct, which also shows no bend. Each of the 4 mucous glands has 2-3 branches. The inner dart sacs are slightly smaller than the outer ones. Inside the vagina there are, laterally, 2 pairs of long plicae. At the exit of the dart sac duct the vaginal plicae do not form any lobes. The flagellum is approximately half as long as the slightly curved epiphallus. A penial membrane, rather weakly developed, is present. The penis is fusiform, slightly bulbous. The penis sheath is smooth inside. The verge is fusiform. The seminal duct is held in place by 2 longitudinal bands. There is a fine channel in the papilla wall at the place of attachment of 1 of the bands. The spermathecal duct is moderately twisting, ending in a wide receptaculum seminis that almost reaches the albumen gland.

Kokotschashvilia tanta Shileyko, sp. nov.
Figs. 57-61; Pl. III, 11

Specimens were collected from alpine meadows near Lebarde village, Gegechkor region, NW Georgia, Grusinian SSR, on 5 July 1962 by M. G. Natsvlishvili and identified by me. Three specimens were examined anatomically. The holotype and 6 paratypes (2 damaged) are at the Zoological Institute, USSR Academy of Sciences, Leningrad.

The shell is lowly conical to lowly top-shaped. Fresh shells are a light horn color.



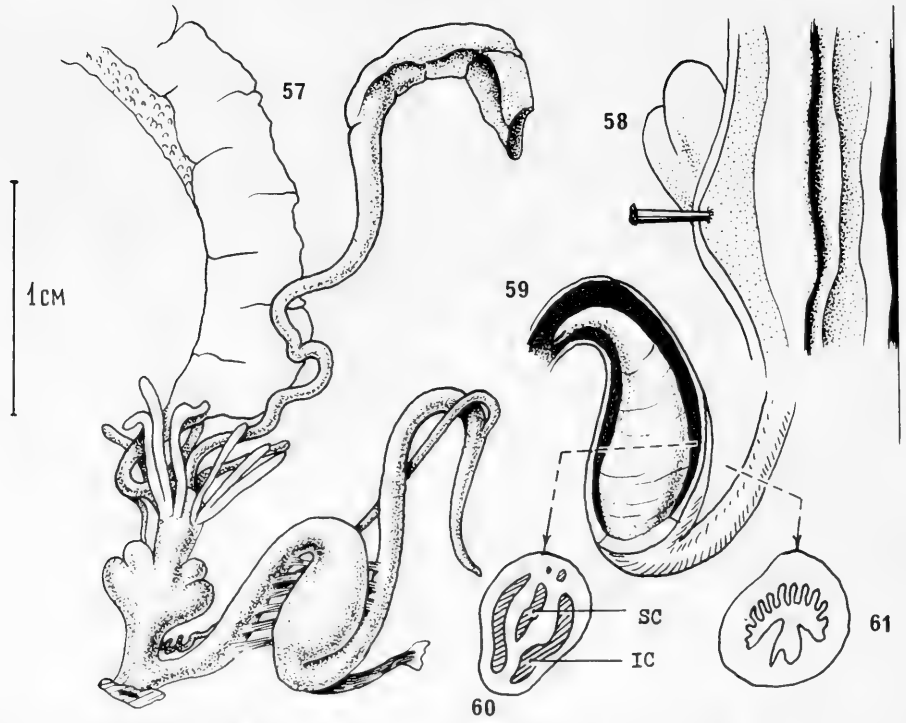
FIGS. 52-56. *Kokotschashvilia makvalae* (Hudec and Lezhawa), Balda village, Gegechkor region, NW Georgia, Grusinian SSR, 3 May 1967. **52,** reproductive tract; **53,** inner structure of vagina in dart sac region; **54,** penis, penis sheath partly removed; **55,** transverse section of verge; the 2 stalks joining the seminal duct to the papillar wall are the longitudinal bands, in cross-section, which attach the duct to the papilla; **56,** cross-section of epiphallus.

The color is darker above and under the periphery of the body whorl, forming vague darker lines. The shell is sculptured with irregular, rather fine radial lines; in places there are vague granulose and spiral lines, more clearly seen on the periphery and the basal part of the shell. A pale line runs above the suture. The aperture is roundly lunate, oblique and deflected. A narrow light spiral line starting a distance back of the aperture runs high on the shoulder of the whorl. The umbilicus is eccentric, and through it the penultimate whorl is fully visible. A pale line on the shell surface corresponds to the lip.

The shell is similar to that of *K. makvalae*, its nearest relative, but differs by its darker color (*K. makvalae* is uniformly white), by the presence of spiral sculpture, by a more oblique aperture, by a larger number of whorls (6.5-7.0 compared to 6) and by its definitely larger size. The largest of our specimens of *K. makvalae* has a diameter of 21.5 mm compared to 22-27 mm in *K. tanta*. It differs from *K. eberhardi* in color and shape (*K. eberhardi* is yellowish and has a wider umbilicus and a higher spire) and by its much larger size. Shell measurements, including 5 of the paratypes, are as follows:

	Holotype (mm)	Paratypes (mm)
Shell height	16.8	16.7 18.0 14.6 16.3 17.8
Shell width	25.5	27.0 24.7 22.2 25.0 26.3

More distinct interspecific differences are in the reproductive anatomy, particularly in the inner structure of the verge. The oviduct makes a smooth bend; it is thin but becomes wider below. There are 4 mucous glands, each with 2-3 branches. The dart sacs are rather massive, globose. The vaginal plicae are heavy and form wide lobes. The flagellum is about half the length of the epiphallus. The latter is



FIGS. 57-61. *Kokotschashvilia tanta* Shileyko, sp. nov., paratype, Lebarde village, Gegechkor region, NW Georgia, Grusinian SSR, 5 July 1962. **57**, reproductive tract; **58**, inner structure of vagina in dart sac region; **59**, penis, penis sheath partly removed; **60**, cross-section of verge; **61**, cross-section of epiphallus.

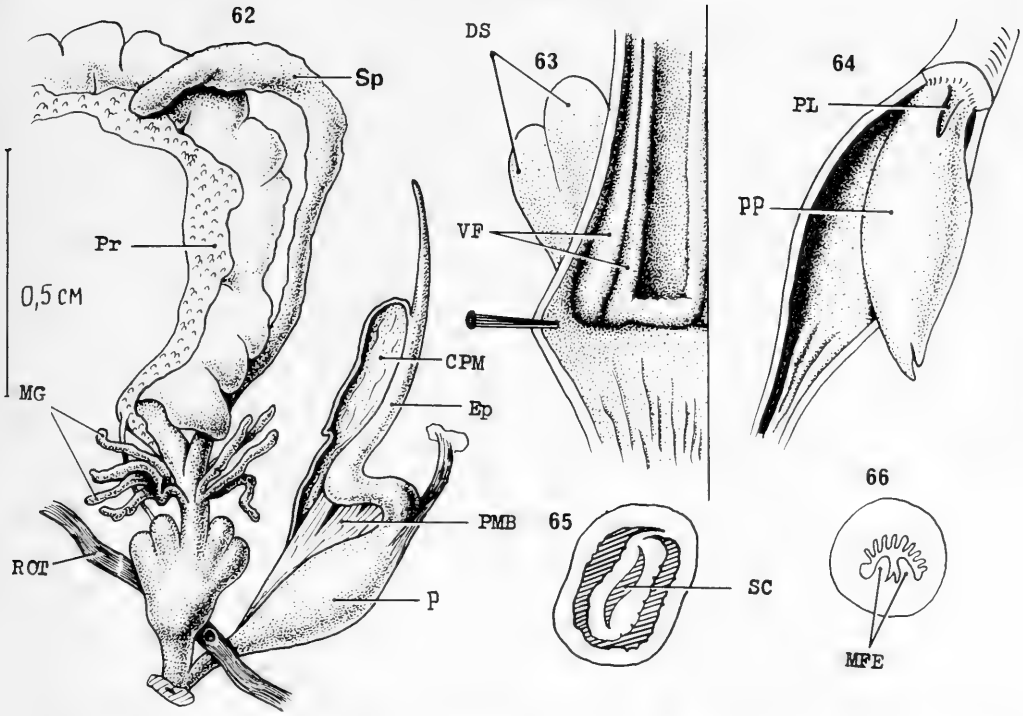
attached to the penis by connective tissue bands. The penis consists of 2 parts: a globose fusiform proximal part and a cylindrical distal one; these are also connected by connective tissue bands (Fig. 57). The verge lies in the former. As in *K. makvalae*, the seminal duct is surrounded by a pair of intrapapillar cavities, but in this species one of the partitions separating the pair is practically absent; thus the seminal duct is closely united with the inner papilla wall on that side (cf. Figs. 55 and 60). As in *K. makvalae*, there is a narrow channel in the papillar wall along the seminal duct, and near it, moreover, a very fine capillary. The spermathecal duct is thin and slightly curving; it gradually merges with the bag-like receptaculum seminis (spermatheca), which was in a flaccid state in all specimens studied. The spermatheca was apparently empty because of the time of collection (June).

It is noteworthy that in *K. holotricha* and *K. eberhardi*, which were collected during June-July also, the spermathecas were empty, whereas in *K. makvalae* and *K. phaeolaema*, collected in early and mid-May, it appeared to be full (cf. Figs. 52, 57, 62, 67 & 71).

Kokotschashvilia holotricha (Boettger, 1884)
Figs. 62-66

Two specimens were examined. They were collected at Tsebelda village, near Sukhumi, in the Black Sea coastal region, Grusinian SSR by G. Lezhawa on 15 June 1968 and identified by V. Hudec. A good shell description is in Likharev & Rammelmeyer (1952).

The spermoviduct curves as it passes to the free oviduct, which itself is straight. There are 4 mucous glands, all with 2 branches. The dart sac region is set off from the lower part of the vagina, which is narrower. The vagina is short. Vaginal plicae occur in pairs and occupy a lateral position; they are abruptly cut off distally and connected by a transverse fold (Fig. 63); the lower part of the vagina and genital atrium is either smooth internally or has irregular fine wrinkles. The flagellum is



FIGS. 62-66. *Kokotschashvilia holotricha* (Boettger), Tsebelda village, near Sukhumi, Black Sea region of Georgia, Grusinian SSR, 15 June 1968. **62,** reproductive tract; **63,** inner structure of vagina in dart sac region; **64,** penis, penis sheath partly removed; **65,** cross-section of verge; **66,** cross-section of epiphallus.

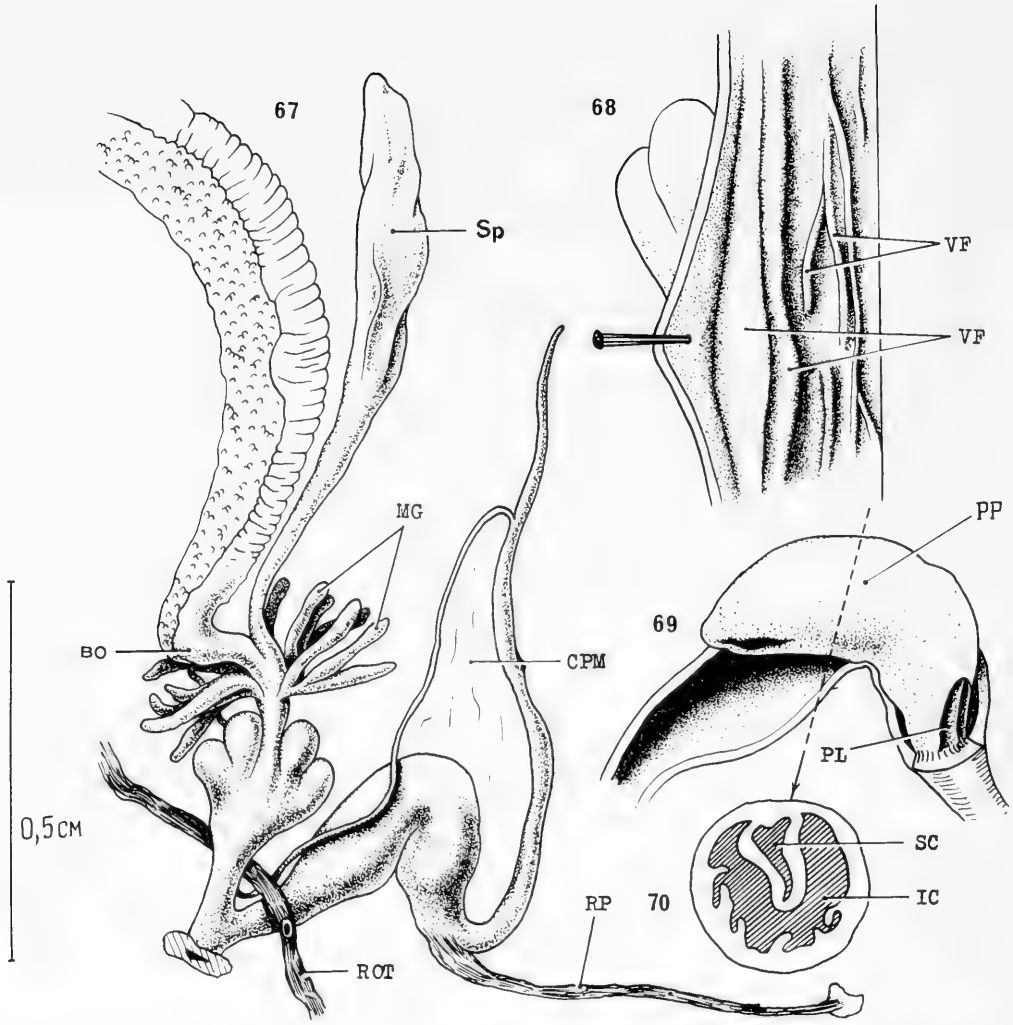
short and conically tapering. It measures about half the length of the epiphallus, which is S-shaped and supplied with particularly well-developed connective tissue bands containing muscle fascicles. The penis is fusiform and has longitudinal folds in the vicinity of the genital atrium. The verge is fusiform; the seminal duct is connected to the inner surface of the papilla wall by 1 single long band and surrounded on all sides by an intrapapillar cavity (Fig. 5); the papilla wall does not include any channels.

Hudec and Lezhawa (1969a, b) did not comment on the S-shaped bend of the epiphallus in this species. Hesse (1931) pointed out that not all the mucous glands have 2 branches; some may be simple.

Kokotschashvilia eberhardi,
Shileyko, sp. nov.
Figs. 67-70; Pl. III, 12

Specimens were collected in July 1969 between the villages of Sioni and Kazbegi, on the S slope of the central part of the main Caucasus range, Grusinian SSR, at an altitude of 2500-3000 m above sea level, by E. Clauss. They were identified by me. Four specimens were examined anatomically. The holotype and 4 of the paratypes are at the Zoological Institute, USSR Academy of Sciences, Leningrad.

The shell is compressedly conical to almost globose. Its coloration is characteristic: the background is a pale straw color that gradually becomes lighter near the sutures: above and under the periphery it may be darker, forming 2 dark bands. It has a sculpture of irregular radial wrinkles, more clearly expressed near the sutures. With a lens it can be seen that in fresh shells the wrinkles are lighter than the background, in fact almost white. There are granulose spiral lines, mostly on the basal part of the shell. The aperture is almost round; at its edge there is a well-developed white lip. This lip is clearly visible from the outside as a whitish-yellow band; 2-3 such lips are formed during the life of the



FIGS. 67-70. *Kokotschashvilia eberhardi* Shileyko, sp. nov., paratype, between Sioni and Kazbegi villages, S slope of central Caucasus, Grusinian SSR, July 1969. **67**, reproductive tract; **68**, inner structure of vagina in dart sac region; **69**, penis, penis sheath partly removed; **70**, cross-section of verge.

mollusk, and these earlier ones are also visible through the shell as light lines. The umbilicus, though narrow, is perspective. There are 6 whorls. The dimensions of our shells are:

	Holotype (mm)	Paratypes (mm)
Shell height	10.3	10.7 11.0 11.7 11.0 10.5 10.7
Shell width	14.5	15.5 15.0 14.1 15.0 14.6 14.8

The shell of this species resembles that of *K. makvalae* and *K. tanta* in shape, though it differs in its smaller size, in color (*K. makvalae* is white and *K. tanta* horn-colored) and also in sculpture. There are clearer distinctions yet in the structure of the reproductive organs.

The spermoviduct makes a sudden but smooth bend as it passes to the free oviduct, which itself is slightly bent. There are 4 mucous glands, each with 3-4 branches. The dart sacs are very massive and globose; the sac region is set off from the vagina by a slight narrowing of the lower vagina. In addition to the usual paired vaginal plicae in a lateral position, there are supplementary smaller vaginal plicae. Usually the basic plicae do not form lobes. The flagel-

lum is thin, and its length is about half that of the epiphallus, which is also thin, cylindrical and weakly curving. The penis is massive, more or less curved. There are no muscle bands, only a fine connective tissue membrane stretched among penis, epiphallus and vas deferens. The inner surface of the penis sheath is smooth. The verge is bulky and bag-like and has a large papillar lacuna. The seminal duct is closely fused to one side of the inner papillar wall and surrounded on all other sides by a wide intrapapillar cavity as in *K. tanta*; however, the inner papillar wall facing this cavity is not smooth, as in *K. tanta*, but bears many lamellae and folds (Fig. 70). The spermathecal duct is almost straight and demarcated from the receptaculum seminis, which has very thin walls. In the specimens studied the receptaculum walls were wrinkled and collapsed, the receptaculum apparently empty. The organ does not reach the albumen gland. The intense pigmentation of the pallial nerve is noteworthy.

Kokotschashvilia phaeolaema
(Boettger, 1886)
Figs. 71-76; Pl. IV, 13

Sixteen specimens were examined anatomically; 14 of these were collected on 14 May 1970 in Chegem Valley, which descends the N slope of the central Caucasus (USSR) as a tributary to the valley of the Terek; they were identified by me. Two specimens were collected from the Khunzah district of Daghestan on 26 August 1955 by T. Khasanov and identified by I. M. Likharev.

As this species is little known, the shell is described here. It is lowly conical and almost globose, light horny, yellowish or chestnut in color, with a diffuse light line at the periphery, radial folds and distinct spiral ridges. The aperture is roundly lunate, slightly deflected and oblique. The umbilicus is narrow and may be half-covered by the reflection of the columellar lip. There is a thick white lip on the inside edge of the aperture. During life the animal forms 3-5 such lips, which can be seen translucently as radial bands of a lighter hue. On the outside, closely joined to them, there is a line darker than the background of the shell. There are 6 whorls. The shell height (from 53 specimens) is 9-12 mm; the width, 10.5-16.0 mm.

Lindholm (1913) described *Helix* (*Fruticocampylaea*) *phaeolaema* Boettger var. *tenuitesta*, which differs by its small, thin-walled, translucent shell (height, 9-10 mm; width, 10.5-12 mm), with a translucent lip and a yellowish or light brown horny color. As these characteristics are all seen in some specimens of our series, it is not necessary to separate this form.

The oviduct makes a sudden sharp bend. There are 4 mucous glands, each usually with 2 branches, but sometimes we find a secondary branching in some particular gland. The dart sacs are well-developed and globose. The vaginal plicae are only moderately developed, but form clear-cut lobes near the opening of the dart sac duct. The length of the flagellum is 1/2 to 3/4 that of the epiphallus. The latter bends twice and is attached to the penis by a well-developed connective tissue membrane (CPM, Fig. 71) in which there are muscle bands. The penis is large, bag-like or globose. Its sheath is smooth inside, except that there are a few longitudinal folds near the genital atrium. The papilla is very inflated, globose or pear-shaped. The seminal duct is suspended from the papillar wall by 2 long bands; i.e., it is surrounded by a pair of intrapapillar cavities. In contrast to other species of this genus, the papillar walls are thick and contain additional intrapapillar cavities in the form of small scattered sinuses in the wall (Fig. 4), which therefore sometimes looks spongy. The receptaculum seminis is very massive and bag-like; its upper edge reaches the lower part of the albumen gland.

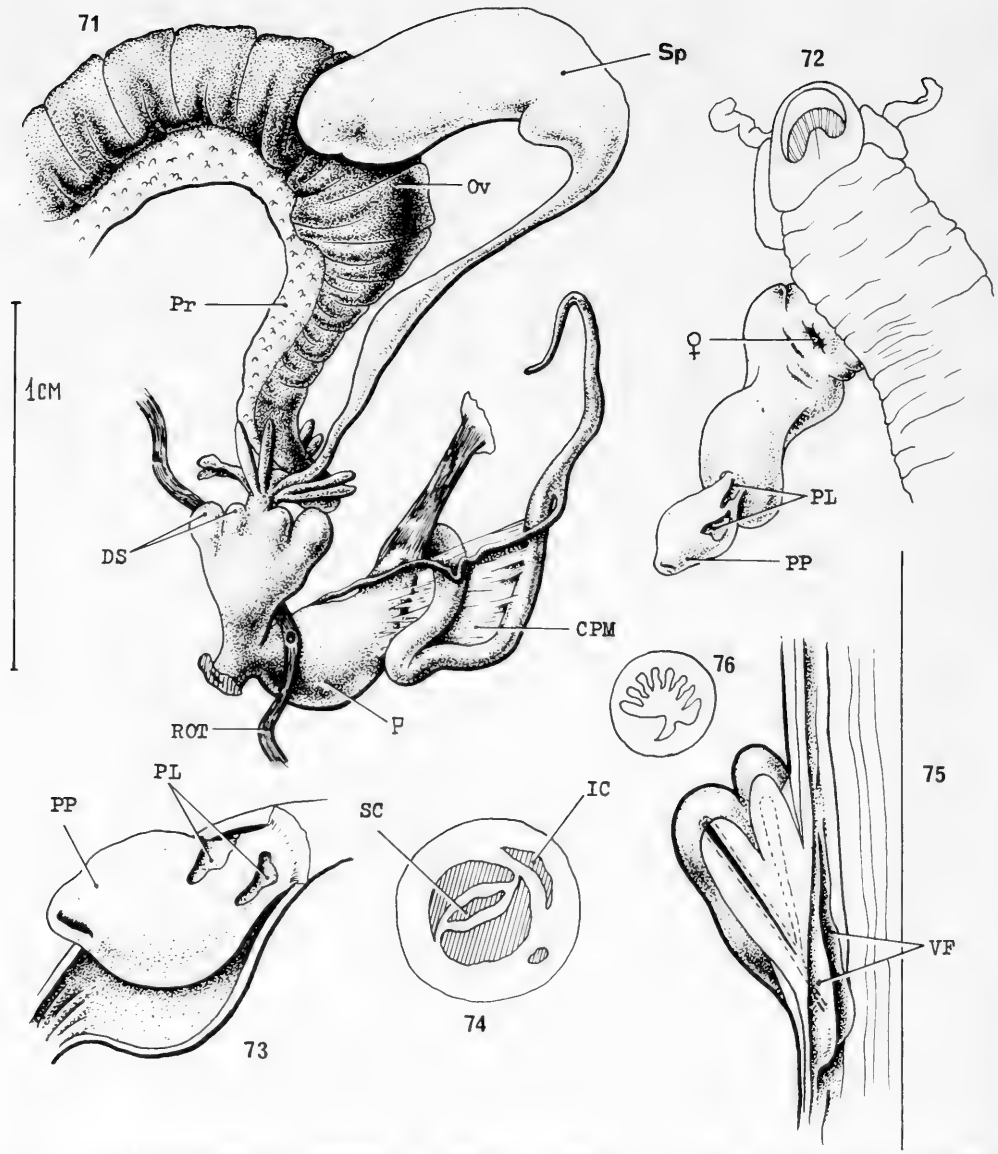
The characteristic peculiarity of this species is the intense pigmentation of the spermoviduct (especially on the oviduct side), of the distal part of the intestine, of the mesentery and of the surface of the circumesophageal nerve ring.

The gross morphology of the genitalia of this species was first described by Kalitina (1958: 159-160).

Genus **Caucasigena** Lindholm, 1927

The shell is almost flat to lowly conical, smooth or ribbed, umbilicate or perforate (subgen. *Dioscuria*). Inside the aperture edge there is a heavy white lip.

There are 3 or 4 mucous glands, each with 2 branches, and a tendency neither for secondary branchings nor for simple,



FIGS. 71-76. *Kokotschashvilia phaeolaema* (Boettger), Chegem Valley, N slope of central Caucasus, USSR, 14 May 1970. **71**, reproductive tract; **72**, penis in copulating position; verge (papilla) is moved out, penis sheath is turned inside out; **73**, penis, penis sheath partly removed; **74**, cross-section of verge; **75**, inner structure of vagina in dart sac region; **76**, cross-section of epiphallus.

nonbranching glands. Inside the papilla (verge) the seminal duct is very narrow. The longitudinal compartments of the intrapapillar cavity that lies on 1 side of the papilla are separated by longitudinal septa from one another and from the cavities (numbering from 3 to 9) that surround the seminal duct. The papillar lacunae are rather large.

Type-species: *Helix eichwaldi* L. Pfeiffer, 1846.

Subgenus **Caucasigena** s. str.

The shell is pale, with brown spiral bands that sometimes are so markedly developed they form nearly the whole background of the shell; in this case there

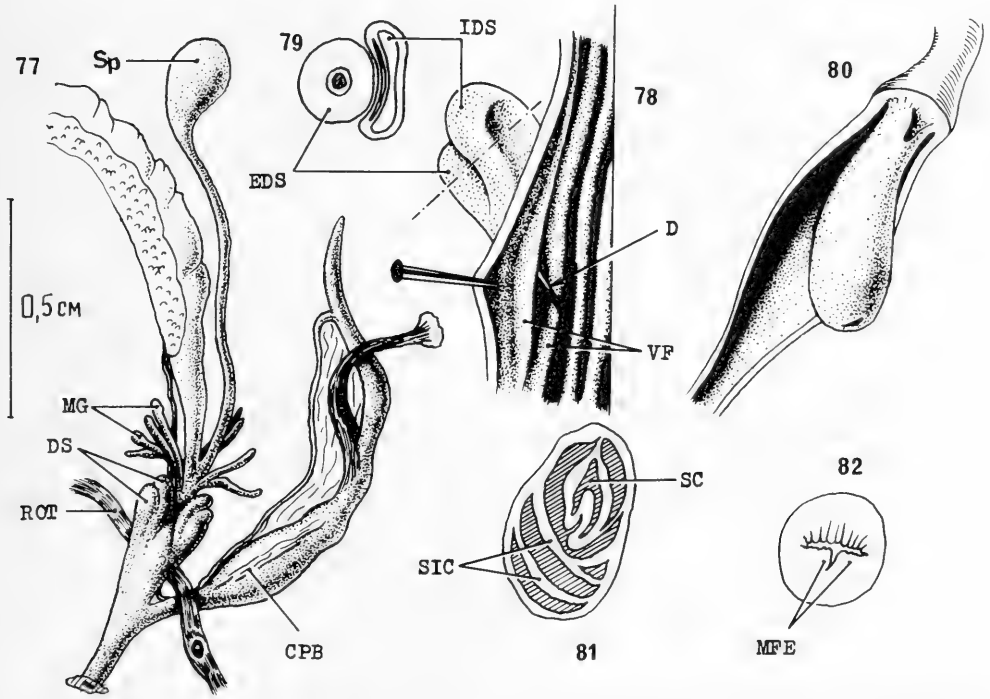
is a lighter line on the periphery. The radial sculpture varies from simple lines (ridges) to very rough ribs. The spiral sculpture appears either as fine striae or as very thin ribs. The flagellum is stout, conical, not more than 1/2 the length of the epiphallus, which is more or less straight. The inner dart sacs are the same size as the outer ones or slightly longer. The seminal duct is attached to the inner wall of the papilla by 1-3 long bands.

Caucasigena (Caucasigena) armeniaca
(L. Pfeiffer, 1846)
Figs. 77-82; Pl. IV, 14

The specimens described are from Mt. Bzovdal (Mt. Todar) in the Stepanavan region, Armenian SSR. They were collected on 18 July 1951 and identified by N. N. Akramovsky. Two of the 6 specimens available were dissected.

Because of some confusion in the literature, it is necessary to give a description of the shells. Records of this species in a number of districts of the main Caucasus range are erroneous: all those from the N slopes of the Great Caucasus are referable to *C. rengarteni*, which Likharev & Rammelmeyer (1952) mistakenly placed in synonymy with *C. armeniaca* and omitted from their index.

The shell is lowly conical with a blunt angle at the periphery; it is relatively thick-walled, of a brownish horny color, with a light line at the periphery. The sculpture is very characteristic and consists of minute, smooth, radial ribs visible only under magnification and spiral periostracal ribs. At first sight they seem like the usual spiral lines, but when magnified 30-40 × and viewed with an oblique beam of light, they are clearly seen to be fine, sparsely but regularly spaced ribs; next to these are fine, rare, weakly curved hairs. The umbilicus is at first wide, funnel-shaped, but it then sharply narrows though it remains perspective. The aperture is roughly lunate, very oblique; on the inner rim there is a somewhat thickened yellowish lip. The shell has 5 gradually increasing whorls. The dimensions of the 6 specimens are:



FIGS. 77-82. *Caucasigena (Caucasigena) armeniaca* (L. Pfeiffer), Mt. Bzovdal (Mt. Todar), Stepanavan region, Armenian SSR, 18 July 1951. **77,** reproductive tract; **78,** inner structure of vagina in dart sac region; **79,** cross-section of dart sacs; **80,** penis, penis sheath partly removed; **81,** cross-section of verge; **82,** cross-section of epiphallus.

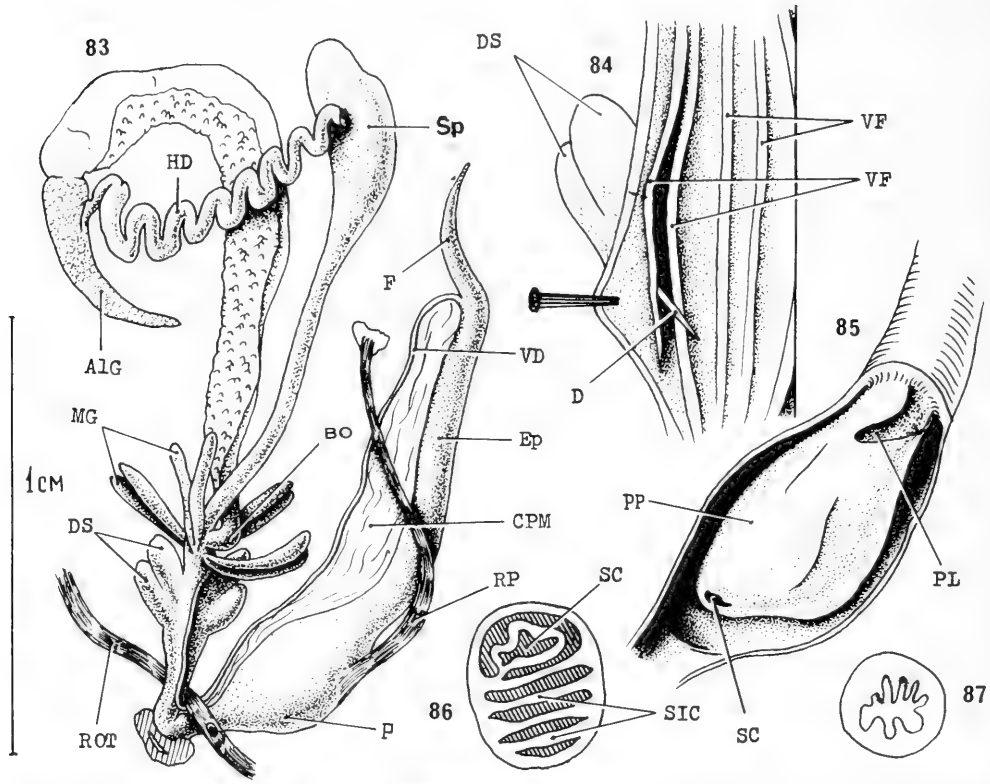
Shell height (mm)	4.0	4.0	4.3	4.5	3.7	5.0
Shell width (mm)	7.9	8.0	8.0	8.5	6.7	8.7

The oviduct is short and straight. There are 4 mucous glands with 2 branches. The dart sacs are characteristic: the upper edges of the inner sacs pass beyond and curve over the elongate outer sacs, and their central portion curves medially (Fig. 77), sometimes almost bent over double. The inner pair of dart sacs is compressed from both sides (Fig. 79). The flagellum and epiphallus are both short; the former is thick and conical and about half as long as the latter, which is cylindrical and weakly curved. The penis is slightly bulbous, fusiform. On the surface of the penis sheath there are slight connective tissue bands (CPB, Fig. 77). Between vas deferens, epiphallus and penis is stretched a fine transparent connective tissue membrane (CPM). The interior of the penis sheath is smooth. The verge is club-shaped or fusiform, with a well-developed papillar lacuna. The seminal duct is attached to the internal papillar wall by 2 long bands; further longitudinal septa divide off another 2-3 cavities (Fig. 81), making a total of 4-5 cavities. The spermathecal duct is almost straight and fine; the spermatheca is small and rounded; it nearly reaches the albumen gland.

Caucasigena (Caucasigena) tschetschenica
(Retowski, 1914)
Figs. 83-87; Pl. IV, 15

Six specimens were dissected. I collected them on a limestone cliff in Kurtatin Valley at about the middle course of Phiagdon River, central part of the N Caucasus, USSR, on 19 September 1970, and I identified them.

The species was described by Retowski (1914) on the basis of 2 specimens collected by Köhnig on Mt. Bonoz-Mta in the Checheno-Ingush ("Chechnya"). Since the



FIGS. 83-87. *Caucasigena (Caucasigena) tschetschenica* (Retowski), Kurtatin Valley, middle course of Phiagdon River, central part of N Caucasus, USSR, 19 September 1970. **83**, reproductive tract; **84**, inner structure of vagina in dart sac region; **85**, penis, penis sheath partly removed; **86**, cross-section of verge; **87**, cross-section of epiphallus.

species is little known, I here repeat its description, supplemented by my own observations.

The shell is small with a rather wide umbilicus, about 1/5 the shell width. The shell shape is almost flat to compressed conical. The color, as a rule, is horny with a pale line on the periphery accompanied by dark horn-colored bands on both sides. There are 5 relatively prominent, slowly increasing whorls, irregularly and roughly ribbed, with fine spiral lines in the spaces among the ribs. The ribs are white. The last whorl is angled or keeled. The aperture is strongly oblique, rounded, with a heavy white lip inside it. The parietal edge of the aperture is short. The columellar edge is widened and slightly reflected. The range of shell measurements from 100 shells is as follows: shell height, 3.2-4.8 mm; width, 6.5-9.8 mm.

Retowski gives the following sizes for his shells:

Shell height (mm) 4-4.5
Shell width (mm) 7.7-8

The oviduct forms a rather smooth bend. There are 4 mucous glands, each with 2 branches. The dart sacs are elongate, and the rather large inner pair points distinctly away from the upper vagina; the outer pair are almost the same size. The flagellum is conical, stout, about half as long as the epiphallus. The penis is fusiform, globose. The male ducts are linked by a fine translucent connective tissue membrane (CPM, Fig. 83). The verge is relatively very massive and bag-like. The seminal duct is attached to the internal papillar wall by only 1 longitudinal band; i.e., it is surrounded by 1 encircling cavity on 1 side of the papilla. In the remaining part of the verge, longitudinal septa standing transversely divide the intrapapillar cavity into 3-5 parts (Fig. 86). The inside of the penis sheath is smooth, with vague partitions in its distal part. The spermathecal duct shows no curves; it ends in an elongate-oval receptaculum seminis, which does not reach the albumen gland.

Caucasigena (Caucasigena) rengarteni
(Lindholm, 1913)
Figs. 88-91; Pl. IV, 16; Pl. V, 17

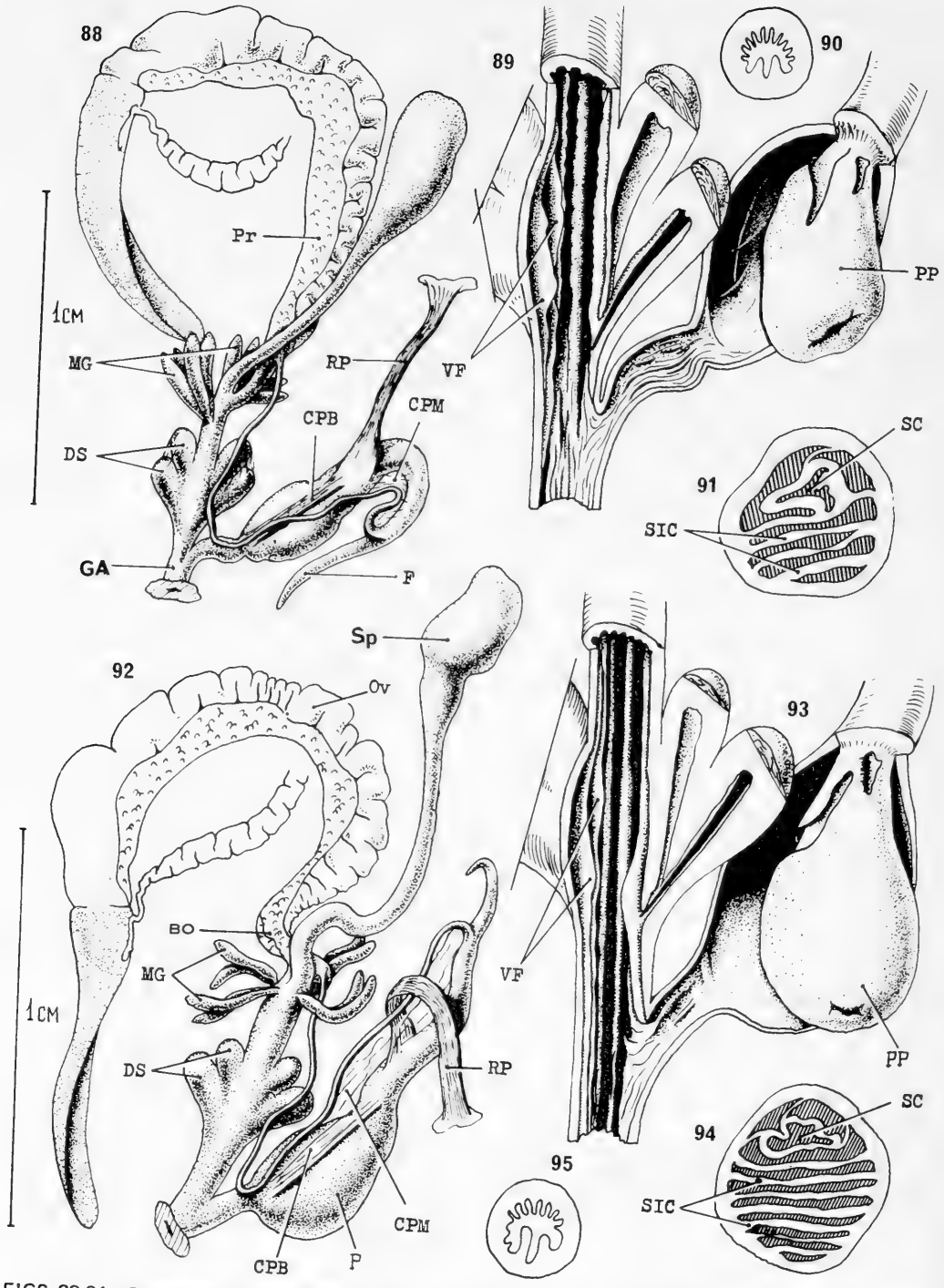
Twenty specimens were dissected. These were collected in the central part of the N Caucasus (USSR) as follows: 10 specimens from the middle course of the Chegem River near Khushtosyrt village, on 14 May 1970; 6 from the upper Chegem near Chegem village, on 19 May 1970; 4 from the old settlement of Ara-Boran between the Chegem and Baksan valleys, on 17 May 1970. These were all identified by me.

The shell characters are well given by Lindholm (1913), but he described the species from 3 specimens, whereas we had about 150 shells. It might now be added that some specimens have rare, wiry, short hairs on the surface of the shell. The dimensions may also be defined more exactly (Lindholm's values were 7 and 14.5 mm, respectively).

Shell height (mm) 5.8- 8.2
Shell width (mm) 7.3-15.5

In the same article Lindholm described *Helix* (*Fruticocampylaea*) *gerassimovi* and outlined the differences between that species and *Helix rengarteni*, but from his description it is not quite clear what characters other than size can distinguish these 2 species. I was able to familiarize myself with the type-series of both of Lindholm's species (i.e., *gerassimovi* and *rengarteni*) and found that the differences between them depended on intrapopulational variability. Most of the specimens from Ara-Boran correspond to the diagnosis of *H. gerassimovi*, whereas in most of the series of *C. rengarteni* from the Chegem Valley one could see continuous gradations among the shells that invalidated the diagnosis of 2 separate species. Thus *H. gerassimovi* is a synonym of *Caucasigena rengarteni*. On Plates IV and V, shell 16 is typical for *"Helix" rengarteni* and shell 17 for *H. gerassimovi.*

The oviduct is almost straight. There are 4 mucous glands, each with 2 branches. The flagellum is thick, pointed and 1/2 to 3/4 the length of the epiphallus. The latter is straight or curved; it is connected with the penis by a very fine translucent connective tissue membrane (CPM, Fig. 88). On the surface of the penis sheath there are connective tissue bands including muscle fibers. The penis consists of a fusiform, globose proximal part, in which the papilla is located, and a slightly curved cylindrical distal part. The proximal part is smooth inside, with only small wrinkles or folds; the distal part bears many long wrinkles



FIGS. 88-91. *Caucasigena (Caucasigena) rengarteni* (Lindholm), Khushtosyrt village, Chegem Valley, central part of N Caucasus, USSR, 14 May 1970. **88**, reproductive tract; **89**, distal part of genitalia, dissected; **90**, cross-section of epiphallus; **91**, cross-section of verge.
FIGS. 92-95. *Caucasigena (Caucasigena) eichwaldi* (L. Pfeiffer), Chmi village, Darial valley, near Ordjonikidze (North Osetia), central part of N Caucasus, USSR, 8 May 1970. **92**, reproductive tract; **93**, distal part of genitalia, dissected; **94**, cross-section of verge; **95**, cross-section of epiphallus.

that merge with the folds in the genital atrium (Fig. 89). There are raised, rather heavy, vaginal plicae that form clear lobes at the mouth of the dart sac ducts. The verge is bulky and bag-like with a foramen in the shape of a wide slit and with large papillar lacunae. The seminal duct is held in place by 3 or 2 longitudinal bands; the intrapapillar cavity in the other half of the papilla is divided into 4-6 compartments by longitudinal septa. The spermathecal duct is straight or very weakly curved; the oval receptaculum seminis lies some distance away from the albumen gland (Fig. 88). For diagnostic characters, see below under *C. eichwaldi*.

Caucasigena (Caucasigena) eichwaldi
(L. Pfeiffer, 1846)
Figs. 92-95; Pl. V, 18

Nineteen specimens were dissected. I collected them in the Darial Valley, central part of the N Caucasus (USSR), near Ordjonikidze (North Osetia) as follows: 10 specimens near Kazbegi village, on 9 May 1970; 9 near Chmi village on 8 May 1970. The identification is mine.

A good shell description is given by Likharev & Rammelmeyer (1952). I nevertheless wish to point out that, although the shell of this species is rather variable as regards color, dimensions and particularly height of whorl, a keel is never found, nor any inclination toward forming an angle at the periphery, a character that distinguishes *C. eichwaldi* from *C. rengarteni.*

As the spermoviduct passes to the oviduct there is a rather sharp curve. There are 4 mucous glands, all with 2 branches. The dart sacs are massive, slightly elongate or globose. The inner vaginal structure is the same as in *C. rengarteni*. The flagellum length is at most half that of the epiphallus. The epiphallus does not make any sudden curve. The connective tissue membrane and penial band are as in *C. rengarteni*. The penis sheath also consists of 2 parts. The verge is massive. In it, the seminal duct is fixed in place by 3 longitudinal bands; i.e., it is surrounded by 3 intrapapillar cavities. The cavity in the remainder of the papilla is divided into 6-9 parts by parallel, transversely arranged, longitudinal septa (Fig. 94). The spermathecal duct forms a marked curve at its base. The receptaculum seminis reaches the lower edge of the albumen gland.

Differential diagnosis of
C. rengarteni and **C. eichwaldi**

The anatomical distinctions between these 2 similar species can now be formulated. In *C. rengarteni* the mucous glands lie approximately at the level of the upper edges of the dart sacs; the uterus is straight; the spermathecal duct is almost straight; the flagellum length is not less, but usually a little more, than half the epiphallus length. The intrapapillar cavity is divided into 4-6 portions. In the type-species, *C. eichwaldi*, the mucous glands lie considerably above the level of the upper edges of the sacs. The uterus is bent and so is the spermathecal duct. The flagellum length is usually less than half that of the epiphallus. The intrapapillar cavity is divided into 6-9 parts.

Subgenus **Anoplitella** Lindholm, 1929

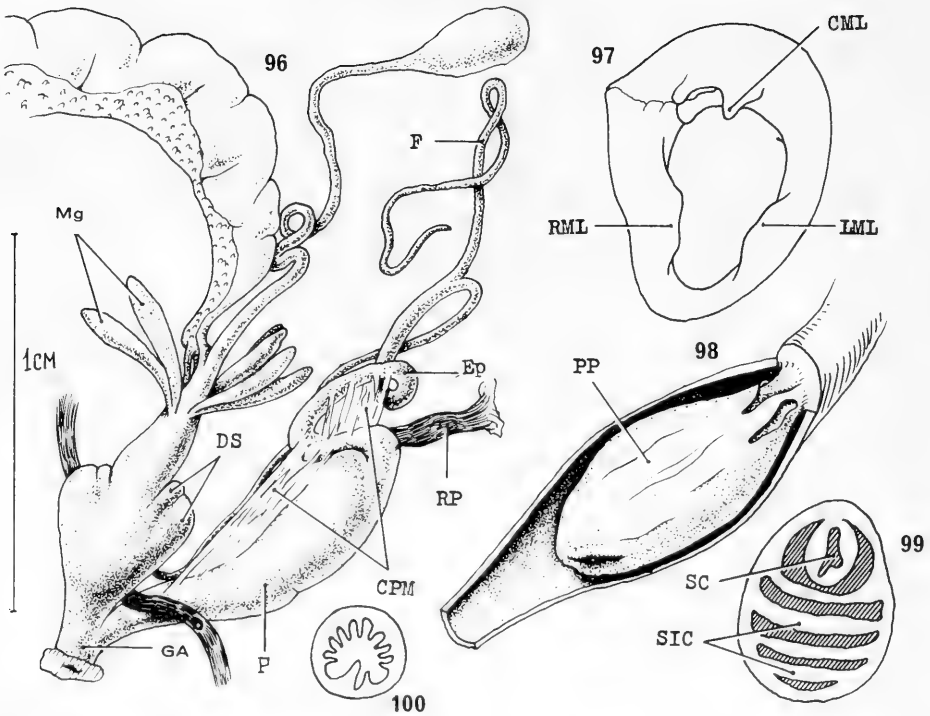
The shell is a light color with lilac spots, lines or marks; usually there are 2 or more less developed brown lines above and under the periphery of the body whorl. If these lines are well developed, the pale purple spots are absent. The shell is thick-walled, with a silky gloss. The umbilicus is more or less perspective. The flagellum is long and thin, a little longer than the curved epiphallus. The spermathecal duct is also long, thin and curved. The seminal duct is fused to the papilla on one side.

Subgenus monotypic.

Caucasigena (Anoplitella) schaposchnikovi
(Rosen, 1911)
Figs. 96-100; Pl. V, 19, 20

Seventeen specimens were dissected. I collected these in the central part of the N Caucasus (USSR) as follows: 10 specimens from the old Ara-Boran settlement between the Chegem and Baksan valleys, on 17 May 1971, and 7 from Chegem Valley near Khushtosyrt village, on 15 May 1970. I identified them.

The shell is flattened, sometimes almost completely flat, smoothly rounded along the periphery and glossy. The umbilicus is rather narrow but perspective: all the previous whorls are visible through it. A little back of the aperture edge there is a thick white lip. The adult has 2-4 earlier lips that are visible through the whorls concerned as light radial lines. The shell is



FIGS. 96-100. *Caucasigena (Anoplitella) schaposchnikovi* (Rosen), Khushtosyrt village, Chegem Valley, central part of N Caucasus, USSR, 15 May 1970. **96**, reproductive tract; **97**, mantle collar; **98**, penis, penis sheath partly removed; **99**, cross-section of verge; **100**, cross-section of epiphallus.

sculptured with fine, irregular, radial striae and in places rare spiral lines. There are 6 whorls.

The dimensions, measured from 65 shells, were as follows:

Shell height (mm)	5.0- 8.5
Shell width (mm)	11.0-15.5

The original description was made from smaller specimens; the shell height was up to 6.5 mm and the width up to 10.5 mm.

In my collection the species is represented by 2 ecological forms that differ in the color and pattern of the shell and that do not occur together. These forms cannot be distinguished by other conchological characters, nor are there any anatomical distinctions. In shady valleys, in which insolation is slight, one finds the form with 2 distinct spiral brown bands of about equal width, 1 of which runs above and the other below the periphery. The distance between them is approximately equal to the width of each band. The upper band is visible along all whorls (Pl. V, 20). In places with intense insolation (e.g., the old settlement of Ara-Boran) the shell color is chestnut to almost lilac; on this background there are more or less numerous bluish-white irregular lines and spots. In some places these spots coalesce into a larger patch or they form a reticulate pattern (Pl. V, 19). At the same time, in most specimens belonging to this second form, there are more or less developed spiral bands that are also characteristic for it. As is evident from study of his material at the Zoological Institute, USSR Academy of Sciences, Lindholm (1929) was dealing with such specimens when he described var. *balkariensis*.

The oviduct is almost straight but may be slightly curved. There are 3 or 4 mucous glands, each with 2 branches. The oviduct and upper vagina gradually increase in bulk from the upper to the lower part, being rather inflated in the region of the dart sacs. The outer sacs are large; the inner sacs are considerably smaller and fused with the upper vagina and the outer sacs. The flagellum is thin, slightly longer than the

epiphallus or equal to it, and coiling. The epiphallus is more or less coiled. There are well-developed connective tissue bands stretched between parts of the epiphallus and between the distal epiphallus and the distal penis. The penis is massive, elongate and smooth on the inside. The verge is sac-like with an opening forming a wide slot and with well-developed lacunae. The seminal duct adheres to the papillar wall on 1 side; the papillar cavity is subdivided into 4-6 parts (Fig. 99). The spermathecal duct is long, thin and coiled. The receptaculum seminis is small and oval; it reaches the albumen gland.

Subgenus **Dioscuria** Lindholm, 1927

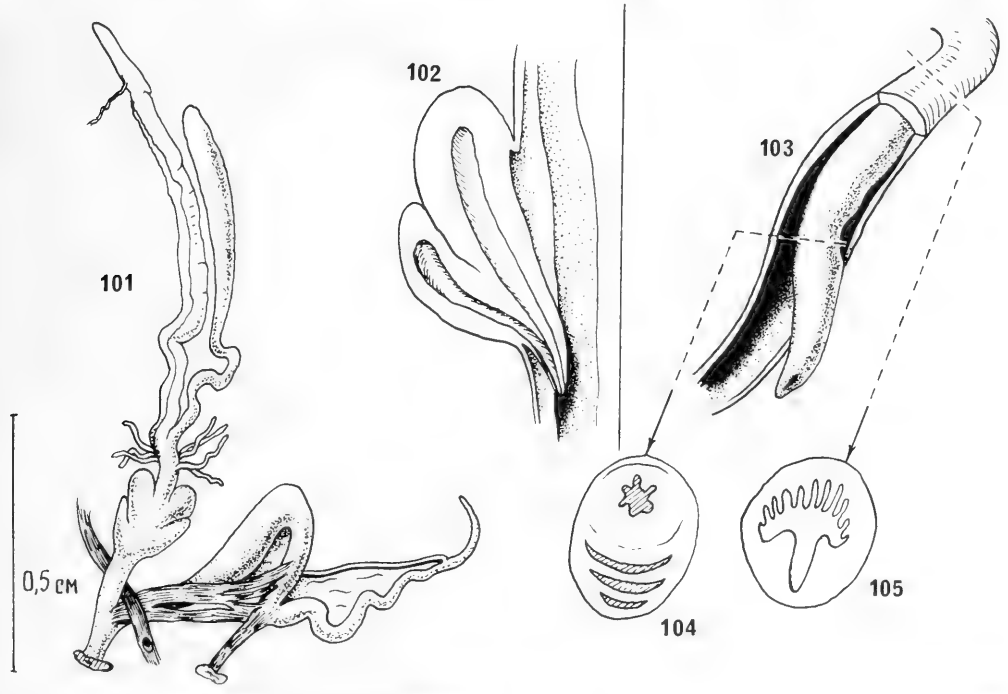
The shell is thin-walled, brittle, with a silky gloss and a large aperture; depressed, conical. The umbilicus is very narrow and almost completely covered by the reflection of the columellar edge. The length of the flagellum is about half that of the epiphallus. The seminal duct is not separated from the papillar wall.

Subgenus monotypic.

Caucasigena (Dioscuria) thalestris
(Lindholm, 1927)
Figs. 101-105; Pl. VI, 22

One not fully mature specimen was dissected. It was collected from Novy Aphon (New Athos), N of Sukhumi, Black Sea region of Georgia, Grusinian SSR, on 2 July 1913, by Nasonov, and identified by Likharev. A shell description is given by Likharev & Rammelmeyer (1952).

The oviduct forms a slight bend. There are 3 mucous glands, each with 2-3 branches. These are situated slightly higher than the dart sacs. The inner dart sacs are a little larger than the outer ones. The vaginal plicae do not form lobes but are relatively well developed. The flagellum is about half the length of the epiphallus, which is wavy and curving. The penis forms 1 U-bend. A heavy muscle band between penis and epiphallus consists of several anastomosing strands. The verge is long and cylindrical. The seminal duct runs in the thickness of the papillar wall. The intrapapillar cavity lying in the wall on 1 side of the verge is divided into 3 parallel



FIGS. 101-105. *Caucasigena (Dioscuria) thalestris* (Lindholm), Novy Aphon, N of Sukhumi, Black Sea region of Georgia, Grusinian SSR, 2 July 1913. Specimen not fully mature. **101**, reproductive tract; **102**, inner structure of vagina in dart sac region, **103**, penis, penis sheath partly removed; **104**, transverse section of verge; broken circle indicates boundary between external and internal layers of papillar tissue; **105**, cross-section of epiphallus.

transverse compartments by longitudinal septa (Fig. 104). The spermathecal duct is tortuous. The elongated receptaculum seminis does not quite reach the albumen gland.

My data differ somewhat from those of Hesse (1931). According to his illustrations, the flagellum is slightly longer than the epiphallus; there is no indication of any penial muscle bands, and the spermathecal duct is short. Unfortunately not all Hesse's drawings are accurate, as he generally ignored muscle and connective tissue attachments. On the other hand, it should be kept in mind that I have examined only 1 not fully adult specimen, and such a character as flagellum length may be influenced by age variation. The same applies to the length and shape of the spermathecal duct.

Genus **Plicuteria** Shileyko, gen. nov.

The shell is depressedly conical with a sharp apex, covered with radial lines, yellowish-white, rarely hirsute; the suture is deep. The lip of the aperture is not thickened but weakly developed. Vaginal plicae appear each as a row of prismatic lamellae of equal size. The conical flagellum is half the length of the epiphallus or less. The epiphallus is almost straight. The spermathecal duct is very short. The receptaculum seminis is irregularly pear-shaped. The verge bears on its surface 2 longitudinal diametrically positioned grooves. In its distal part the seminal duct hangs from the inner walls of the papilla on 2 long bands, and in the proximal part it is fused to 1 side of the papillar wall.

Genus monotypic.

It is not clear why Polinski (1924) did not single out this snail as a separate species or subspecies. According to his illustrations and description, he took into account features of the outer genitalia such as the sturdy flagellum, spermathecal duct and receptaculum seminis, which are distinct enough to separate it from other European *Trichia*. The shell of this snail is evidently distinctive also.

At any rate, the new genus is unique in that the longitudinal vaginal plicae are subdivided by regular transverse prismatic folds that form a dense pattern on the internal wall of the vagina, and the spermathecal duct is shorter than in any other form of this group.

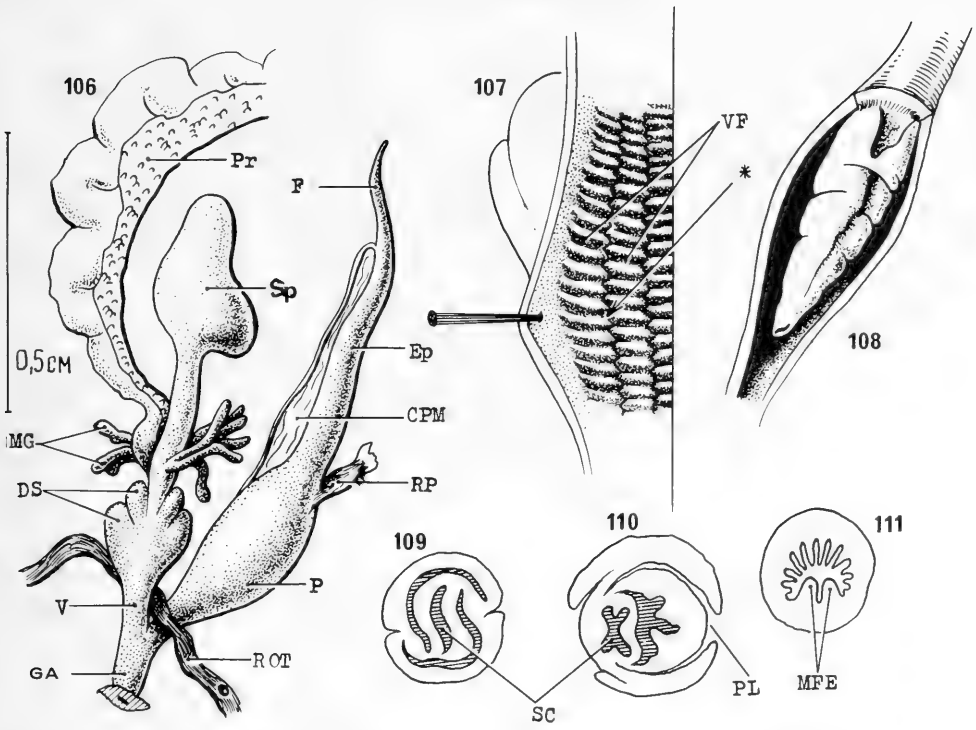
Plicuteria lubomirski (Slóssarski, 1881)
Figs. 106-111; Pl. VI, 23

One specimen was examined. It was collected near the town of Olomouc, Moravia, Czechoslovakia, on 16 April 1964 and identified by V. A. Hudec.

As it emerges from the spermoviduct, the oviduct forms a definite bend. There are 4 mucous glands, each with 2-3 branches. The outer dart sacs are somewhat more massive than the inner ones, whose upper tips reach well beyond those of the outer sacs. The lower vagina is rather long and cylindrical. Its inner structure is most characteristic and unlike that of any other species; the vaginal plicae are clearly divided into regular rows of prismatic lamellae (Fig. 107). The male duct is elongate, neither curving nor twisted. The length of the conical flagellum is less than that of the thick, cylindrical epiphallus, which is connected to the vas deferens by a very fine membrane. The penis is fusiform; the penis sheath is smooth inside. The verge bears 2 shallow longitudinal furrows on its surface (Figs. 108, 109), as well as a few incomplete ring grooves (Fig. 108). In the proximal part of the verge the seminal duct is adjacent to the papillar wall on one side. On the opposite side it borders on a large intrapapillar cavity. Deep folds here reach into the verge wall from the papillar lacunae (Fig. 110). Distally (Fig. 109), the seminal duct lies encircled by 2 cavities of sickle-shaped cross-section. Their position, in conjunction with the thickness of the wall, supports the idea that cavities are thus formed during ontogenesis, most likely in the thickness of the papillar walls. The spermathecal duct is very thick, straight and short. The receptaculum seminis is irregularly pear-shaped and quite distant from the lower edge of the albumen gland.

Genus **Trichia** Hartmann, 1840

The shell is low to depressed conical, rather brittle, of a brownish horn color, with a more or less narrow umbilicus. As a rule there are hairs on the shell surface, which are absent in the adult specimens of some species. A thickened lip is either absent or occupies the basal edge of the aperture. There are 4 mucous glands, usually with 2 branches each. The dart sacs are elongate and more or less club-shaped. Inside the verge the seminal duct is en-



FIGS. 106-111. *Plicuteria lubomirskii* (Slóssarski), Olomouc, Moravia, Czechoslovakia, 16 April 1964. **106,** reproductive tract; **107,** inner structure of vagina in dart sac region; *stylophore opening; note that longitudinal folds have become rows of prismatic lamellae; **108,** penis, penis sheath partly removed; **109, 110,** cross-sections of verge at different levels; **111,** cross-section of epiphallus.

circled by a pair of intrapapillar cavities. There may be 1 further narrow slit-like cavity in the papillar wall.

The type-species is *Helix hispida* Linné. It was formerly thought to be *T. filicina* L. Pfeiffer, but Forcart (1958) proved that Hartmann, when he determined the genus *Trichia* with *T. filicina* as the type-species, really meant *T. hispida.*

Subgenus **Petasina** Beck, 1847

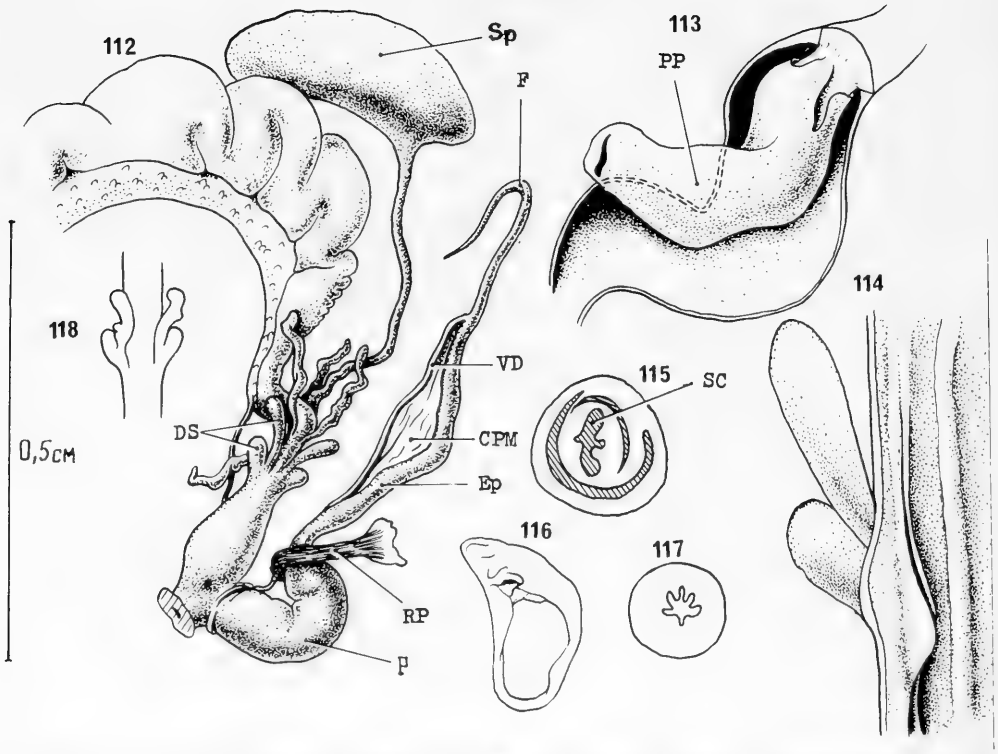
The shell is dome-like, with fine short hairs and a very narrow umbilicus. On its basal edge the lip sometimes bears a heavy swelling. The 4 mucous glands are simple, not branched, and some of them are only partly divided into 2. The inner pair of dart sacs is quite separate from the outer pair as well as from the upper vagina: the upper tips of the inner sacs reach well beyond the place of attachment of the mucous glands. The receptaculum seminis is very bulky and of a characteristic hammer shape.

Type-species: *Helix unidentata* Draparnaud, 1805.

Trichia (Petasina) unidentata
(Draparnaud, 1805)
Figs. 112-118; Pl. VI, 24

Five specimens were examined anatomically: 2 from central Czechia, Czechoslovakia, collected on 8 September 1969 by J. Buchar and identified by myself, and 3 from the High Tatra Mountains, Slovakia, Czechoslovakia, collected on 20 July 1964 by V. Hudec and identified by him.

As the spermoviduct passes into the oviduct, it curves weakly; the free oviduct itself is also slightly bent. There are 4 mostly unbranched, somewhat kinky mucous glands. The inner dart sacs are long, well separated, club-shaped; the outer ones are considerably shorter. One of the specimens had a distinct knobby thickening on the inner sacs (Fig. 114). The lower vagina is inflated and fusiform. The vaginal plicae are weakly developed, but there



FIGS. 112-118. *Trichia (Petasina) unidentata* (Draparnaud), central Czechia, Czechoslovakia, 8 September 1969. **112**, reproductive tract; **113**, penis, penis sheath partly removed; **114**, inner structure of vagina in dart sac region; **115**, cross-section of verge; **116**, mantle collar; **117**, cross-section of epiphallus; **118**, dart sacs of another specimen from same location.

are distinct lobes at the openings of the dart sac ducts. The flagellum is about 2/3 the length of the cylindrical, almost straight epiphallus. The membrane connecting the distal parts of the male duct is very fine and translucent. The penis is cylindrical, straight or curved. The seminal duct is attached to the papillar wall by 3 longitudinal bands and surrounded by a pair of intrapapillar cavities with a crescent-shaped cross-section. The spermathecal duct is fine and slightly wavy; it does not join the massive receptaculum seminis apically but from below (hammerhead shape). It is of interest that in specimens collected in July and September the spermatheca was full.

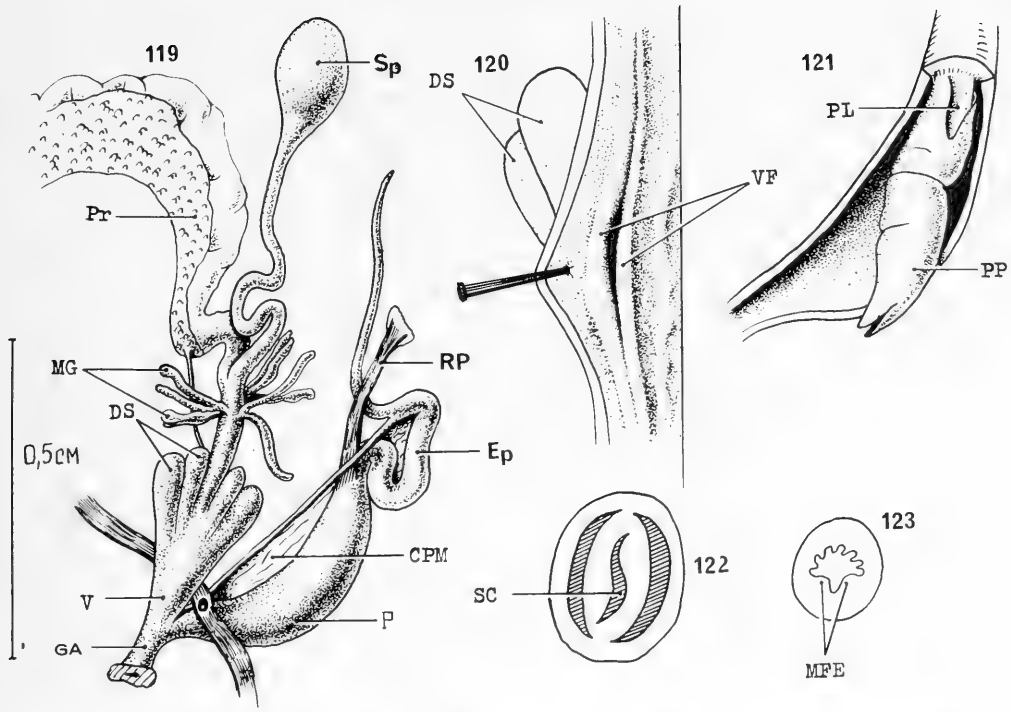
Subgenus **Trichia** s. str.

The shell is flattened; the contour of the whorls is not dome-like but conical. The umbilicus is wider than in representatives of the subgenus *Petasina*. The inner dart sacs are not so sharply separated. There are 4 mucous glands, each with 2 branches; in some glands the second branch is reduced. The mucous glands are attached considerably above the tips of the inner dart sacs. The receptaculum seminis is oval and rather small.

Whether the conchological distinctions on which the *Trichia hispida* species group (*T. plebeia*, including *"sericea," septentrionalis, concinna*) is based (Forcart, 1965) are justified could not be investigated here for lack of sufficient material. Two species of this group (*T. plebeia, T. concinna*) are discussed as separate species in this paper, although it has not been possible to determine whether the anatomical distinctions found are species-specific or just due to seasonal variation.

Trichia (Trichia) plebeia
(Draparnaud, 1805)
Figs. 119-127; Pl. VII, 25, 26

Four specimens were dissected that had been collected from Grdlorez village, near Prague, Czechoslovakia, on 18 September



FIGS. 119-123. *Trichia (Trichia) plebeia* (Draparnaud), Grdlorez village, near Prague, Czechoslovakia, 18 September 1968. **119**, reproductive tract; **120**, inner structure of vagina in dart sac region; **121**, penis, penis sheath partly removed; **122**, cross-section of verge; **123**, cross-section of epiphallus.

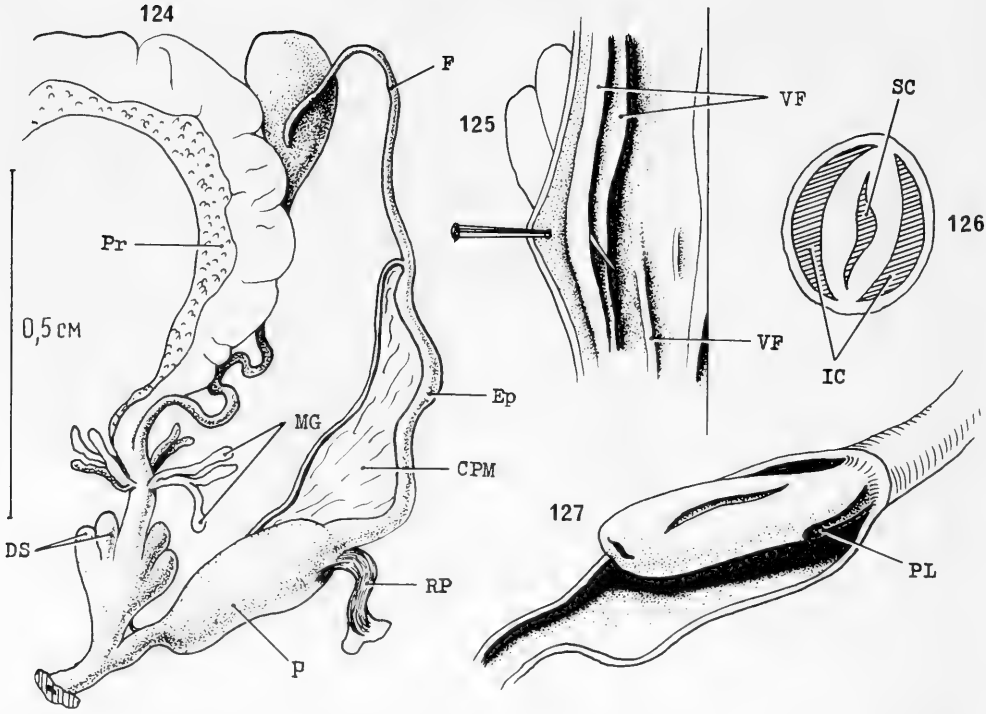
1968 by V. Hudec and identified by him (Figs. 115-119; Pl. V, 25). Another 4 specimens originated from Bodetal in the Harz region, German Democratic Republic. These were collected in June 1969 by E. Clauss and identified as *Trichia "sericea"* (Figs. 124-127; Pl. VII, 26).

The Czech specimens are as follows: there is a smooth curve where the sperm-oviduct continues as the free oviduct, which is also gently bent. There are 4 mucous glands, each with 2 or 3 branches. The dart sacs are elongate, opposite to one another and to the upper part of the vagina. The dart sac region is not set off but passes smoothly into a conically tapering lower vagina. The vaginal plicae are relatively well developed but form no lobes. The flagellum is about the same length as the epiphallus, which bends sharply 2-3 times; its various parts are connected by a membrane stretched between it, the penis and the vas deferens. The penis is fusiform and slightly curved. The verge is marked by a few incomplete ring grooves. The seminal duct is not attached to the inner walls of the papilla by thin longitudinal bands but is fused to the wall at 2 diametrically opposed spots, and the 2 nonadhering sides between them constitute the 2 intrapapillar cavities (Fig. 122). The spermathecal duct is somewhat convoluted; the spermatheca is small and oval and almost reaches the albumen gland.

The German specimens agree in most respects. Note, however, that they rather resemble *T. concinna* in others, such as the unwound position of the epiphallus, which is not held in the bent state by the connective tissue membrane; the smooth outer aspect of the papilla, which lacks ringed grooves; and by the presence of short additional vaginal plicae. The spermatheca was larger in these specimens than in *T. plebeia* from Czechoslovakia and did not reach the lower edge of the albumen gland.

Trichia (Trichia) concinna (Jeffreys, 1862)
Figs. 128-131; Pl. VII, 27

Four specimens were dissected. They were collected in the vicinity of Rožnave,



FIGS. 124-127. *Trichia (Trichia) plebeia* (Draparnaud), Bodetal, Harz region, German Democratic Republic, June 1969. **124**, reproductive tract; **125**, inner structure of vagina in dart sac region; **126**, cross-section of verge; **127**, penis, penis sheath partly removed.

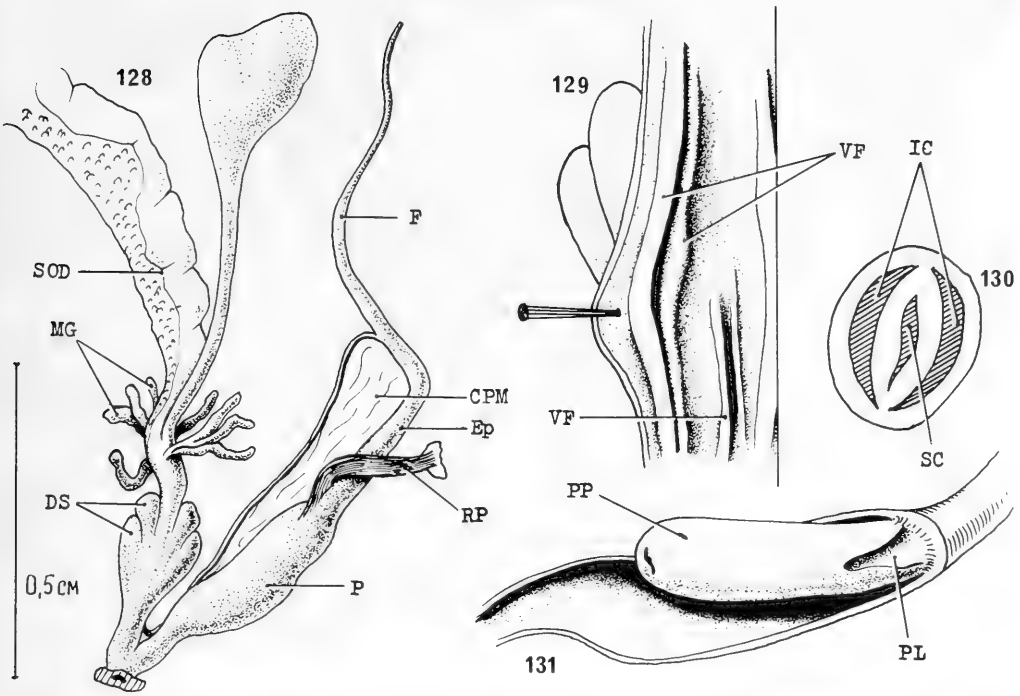
Slovakia, Czechoslovakia, by V. Hudec on 15 July 1962 and identified by him.

The spermoviduct curves weakly at the emergence of the oviduct and at the bend of the oviduct. There are 4 mucous glands, each with 2-3 branches. The dart sacs are short and club-shaped, closely pressed together and partly fused. The dart sac regions are vaguely offset from the cylindrical vagina. The flagellum is almost 1.5 times longer than the cylindrical, weakly curved epiphallus. As in both preceding species (*T. plebeia, T. unidentata*), the male ducts are bound by a fine, transparent, connective tissue membrane. The verge is cylindrical or slightly fusiform, with a large papillar lacuna. The inner structure of the papilla is as in *T. plebeia.* The spermathecal duct is almost straight. The receptaculum seminis is rather bulky and irregularly shaped; it just fails to reach the albumen gland.

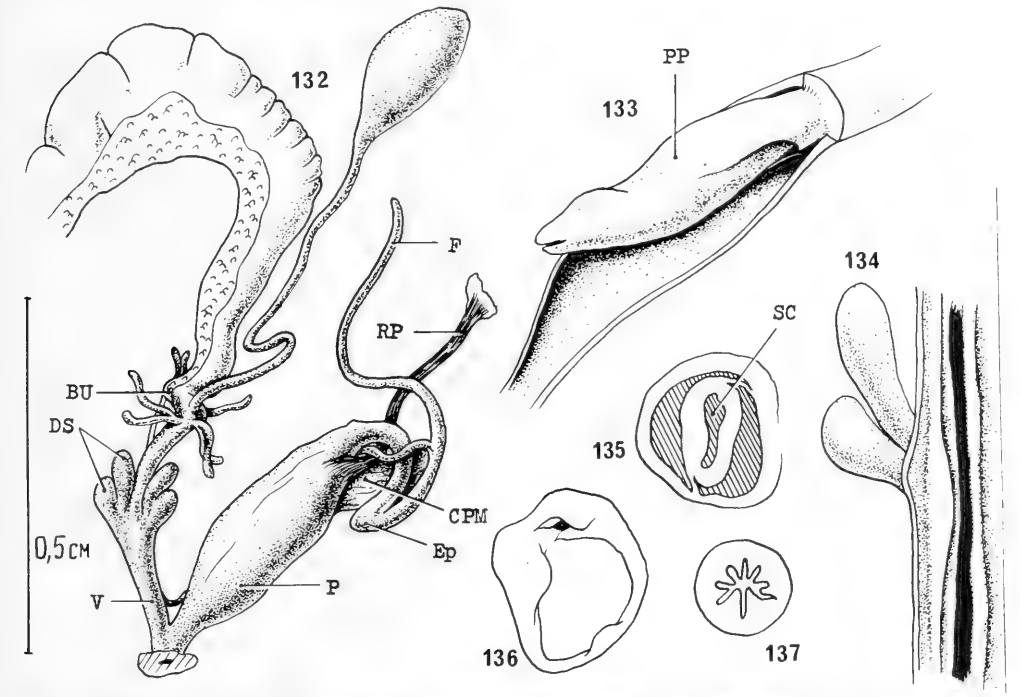
Trichia (Trichia) hispida (Linné, 1758)
Figs. 132-137; Pl. VII, 28

Forty specimens were examined. They were collected as follows: in the USSR, 11 specimens from the Lenin Mountains in Moscow on 12 July 1970; 13 specimens from the Ilyich Foundation settlement near the Serebryanka River, Moscow region, on 15 August 1970; 4 specimens from the garden of the Zoological Institute, USSR Academy of Sciences, Leningrad, on 28 September 1965; and 5 specimens from Stryisky Park, Lvov (Eastern Ukraine), on 25 September 1969. All these were collected and identified by me. Further, 4 specimens were collected from a garden in the town of Quedlinburg, German Democratic Republic in April 1969 by E. Clauss and identified by him. Lastly, 3 specimens from the region of Cologne, German Federal Republic, were collected by H. Nordsieck and identified by V. Hudec.

At the point where the oviduct emerges from the spermoviduct, there is a smooth curve; the oviduct is also slightly bent. There commonly are 4 mucous glands, rarely 3; usually each has 2 branches but secondary branching is not uncommon, so the total number of branches may amount to 12. On the other hand, some branches may be reduced, bringing down the total to 4 or 5. The inner pair of dart sacs may be



FIGS. 128-131. *Trichia (Trichia) concinna* (Jeffreys), Rožnave, Slovakia, Czechoslovakia, 15 July 1962. **128,** reproductive tract; **129,** inner structure of vagina in dart sac region; **130,** cross-section of verge; **131,** penis, penis sheath partly removed.



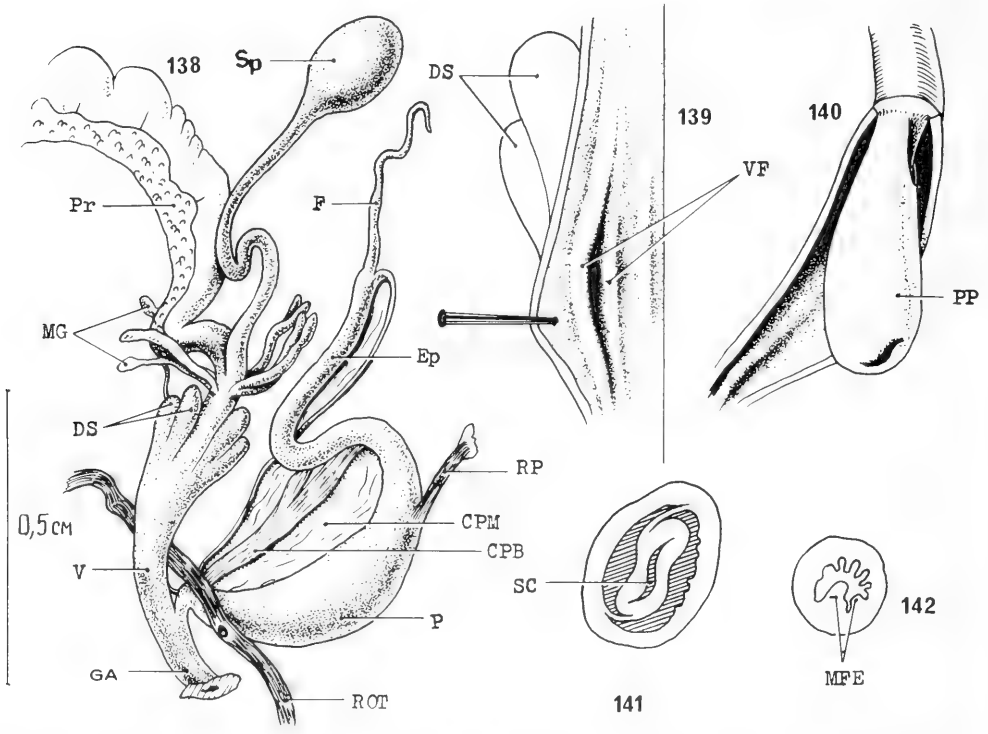
FIGS. 132-137. *Trichia (Trichia) hispida* (Linné), Lenin Mountains, Moscow, USSR, 12 July 1970. **132,** reproductive tract; **133,** penis, penis sheath partly removed; **134,** inner structure of vagina in dart sac region; **135,** cross-section of verge; **136,** mantle collar; **137,** cross-section of epiphallus.

developed to approximately the same degree as the outer, or they may be a little less developed. Their upper tips always considerably surpass those of the outer sacs, the mouth of the mucous glands is always considerably above the upper ends of the inner dart sacs. The lower vagina is rather long and cylindrical. The vaginal plicae are narrow but distinct. There are small lobes at the opening of the dart sacs. The length of the flagellum is equal to or greater than that of the epiphallus. Usually the latter is sharply curved and sometimes forms a loop. Between penis and epiphallus a connective tissue membrane attaches the epiphallus to the penis sheath. The penis is massive and fusiform. Its sheath is smooth inside. The verge is also fusiform. The seminal duct is more clearly separated from the papillar| wall than usual; i.e., it is suspended by 2 fine longitudinal bands. The spermathecal duct is gently curving and sometimes weakly twisting. The receptaculum seminis is oval and almost reaches the albumen gland.

Trichia (Trichia) villosula
(Rossmaessler, 1838)
Figs. 138-142; Pl. VIII, 29

Three specimens were dissected. These were collected from Babice, near Brno, Moravia, Czechoslovakia, on 16 August 1968 by V. Hudec and identified by him.

The oviduct makes a rounded bend. There are 4 mucous glands, of which some have 2 branches. The dart sacs are very elongate, closely opposed to each other and to the upper vagina. They are massive; the tips of the inner sacs extend beyond those of the outer sacs. Anatomically this species stands apart from others of the subgenus *Trichia* because the tips of the inner dart sacs reach the base of the mucous glands. The vagina is long, slowly narrowing to the genital atrium. The vaginal plicae are relatively well developed; they do not form lobes. The flagellum is somewhat shorter than the epiphallus, which is smoothly curving and linked to the penis by connective tissue bands in which there are muscle



FIGS. 138-142. *Trichia (Trichia) villosula* (Rossmaessler), Babice, near Brno, Moravia, Czechoslovakia, 16 August 1968. **138,** reproductive tract; **139,** inner structure of vagina in dart sac region; **140,** penis, penis sheath partly removed; **141,** cross-section of verge; **142,** cross-section of epiphallus.

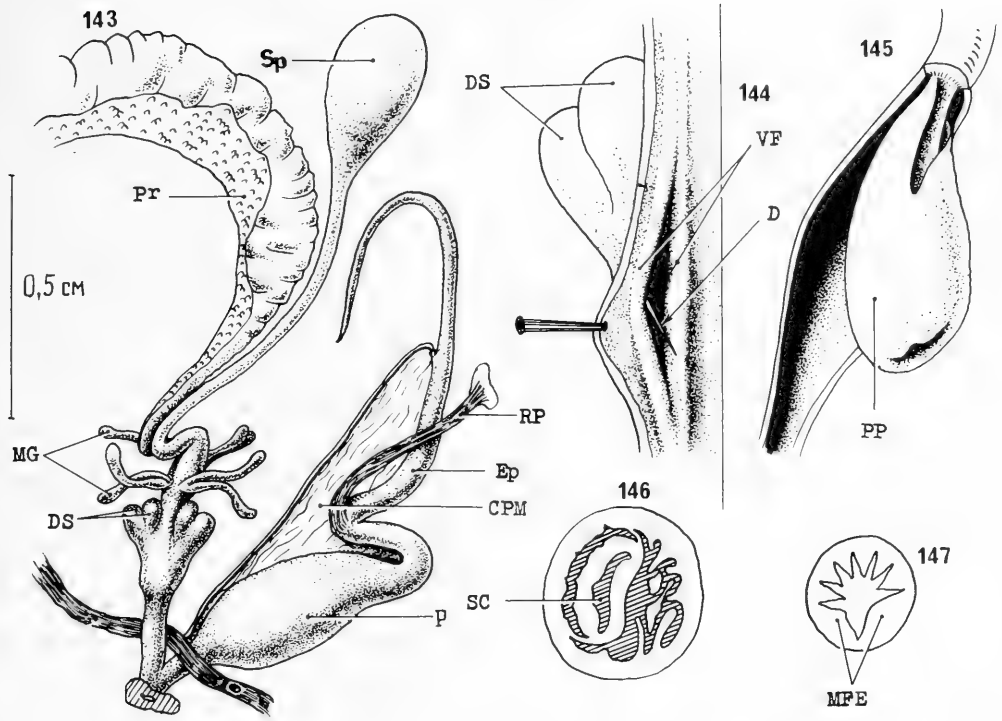
fibers. In addition, the vas deferens, epiphallus and penis are bound together by a fine translucent membrane. The penis is fusiform and slightly curved. On approaching the genital atrium it rapidly narrows and forms a distinct bend. The verge is club-shaped, with large papillar lacuna and a wide slit for its outlet. Inside the penis sheath at the junction with the genital atrium there is a long fold. The seminal duct is attached to the papillar wall by 2 fine longitudinal bands. The spermathecal duct is thin, with an S-shaped bend. The receptaculum seminis is rounded and almost reaches the albumen gland.

Judging from Polinski's (1924) drawings, the specimen from the town of Zakopane, Galicia, in the High Tatra region of S Poland near the Czechoslovakian border, had a flagellum approximately equal in length to the epiphallus or even a little longer. A penial band was not present.

Trichia (Trichia) striolata
(C. Pfeiffer, 1828)
Figs. 143-147; Pl. VIII, 30

Four specimens were examined. They were collected in the Cologne area, German Federal Republic, on 30 May 1963 and identified by V. Hudec.

The free oviduct twice bends sharply, in the shape of an S. There are 4 mucous glands, of which some have 2 branches. Both the inner and outer dart sacs are of equal size. The inflated dart sac region is sharply offset from the thinner, lower part of the vagina. The vaginal plicae, which arise rather suddenly, do not form any lobes. The flagellum is about as long as the epiphallus or a little longer. The connective tissue membrane between the distal portions of the male duct is well developed. The penis is fusiform, massive and bulbous; the penis sheath is smooth inside; the distal



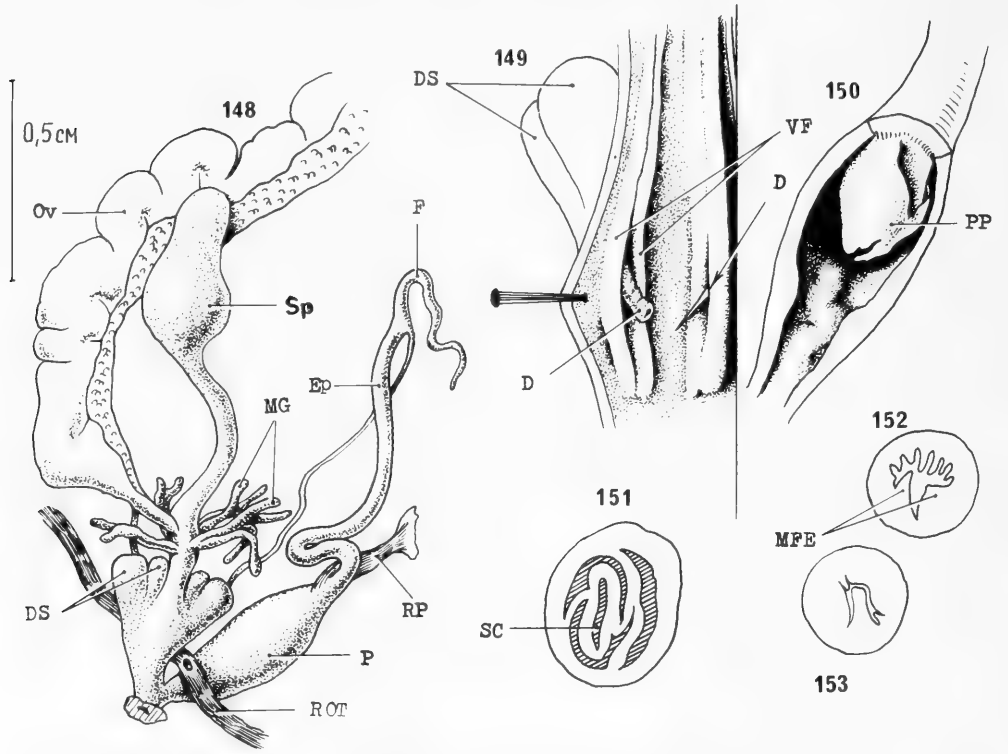
FIGS. 143-147. *Trichia (Trichia) striolata* (C. Pfeiffer), Cologne area, German Federal Republic, 30 May 1963. **143**, reproductive tract; **144**, inner structure of vagina in dart sac region; **145**, penis, penis sheath partly removed; **146**, cross-section of verge; **147**, cross-section of epiphallus.

part of the penis is cylindrical. The verge is club-shaped, with a very widely split foramen and a large papillar lacuna. The seminal duct is attached to the papillar wall by 2 longitudinal bands. It differs from the other members of the subgenus in that 1 of the 2 intrapapillar cavities surrounding the seminal duct is larger than the other and has very friable, corroded walls with many hollows and lamellae (Fig. 146). The spermathecal duct repeats the bend of the oviduct in its basal part. It is thin and ends in an oval receptaculum seminis that almost reaches the lower edge of the albumen gland.

Trichia (Trichia) danubialis
(Clessin, 1874)
Figs. 148-153

Two specimens were examined. They were collected near Petržalka village, near Bratislava, Slovakia, Czechoslovakia, on 15 April 1965 by V. Hudec and identified by him.

The oviduct is straight or faintly curving. The mucous glands, originally 4, may be reduced to 2; some of them show multiple secondary branchings. The dart sacs are well developed and massive; the outer pair is larger than the inner. The lower vagina is very short and not set off externally. The 2 pairs of vaginal plicae occupy lateral positions and abruptly stop below the mouth of the dart sac ducts and before the genital atrium (Fig. 150); they do not show any lobes. The flagellum is 2/3 the length of the epiphallus and is thin and curving. In its distal part the epiphallus sharply curves twice. The penis sheath has an internal longitudinal fold. The verge is short and fusiform; it occupies only the proximal part of the cavity of the penis sheath. The seminal duct is attached to the papillar wall by 2 longitudinal bands. In addition to the 2 intrapapillar cavities thus surrounding it, there is another unpaired cavity in the papillar wall (Fig. 151). The papillar lacuna is very large. The spermathecal duct is short and thick, only weakly curving. The receptaculum seminis is pear-shaped and does not reach the lower part of the albumen gland.



FIGS. 148-153. *Trichia (Trichia) danubialis* (Clessin), Petržalka village, Bratislava area, Slovakia, Czechoslovakia, 15 April 1965. **148,** reproductive tract; **149,** inner structure of vagina in dart sac region; **150,** penis, penis sheath partly removed; **151,** cross-section of verge; **152,** cross-section of epiphallus near penial retractor; **153,** cross-section of epiphallus in proximal part.

This species is usually treated as a subspecies of *T. striolata*, but the conchological as well as anatomical distinctions are without doubt important enough to warrant full species status. It should be noted that the photograph of *T. striolata* in Ložek's book (1956) depicts a specimen taken from the same place as the *T. danubialis* we have studied. Unless one takes these forms to be sympatric, one may suppose that Ložek's *T. striolata* is the snail here identified as *T. danubialis*.

The peculiarities of the outer morphology of the genitalia of *T. danubialis* have been previously noted by Hudec (1964).

Genus **Edentiella** Polinski, 1929

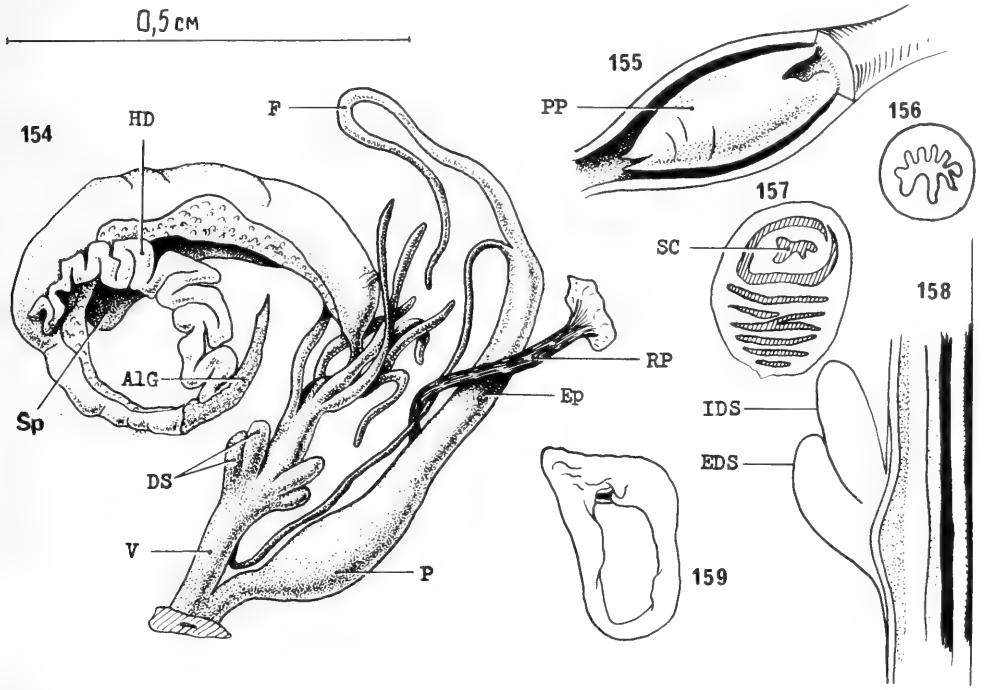
The shell is similar to that found in the representatives of the subgenus *Petasina* of *Trichia* but is distinguished by narrower umbilicus and by the absence of a tooth in the aperture. The mucous glands are long and rather well developed. The flagellum length is equal to the joint length of penis and epiphallus or a little shorter. The inner structure of the verge is as in the Caucasian *Caucasigena*; i.e., there is a system of intrapapillar cavities that are divided by longitudinal septa. The seminal duct is attached to the interior papillar wall by a single longitudinal band.

Type-species: *Helix edentula* Draparnaud, 1805.

Edentiella bakowskii (Polinski, 1924)
Figs. 154-159; Pl. VIII, 31

Five specimens were examined. I collected them in a beech forest in the vicinity of Kvasi village, near the town of Rakhov, in the Transcarpathians (Ruthenia), Ukrainian SSR on 14 September 1969, and I identified them.

As the spermoviduct passes to the oviduct, it suddenly narrows and makes a slight curve. Further down the oviduct gradually widens. The mucous glands are very well developed; they are about as long as the female tract between the attachment of the spermathecal duct and the genital atrium, or a little shorter. According to my observations, the glands, usually 6-8 in number, are not grouped in bundles but are located around the oviduct at the level of the spermathecal duct takeoff. Hudec



FIGS. 154-159. *Edentiella bakowskii* (Pokinski), Kvasi village, Rakhov district, Transcarpathian region, Ukranian SSR, 14 September 1969. **154**, reproductive tract; **155**, penis, penis sheath partly removed; **156**, cross-section of epiphallus; **157**, cross-section of verge; **158**, inner structure of vagina in dart sac region; **159**, mantle collar.

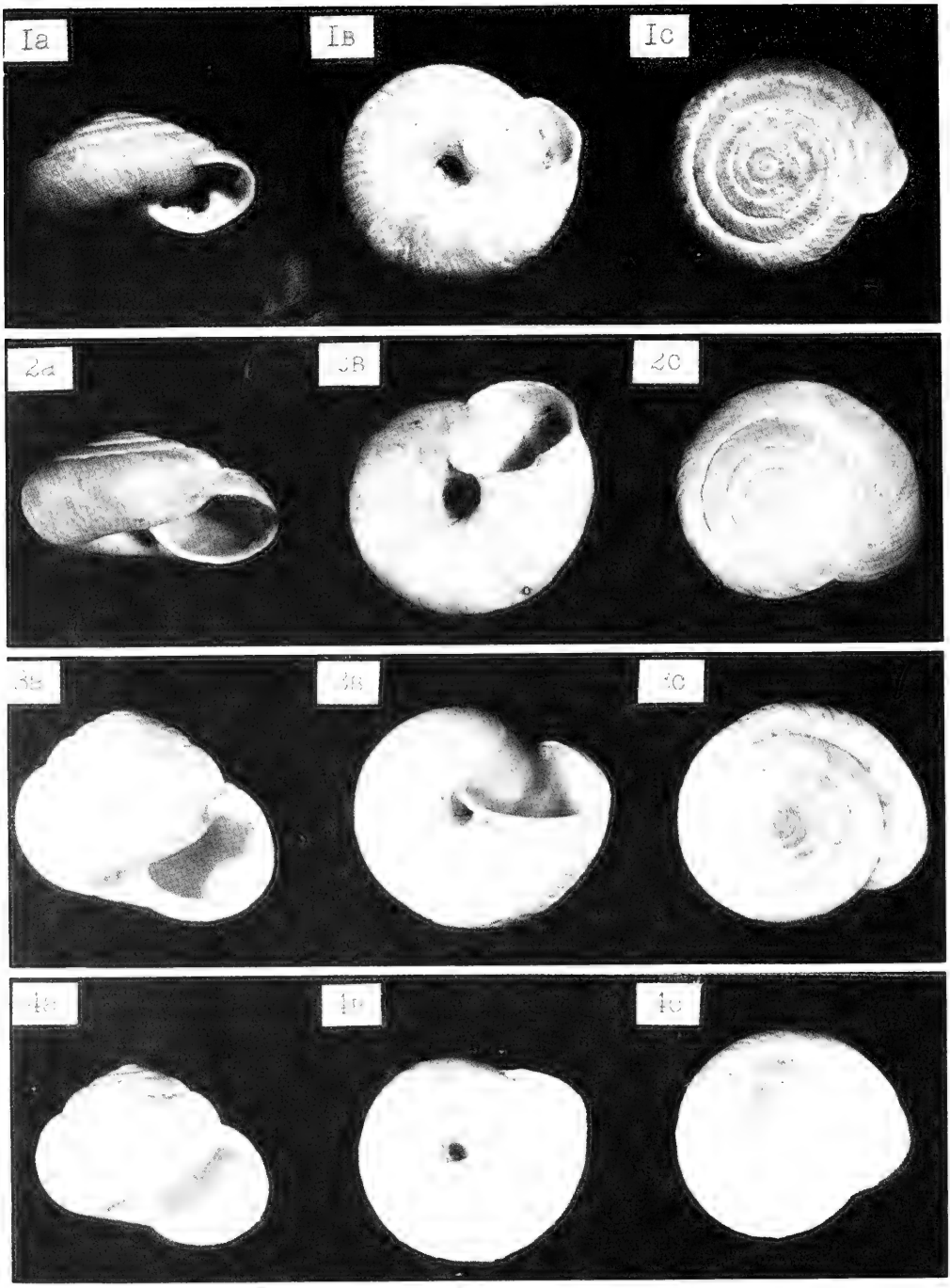


PLATE I (not actual size). 1a, b, c, *Odontotrema diplodon* Lindholm, Chatkal Range, NW Tian-Shan Mountains, Kirghiz SSR, May 1972. 2a, b, c, *Leucozonella ferghanica* Lindholm, Sary-Chileck Nature Reserve, Chatkal Range, NW Tian-Shan Mountains, Kirghiz SSR, 6 July 1966. 3a, b, c, *Leucozonella caryodes* (Westerlund), Talas Range, NW Tian-Shan Mountains, Kirghiz SSR, 4 June 1972. 4a, b, c, *Leucozonella rubens* (Martens), foothills of the Kirghiz Range, NW Tian-Shan Mountains, Kirghiz SSR, 15 June 1972.

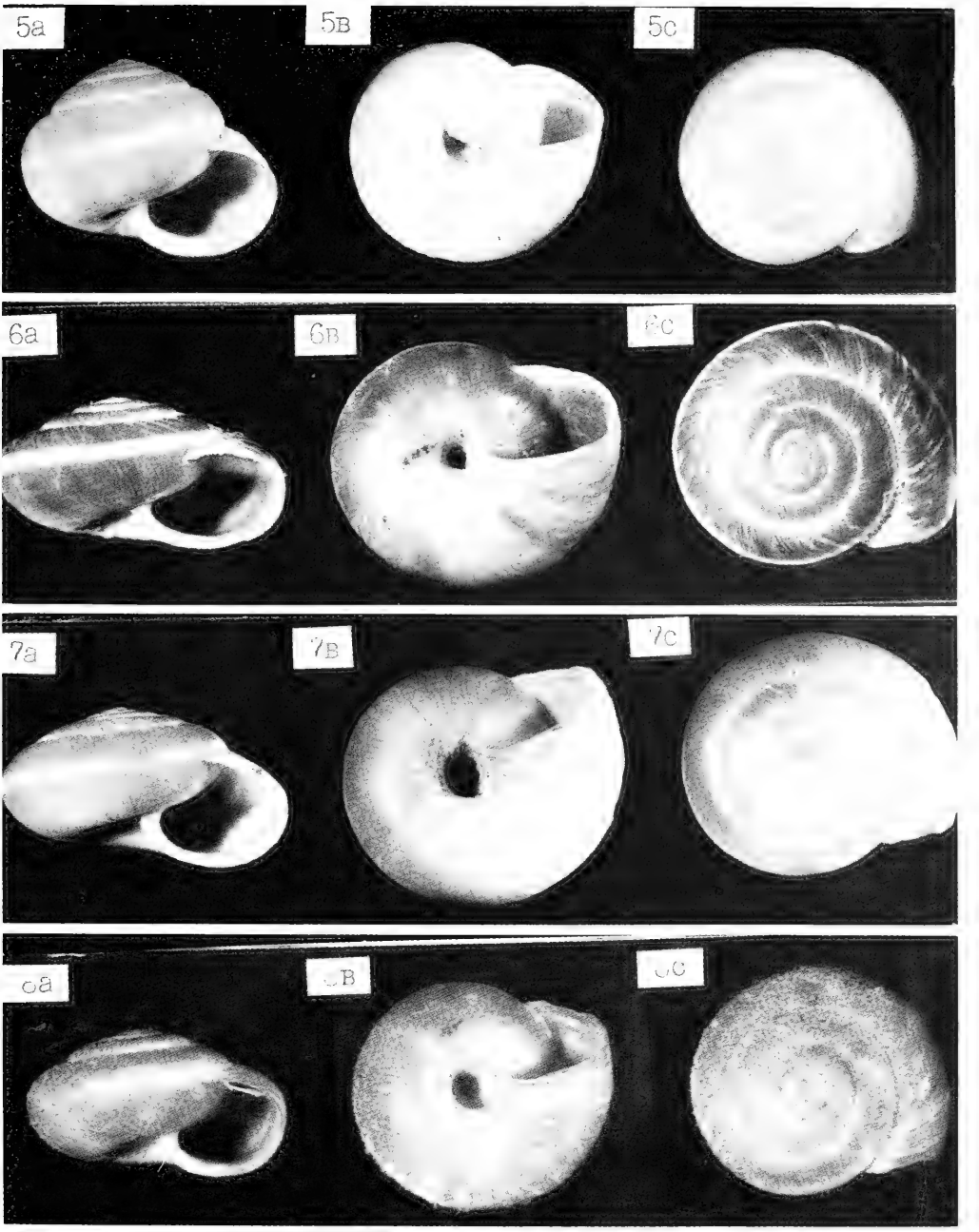


PLATE II (not actual size). 5a, b, c, *"Euomphalia" regeliana* (Martens), foothills of the Kirghiz Range, NW Tian-Shan Mountains, Kirghiz SSR. 6a, b, c, *Leucozonella rufispira* (Martens), Anzob pass, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 28 July 1968. 7a, b, c, *Leucozonella retteri* (Rosen), Kandara Valley, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 2 July 1967. 8a, b, c, *Leucozonella caria* Shileyko, sp. nov., holotype, Khodzcha-Obi-Garm Rest Home, Hissar Range, W Tian-Shan Mountains, Tadzhik SSR, 28 May 1968.

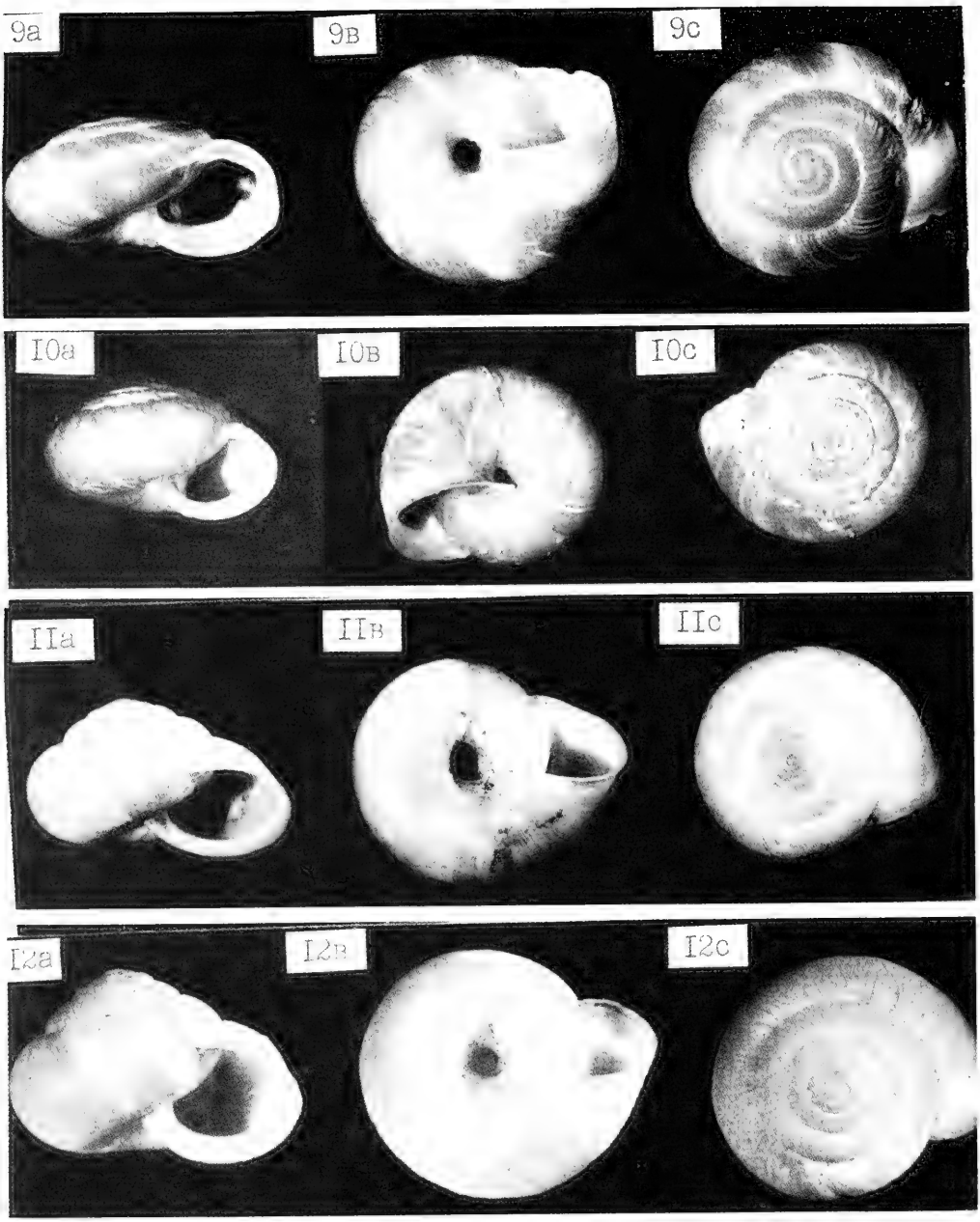


PLATE III (not actual size). 9a, b, c, *Hygrohelicopsis darevskii* Shileyko, sp. nov., holotype, Chegem Valley, N slope of central Caucasus, USSR, 10 August 1965. 10a, b, c, *Teberdinia zolotarevi* (Lindholm), topotype, Teberdia Nature Reserve, NW Caucasus, USSR, 24 July 1958. 11a, b, c, *Kokotschashviia tanta* Shileyko, sp. nov., paratype, between Sioni and Kazbegi villages, slope of central Caucasus, Grusinian SSR, July 1969.

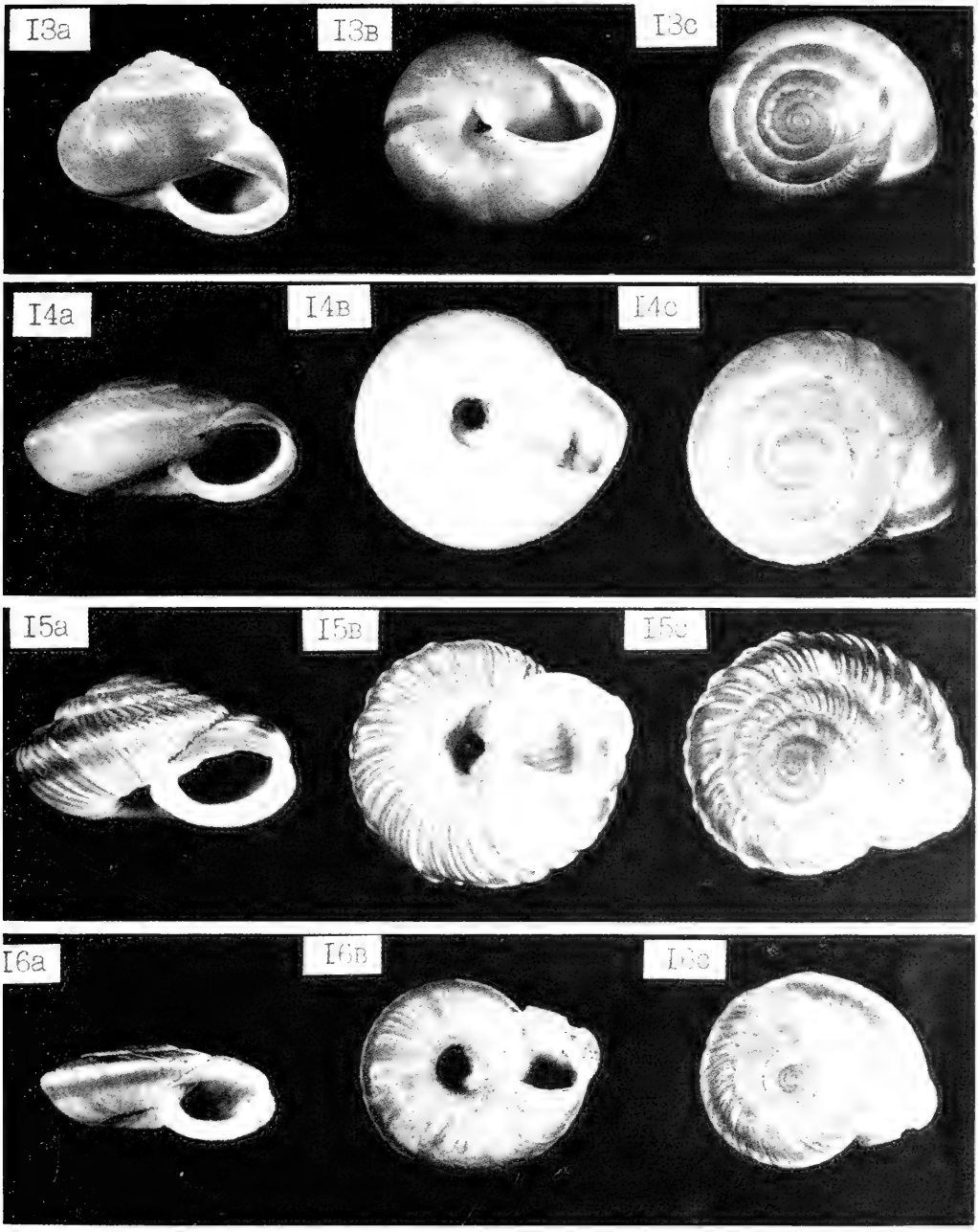


PLATE IV (not actual size). 13a, b, c, *Kokotschashvilia phaeolaema* (Boettger), Chegem Valley, N slope of the central Caucasus, USSR, 14 May 1970. 14a, b, c, *Caucasigena (Caucasigena) armeniaca* (L. Pfeiffer), Mt. Bzovdal (Mt. Todar), Stepanavan region, Armenian SSR, 18 July 1951. 15a, b, c, *Caucasigena (Caucasigena) tschetschenica* (Retowski), Kurtatin Valley, middle course of Phiagdon River, central part of N Caucasus, USSR, 19 September 1970. 16a, b, c, *Caucasigena (Caucasigena) rengarteni* (Lindholm), Khushtosyrt village, Chegem Valley, central part of N Caucasus, USSR, 14 May 1970.

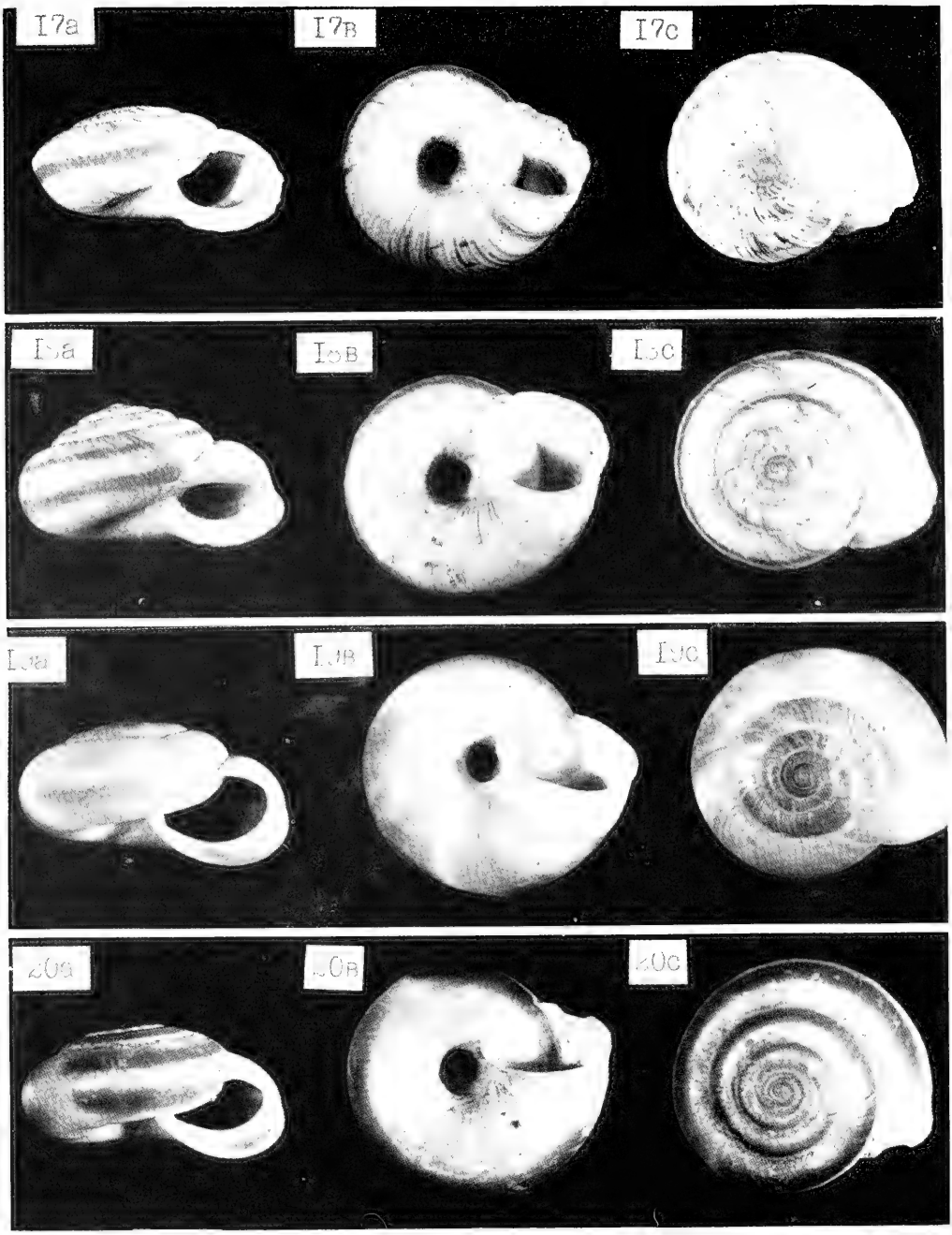


PLATE V (not actual size). 17a, b, c, *Caucasigena (Caucasigena) rengarteni* (Lindholm), old settlement of Ara-Boran, between Chegem and Baksan valleys, central part of N. Caucasus, USSR, 17 May 1970. The form described by Lindholm under the name *Helix gerassimovi*. 18a, b, c, *Caucasigena (Caucasigena) eichwaldi* (L. Pfeiffer), Chmi village, Darial Valley, near Ordjonikidze (North Osetia), central part of N Caucasus, USSR, 8 May 1970. 19a, b, c, *Caucasigena (Anoplitella) schaposchnikovi* (Rosen), old settlement of Ara-Boran, between Chegem and Baksan valleys, central part of N Caucasus, USSR, 17 May 1970. 20a, b, c, *Caucasigena (Anoplitella) schaposchnikovi* (Rosen), Chegem Valley (damp, shady slope) near Khushtosyrt village, central part of N Caucasus, USSR, 15 May 1970.

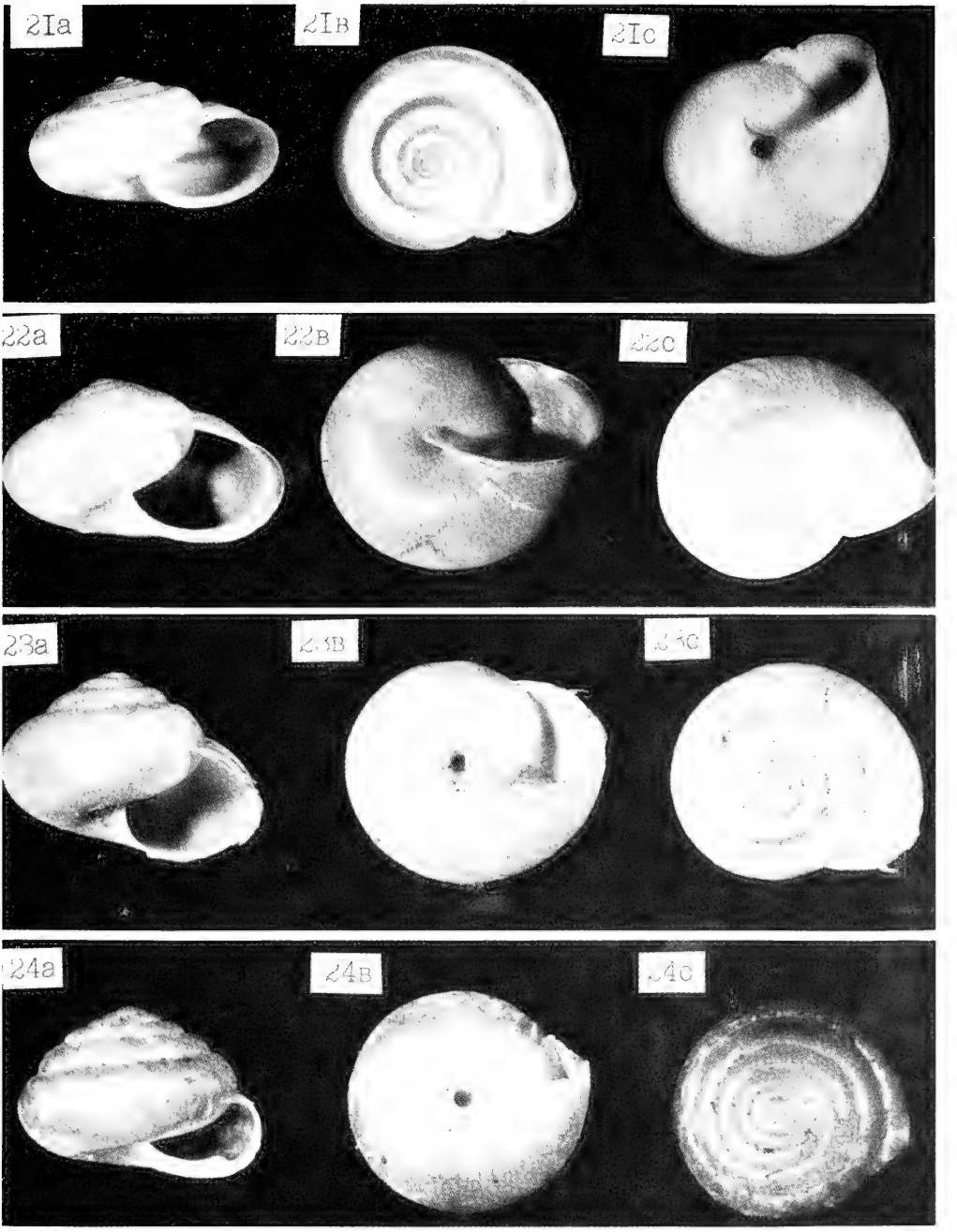


PLATE VI (not actual size). 21a, b, c, *Xerocampylaea zelebori* (L. Pfeiffer), Roumania. 22a, b, c, *Caucasigena (Dioscuria) thalestris* (Lindholm), Novy Aphon, N of Sukhumi, Black Sea region of Georgia, Grusinian SSR, 2 July 1913. Specimen not fully mature. 23a, b, c, *Plicuteria lubomirskii* (Slóssarski), Olomouc, Moravia, Czechoslovakia, 16 April 1964. 24a, b, c, *Trichia (Petasina) unidentata* (Draparnaud), central Czechia, Czechoslovakia, 8 September 1969.

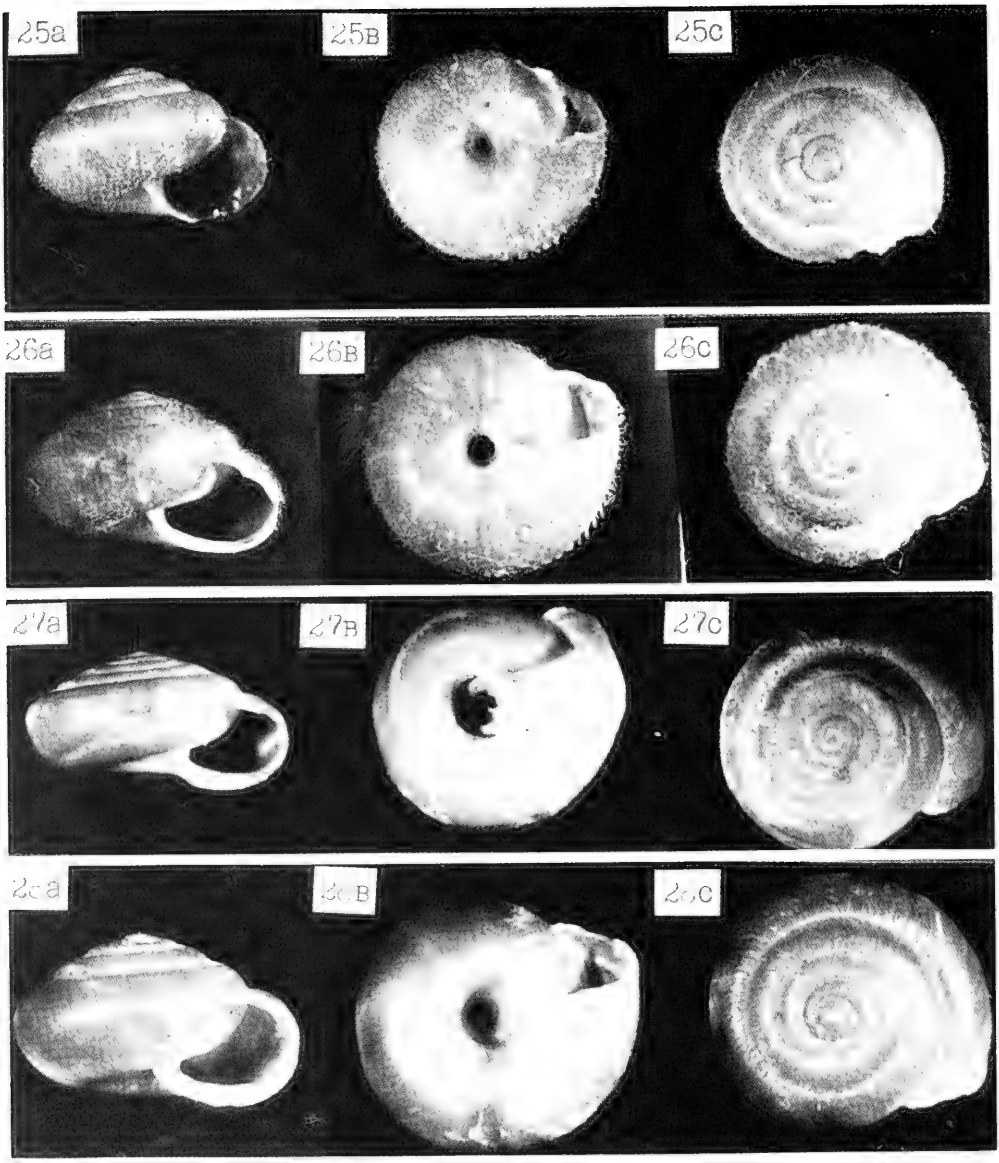


PLATE VII (not actual size). 25a, b, c, *Trichia (Trichia) plebeia* (Draparnaud), Grdlorez village, near Prague, Czechoslovakia, 18 September 1968. 26a, b, c, *Trichia (Trichia) plebeia* (Draparnaud), Bodetal, Harz region, German Democratic Republic, June 1969. 27a, b, c, *Trichia (Trichia) concinna* (Jeffreys), Roznave, Slovakia, Czechoslovakia. 15 July, 1962. 28a, b, c, *Trichia (Trichia) hispida* (Linné), Lenin Mountains, Moscow, USSR, 12 July 1970.

(1965) notes the presence of 2 glands, each with 4 branches. Presumably these glands may either be sited independently or be grouped in 2 fascicles. The dart sacs are elongate, well separated from the upper vagina. The outer and inner pairs are developed to approximately the same degree. The lower vagina is cylindrical. The vaginal plicae are clearly formed. They may be weakly inflated near the outlet of the dart sac ducts, but they do not form lobes. The flagellum is 1.5-2 times longer than the straight epiphallus (in Hudec's work the epiphallus is described as being curved); this organ passes smoothly into the penis without any demarcation. There is either no membrane between vas deferens, epiphallus and penis, or it is insignificant. The penis is

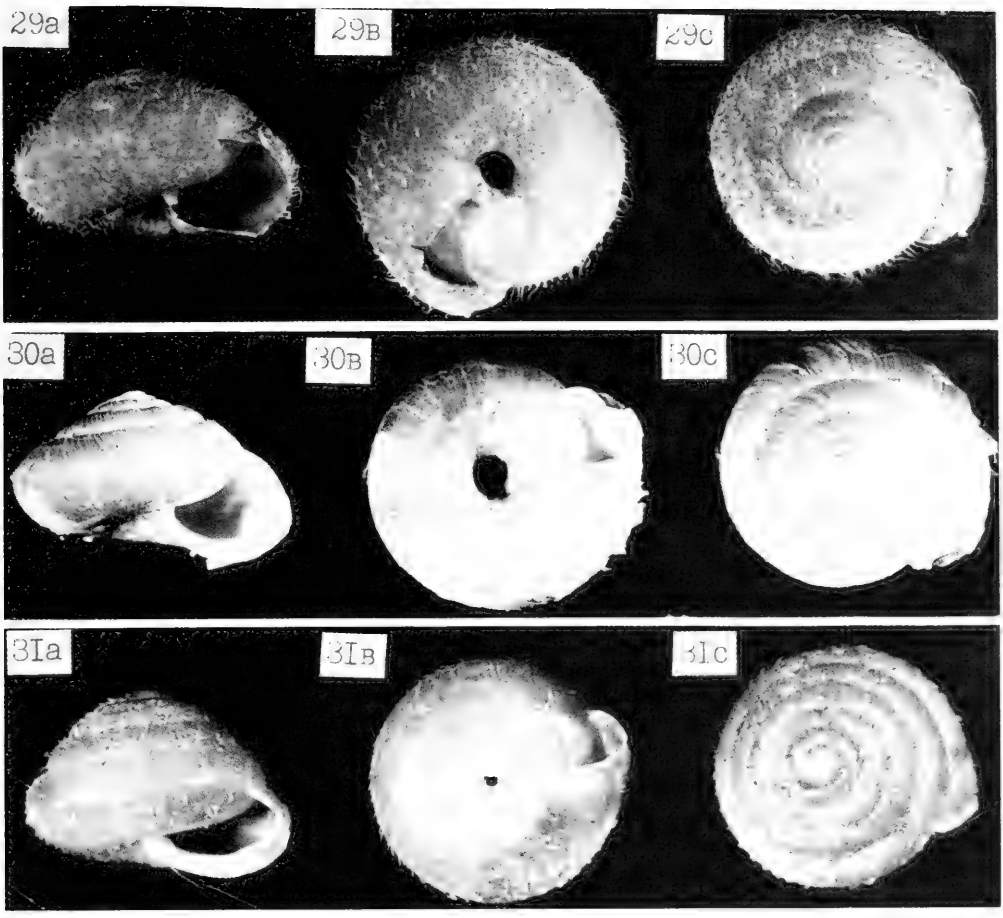


PLATE VIII (not actual size). 29a, b, c, *Trichia (Trichia) villosula* (Rossmaessler), Babíce, near Brno, Moravia, Czechoslovakia, 16 August 1968. 30a, b, c, *Trichia (Trichia) striolata* (C. Pfeiffer), Cologne area, German Federal Republic, 30 May 1963. 31a, b, c, *Edentiella bakowskii* (Polínski), Kvasi village, Rakhov district, Transcarpathian region, Ukranian SSR, 14 September 1969.

slightly bulbous; its distal part is cylindrical. The verge is perfectly fusiform. The seminal duct is attached to 1 side of the inner papillar wall by a longitudinal band; i.e., it is encircled by 1 cavity. The opposite papillar wall encloses a system of intrapapillar cavities divided by septa (Fig. 157). These more or less parallel cavities anastomose and are also all connected with the lumen of the penis sheath by the papillar lacuna. The spermathecal duct is fine and passes very smoothly to the elongate-oval receptaculum seminis, which ends quite a distance short of the lower part of the albumen gland (Fig. 154). In 1 specimen studied, the receptaculum seminis had an isolated position.

Taking into consideration the marked conchological similarity, one may suppose that *T. edentula* (Draparnaud) and *T. bielzi* (Schmidt) [see also Soós, 1917] are close to our species. Likharev & Rammelmeyer (1952), following Polínski (1924), classify it as a variety of *T. bielzi*, noting that the dart sacs attach in the middle of the "uterus" (i.e., the part here differentiated into upper and lower vagina), which is characteristic for Polínski's section *Filicinella*. Our material, however, shows that the dart sacs are so placed in many European *Trichia*. Thus the validity of *Filicinella* becomes questionable. The final answer to this question will depend on detailed investigation of the inner structure of *T. filicina*, the type-species of this section. Note that conchologically *T. filicina* is nearer to *Trichia* s. str. than to *Edentiella*.

IDENTIFICATION KEY

1. Species inhabiting central Asia	2.
Species inhabiting the Caucasus or Europe	9.
2. Shell aperture with 2 teeth	*Odontotrema diplodon*
Shell aperture without teeth	3.
3. Shell globose	4.
Shell more or less depressed	6.
4. Shell white, thick; body whorl lighter in color than other whorls; penis papilla without appendix near base	*Leucozonella caryodes*
Shell horny to reddish, uniformly colored, with white spiral band; walls moderately thick; penis papilla with appendix near base	5.
5. Shell diameter 14 mm or more	*Leucozonella rubens*
Shell diameter 11 mm or less	*Leucozonella mesoleuca*
6. Shell surface with distinct periostracal hairs; diameter 9 mm or less	*Leucozonella caria*
Shell surface without periostracal hairs or diameter more than 9 mm	7.
7. Shell surface coarsely wrinkled	*Leucozonella ferghanica*
Shell surface rather smooth	8.
8. Shell diameter 15 mm or more, surface with spiral lines; no groove on the penis papilla (i.e., it has become closed and is now an intrapapillar cavity)	*Leucozonella retteri*
Shell diameter 14 mm or less, surface without spiral lines; there is a deep, open groove on the penis papilla	*Leucozonella rufispira*
9. Species inhabiting the Caucasus	10.
Species inhabiting Europe	23.
10. Shell distinctly hirsute	*Kokotschashvilia holotricha*
Shell without hairs or with very short hairs	11.
11. Shell with coarse, prominent radial ribs	*Caucasigena tschetschenica*
Shell not ribbed or sculptured with rib-striae	12.
12. Shell diameter 9 mm or less	*Caucasigena armeniaca*
Shell diameter 10 mm or more	13.
13. Shell pale greenish, with large aperture, body whorl voluminous, umbilicus minute	*Caucasigena thalestris*
Shell does not have all features enumerated	14.
14. Shell periphery angular	*Caucasigena rengarteni*
Shell periphery rounded	15.
15. Shell yellowish-brown, almost uniform in color; on the penis papilla there is a longitudinal groove	*Teberdinia zolotarevi*
Shell color is other; penis papilla without groove	16.
16. Shell depressed, with washed-out radial spots; inner pair of dart sacs not visible on external inspection of upper vagina	*Hygrohelicopsis darevskii*
Shell with more or less prominent spire; inner pair of dart sacs visible without dissection of upper vagina	17.
17. Shell nearly globose, diameter barely exceeding the height, uniformly brown or chestnut in color	*Kokotschashvilia phaeolaema*
Shell more or less depressed, diameter markedly	

exceeding the height, gray or white, often with spiral bands	18.
18. Spiral bands are very faint or absent	19.
Spiral bands are distinct	21.
19. Shell diameter 19-22 mm; in the limits of penis papilla, seminal duct attached to inner papilla wall by 2 longitudinal membranes	*Kokotschashvilia makvalae*
Shell diameter either less than 17 mm or more than 22 mm; seminal duct closely adhering to 1 side of inner wall of papilla	20.
20. Shell diameter 17 mm or less; inner wall of penis papilla with numerous plicae	*Kokotschashbilia eberhardi*
Shell diameter 22 mm or more; inner wall of penis papilla is smooth	*Kokotschashvilia tanta*
21. Umbilicus is narrow	*Caucasigena abchasica*
Umbilicus is wide, perspective	22.
22. Shell rather strongly striate	*Caucasigena eichwaldi*
Shell nearly smooth	*Caucasigena schaposchnikovi*
23. Shell yellowish-white, spire conical, aperture with fragile edges, almost without lip; the vaginal plicae are split into patterns of regular prismatic lamellae	*Plicuteria lubomirskii*
Shell does not have all features enumerated	24.
24. Shell of mature specimen with distinct periostracal hairs	25.
Shell of mature specimen without hairs or with rare faint hairs	26.
25. Hairs long	*Trichia villosula*
Hairs rather short	*Trichia plebeia*
26. Shell aperture with basal tooth	*Trichia unidentata*
Shell aperture toothless	27.
27. Shell perforate; wall of penis papilla contains system of cavities, separated by septa	*Edentiella bakowskii*
Shell umbilicate; wall of penis papilla contains pair of symmetrically disposed cavities	28.
28. Shell diameter 6-9 mm	29.
Shell diameter 10-13 mm	30.
29 Shell widely umbilicate	*Trichia concinna*
Shell moderately umbilicate	*Trichia hispida*
30. Shell distinctly angulate; in addition to paired intrapapillar cavities, there is a narrow unpaired cavity	*Trichia danubialis*
Shell obtusely angulated; 1 intrapapillar cavity larger than the other and with corroded internal wall	*Trichia striolata*

DISCUSSION

Analyzing the results obtained, I have concluded that the ancestral trichiae developed in 2 ways, 1 taken by the Asian forms and the other by the Caucasian-European forms.

Asian forms: by eastward migration, some species reached the mountainous regions of central Asia. These formed shells the common shape of which is now characteristic for most of the central Asian Helicoidea (*Leucozonella*). Conchologically separate from these are *Odontotrema diplodon* and *Leucozonella caria*, the latter strongly resembling European *Trichia* s. str.

We may assume that the verge of the initial primitive forms was a simple tube without the longitudinal groove on its surface and without any intrapapillar cavity (Fig. 160, I). In Recent Asiatic species, all the basic phases of intrapapillar cavity formation can be observed (Fig. 160, II-IV). At first the longitudinal groove on the surface of the verge is formed in the proximal part only (Fig. 160, II, *Leucozonella ferghanica);* then it extends along the whole papillar length (*Odontotrema diplodon*), deepens (*Leucozonella caryodes*; Fig. 160, III) and tends to become closed (*L. rubens, L. rufispira*); the final phase is the fully closed groove, which now forms an intrapapillar cavity (*L. retteri, L. caria*; Fig. 160, IV). In these forms the seminal duct adheres closely to the inner papillar wall on 1 side, and the intrapapillar cavity embraces it from all other sides. A remnant of the groove is the papillar lacuna that exists in all species discussed and by means of which the intrapapillar cavity connects with the cavity of the penis sheath. The circumstance that most Asiatic species have an intrapapillar cavity that remains open testifies to their relatively primitive state. This conclusion is strenghtened by the fact that other Asiatic Hygromiidae are also the most primitive representatives of their groups (Shileyko, 1970).

We must further conclude that, on the whole, the "Helicidae" auct. had a wider distribution in the past than now and that Recent Asiatic representatives of the group are relics from the Tertiary period.

Caucasian-European forms: the second path of development was the formation of 2 groups: the Caucasian and European groups. In these the formation of the intrapapillar structures evolved by another principle than in the Asiatic group. Basically, intrapapillar cavities occur in pairs in the papilla walls, embracing the centrally placed seminal duct from 2 opposite sides; the papillar lacuna forms in a parallel manner. In other words, it is necessary to assume that the papillar lacunae of Asiatic and European-Caucasian species are not homologous. Similar types of papillar structure exist in such clearly independent groups as *Hygrohelicopsis, Teberdinia, Plicuteria, Trichia* s. str. and in some species of the genus *Kokotschashvilia* (Fig. 160, V).

The very complex system of septate intrapapillar cavities such as are found in *Edentiella* and *Caucasigena* (Fig. 160, IX) might possibly have formed in the way shown by species such as *Trichia striolata* and *T. danubialis* (Fig. 160, VII and VIII, respectively). It consists of the fragmentation of 1 papillar wall, in which is thus formed a tertiary cavity, i.e., a derivative of the secondary cavity, which then further disintegrates into a series of narrower cavities. Nevertheless, the totality of characters of another order makes it necessary to recognize an independent origin for the European *Edentiella* and the Caucasian *Caucasigena*.

Within the genus *Kokotschashvilia* one can observe a number of variants in papilla structure that we are here attempting to derive from the same initial point of departure (Fig. 160, V). The papilla structure in *K. makvalae* (Figs. 55; 160, V) is nearest to this initial point: the seminal duct is surrounded by a pair of intrapapillar cavities. A capillary runs in the papilla wall along 1 of the longitudinal tissue bands attaching the seminal duct to the inner papillar wall. In *K. tanta* this capillary is retained. The seminal duct is displaced toward 1 of the papillar walls and adheres to it closely; on the opposite side there still is a band of tissue separating the 2 cavities (Fig. 160, X). This division is absent in *K. eberhardi,* in which the cavity, now single, has a crescent-shaped cross-section, smooth-walled in not fully mature specimens and sinuously folded in mature specimens (Fig. 160, XI, XII). As a result, the type of papillar structure of *K. eberhardi* is formally the same as in the Asiatic *Leucozonella retteri* and *L. caria* (Fig. 160, IV). To interpret this similarity as a purely formal one is justified from 2 considerations. In the first place, the formation of the intrapapillar cavity embracing the seminal duct in *K. eberhardi* has been traced in allied species of *Kokotschashvilia* (see above). In the second place, I have made a series of dissections of *K. eberhardi* at various stages of maturity which demonstrates that in specimens not fully adult the seminal duct is still attached by a longitudinal tissue band to the papilla wall opposite to the wall it adheres to, the band dividing the intrapapillar cavity into 2 chambers (Fig. 161) just as in adult *K. tanta*.

In *K. holotricha*, the seminal duct does not adhere to the papillar wall on one side but is well removed, being held in a central position by a thin longitudinal band and

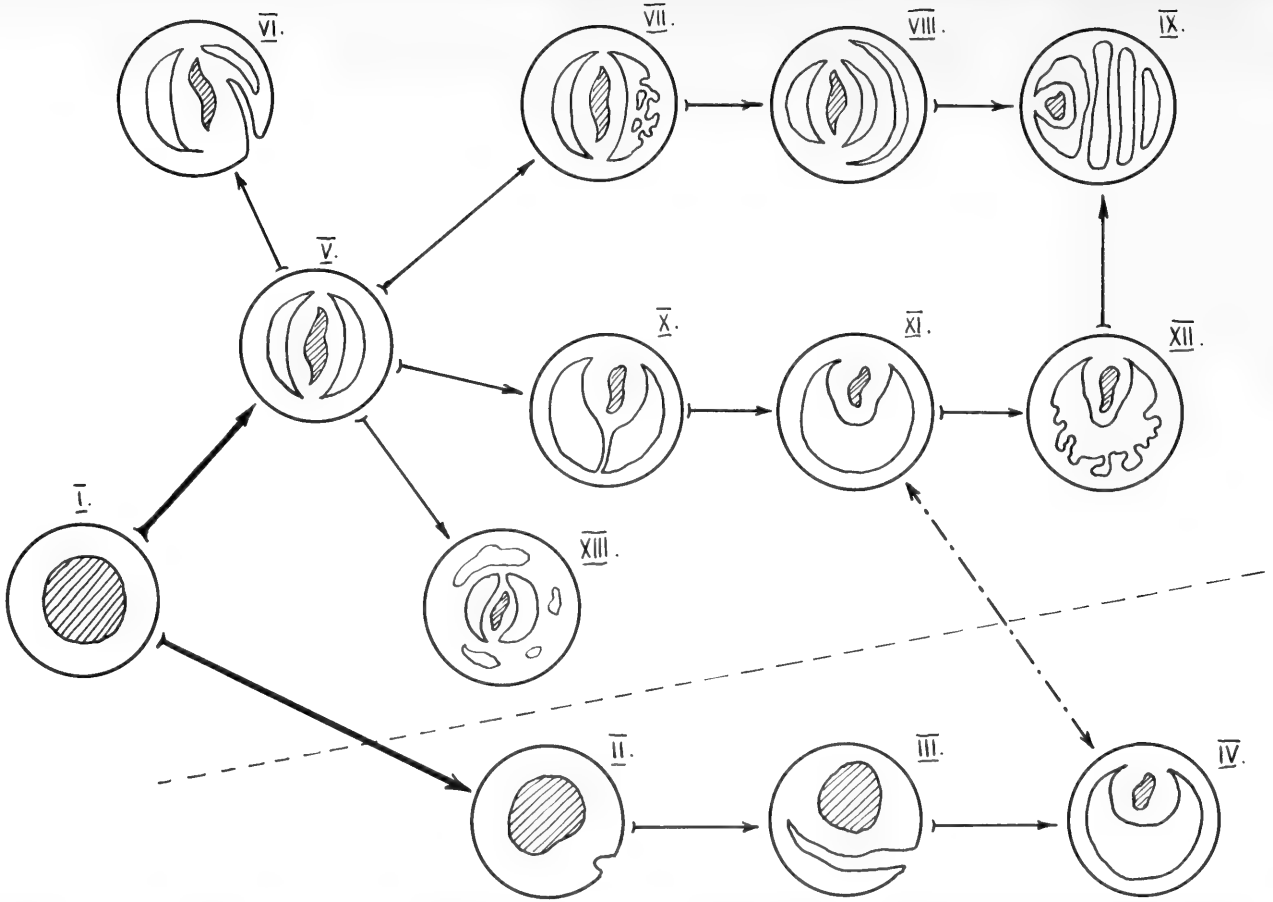


FIG. 160. Variation in verge structure in the various groups of *Trichia* s. lat. and their relationships. Cross-section of seminal duct is shaded. I, initial papilla type as a simple tube. II-IV, gradual formation of the intrapapillar cavity from a papillar groove in Asiatic species. V-XIII, various modes of formation of intrapapillar cavities from a paired cavity in European and Caucasian species, as exemplified by the following forms: I, *Leucozonella ferghanica,* distal part; II, *Odontotrema diplodon; Leucozonella ferghanica,* proximal part; III, *L. caryodes;* IV, *L. retteri, L. caria;* V, *Hygrohelicopsis darevskii, Plicuteria lubomirskii, Trichia plebeia, T. concinna, T. hispida,* etc.; VI, *Teberdinia zolotarevi;* VII, *Trichia striolata;* VIII, *T. danubialis;* IX, *Edentiella bakowskii, Caucasigena armeniaca, C. rengarteni, C. eichwaldi, C. schaposchnikovi, C. thalestris;* X, *Kokotschashvilia tanta;* XI, *K. eberhardi,* immature specimen; XII, *K. eberhardi,* mature specimen; XIII, *K. phaeolaema.*

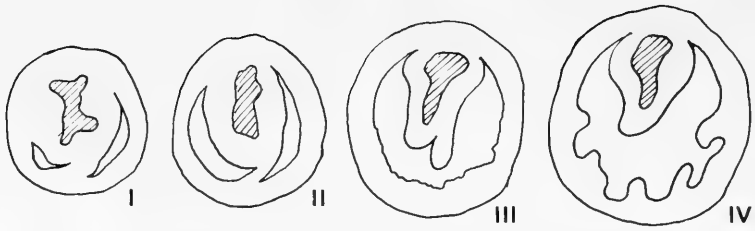


FIG. 161. Formation of intrapapillar cavity in *Kokotschashvilia eberhardi* during ontogenesis (in cross-section). I, Initial paired cavities in papilla wall and broad contact between seminal duct (shaded) and inner papilla wall. II, The cavities grow and there develops between them a longitudinal band that attaches the seminal duct to the papillar wall opposite the area of fusion with the wall. III, Connection of seminal duct and papillar wall by the band is lost by coalescing cavities. IV, Except for the one point of adherence, the seminal duct is now completely isolated from the surrounding papillar wall.

encircled by the intrapapillar cavity (Fig. 65). Finally, in *K. phaeolaema* additional sinuses have formed in the papilla wall (Fig. 160, XIII).

Just as the papillar structures in *Edentiella* (Fig. 160, IX) are derived from *Trichia striolata* and *T. danubialis* (Fig. 160, VII, VIII), those of *Caucasigena* (Fig. 160, IX) are derived from *Kokotschashvilia* in a parallel and independent manner (Fig. 160, XI, XII, IX): in *K. eberhardi* the inner papillar wall opposite to the seminal duct shows clear traces of dissolution (Fig. 70; 160, XII).

There still is 1 more variant in papillar structure: in *Teberdinia* there are both a pair of intrapapillar cavities and a deep groove on the penis surface; 1 of the cavities reaches into the lobe separated off by the groove (Fig. 160, VI). At the present time it is difficult to give any comparative morphological estimation of this variant as we do not yet know any related types of papilla structure. We might imagine the formation of a third cavity from a closing groove, in which case we would get a variant corresponding to *T. danubialis* (Fig. 160, VIII). One might suppose that with the groove closing and with a simultaneous reduction of the band attaching the seminal duct to the papilla, there might arise the variant answering to *Edentiella* and *Caucasigena* (Fig. 160, IX). Assessment must be left to a later date, although the second of the modes discussed seems quite likely.

If I have paid so much attention to the details of verge structure, it is because the richness and variability of intrapapillar structures will display and reflect the common evolution of the group. No other feature offers us so rich a material for phylogenetic reconstruction. However, while considering variants of the papilla structure in detail, I am not proposing to attach excessive importance to the characters of the papilla. The phylogenetic scheme (Fig. 162) here submitted rests as far as possible on the feature complex valuable for practical taxonomy. In the diagnosis of genera (see Systematic Part) various categories of characters are given, some of which need to be considered in discussing the proposed scheme of classification.

In addition to the anatomical features treated in connection with the Asiatic group, we shall also consider the 4 conchological types occurring in that geographical area. The first type, exhibited by *Odontotrema diplodon* (Pl. I, 1), is very clearly distinguished by its aperture fold; the second type is the almost globose shell of *Leucozonella caryodes, L. rubens* and *L. mesoleuca*; third, we have the flattened shell type in *L. ferghanica, L. rufispira* and *L. retteri*; and the fourth is a purely European conchological type: *L. caria* (European *Trichia* are relatively small, thin-shelled, usually brown, usually hirsute).

The primitive species obviously is *Odontotrema diplodon*. Apart from the characters of the penial papilla (open groove) already mentioned, we must consider the high degree of isolation of the dart sacs. Taking into consideration the oligomerization principle of homologous organs (Dogel, 1954), and the starting point of this principle, i.e., the multiple inception of newly formed organs, we must conclude that the ancestral forms had 4 equivalent dart sacs, each of which included a dart. The first phase of oligomerization was the

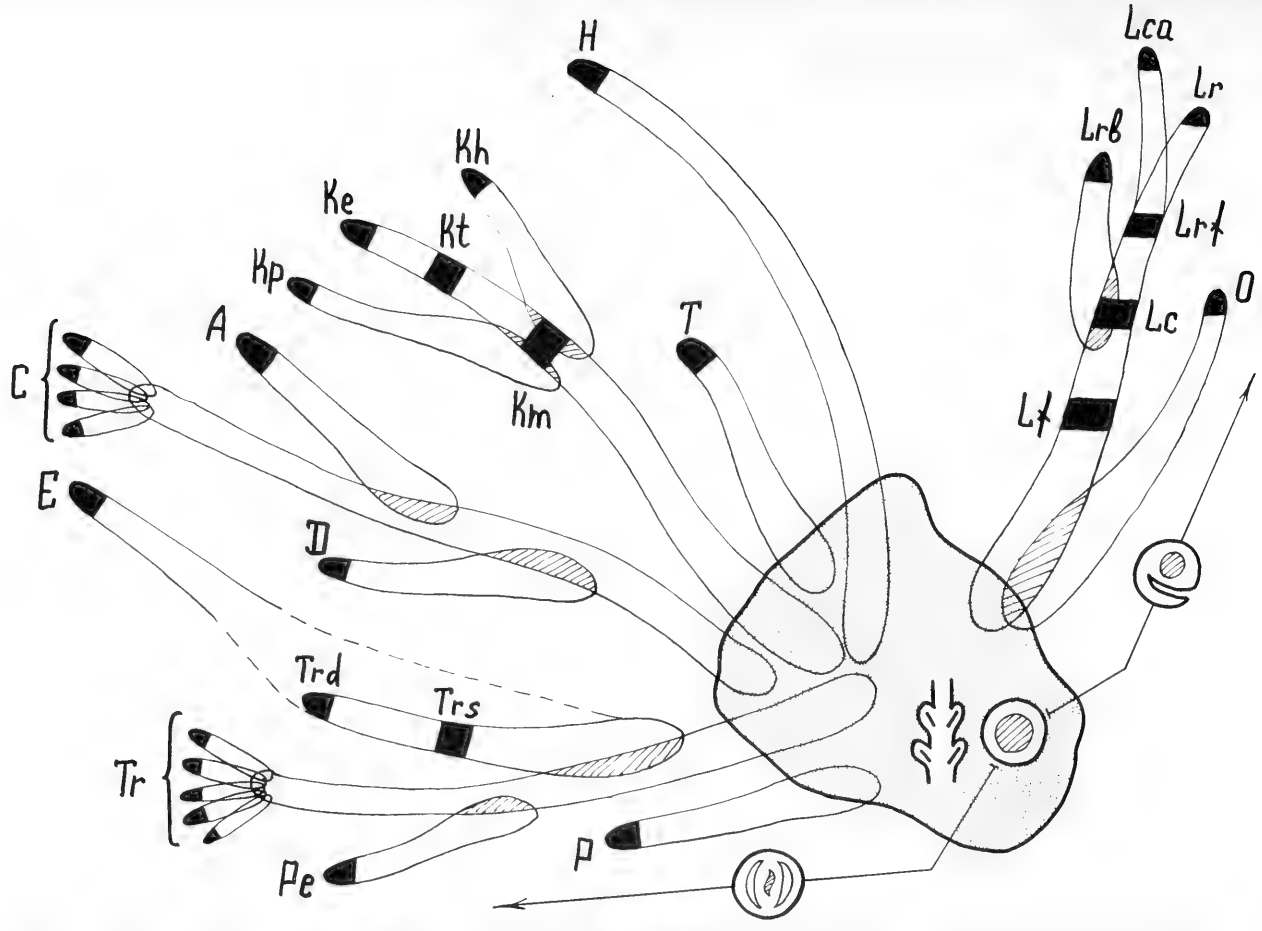


FIG. 162. Phylogenetic scheme of the *Trichia* s. lat. group. The stippled area corresponds to the complex of ancestral forms characterized by the presence of 4 equal dart sacs and a verge in the form of a simple tube. Arrows show 2 main evolutionary paths of the verge. Places corresponding to actual species are marked in black. A. *Caucasiaena (Anoplitella);* C, *Caucasigena (Caucasigena);* D. *Caucasigena (Dioscuria);* E, *Edentiella;* H. *Hvarohelicopsis;* Ke, *Kokotschashvilia eberhardi;* Kh, *K. holotricha;* Km, *K. makvalae;* Kp *K. phaeolaema;* Kt, *K. tanta;* Lc, *Leucozonella caryodes;* Lca, *L. caria;* Lf, *L. ferghanica;* Lr, *L. retteri;* Lrb, *L. rubens;* Lrf, *L. rufispira;* O, *Odontotrema;* P, *Plicuteria;* Pe, *Trichia (Petasina);* T, *Teberdinia;* Tr, *Trichia (Trichia),* in part; Trd, *T. danubialis;* Trs, *T. striolata.*

loss of darts in the inner (upper) sacs. Further oligomerization of adventitious organs in the female tract causes their gradual reduction (maximal degree of reduction observed in *Hygrohelicopsis*). The presence of well-developed, clearly separated inner dart sacs approximately the same size as the outer sacs reaffirms the primitive condition of *O. diplodon.* The shell also shows the isolation of this species.

From papillar and other features it is logical to consider *Leucozonella ferghanica* to be a primitive type in respect to other Asiatic species. The groove on the surface of the verge is only weakly traced, and the upper vagina is very long. *L. caryodes* is then a derivative; the groove on the verge surface is much longer and the upper vagina less so. The shell is more globose. *L. rubens* retains a globose shape but shows reduction of the inner dart sacs and a closer connection with the surface of the upper vagina. At the same time this species has acquired a number of quite important features such as a markedly shorter flagellum, more branched mucous glands, and the presence of an intrapapillar appendix, the purpose and origin of which is not clear.

Leucozonella rufispira, another derivative of *L. caryodes* independent of *L. rubens*, is a distinct form by reason of other features. The flagellum length and degree of isolation of the dart sacs have not changed; the shape of the sacs has changed. The depth and general character of the papillar groove correspond to what we see in *L. caryodes*. The shell has acquired a more flattened form.

Judging mainly by the shell, *Leucozonella retteri* is a derivative of *L. rufispira*. It has a qualitatively important new evolutionary feature, i.e., the final formation of the intrapapillar cavity, but generally retains other characters of *L. rufispira.*

In the series of the central Asiatic forms, *Leucozonella caria* is prominently distinct by its shell, which, in my view, like the shells of the European trichiae, retains the initial features characteristic for the ancestral forms. At the same time the species has the essential character that relates it to the true Asiatic group: its verge structure completely corresponds to *L. retteri* (Fig. 160, IV) and has nothing to do with European species. It is necessary to point out here that study of the external morphology of the genitalia does not engender understanding of the real essence of this species; using only the character of outer shape, we should regard *L. caria* as a member of European *Trichia* s. str., which would be very wide of the mark.

To finish the discussion of the Asiatic group, we need to point out 1 further obviously primitive feature common to all these species: the regular internal longitudinal folding of the epiphallus which, in cross-section, gives to its lumen the shape of a multiradial regular star. Subsequently 2 neighboring longitudinal folds developed more strongly while the other folds were grouped on the opposite epiphallus wall. As a result there formed 2 main spermatophore guide "rails" and a number of small ribs above them; in the furrows between these fit the rows of thorns on the spermatophore surface (Fig. 163). Such a development of the 2 longitudinal folds is observed in the Caucasian and European species. As for the Asiatic species, the differentiation of the equal folds in the epiphallus lumen is only beginning, and distinctions between these 2 groups of folds are sometimes hard to make. The lumen of the epiphallus in cross-section retains in most cases the original type of multiradial, almost regular star (see Figs. 15, 22, 32).

The European-Caucasian group of genera does not form one single developmental line, as is so well seen in the Central Asiatic forms, but is the product of some parallel and, to a certain degree, independent evolution, with the retention of a more or less definite complex of similar features. We do not find any anatomical characters permitting judgment on the evolutionary tendencies of the Caucasian and European groups. But the conchological distinctions between the European and Caucasian species are clearly seen. Generally characteristic for the European forms is a rather fragile, small- or medium-sized, usually hirsute shell of either a uniform brown color or marked with a faint white line at the periphery, whereas the Caucasian forms are, as a rule, more solid, larger, usually not hirsute and show a variety of color besides brown (chestnut, white, lilac, greenish, yellowish, etc.). There are exceptions among the European forms, such as *Plicuteria lubomirskii* and *Xerocampylaea zelebori* (L. Pfeiffer) (Pl. VI, 21). The former also stands out anatomi-

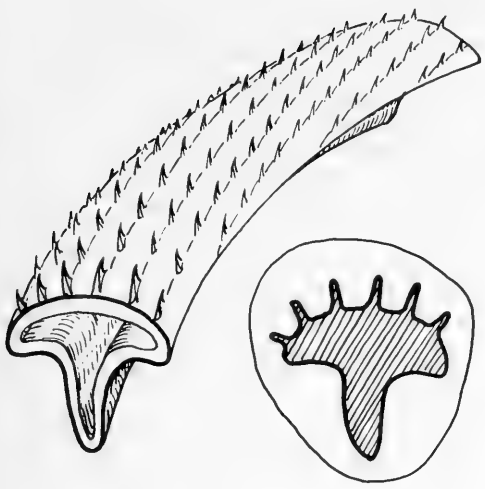


FIG. 163. Part of the spermatophore and transverse section of epiphallus (at right side) of *Caucasigena (Caucasigena) eichwaldi.*

cally from the general run of species. The latter is discussed below. Almost all the European trichiae are forest animals.

The Caucasian species are primarily connected with 2 types of biotope: (1) with rocks (*Caucasigena* s. str.) and (2) with either open and dry or damp slopes (the other forms, i.e., *Hygrohelicopsis, Teberdinia, Kokotschashvilia* and the other 2 subgenera of *Caucasigena*). According to these habitats, we see 2 shell types: (1) either more or less flattened, ribbed shells, or (2) high to almost globose shells with relatively vague sculpture. The presence or absence of periostracal hairs on the shells of representatives of different species is not a constant feature: as a rule *Kokotschashvilia holotricha* almost always is hirsute; *Caucasigena rengarteni* is generally not hirsute, but there are some specimens with short hairs. I consider the presence of hairs to be a primitive character and believe that the disappearance of this feature in the Caucasian species is an independent and parallel development.

Another conchological exception among European species is *Xerocampylaea zelebori*, a species living among rocks as does the Caucasian group of *Caucasigena* s. str. It is now known that a rocky habitat usually produces a marked imprint on the aspect of the shell. The conchological similarity between these forms (flat, ribbed) caused earlier authors to regard *Caucasigena* as a separate section of the genus (or subgenus) *Xerocampylaea* Kobelt, 1871. As *X. zelebori* has not been carefully investigated anatomically, and its distribution (Serbia, in part Roumania) is quite far from the Caucasus, I do not connect these groups, particularly as I am trying to establish the independence of the genus *Caucasigena*. Other evidence is that the shell of *X. zelebori* is otherwise quite clearly distinct from that of *Caucasigena*; the apex is acute and not obtuse, and the contour of the whorls is not dome-shaped.

Lastly we must take into account similar features among all the species examined. Except for the presence of 2 pairs of dart sacs, the only other common character is the presence of a papillar lacuna (in species with a completely formed intrapapillar cavity), by means of which the intrapapillar cavity communicates with the cavity of the penis sheath. We assume that when the opening into the cavity of the genital atrium is closed, the increased pressure of the intrapenial liquid helps to squeeze the papilla outside, in part owing to the presence of this lacuna. In copulation the papilla is not turned inside out but is moved forward (Fig. 72). These 2 features are the only ones common to all species discussed. Even the topography of the right-hand ocular retractor, which used to be taken as the basic anatomical criterion for the assignment of any species to either the "Helicellinae" auct. or the "Hygromiinae," is not constant in the group studied. In the hygromiids living under arid conditions, the right ocular retractor passes alongside and not between the penis and vagina, and the shells of such groups have adaptive features such as very light color and strong calcification. Thus the retractor in question only passes near the distal part of the genitalia and not between them in *Hygrohelicopsis darevskii* (Fig. 42). Nevertheless, the sum total of characters of another nature (shell, penial papillar structure, pigmentation of integuments, ecology) indicates that *H. darevskii* obviously is a representative of the Trichiinae.

From the above it is clear that, for the elucidation of a true and objective system, it is necessary to enlist as many and various characters as possible. This was attempted in the present work.

ACKNOWLEDGMENTS

It is my pleasant duty to acknowledge gratefully the help of all those without whose kind assistance the present research would not have been completed. The scientists of the Zoological Institute, USSR Academy of Sciences, Leningrad, Drs. I. M. Likharev and Ya. I. Starobogatov, gave much helpful advice and instructions during the preparation of this manuscript. N. N. Akramovsky of the Zoological Institute, Armenian Academy of Sciences, furnished most valuable information with respect to *Caucasigena armeniaca*. I am greatly indebted to Dr. V. Hudec, of the University of Prague, Czechoslovakia; Dr. E. Clauss, of the Institut für Pflanzenzüchtung at Quedlinburg, German Democratic Republic; and Mr. Z. Izzatulaev, of the Institute of Zoology, Academy of Sciences of Tadzhikistan at Dushanbe, for the important material they have provided. Some separate points of the discussion were constructively criticized by L. V. Shileyko and J. P. Ghubar, whose constant assistance and support I received while writing this paper.

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MALACOLOGIA, 1978, 17(1): 57-61

A LATITUDINAL PATTERN IN BIVALVE SHELL GAPING

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ABSTRACT

Bivalves with persistent posterior shell gapes comprise an increasingly smaller proportion of infaunal bivalve assemblages from Arctic to temperate to tropical coasts. This trend is not a taxonomic artifact, since it can be shown that deep-burrowing cold-water gapers are replaced in the tropics largely by tightly closing lucinids. We interpret the increasing equatorward emphasis on complete shell closure among bivalves to reflect an increase in the intensity of predation. The latitudinal trend in predation and bivalve shell architecture is consistent with the microgeographical reduction in proportion of gaping from deep to shallow sediment layers.

INTRODUCTION

Although latitudinal gradients in diversity are well known and of extremely wide occurrence among both marine and nonmarine organisms (see e.g. Fischer, 1960), much less is known about latitudinal variations in animal architecture. Bakus (1969) has summarized gradients in algal morphology, and has related these to an equatorward increase in grazing intensity. Bakus (1974) and Bakus & Green (1974) have described a gradient of increasing toxicity toward the tropics among holothurians and sponges. An increasing emphasis on calcification (thicker shells, stronger sculpture, etc.) among tropical as compared to cold-water species has been recognized both in gastropods (Graus, 1974; Vermeij, in press) and bivalves (Nicol, 1967). Besides the increase in the aragonite:calcite ratio in some bivalve shells toward the tropics (Lowenstam, 1954; Waller, 1972), no other latitudinal variations in shell architecture of bivalves have been described to our knowledge.

In this paper, we point out that bivalves with persistent posterior gapes are primarily polar and temperate in distribution, and suggest that the more complete shell protection provided by tight valve closure in most tropical bivalves is related to an equatorward increase in predation intensity.

METHODS

The latitudinal gradient in shell gaping may be documented in 2 ways. First, we can estimate the number of species in each faunal province possessing a permanent posterior gape, and then compare this number with the total number of bivalve species in that province. Such estimates may be obtained by consulting major faunistic works; in our study, we have used Abbott (1974) for temperate North America, Keen (1971) for tropical West America, and Abbott (1974) and Humfrey (1975) for the tropical western Atlantic. We have excluded from our analysis all hard-bottom, nestling, and rock-boring bivalves, and have restricted our estimates to shallow-water soft-bottom clams living no deeper than 100 fathoms (278 m). Even within genera and families, we have rejected species which bore into rocks while retaining those which are found in soft sediments.

Provincial boundaries used in this study conform to those proposed by Briggs (1974). The Arctic region is defined to extend south to the Gulf of St. Lawrence on the Atlantic coast, and (as the Aleutian Province) to Puget Sound on the Pacific coast. The Oregonian Province extends from Puget Sound to Point Conception, California; it is succeeded in a southward direction by the Californian Province, which extends to Magdalena Bay, on the outer coast of Baja California. The Panamic Province includes the outer coast of Baja California south of Magdalena Bay, and the mainland coast from the mouth of the Gulf of California to northernmost Peru. We have excluded from our analysis all species which are endemic to the offshore islands (Galapagos, Clipperton, Cocos, Revillagigedo), or to the Gulf of California.

In the Atlantic, the Boreal Province extends from the Gulf of St. Lawrence and Newfoundland to Cape Cod, Massachusetts; the Virginian Province succeeds this province southward to Cape Hatteras, North Carolina. The Carolinian Province is here regarded as extending from Cape Hatteras to the area of Palm Beach, Florida. Although the northern Gulf of Mexico also belongs to the Carolinian Province (see e.g. Hedgpeth, 1953), we have not included bivalves from that area in our analysis. The West Indian Province comprises the east coast of Florida south of Palm Beach, as well as the Bahamas, West Indies, and the mainland coast of America from eastern Yucatan south. The Brazilian region faunistically belongs to the West Indian Province (see e.g. Vermeij & Porter, 1971), but is not included here because of insufficient faunistic data.

A second way of describing the latitudinal pattern in posterior gape is to estimate the relative number of species with persistent posterior gapes at variously latitudinally separated localities. Species lists from various regional studies have been supplemented with collections made by the senior author from various parts of the world.

RESULTS

The genera in North American waters found to have persistent posterior gapes and included in our study are listed in Table 1. As Stanley (1970) and Runnegar (1974) have already pointed out, most gapers are deep-burrowing forms whose rate of burial varies from rapid (*Ensis*) to very slow (*Mya, Cyrtopleura*). Some species, however, are shallow burrowers; thus, *Labiosa lineata* (Say) is common on certain *Thalassia* flats in west Florida, where it is shallowly buried in muddy sand (Vermeij, personal observations). The cardiid *Lophocardium* is probably also a shallow burrower.

Table 2 presents the proportion of species with posterior gapes in each of the generally recognized biogeographical provinces on the Pacific and Atlantic coasts of North America, as estimated from the taxonomic works cited above. In general, tropical faunas have a much lower proportion of species with posteriorly gaping valves than do temperate faunas, but the steepness of the gradient is quite different on the 2 coasts. In the eastern Pacific, there is no significant difference between the Aleutian, Oregonian, and Californian Provinces in the incidence of gaping; but all 3 provinces show a sharply higher incidence of gaping than does the tropical Panamic Province ($p < 0.005$, chi-square tests). Differences between adjacent provinces along the Atlantic coast are not significant ($p > 0.05$, chi-square tests), but there is an unmistakable and rather uniform decline in the proportion of gaping species from north to south. To achieve the 0.05 level of significance between provinces, one must move at least 2 and sometimes 3 provinces depending on the location within the gradient.

Even within particular families, there is a trend for gaping species to be temperate

TABLE 1. North American genera of persistently gaping, infaunal clams.

Solemyidae: *Solemya*
Nuculanidae: *Nuculana* (only *N. pernula* Müller)
Malletiidae: *Tindaria*
Cardiidae: *Papyridea, Lophocardium*
Veneridae: *Saxidomus*
Semelidae: *Cumingia*
Sanguinolariidae: *Sanguinolaria* (not *S. nuttalli* Conrad), *Tagelus, Gari*
Solenidae: *Siliqua, Solen, Ensis, Solecurtus*
Mactridae: *Mactra, Spisula* (only *S. dolabriformis* Conrad), *Labiosa, Tresus, Mactrellona* (only *M. clisia* Dall)
Hiatellidae: *Cyrtodaria, Panomya, Panopea*
Myidae: *Mya, Sphenia, Paramya, Cryptomya*
Pholadidae: *Cyrtopleura*
Lyonsiidae: *Lyonsia* (not *L. arenosa* Möller), *Entodesma*
Thraciidae: *Cyathodonta, Asthenothaerus*
Poromyidae: *Poromya*

TABLE 2. Incidence of persistent posterior gaping in shallow-water infaunal bivalves from North America (data compiled from Keen, 1971; Abbott, 1974; Humfrey, 1975).

Province	Number of species		Incidence of gaping %
	Gapers	Total infaunal	
Eastern Pacific			
Aleutian	23	79	27
Oregonian	47	160	29
Californian	32	114	29
Panamic	58	448	13
Western Atlantic			
Arctic	11	36	31
Boreal	16	57	28
Virginian	17	70	24
Carolinian	20	137	15
West Indian	23	193	12

or polar in distribution. Most mactrids with tightly closing valves (e.g. *Rangia,* most species of *Spisula, Mulinia*) are warm-temperate to tropical in distribution, while such typically gaping genera as *Tresus* and the European *Lutraria* are cold-water forms. The cold-water eastern Pacific *Saxidomus* is one of the very few venerids with a persistent (albeit small) posterior gape.

The gradient as described from faunistic studies is again evident when the clams from restricted localities are considered (see Table 3). The high percentage of gapers at Garrison Bay, an intertidal mud flat on San Juan Island in Washington State (48.5° N) (44%) contrasts sharply with the percentages in tropical Jamaica (9.1%) and the Seychelles (2.6%).

DISCUSSION

The observed equatorward decline in the incidence of gaping clams could be due to several causes, some trivial and others more interesting. Since gaping clams tend to be deep burrowers, it might be argued that tropical clams do not, on the whole, burrow as deeply as temperate clams. This seems unlikely; not only do deposit-feeding tellinids often burrow deeply in the soft bottoms of both temperate and tropical regions, but the lucinids are a predominantly tropical group characteristically found at great sediment depths (see Allen, 1958; Jackson, 1972, 1973). Both tellinids and lucinids are normally completely enclosed in their shells, and do not possess persistent gapes. Thus, the latitudinal gradient in gaping is not an artifact of taxonomy, nor does it reflect a restriction of tropical bivalves to shallow sediment depths.

We must, therefore, conclude that the latitudinal gradient in gaping is a biological response to an external factor which is correlated with temperature. The close correlation with mean maximum temperature is evident from the difference in the steepness of the gradient on the two coasts of North America. In the eastern Pacific, water temperatures are cool and fluctuate little, and the north-south increase in temperature is comparatively small from Alaska to northern Baja California. On the east coast of North America, temperatures fluctuate wildly from winter to summer, and there is a steady rise in summer but not in winter temperatures from Canada to Florida.

How might temperature gradients influence the incidence of gaping? Following the arguments of Bakus (1969, 1974), Bakus & Green (1974), and Vermeij (1977, in press) we believe that the increased intensity of various types of predation from high to low latitudes can account for the relative decline in species with gaping valves. It is becoming well established that the intensity of crushing predation (by fishes, crabs, stomatopods, lobsters, etc.) increases toward warmer latitudes (for data see Menzel & Hopkins, 1955; Vermeij, 1977a). Data on other types of predation are scantier, but the diversity of predators which drill, swallow, or pry open their bivalve prey increases sharply toward the tropics. Among the important predators on bivalves, only the asteroids and birds are more prominent at high latitudes than in the tropics (Vermeij, in preparation).

Several types of predators apparently take less time and require less specialized equipment to prey on gaping clams than on more tightly closing bivalves. Naticid gastropods do not normally drill gaping spe-

TABLE 3. Incidence (I) of bivalve species with a persistent posterior gape (G) from total considered bivalve species (T) in selected localities.

Locality	Lat.	Spp G	Spp T	I%	Source
Garrison Bay, Washington (intertidal)	48.5° N	4	9	44	GJV
San Juan Islands, Washington (subtidal)	48.5° N	5	20	25	GJV
Mugu Lagoon, California	34° N	3	15	20	Peterson, 1975
Alligator Harbor, Florida	30° N	3	18	17	GJV
Sanibel Island, Florida	27° N	3	22	14	GJV
Jamaica (*Thalassia* beds)	17° N	4	44	9	Jackson, 1973
Venado Beach, Panama Canal Zone	7° N	2	21	10	GJV
Singapore	1° N	4	27	15	Vohra, 1971
Mahé, Seychelles	3° S	2	76	3	Taylor, 1968

cies of *Tagelus,* but rather can attack them through the open anterior or posterior ends of the shell (Stump, 1975); they must resort to drilling in order to prey on venerids and other tightly closing clams. Gaping clams are digested more rapidly by the sand-dwelling asteroid *Astropecten* than are many tightly closing corbulids and venerids (Christensen, 1970; Massé, 1975). Extraorally digesting sea stars may not need to pry open clams with a persistent gape, since the digestive juices from their stomachs can be introduced into the victim by way of the siphon. Gastropods of the genus *Busycon* require a strong outer lip in order to pry open clams with tight valve closure, but could do the job with a much thinner lip when preying on *Ensis* and other gaping bivalves (Carriker, 1951; Paine, 1962, 1963). The North Atlantic snail *Buccinum undatum* Linn. must chip the valves of tightly closing clams, but need not employ this tactic in attacking gapers (Nielsen, 1975). Siphon-cropping fishes can readily snip off siphons from posteriorly gaping clams, but not so readily from tightly closing forms (Nesis, 1965; Edwards & Steele, 1968). Tropical acanthurid, scarid, and other fishes graze, scrape, and browse exposed rocky surfaces and grass flats extensively (see e.g. Randall, 1967), and could have a disastrous impact on permanently gaping bivalves even if these were eaten or attacked only incidentally.

Carriker & Van Zandt (1972) have pointed out that tight valve closure prevents metabolic products from escaping in the presence of predators, and could thus aid in preventing detection. This is obviously not possible in bivalves with permanent gapes.

The relative decline in gaping clams toward the tropics thus appears to go hand in hand with increasing protection from predators, and is repeated on a microgeographical scale from deep| to shallow sediment layers. Relatively few predators can attack deep-burrowing bivalves; some naticid gastropods, temperate asteroids, and warm-water rays can exploit deeply buried prey. Long-billed birds such as the curlew (*Numenius*) are generally incapable of taking deeply buried clams (see Burton, 1974). From the point of view of predation, then, moving down into the sediment is akin to heading away from the equator.

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MALACOLOGIA, 1978, 17(1): 63-72

THE HABITAT AND FEEDING BEHAVIOR OF THE WENTLETRAP *EPITONIUM GREENLANDICUM*

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ABSTRACT

Epitoniids (wentletraps) are mesogastropod prosobranchs which forage for and/or parasitize a variety of benthic coelenterates. *Epitonium greenlandicum* (Perry) is circumboreal in distribution and occurs subtidally in the northwestern Atlantic.

Laboratory and field observations indicate that this wentletrap feeds infrequently, and only on anemones. After feeding, *E. greenlandicum* burrows into soft mud and can remain inactive for as long as 80 days. Wentletraps are able, at least over short distances, to locate anemones by chemotaxis.

Although *E. greenlandicum* can feed on at least 6 species of anemone, it shows a preference for *Metridium senile* and tends to parasitize this large anemone under laboratory conditions. In the shallow waters of the Bay of Fundy, *M. senile* occurs on exposed rocks and ledges and is not available to the primarily infaunal wentletraps. Consequently, *E. greenlandicum* must forage for small anemones on a mixed mud-cobble bottom.

It is suggested that epitoniids are able to occupy a purely ectoparasitic niche only in areas where there is both a stable supply of the host coelenterate and a nearby refuge from visual predators.

INTRODUCTION

The various groups of prosobranch gastropods which have evolved methods of feeding on coelenterate prey are listed by Robertson (1966, 1970) and by Perron & Turner (in press). Among these gastropods, wentletraps (family Epitoniidae) are cosmopolitan in distribution and range from the intertidal zone to abyssal depths. Detailed anatomical studies of epitoniids have been published by Taki (1956, 1957) and by Hochberg (1971), but surprisingly little information is available on wentletrap natural history and ecology.

The first significant contribution to our knowledge of feeding in epitoniid gastropods was Thorson's (1957) suggestion that the entire family possibly is parasitic on sea anemones. Thorson based his statement on the behavior of the wentletrap *Opalia crenimarginata* (Dall), which lives in close association with, and feeds on, the anemone *Anthopleura xanthogrammica* (Brand). Robertson (1963), in a review of the literature on epitoniid feeding, noted that few wentletraps are found in association with coelenterates, and so modified Thorson's statement by suggesting that wentletraps may grade from permanent ectoparasites to foraging predators on coelenterates.

The available literature on wentletrap natural history consists of fragmentary accounts and brief reports on a variety of species. The present study, however, is a more detailed examination of the habitat and feeding behavior of a single species, *E. greenlandicum* (Perry). The data presented here contribute to a broader understanding of the ecology of wentletraps, an interesting group of gastropods.

METHODS

Field data on *E. greenlandicum* were obtained using SCUBA techniques in 10-25 m of water at Eastport, Maine (44°54'N, 66°59'W). A total of approximately 12 hours of daytime underwater observations was distributed throughout the year (every other month from May, 1973–May, 1974 with a single observation in May, 1976). One nighttime dive was made in May, 1974. During these dives, notes were taken on the behavior of *E. greenlandicum* and

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on the distributions and densities of several species of anemone. Anemone densities were determined from bottom transects and analysis of underwater photographs. Some specimens of *E. greendlanicum* were fixed in 10% formaldehyde solution immediately after collection and saved for subsequent gut content analysis. Live wentletraps and anemones were collected and maintained 1st in a recirculating sea water system at the University of New Hampshire, and later in a flowing system at the Marine Biological Laboratories at Woods Hole, Massachusetts.

Ten specimens of *E. greenlandicum* were kept in a sea table arranged to approximate their natural habitat. Mud and rocks from the Eastport study site were placed in the sea table along with several specimens of the anemone *Metridium senile* Linn. Each of the 10 wentletrap shells was numbered and daily records were kept of feeding frequencies, duration of feeding episodes, and general feeding behavior.

The feeding preferences of *E. greenlandicum* were experimentally determined by prey presentation trials similar to those described by Edmunds et al. (1974) for the nudibranch *Aeolidia papillosa* (Linn.). In these trials, wentletraps were placed 1 cm away from and facing single anemones. If the wentletrap everted its proboscis and attached its jaws to the anemone within 15 min, a positive feeding response was scored. A negative response was scored if no feeding took place within this time. The anemones presented to *E. greenlandicum* were *M. senile, Gonactinia prolifera* (Sars), *Stomphia coccinea* Mueller and *Tealia felina* Linn. Ten anemones of each species were presented, 1 at a time, to each of 6 wentletraps. During these experiments, responses of anemones to predation were noted.

The hypothesis that *E. greenlandicum* may locate prey through chemoreception was tested using 2 types of olfactometers. First, specimens of *E. greenlandicum* were tested using a 4-chambered olfactometer designed by Wood (1968). In this device, water from peripheral prey cells flows down ramps to a predator chamber, and exits through a central drain pipe. A second set of tests was run using a simple plexiglas Y-maze 30 cm in length. Water flow rates were adjusted to 250 ml/min in both the 4-chambered olfactometer and the Y-maze.

Wentletraps were run in groups of 8 in both sets of experiments. At the end of 8 hr, positive responses were scored for all wentletraps which had entered the stimulus arm of the Y-maze or the stimulus chamber of the 4-chambered olfactometer. Individual snails were removed from the olfactometer as they prepared to feed on the prey anemone. Stimulus and control chambers were randomly designated at the start of each trial. Only specimens of *M. senile* were used as prey.

Preliminary Y-maze trials were run without prey to determine whether *E. greenlandicum* schools or trails. Such behavior patterns would bias the results of olfactometer trials in which groups of predators were run simultaneously.

RESULTS

Field observations

The substrate at the Eastport, Maine study site consisted of exposed rocks and ledges surrounded by cobble encrusted with coralline algae in a fine mud matrix. Water temperatures ranged from 2.1°C in January to 12.5°C in August. Tidal currents were strong.

Metridium senile was the most obvious anemone at Eastport. On rocky ledges, these suspension-feeding anemones often exceeded 20 cm in height, and formed aggregations of up to 160 individuals/m^2. *Metridium senile* was rare (less than 0.1/m^2) on the less exposed cobble bottom.

In contrast to *M. senile,* the tiny (up to 6 mm in height) *Gonactinia prolifera* was common in the cobble-mud areas, but rare on exposed rock surfaces. *G. prolifera* prefers the undersides of rocks and dead mollusk shells where some space remains for water circulation. The distribution of this predatory anemone was extremely patchy and related to the availability of suitable habitats. Abundances ranged from 0 to 50 individuals/m^2.

Tealia felina and *Stromphia coccinea* were found with *M. senile* on the rock ledges, but also occurred on the cobble. Neither of these anemones forms aggregations, and their densities were roughly 0.2/m^2.

Thirty specimens of *E. greenlandicum* were collected during the study, and an

average of 5 snails could be located per hr of intense searching. There were no apparent seasonal variations in the abundance of *E. greenlandicum.* Twenty-eight specimens were found crawling on or among the cobbles and 2 were in the mud at the base of 1 large *M. senile.* Large anemones (*M. senile* and *T. felina*) were always carefully checked for associated wentletraps, but none were ever found with them on exposed rocks or ledges either during daylight or at night.

Three specimens of *E. greenlandicum* were observed feeding in the field. In each case, the anemone being attacked was a specimen of *G. prolifera* and the wentletrap swallowed the small anemone whole.

Wentletraps collected in the field generally had empty guts. Of 12 animals collected throughout the year and examined in the laboratory, only 1 (collected May, 1973) had ingested material in its gut. This material contained nematocysts and was presumed to be anemone tissue.

Laboratory observations and experiments

Locomotion

The locomotion of *E. greenlandicum* consists of 2 alternating movements. In the first, the shell remains stationary while the foot crawls forward and obtains a purchase in the substrate. In the second movement, the foot remains stationary while the columellar muscle contracts and pulls the shell forward. Miller (1974) showed that this "discontinuous" form of locomotion, common to many burrowing gastropods such as the Turritellidae and the Terebridae, is related to slow locomotory rates and to a reduced ability of the foot to adhere to hard surfaces. Using Miller's techniques, I measured the maximum adhesion (tenacity) of the foot of *E. greenlandicum* at less than 10 g/cm^2 foot area. In contrast, Miller (1974) measured tenacities of up to 2792 g/cm^2 foot area for epifaunal gastropods.

Prey preferences and anemone defensive reactions

In the laboratory, *E. greenlandicum* fed only on anemones [*M. senile, G. prolifera, S. coccinea, Bunodactis stella* (Verrill), *Actinauge verrilli* McMurrich and *Diadumene leucolena* (Verrill)], even though soft-bodied polychaetes, holothurians and tunicates were frequently available to them. The anemones *B. stella, A. verrilli* and *D. leucolena* were not found at the Eastport study site and therefore were not included in the prey preference experiments.

When feeding on large anemones, wentletraps always attached their jaws to the column or base of the anemone (Fig. 1). Anemone tentacles were never bitten off and swallowed individually as has been reported for *E. tinctum* (Carpenter) by Hochberg (1971). Small anemones (less than 1 cm in height) were simply grasped and swallowed whole. During feeding, anemone tissues could be seen slowly moving through the extended proboscis.

Although *E. greenlandicum* is capable of feeding on several species of anemone, repeated laboratory observations suggested a strong preference for *M. senile.* This suspected preference was confirmed by the results of prey presentation trials involving 4 species of anemone (Table 1). Out of 240 such trials, only 77 positive feeding responses were scored. *Metridium senile* accounted for 75% of the positive responses, *G. prolifera* was 2nd in preference, and both *T. felina* and *S. coccinea* were essentially ignored.

Metridium senile responded to wentletrap attacks by (1) bending away from the predator, (2) extruding nematocyst-laden acontia, and (3) moving slowly away at approximately 1 cm/hr. *Epitonium greenlandicum* was never deterred by these defensive reactions. Crawling at a mean rate of 45 cm/hr (N = 5), *E. greenlandicum* readily overtook its prey, and once its jaws were attached to the anemone the wentletrap was not easily dislodged.

While the head and foot of *E. greenlandicum* recoil violently from the sting of nematocysts, the outer surface of the proboscis appears less sensitive. When *M. senile* extruded acontia toward a feeding wentletrap, the operculum was withdrawn and the snail continued feeding with only the proboscis exposed to nematocysts.

The defensive reactions of *G. prolifera* consisted of (1) using its tentacles to sting the wentletrap on the head or foot, (2) "walking" away, or (3) swimming. Stinging was an effective defense only when the anemone's tentacles came into direct contact with the head or foot of the snail. Such stings caused wentletraps to recoil violently and lie inert for several min. During this period, *G. prolifera* would sometimes walk away from the wentletrap

FIG. 1. Two specimens of *Epitonium greenlandicum* attacking a large (height 8 cm) *Metridium senile.* One wentletrap has already attached its jaws to the anemone, while the other is approaching with its proboscis partially everted.

TABLE 1. The number of positive feeding responses scored for each speimen of *Epitonium greenlandicum* (A-F) when presented with different species of anemone.

Anemone	Wentletrap						Total
	A	B	C	D	E	F	
Metridium senile	10	10	8	10	9	10	57
Gonactinia prolifera	2	0	2	5	6	4	19
Stomphia coccinea	0	0	1	0	0	0	1
Tealia felina	0	0	0	0	0	0	0

in the manner described by Robson (1966, 1971). In only 2 out of 60 trials did swimming occur in response to contact with the wentletrap's proboscis. One anemone swam 5 cm from the wentletrap and successfully avoided predation, while the other was eaten in spite of its attempts at escape.

Tealia felina was never approached by wentletraps and showed no defensive reac-

tions to the presence of *E. greenlandicum.* The single *S. coccinea* which was attacked retracted its tentacles but did not swim.

Feeding frequency and duration

The 10 marked specimens of *E. greenlandicum* maintained in the laboratory sea table fed a combined total of 25 times during 6 months. Four specimens died after feeding only once. Table 2 gives the intervals between, and durations of, feeding episodes for the remaining specimens. Since the *M. senile* anemones in the sea table were all large, they could not be swallowed whole and usually survived several wentletrap attacks.

Each feeding episode lasted 4-10 hr (Table 2), after which the wentletrap burrowed in the mud at the base of the anemone on which it had just fed. Emerging from the mud 12-80 days later, the wentletrap usually attacked the same anemone.

Location of prey through chemotaxis

In the laboratory, wentletraps showed a tendency to gather about injured anemones, and alternately everted and retracted their proboscises when approaching specimens of *M. senile* (Fig. 1). Eversion of the proboscis was never observed in the absence of anemones. Such observations suggest that, at least in the laboratory, *E. greenlandicum* may be able to locate prey through chemosensory means, and the results of Y-maze olfactometer tests (Table 3) support this hypothesis. All responding animals showed a strong tendency to move toward the prey, and periodically everted their proboscises throughout the trials. In the control experiments, where no prey was placed in either arm of the Y-maze, nearly equal numbers of wentletraps moved to the right and left arms of the maze (Table 3). Wentletraps did not move in "schools," nor did they appear to follow each others' mucous trails as has been reported for *Littorina irrorata* by Hall (1973).

Seventy-three percent of the wentletraps entering the prey arm of the Y-maze attempted to feed during the 8-hr trials. These animals required 2.5-8 hr ($\bar{x}$= 4.9 hr) to locate the prey in the 30 cm long Y-maze.

Experiments using a 4-chambered olfactometer produced data which, when subjected to a χ^2-test, was random. In these trials, wentletraps tended to move about the central chamber everting their proboscises, but less than 5% of the animals attempted to climb the ramps leading to either prey or control chambers.

TABLE 2. Durations of, and intervals between, laboratory feeding episodes of *Epitonium greenlandicum.*

Wentletrap	Duration (hr)	Interval (days)
A	7,9	18
B	4,7	80
C	7,5,5,10,5,4	59,13,12,22,12
D	8,10,5	25,45
E	6,8,6,5	13,8,24
F	6,5,6,9	20,24,21

TABLE 3. Results of Y-maze prey and control trials, showing the numbers of *Epitonium greenlandicum* snails moving into the stimulus arm, the non-stimulus arm, and the number not responding during the 8-hour trials.

	N*	Stimulus arm	Non-stimulus arm	Not responding
Prey trials	88	68	7	13
Control trials	88	13**	16	59

*Wentletraps were run in groups of 8 in a total of 11 prey trials and 11 control trials.
**Since no prey was used in the control trials, both arms are non-stimulus arms.

DISCUSSION

Robertson (1963) stated that epitoniids grade from more or less permanent ectoparasites, through temporary ectoparasites, to foraging predators, and he predicted that foraging species would feed infrequently and show high flexibility in their choice of prey. The laboratory and field observations in the present study generally support these predictions, and also elucidate some of the ecological factors which may determine feeding strategies within the family Epitoniidae.

Function of chemoreception

In 1963, Robertson commented that the wentletrap *E. rupicola* (Kurtz) seemingly foraged at random and appeared unable to

locate anemones by chemosensory means. To my knowledge, the Y-maze data in the present study are the first reported evidence of chemotaxis in any epitoniid. However, simple Y-mazes are rare in nature, and it should also be remembered that in these experiments wentletraps required a mean of 4.9 hr to find anemones only 30 cm away. Clearly, the results of these Y-maze tests do not prove that foraging wentletraps can locate distant anemones in their natural habitat.

Wood (1968) designed the 4-chambered olfactometer to test rigorously the chemosensory abilities of the foraging neogastropod *Urosalpinx cinerea* (Say). The water currents in this olfactometer are swifter and more complex than would be encountered in a Y-maze, and the inability of *E. greenlandicum* to locate prey in this system may reflect the wentletrap's actual performance in the turbulent bottom currents at the Eastport study site.

The most important site of chemoreception in prosobranch gastropods is probably the osphradium (Kohn, 1961). In the carnivorous Neogastropoda, a relatively long siphon directs water currents onto a bipectinate osphradium, and many of these gastropods are known for their chemosensory prey-finding abilities (Kohn, 1961; Hathaway & Woodburn, 1961; Wood, 1968). *Epitonium,* a mesogastropod, has only a monopectinate osphradium, lacks an extended siphon, and therefore seems poorly adapted for locating distant prey through chemotaxis.

Chemoreception may, however, serve important functions for both ectoparasitic and foraging wentletraps. When a foraging *E. greenlandicum* senses a nearby anemone through chemoreception, the proboscis is everted and moved about, apparently at random. When the proboscis comes into contact with the anemone, random movement ceases and feeding begins. Therefore, although precise location of anemones may be through tactile stimulation of the everted proboscis, proboscis eversion itself is chemically stimulated. Everting the proboscis may also prevent the sensitive head and foot of the wentletrap from directly encountering the tentacles of a stinging anemone such as *G. prolifera.* Ectoparasitic wentletraps probably remain close enough to their prey so that only limited chemosensory abilities are necessary to prevent loss of contact with the host anemone.

Dietary flexibility

No epitoniid has ever been found to feed on other than coelenterate prey. Couthouy's (1838) observation that *Scalaria subulata* Couthouy [= *Epitonium greenlandicum*] feeds on beef is almost certainly in error since he saw no proboscis everted during "feeding." Fretter & Graham (1962) suggested that wentletraps may have an alternate method of feeding on an active animal such as an annelid or nemertine. Robertson (1963) considered this possibility unlikely, and I must concur in light of the fact that specimens of *E. greenlandicum* consistently rejected all non-coelenterate prey offered to them during the present study.

Although several species of wentletraps are known to associate with, and feed on, anemones (Thorson, 1957; Robertson, 1963, 1970), little is known about the specificity of these associations. *Epitonium greenlandicum* can feed on at least 6 species of anemone, while the ectoparasites *Opalia crenimarginata* (Dall), *E. tinctum, E. ulu* Pilsbry and *E. albidum* (Orb.) have as yet been reported only on 1 or 2 hosts.

Emlen (1968) stated that predators may be expected to specialize when food is abundant, and to feed more indiscriminately as food becomes scarce (see also Menge, 1972). One extension of this prediction is that foragers should show lower prey specificity than do parasites. In terms of relative prey specificity within the Epitoniidae, *E. greenlandicum* seems to show the characteristics of a foraging generalist. However, since only fragmentary data exist on the diets of most epitoniids, other wentletraps may be able to feed on more species of coelenterates than is presently known.

Wentletrap predation and anemone defenses

The behavioral aspects of the relationship between *E. greenlandicum* and its prey compare interestingly with published accounts of nudibranch-anemone associations.

Although the nudibranch *Aeolidia papillosa* feeds on both *T. felina* and *M. senile* in the northwestern Atlantic (Harris, personal communication), Waters (1973) and Edmunds et al. (1974) have demonstrated that *A. papillosa* shows a distinct preference for *T. felina* over *M. senile.* In contrast to *E. greenlandicum, A. papillosa*

is often effectively repelled by the acontia of *M. senile,* and Harris (1973) reports that this nudibranch may be fatally stung when enveloped in *M. senile* acontia.

In the laboratory, *E. greenlandicum* strongly preferred *M. senile* over any of the other anemones tested. *Epitonium greenlandicum* is protected from acontia by its shell and operculum, and Clench & Turner (1952) report that the esophagus of epitoniids is lined with cuticle. However, the outer surface of the proboscis does not appear to be cuticularized, and the nature of its seeming immunity to nematocysts remains to be studied.

Robson (1971) reported that the anemone *G. prolifera* swims in response to chemicals secreted by *A. papillosa, Coryphella rufibranchialis* (Johnston) and two other unidentified nudibranchs. Although Robson found no direct evidence that these nudibranchs feed on *G. prolifera,* the swimming response is probably an escape reaction to a predatory nudibranch. Conversely, *E. greenlandicum* rarely elicits a swimming response from *G. prolifera* even when physically disturbing the anemone.

Stomphia coccinea swims to escape predation by *A. papillosa* and certain starfish predators (Yentsch & Pierce, 1955; Robson, 1961). In the present study, however, swimming was not observed in *S. coccinea,* and the results of prey presentation experiments suggest that *E. greenlandicum* rarely, if ever, feeds on this anemone in nature.

Finally, *T. felina,* although consistently ignored by *E. greenlandicum* in the present study, ranked 4th out of 11 anemones in the prey preference hierarchy of *A. papillosa,* and showed distinct defensive behavior in response to this nudibranch (Edmunds et al., 1974, 1976).

These descriptions of anemone defensive reactions and the more extensive discussion of the subject in Edmunds et al. (1976) suggest that most of these behavior patterns are adaptations primarily directed against predatory nudibranchs rather than wentletraps. Nudibranchs are relatively fast [500 cm/hr for *A. papillosa* (Edmunds et al., 1976)] voracious predators, and Harris (1973) reports that predation by *A. papillosa* has a marked effect on the densities and age structures of *M. senile* populations. Wentletrap attacks, however, rarely result in the deaths of any but the smallest anemones, and even when small anemones such as *G. prolifera* are involved, wentletraps move so slowly that they probably account for only a small fraction of anemone mortalities.

Habitat and foraging behavior

The laboratory data on *E. greenlandicum* prey preferences suggest that this wentletrap is a facultative ectoparasite on the anemone *M. senile.* Although *E. greenlandicum* may feed heavily on *M. senile* in some areas (perhaps in deep water), field observations at Eastport, Maine reveal that in shallow subtidal places, wentletraps probably spend most of their time foraging for small anemones (*G. prolifera*) in a habitat where *M. senile* is rare.

The absence of wentletraps from the groups of *M. senile* anemones on exposed rocky ledges is the result of a combination of factors. *Epitonium greenlandicum* is essentially an infaunal animal and its type of locomotion is common to many burrowing gastropods. The low adhesion values obtained for the foot of *E. greenlandicum* in the present study show that this gastropod is poorly adapted for clinging to exposed rocks in strong currents.

At a mean speed of 45 cm/hr, *E. greenlandicum* requires several hr to cross a few meters. In order to prey on the ledge-dwelling anemones at Eastport, wentletraps would have to cross wide expanses of bare rock on which they could be easily perceived by visual predators such as fish or crabs. Fish are known to feed on wentletraps (Clapp, 1912; Wigley, 1956), and Homans & Needler (1944) found "large quantities" of *E. greenlandicum* in the guts of haddock caught off Nova Scotia. Also, since there is no mud on the rocky ledges, satiated wentletraps would not be able to conceal themselves by burrowing after each feeding episode.

Thus restricted to the cobble-mud habitat, *E. greenlandicum* must engage in a mixed strategy of foraging and temporary ectoparasitism. Wentletraps which encounter large anemones on the proper substrate probably remain associated with them for some time. The 2 specimens of *E. greenlandicum* found burrowed near 1 large *M. senile* are evidence that such associations exist in nature as well as in the laboratory. However, the sparse distribution of *M. senile* individuals on the cobble-mud bottom, the empty guts of nearly all the snails examined, and the fact that 28 of the 30

wentletraps observed in the field were not near large anemones, indicates that *E. greenlandicum* is primarily a forager, at least at the Eastport study site.

Epifaunally foraging wentletraps must remain exposed to visual predators during each foraging period, and although *E. greenlandicum* can subsist infaunally for up to 80 days after being satiated on a large anemone (Table 2), intervals between feeding episodes may be considerably shorter when only small anemones are available. At least in terms of predator avoidance, ectoparasitsm would seem to be a more nearly optimal feeding strategy than is foraging.

CONCLUSIONS

It may now be possible to identify ecological factors which determine whether a given epitoniid will function as an ectoparasite or as a forager. All known ectoparasitic wentletraps occur where there is both a stable supply of the host coelenterate and a nearby refuge from visual predators. On the west coast of the United States, the epitoniid *Opalia crenimarginata* feeds on the large anemone *Anthopleura xanthogrammica* and hides itself in the shell-gravel at the anemone's base (Thorson, 1957). *Epitonium tinctum* is also reported to "live in close association with sea anemones in sand pockets and sand-filled crevices . . ." (Strong, 1941). In the Caribbean, Robertson (1963 and personal communication) reports that *E. albidum* burrows in sand pockets at the base of *Stoichactis helianthus* (Ellis). Off the Hawaiian Islands, *E. ulu* is a temporary parasite on the coral *Fungia scutaria* Lamarck. According to Bosch (1965), this wentletrap avoids direct light and feeds on the underside of the coral. Similar behavior is reported for *E. costulatum* (Kiener) by Root (1958), as quoted in Robertson (1963). This Philippine wentletrap was always found on the underside of the coral *Fungia* or in the sand at the base of the coral.

In the laboratory, specimens of *E. greenlandicum* behaved like temporary ectoparasites, remaining associated with the same anemone for long periods. These wentletraps had a relatively concentrated and stable supply of large anemones available to them and could easily burrow in the mud between feeding episodes. At the Eastport, Maine study site, however, groups of large anemones occurred only on hard substrates. Habitat restraints, especially the lack of hiding places near these anemones, prevented the wentletraps from being ectoparasitic.

The case of *E. greenlandicum* suggests that epitoniids forage only when the necessary prerequisites for ectoparasitism [(1) a stable food supply, and (2) a convenient refuge from predators] do not exist. All wentletraps may, as Thorson (1957) originally predicted, be facultative ectoparasites.

ACKNOWLEDGEMENTS

I thank Larry Harris and Ruth Turner for their support of this research. I also thank Brian Rivest and Alan Hulburt for diving assistance. Special thanks for technical assistance are also due to Irene Thompson.

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RÉSUMÉ

L'HABITAT ET LE COMPORTEMENT PRÉDATEUR D'*EPITONIUM GREENLANDICUM*

Frank Perron

Les epitoniidés sont des mollusques mésogastéropodes prosobranches qui fouillent pour trouver et/ou qui vivent en parasite chez une variété de coelentérés benthiques. *Epitonium greenlandicum* (Perry) est de distribution circumboréale et se présente au-dessous du niveau de la marée basse dans l'Atlantique du Nord.

Des observations au laboratoire et sur place indiquent que ces gastéropodes ne se nourrissent que rarement, et seulement d'anémones. Une fois nourri, *E. greenlandicum* se terre dans de la boue molle et peut y rester inactif pendant une période de jusqu'à 80 jours. Les epitoniidés ont la capacité, du moins dans de petites distances, de repérer les anémones par chémotaxis.

Quoque *E. greenlandicum* puisse se nourrir d'au moins 6 espèces d'anemone, il témoigne d'une préférence pour *Metridium senile* et tend à vivre en parasite ches cette grande anémone sous des conditions de laboratoire. Dans les eaux peu profondes de la Baie de Fundy, *Metridium senile* se trouve sur des rochers et des corniches déchaussés, et n'est pas accessible aux epitoniidés qui sont principalement infaunales. Par conséquent, *E. greenlandicum* doit fouiller pour trouver de petites anémones sur un vasard caillouté.

On suggère que les epitoniidés ne peuvent se nicher d'une manière purement ectoparasitaire que dans des régions où il y a à la fois une provision stable des coelentérés hôtes et à proximité un refuge des prédateurs.

J. D. C.

АБСТРАКТ

МЕСТО ОБИТАНИЯ И ПОВЕДЕНИЕ ПРИ ПИТАНИИ EPITONIUM GREENLANDICUM

Франк Е. Перрон

Epitoniidae (Mesogastropoda:Prosobranchia) - это моллюски, питающиеся различными морскими анемонами. Epitonium greenlandicum (Перри) по распределению циркумбореальный и встречается в северо-западной части Атлантического океана.

Лабораторные и полевые наблюдения указывают на то, что этот моллюск питается редко и только анемонами. После питания E. greenlandicum погружается в мягкую тину, где он может оставаться неподвижным вплоть до 80-ти дней. E. greenlandicum обладает способностью находить анемоны при помощи химического ощущения по крайней мере в пределах короткого расстояния.

Несмотря на то что E. greenlandicum может питаться по крайней мере шестью видами анемон, он предпочитает Metridium senile и часто паразитирует эту крупную анемону в лабораторных условиях. В мелких водах залива Фунди M. senile, встречающийся на обнаженных скалах и выступах, недоступен E. greenlandicum, главным образом обитающему в тине. Следовательно Epitonium greenlandicum вынужден охотиться на мелких анемон на дне, покрытом тиной.

Можно заключить, что Epitoniidae обладают способностью занимать чисто паразитическую нишу только там, где существует постоянный запас анемон и где имеется ближележащее убежище от зрячих хищников.

I.T.

MALACOLOGIA, 1978, 17(1): 73-97

SEASONAL REPRODUCTIVE PATTERNS IN 3 VIVIPARID GASTROPODS

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ABSTRACT

Seasonal reproductive cycles in *Campeloma geniculum* (Conrad), *Lioplax pilsbryi* Walker and *Viviparus georgianus* (Lea), based on 12 consecutive monthly collections, are described in terms of activity levels of reproductive organs and brood characteristics.

Gametogenic activity and occurrences of mature gametes showed seasonal patterns in each species as did secretory activity of accessory reproductive organs. In *C. geniculum* commencement of fertilization, as judged by the appearance of the smallest young in the pallial oviduct, followed maximum spermatogenic activity, maximum abundance of eupyrene sperm in the testis and eggs in the ovary, and the highest level of secretory activity in the albumen gland. Similar relationships between reproductive organ activity and brood production were seen in *L. pilsbryi* and *V. georgianus* (but not as clearly due to a greater degree of individual variation in these 2 species).

Although reproduction by individuals was similar, the relative degree of synchronization of the individuals sometimes provided for different patterns in the populations. Animals of *C. geniculum* showed the least amount of individual variation and greatest synchronization, with fertilization and birth periods occurring during comparatively short periods of time in the population. In contrast, animals of *L. pilsbryi* and especially *V. georgianus* were unsynchronized, with the population exhibiting fertilization and birth at any time during the year. As a consequence, individual and population patterns were similar in *C. geniculum* but different in *L. pilsbryi* and *V. georgianus*.

Greater synchronization in *C. geniculum* most clearly allowed observation of seasonal brood development. Initial fertilizations provided a brood of small-sized young, and continued fertilizations added new members to the brood; as a result of the latter, the brood was not only larger but consisted of individuals in a size series. Subsequent birth of the larger members of the brood, generated from the earlier fertilizations, reduced the brood size; the remaining young in the brood increased in size prior to their birth. A few individuals contained both large and small young near the end of the birth period, indicating that more than 1 breeding cycle occurs in the life span. This pattern was also shown by individuals of *L. pilsbryi* and *V. georgianus*, but its appearance in their populations was obscured by lack of synchrony and smaller brood sizes.

Specific details on fertilization, incubation and birth periods, individual reproductive habit and population pattern, number of young per brood and size of newborn are tabulated and compared to prior reports on congeners.

Brood production is also shown in terms of comparative size relationships. *Viviparus georgianus* has the largest gravid adults (average diameter: 26.9 mm) and the largest newborn (⩾ 7.5 mm diameter) from broods averaging 11.2 young per female. *Lioplax pilsbryi* has the smallest adults (average diameter: 15.7 mm) and moderate-sized newborn (⩾ 4.5 mm diameter) from the smallest broods (average: 9.0 young per female). The intermediate-sized *C. geniculum* snails (average diameter: 18.2 mm) contained the smallest newborn (⩾ 3.5 mm diameter) from the largest broods (27.7 young per female). The average number of young per female recorded here is the largest observed for these 3 species in this study; in each case the largest broods were comprised of young in a size series.

INTRODUCTION

Various aspects of reproduction among viviparid gastropods have received prior attention, but the findings have not yet provided a complete understanding of the process and events. Although many descriptive accounts of the structural reproductive system exist for these snails (cf. Vail, 1977), specific functions of the reproductive organs have not yet been defined in detail. Moreover, virtually nothing is known of seasonal activity cycles of the individual organs in relation to each other in each sex and to the ultimate production of young.

Except for the works of Popoff (1907), Mattox (1937) and Bottke (1972, 1973), oögenic phenomena have been relatively

neglected. However, viviparid spermatogenesis has received considerable attention. Emphasis on the latter has been placed upon the mechanism of production of typical (eupyrene) and atypical (oligopyrene) spermatozoa and morphology of mature sperm types (cf. Meves, 1903; Pollister, 1939; Pollister & Pollister, 1943; Hanson et al., 1952; Gall, 1961). Only Rossi (1968) and Barbato (1971) considered seasonal occurrences of the 2 types of sperm.

The functions and seasonal cycles of the accessory reproductive organs (viz., seminal vesicle and prostate gland in males; albumen gland and seminal receptacle in females) have not been investigated for viviparids. However, these organs have been investigated in some oviparous pulmonates and their seasonal activities and hormonal regulation described (cf. Duncan, 1958; Smith, 1966, 1967; Runham & Laryea, 1968; Goudsmit, 1973).

Numerous workers have published brief notes, observations which do not take into account seasonal changes, on size dimorphism between adult male and female viviparid shells (e.g., Wood-Mason, 1881), sex ratios (Wood-Mason, 1881; Hubricht, 1943), birth (Frömming, 1928; Crabb, 1929) and size of newborn (Baker, 1928). Other workers conducted more detailed, quantitative, seasonal population studies that provided information on fertilization periods, duration of incubation and life spans, as well as on the aforementioned features (Van Cleave & Lederer, 1932; Van Cleave & Chambers, 1935; Van Cleave & Altringer, 1937; Medcof, 1940; Chamberlain, 1958; Berry, 1974). Aspects of fecundity were considered by Annandale & Sewell (1921), Rohrbach (1937), Miroshnitshenko (1958), Stánczykowska (1960), Stánczykowska et al. (1971), Samochwalenko & Stánczykowska (1972) and Berry (1974). Only Frömming (1931, 1940, 1956) reported general natural history observations.

This report provides, for examples of each of 3 Nearctic viviparid genera, viz., *Campeloma geniculum* (Conrad), *Lioplax pilsbryi* Walker and *Viviparus georgianus* (Lea), a description of seasonal activity levels of gonads and accessory organs, analysis of seasonal brood chracteristics and an interpretation of the data showing how these viviparids successfully produce offspring. The study was conducted on only 1 population of each species, and intraspecific and congeneric variations are unknown. However, the results offer a base line to which future studies can be compared.

MATERIAL STUDIED

The animals of *Campeloma geniculum, Lioplax pilsbryi* and *Viviparus georgianus* were from the same Florida populations studied by Vail (1977), who previously noted deposition of voucher specimens in specific museums. The former 2 species occurred at the same site and the latter 1 at a 2nd.

METHODS

Attempts were made to obtain consecutive monthly collections of live animals over the span of an entire year: *C. geniculum* and *L. pilsbryi* snails from December, 1971 through November, 1972, and *V. georgianus* individuals from February, 1972 through January, 1973. However, high water levels occasionally prevented an adequate collection. April and July samples of *C. geniculum* and *L. pilsbryi* were unobtainable until 1974. Specimens of *L. pilsbryi* were not available in February of 3 consecutive years, and for that same reason it was necessary to treat a collection of *V. georgianus* snails made on June 30, 1972 as the July sample. Individuals were sexed in the field on the basis of asymmetric, right tentacles (the right one being the copulatory organ of males) and treated as detailed below.

Each monthly collection of each species contained up to 30 females which were immediately isolated in individual jars of water to assure accurate analysis of individual broods regardless of possible abortion of young prior to laboratory study. Collecting was purposefully biased in that females of large size (i.e., of more probable reproductive age) were selected for brood analysis. Thus not all females encountered were included. Nevertheless, on occasion (particularly during high waters) a higher proportion of smaller females was obtained in the sample, and many of these animals were too young to be gravid. These isolated females were conchologically measured, deshelled, fixed and preserved with any

aborted young in the individual jars. The females were later examined for incubating young. Shell measurements were obtained from all young incubated in 10 randomly chosen gravid females.

In addition, 5 females and 5 males per species per month were randomly chosen for histological examination of their gonads and accessory reproductive organs.

Narcotization was accomplished with Diabutal (= sodium pentobarbital), fixation with Lavdowsky's fluid (= AFA) and preservation with 70% ethyl alcohol. Histological preparations involved paraffin embedding, sectioning at 10 μm thickness, and staining with Harris' hematoxylin and alcoholic eosin.

Measurements of adult male and female shells were made with Vernier calipers, and of shells of incubating young with a calibrated ocular micrometer. Other workers have employed length to denote shell size, but, because of frequent apical decollation among adult specimens examined in this study, only diameter was used herein as an index of adult size and also, to be consistent, size of incubating young. For convenience, all incubating young (usually previously termed "embryos" regardless of developmental state) are hereafter designated as "F_1" snails and all post-birth individuals as "P_1" animals (or "adults") regardless of size or age. Thus, reference to F_1 size is to the dimension of the F_1 shell diameter; however, reference to brood size is to the total number of F_1's in the pallial oviduct.

References are made later in this report to different seasons in northern Florida as follows: December, January and February constitute winter; spring includes March, April and May; summer contains June, July and August; and September, October and November comprise fall.

SEASONAL ORGAN ACTIVITY

An arbitrary percentage value was assigned to the state or level of activity of the different reproductive organs in each of the 10 individuals in the monthly samples and an "average state" was computed for each month to evaluate the composite activity in the population: low activity = 0%, moderate activity = 50%, and high activity = 100%. The findings are described in this section and illustrated in Figs. 1-3.

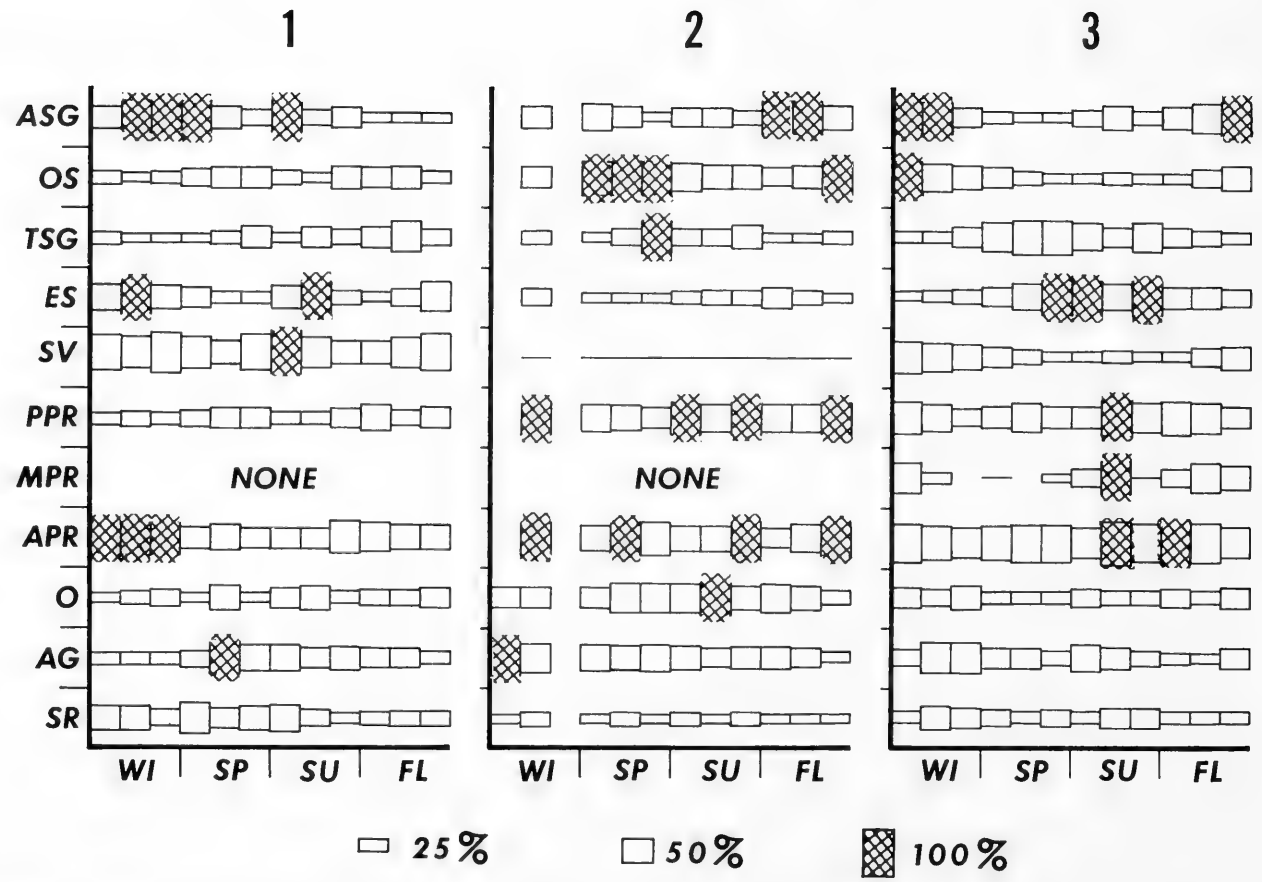
Stages of both typical and atypical spermatogenesis were identified according to Meves' (1903) descriptions. Levels of testicular activity were estimated on the basis of relative abundances of typical and atypical spermatogenesis and spermatozoa in histological sections. Possible sperm storage was judged by the presence or absence of sperm in the various male and female accessory organs, and, when present, by whether the gametes were free in the lumen or oriented with heads against cells lining the lumen.

Levels of glandular activity in the different accessory organs were evaluated in terms of the relative abundance of granular secretory products (cf. secretory phases of the albumen gland as described by Smith, 1966). The granular appearance of the secretory products may not be natural but an artifact resulting from reagents used for fixation and/or histological preparation (cf. Goudsmit & Ashwell, 1965).

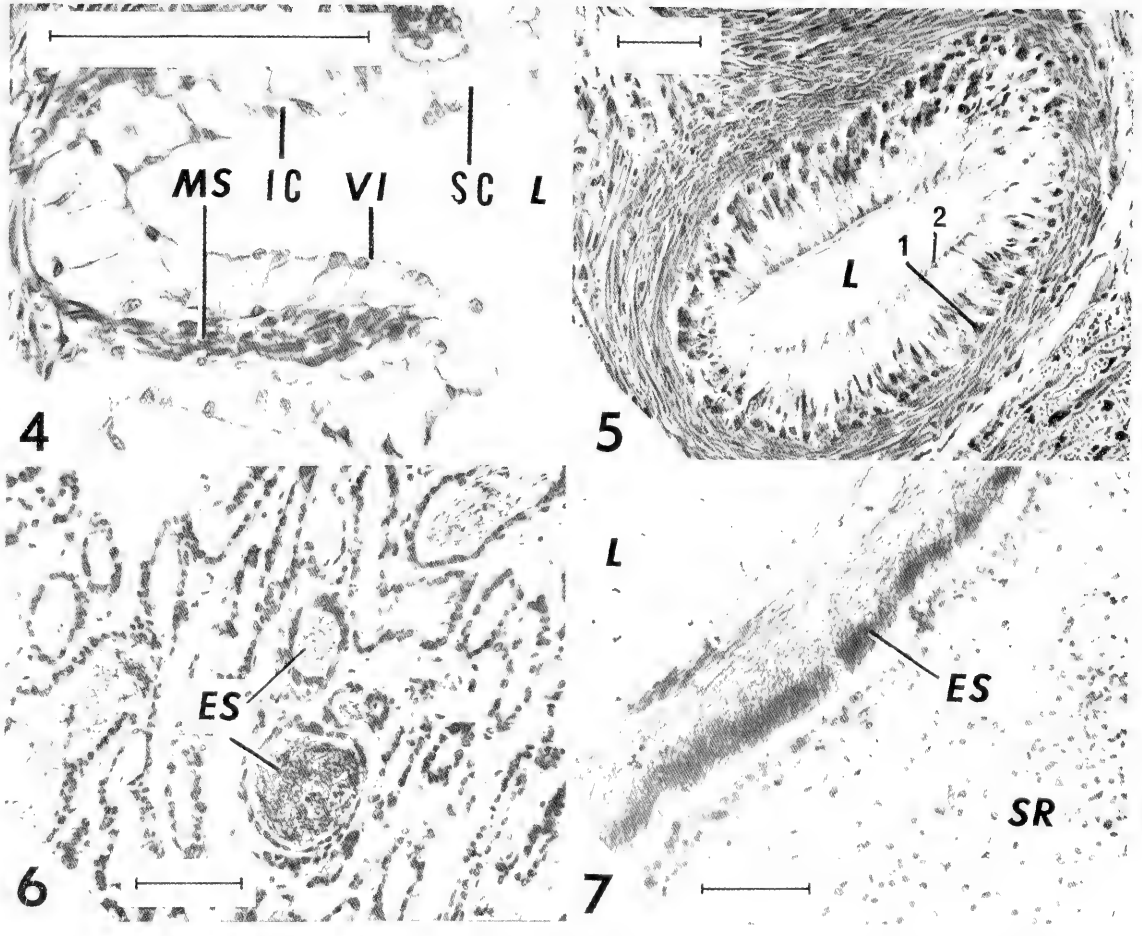
Campeloma geniculum

Males. *Testis* (Fig. 1). Levels of spermatogenic activity and abundance of spermatozoa varied seasonally in the population. Atypical spermatogenesis (ASG) occurred at highest levels during most of winter and early spring, and oligopyrene sperm (OS) were most abundant in mid- and late spring and late summer through mid-fall. Typical spermatogenesis (TSG) was most common from midsummer through fall, with a peak in mid-fall; eupyrene sperm (ES) were abundant during late fall and throughout winter, with a 2nd pulse in midsummer. They were at lowest level in mid-fall.

Seminal vesicle (Figs. 1, SV; 4). The numerous villi (VI) are comprised of secretory cells surrounding a central stalk of muscle fibers (MS) extending from the outer circular muscle layer, and small, triangular, ciliated interstitial cells (IC) that alternate with the tips of the non-ciliated, columnar secretory cells (SC) adjacent to the lumen. Secretory products of unknown constitution and function appeared as faintly outlined bodies and were neither acidophilic nor basophilic. They were present throughout the year, although peak abundance was observed in mid-fall through winter and again in late spring and early summer (Fig. 1). Vacuoles, usually an indication of relative glandular inactivity, were consistently present. No sperm, either free



FIGS. 1-3. Seasonal reproductive organ activities in *Campeloma geniculum* (1), *Lioplax pilsbryi* (2) and *Viviparus georgianus* (3). Seminal receptacle (SR) variations are based on occurrences of eupyrene sperm. In *L. pilsbryi* (2) the apparent lack of any seasonal secretory activity in the seminal vesicle (SV) is indicated by a solid line (see text). AG, albumen gland; APR, anterior region of prostate gland; ASG, atypical spermatogenesis; ES, eupyrene sperm; FL, fall season; MPR, middle region of prostate gland; O, ovary; OS, oligopyrene sperm; PPR, posterior region of prostate gland; SP, spring season; SR, seminal receptacle; SU, summer season; SV, seminal vesicle; TSG, typical spermatogenesis; WI, winter season.



FIGS. 4-7. Parasagittal sections of *Campeloma geniculum* seminal vesicle (4), proximal region of prostate gland (5), albumen gland containing eupyrene sperm (6), and seminal receptacle with oriented eupyrene sperm (7). 1, nucleus of secretory cell; 2, nucleus of interstitial cell; ES, eupyrene sperm; IC, interstitial cell; L, lumen; MS, muscle; SC, secretory cell; SR, seminal receptacle; VI, villus. All scales: 10 μm.

in the lumen or attached to villi, were seen in any of the 60 glands studied.

Prostate gland (Figs. 1, 5). Externally appearing to be a convoluted, undifferentiated duct, the prostate was found to be comprised of 2 histologically distinct regions: a basophilic, proximal part (Fig. 1, PPR) posterior to the tentacular base, and a strongly acidophilic part distal to that base (Fig. 1, APR). Common to both regions are large, unciliated, columnar secretory cells that extend from the walls to penetrate the cylindrical lumen, and small, triangular, ciliated interstitial cells alternating with the tips of these secretory cells adjacent to the lumen (Fig. 5). Activity levels in the 2 regions overlapped seasonally but showed different patterns. Granules in the proximal region were uncommon, although they were maximally abundant during early fall; the distal region displayed a longer period of maximum activity, viz., during the entire winter, although the granules were generally abundant at all times. No sperm were observed anywhere in the 60 prostates studied.

Females. *Ovary* (Fig. 1, 0). Oocytes were most abundant in mid-spring and again in midsummer, least numerous in early winter and late spring, but common at all other times. The largest oocytes were 38.6 ± 3.9 μm (N = 10) in diameter.

Albumen gland (Figs. 1, AG; 6). The secretory cells lining the central lumen of very numerous acini showed distinct seasonal variation in the abundance of strongly acidophilic granules. Little or no activity occurred from late fall through winter. Activity subsequently increased to a peak in mid-spring and then progressively declined in summer and fall.

Eupyrene (never oligopyrene) sperm (Fig. 6, ES) were found in the albumen gland of some specimens throughout the year. Inasmuch as they were always relatively few in number, their presence could not be confidently evaluated on a seasonal basis. However, most of the P_1's from late fall through early spring collections contained sperm in the acini, and most P_1's from midsummer through mid-fall did not. When present, the sperm were usually oriented in the acini lumens, with heads at least against (penetrating?) the apical ends of the secretory cells, giving the appearance of being stored.

Seminal receptacle (Figs. 1, SR; 7). The folds of the glandular lining of this organ include unciliated, columnar cells that contain small secretory granules that are only faintly acidophilic. These granules did not exhibit a seasonal occurrence, but there was a marked vacuolization among the cells from late summer through early winter. The presence of spermatozoa was highly seasonal. Most females contained large quantities of predominantly oligopyrene sperm free in the large lumen during the early winter and in the spring and early summer; also during the latter period, some (but relatively few) eupyrene sperm were oriented against the cells of the glandular folds (Fig. 7, ES). Few females contained sperm in the seminal receptacle during the rest of the summer and throughout the fall.

Pallial oviduct. Histological sections of this organ were not made from material in the monthly collections, but the glandular walls of this organ visibly appear thicker during the presence of younger incubating F_1's (in general, mid-spring to mid-fall) and become thinner as the F_1's advance in development (late fall through early spring).

Lioplax pilsbryi

Males. *Testis* (Fig. 2). The seasonal variation in levels of spermatogenic activity and spermatozoan abundance in this population differed slightly from that of *C. geniculum.* Atypical spermatogenesis (ASG) was most active during early and mid-fall; oligopyrene sperm (OS) displayed greatest abundance in spring and late fall. Typical spermatogenesis (TSG) was most active in late spring; eupyrene sperm (ES) were most abundant in early fall and least abundant in late fall.

Seminal vesicle (Fig. 2, SV). Secretory products were neither acidophilic nor basophilic, and large vacuoles comprised a major feature of the secretory cells. Vacuoles were abundant throughout the year, and no seasonality in secretory activity could be ascertained. Sperm were not observed in any of the 50 organs examined.

Prostate gland (Fig. 2). Externally appearing to be an undifferentiated duct, the prostate has a posterior, proximal region (PPR) that is less acidophilic than the distal region (APR). This differential staining may be the result of different amounts of a single secretion or of different secretions. In both regions nonciliated, tall-columnar secretory cells line

the cylindrical central lumen, and small, triangular, ciliated interstitial cells alternate with the tips of the secretory cells. Secretory granules were always abundant in both regions, and no significant seasonal variation in secretory activity was perceived in the population sample. Neither eupyrene nor oligopyrene sperm were found in any of the 50 glands examined.

Females. *Ovary* (Fig. 2, O). Although common during the rest of the year, oöcytes were most abundant in the population in mid-spring through midsummer and again in early fall in contrast to peaks of shorter duration in *C. geniculum.* The average diameter of the largest oocytes was 75.1 ± 3.5 μm (N = 10).

Albumen gland (Fig. 2, AG). Seasonal variation in the abundance of granules in the secretory cells contrasted to that observed in *C. geniculum.* In *L. pilsbryi* inactive acini were found in the population only in late fall, and maximum secretory activity occurred in early winter through spring.

Eupyrene (never oligopyrene) sperm were found each month in some P_1's in albumen glands of all activity levels. Although accurate estimation of abundance was impossible, it was noted that proportionately more females contained sperm there during mid- and late spring, midsummer and late fall. The sperm were located within the lumen of the acini, oriented with heads against (into?) the secretory cells. Sperm were also found in the albumen gland duct, and in both arms of the oviduct, in which they were unattached but with heads directed toward the albumen gland.

Seminal receptacle (Fig. 2, SR). Although small, faintly acidophilic granules were observed, no perceptible seasonal secretory activity pattern was found in the population. Also, neither presence nor absence of sperm was seasonal. Indeed, only 4 of 55 P_1's contained sperm (mostly eupyrene, but some oligopyrene), and of those only 2 had large numbers of these sperm.

Pallial oviduct. The observed seasonal changes in this *L. pilsbryi* population were similar to those of *C. geniculum.*

Viviparus georgianus

Males. *Testis* (Fig. 3). The seasonal variation of spermatogenic activity in this population contrasts with that observed in *C. geniculum* and *L. pilsbryi* as follows. In *V. georgianus,* atypical spermatogenesis (ASG) showed greatest activity in late fall through mid-winter, thereafter progressively decreasing to lowest levels in mid- and late spring, and then gradually increasing during the summer through mid-fall. An August decline is interpreted as reflecting inherent individual variation rather than an actual decrease in activity. Oligopyrene sperm (OS) were most abundant during early winter, only moderately abundant in mid-winter through early spring, and least abundant in late spring through early fall, after which time they began to increase in number. Typical spermatogenesis (TSG) was at highest levels during mid- and late spring and least active from late fall through mid-winter. Eupyrene sperm (ES) were most abundant from late spring through the summer, and least abundant in early winter.

Seminal vesicle (Fig. 3, SV). Neither ciliated interstitial cells nor spermatozoa were seen in any of the 60 organs examined. Secretory granules were most abundant in early winter, with only a slight decrease in relative numbers during mid- and late winter. Least secretory activity occurred from late spring through early fall. Vacuoles in the secretory cells became more abundant as secretory activity decreased.

Prostate gland (Fig. 3). Secretory activity and also a peculiar regional differentiation showed seasonal variation. Internal regionalization of this gland was not noticeable in late winter specimens, and individuals then showed variable, but generally low to moderate, secretory activity. Two distinct regions of the prostate were discernible in most early and mid-spring specimens: the posterior 2/3 (PPR) contained moderately acidophilic granules, whereas the anterior 1/3 (APR) contained only strongly acidophilic granules. At this time both regions displayed moderate to high levels of secretory activity. During maximum prostate secretory activity, in late spring through early winter, 3 regions were discernible: a posterior region (PPR) with large, coarse, moderately acidophilic granules; a middle part (perhaps corresponding to the red region in live animals; cf. Vail, 1977) (MPR) containing equally acidophilic but smaller, finer granules; and a strongly acidophilic anterior

section (APR) containing medium-sized granules. During the subsequent period of decreasing activity, only 2 regions were observed, viz. a moderately acidophilic posterior 2/3 and a strongly acidophilic anterior 1/3. Regional activity levels were almost always synchronous. Fig. 3 shows seasonal secretory activity patterns of the prostate regions for the population, but does not reveal individual variation within a month. Neither eupyrene nor oligopyrene sperm were found in any of the 60 organs studied.

Females. *Ovary* (Fig. 3, O). In contrast to those of *C. geniculum* and *L. pilsbryi,* oöcytes in *V. georgianus* were in greatest abundance in late winter, only slightly less abundant in late fall and, although still common throughout the rest of the year, showed a slight decrease in numbers in the spring. The diameter of the largest oocytes was 51.8 ± 4.8 μm (N = 10).

Albumen gland (Fig. 3, AG). Maximum secretory activity in the population occurred in mid- and late winter, with a 2nd minor peak in early summer. Least activity occurred in late summer through mid-fall. Unlike the occurrences in *C. geniculum* and *L. pilsbryi,* none of the 60 albumen glands examined of *V. georgianus* snails contained either eupyrene or oligopyrene sperm. That observation may be erroneous, however, because the mature glands undergoing greater secretory activity sectioned poorly, with the tissue usually shredding. This problem was less severe in *C. geniculum* and *L. pilsbryi.*

Seminal receptacle (Fig. 3, SR). Ciliated, columnar secretory cells, surrounding muscle fibers in the villi that project into the lumen of this organ, showed no seasonal variations in activity levels; secretory granules were common throughout the year. At least some P_1's from each month contained both eupyrene and oligopyrene sperm in the seminal receptacle; most P_1's contained these sperm in mid- and late winter and mid- and late summer; fewest P_1's contained them in early summer and fall. Both oligopyrene and eupyrene sperm were found in the lumen, whereas only the latter occurred attached to the walls.

Pallial oviduct. Monthly sections of the posterior portion of this organ were included in histological preparations of the albumen gland and seminal receptacle, but quantitative histological evaluation of the whole organ was not attempted. Histological sections have provided some qualitative information about the posterior, proximal end. Ciliated, tall-columnar secretory cells lining the lumen centrally contained many vacuoles; some of the vacuoles contained a subelliptical secretory body, whereas others contained what appeared to be degenerating sperm (both types?). Gross dissections provided the visual impression that the walls varied in thickness seasonally in a manner similar to that in *C. geniculum* and *L. pilsbryi.*

BROOD PRODUCTION

Information presented in this section was obtained from monthly size-frequency histograms of F_1's, seasonal frequencies of the different stages of brood development and both seasonal and non-seasonal analyses of the P_1's.

Different numbers of gravid P_1's and of F_1's occurred from month to month, and therefore total numbers in each sample were converted to percentages; the latter serve as "frequency" in the histograms (Figs. 8, 15, 16) and in the figures (9-11) denoting the seasonal distribution of broods in different developmental stages.

The constitution of individual broods varied both within a single month as well as seasonally. Some broods contained either only small F_1's or only large F_1's, others contained a size series of smaller to larger F_1's and still others contained F_1's of 2 different broods, each from a different fertilization as evidenced by different size classes. As shown later, these different conditions are seasonal stages in brood development.

Campeloma geniculum

Fertilization, incubation and birth periods, and brood (F_1) characteristics were highly seasonal. Monthly size-frequency histograms of the F_1 population (Fig. 8) indicated that fertilizations, interpreted according to the presence of smallest, youngest F_1's in the pallial oviducts, began about mid-spring and, although decreasing in frequency after midsummer, continued to early fall. The histograms suggested that (1) incubation occurred over the winter, and birth, judged by the gradual disappearance of the largest,

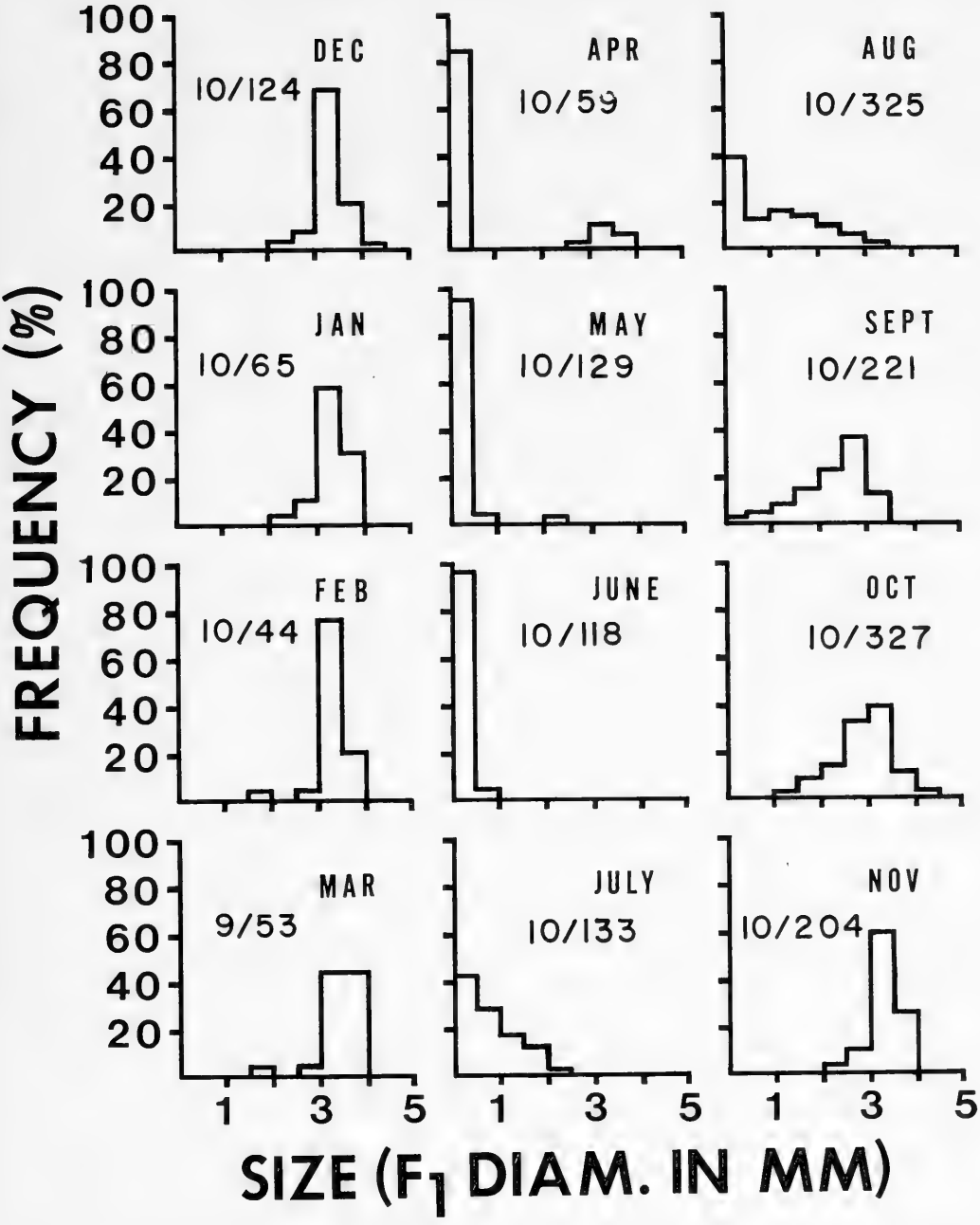
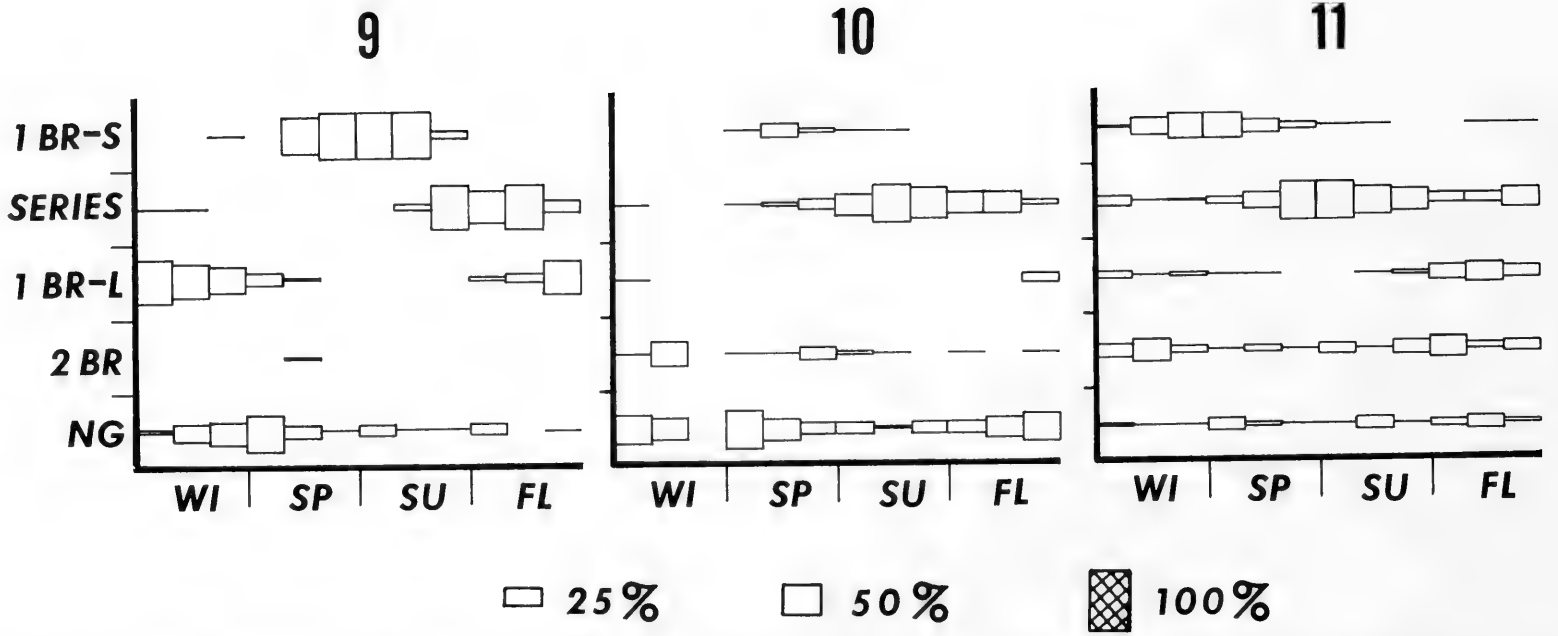


FIG. 8. Monthly size-frequency histogram of incubating *Campeloma geniculum* snails. Diam., diameter; F_1, young developing within the pallial oviduct. Frequency: % of sample; fraction numerator: number of P_1's examined; denominator: number of F_1's examined.



FIGS. 9-11. Seasonal frequencies (% of sample) of different stages of brood development in *Campeloma geniculum* (9), *Lioplax pilsbryi* (10) and *Viviparus georgianus* (11). Ordinate: stages of brood development. Abscissa: seasons. FL, fall; NG, non-gravid female; SERIES, single brood in a size series; SP, spring; SU, summer; WI, winter; 1 Br-L, only large young in a single brood; 1 BR-S, only small young in a single brood; 2 BR, simultaneous occurrence of 2 broods in 1 female.

oldest F_1's from the pallial oviducts, occurred in the spring; (2) only 1 brood, with an incubation period of perhaps as long as 12 months, was generated in a year; (3) birth of that brood was not completed before a 2nd fertilization period provided for a subsequent brood, i.e. there was no interim period of non-gravidity among the P_1's. Seasonal analyses of the nature of the broods and also of brood sizes provided a more detailed, modified interpretation of some of these initial, provisional conclusions.

Most females with only small F_1's (arbitrarily, diameter < 1.0 mm) occurred in mid-spring through midsummer, those with F_1's in a size series from mainly late summer through mid-fall (0.5-1.5 mm in August, 1.0-2.5 mm in September, and 2.0-3.5 mm in October) and those with just large F_1's (> 3.0 mm) principally in late fall throughout the winter (Fig. 9). Also, only 9 of the 30 P_1's (i.e., 30.0%) in the March collection, all of reproductive size, were gravid, and just 2 (1 each in April and May) of the total of 279 gravid females simultaneously contained 2 broods, 1 with small F_1's and 1 with large F_1's.

Following early development of an initial number of F_1's in the spring and early summer, continued fertilizations during the rest of the summer and early fall added new members to the brood, thus providing for a single brood in a size series during the latter period. The largest F_1's in size-series broods, generated by fertilization in mid-spring to early summer, were born during the winter after an incubation period of 8-10 months. The smallest F_1's in size-series broods, generated by fertilization in midsummer to early fall, matured to become the late fall and winter broods of only large F_1's and were born in the spring after an incubation period of 8-10 months. Comparison of seasonal brood sizes supports these conclusions.

Beginning with the appearance of small F_1's in the pallial oviduct (cf. April, Fig. 8), the mean brood size (i.e. the mean number of young per brood) in P_1's with only small F_1's gradually increased. The mean brood size increased markedly when F_1's in a size series predominated (cf. Fig. 12). In late fall, when broods of large F_1's began to appear, the mean brood size decreased gradually as births commenced, reaching a low in the late winter and spring as births were concluded.

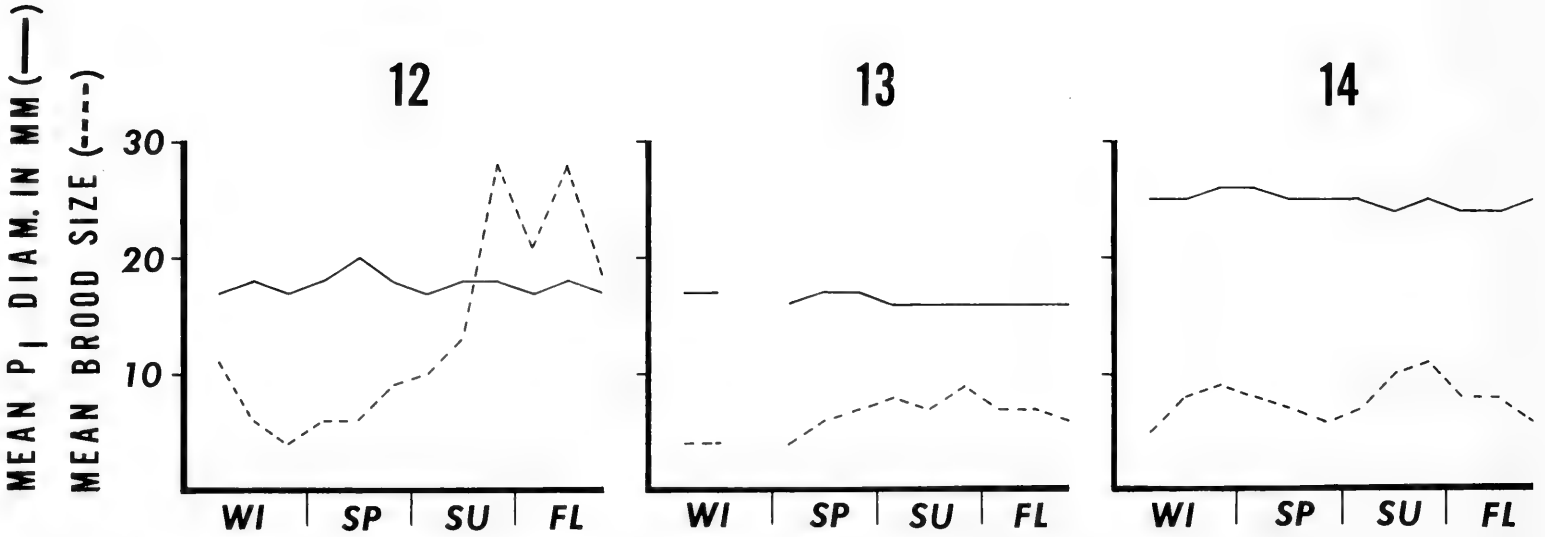
Although the gravid P_1's examined throughout the study were of similar mean diameter, the mean brood size varied in a seasonal pattern, increasing from a low in February (large F_1's) to highs in August and October (F_1's in size series) and then decreasing thereafter (Fig. 12, Table 1). The unexpected lower mean brood size in September is attributed to the F_1's (size series) occurring in smaller P_1's than did larger broods immediately before and after. In contrast to that seasonal independence of mean brood size in similar-sized P_1's, analysis of the August P_1 collection (month of the maximum mean brood size) showed that mean brood size correlated with the size of the P_1's ($r_p = 0.742$, $P < 0.01$). In Table 2, composite data from all monthly collections show brood sizes in relation to P_1 size classes.

The smallest gravid P_1 was 13.2 mm in diameter, and just 7 of 21 P_1's < 14.0 mm were gravid. The F_1's in those 7 P_1's were probably generated by fertilization in the prior spring to summer. The P_1's came from fall (F_1's in size series) and early winter (only large F_1's) collections and had brood sizes of 2-7. The P_1's themselves may have been born as recently as the previous winter. Unfortunately, possibly gravid P_1's were not saved in the other collections because enough larger females were present. Judged by sizes of largest F_1's in the pallial oviduct (cf. Fig. 8), newborn were about $\geqslant 3.5$ mm in diameter.

Lioplax pilsbryi

Fertilization, incubation and birth periods, as well as brood (F_1) characteristics, were again of seasonal nature. Monthly size-frequency histograms of the F_1 population (Fig. 15) indicated that (1) fertilization took place throughout the year, with highest frequency in the spring, decreasing to lowest values in late fall and then increasing during winter; (2) births began in spring and lasted until midsummer; (3) only 1 brood was produced in a year, after an incubation period of up to 12 months; (4) fertilization of a later brood began before birth of the previous brood was completed. Clarification of these interpretations is provided by analyses of seasonal ontogenetic brood stages and of brood sizes.

Broods comprised solely of small F_1's (diameter < 1.0 mm), in 6.1% of all gravid



FIGS. 12-14. Seasonal relationship between mean brood size and mean size of gravid P_1 females in *Campeloma geniculum* (12; also see Table 1), *L. pilsbryi* (13) and *V. georgianus* (14). FL, fall; P_1, adult individual; SP, spring; SU, summer; WI, winter.

TABLE 1. Monthly sizes of gravid *Campeloma geniculum* P_1's and their broods. (Also see Fig. 12.)

	Month											
	D	J	F	M	A	M	J	J	A	S	O	N
P_1 diam. (mm)												
$\bar{x}$	16.8	18.1	17.1	17.8	19.6	17.6	16.7	17.8	18.0	16.9	17.7	17.0
S.D.	2.0	1.8	4.2	1.3	1.4	2.1	3.4	1.2	1.6	1.2	1.5	1.8
Range	13.2-21.0	14.8-20.9	14.7-20.9	15.2-19.5	17.6-22.3	15.0-25.4	15.0-21.0	16.2-20.0	14.1-20.5	13.5-19.3	14.9-19.3	13.8-20.9
No. P_1's	27	21	19	9	10	27	26	29	29	24	30	28
Brood size (mean number of young per brood)												
$\bar{x}$	10.7	6.1	4.1	5.9	5.9	9.2	9.7	13.3	27.7	20.8	27.2	19.2
S.D.	8.8	4.5	3.1	4.9	3.9	7.0	5.7	7.7	11.9	8.9	11.1	9.1
Range	2-37	1-20	1-11	1-14	1-14	1-37	3-22	1-31	8-55	6-43	10-51	3-38
Total F_1's	200	128	78	53	59	248	252	385	804	498	817	538

P_1's, occurred only in spring through mid-summer, and then only in low frequencies (Fig. 10). The F_1's in a size series (1.5-4.4 mm in winter, $<$ 0.5-4.2 mm in spring, $<$ 0.5-4.8 mm in summer and 1.4-4.9 mm in fall), were observed in 43.3% of all gravid P_1's, and occurred in all months except January, but were most frequent from early summer through mid-fall. Broods of only large F_1's ($>$ 3.0 mm) were rare, in just 3.2% of all gravid P_1's, and occurred only in mid-fall through early winter. The P_1's simultaneously incubating members of 2 different broods were uncommon (9.7% of all gravid P_1's) and of wide temporal occurrence, but showed greatest frequency in mid-winter through spring. Non-gravid P_1's (13.3-63.2% of the monthly collections, and 48.6% of the total number of females) were most frequent in mid-fall through mid-winter, but these were often too small (young) to be reproductively mature. Continued development of the 1st F_1's formed plus subsequent fertilization occurring at intervals caused broods in a size series. Broods of only large F_1's were composed of the formerly younger F_1's of the size-series condition remaining after the birth of the older F_1's. Broods with 2 size groups of F_1's occurred in P_1's that underwent fertilization to begin a new brood before the large F_1's of the existing brood (i.e. the youngest F_1's of the size-series condition) were born. On the basis of seasonal frequencies of P_1's with 1 brood of just large F_1's, and of those with 2 broods, it is concluded that birth occurred in the winter after an incubation period of 10-12 months; this interpretation is supported by a seasonal comparison of brood sizes (see below). According to monthly size-frequency histograms (cf. Fig. 15), births continued into spring after an incubation period of like duration.

The mean brood size was always comparatively small, but still varied seasonally in similar-sized P_1's (cf. Fig. 13). Smallest broods occurred during the winter and early spring, after which the brood size increased to a peak in late summer before decreasing during fall. Analysis of different-sized P_1's in the August sample (which had the largest brood sizes) showed there was no significant increase in brood size with the increase in the size of P_1's (r_p = 0.244, $P > 0.05$). In Table 2 a composite of data from all monthly collections show mean brood size in relation to P_1 size.

The smallest gravid P_1 was 13.8 mm in diameter, and only 4 of 69 P_1's $<$ 15.0 mm were gravid. Such relatively small P_1's occurred in each monthly collection, whereas those 4 gravid P_1's were found only in midsummer into early fall. The F_1's in the 4 gravid P_1's, probably generated by fertilization in the prior spring, constituted brood sizes of 3-10, all in a size series; the P_1's themselves may have been born a year before (i.e., in the preceding winter or spring), and were nearly full-sized P_1's. Judged by sizes of largest F_1's (Fig. 15), newborn were about $\geqslant$ 4.5 mm in diameter.

Viviparus georgianus

The majority of size-frequency histograms of the F_1 population (Fig. 16) dis-

TABLE 2. Composite data, combined from all monthly samples, showing average number of F_1's in *Campeloma geniculum, Lioplax pilsbryi* and *Viviparus georgianus* in relation to size of gravid P_1's.

P_1 diameter (mm)	*C. geniculum*		*L. pilsbryi*		*V. georgianus*	
	No. P_1's examined	No. F_1's $\bar{x}$±S.D. (range)	No. P_1's examined	No. F_1's $\bar{x}$±S.D. (range)	No. P_1's examined	No. F_1's $\bar{x}$±S.D. (range)
30.1-31.0					2	12.5 ±0.7 (12-13)
29.1-30.0					9	11.33±4.35(6-18)
28.1-29.0					16	12.43±7.18(1-23)
27.1-28.0					20	10.35±6.21(1-23)
26.1-27.0					37	9.56±6.16(1-25)
25.1-26.0	1	37.0± 0.0(-37-)			65	7.40±5.04(1-20)
24.1-25.0					73	7.40±4.80(1-24)
23.1-24.0	0	- - - - - - - -			58	7.10±4.24(1-17)
22.1-23.0	1	1.0±0.0(-1-)			28	5.25±3.54(1-14)
21.1-22.0	0	- - - - - - - -			10	3.81±1.83(1-6)
20.1-21.0	20	22.2±18.9(1-55)			0	- - - - - - - - -
19.1-20.0	31	21.3±12.1(1-38)			0	- - - - - - - - -
18.1-19.0	56	16.0±12.0(1-50)	5	6.4 ±3.17(4-13)	0	- - - - - - - - -
17.1-18.0	60	16.0±12.0(1-45)	27	6.24±2.71(2-13)	0	- - - - - - - - -
16.1-17.0	57	13.5± 8.7(1-36)	74	6.48±2.75(2-13)	0	- - - - - - - - -
15.1-16.0	34	8.0± 4.7(1-17)	44	6.57±2.82(2-12)	1	15.0±0.0(-15-)
14.1-15.0	12	7.2± 4.0(1-14)	3	8.0 ±2.60(5-10)	1	5.0±0.0(-5-)
13.1-14.0	7	3.9± 2.1(2-7)	1	3.0 ±0.0 (-3-)	0	- - - - - - - - -

played a bimodal distribution that perhaps initially suggests the production of 2 broods in a year, with P_1's typically becoming gravid with a new brood prior to the birth of an existing brood and simultaneously incubating the 2 broods for most of the year. However, 2 periods of fertilization and of birth are not evident in Fig. 16. Fertilization began in late fall, increased in frequency over the winter to a maximum in mid-spring, and then decreased into early fall. Births began in mid-winter and continued through mid-summer. A clearer picture of seasonal pattern(s) is provided by an evaluation of brood characteristics.

Gravid P_1 individuals contained F_1's in 1 of 4 developmental conditions (Fig. 11). Broods of only small F_1's (diameter < 1.0 mm) occurred in all months except October and November, but were most frequent in spring and early summer. The F_1's in a size series, found in some P_1's each month ($<$ 1.0-7.0 mm in winter, $<$ 1.0-6.0 mm in spring, $<$ 1.0-7.5 mm in summer and 3.0-7.5 mm in fall), were most frequent in midsummer through mid-fall and least common in spring. Broods of just large F_1's ($>$ 5.0 mm) were most numerous in late fall into mid-winter, decreased in frequency into early summer, and were lacking in mid- and late summer. Some P_1 individuals simultaneously contained members of 2 different broods (large F_1's and small F_1's) in each monthly collection, but they were most abundant in late winter and early spring, and again in mid- and late fall. A few non-gravid females were found each month, but were only sporadically more common, viz., in November, December and May (non-gravid P_1's of reproductive size; see below) and in September (all non-gravid P_1's of less than reproductive size). According to those findings, most P_1's underwent initial fertilization in spring and early summer; continued fertilization into fall added new members to the brood, which now appeared as F_1's in a size series. The subsequent appearance of broods containing only large F_1's (in late fall to mid-winter) was a result of the birth of the largest F_1 members in the previous size series. The presence of 2 broods in some P_1's at that same time indicates that the large F_1's were joined by small F_1's from uncommon early fall fertilizations. Nevertheless, some P_1's exhibited lower frequencies of these different brood conditions during other seasons, indicating that fertilizations, sequential developmental stages and births evidently were able to occur at almost any time. Moreover, it is possible that, in some instances, some P_1's did not undergo continued fertilzations, and broods of only small F_1's may have directly become broods of just large F_1's. Comparisons of seasonal brood sizes in the majority of P_1's that display the most frequent pattern in general support those conclusions.

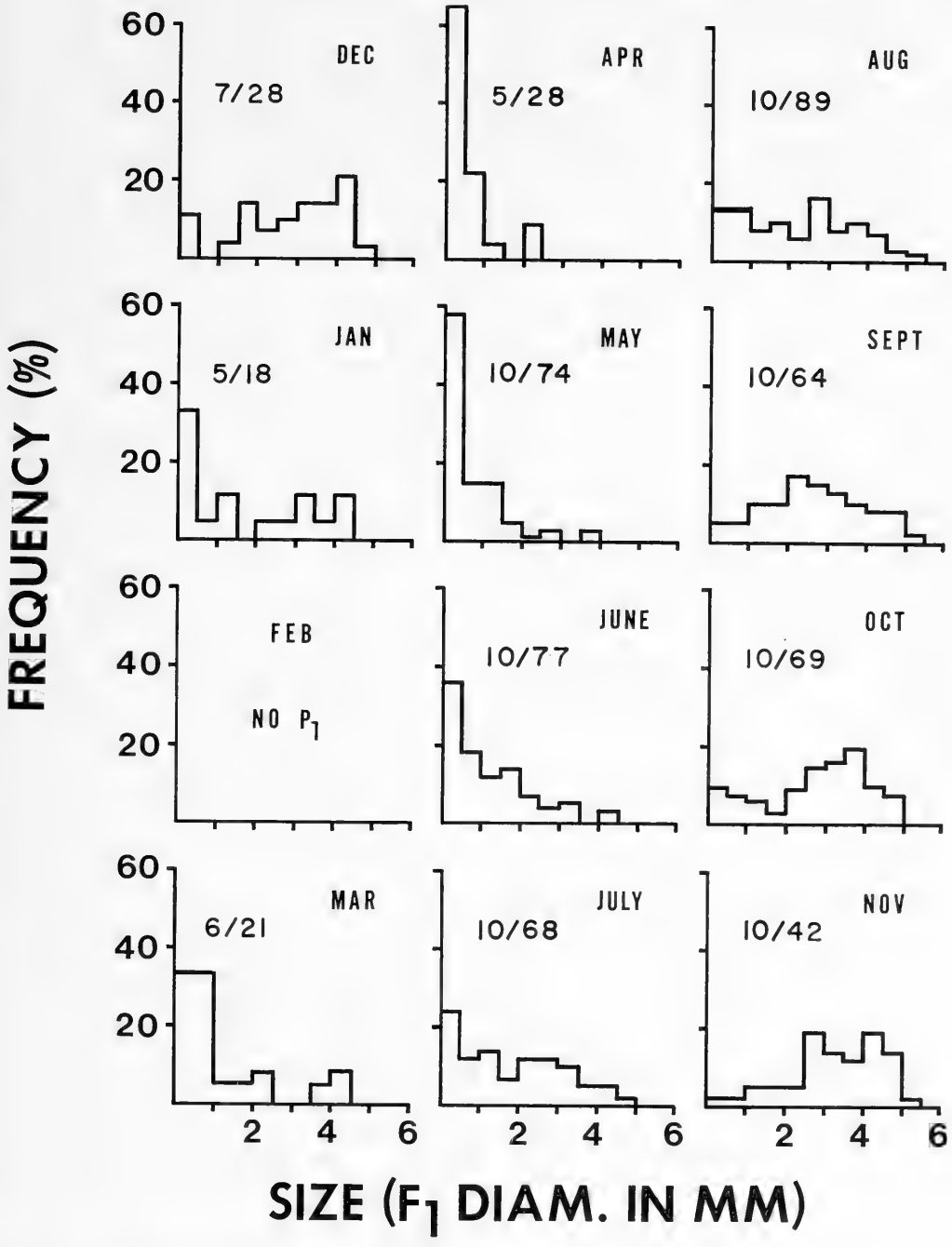


FIG. 15. Monthly size-frequency histograms of incubating *Lioplax pilsbryi* snails. Diam., diameter (mm); F_1, young developing within the pallial oviduct. Frequency: % of sample; fraction numerator: number of P_1's examined; denominator: number of F_1's examined.

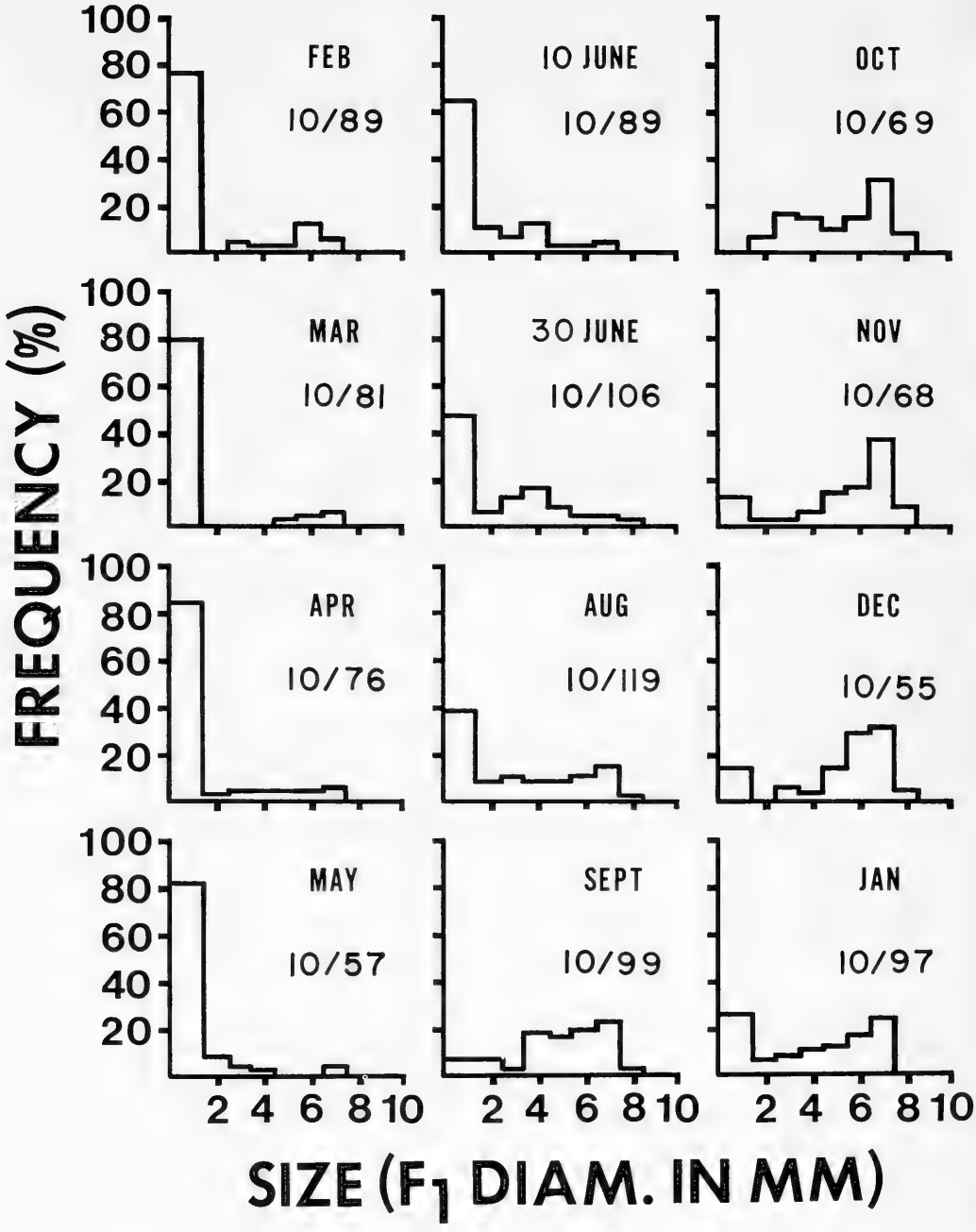


FIG. 16. Monthly size-frequency histograms of incubating *Viviparus georgianus* snails. Diam., diameter (mm); F_1, young developing within the pallial oviduct. Frequency: % of sample; fraction numerator: number of P_1's examined; denominator: number of F_1's examined.

Among P_1's incubating only small F_1's, the greatest mean brood size (8.6 F_1's/P_1) occurred in the spring. As brood development progressed and P_1 with only size-series broods were prevalent, the mean brood size increased over the summer (to 11.6-12.5 F_1's/P_1) and then decreased to low levels in early and mid-fall. As births occurred, brood sizes decreased. Among P_1's with just large F_1's, brood sizes were largest in late fall into mid-winter (3.8-4.4); P_1's with 2 broods had smaller "composite" brood sizes in mid- and late fall (6.6 and 5.4, respectively), and showed larger values in late winter and early spring (9.2 and 11.6). Seasonal variation in those values reflects a combination of additions of small F_1's by fertilizations and subtractions due to births of large F_1's.

Gravid P_1's were of similar mean size (diameter) throughout the study, although the mean brood size, without consideration of different kinds of developmental stages, varied in a seasonal pattern (Fig. 14). The mean brood size increased from its lowest value in early winter to an initial peak in late winter, then decreased to a low in late spring, after which it rose to a maximum value in late summer and then steadily decreased during the fall. This pattern, like that seen in the F_1 size-frequency histograms (Fig. 16), is one of bimodal distribution, and suggests that births occurred within the entire population mostly in mid-spring into early summer and again in late fall and early winter. The earlier period, clearly identified in the histograms by low frequencies of the largest F_1's, is characterized principally by the presence of small broods of only small F_1's generated by recent fertilizations; the scarcity of large F_1's here is indicative of the birth period. The latter period, characterized mainly by little broods of large F_1's, is not a birth period; rather the small brood sizes are the result of the absence of small F_1's from the recent fertilizations. Largest brood sizes were found in mid- and late summer (mainly F_1's in a size series), with a secondary pulse in late winter and spring (high frequencies of P_1's containing 2 broods). Analysis of the August P_1 sample (having the maximum brood size for the year) showed brood size and P_1 size were not correlated ($r_p = 0.057$, $P > 0.05$). Table 2, listing composite data from all monthly collections, shows mean brood sizes in relation to P_1 size classes.

The smallest gravid P_1's were 15.6 and 15.0 mm in diameter; those were the only individuals among 19 P_1's < 21.0 mm that were gravid, and just 12 of 31 P_1's < 22.0 mm were gravid. The F_1's in the 2 smallest gravid P_1's, from the collection of June 30 constitute brood sizes of 15 (size series) and 5 (only small F_1). Judged by sizes of the largest F_1's in the pallial oviduct (Fig. 16), newborn were about $\geqslant 7.5$ mm in diameter.

DISCUSSION

Relationship of Reproductive Organ Activity and Brood Production

When fertilizations and births occur in all individuals during a comparatively discrete period of time, the population reflects a relative synchronization of individual members. Lesser individual variation in fertilization or birth periods presents a more synchronized pattern for the population than does greater individual variation. Moreover, the identification and description of any coordination (either simultaneous or sequential) between organ activities and brood production at both the individual and the population level becomes more difficult with increasing individual variation. Some variation observed here within each monthly sample may have occurred because the animals were born at different times and underwent different rates of maturation.

Among the 3 viviparids described herein, *C. geniculum* individuals exhibited the greatest synchronization, with fertilizations and births occurring in the population at discrete periods of time. Peak typical spermatogenesis, maximum abundance of eupyrene sperm in the testis, greatest abundance of oöcytes in the ovary, and highest levels of secretory activity by the albumen gland occurred at or prior to the commencement of fertilization, i.e. prior to the appearance of the smallest F_1's in the pallial oviduct. Although not conclusively shown by the available data, copulation (circumstantially identified here by the presence of abundant eupyrene and oligopyrene sperm in the seminal receptacle and by eupyrene sperm in the albumen gland) is thought to have occurred during late fall through mid-winter in larger, older females, and in the other females in the

spring. Mating pairs were never seen in the field; higher water levels in winter and spring preclude their detection. Moreover, during periods of low temperatures the animals burrow into the substrate, where copulation in *L. pilsbryi* was observed to occur (see below). Following initial fertilizations, organ and gland activities decreased somewhat (Fig. 1), even as fertilizations continued (at lower frequencies) (Fig. 8) to create broods with F_1's in a size series. The albumen gland remained relatively active, providing sufficient quantities of perivitelline fluid for new embryos during that period.

In contrast, greater variation between individuals of *L. pilsbryi* and *V. georgianus* contributed to longer periods of fertilization and birth in those populations, and thus maturation rates and the time of the 1st fertilization varied.

Peak abundance of oöcytes in the ovary of *L. pilsbryi* occurred during the time of highest fertilization frequencies, and the albumen gland was very active only during the earlier part of the long fertilization period (Figs. 2, 15). However, eupyrene sperm in the testis appeared to be uncommon during this time. Mating pairs, partly embedded in the substrate, were found in the field in May and October. These observations support the conclusion that spring and fall are copulatory periods. This is suggested by the occurrence of eupyrene sperm at those times in the albumen gland (but not in the seminal receptacle), but do not explain the relative lack of sperm in that gland during the winter or their presence there in midsummer. If sperm present in the albumen gland in July were received during copulation in the prior spring and stored there, it would appear possible that sperm received in the fall copulation could similarly be stored in the albumen gland in the winter. However, few P_1's in the winter contained sperm in the albumen gland, but that lack may reflect failure of most of the investigated specimens to have mated in the fall. Some of the smallest F_1's occurred in the pallial oviduct in all months, but times of lowest frequencies of those F_1's (fall and early winter) may be considered a post-fertilization period in most of the population; organ and gland activities were diminished then in the monthly samples.

Least synchronization occurred in *V. georgianus,* where P_1 individuals showed comparatively long fertilization and birth periods. Mating pairs were never seen in Holmes Creek, although copulation in other populations was observed in the spring and fall (also during winter among snails in aquaria). Judged by frequencies of P_1's with sperm in the seminal receptacle (but not in the albumen gland), copulation in Holmes Creek must have occurred immediately prior to a 1st fertilization period. At the same time, immediately preceding the highest frequencies of the smallest F_1's in the pallial oviduct, eupyrene sperm were most abundant and greatest secretory activity of the albumen gland occurred (Fig. 3). Eggs were most numerous at 2 times in the population, once in the fertilization period and again in the post-fertilization period (identified by low frequencies of the smallest F_1's). Eupyrene sperm were least abundant and albumen gland activity was lowest during the post-fertilization period (Fig. 3). Nevertheless, at least some individuals, both male and female, appeared to display a high level of gonadal and glandular activity in each month.

Reproductive Organ Function

It is generally conceded that eupyrene sperm from typical spermatogenesis are capable of fertilization. However, a variety of other functions have been hypothesized for the oligopyrene sperm from atypical spermatogenesis (Meves, 1903; Ankel, 1924, 1930; Gall, 1961; Nishiwaki, 1964; Rossi, 1968). Inasmuch as atypical spermatogenesis and oligopyrene sperm exhibit high seasonal levels prior to those of typical spermatogenesis and eupyrene sperm (Rossi, 1968; Barbato, 1971; partly confirmed here), the hypothesis that oligopyrene sperm, by catabolic breakdown, provide a nutrient-energy source for eupyrene sperm (Hanson et al., 1952; Yasuzumi & Tanaka, 1958; Dembski, 1968) might be the most logical. Dembski (1968) also suggested that disintegrated atypical sperm in the seminal receptacle and pallial oviduct might provide nutrient matter for the female. However, the actual function of oligopyrene sperm has not yet been clearly demonstrated.

Although Rossi (1968) and Barbato (1971) reported atypical spermatogenesis and oligopyrene sperm to be prevalent during colder, winter months, findings for

all 3 species studied here showed atypical spermatogenesis and spermatozoa to be common during warmer periods also (Figs. 1-3). Moreover, oligopyrene and eupyrene sperm are not mutually exclusive in occurrence either within the population or within a single individual, and both types have simultaneously been found in the pallial oviduct and seminal receptacle of females (Popoff, 1907; Hanson et al., 1952; Dembski, 1968; verified here).

Seminal vesicle function in gastropods is commonly considered to be the storage of autosperm (i.e. sperm produced by the individual). Secretory activity to provide "nutrients" for the sperm would seem to be associated with the provision of such space. However, no individual of the 3 viviparids studied here contained sperm in that organ. The secretions might contribute to a kind of "culture medium" for sperm passing through the tract. *Lioplax pilsbryi* has a seminal vesicle of somewhat different structural organization from that in *C. geniculum* and *V. georgianus* (Vail, 1977), and that gland's secretions in *L. pilsbryi* may or may not be different from those in the other 2 species. The same process may occur in the prostate gland in *V. georgianus* (Vail, 1977), and indeed in this species the seasonal regionalization shown by differential staining properties suggests that the same region of the prostate might secrete different products through time. These secretions presumably serve in the maintenance of autosperm, none of which were found stored in this gland. However, Kamaloney (1968) reported sperm stored in the prostate gland of *Bellamya sumatrensis* (Viviparidae: Bellamyinae).

Following copulation, sperm deposited in the pallial oviduct are conveyed to the seminal receptacle along the sperm groove, although the mechanism for keeping at least most of these oligopyrene and eupyrene gametes in that open groove is unknown. As previously noted, the walls of the pallial oviduct are seasonally thicker or thinner with glandular tissue, and the secretions may in part serve for sperm maintenance.

The seminal receptacle in gastropods is considered the usual site for storage of allosperm (i.e., sperm received during copulation) in part because it produces a "nutritive" secretion. However, few females of any of the 3 species studied here contained either oligopyrene or eupyrene sperm oriented with their head against (penetrating?) the cells of the inner surface of this structure. Very few *L. pilsbryi* females contained any sperm in the seminal receptacle, and the majority of *C. geniculum* and *V. georgianus* females with sperm in this organ contained them free in the lumen. This latter condition does not suggest prolonged sperm storage in this organ in these species.

In *C. geniculum* and *L. pilsbryi,* but not in *V. georgianus* (perhaps due solely to shredded sections), eupyrene sperm were commonly found in the oviduct and in the albumen gland. Sperm were possibly being stored there as they were in the albumen gland and oviduct of *V. contectus* according to Dembski (1968). Copulation in some *C. geniculum* animals may occur several months prior to fertilization, necessitating a mechanism for comparatively long-term sperm storage in females. Such a condition would explain the availability of sperm that contribute to sequential fertilizations to provide broods with F_1's in a size series. Sperm storage in the albumen gland would allow for simultaneous discharge of sperm and albumen (termed "perivitelline fluid" when surrounding a developing F_1 within an acellular capsule) into the oviduct near the junction of the ovary and oviduct. Whereas Dembski (1968: 151) suggested for *V. contectus* that "it is possible that the disintegrated and ingested (eupyrene) sperm which were found in the albuminous gland are converted into yolk by the glandular cells of this gland." Annandale & Sewell (1921: 235) previously reported (eupyrene?) sperm in the perivitelline fluid of *Idiopoma bengalensis* (Bellamyinae). The presence of an egg in the proximal arm of the oviduct might initiate such a sperm-albumen discharge, or an initial discharge from the albumen gland might stimulate the release of oöcytes from the ovary to the oviduct. Similarly, fertilization in *L. pilsbryi* occurred throughout the year in the population, at irregular intervals in P_1 individuals resulting in numerous occurrences of broods with F_1 in a size series. This condition is likewise attributed to the storage of sperm for intermittent fertilization. It is nevertheless enigmatic that, while apparently containing more eggs in the ovary, *L. pilsbryi* P_1's release fewer eggs at a time and at apparently greater intervals than do *C. geniculum* and *V. georgianus* P_1's.

Few details of the nature of viviparid albumen and its functions(s) as perivitelline fluid are known. Annandale & Sewell (1921: 235) noted presumably calcareous spicules in the perivitelline fluid of *Idiopoma bengalensis* and suggested that the F_1's use these spicules in their shell production. In his study on the "nutrition" of *V. viviparus* F_1's, Charin (1926: 66) recorded mucus with bound carbohydrates (mucopolysaccharides?) in the perivitelline fluid and the absence of ". . . frei Kohlenhydrate (auch Glykogen) und Lipoiden." He also noted that the surrounding capsule, rich in lipids, was permeable to carbohydrates and lipids, and suggested that the gravid P_1 continues to supply those substances throughout development. Bottke (1972, 1973: 239) also indicated that the small, nearly yolkless (fertilized) eggs of *V. contectus* necessarily depend on ". . . large amounts of secondary yolk provided by the albumen gland and glandular cells of the (pallial) oviduct." Alyakrinskaya (1969) and Samochwalenko & Stánczykowska (1972) noted that the capsules of *V. fasciatus* and *V. viviparus* contain proteins. Finally, Chatterjee & Ghose (1973) reported seasonal accumulations and declines of glycogen and lipids in "genital organs" of *I. bengalensis* (viz. gonad probably only testis, albumen gland and prostate gland) in relation to the breeding season (undescribed).

Identification of specific viviparid accessory organ secretions and information on their functions such as Goudsmit & Ashwell (1965), Goudsmit & Neufeld (1966) and Goudsmit (1973) provided for *Helix pomatia* (Linn.), a terrestrial pulmonate, are presently lacking.

The site of fertilization in viviparid females has been previously reported to be the seminal receptacle (von Siebold, 1836; Mattox, 1938) and the sperm groove on the floor of the pallial oviduct (Speyer, 1855). However, because sperm were observed stored in the albumen gland as well as present in the oviduct, and because encapsulated, very small F_1's were found within the distal arm of the oviduct in all 3 species studied here (most commonly in *V. georgianus*), it is concluded that fertilization and capsule formation occur in the oviduct. Kamaloney (1968) also hypothesized the occurrence of fertilization in this site for *Bellamya sumatrensis*, but considered fertilization in the seminal receptacle equally possible. This conclusion also concurs, in part, with that of Leydig (1850) for *V. viviparus*.

Capsule form, with a chalaza or funiculus (cf. Leydig, 1850: pl. 11, fig. 16), is similar in the 3 species studied here. The opening from the narrow oviduct into the comparatively spacious seminal receptacle is very small, and the chalaza perhaps results from a "pinching" effect on the acellular capsular envelope as the F_1 and perivitelline fluid contained therein are forced through that opening.

Brood Production

Seasonal reproductive patterns have been described for some viviparids, but the available information does not document sufficient possible conspecific variations to permit adequate congeneric or conspecific comparisons at this time. This study, like most others, provides information on the reproductive cycle from observations of just 1 population of each species. Preliminary observations (Vail, unpubl.) on many other populations of *C. geniculum* and *V. georgianus* indicate that maximum size of P_1's, mean brood size and F_1 birth size, while relatively uniform within a population, are highly variable among populations. It is not yet known whether these other populations vary in the number of broods produced per year. Environmental factors influencing P_1 size, brood size and F_1 birth size are, at best, poorly known. Until these factors are understood, their effects cannot be distinguished from possible generic or species specific traits (if any). Information on the 3 viviparids studied here is presented in Tables 3 and 4 with similar data from the previous studies as a summary of current information rather than a comparison of genera or species. Such comparisons should not be attempted until intraspecific variation and environmental influences have been studied.

Previous studies on viviparid reproductive cycles emphasized analysis of the P_1 population instead of the F_1 population as was done here. Birth periods, age classes, life spans and incubation periods were extrapolated from seasonal size frequency histograms based on P_1 shell length. Brood sizes (total number of incubating young) and the size of F_1 individuals were frequently obtained from observations made without regard to season. In this study, it

TABLE 3. Life history features of North American Viviparidae. Data drawn from Chamberlain (1958), Medcof (1940), Van Cleave & Altringer (1937), Van Cleave & Chambers (1935), Van Cleave & Lederer (1932) and the present study. L, shell length; D, shell diameter.

Feature	*Campeloma decisum*	*C. geniculum*	*C. rufum*	*C. tannum*
Smallest gravid P_1's	15.0 mm L	13.2 mm D	20 mm L	17.5 mm L
Fertilization period	at least March-May	mid-spring-early fall	May-June	"at all seasons
Incubation duration	?	8-10 months	11-12 months	all seasons"
Birth period	March-June	Feb.-April	May(peak)-Sept.	"summer"
♀ age at 1st birth	2 years	?	2 years	3 years
Non-gravid interval				
individual	?	yes	?	?
population	?	mostly	?	?
Sex. size dimorphism	no males	mostly	no males	no males
Life span (♂:♀)	0:3 years	?:?	0:2-3 years	0:5 years

Feature	*Lioplax pilsbryi*	*L. sulculosa*	*Viviparus contectoides*	*V. georgianus*
Smallest gravid P_1's	13.8 mm D	10-12 mm L	16-19 mm L	15.0 mm D
Fertilization period	continuous, peak in spring	spring (?)	early summer	mid-spring-early fall
Incubation duration	10-12 months	12 months	8-10 months	10-12 months
Birth period	Dec.-April	May-June	Feb.-June	Jan.-July
♀ age at 1st birth	?	2 years	2 years	?
Non-gravid interval				
individual	no	?	yes	possibly
population	no	no	yes	no
Sex. size dimorphism	no	yes	yes	no
Life span (♂:♀)	?:?	1:2 years	1:3 years	?:?

TABLE 4. Sizes of large North American viviparid P_1 females, newborn and broods. Data drawn from references cited in Table 3.

Species	P_1 length (mm)	Newborn length (mm)	Mean no. of young per P_1[b]
C. decisum	modes: 17-20 (up to 27.3)	about 3.0	?
C. geniculum[a]	modes: 24-26 (up to 34.2)	⩾4.0 (up to 4.6)	4.1-27.7 (up to 55)
C. rufum	modes: 29-31 (up to 40)	mode: 4.0 (2.5-6.0)	3.0-42.1[c] (up to 90)
C. tannum	modes: 16-20 (up to 47)	?	2.0-15.0[c] (up to 26)
L. pilsbryi[a]	modes: 23-24 (up to 27.8)	⩾5.0 (up to 6.1)	3.5-9.1 (up to 13)
L. sulculosa	modes: 16-17 (up to 23.3)	about 2.5 (up to 2.7)	(up to 46)
V. contectoides	modes: 27-29 (up to 40.7)	about 3.8	?
V. georgianus[a]	modes: 32-34 (up to 38.1)	⩾7.4 (up to 8.2)	5.2-10.4 (up to 25)

[a]Sizes of newborn judged from those of large F_1's in pallial oviduct.
[b]Brood sizes shown as range of monthly means.
[c]Means from different P_1 age classes.

was seen that using shell length as an indication of P_1 size is inadequate for these perennial animals because the apex can become eroded. It is possible that, due to erosion, 2 P_1's could be in the same length-size class but be in different diameter-size classes. Thus, shell diameter, rarely modified by erosion, is a more reliable index of P_1 size. Information on brood production obtained only from

analyses of the P_1 population without consideration of the F_1 population can be incomplete and misleading. Lacking would be data showing (1) whether P_1 individuals undergo fertilization of a new brood prior to birth of the existing brood or experience a non-gravid interim between consecutive broods, (2) if members of a single brood are born within a brief period of time or sporadically over a longer period of time, or (3) how the brood production cycle of an individual compares to the cycle shown by the population, i.e., an individual might give birth to all her young at one time but it might be several months before all females in the population had given birth. Also, brood sizes and F_1 dimensions obtained from samples taken without regard to season could be misleading as both change during the course of brood development (Figs. 8, 12-16).

The best documented prior information on North American viviparid reproductive cycles (Tables 3, 4) concerns *Campeloma rufum* (Van Cleave & Altringer, 1937), *C. tannum* (Medcof, 1940), *C. decisum* (Chamberlain, 1958), *Lioplax sulculosa* (Van Cleave & Chambers, 1935) and *Viviparus contectoides* (Van Cleave & Lederer, 1932). The former 3 studies were based on seasonal (but not consecutive monthly) samples from the same population each, and the latter 2 each combined data from several populations. The findings of the present study resemble those from the aforementioned investigations only for a few features (Table 3, *Campeloma:* fertilization period; *Lioplax:* incubation duration; *Viviparus:* birth period). All but lacking from those prior studies are data on age structure, life spans and seasonal brood production patterns.

On the basis of current information the basic pattern of brood production appears to be similar in these 8 species representing 3 genera. In each there is 1 fertilization period and 1 birth period per year and incubation of young lasts nearly 1 year. Differences appear to occur only in the specific time and duration of each event (Table 3). However, information from the prior studies suggests that fertilization and birth periods overlap in individuals as well as in the population. While it is possible that 2 broods are simultaneously incubated, it is also possible that individual females experience a non-gravid interim between broods. Here a non-gravid interim was seen in individual *C. geniculum* but not in the population; simultaneous incubation of 2 broods was observed in *L. pilsbryi* and *V. georgianus.*

Van Cleave & Lederer (1932) also reported that newborn male *V. contectoides* ($<$ 4 mm in shell length) from several populations in Illinois and New York possessed a copulatory tentacle. Observations of *V. georgianus* P_1's from Holmes Creek indicated that tentacular asymmetry was not evident in individuals $<$ 11 mm in diameter (newborn: $\geqslant$ 7.5 mm). This delayed tentacular asymmetry was also observed in *C. geniculum* and *L. pilsbryi.*

Sexual size (and age) differences have been reported in *L. sulculosa* and *V. contectoides* with females attaining a larger size and living 1 year longer than males (Van Cleave & Chambers, 1935; Van Cleave & Lederer, 1932). Sexual size dimorphism does not seem to occur in either *L. pilsbryi* or *V. georgianus*, but *C. geniculum* females are generally larger than the males (nonquantitative personal observations). The occurrence of sexual size dimorphism in *C. geniculum* is the 1st such record for *Campeloma*, but only because this is the 1st dioecious population so studied.

Viviparid brood size has previously been considered in relation to season, P_1 size and age, size of developing F_1's and P_1 habitat. Brood size in *C. geniculum, L. pilsbryi* and *V. georgianus* increases seasonally following the onset of fertilization to a maximum and subsequently decreases as births occur (Figs. 12-14). This seasonal fluctuation is especially noticeable in *C. geniculum*, and is a consequence of progressive brood development (Figs. 9-11). This is in agreement with those observations by Van Cleave & Altringer (1937), Miroshnitshenko (1958), Stánczykowska (1960) and Samochwalenko & Stánczykowska (1972) on *C. rufum, V. viviparus, V. fasciatus* and *V. fasciatus* and *V. viviparus*, respectively.

Brood size has also been related to P_1 size and age. Observations on different-sized *C. geniculum* and *V. georgianus* P_1's show brood size increased with increasing P_1 size (and age). These observations concur with those of Medcof (1940), Miroshnitshenko (1958), Stánczykowska et al. (1971) and Samochwalenko & Stánczykowska (1972). In contrast, brood sizes in *L. pilsbryi* remained relatively constant among P_1's of different sizes and ages.

TABLE 5. Approximate diameter (mm) of *Campeloma geniculum, Lioplax pilsbryi* and *Viviparus georgianus* at birth and maturation plus average and maximum diameter of gravid P_1 in each population.

	C. geniculum	*L. pilsbryi*	*V. georgianus*
Birth size	⩾3.5	⩾4.5	⩾7.5
Smallest gravid P_1	13.2	13.8	15.0
$\bar{x}$ diameter of gravid P_1's	18.2±1.3	15.7±1.0	26.9±1.6
Largest P_1 collected	25.6	18.3	30.4

Also, a post-reproductive senescence, as reported by Annandale & Sewell (1921), Van Cleave & Lederer (1932) and Van Cleave & Altringer (1937) for *Idiopoma bengalensis, V. contectoides* and *C. rufum*, respectively, was not observed in the 3 species studied here. Table 2 shows average brood size in relation to P_1 size for each of these species. A composite sample was used in each instance because it was not possible to obtain a large sample containing P_1 in each size class during any 1 month.

Campeloma geniculum and *L. pilsbryi* snails are approximately the same size at birth and both approximately triple their diameter before first becoming gravid. However, *C. geniculum* snails can grow to be much larger than *L. pilsbryi* individuals with a 7-fold increase of birth size (compared to only a 4-fold increase in the latter species). *Viviparus georgianus* animals approximately double their diameter between birth and first becoming gravid, and potentially can increase 4-fold in size. Table 5 summarizes these relationships. It is not known whether these relationships are a reflection of inherent differential growth rates, maturation rates, life spans or environmental factors or a combination of these factors.

Reports relating brood size to P_1 habitat were not substantiated in this study. Annandale (1924), Prashad (1928), Rohrbach (1937) and Samochwalenko & Stánczykowska (1972) observed that females in lotic populations carried more F_1's per brood than did females in lentic situations. The 3 populations studied here showed wide variation in brood size even though all were from lotic habitats (*C. geniculum* and *L. pilsbryi* being from the same locality). In addition, preliminary analyses of qualitative samples from lentic populations have provided a further comparison. A collection made in December, 1973 of *C. geniculum* snails (number of P_1's: 18; mean diameter: 18.0 mm), *L. choctawhatchensis* individuals (15; 11.0) and *V. georgianus* snails (13; 24.5) from Lake Talquin, a reservoir in Leon Co., Florida, yielded mean brood sizes of 80.1, 48.5 and 23.4, respectively. These values are all greater than those reported here for the Chipola River and Holmes Creek congeners.

ACKNOWLEDGEMENTS

This study was funded, in part, by a Gerald W. Beadel Grant from Tall Timbers Research Station. The author expresses appreciation to William H. Heard of Florida State University for providing assistance and reviewing this manuscript, and to E. V. Komarek, Sr. of Tall Timbers Research Station for providing facilities and promoting basic research.

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MALACOLOGIA, 1978, 17(1): 99-109

POSITION OF THE CLASS APLACOPHORA IN THE PHYLUM MOLLUSCA[1]

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ABSTRACT

The Aplacophora are shell-less, vermiform mollusks found from the continental shelf regions of the world to depths of 9,000 m. They are grouped into a single class with 2 subclasses: Chaetodermomorpha (= Caudofoveata) and Neomeniomorpha (= Ventroplicida; Solenogastres sensu Salvini-Plawen), a classification which preserves early nomenclature based on *Chaetoderma* Lovén, validated by the International Commission on Zoological Nomenclature. The name "solenogaster" is reserved as a common noun like "clam" or "snail."

The Aplacophora have several typical molluscan characters, namely a radula with associated buccal structures; a style sac and mucoid style; a coelom restricted to gonads, a dorsal pericardium, and kidneys; a heart consisting of a ventricle and 2 auricles; laterodorsal-ventral musculature; ventral musculature that bends the body and contained organs dorsally; a dorsal gut; mantle cavity and gills; a vestigial foot which secretes a slime trail; a nervous system of paired ganglionated cords, ladderlike, with pharyngeal ring and buccal ganglia; and finally a development which includes spiral cleavage and a protobranch-like larva. Most of these molluscan characters are not structurally like those of chitons with which they have often been classified; therefore, the Aplacophora are classified separately from the Polyplacophora.

The 2 aplacophoran taxa resemble each other in their nervous system, coelom, haemocoele, musculature, and shape. Differences in integument between the 2 taxa may be due to reduction in the burrowing Chaetodermomorpha, and in the digestive system due to the obligate coelenterate feeding in the Neomeniomorpha.

It is of phylogenetic importance that several characteristic molluscan structures have evolved in the Aplacophora independent of a shell.

INTRODUCTION

The Aplacophora are worm-shaped mollusks surrounded by a cuticle bearing calcareous spicules; they inhabit the deep ocean basins and continental shelf and slope regions of the world. Observations from more than 380 collections made by the Woods Hole Oceanographic Institution, Oregon State University, Scripps Institution of Oceanography, and Centre National de Tri d'Océanographie Biologique show that their greatest species radiation has been in the deep sea. They burrow into or creep on mud; some wrap themselves around alcyonarians upon which they feed. They are common in the deep sea, occurring in nearly all small-meshed epibenthic dredge hauls (Hessler & Sanders, 1967) and box cores taken from all depths to 9,000 m. In recent years the number of biological surveys of the deep sea has increased, and the discovery of numerous new aplacophoran species has rekindled interest in this group. Although most species are still to be described, some recent taxonomic works are those of Salvini-Plawen (1972, bibliography, for his papers), Schwabl (1963) and Scheltema (1976).

CLASSIFICATION

The 2 classifications given in Table 1 are currently in use for living mollusks. Both classifications retain the 2 distinct taxa that have been recognized since the 19th century: one taxon (Neomeniomorpha = Ventroplicida = Solenogastres sensu Salvini-Plawen) is distinguished by a ventral groove containing a narrow foot, the other (Chaetodermomorpha = Caudofoveata) lacks a ventral groove and has an oral shield and a pair of ctenidia. It should be stressed that there has been no modification of membership in the 2 aplacophoran taxa in either of

[1]Contribution No. 3763 from the Woods Hole Oceanographic Institution.

the classifications; only the hierarchical rank has been shifted, and in the second, names have been changed.

The first classification is recommended for 3 reasons: 1) it conserves nomenclature in use since the early 1900's; 2) it separates the Aplacophora and Polyplacophora; and 3) it gives a hierarchical ranking to the 2 aplacophoran taxa that fits well with the arrangement of equally similar taxa in other molluscan classes.

NOMENCLATURE

Nomenclatural changes in the Aplacophora started in the 1930's with the belief that the genus *Chaetoderma* Lovén 1844 was a junior homonym of *Chaetoderma* Swainson 1839 (Pisces). The next available name was *Crystallophrisson* Möbius 1875, an orthographic horror variously spelled in the literature. Heppell (1963), who showed that Swainson used multiple spellings, requested and obtained from the International Commission on Zoological Nomenclature (ICZN) validation of the genera *Chaetoderma* Lovén 1844 and *Chaetodermis* Swainson 1839 (Opinion 764, ICZN, 1966).

Previous to the ICZN ruling, Boettger (1956), wishing to avoid an ordinal name based on "*Crystallophrisson,*" used Caudofoveata to replace Chaetodermatoidea; he also changed the name Neomeniida to Ventroplicida. As *Chaetoderma* Lovén is a valid name, there is historical reason for preserving Pelseneer's names Chaetodermomorpha and Neomeniomorpha for the 2 major taxa of Aplacophora. The use of Solenogastres Gegenbaur 1878 for the Neomeniomorpha alone is particularly confusing, as Gegenbaur included the only 2 genera then known, *Chaetoderma* and *Neomenia,* under this name, which appears throughout the literature as synonymous with Aplacophora. "Solenogaster" is best used with lower case and anglicized spelling, a term equivalent to "clam" or "snail."

APLACOPHORA AS MOLLUSKS

The molluscan affinities of the Aplacophora, first noticed in the mid-1870's, have long been discussed (see Hyman, 1967: 2-3, 68-70, for a review). The Aplacophora have been placed back and forth within and outside the phylum Mollusca for nearly a century; when considered true mollusks, it has usually been because of supposed similarities to chitons. Even Hyman, who recognized the chitons and aplacophorans as separate classes, stated that such similarities "justify the inclusion of solenogasters in Mollusca" (1967: 69), and Fretter & Graham considered that the solenogasters are "undoubtedly related in some way to the chitons" (1962:8), although they placed them outside the Mollusca. The latter returned them to Mollusca in 1976 (p. 548) without discussion. Salvini-Plawen (Table 1) has grouped chitons and aplacophorans as a separate subphylum. The chitons and aplacophorans together have been known as Amphineura, Aculifera, or Isopleura.

At the time that Hoffman (1949) carefully described the integument of the Aplacophora, they had not been considered mollusks by Thiele (e.g., 1925: 12-14), one of the strongest voices of the preceding decades, because they seemed to him to lack the important molluscan characters of "shell, mantle, foot, and nephridia; further, in other mollusks the gonoducts do not issue from the pericardium" (as summarized by Hyman, 1967: 68). Hoffman compared chiton and aplacophoran integuments in both mantle and foot, and by certain homologies between them, brought the 2 aplacophoran taxa back within the concept of mollusks. Two doubtful homologies that he proposed are (1) that the slime tracts of chitons equal the "shell glands" of aplacophorans, a homology that calls for an impossible reorientation of gills in the aplacophorans as noted by Hoffman himself (1949: 407), and (2) that the gland cells of the chiton foot equal the glands of the chaetodermomorph oral shield. However, he concluded that "In spite of my view that the Neomenioidea and Chaetodermatida were thus early separated from each other and that their common stem was chiton-like in respect to the mantle and foot, I do not feel justified in regarding the Neomenioidea, Chaetodermatida, and chitons as 3 equivalent groups in the class Amphineura. The Neomenioidea and Chaetodermatida resemble each other exactly in more respects (e.g., in regard to the nervous system and coelom) than either resembles the chitons" (1949: 424, here translated).

Boettger (1956) elaborated on Hoffman

TABLE 1. Classification of the living Mollusca.[1]

I Phylum Mollusca	II Phylum Mollusca[2]
	Subphylum Aculifera Hatschek 1891 [= Amphineura von Ihering 1876]
Class Monoplacophora Wenz 1940	
Class Aplacophora von Ihering 1876 [= Solenogastres Gegenbaur 1878]	
Subclass Chaetodermomorpha Pelseneer 1906	Class Caudofoveata Boettger 1956
Subclass Neomeniomorpha Pelseneer 1906 [= Ventroplicida Boettger 1956]	Class Solenogastres Gegenbaur 1878 [*partim*]
Class Polyplacophora [*ex* Polyplacophores] de Blainville 1816	Class Placophora von Ihering 1876
	Subphylum Conchifera
	Class Tryblidiida Wenz 1939 [= Monoplacophora]
(Classes Gastropoda, Pelecypoda, Scaphopoda, Cephalopoda)	(Classes Gastropoda, Bivalvia, Scaphopoda, Cephalopoda)

[1]A recent classification including the extinct mollusks is in Runnegar & Pojeta (1974), who place the Aplacophora and Polyplacophora by themselves in 2 separate subphyla.
[2]From Salvini-Plawen (1972).

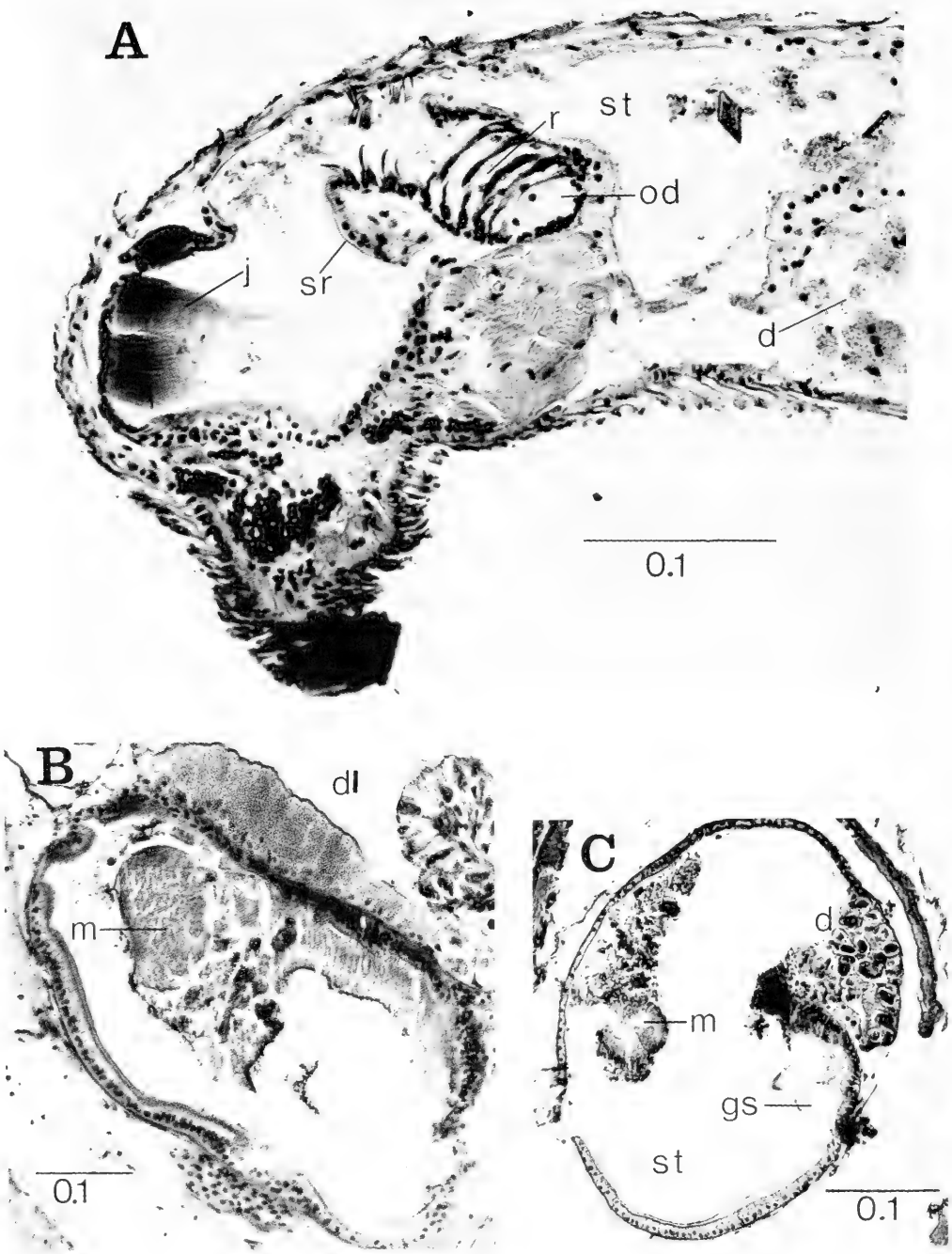
in a theoretical paper on early mollusks, and Salvini-Plawen (1967: 399) stated that from Hoffman's work "der Placophoran-Verwandtschaft der aplacophoran Gruppen nicht mehr zu Zweifeln ist."

The Aplacophora bear many preeminently molluscan characters other than an integument that may or may not be homologous to that of the chitons (see below). Despite their specialized vermiform shape, they have many archaic, or conservative, molluscan characters.

A typical radula exists in several genera with a radular membrane issuing from a radula sac and bearing rows of teeth formed by odontoblasts (Figs. 1A, 2; Scheltema, 1972; Hyman, 1967, fig. 15). Although much of the published work indicates no more than serrate cuticularized pharyngeal epithelium, critical examination of some Chaetodermomorpha has shown the existence of a subradular as well as a radular membrane (Figs. 1A, 2; also Scheltema, 1972). However, Salvini-Plawen (1972: 240) considered that there is only a single basal cuticle. There are paired bolsters, which are chondroid in some genera (Fig. 3B). There is no docoglossate, chiton-like dentition present in any Aplacophoran genus so far described.

A style sac occurs in the Chaetodermomorpha; a style in the form of a mucoid rod and gastric shield are present as well in the family Chaetodermatidae (Fig. 1) and in the genus *Limifossor.* (A description of this uniquely molluscan character is to be made more fully elsewhere.) There is no rod in the chiton style sac (Fretter, 1937). The Chaetodermomorpha also have a blind digestive gland, which empties into the posterior stomach (Fig. 1C; Wirén, 1892). The Neomeniomorpha, many of which are obligate predators on coelenterates (Salvini-Plawen, 1967), have a very different and probably specialized mid-gut system consisting of a wide tube thrown into folds and lack a separate digestive gland. There are no chiton-like esophageal glands ("sugar-glands") in the Aplacophora, although salivary glands are found.

The aplacophoran coelom, as in all mollusks is restricted to paired gonads, pericardium, kidneys, and the ducts therefrom; unlike other mollusks, the gonads empty directly into the pericardial cavity and gametes pass out of the pericardial cavity through coelomoducts (Fig. 4A). The heart and pericardial cavity are dorsal and molluscan in organization (Scheltema, 1973; and others, see Hyman, 1967). They may indicate that the early molluscan condition was a pair of ventricles and a paired pericardium, as found in *Neopilina,* for the heart is bilobed during diastole and there are 2 large V-shaped lateral extensions of the pericardium (Chaetodermatidae: Fig.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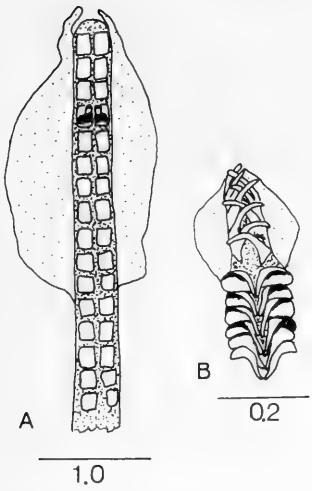

FIG. 2. Radula ribbon (heavy stippling) and subradular membrane (light stippling) of the limpet *Acmaea testudinalis* (A) and *Prochaetoderma* sp. (B; cf. Fig. 1). The position of the teeth in *Acmaea* is indicated; one transverse row is drawn. Scales in mm.

4C; also Scheltema, 1973, figs. 1, 2). The chiton pericardial cavity, also large, does not receive gametes from the gonads.

Histological evidence suggests functional kidneys in the Chaetodermatidae; the cells of the C-shaped coelomoducts, emptying the pericardium to the outside, are similar to the kidney cells in the protobranch *Nucula* (Fig. 3A, 3C). There are no experimental data on the function of the aplacophoran coelomoducts.

Laterodorsal-ventral musculature, expressed serially in the Neomeniomorpha and in restricted body areas in the burrowing Chaetodermomorpha, may preserve the condition leading to reduction in pedal retractors of shelled mollusks including chitons (Salvini-Plawen, 1969). Ventral longitudinal muscles, found in both chitons and aplacophorans, produce a dorsal bend (Fig. 5A-D). In the burrowing chaetoderms these muscles are weak (Fig. 4B). The digestive system is dorsally placed with a ventral mouth and anus (Figs. 1A, 3A, 4B). The dorsal bend appears during development in the late embryos of 2 species of neomeniomorphs (Pruvot, 1890; Thompson, 1960).

The molluscan head-foot is not in great evidence in the vermiform Aplacophora. However, vestiges remain in both taxa. The Neomeniomorpha produce a sticky slime track along which they creep by the ciliary action of a greatly reduced foot; the head is free of the substrate and moves about by hydrostatic action (personal observation). The Chaetodermomorpha retain a ventral (= pedal) sinus (Fig. 4B), and the genus *Scutopus* still has an external indication of a lost foot (Salvini-Plawen, 1972). In both taxa there is "a malleable 'haemoskeleton' that can be manipulated by the muscles of the body wall" as described by Morton (1967: 17) for the ancestral condition.

A mantle is present in the sense that the epithelium which covers the outer body secretes a mucoid substance and calcium carbonate and forms a fold over a mantle cavity (Fig. 4A, 4C). Histochemical staining indicates that the cuticle of *Proneomenia* is composed of a glucoprotein complex "tentatively equated with an early mucoid stage in the evolution of the molluscan shell" but it is not the same as chiton cuticle, which is more specialized (Beedham & Trueman, 1968: 443). Both Hoffman (1949) and Salvini-Plawen (1972) considered the spicular part of the chiton cuticle to be homologous to aplacophoran cuticle; however, Stasek (1972: 18) and Stasek & McWilliams (1973) pointed out that the homologous parts may be the spiculose integument of the dorsally turned mantle of the chitons and the nonspiculose mantle integument bordering the foot-fold

FIG. 1. A: Sagittal section, anterior of *Prochaetoderma* sp. The most recently formed radula tooth lies adjacent to the odontoblasts at the blind end of the radula sac. The empty space in the head is that part of the pharynx lying between the paired "jaws" that protect and open the ventral mouth (not shown in section). B: Cross-section through mucoid style lying at anterior end of the style sac in *Chaetoderma nitidulum*. Two digestive cell types line the digestive gland lumen ("Körnerzellen" and "Keulenzellen"; Wirén, 1892). C: Cross-section of anterior end of 100 μm mucoid style projecting from style sac (not shown) and gastric shield in *Falcidens caudatus*; the digestive gland opens into the stomach. Scales in mm.

d	digestive gland	od	odontoblasts
dl	digestive gland lumen	r	radula sac
gs	gastric shield	sr	subradular membrane
j	"jaws"	st	stomach
m	mucoid style		

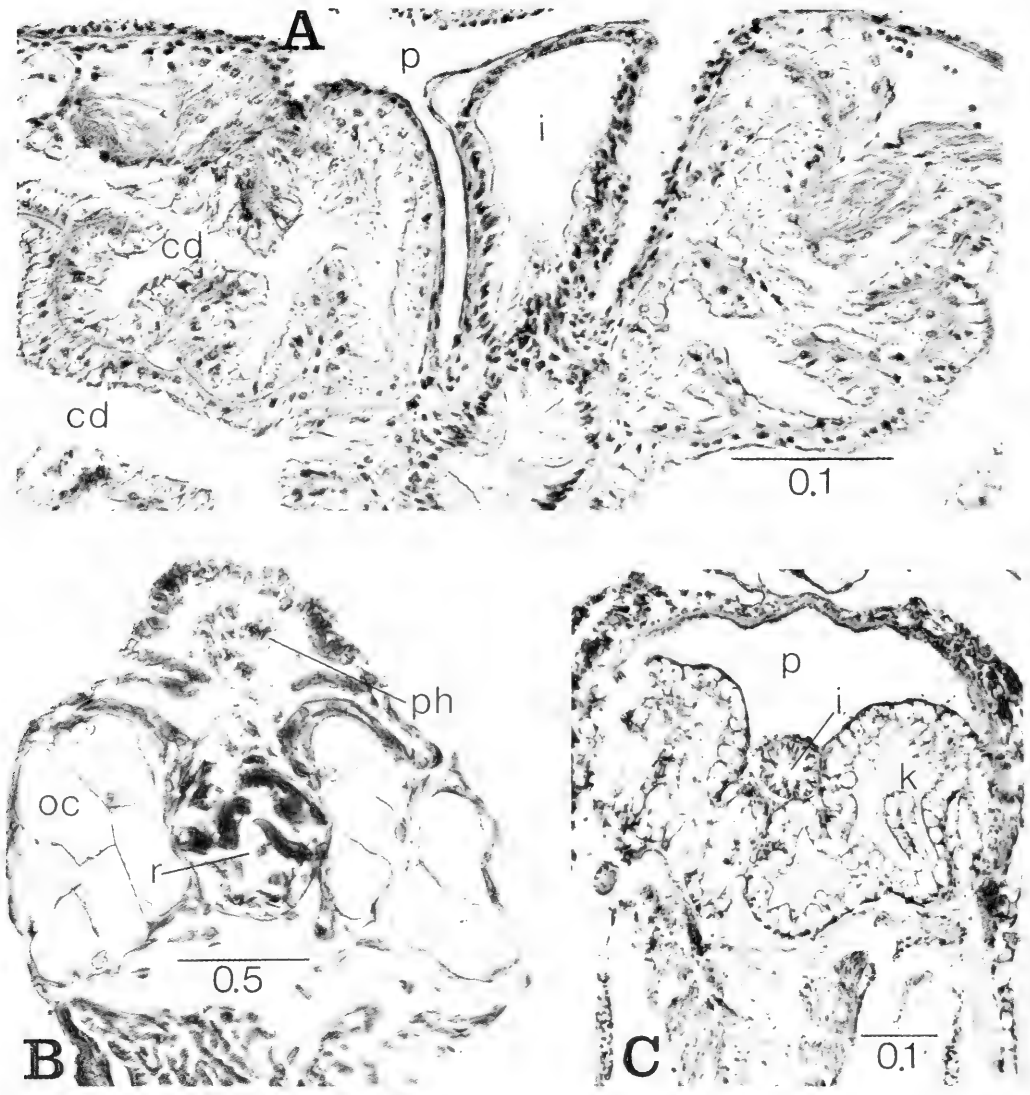

FIG. 3. A: Coelomoducts of *Chaetoderma nitidulum* show great histological similarity to the kidneys of the protobranch *Nucula annulata* (C). Both upper and lower limbs of the C-shaped coelomoducts of *Chaetoderma* are evident, as well as the ventral bend of the intestine. B: Paired odontophore cartilages lie on either side of the radula sac with radula teeth in *Prochaetoderma* sp. C: Kidneys of the protobranch *Nucula annulata*. Scales in mm.

cd	coelomoducts	p	pericardial cavity
i	intestine	ph	pharynx
k	kidney	r	radula sac
oc	odontophore cartilage		

and lying within the ventral groove of the Neomeniomorpha.

The shape of the aplacophorans has greatly affected the extent of the mantle cavity, but typical molluscan characters are still evident in the posterior cavity into which the anus and coelomoducts empty and which contains paired gills in the Chaetodermomorpha. The Neomeniomorpha also retain the mantle cavity in the form of a groove on either side of the vestigial foot.

The nervous system is composed of paired cerebral ganglia and paired lateral and ventral cords with cross commissures, a

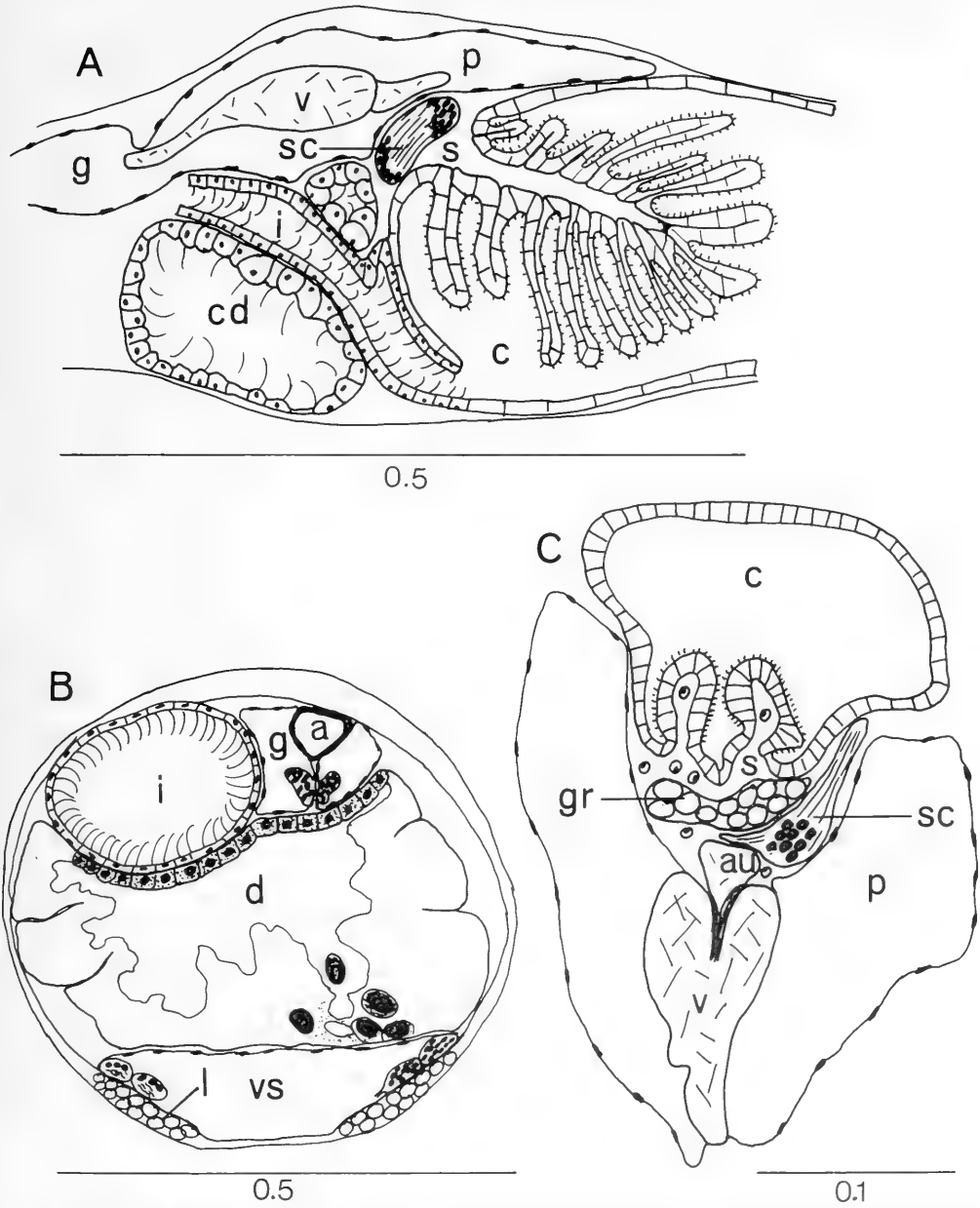

FIG. 4. A: Sagittal section through posterior region of *Falcidens caudatus.* The gonad empties into the pericardial cavity, which is emptied by C-shaped coelomoducts (connection not shown). The intestine, above which lies a suprarectal commissure, bends ventrally to empty into the mantle cavity between a pair of gills. Muscles are not shown. B: Cross-section through the posterior half of a *Chaetoderma nitidulum* juvenile. The gonads, fused in adults, are still paired; the intestine is dorsal. There are 2 types of cell in the digestive gland (cf. Fig. 1B). The ventral sinus is probably homologous to the pedal sinus; a slight thickening of the muscle wall on either side is weakly expressed ventral longitudinal bands causing dorsal bending (Fig. 5). C: Dorsal frontal section through the posterior end of *Falcidens caudatus.* Both the ventricle in diastole and the pericardial cavity are bilobed. Scales in mm.

a	aorta	g	gonad	s	gill sinus
au	auricle	gr	gill retractor	sc	suprarectal commissure
c	mantle cavity	i	intestine	v	ventricle
cd	coelomoducts	l	longitudinal bands	vs	ventral sinus
d	digestive gland	p	pericardial cavity		

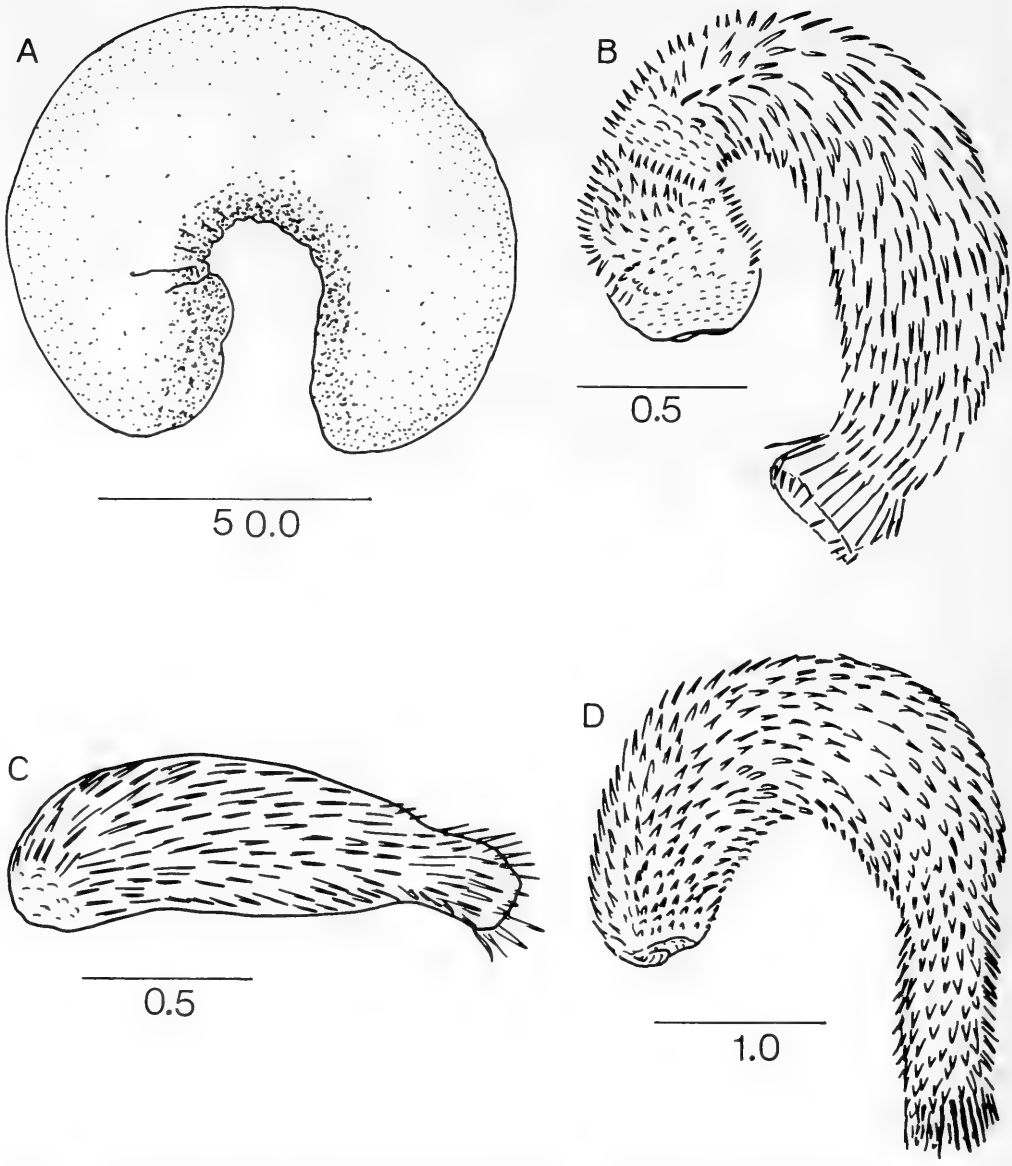

FIG. 5. Flexed body in A: *Neomenia herwigi* Kaiser, 1976, a giant neomeniomorph from 130 m off the Falkland Islands (Kaiser, 1976); B: *Chaetoderma* sp. from 67 m S of Woods Hole, Massachusetts; C: *Prochaetoderma* sp. from 1600 m S of Woods Hole; D: *Falcidens* sp. from 650 m off Cape Hatteras, North Carolina. Scales in mm.

primitive ladderlike plan as in chitons and Monoplacophora, but it is ganglionated rather than medullary (see review in Bullock & Horridge, 1965). The differences in the 2 aplacophoran taxa are given in Salvini-Plawen (1972). There is a typical molluscan buccal innervation and, like chitons, a suprarectal commissure.

Thompson (1960), who observed the development of the late egg, early embryo and settlement stages of *Neomenia,* has published the most comprehensive account of the later development of an aplacophoran, which is remarkably like that of protobranchs and scaphopods and not at all like that of chitons. Baba (1951) described and

illustrated early spiral cleavage for a neomeniomorph.

DISCUSSION

Aplacophora are true mollusks by the present definitions of that phylum, a member of which must express one or more of several diagnostic traits. No single key character defines the phylum (Stasek, 1972). It is unfortunate that the Aplacophora have been considered mollusks chiefly by their superficial resemblance to chitons, for there are few shared characters between them except their archaic nervous systems (the "Amphineura") and primitive mucoidal mantles with spicules ("Aculifera"). Other proposed structural homologies seem highly improbable and have been influenced by the idea that aplacophorans really cannot be considered mollusks unless (1) an affinity to chitons can be shown (Hoffman, 1949) or (2) their position as a stem form of the Mollusca can be proven (Salvini-Plawen, 1969, 1972).

From the structures briefly described in the previous section, the Aplacophora can certainly be included among the Mollusca on the basis of characters shared with other molluscan classes. To place the 2 taxa as subclasses in a single class makes a classification that gives a hierarchical ranking consistent with that of other equally similar molluscan subclasses. The differences between the Chaetodermomorpha and Neomeniomorpha in integument, form of mantle cavity, and digestive system set them apart from each other, but no more so than are prosobranch and opisthobranch gastropods. The differences in nervous systems stressed by Salvini-Plawen (1972: 320, fig. 50) are not as great as the differences among the prosobranch gastropods with their separated or fused pleural and cerebral ganglia and their ladderlike or unconnected pedal cords (Fretter & Graham, 1962). In fact, it is exactly the similarity in nervous systems in the 2 aplacophoran taxa—ladderlike paired lateral and ventral cords which are ganglionated, and a suprarectal commissure (Fig. 4A, 4C)—that shows the close relationship between them; moreover, there is a dorsoterminal sensory organ in both.

Equally important indications of similarity are the connection between gonads, pericardium, and coelomoducts in both taxa and their vermiform shape, conditions unique among the mollusks. The lack of a foot groove in the Chaetodermomorpha is most parsimoniously explained by loss owing to their burrowing habit. It is not clear that the simpler, thinner integument of the Chaetodermomorpha is the more archaic of the 2 taxa (Hoffman, 1949); it could just as well be that their cuticle is a secondarily derived state related to burrowing.

The basis for Salvini-Plawen's 2 aplacophoran classes is the rigid application of Hennig's cladistic methods. The method itself has several serious failings (see Szalay, 1977, for a critique); one is that it precludes finding new phylogenetic relationships from the fossil record. Thus, the newly recognized fossil molluscan class Rostroconchia (Pojeta & Runnegar, 1976), probably ancestral to Pelecypoda and descendant from Monoplacophora, can have no place in a predetermined phylogeny such as devised by Salvini-Plawen (1968, 1969, 1972).

Hennig's method is inappropriate as applied to such a large, diverse group as the Mollusca with an unknown number of extinct and unrecognized ancestral forms. It has led Salvini-Plawen (1972) to propose several evolutionary improbabilities. Consider 2 examples.

First, according to Salvini-Plawen (1972: 284, fig. 35), the molluscan coelom was first (hypothetically) a pair of gonads and a small ductless pericardium. Then the next evolutionary steps were a (hypothetical) joining of gonad to pericardium and development of a pair of coelomoducts emptying the pericardium. Although this is the condition which presently exists in all Aplacophora except *Phyllomenia* (Salvini-Plawen, 1970), it is said to be secondarily derived after a (hypothetical) separation of gonads and pericardium, each with paired ducts, which gave rise to the condition found in all other living mollusks; replication of the coelom then took place in the Monoplacophora. Only in the aplacophoran taxa are the gonads considered again to open into the pericardium in 2 separate evolutionary events.

The original state of the molluscan coelom is conjectural, and its organogenesis is unknown in the Aplacophora and Monoplacophora. However, the large pericardium of the Monoplacophora, Aplacophora, and Polyplacophora, the bilaterality of all coe-

lomic spaces in the former 2, and the paired monoplacophoran ventricles, seem to argue against an original small, single coelomic widening simply to protect the heart. The matter of where the gonads emptied originally in the evolutionary history of the mollusks is left open; it may be that the unique gonad-pericardium connection of the Aplacophora is a derived state arising from their vermiform shape. In that case, *Phyllomenia* would be the 1 genus retaining a more ancestral condition.

The second example of an improbable evolutionary event proposed by Salvini-Plawen (1972) is that the oral shield of the Chaetodermomorpha is a remnant of the molluscan foot. This singular homology forms the basis for making 2 classes of the Aplacophora and relates the occurrence of mucous gland cells that open beside the oral shield in *Chaetoderma* to those along the foot groove in *Proneomenia* (Hoffman, 1949). The chaetoderms are burrowing forms, moving headfirst by hydrostatic action. Gland cells are ubiquitous in the outer epithelium throughout the mollusks and appear wherever lubrication is needed functionally. The specialized innervation (Salvini-Plawen, 1972) and cuticularization of the chaetoderm oral shield seem to discount that it is part of an original gliding sole; one should not argue generalized gland cells beside the oral shield and at the same time specialized innervation to it.

The phylogenetic position of the Aplacophora is not easy to ascertain. There is general agreement that the group is primitive and probably very old geologically. There must have been innumerable trials at calcification of a mucoid integument among precambrian forms for which we now have no direct evidence. The Aplacophora were the only forms to survive that did not produce a point—or points—of calcification from which a shell could grow; each calcareous spicule is produced by a single cell (Hoffman, 1949). However, the aplacophoran integument with its spicules, although primitive, should not be considered identical with the original molluscan integument, for it, too, must be the result of selection.[2]

The most interesting phylogenetic information to be obtained from the Aplacophora is that several characteristic molluscan structures have evolved independent of a shell. The notion that the adaptive functional possibilities of the mantle cavity have led to great diversity in the mollusks is upheld, for in becoming worms, aplacophorans have not exploited this structure and retain quite a uniform morphological pattern. However, their unique specialization of a worm shape has been well adapted to the deep sea benthos.

ACKNOWLEDGEMENTS

The conclusions in this paper are based on studies made of organisms dredged and sorted under National Science Foundation grants GB 6027X, GA 31105 and GA 36554; the studies were initiated under a grant from the Radcliffe Institute. I thank Rudolf Scheltema, Kristian Fauchauld, Judith Grassle, and Alison Stone Ament for carefully reading the manuscript. Peter Riser and Patricia Morse kindly provided the opportunity to observe a living neomeniomorph on a recent visit to the Northeastern University laboratory at Nahant, Massachusetts.

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MALACOLOGIA, 1978, 17(1): 111-115

GENETIC STUDIES ON *BIOMPHALARIA STRAMINEA*: OCCURRENCE OF A FOURTH ALLELE OF A GENE DETERMINING PIGMENTATION VARIATIONS

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ABSTRACT

Three alleles of a gene regulating pigmentation in *Biomphalaria straminea* have been described. Studies on their relationship to a 4th allele of the same gene, determining a phenotype in which black spots occur in the mantle but body and eyes lack black pigment, are reported. The spotted-mantle allele is recessive to wildtype and blackeye alleles, dominant over albino.

Occurrence of 3 alleles of a gene determining pigmentation variations in *Biomphalaria glabrata* (Say) was reported by Richards (1967). These variations, which show simple Mendelian inheritance, have provided excellent markers for genetic studies. Three similar pigmentation phenotypes (black wildtype pigmentation, blackeye, and albino) also occur in *Biomphalaria straminea* (Dunker) determined by 3 alleles (Richards, 1972, 1975). This has facilitated studies on variations in susceptibility to infection with *Schistosoma mansoni* in *B. straminea.* A 4th pigmentation phenotype, lacking black pigment in foot and eyes but with black mantle spots was observed in a colony of *B. straminea* (Richards, 1972). Studies on the genetics of this phenotype are here described.

MATERIALS AND METHODS

In 1971 a shipment of *B. straminea* from Sete Lagoas, Minas Gerais, Brasil, was received from Dr. Lobato Paraense. This sample of about 30 surviving snails included both albino and blackeye pigmentation phenotypes (Richards, 1975). After isolating some of the snails of each pigment type the remainder were separated into an albino and a blackeye population in two jars (Fig. 1). When the albino colony was examined several months later, it was noted that some of the descendants of the original population had black pigmented mantle spots, although as in the albinos the eyes and body lacked black pigment.

Thirteen (only 3 included in Fig. 1) of these spotted mantle snails were isolated as juveniles and observed through several generations of selection and selfing, and controlled mating experiments were performed. Snails from which offspring by selfing had previously been obtained were mated for 1 week and then reisolated.

The 3 pigment alleles similar to those in *B. glabrata* are: (C) wildtype with black-pigmented body, eyes, mantle collar, and mantle spots; (c^B) blackeye with black-pigmented eyes and mantle spots but lacking black body or mantle collar pigment; and (c) albino with no black pigment. The C is dominant, c^B recessive to C but dominant over c, and c recessive. The 4th allele in *B. straminea* is designated (c^M), with black mantle spots but lacking black body, eyes, or mantle collar pigment (Fig. 2).

RESULTS

Albino and blackeye stocks established by isolation of snails from the original sample in 1971 and selected for homozygosity have bred true for these phenotypes for 5 years. A wildtype black-pigmented laboratory colony of *B. straminea* at The National Institutes of Health has bred true for this phenotype during 15 years of

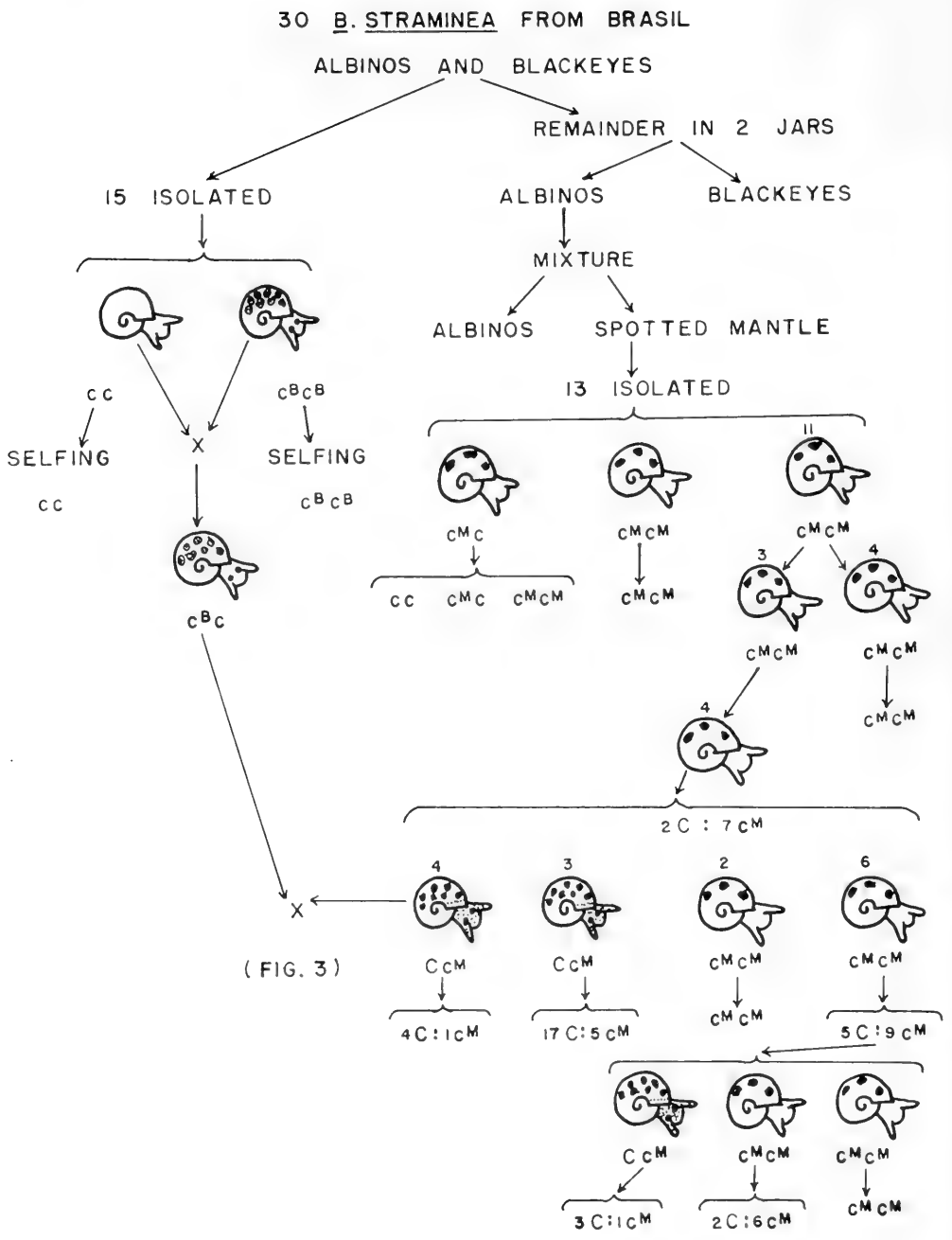

FIG. 1. Origin of spotted mantle *Biomphalaria straminea.* Many of the snails isolated and observed are omitted from the diagram. Phenotypes are depicted as in Fig. 2.

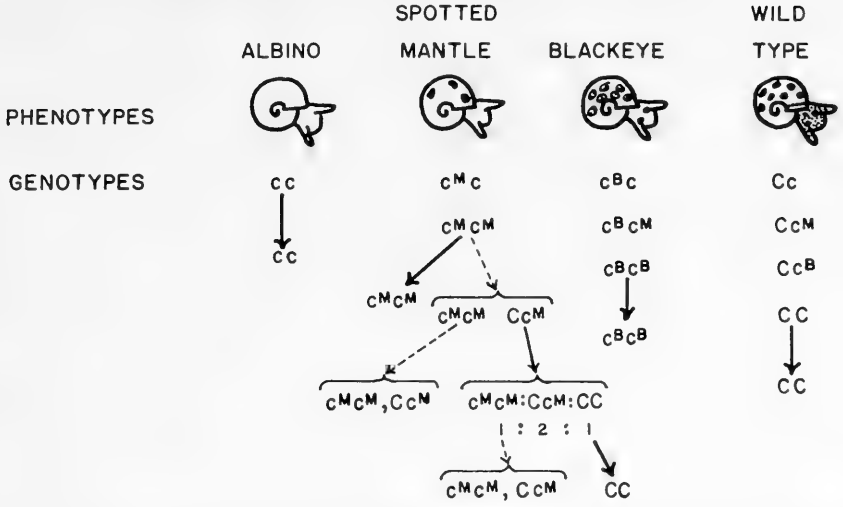

FIG. 2. The 4 pigmentation phenotypes and their genotypes. Heavy arrows indicate true breeding lines.

observation. When snails of the spotted mantle phenotype were observed in the interbreeding colony of albino *B. straminea*, 13 were isolated. Eleven of these produced only spotted mantle descendants (or spotted-mantle and albino descendants if heterozygous) for as many generations as observed. Albino lines derived from these snails bred true for the albino phenotype. Among the descendants of 2 of the spotted mantle snails originally isolated (including #11 in Fig. 1) sporadic wildtype snails occurred. In each case when a wildtype offspring of a spotted mantle snail was isolated, it proved to be heterozygous (Cc^M), producing offspring of both C and c^M phenotypes in approximately 3:1 ratio. Homozygous wildtype snails derived from these heterozygotes bred true for the wildtype phenotype thereafter.

The relationships among the 4 pigment alleles are illustrated in Fig. 3, which includes 2 crosses, each involving all 4 alleles but in different mating combinations. From the cross $c^Bc \times Cc^M$ 14 F_1s were obtained, in phenotypic ratio $6C:4c^B:4c^M$. From the cross between 2 of the F_1s, $Cc \times c^Bc^M$, 39 offspring were obtained, in phenotypic ratio $22C:8c^B:9c^M$. Heterozygotes from these and other crosses were selfed with the following results: 16 Cc^M snails yielded $108C:43c^M$ offspring (2.51:1) 12 c^Bc^M snails yielded $85c^B:30c^M$ offspring (2.83:1); and 19 c^Mc snails yielded $80c^M:49c$ offspring (1.63:1).

Although blackeye pigment phenotype *B. straminea* typically produced mantle spots, these generally have less dense black pigmentation than wildtype *B. straminea*. *Biomphalaria straminea* snails of the spotted-mantle phenotype have fewer mantle spots (sometimes only 1) but these have dense black pigment like the wildtype *B. straminea*. This is particularly evident when a blackeye-spotted-mantle (c^Bc^M) heterozygote is selfed; the blackeye offspring have many pale mantle spots, while the spotted-mantle offspring have fewer but darker black spots.

Like the wildtype and blackeye pigment types in B. *glabrata* (Richards, 1972), these phenotypes and the spotted-mantle in *B. straminea* have a network of cells with black pigment lining the hemocoel. These pigmented cells, like the mantle spots, are fewer in number in the spotted mantle snails than in the wildtype.

DISCUSSION

Data, including that from other selfed *B. straminea* snails and crosses in addition to those presented here, suggest that the spotted-mantle phenotype is determined by a 4th allele of the gene for pigmentation, and that this allele is recessive to wildtype

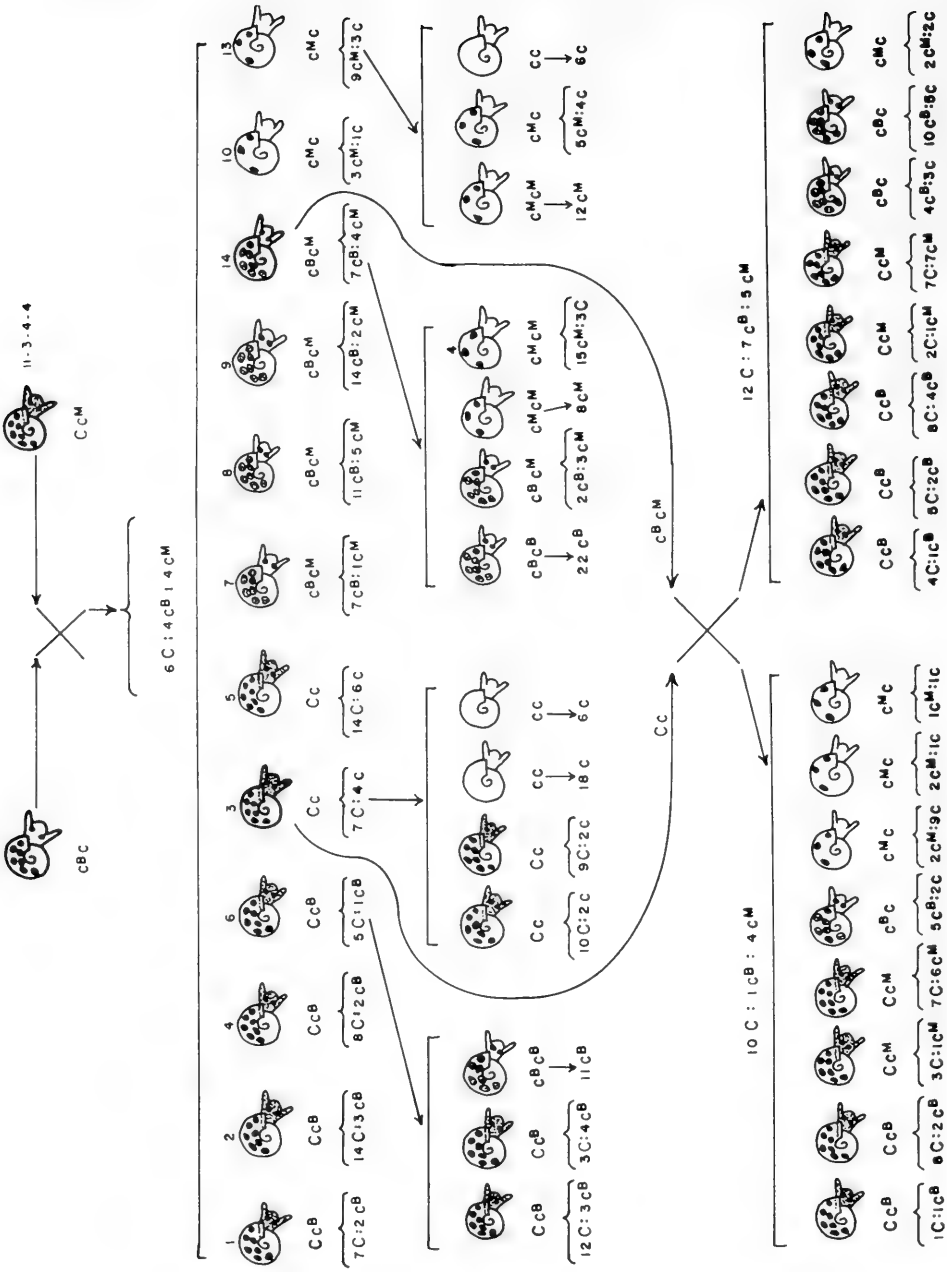

FIG. 3. Two crosses, each involving all 4 pigment alleles, but in different combinations.

and blackeye alleles, dominant over albino.

Spotted mantle is not as dependable a genetic marker as the other 3 pigment alleles. In some homozygous spotted-mantle lines, the mantle spots are distinguishable in juvenile snails and spotting occurs in essentially all snails. In other homozygous spotted-mantle lines, however, due either to delayed development or minimal expression of spotting, some snails cannot be distinguished from albinos.

In both aquarium populations and clonal stocks maintained by selfing, homozygous wildtype, blackeye, and albino stocks of *B. straminea* have bred true through many generations. Eleven of 13 juvenile spotted-mantle snails originally isolated bred true for the spotted mantle, or if $c^{M}c$ heterozygotes, produced only spotted-mantle and albino offspring. The other 2 gave rise to spotted-mantle stocks in which wildtype snails continued to appear sporadically. In some spotted mantle stocks it is possible that the pigment gene is unstable or that the pigmentation is modified by other genetic factors. If a similar change to blackeye or albino cccurs, this has not been recognized.

Several different types of black pigmentation in *B. glabrata* have been reported (Richards, 1969, 1972). Occurrence of the spotted-mantle phenotype in *B. straminea* suggests an additional distinction between eye and mantle spot pigmentation. Based on the tissues involved and the distribution of pigment granules at least 5 forms of black pigmentation can be distinguished: mantle spots consisting of groups of epithelial cells with concentrated pigment, a loose reticulum of pigmented cells lining the hemocoel, pigmented eyes, diffuse pigment granules in the mantle epithelium (in *B. glabrata*), and pigment granules in the connective tissue of mantle collar and head-foot regions. It remains to be determined if these pigment manifestations represent chemical differences or merely genetic regulation of the locations of pigment formation.

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MALACOLOGIA, 1978, 17(1): 117-124

LE SYSTÈME CIRCULATOIRE ET LE JEU DES SIPHONS CHEZ *DONAX TRUNCULUS,* MOLLUSQUE LAMELLIBRANCHE

Marcel Mouëza[1] et Liliane Frenkiel[2]

RÉSUMÉ

Après avoir étudié le système circulatoire de *Donax trunculus* Linn. à l'aide d'injections directes et récurrentes, les expériences de Chapman & Newell (1956) ont été reprises sur ce Mollusque, ainsi que des expériences complémentaires. Celles-ci démontrent que, contrairement à ce qui a lieu chez *Scrobicularia plana*, l'intervention d'un flux sanguin extérieur est nécessaire à l'extension des siphons chez *Donax trunculus.*

INTRODUCTION

Dans son étude sur les Tellinacea, Yonge (1949) postule que l'extension des siphons a lieu par un afflux de sang de la circulation générale—forcé dans les espaces hémocoeliens des parois siphonales—tandis que la rétraction met en oeuvre les muscles rétracteurs très développés dans cette super-famille. Chapman & Newell (1956), ayant effectué une série d'expériences sur *Mya arenaria* et *Scrobicularia plana*, estiment, dans le cas de cette dernière espèce, et contrairement à Yonge, que le sang extérieur aux espaces hémocoeliens des parois siphonales n'intervient pas dans les mouvements des siphons. Pour eux, l'extension est conditionnée par le jeu des muscles radiaires. Ces affirmations contradictoires nous ont amenés à reprendre l'analyse du jeu des siphons chez un Tellinacea, *Donax trunculus* Linn., après avoir effectué une investigation méthodique du système circulatoire.

TECHNIQUES D'ÉTUDE

La resection de la coquille a été pratiquée au disque à séparer monté sur tour rapide. Réalisée entre les adducteurs antérieur et postérieur et le rétracteur des siphons, elle permet de libérer le manteau. Effectuée autour de la charnière, elle permet d'accéder au coeur après avoir découpé les expansions suprabranchiales et le péricarde.

Des injections de bleu d'aniline, ou d'une solution préparée selon la technique de Ko Bun Hian (1973) ont été pratiquées dans le ventricule et la veine viscéro-pédieuse. L'exploration du système lacunaire a été complétée par des injections dans le velum, les siphons et la lacune palléale située en arrière du muscle rétracteur des siphons.

Des ligatures des siphons ont été réalisées sur des animaux placées à des températures comprises entre 0°C et 5°C.

Des destructions partielles ou totales du coeur ont été réalisées; la lacune postérieure au rétracteur des siphons a été supprimée par destruction de sa paroi externe au thermocautère. Les résultats de ces diverses interventions ont été observées après un délai de 24 heures.

Les techniques histologiques employées pour l'étude de la structure des siphons sont classiques: fixation au Bouin-Hollande, coloration par une variante du trichrome de Gomory citée par Gabe (1968) et par l'Azan.

OBSERVATIONS

Système Circulatoire

Le coeur, situé en arrière et hors de la masse viscérale, est enveloppé par le péricarde en relation avec des glandes péricardiques bien développées que White (1942) n'a pas trouvées chez *Donax vittatus*.

Sensiblement fusiforme, le ventricule qui possède de fortes parois musculaires, donne

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naissance à l'aorte antérieure (Fig. 1). Les oreillettes en position latéro-ventrale, communiquent entre elles au niveau de leur partie antérieure, sous le ventricule. Chaque oreillette communique sur sa face ventrale avec un gros tronc vasculaire, somme des vaisseaux branchiaux, et sur sa face interne, avec le ventricule.

Le système artériel débute par l'aorte antérieure qui passe sur la paroi dorsale de l'estomac, plonge dans la cavité viscérale, à gauche de l'oesophage, et donne naissance à toute une série d'artères dont le trajet a été figuré pour l'essentiel. Deshayes (1844) signale qu'il n'a pas réussi à faire pénétrer l'injection dans l'aorte postérieure. De nombreuses injections ne nous ont pas permis de mettre en évidence une vascularisation partant de la région postérieure du ventricule, ni d'artère récurrente du système artériel antérieur.

Le système veineux est aussi développé que le système artériel, dense dans la masse viscérale, particulièrement au niveau de l'estomac et de la glande digestive. Son collecteur principal, la grosse veine viscéro-pédieuse s'achève dans le sinus veineux. Ce dernier communique avec le rein par un orifice situé entre les deux connectifs cérébro-viscéraux au niveau où ils sortent de la masse viscérale.

Les injections dans les siphons, dans les lacunes palléales situées en arrière du rétracteur des siphons, dans les lacunes du bord palléal en avant du muscle cruciforme, concordent à démontrer l'existence d'un vaste réseau de lacunes périphériques, communiquant entre elles, dont 2 sont importantes. La première, impaire, située entre l'adducteur antérieur, le rétracteur antérieur et le protracteur (Mouëza & Frenkiel, 1974, fig. 1), communique en bas, en avant avec les lacunes du manteau viscéral, en arrière avec le vaisseau supérieur des branchies. Elle entre aussi en relation, en haut, avec les lacunes des bords du manteau. La seconde lacune, paire, située en arrière du rétracteur des siphons, communique largement avec les siphons, les lacunes du bord palléal, les branchies, le rein, et, par son intermédiaire, le sinus veineux. Les lacunes du bord palléal forment un système qui court tout autour du manteau. Elles communiquent avec les lacunes du siphon inhalant (Fig. 3), en arrière du muscle cruciforme, ainsi qu'avec la lacune impaire située en arrière de l'adducteur antérieur. Elles sont en outre en relation avec la lacune située entre les dents de la charnière, ainsi qu'avec le réseau lacunaire des adducteurs antérieur et postérieur.

Les Siphons

Les siphons des Tellinacea sont entièrement séparés et très mobiles, car ils proviennent, selon Yonge (1957), de la soudure des plis palléaux internes et, de ce fait, ne possèdent pas de tentacules. Ils laissent donc pénétrer les dépots du fond, sans discrimination, dans la cavité palléale. Cet état de fait se trouverait réalisé chez les Donacidae pour *Donax vittatus* (Yonge, 1949) et *Egeria radiata* (Purchon, 1963). Mais chez *Donax gouldi* (Pohlo, 1967) et *D. denticulatus* (Wade, 1969), le siphon inhalant est pourvu d'une couronne de tentacules qui joue un rôle de filtre. Tel est aussi le cas de *D. trunculus* (Fig. 2).

La paroi du siphon inhalant a une structure qui se retrouve, à quelques variantes près, dans tout le groupe des Tellinacea, structure décrite par Rawitz (1892) chez *Psammobia vespertina*, puis par Yonge (1949) chez *Scrobicularia plana* et *Donax vittatus*. Depuis, Chapman & Newell (1956) ont signalé que les couches de muscles circulaires décrites par Yonge sont en réalité formées de conjonctif riche en fibres collagènes et pauvres en cellules musculaires. Ces fibres, qui, en fait, ne sont pas circulaires forment un lacis et s'insèrent obliquement sur les épithéliums interne et externe. Chez *Donax trunculus* (Fig. 4-8), la paroi du siphon inhalant est constituée par 2 couches musculaires épaisses, L_1 et L_2, entourées par les assises conjonctives C_1, C_2 et C_3 contenant de rares fibres musculaires. Une couche de fibres musculaires longitudinales L_3 s'individualise dans la couche C_3 au contact de l'épithélium externe. Une couche similaire existe dans la couche C_1 au contact de l'épithélium interne, mais est moins bien individualisée. Des cloisons radiaires—muscles et fibres collagènes—courent d'un épithélium à l'autre. L'assise C_4, bien individualisée chez *Scrobicularia plana*, ainsi que chez *Donax vittatus* (Yonge, 1949), est irrégulière chez *D. trunculus*.

La répartition des hémocoeles est sensiblement différente dans un siphon rétracté ou en extension. Dans le siphon rétracté (Fig. 6) elles sont limitées à la couche C_2. Les hémocoeles longitudinales dorsale et ventrale, décrites par Duval (1963) chez

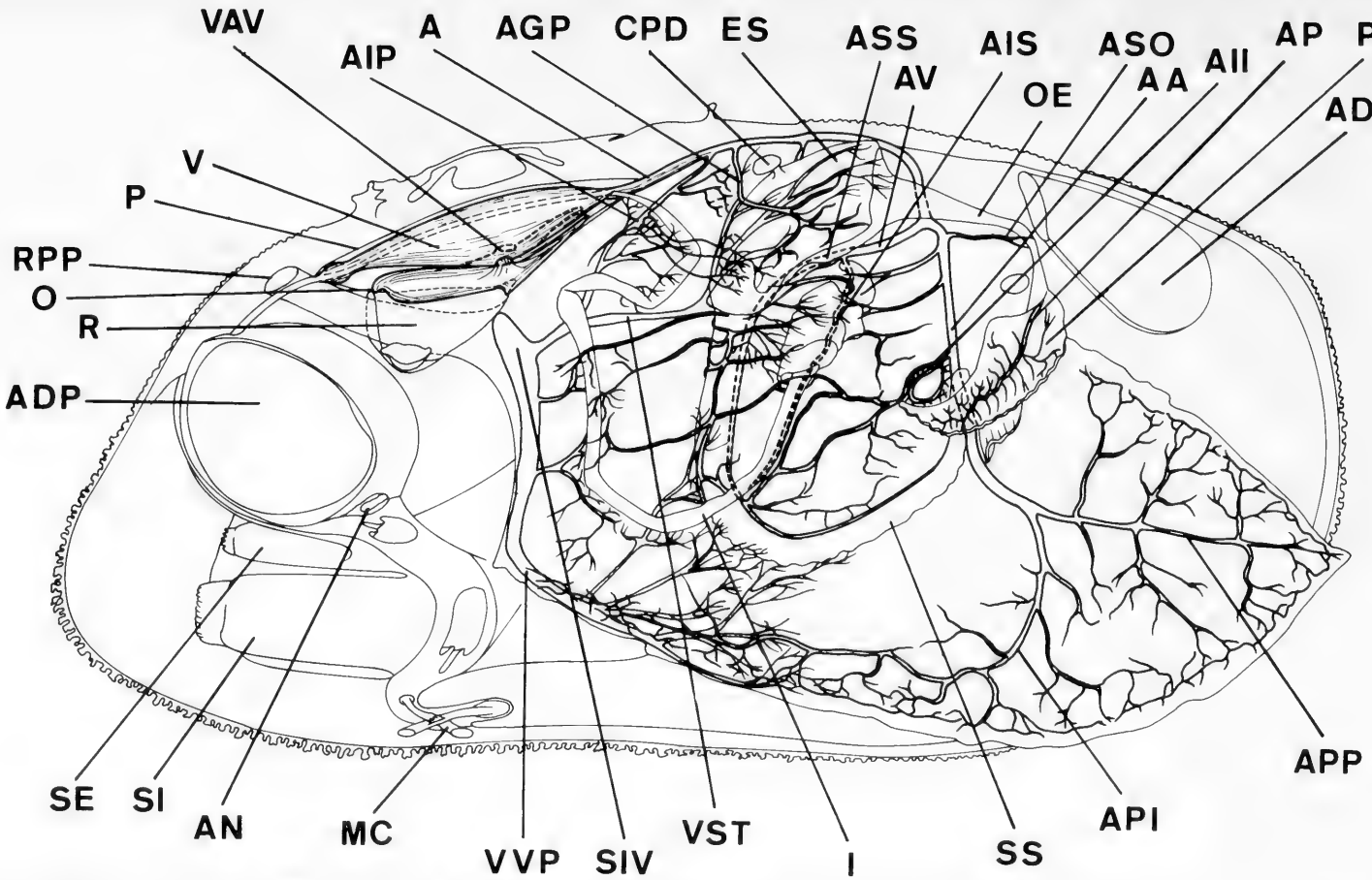

FIG. 1. Système circulatoire de *Donax trunculus*. A, aorte antérieure; AA, artère antérieure; ADA, adducteur antérieur; ADP, adducteur postérieur; AGP, artère gastrique postérieure; AII, AIP, AIS, artères intestinales inférieure, postérieure et supérieure; AN, anus; AP, artère des palpes; API, APP, artères pédieuses inférieure et principale; ASO, artère sous-oesophagienne; ASS, artère du sac du stylet; AV, artère viscérale; CPD, caecum postéro-dorsal; ES, estomac; I, intestin; MC, muscle cruciforme; O, oreillette; OE, oesophage; P, péricarde; PA, palpe; R, rein; RPP, rétracteur postérieur du pied; SE, siphon exhalant; SI, siphon inhalant; SIV, sinus veineux; SS, sac du stylet; V, ventricule; VAV, valvule auriculo-ventriculaire; VST, veine stomacale; VVP, veine viscéro-pédieuse.

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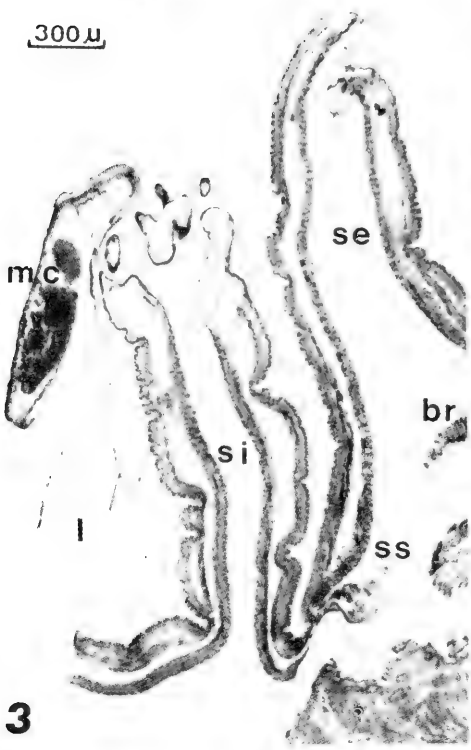

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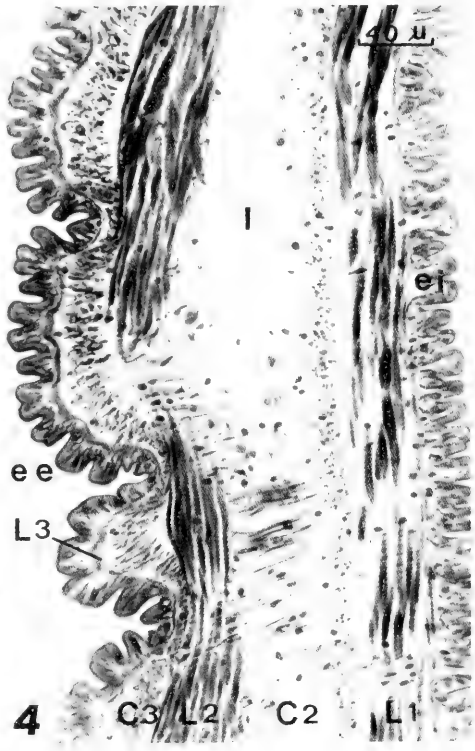

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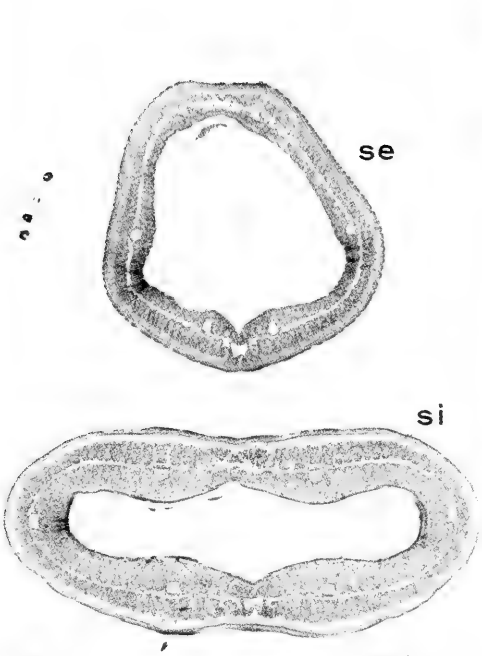
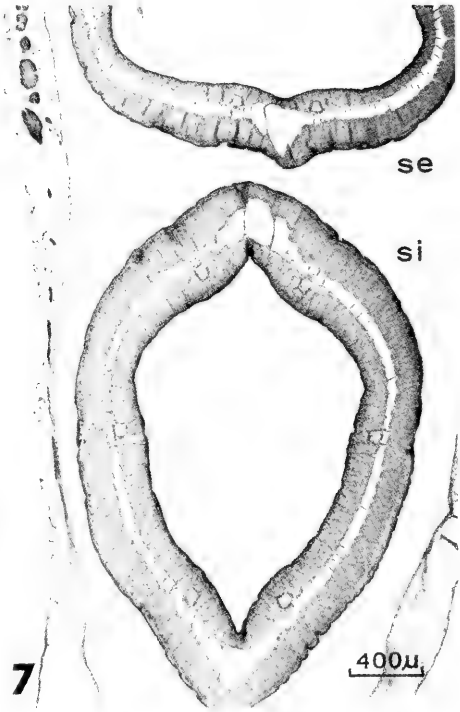
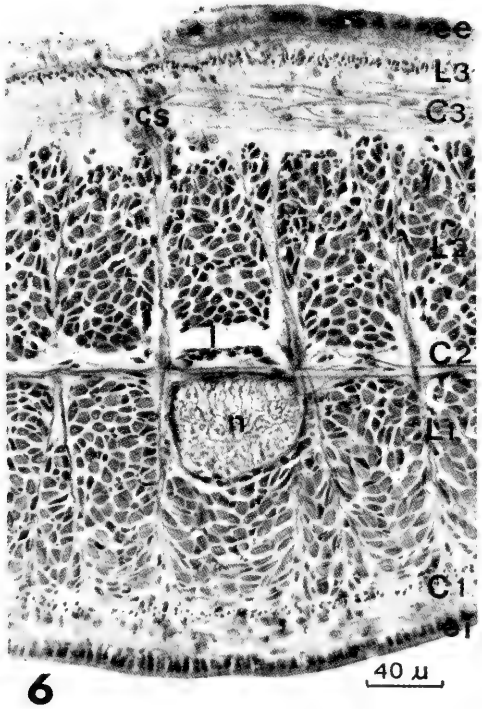
FIG. 2. Extrémité du siphon inhalant montrant la lacune périphérique à la base des tentacules.
FIG. 3. Coupe parasagittale des siphons montrant les relations des lacunes des siphons avec celles du velum. La coupe passe par les lacunes dorsale et ventrale du siphon inhalant.
FIG. 4. Coupe longitudinale de la paroi du siphon inhalant. L'épithélium interne se plisse régulièrement tandis que l'épithélium externe présente un plissement mineur produit par les muscles L_3 et un plissement majeur produit par les muscles L_2. La répartition des faisceaux musculaires est à comparer avec celle observée sur coupe transversale (Fig. 6 et 8).

Abréviations utilisées dans les figures 2 à 8: br, branchie; C_1, C_2, C_3, couches conjonctivo-musculaires circulaires; cs, cellules sensorielles; ee, épithélium externe; ei, épithélium interne; l, lacune; L_1, L_2, L_3, couches musculaires longitudinales; mc, muscle cruciforme; mr, muscle radiaire; n, nerf; se, siphon exhalant; si, siphon inhalant; ss, septum siphonal; t, tentacules siphonaux.







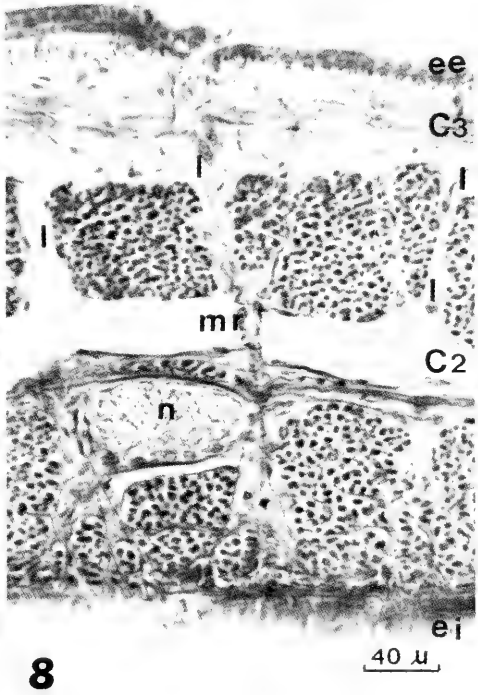


FIG. 5. Siphons exhalant et inhalant rétractés.
FIG. 6. Paroi du siphon inhalant rétracté.
FIG. 7. Siphons exhalant et inhalant en extension.
FIG. 8. Paroi du siphon inhalant en extension.
Abréviations: voir sous Fig. 4.

plusieurs espèces, mais que cet auteur n'a trouvées chez *D. trunculus*, sont pourtant bien développées (Fig. 5 & 7). A l'extrémité des siphons, elles s'ouvrent largement dans l'hémocoele circulaire, qui occupe toute l'épaisseur de la paroi siphonale dont la musculature est réduite et les différentes couches musculaires non individualisées à ce niveau (Fig. 2). L'hémocoele circulaire qui occupe une place importante à la base des siphons, se résoud à leur extrémité dans les tentacules. Chez un animal anesthésié, dont les siphons sont en extension partielle, des expansions de l'hémocoele circulaire pénètrent le long des faisceaux de muscles radiaires (Fig. 8). Lorsque le siphon en extension a été ligaturé à sa base, on met en évidence des lacunes dans la couche C_3 au contact de la couche L_2. Ces lacunes communiquent avec les hémocoeles de la couche C_2 par l'intermédiaire des lacunes radiaires. Le siphon exhalant a une paroi plus mince que celle du siphon inhalant. Construite sur le même plan, elle comporte les mêmes couches musculaires et conjonctives, cependant moins bien individualisées.

L'observation montre que l'extension des siphons est progressive, sans à-coups, comme l'ont noté Chapman & Newell (1956), au contraire de la rétraction qui peut être rapide, voire brusque. Normalement, *Donax trunculus* ne sort jamais ses siphons au maximum de leur longueur. Toutefois, lorsque l'animal est placé dans des conditions défavorables, en particulier dans une eau insuffisamment oxygénée où la nourriture se fait rare, les siphons deviennent très longs et turgescents. Trevallion (1971) a montré expérimentalement que des conditions défavorables entrainaient les mêmes effets chez *Tellina tenuis*. Dans les conditions normales d'existence, la turgescence peut intervenir chez *D. trunculus* à la fois pour provoquer l'extension et l'élargissement des siphons, surtout du siphon inhalant. En augmentant le diamètre de ses siphons, du simple au double, *D. trunculus* renforce les moyens qu'il a de s'opposer aux déplacements sous l'action des courants ou des vagues. Avec son pied et ses siphons étalés, il offre le maximum de résistance au transport (Mouëza, 1972). Ceci est en contradiction avec l'idée de Yonge (1949), selon laquelle le volume sanguin n'est pas suffisant pour permettre, chez les Tellinacea, à la fois l'extension du pied et des siphons, et avec la théorie de Chapman & Newell (1956) qui lient l'extension des siphons à l'amincissement de leur paroi. De plus le fait qu'un animal anesthésié par le froid puisse garder ses siphons largement sortis, s'oppose à l'idée selon laquelle la contraction des muscles radiaires est le moteur principal de l'extension.

EXPÉRIMENTATION

Après ablation d'une valve de la coquille, le siphon exhalant est à peine sorti et le siphon inhalant s'allonge peu—5 mm environ—avec une forte tendance à se redresser du côté de la valve absente. Le jeu des siphons apparaît donc faussé, aussi a-t-on eu recours à une autre méthode pour montrer sans équivoque que la pression de l'eau contenue dans la cavité palléale n'intervient pas dans l'extension des siphons. Il suffit de réséquer la plus grande partie de la valve en laissant en place les surfaces d'insertion des adducteurs, ainsi que celle des rétracteurs des siphons, ce qui équivaut à l'ablation de la valve. Dans de telles conditions, les siphons sortent largement et leur jeu extension-rétraction est normal.

La destruction du coeur a été partielle—suppression du ventricule, suppression des oreillettes—ou totale. Dans le premier cas, le jeu des siphons demeure normal, tandis que dans le dernier, il n'y a jamais extension des siphons.

La ligature des siphons a été pratiquée sur l'un ou l'autre ou sur les 2 siphons à la fois. Dans le cas de la ligature d'un seul siphon, quel qu'il soit, le jeu de l'autre demeure normal. La ligature des 2 siphons à la fois entraine les mêmes effets que ceux observés dans le cas de ligature d'un seul siphon. La ligature est suivie d'une rétraction qui met en jeu seulement la partie proximale, la partie distale isolée par la ligature gardant même longueur et même diamètre. L'allongement reste possible; il a lieu sans à-coup et de manière progressive, par étirement de la partie proximale. Dans le cas d'une ligature du siphon inhalant en extension sub-totale, lorsque la ligature est proche de la coquille, l'animal ne peut que difficilement rentrer la partie distale, qui a gardé une longueur constante. Au prix d'une rétraction forcée, il peut n'en laisser dépasser qu'une très faible portion, mais il doit pour cela, rétracter la partie antérieure du siphon, en avant du chiasma du muscle cruciforme, ce qui n'est pas normal chez les *Donax*.

Après destruction de la lacune située en arrière du rétracteur des siphons, les siphons ne s'allongent plus du tout, même au cours de l'enfouissement qui reste possible.

DISCUSSION

Chapman & Newell (1956) estiment que le jeu des siphons chez *Scrobicularia plana* s'explique par l'action des fibres musculaires radiaires dont la contraction provoque l'élongation par redistribution du liquide antagoniste dans les espaces hémocoeliens et l'amincissement des parois siphonales. L'extension, pour eux, apparait indépendante du flux sanguin et les parois gardent un volume constant à tous les stades du jeu des siphons, de telle sorte que, lors de la rétraction, l'espace hémocoelien longitudinal qui participe au stockage du liquide antagoniste s'élargit.

Chez *Donax trunculus* le jeu des siphons présente une certaine analogie avec celui de *Scrobicularia plana*, l'accent doit cependant être mis sur les différences. L'expérimentation amène à tenir pour certaine l'intervention du flux sanguin extérieur aux siphons, et, pour cela, le pompage du coeur semble obligatoire et l'intégrité des lacunes situées en arrière des rétracteurs des siphons doit être conservée. Ceci suppose une large communication entre ces lacunes et les siphons, communication qui existe effectivement. L'injection du système artériel permet exceptionnellement de mettre en évidence ces relations, ce qui, par contre, peut être réalisé à partir d'injections dans le système lacunaire palléal ou dans la veine viscéropédieuse. L'histologie apporte la preuve incontestable des liaisons entre les lacunes postérieures au rétracteur des siphons et celles des siphons inhalant et exhalant, ainsi que des liaisons existant entre les lacunes du velum et du siphon inhalant. Enfin la meilleure preuve d'un apport extérieur de sang réside dans le fait qu'après ligature, le tronçon proximal s'allonge et repousse progressivement le bout distal qui garde une longueur constante.

Lors de la rétraction, contrairement à ce qui a été observé chez *Scrobicularia plana*, les espaces hémocoeliens de la couche C_2 sont plats. Seules les hémocoeles longitudinales dorsale et ventrale gardent une certaine importance. Dès que l'extension a lieu, les espaces hémocoeliens deviennent turgescents. Les lacunes de la couche C_2 augmentent de volume; celles de la couche C_3 deviennent apparentes, l'épithélium externe se déplisse. Le volume sanguin admis peut entrainer ou non une augmentation du diamètre, très variable à longueur égale. L'épithélium interne du siphon inhalant offre peu de possibilités de déplissement. Il se pourrait que le diamètre de la lumière soit quand même accru par le jeu des fibres radiaires comme le postulent Chapman & Newell. Il n'a pas été possible d'apporter d'arguments sur ce point. L'épithélium interne du siphon exhalant possède la même structure que l'épithélium externe, argument en faveur d'une possiblité plus grande d'extension de sa lumière.

L'extension résulterait chez *Donax*, dans un premier temps, d'un relâchement des fibres musculaires longitudinales. Il existe une position d'équilibre, nécéssitant une dépense d'énergie minimale, au cours de laquelle les siphons et le pied sont légèrement allongés, position dans laquelle on trouve fréquemment les animaux sur le sédiment. Le deuxième temps de l'allongement est actif, et le coeur semble intervenir dans ces mouvements de fluide. Le fait que la destruction partielle du coeur n'entraine pas de disfonctionnement demeure inexplicable dans l'état actuel. L'extension sans à-coups, qui peut être lente ou rapide s'explique par la présence d'un réservoir. De fait, la destruction des lacunes situées en arrière des rétracteurs des siphons empêche toute extension. Le jeu des muscles radiaires et longitudinaux intervient, par contre, pour permettre les mouvements des siphons et leur rétraction. Celle-ci, rapide et brusque, se conçoit aussi par la présence d'un réservoir, prêt à recevoir le sang brutalement chassé des siphons sous l'action de la contraction des muscles.

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ABSTRACT

The circulatory system of *Donax trunculus* Linn. is described, having been investigated by means of direct and recurrent injections, together with experiments on the movements of the siphons using Chapman & Newell's (1956) methods. Our experiments show that a flow of blood into the siphons is necessary for their extrusion in *D. trunculus*. This is different in *Scrobicularia plana*.

MALACOLOGIA, 1978, 17(1): 125-142

PRELIMINARY CHARACTERIZATION OF THE SECRETION OF THE ACCESSORY BORING ORGAN OF THE SHELL-PENETRATING MURICID GASTROPOD *UROSALPINX CINEREA*[1]

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ABSTRACT

Results of a preliminary characterization of the secretion of the accessory boring organ (ABO) of the shell-boring, predatory, prosobranch gastropod *Urosalpinx cinerea follyensis* Baker are reported. The secretion was examined on ABOs of live snails normally extended through boreholes in valve models, and was studied ultrastructurally, histochemically, and physiologically. Hydrogen and chloride ions were monitored with microelectrodes.

The ABO emerged from the foot of the snail bearing on its free distal surface most of the secretion to be used during the subsequent period of dissolution; only small amounts were released during ABO activity in the borehole. Blots of secretion from large snails touched to fragments of cover glass measured 1.5 to 2.0 mm in diameter, and contained an estimated 1 to 2 μg of dry material. Roughly a 3rd of the volume of the secretion evaporated during drying on blots, leaving a highly hygroscopic residue. Ultrastructurally the secretion contained minute particles resembling crystals and secretion granules.

No acid mucopolysaccharides were identified in the secretory cells of ABOs stained with alcian blue and astra blue following fixation in several separate fixatives. The periodic acid-Schiff reaction was strongly PAS-positive.

ABOs heated to 80°C did not etch. Those in a solution of papain produced no etchings, whereas others immersed in a trypsin solution etched conspicuously. The range of pH of the secretion was 3.8 to 4.0, whereas secretion free of seawater on the gland did not fall below 5.2. Maximum chloride ion concentration in the secretion ranged from 0.79 M to 1.71 M. Chloride ion concentration increased stepwise from the time of extension of the ABO generally to withdrawal from the borehole. Qualitative analysis of dry secretion by energy dispersive x-rays confirmed the presence of chloride and also disclosed sodium. Minute quantities of organic matter in the secretion were volatilized by heating.

INTRODUCTION

Although the pace of research on the biology of calcibiocavites (organisms that hollow out spaces in hard calcareous substrata) has quickened in the last decade (Carriker, Smith & Wilce, 1969; Carriker & Van Zandt, 1972b; Milliman, 1974; Golubic et al., 1975; Warme, 1975), studies on the physiological and chemical phases of penetration of hard calcareous substrates by organisms have lagged seriously behind the systematic, morphological, behavioral, and ecological aspects.

In this paper we report the results of a preliminary characterization of the secretion of the accessory boring organ (ABO) of the shell-boring, predatory, prosobranch gastropod *Urosalpinx cinerea follyensis* Baker.

Background for this investigation is found in papers by the following authors, who reported on preliminary physiological and chemical aspects of shell penetration in the following species. On *Urosalpinx cinerea:* Carriker & Chauncey, 1973; Carriker & Van Zandt, 1964; Carriker, Scott & Martin, 1963; Carriker, Van Zandt & Charlton, 1967; Carriker, Van Zandt & Charlton, 1972; Person et al., 1967; Smarsh

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et al., 1969; Zottoli & Carriker, 1974; on *Nucella lapillus*: Chétail & Fournié, 1969, 1970; Chétail, Binot & Bensalem, 1968; on *Polinices lewisi*: Bernard & Bagshaw, 1969; and on *Argobuccinum argus*: Day, 1969. This literature is reviewed in detail by Carriker & Williams (1978) with reference to an hypothesis on the chemical mechanism of shell dissolution by predatory boring gastropods.

MATERIALS AND METHODS

Animals

Urosalpinx cinerea follyensis snails were collected in Wachapreague, Virginia, and were maintained in the laboratory with rapidly flowing seawater and oysters, *Crassostrea virginica* (Gmelin), or mussels, *Mytilus edulis* Linné. Large, actively feeding adult snails were employed in the study. During the winter, snails were maintained in running seawater warmed (17-19°C) and allowed to reach gas phase equilibrium in a heat exchanger (Carriker & Van Zandt, 1973); maximum temperature in the summer was 24°C. Salinity of the seawater varied from 30.4 to 32.0 °/oo, and the pH ranged from 8.0 to 8.2. Standard flourescent ceiling lights illuminated the seawater trays during the day and occasionally during the early evening, providing about 35 footcandles of light at the level of the trays. Only limited indirect daylight came to the front sides of trays through windows in adjoining cubicles.

Collection of Secretion

Collection of secretion from the normally extended ABO of *Urosalpinx cinerea* was carried out on a valve model under a dissecting microscope (Fig. 1). Models were prepared as follows (Carriker & Van Zandt, 1972b: 178-182): on a Friday, snails deprived of food for about a week were placed in running seawater among well-shaped actively feeding oysters and mussels about 10 cm long. On the following Monday approximately 20 of the bivalves on which snails were boring were set aside for opening. The free valve and flesh were gently removed under water, and the remaining half-shell boring-snail preparation was then placed, inner surface of the valve facing up and the snail suspended underneath, on a support cut from a plastic dish (fig. 6 in Carriker & Van Zandt, 1972b). Most of the snails completed penetration of the valve during the Monday-Friday period. Boring rate was about 0.3-0.5 mm per day, and it took snails about 8 hr to excavate a borehole completely once the emerging incomplete hole was visible through the translucent interior portion of the valve. Valve models were positioned one at a time on a manipulated stage in running seawater under a binocular microscope just prior to breakthrough, the inside surface of the valve elevated slightly above the meniscus of the seawater. Care had to be taken to position the inhalant siphon of the snail under water, or the snail would promptly abandon the borehole. The inner surface of the valve was then rinsed with distilled water and air-dried. Elevation of the model out of the seawater reduced the possibility of welling of seawater through the borehole during exchange of the ABO and the proboscis. When occasionally seawater did spill onto the inner surface of the shell, the surface was rinsed again with distilled water and blotted dry with absorbent paper. For the most part, however, the ABO (surrounded by a sleeve of the stalk epithelium) generally extends from the foot into the borehole, and emerges from this sleeve as it comes close to the bottom of the borehole. This manner of extension of the ABO, in addition to voidance of seawater from the borehole by the propodium prior to entrance of the ABO, insured minimal, if any, dilution of the secretion by seawater (Carriker & Van Zandt, 1972b). This facilitated collection of secretion free, or relatively free, of seawater. Samples contaminated with seawater were discarded.

When the inner surface of the valve was coarsely textured, we coated the surface with a thin layer (0.2-0.3 mm) of hot paraffin just before breakthrough by the snail to reduce capillary creeping of seawater over the shell. Snails readily rasped through this thin layer of paraffin.

During very dry atmospheric conditions, when the secretion tended to dry on the gland, we increased atmospheric moisture in the vicinity of the gland by placing strips of cheesecloth across the valve a short distance from the borehole. Ends of the cloth immersed in running seawater around the model served as wicks to draw seawater over the shell and accelerated evaporation. Seawater did not wet the shell

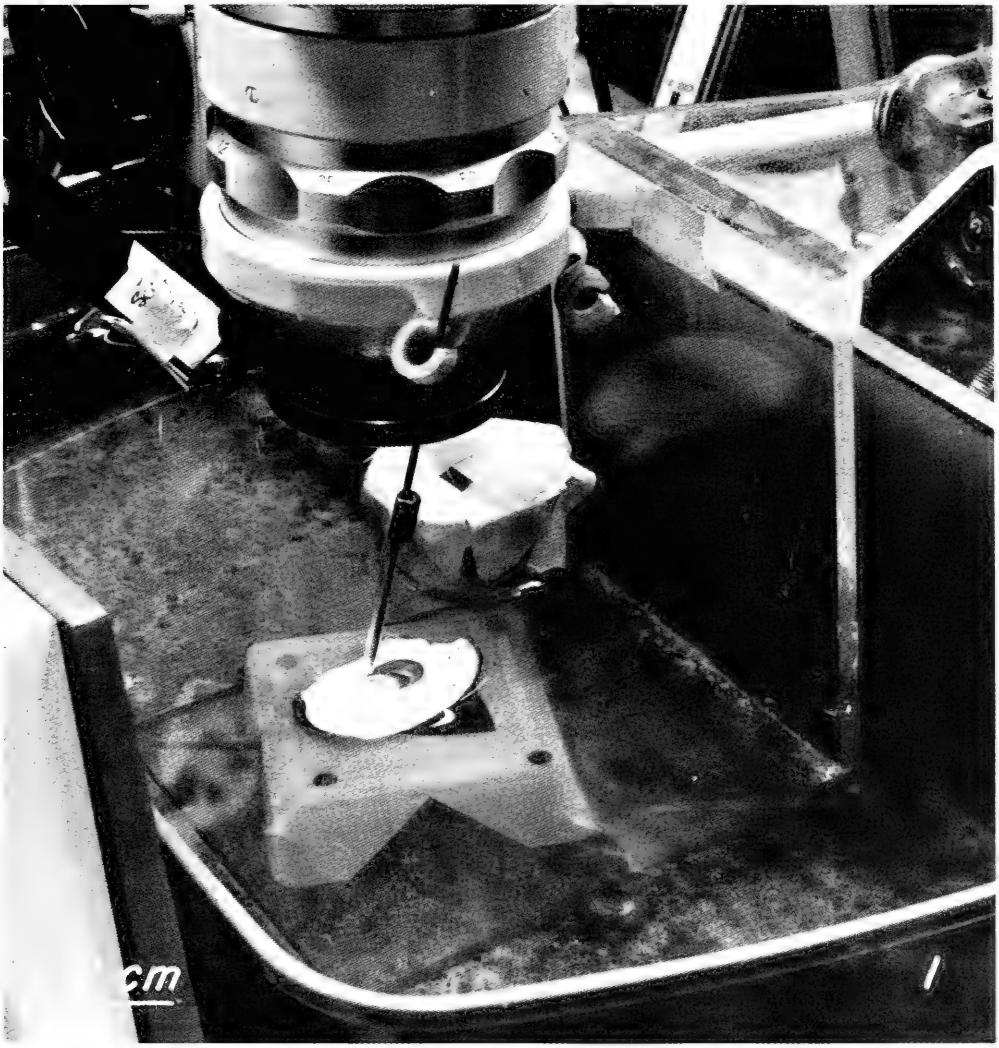


FIG. 1. Valve model for collecting secretion. Snail is attached to underside of valve, and glass bead on end of a glass stem is positioned in borehole to prolong use of hole by snail. Seawater is flowing slowly through the container.

surface immediately around the borehole. Completion of boreholes was accelerated artificially by 1 of 2 methods: (a) by drilling into the shell almost to the emerging borehole with a 1 mm bit and dental drill, and (b) by application of 1N HCl with a small pipette to the shell over the emerging borehole. Action of the acid was immediately stopped by addition of seawater. The surface of the shell was rinsed with distilled water just before artificial breakthrough. Accidental spillage of acid onto the ABO caused no response from the snail, whereas addition of distilled water to dilute the secretion for easier removal resulted in violent withdrawal of the gland. Glucose of approximately the same osmotic pressure as seawater was tolerated by the snail, but caused the secretion to form a film over the ABO.

Occasionally for experimental reasons it was necessary to delay breakthrough for a few hours. This was done by coating the shell surface over the emerging borehole

with a relatively thick layer of molten paraffin. When ready to expose the borehole, we scraped the layer of paraffin off the valve.

Abandonment of the borehole usually took place when the hole was large enough for the snail to extend its proboscis fully through it into the air; after unsuccessful attempts to locate flesh over the surface of the valve, the snail withdrew its proboscis and crawled away. In rare cases, snails continued to enlarge the borehole without extending the proboscis through it, apparently because of the lack of stimulus required to initiate a feeding response and terminate shell boring. In order to prolong the use of the model for collecting secretion and provide as large a borehole as possible for full extension of the ABO, we frequently delayed abandonment of the hole by the snail. This was accomplished by gently positioning a tear-shaped glass bead or rod mounted on a stem over the borehole each time the ABO was withdrawn and before the proboscis was extended (Fig. 1); the false bottom over the borehole prevented discontinuation of shell boring.

In the event that a snail deserted its borehole, the snail could often be attracted back by placing a small piece of fresh oyster or mussel flesh over the borehole. This procedure although of behavioral interest, interrupted the normal sequence of ABO-proboscis activity, and made collection of secretion difficult.

When the ABO bulged out of the borehole a distance at least its own diameter (about 1 mm in large snails), secretion was ready for collection. This was done by touching bits of cover glass, filter paper, glass hooks, or capillary tips to the secreting disc with the aid of a micromanipulator under a binocular microscope. Touching the surface of the ABO had to be done gently because the gland is heavily innervated (Nylen et al., 1969) and very sensitive to mechanical irritation and gradual changes in heavy pressure. A small fragment of mirror set at an angle beside the borehole and ABO, and visible through the binocular microscope, facilitated placement of the collecting device on the center of the secretory disc.

Secretion was collected by 3 separate techniques: *Capillaries.* The tip of a capillary (Drummond microcap, 2 μl), directed by a micromanipulator and held on a hypodermic needle attached to a screw-driven suction apparatus, was touched to the exposed surface of the moist ABO. By capillarity, secretion rose in the capillary about a mm. The drop of secretion was then drawn to the middle of the capillary by aspiration and the ends of the capillary were sealed by flaming. In high humidity, the only condition under which the technique works, it was possible to collect 2 or 3 successive samples of secretion in the same capillary during successive extensions of the ABO.

Glass hooks. A 2-μl capillary was drawn to a fine, slightly hooked point in a small flame. After the ABO was extended in a relatively dry atmosphere, the partly jelled secretion was picked off the gland. The secretion, being viscid, formed a drop and soon dried, roughly 2/3 of it evaporating. In some cases it was possible to make 2nd and 3rd collections on the same glass hook on successive extensions of the ABO of 1 snail.

Cover glass fragments. This proved the most successful procedure, and maximal quantities of secretion were obtained by it. After the ABO billowed fully out of the borehole, the flat surface of a fragment of cover glass (approximately 3 X 4 mm) held on opposing edges by fine forceps, was touched quickly to the disc of the extended ABO. Snails did not seem to be irritated by sudden contact, and a thick blot of the full area of the exposed gland was generally collected on the glass surface. The surface of the glass picked up most of the secretion on the gland; a 2nd application of glass to the same gland before withdrawal resulted in very little additional secretion.

This, and other observations, indicated that most of the secretion was released by the ABO when 1st extended. Secretion was exuded in a uniform layer over the entire crown of the gland, resulting in a thin cap-like sheet which tended to retain its form in seawater and in air. Because of their highly hygroscopic nature, collections of dry secretion were stored in a vacuum desiccator.

Electron Microscopy

For examination at low magnifications with the scanning electron microscope (SEM), ABO secretion was blotted on fragments of cover glass and dried in air. Each

glass fragment was secured to a SEM stub with silver paint and coated with carbon and gold in a vacuum. For study of the ultrastructure of the secretion at higher magnifications, blots were fixed in osmium vapor immediately after collection, dehydrated in an acetone-ethanol series, dried in a critical point dryer, and coated with carbon and gold. Secretion in freshly collected incomplete boreholes was prepared as follows: snails were allowed to penetrate valve models of *Mytilus edulis* until radular and ABO activity were visible through the translucent inner aragonitic layer of the shell; snails were removed after the ABO had been in the hole about 30 min, and boreholes were dried immediately with a stream of air. Most of the shell surrounding the borehole was then removed with cutting pliers, and the shell preparation was mounted on a stub with silver paint, dried in an oven at 60°C overnight, coated with carbon and gold, and examined in the SEM.

For ultrastructural examination by transmission electron microscopy (TEM), a very thin layer of ABO secretion was blotted on parlodion-coated copper EM grids. Grids were held one at a time with fine curved forceps and touched very lightly to the moist secretory disc of the ABO. Some grids were examined in the EM without coating under an electron beam of low intensity, while others were coated with carbon before viewing.

Histochemistry

ABOs were excised from the foot of active unanesthetized snails (Carriker & Van Zandt, 1972a describes the method). Glands were dropped immediately in one of the following histological fixatives: Bouin's, Campy, formalin in seawater, Gilson, glutaraldehyde in seawater, Heidenhain's Susa, Petrunkewitsch, and Perenyi. Paraffin tissue sections, cut 4 μm thick, were mounted on microscope slides and stained with (a) alcian blue and safranin-O, and (b) astra blue and Kernechtrot, to determine if acid mucopolysaccharides were present. Histochemical identification of neutral mucopolysaccharides and mucoproteins in sections of ABOs was carried out with the periodic acid-Schiff (PAS) reaction (Barka & Anderson, 1963). For general differentiation of tissues, sections were stained with Lillie's modification of Masson's trichrome stain and mounted in balsam saturated with salicylic acid.

Physiology

Heat. Since nearly all enzymes are irreversibly destroyed by heating to 80°C, we applied heat to determine if this affected the capacity of ABOs to etch shell. Fourteen ABOs were excised from large snails. Seven ABOs were placed in a small screw-capped vial in 4 drops of clean seawater of pH 7.8, and the other 7 were left in 4 drops of seawater in a 1 cc dish. The vial, tightly capped, was then heated in a water bath to 85°C and left at this temperature for 2 min. Then heated ABOs were cooled. Both heated and control ABOs were transferred with a little of their own seawater to polished shell preparations in a moist chamber at about 24°C and left there for 18 hr. At the end of this time ABOs were flushed from the shell fragments, and shell surfaces were air-dried and examined with a light microscope for evidence of dissolution.

Enzymes. The gross effect of the proteolytic enzymes, papain and trypsin, on the etching capacity of the secretion of excised ABOs was determined as follows. Nine glands were excised from adult snails. Three ABOs were placed in a 1% solution of papain (w/v in glass-filtered seawater), and 3 were put in a 1% (w/v in seawater) solution of trypsin. In each case, glands were allowed to remain in the enzyme solutions for about 2 sec and were then transferred individually to polished shell with a small drop of the enzyme-seawater solution. The 3 remaining ABOs, controls for the effects of enzyme solutions on ABO etching, were placed in filtered seawater and similarly transferred to polished shell with a drop of seawater. Drops of enzyme solutions were also placed on polished shell as controls for the effects these enzymes might have on shell dissolution independent of ABOs. Shell-gland-enzyme preparations were held in a moist chamber for about 15 hr. Shell surfaces were then rinsed, dried, coated with chromium in vacuum, and examined optically with incident illumination.

pH. The pH of secretion of intact, normally functioning ABOs of *Urosalpinx cinerea* was determined by Carriker, Van Zandt & Charlton (1967) in an oyster model. This device, however, presented

problems which were not possible to solve: seepage of fluids through the shell-glass juncture from the dying oyster within the model into the artificially bored hole, access to the disc of the ABO in the artificial borehole only from the side with the pH glass microelectrode, and extreme sensitivity of the system to electric fields which forced us to carry out determinations in still seawater.

Development of the valve model (Carriker & Van Zandt, 1972b) presented an opportunity to check our earlier determinations of the pH of the ABO secretion in a normally functioning ABO under relatively normal conditions. One at a time adult snails which had completed a borehole in a *Mytilus edulis* valve were positioned on a submerged, mechanically manipulated stage in a container of slowly running seawater, the surface of the valve raised above the meniscus of the water and illuminated under a binocular microscope. A pH glass microelectrode (50 millivolts per pH unit, and relatively temperature independent) was held in a micromanipulator. The pH was recorded with a Beckman research pH meter and strip chart recorder, and the standard reference electrode was inserted in seawater flowing beside the valve model. The system was stable, and once calibrated, remained constant for several hours at a time. Eight series of complete recordings were made of the pH of secretions from the propodium, propodial transverse pedal furrow, buccal cavity, and ABO as they were moved into the borehole by snails.

Chloride ion. In view of the low pH of the ABO secretion (Carriker, Van Zandt & Charlton, 1967) and dissolution of molluscan shell by it, the large quantities of NaCl in dried ABO secretion (Carriker, Van Zandt & Grant, 1972), and the lack of response of ABOs to the application of HCl, we decided to test for the presence of chloride ion activity. However, the small quantities of secretion released during shell penetration and its highly viscid, volatile nature prevented its examination by standard analytical techniques.

Accordingly, we selected an intracellular chloride ion microelectrode (Microelectrodes, Inc.) with a tip 0.5 μm in diameter for the determination. The small size of the electrode permitted precise placement of the electrode tip on various parts of extended ABOs. The electrode consisted of a silver-silver chloride-coated platinum wire, and was filled with 0.5 M KCl saturated with AgCl. Possible interfering ions were bromide and sulfide. Potential was recorded with a Beckman research pH meter and strip chart recorder. A Faraday cage was used to shield the snail-valve preparation and electrodes from electrical interference. A 0.5 M KCl solution was arbitrarily designated as the standard reference solution because a 0.5 M chloride ion concentration approximates the chloride ion concentration in seawater and in tissue fluids in marine invertebrates. Thus a difference in electrical potential, as measured with the chloride ion microelectrode, between the 0.5 M KCl standard and the ABO secretion would be proportional to the log of the chloride ion concentration of the ABO secretion. Chloride ion microelectrodes and strip chart recorder were calibrated by titration with standard KCl solutions (Whitfield, 1971). Fixed point calibration of the recorder was performed both before and after each recording period. During the course of recording from the ABO secretion, we used seawater flowing around the snail-valve preparation as a secondary standard. This allowed a continual check on stability and drift of the recording apparatus.

Individual *Urosalpinx cinerea* which had bored a hole in a valve of *Crassostrea virginica* were placed one at a time on a submerged mechanically manipulated stage in a container of slowly running seawater, and the surface of the valve, with the snail beneath it, was elevated above the meniscus of the water. A binocular microscope was positioned above the valve model and the boresite was illuminated by a microscope lamp. The double junction reference electrode was immersed in the seawater beside the snail. The surface of the valve model was rinsed and dried prior to use, and any seawater welling through the borehole as the proboscis withdrew was carefully blotted off with absorbent paper.

As soon as the snail withdrew its proboscis and extended the ABO through the borehole, we applied the tip of the chloride ion microelectrode to the center of the gland. Maximum secretion was present at this point and there was least chance of contamination with chloride ions from seawater.

Presence of chloride ions was supported by the use of qualitative energy dispersive

x-ray analysis (EDAX) in combination with scanning electron microscopy. Employing the snail-valve model used for the chloride ion microelectrodes, we collected 6 successive blots of pure ABO secretion on the tip of a fragment of pure carbon. The fragment was supported on a micromanipulator, and the tip was applied to the crown of the ABO for a few seconds when it was 1st extended. After each collection, and prior to the next extension of the ABO, the secretion was allowed to dry on the carbon. In preparation for viewing in the SEM and the elemental scanning analysis (EDAX), the carbon fragment was coated with carbon in a vacuum.

RESULTS

Release of Secretion by ABO

Behavioral observations suggested that most of the secretion utilized in the incomplete borehole during chemical activity collects over the secretory epithelium inside the stalk sleeve of the ABO while it is withdrawn within the foot during the resting phase. As the gland slides into the incomplete borehole, it is initially enveloped by the stalk sleeve, but billows quickly out of the sleeve and presses the drop of accumulated secretion against the bottom of the incomplete borehole. The suggestion that the ABO emerges from the foot holding most of the released secretion to be used during the subsequent period of dissolution was examined experimentally by separating different snails from their boreholes at short and long intervals after initiation of shell dissolution. Duration of chemical and rasping periods prior to final dissolution period, and duration of chemical dissolution period before snail removal, are tabulated in Table 1.

TABLE 1. Duration of chemical and rasping activities of *Urosalpinx cinerea* in incomplete boreholes prior to removal of snails from the shell for determination of amount of secretion in the boreholes.

Snail	ABO in hole	Rasping in hole	ABO in hole prior to removal of snail
1	35 min	10 min	75 sec
2	40	1.5	60
3	30	1.0	60
4	40	1.3	20 min
5	35	1.0	25
6	30	1.5	30

Boreholes were removed quickly, air-dried, coated with platinum-palladium, and examined with the SEM. The thickness and appearance of dried secretion visible under the SEM was similar for boreholes removed approximately 1 min and 20-30 min after initiation of dissolution. These observations support the hypothesis that most of the active non-volatile components of the secretion associated with shell dissolution are supplied from the start. The hypothesis was confirmed by the fact that we were able to collect only small additional amounts of secretion from the surface of the ABO in capillaries or by blotting on glass after initial protraction of the ABO.

That small amounts of secretion continue to be released during ABO activity in the borehole is suggested by the following experiment. Three ABOs were excised in rapid succession from resting snails, placed in 0.01 ml of seawater with a 1 ml hypodermic needle in a covered microdish and left there for 0.5 hr. At the end of this time, ABOs were washed vigorously in seawater, and then placed in a drop of fresh seawater on polished shell in a humidity chamber for 15 min. A second set of 3 ABOs was treated similarly, except that they were soaked in seawater for 1 hr before washing and placing on polished shell. Pronounced etching occurred under all 6 ABOs. The continuous gentle pulsatory movements of the ABO in the borehole, ranging from 21 to 28 per min at room temperature, could be related to release of secretion.

Physical Characteristics of the ABO Secretion

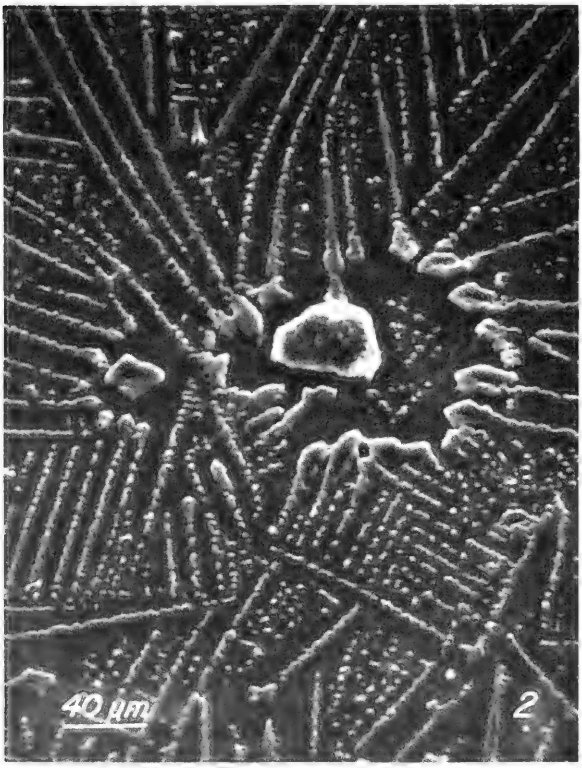
Upon exposure to relatively dry air, the highly viscid secretion of normally extended ABOs in a valve model jelled on the surface, the clear glaze increasing in firmness with increasing dryness. Secretion under the glaze in contact with microvilli remained fluid, facilitating removal of secretion films with glass hooks. Jelling also occurred under seawater, though not to the extent that it did in air, the film of secretion becoming a light translucent milky color in air.

Dry secretion collected on glass or paper was highly hygroscopic and quickly accumulated water under conditions of

high atmospheric humidity. Dry secretion in incomplete boreholes rinsed with distilled water as soon as removed from boring snails appeared to be unaffected ultrastructurally when examined with the SEM at mangifications up to 6,000 X.

Full blots of secretion from large snails (40-45 mm in shell height) touched to fragments of cover glass from extended ABOs measured 1.5 to 2.0 mm in diameter, and contained approximately 1 to 2 μg of dry material, estimated from an approximation of the volume of the dried secretion. The pattern of dried secretion appeared to vary with speed of drying, humidity, temperature, thinness of the secretory film, and drafts of air over the sample. Figure 2 illustrates the dendritic pattern commonly observed on thick blots allowed to stand at normal atmospheric conditions and room temperature for 1 or more days. Size of particles ranged from 0.7 μm to 30 μm. Smaller particles appeared crystalline in form (Fig. 3). About 65% of the secretion was volatile and evaporated on drying. Assuming that blots picked up about 80% of the free secretion, we estimated that the amount of secretion released by the ABO during 1 application in the borehole was roughly 2.5 to 5 μg.

Appearance of dry secretion in an incomplete borehole in the shell of *Mytilus edulis* taken from a snail at the end of a normal period of chemical activity is illustrated in Figure 4. Thickness of the secretion coat ranged from 0.03 to 1.7 μm, and varied with the amount of secretion applied to the borehole by the snail. As a result of drying during preparation for scanning electron microscopy, the secretion coat frequently peeled back and partially fragmented, exposing incompletely dissolved shell prisms and lamellae beneath (Fig. 4, 5, 6). The crystalline-like particles present in the secretion blots were also commonly found on the secretion coat in boreholes



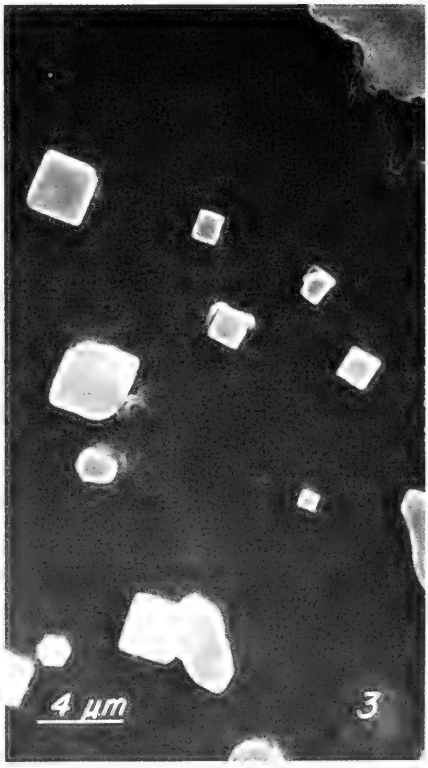


FIG. 2. Blot of ABO secretion of *Urosalpinx cinerea* on surface of glass illustrating pattern of drying. Blot remained in open room for 3 days prior to vacuum-drying and coating with metal. Scanning electron micrograph.

FIG. 3. Magnification of smallest "crystals" in the secretion blot illustrated in Fig. 2.

(Fig. 6, 7), where, however, because of previous rasping activity by the snail, it was difficult morphologically to separate particles from partly dissolved fragments of shell units dislodged by the radula during the previous rasping period. At high magnifications the dry secretion coat in the borehole resembled a relatively homogeneous film (Fig. 7). A pattern of small particles ranging in diameter from about

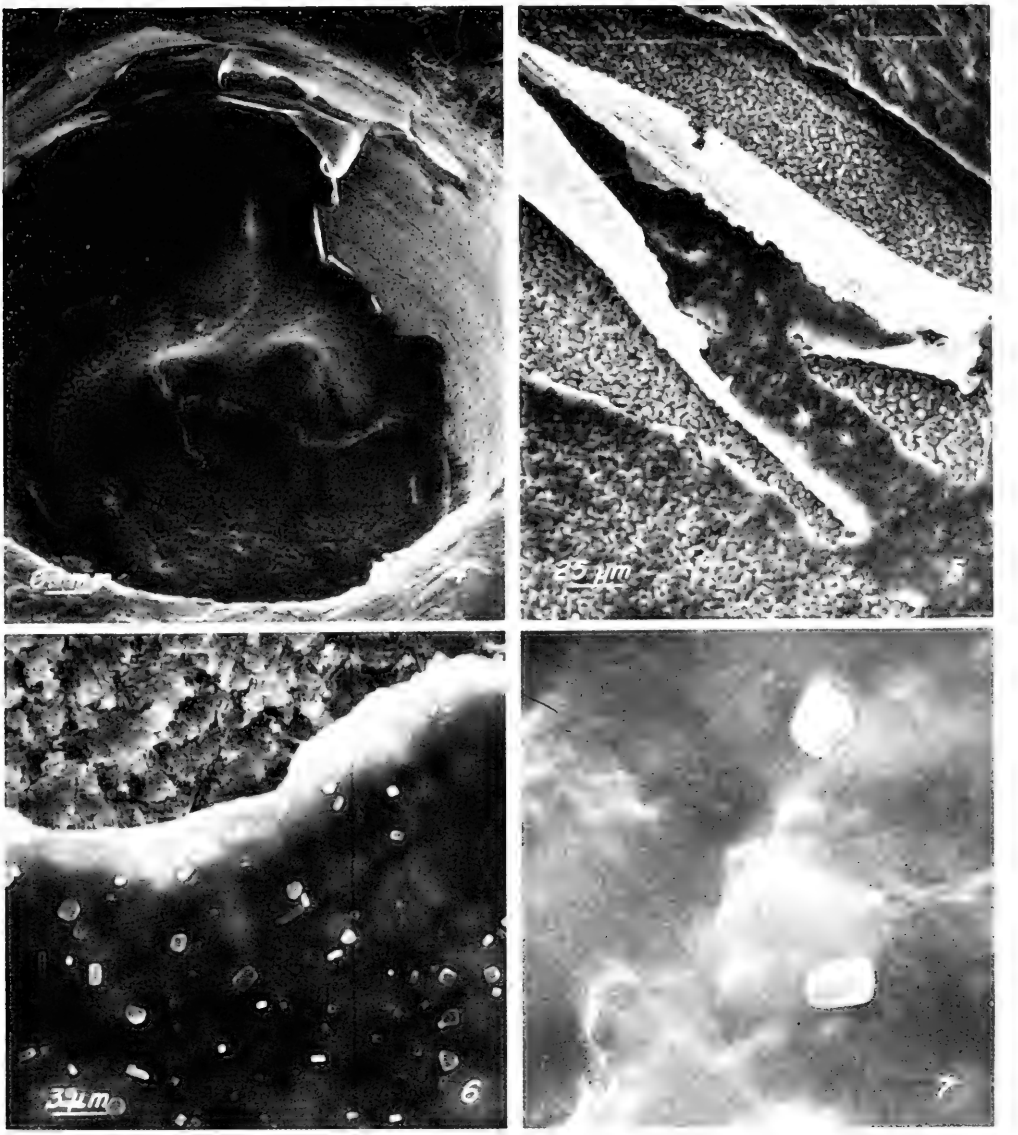


FIG. 4. Incomplete borehole of *Urosalpinx cinerea* in shell of *Mytilus edulis,* taken quickly from the snail after ABO was in borehole for 26 min preceded by a 2-min rasping period. The secretion coat curled away from walls of hole on drying, exposing portions of attached shell beneath. Scanning electron micrograph.

FIG. 5. Higher magnification of borehole shown in Fig. 4 to show relative thickness of the secretion coat and the cleanly dissolved shell units beneath. Scanning electron micrograph.

FIG. 6. Higher magnification of secretion coat and "crystals" in borehole shown in Fig. 4. "Crystals" are comparable in size to smaller ones in Fig. 3. Scanning electron micrograph.

FIG. 7. Enlargement of 2 "crystals" shown in Fig. 6. Scanning electron micrograph.

0.02 μm to 0.05 μm was observed in scanning electron micrographs of a thin blot of ABO secretion treated with osmium vapor.

Transmission electron microscopy revealed additional morphological detail on the characteristics of secretion blots. After secretion was removed from an ABO by 1 or 2 successive blottings, microvilli from the ABO, unprotected by the lubricating secretion, adhered to the parlodion surface on the next application of a copper grid and were pulled off (Fig. 8). Flattened by adhesion and drying, microvilli (normally about 0.1 μm in diameter) measured about 0.2 μm in diameter. Free distal ends were smooth and slightly enlarged. Remaining microvillar surfaces were covered with prominent nodules; vesicles (about 0.04-0.06 μm in diameter), diffuse in appearance, were abundant close to microvillar membranes, and some appeared in various stages of fusion with the nodules as if in the process of release.

A thin secretion blot collected on a dry day on a parlodion-coated grid, immediately dried further in a vacuum desiccator, and coated with carbon, consisted principally of a background of finely granular to homogeneous material with occasional prominent fragmented bodies about 0.6 to 0.7 μm long (Fig. 9). The latter, prominently membrane-bound, when not fragmented and not flattened by drying would correspond approximately to the size of the secretion granules in the secretory cells of the ABO (Nylen et al., 1969). Because of this similarity we will tentatively name these particles "secretion granules" in this report.

Thin secretion blots on parlodion-coated grids, likewise collected on a dry day, but held in a moist chamber for 1.5 hr before drying and coating with carbon, presented a rather different appearance (Fig. 10). Secretion granules were intact, and abundant, and scattered among what resembled crystallization of finely granular material into rosette patterns organized for the most part around the secretion granules. The latter ranged in diameter from 0.17 to 0.40 μm, corresponding well in size with the granules described by Nylen et al. (1969).

A final set of secretion blots on parlodion-coated grids was collected and held in a moist chamber at room temperature for about 18 hr, then dried and examined ultrastructurally. The secretion dried and "crystallized" in dendritic patterns approximating the low magnifications photographed in Fig. 2. Individual "crystals" ranged in size from 0.04 to 1.3 μm and their shapes were similar to those photographed in Fig. 3. No secretion granules were evident, probably deteriorating during the long exposure to condensation water in the moist chamber. At high magnifications

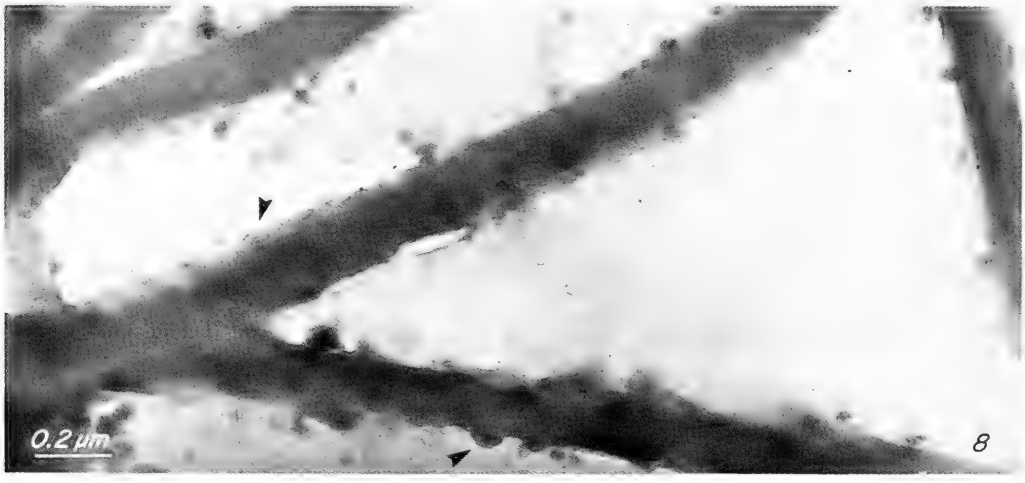


FIG. 8. Microvilli, diameter 0.17 to 0.22 μm, of ABO pulled off on a parlodion-coated grid. Preparation placed immediately in vacuum desiccator, then coated with carbon. Arrows point to nodules on microvilli which are clearly in focus. Vesicles are located along surface of microvilli and free around them. Transmission electron micrograph.

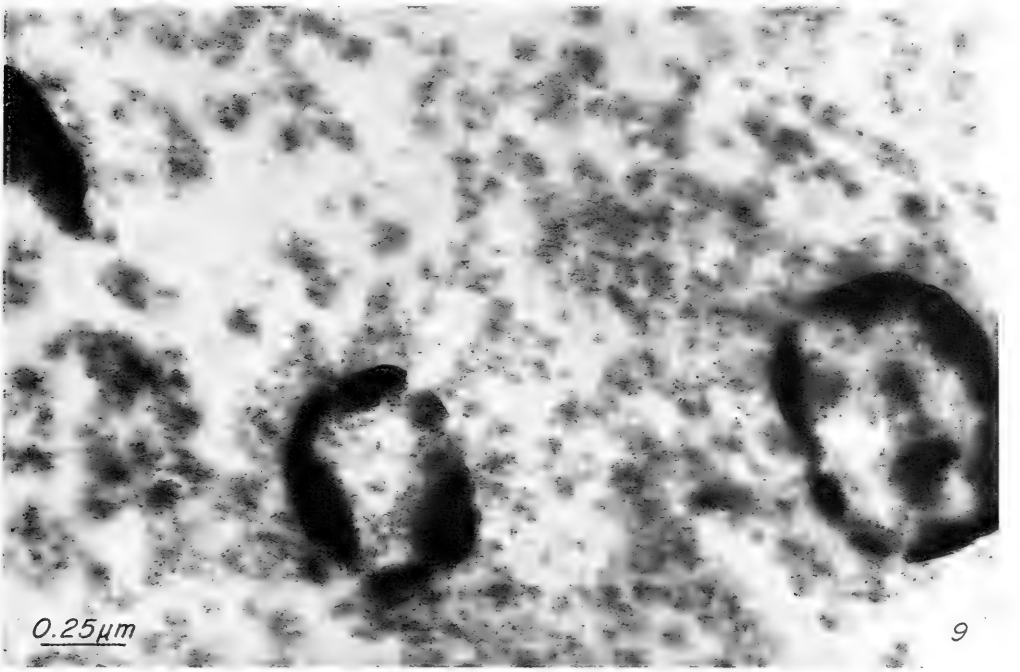


FIG. 9. Thin blot of secretion of the accessory boring organ on a parlodion-coated grid dried immediately after removal from the organ; coated with carbon. Large dark bodies, about 0.67 μm long, appear like secretion granules. Remainder of dry secretion varies from finely granular to homogeneous in texture. Transmission electron micrograph.

individual "crystals" exhibited a complex, fine, intracrystalline structure of variable design (Fig. 11).

Histochemistry of ABO Secretory Epithelium

The alcian blue test for acid mucopolysaccharides was reliable after fixation of tissues in Bouin's, buffered formalin, Gilson's, buffered glutaraldehyde, and Heidenhain's Susa fluids. Mucous cells of the proboscis integument, esophagus, radular sac, salivary glands and ducts, pharynx of Leiblein, and pedal epithelium of *Urosalpinx cinerea* gave strong positive results, whereas secretory cells of the ABO produced a negative reaction. These results were confirmed by use of the stain astra blue. A pronounced negative reaction was obtained with this stain in the secretory cells of the ABO after fixation of tissues in Champy's, Gilson's, Perenyi's, Petrunkewitsch's, and buffered formalin fluids.

Further evidence for the absence of acid mucopolysaccharides in the secretory cells of the ABO was obtained with the periodic acid-Schiff reaction applied to tissue sections fixed in Gilson's, Heidenhain's Susa, and buffered formalin solutions. Most of the secretion in the epithelium of the ABO was strongly PAS-positive, the microvillar zone especially being distinctly rose colored after the alcoholic fixatives. Acid mucopolysaccharides are generally PAS-negative, or give only a weak reaction of questionable specificity. The PAS test identified primarily neutral mucopolysaccharides and mucoproteins.

In ABOs fixed in Perenyi's fluid and stained with Lillie's modification of Masson's trichrome stain, we observed conspicuous leucocyte-like cells packed with red spherules averaging 0.6 to 1.3 μm in diameter. In several cases the leucocyte-like cells seemed to have burst and the red spherules were scattered in spaces adjacent to the cells. Spherules of similar size and color were distributed in short string-like rows in minute channels oriented radially from the proximal to the microvillar periphery of the secretory epithelium;

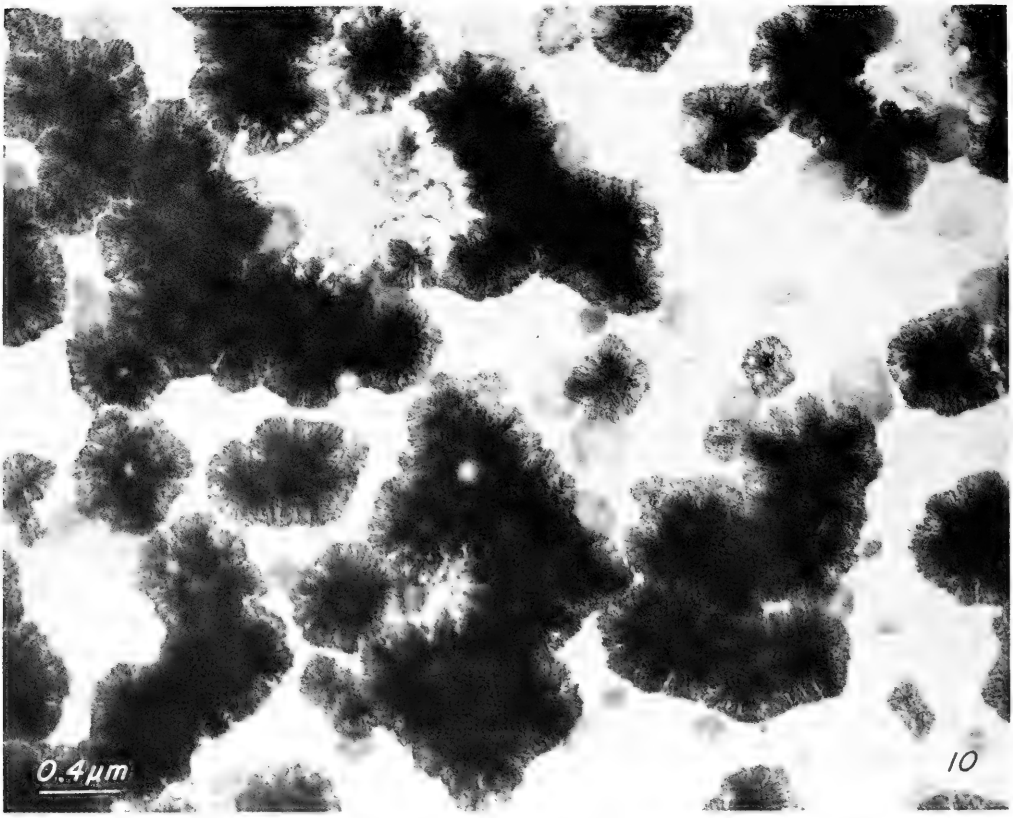


FIG. 10. Thin blot of secretion of the ABO on a parlodion-coated grid held in a moist chamber at room temperature for 1.5 hr, then dried and coated with carbon. Clear bodies, "secretion granules," range in diameter from 0.17 to 0.40 μm (average 0.33 μm). Finely granular patterns appear to represent "recrystallization" of the secretion during the period in the moist chamber. Transmission electron micrograph.

although continuous strings could not be traced from sinus to periphery, they were visible in sections over various parts of the secretory epithelium, collectively giving the impression of continuous connection. Association, if any, between the spherules in the leucocyte-like cells and in the minute channels, is uncertain. The same pattern and distribution of red spherules were observed in other ABOs fixed in the other nitric acid fluids (Gilson's and Petrunkewitsch's) and like-wise stained with the trichrome solution. Trichrome stains are not histochemical in nature, so the color gave no clue to the identity of the spherules. That they are probably not secretion granules may be inferred from the considerably larger size of the spherules than the secretion granules.

Physiology

Heat. None of the 7 heated ABOs etched polished shell, whereas all 7 control ABOs produced moderate etchings. Heated ABOs were firm and shrank from the effect of the heat, and control ABOs remained flaccid and soft. A preliminary experiment on inactivation of etching by heat was reported by Carriker, Scott & Martin (1963), and was repeated because of improved methods for testing the shell-penetrating capacity of excised ABOs. Present results confirm earlier findings.

Enzymes. The effect of proteolytic enzymes on the etching capacity of the secretion of excised ABOs was tested with papain and trypsin. These results are summarized in Table 2. The 3 ABOs placed in

FIG. 11. Thin blot of secretion of the ABO on a parlodion-coated grid, held in moist chamber at room temperature for about 18 hr, then dried and examined. Specimen was not coated with carbon, so electron beam intensity was kept low. "Crystals" are arranged in dendritic patterns, similar to those shown in Figs. 2 and 3. Transmission electron micrograph.

TABLE 2. Effect of enzymes on etching activity on polished shell of ABO excised from *Urosalpinx cinerea:* + minimal, ++++ maximal etching.

Treatment	Etching Activity		
	1	2	3
3 ABOs in seawater only	+++	++	++++
3 ABOs in papain solution	0	0	0
3 ABOs in trypsin solution	++++	++	+++
Papain solution only	0	0	0
Trypsin solution only	0	0	0

the papain solution did not etch polished shell, whereas ABOs immersed in the trypsin solution produced strong etchings comparable to those of control ABOs in seawater (Carriker, Scott & Martin, 1963). Control drops of enzyme solutions did not etch. The ABOs were slightly mushy at the end of the experiment.

pH. Hydrogen ion concentration of the ABO secretion, when the ABO extended normally through the borehole in a valve model and was covered with traces of seawater, dropped consistently to levels ranging from 3.8 to 4.0. When the secretion was free of seawater, however, the pH did not fall below 5.2. Minimal pH of fluid in the buccal cavity when the snail took the electrode deep into its mouth was 5.7. The pH of the mucoid secretion within the transverse pedal furrow ranged between 7.0 and 7.8, the level seemingly more dependent on environmental conditions than on the secretion of the furrow itself.

Chloride ion. Ten recordings were made of chloride ion concentration in secretion on ABOs extended normally through the borehole in a valve model. Duration of recordings ranged from 3 to 8 min, the time the ABO remained in the borehole. Temperature of the seawater during observations was about 22°C. Recording 5 was aborted when the ABO was withdrawn, and the 10th recording was discarded because of electronic drift in the instruments.

Maximum chloride ion concentration from the remaining 8 recordings ranged from 0.79 M to 1.17 M, levels conspicuously above the standard 0.5 M concentration in seawater. Increase in chloride ion concentration on the surface of the ABO with time followed a stepwise course until the ABO was withdrawn, generally after the maximum chloride ion concentration was reached (Fig. 12). In 1 case, the ABO remained in position in the borehole after maximum concentration was reached and thereafter for approximately 6 min the

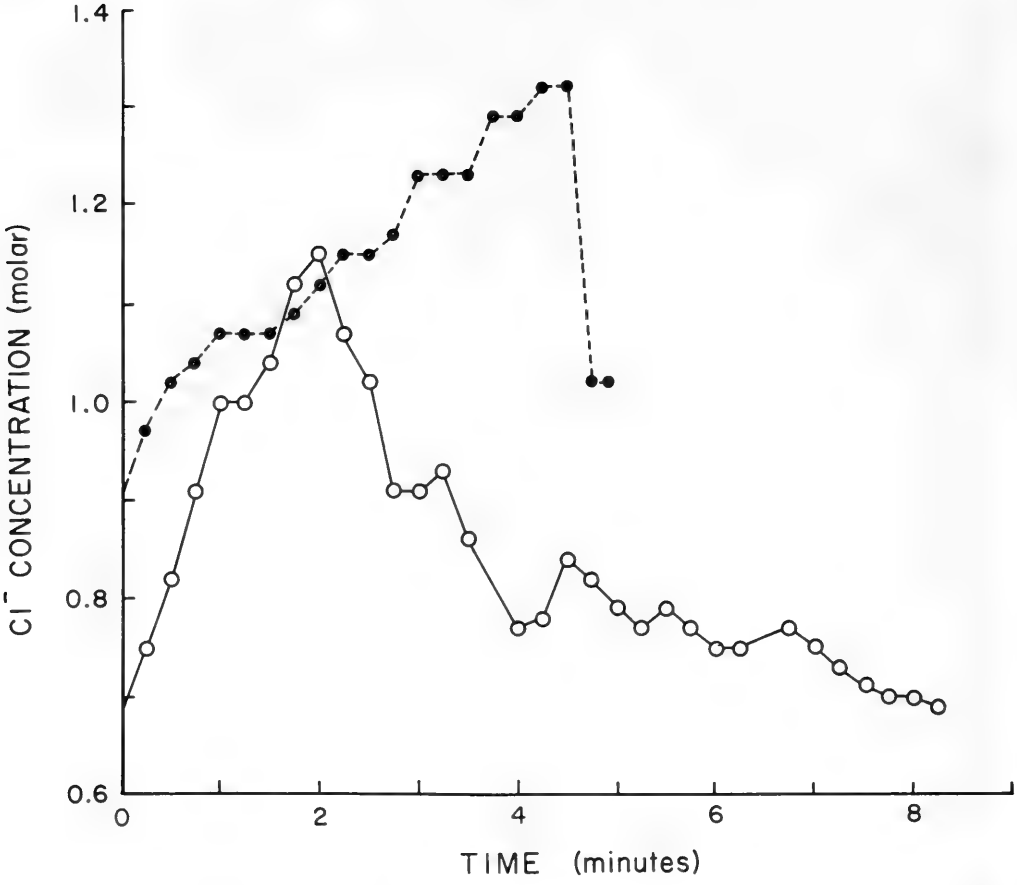


FIG. 12. Chloride ion concentration in secretion of the accessory boring organ measured over time. Chloride ion concentration was calculated from potential recordings at 15-sec intervals and is presented here as discrete data points. Dashed line and solid circles show stepwise increase in chloride concentration observed in Recording 7. This pattern was typical of the other runs with the exception of Recording 8 which is depicted here by solid line and open circles. In this case, a rapid increase in chloride ion concentration was followed by a much longer period of decline in concentration.

chloride ion concentration showed a stepwise decrease (Recording 8, Fig. 12). Five of the recordings were of sufficient duration and free of artifacts to allow transposition of the data at approximately 15-sec intervals from the recorder paper to graph form; all recordings demonstrated stepwise changes in chloride concentration. Of the total of 8 recordings, 6 demonstrated a continuous increase in concentration of chloride during the period of recording, and 2 showed a drop at the end.

During most recordings, the tip of the electrode was immersed in a small pool of ABO secretion made by a slight depression in the surface of the ABO by the tip of the electrode. While extended in the borehole, the ABO consistently underwent gently

rhythmic undulations. However, correlation between the frequency of undulations and stepwise changes in chloride ion concentrations was not clear. Accidental jarring of the electrode against the ABO, or other mechanical irritations, caused premature withdrawal of the gland, and accounted for the brevity of the recordings. Ordinarily the ABO is extended for an average of 20 to 30 min or more (Carriker & Van Zandt, 1972b).

Qualitative analysis by energy dispersive x-rays (EDAX) of dry ABO secretion on a carbon stub supported the presence of chloride, and in addition revealed the presence of sodium. Peaks on the screen disclosed a high concentration of chloride and a moderate concentration of sodium, and trace amounts of calcium and potassium. The carbon control showed none of these. Comparison by Emmett Smith (personal communication) of the index of refraction of dried ABO secretion with standard NaCl also confirmed that NaCl was the major constituent (at least 90%) in the secretion. Spot analysis in the scanning electron microscope with EDAX of dried ABO secretion blotted on fragments of cover glass demonstrated that chloride and sodium found on the carbon stub were localized in the secretion particles described earlier (Figs. 2-3).

Emmett Smith (personal communication) in additional optical tests slowly heated dried secretion to 340°C and observed a slight browning suggestive of decomposable organic matter starting at 240°C. A portion of the secretion was then fumed over concentrated HCl, allowed to stand until no odor of HCl was evident, and treated with ninhydrin in ethanol followed by heating. There was no evidence of color change, suggesting that organic matter present in the secretion of the ABO was volatile and evaporated along with the solvent, presumably water.

DISCUSSION

By carrying a full load of secretion on the distal surface of the ABO into the borehole at the beginning of the chemical phase of penetration (Carriker & Van Zandt, 1972b), the snail is able to commence dissolution of shell immediately over the entire surface of the bottom of the incomplete borehole. Smaller quantities secreted thereafter could be necessary to supplement the initial amount of shell-dissolving chemicals, or could provide a vehicle for discharging ions (such as chloride) from the gland as other ions (calcium, for example) pass into the gland (Carriker & Williams, 1978). Pulsatory movements of the ABO in the borehole should facilitate such ionic exchange. These movements possibly also serve as a pump to work the secretion into the dissolving shell surface, and mix the dissolving mineral and organic components of shell with the secretion.

Because of its viscid nature, the ABO secretion is more readily applied and held at the surface of the shell in the borehole by the ABO than a watery fluid, which would tend to drain out of the borehole. Simultaneously the secretion probably protects the delicate microvillar surface of the ABO (Nylen et al., 1969) from possible abrasion against the surface of the shell of the borehole during pulsatory movements of the ABO. Furthermore, the secretion undoubtedly serves as a radular lubricant during rasping of the shell surface, as well as a wet adhesive agent, which facilitates removal by radular cusps of loosened shell fragments from the surface of the borehole prior to swallowing. A further advantage is that the secretion coats shell fragments prior to swallowing in order to minimize laceration of epithelial surfaces of the alimentary canal as the fragments pass down the esophagus to the stomach and out the intestine (Carriker, 1977). Some of the secretion is probably lost in this way.

The uniformly repeating pattern of small particles ranging in diameter from about 0.02 to 0.05 μm in osmium-treated thin blots of ABO secretion is similar to the pattern of particles of approximately similar size (0.04-0.06 μm) seen close to and on microvilli (Fig. 8). Whether these are identical is problematical, though the similarity in size and close association of ABO secretion and microvilli would suggest that they could. Freshly released ABO secretion dried on filter paper likewise presents a granular appearance, though the size of the particles appears larger than that of particles associated with the microvilli.

The larger bodies observed in dried secretion blots and ranging in diameter from about 0.17 to 0.40 μm (Fig. 9, 10), because of their membrane-bound nature

and roughly comparable size (allowing for an increase in diameter due to flattening during drying), are suggestive of the secretion granules (average diameter 0.2 μm) described by Nylen et al. (1969) in transmission electron micrographs of secretory cells of the ABO. These authors suggested that these dense, membrane-bound secretion granules possibly constitute the patent cell constituent for extracellular use, and that vesicles could be other possible contributors of shell-dissolving chemicals. The appearance of these bodies outside of the secretory cells of the ABO in secretion blots supports the suggestion of Nylen et al. (1969).

In view of the high concentration of chloride and sodium ions in the secretion, as well as the presence of some organic matter such as carbonic anhydrase, it is likely the crystal-like bodies, which ranged in size from about 0.04 to 1.3 μm in dry secretion, were NaCl crystals whose structure had been modified by organic and possibly other substances in the secretion (Fig. 11).

Neutral mucopolysaccharides and mucoproteins were also found in the ABO of the naticid gastropod *Polinices lewisi* (Bernard & Bagshaw, 1969) and in the burrowing bivalve *Lithophaga lithophaga* (Jaccarini et al., 1968). The latter proposed that penetration of shell could be facilitated by complexing of calcium by neutral mucoproteins, and this possibility should be kept in mind as an aspect of the shell-penetrating mechanism of *Urosalpinx cinerea.*

When ABOs of live snails extended normally through the borehole in valve models, the pH of the secretion ranged between 3.8 and 4.0. This range was similar to that obtained earlier by Carriker, Van Zandt & Charlton (1967) in oyster models under less favorable instrumental conditions. The pure secretion on the surface of the ABO, free of seawater, however, gave a pH reading of 5.7. Whether the viscid nature of the pure secretion modified the reading, or the acidic element of the secretion was not released until the secretion came in contact with seawater, has to be determined.

The observation that heated excised ABOs did not etch polished shell suggests that heat-inactivated enzymes which might hydrolyze organic matrix of shell, or volatilized, or otherwise altered, inorganic chemicals which might solubilize the mineral crystals.

Experiments treating ABOs with enzymes confirm the proteinaceous nature of at least 1 necessary component of ABO secretion. Trypsin has a pH optimum of 7.8. The presumed catalytic mechanism of trypsin requires a substrate cationic group to react with an anionic group of the enzyme (White et al., 1968). Since ABO secretion maintains its pH at 5.0 when uncontaminated with seawater (pH about 8.0) and at 4.0 when in contact with seawater, the effectiveness of trypsin in hydrolyzing an active protein component of the ABO secretion would be greatly reduced if not completely neutralized. The relative activity of papain has a pH optimum in the range of 6.0 to 7.0 unless the substrate is electrically neutral or substrate charge is not involved in catalysis (Lehninger, 1970). In the latter case, relative activity of papain is independent of pH. The relative efficiency of papain, as compared to trypsin, in inactivating the etching ability of ABOs thus could be attributed to the pH characteristics of the ABO secretion and the charge characteristics of the active protein component in the ABO secretion. It is unlikely that this component is carbonic anhydrase because carbonic anhydrase requires a Zn^{++} co-factor which is catalytically active. The enzymatic or ionic exchange functions of this protein substance remain to be explored.

Discovery of substantial quantities of NaCl in the dry secretion (Smith, personal communication), and approximately 3 times the quantity of chloride ions in freshly released secretion than in seawater (confirmed independently by energy dispersive x-ray analysis of dry secretion), was unexpected. The fact that all recordings of the secretion demonstrated stepwise increases in the concentration of chloride ions, supports our observations that the ABO continues to release small amounts of secretion after its placement in the borehole at least during the initial chemical phase of penetration. It has not yet been determined, as suggested in the case of Recording 8, whether decline of chloride ion concentration during the latter part of each chemical activity in the borehole is characteristic. Although correlation between the frequency of rhythmic undulations of the ABO in the borehole and stepwise changes in chloride ion concentration was not obvious, it is likely that the 2 functions are related.

That ABO secretion might not be the

only biological fluid involved in shell-penetration is suggested by the functional involvement of the proboscis and transverse pedal furrow in hole-boring (Carriker, 1977; Carriker & Van Zandt, 1972b). The accessory salivary glands empty into the buccal cavity and the anterior pedal mucous gland drains into the transverse furrow. The composition of these secretions and their role in shell-dissolution have yet to be determined. It is likely, however, that their function, if any, is minor as snails deprived of their ABO are unable to penetrate shell (Carriker & Van Zandt, 1972a). Rigorous proof of this assertion would require surgical or pharmacological inactivation of the salivary and pedal glands.

Physiological and chemical results obtained in this investigation are discussed further by Carrriker & Williams (1978) with reference to an hypothesis on the chemical mechanism of shell-penetration by boring gastropods.

ACKNOWLEDGMENTS

Michael Castagna generously supplied live *Urosalpinx cinerea follyensis* from the eastern shore of Virginia. Special thanks go to Marie U. Nylen for collaboration in preliminary studies of the ABO secretion, and to Philip L. Levins for helpful suggestions in the early phases of research on the ABO secretion. Frank Medeiros photographed Fig. 1; scanning electron micrographs in Figs. 2-7 were taken in collaboration with Virginia Peters; transmission electron micrographs in Figs. 8-10, in collaboration with David Harling; and that in Fig. 11, with Peter Schaefer. Emmett M. Smith carried out optical tests on dry ABO secretion which we provided for him. Gary Charlton kindly prepared and contributed the pH glass microelectrode. Richard Srna provided helpful suggestions on the calibration and use of the chloride microelectrode. John Sherman and Takako Nagasi assisted with the EDAX analysis of dry secretion. Walter S. Kay prepared final prints of Fig. 1-11 for publication.

Howard H. Chauncey, Julius Gordon, Myroslaw G. Harasewych, Philip Person, and Karl M. Wilbur kindly reviewed the manuscript and offered many valuable suggestions.

Research was supported in part by Public Health Service Research Grant DE 01870 from the National Institute of Dental Research, and in part by a grant from the University of Delaware Research Foundation. Part of the Research was performed during the tenure of the senior author in the Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts.

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MALACOLOGIA, 1978, 17(1): 143-156

THE CHEMICAL MECHANISM OF SHELL DISSOLUTION BY PREDATORY BORING GASTROPODS: A REVIEW AND AN HYPOTHESIS[1]

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ABSTRACT

The results of investigations of the authors and other researchers on predatory boring gastropods reported in earlier publications are analyzed to formulate a preliminary hypothesis on the physiological-chemical mechanism of shell penetration by boring gastropods.

The authors hypothesize that boring muricid and naticid gastropods employ a combination of enzymes, an inorganic acid, and chelating agents in a hypertonic medium to facilitate dissolution of shell and intracellular transport of calcium during the chemical phase of valve penetration. Other chemicals, as yet unidentified, possibly also take part.

Secretory granules and vesicles in the secretory epithelium of the accessory boring organ and in secretion released externally, organic matter in the secretion, and dissolution by the secretion of the insoluble fraction of the organic matrix of shell suggest the involvement of at least 1 enzyme in the hydrolysis of the organic portion of the shell. The enzyme carbonic anhydrase catalyzes the hydration of $C0_2$ and is present in both the secretory epithelium and the free secretion. Hydrogen and chloride ions identified with microelectrodes, the chloride ions confirmed with energy dispersive x-ray analysis, suggest the role of HCl as one of the solubilizers of the mineralized component of shell. A chelating agent, localized in the secretory epithelium but not yet chemically identified, and a mucoprotein, also in the secretory epithelium, perhaps participate in sequestering calcium and other cations in the shell. Chelators possibly also function in removing soluble fractions of the organic matrix of shell.

INTRODUCTION

In other publications (Carriker, Van Zandt & Grant, 1972; Carriker, Williams & Van Zandt, 1978) we reported the results of a preliminary characterization of the secretion of the accessory boring organ (ABO) of the shell-boring prosobranch gastropod *Urosalpinx cinerea follyensis* Baker. In this paper we have drawn on these findings and those of earlier investigators to formulate an hypothesis on the physiological-chemical mechanism of shell penetration by boring gastropods (Carriker & Smith, 1969; Carriker & Van Zandt, 1972a; Carriker, Smith & Wilce, 1969; Carriker, Person, Libbin & Van Zandt, 1972). As background for this synthesis we include here a summary of existing knowledge of the secretory function of the ABO, chemical aspects of penetration by other invertebrate calcibiocavites, and dissolution of bone by vertebrate osteoclasts.

The only shell-boring gastropods in which the ABO has been examined in any detail are *Urosalpinx cinerea* (Say), *Nucella lapillus* (Linné), and *Polinices lewisi* (Gould). Probably because of the difficulty of the subject and the small size of the organisms, no one has yet investigated the physiological mechanism of penetration of calcareous substrates by Algae and Fungi (Golubic et al., 1975).

LITERATURE REVIEW

Cytology

The secretory disc of the ABO of boring gastropods is composed of tall columnar epithelial cells arranged in groups. Each cell within a group undergoes primary branching to form slender interlocking projections (Nylen et al., 1969). These projections undergo secondary branching to form a

[1]University of Delaware College of Marine Studies Contribution No. 112.

brush border of unusually long, densely packed microvilli (each about 0.1 μm in diameter) at the surface of the secretory disc (Fig. 1). Cell groups are further characterized by nerve fibers which penetrate the group basally and terminate in the region of primary branching. Each cell group is delimited by a membrane, the basal lamina, and is distinguished from neighboring cell groups by an interstitial space. The interstitial spaces contain muscles, capillaries, and nerve fiber bundles. Individual nerve fibers terminate on the muscles but not on the capillaries. The interstitial space in active ABOs widens to form a distal sinus at the level of primary branching (Carriker, 1943; Carriker & Van Zandt, 1972a; Fretter, 1946; Nylen et al., 1969; Derer, 1975).

The secretory epithelium of the ABO possesses cytological features characteristic of highly active tissues. Although the fundamental architecture of the secretory epithelium is the same in both active and resting ABOs, the epithelium of active ABOs is greatly amplified and more highly differentiated than their resting counterparts. Active ABOs are characterized by increased length of the epithelial cells and their microvilli as well as by enlargement of the distal interstitial sinus (Nylen et al., 1969). Dense populations of mitochondria, especially prominent towards the distal end of secretory cells, are more abundant in active than in resting ABOs. In active ABOs most of the mitochondria in the distal portion of the cell are distributed in an area of cell contact with the hemocyanin-packed distal sinus while some smaller mitochondria are located subjacent to the zone of primary branching. There is increased differentiation, especially of Golgi complexes, during periods of active boring. Conspicuous in the cytoplasm are dense

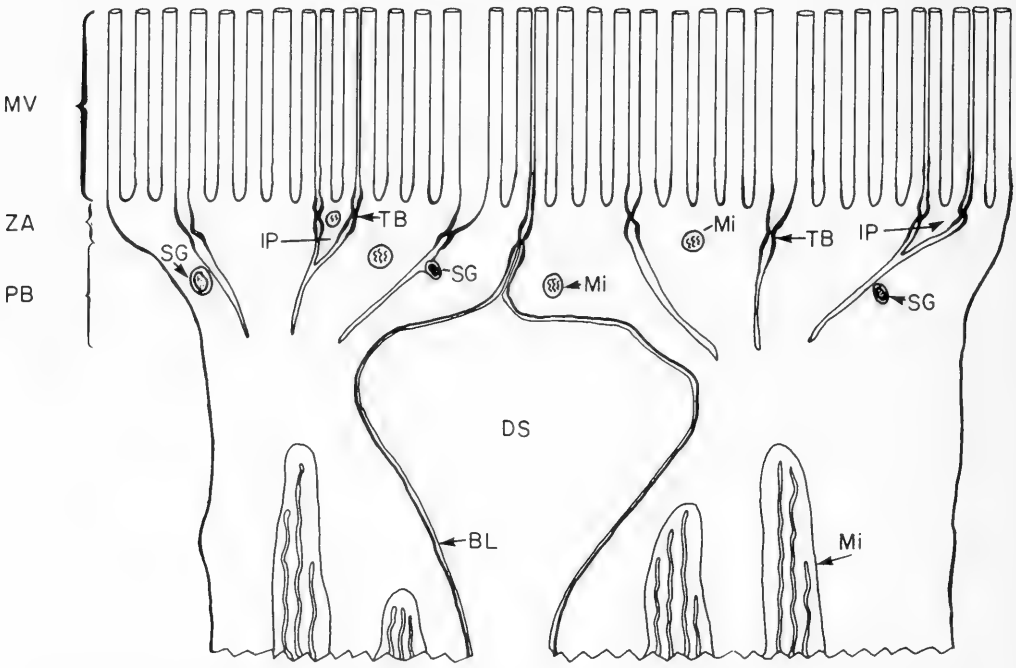

FIG. 1. Schematic diagram of distal portions of 2 cells in adjacent cell groups of the secretory epithelium of the accessory boring organ of *Urosalpinx cinerea.* Individual cell groups are bound by a membrane, the basal lamina (BL). An interstitial space and distal sinus (DS) separate cell groups from one another. The labeled interdigitating projections (IP) in the zone of primary branching (PB) originate from parent cells out of the plane of focus in the diagram. Terminal bars (TB) are areas of cellular contact between interdigitating projections. The region of formation of terminal bars is proximal to the microvillar zone (MV) and is designated as the zona adherens (ZA). Mitochondira (Mi) are distributed next to the distal sinus and in the region of primary branching. Secretion granules (SG) are depicted in the region of primary branching and are presumably transported to this region from the proximal end of the cell. Nomenclature after Nylen et al. (1969).

membrane-bound secretory granules of rather uniform size, averaging about 0.2 μm in diameter; other inclusions comprise multivesicular bodies and single vesicles. Fusion of the secretory granules occurs in the zone of primary branching (Nylen et al., 1969).

Histochemistry

The following reports deal with intracellular substances in the gastropod ABO which seem to be concerned with secretion of shell-dissolving substances. In histochemical studies of the ABO of *Urosalpinx cinerea,* Person et al. (1967) discovered that cytochrome oxidase, succinate dehydrogenase, and lactate dehydrogenase activities are localized in the secretory cells. Oxidase activities of mitochondria-rich particulate fractions isolated from homogenates of whole active ABOs are exceedingly high and almost twice those of inactive ABOs. Chétail & Binot (1967) and Smarsh et al. (1969) determined histochemically (Häusler's method) that in *Nucella lapillus* and *U. cinerea* (respectively) carbonic anhydrase is present both in active and inactive ABOs as well as in other secretory tissues of the snails. The greatest carbonic anhydrase activity occurs in the proximal and microvillar zones of the secretory cells. Carbonic anhydrase activity is inhibited by sodium acetazolamide. Initial histochemical research by Chétail & Fournié (1970) demonstrated the presence of adenosine triphosphatase in the distal rim of secretory cells and adjacent to limiting membranes of cell groups. Preliminary histochemical studies by Smarsh (personal communication) revealed strong aminopeptidase activity primarily in the epithelial cells of the ABO stalk. Chétail, Binot & Bensalem (1968) found, also histochemically, that lipase and alkaline phosphatase are present in both active and inactive ABOs. Zottoli & Carriker (1974), using hide powder azure to search for a possible protease, did not find convincing amounts in whole excised ABOs of *U. cinerea.*

Also histochemically, Smarsh et al. (1969) and Chétail & Fournié (1970) found calcium in red-staining granules, or microbodies, in active ABOs, but generally not in inactive ABOs, of *Urosalpinx cinerea* and *Nucella lapillus,* respectively. The calcium deposits found in *N. lapillus* are located at the base of the microvilli and in the zone of primary branching. A strong cobalt-binding material, possibly a chelator, was localized histochemically by Smarsh et al. (1969) in the microvillar region of the ABO of *U. cinerea.* The staining reaction is strongest in active ABOs, and there is little or no staining in inactive ones.

Studies of the ABO of *Nucella lapillus* showed that secretory cells of the inactive gland are rich in glycogen, whereas those of the active gland are poor in this storage carbohydrate (Chétail, Binot & Bensalem, 1968). Nylen et al. (1969) in an ultrastructural investigation of the ABO of *Urosalpinx cinerea* confirmed these observations.

Bernard & Bagshaw (1969) established histochemically that the ABO of the naticid snail *Polinices lewisi* consists of 2 distinct epithelial regions, a peripheral one producing mucin and a central one elaborating a mucoprotein. This anatomical condition contrasts with that of the muricid ABO which contains only the central secretory disc (Carriker & Van Zandt, 1972a).

ABO Secretion

The following papers are concerned with substances in the released secretion of the gastropod ABO which are possibly involved with shell dissolution.

Carriker, Van Zandt & Grant (1972) reported that freshly released secretion from normally extended ABOs of *Urosalpinx cinerea* is highly viscid, microscopically granular, varies in color from colorless to pale violet depending on the individual and that much of the secretion does not dissolve in seawater.

The pH of ABO secretion was measured in *Urosalpinx cinerea* with a glass microelectrode (Carriker, Van Zandt & Charlton, 1967). Continuous recording shows a pH range of 3.8 to 4.1. The cymatiid gastropod *Argobuccinum argus* secretes H_2SO_4 (0.4 to 0.5 N) for boring into tubicolous polychaetes (Day, 1969). In this case, the acid is produced not by an ABO but by a large proboscis gland.

Blots of secretion from normally extended ABOs of *Urosalpinx cinerea* collected on filter paper, as well as secretion on the exterior of the ABO in histochemical sections of the foot, test positively for carbonic anhydrase. The carbonic anhydrase activity of the secretion blots is

inhibited by sodium acetazolamide (Carriker & Chauncey, 1973). Secretion also contains cytochrome oxidase, a finding of considerable interest since this enzyme, as well as carbonic anhydrase, is almost always intracellular (Person et al., 1967).

Secretion of whole active excised ABOs and halves of active excised ABOs etch polished mollusc shell. Dissolution commences as soon as the excised gland is applied to polished shell and continues at a greatly reduced rate for several hours. Control tissues from other parts of the snail do not dissolve polished shell (Carriker, Scott & Martin, 1963; Carriker & Van Zandt, 1964). Excised ABOs heated to about 80°C (Carriker, Williams & Van Zandt, 1978), and purified bovine carbonic anhydrase likewise do not etch shell (Carriker & Chauncey, 1973). Excised ABOs treated with papain did not etch polished shell while those treated with trypsin retained their etching capabilities. Papain and trypsin solutions alone did not etch polished shell (Carriker, Williams & Van Zandt, 1978).

Behavioral Experiments

Carriker & Van Zandt (1972b) demonstrated during normal boring in prey shell that the ABO of live *Urosalpinx cinerea* dissolves shell in the incomplete borehole for periods ranging from a few minutes to about an hour. The relatively long periods of chemical activity alternate with brief periods of radular rasping. Rate of normal shell penetration by live snails, 0.3-0.5 mm per day, is reduced by sodium acetazolamide, an inhibitor of carbonic anhydrase, in both *U. cinerea* (Carriker & Chauncey, 1973) and *Nucella lapillus* (Chétail & Fournié, 1969) when the inhibitor is added to seawater in which the gastropods are maintained. Addition one at a time of $C0_2$, NaCl, and KCl to the seawater accelerates the rate of hole-boring by *N. lapillus* (Chétail & Fournié, 1969).

Comparative Calcibiocavitology

Other Invertebrates. Chemical aspects of penetration of calcareous substrata by other invertebrate calcibiocavites have also received some attention. In the bivalve *Lithophaga lithophaga* histochemical evidence points to secretion by the pallial glands of a neutral mucoprotein, which is believed to complex calcium; apparently no acid mucopolysaccharides occur, and there is no indication of an acid (Jaccarini et al., 1968). Carbonic anhydrase is implicated in the mechanism of shell penetration by the burrowing sponge *Cliona celata* (Hatch, 1975) and by the burrowing barnacle *Trypetesa nassarioides* (Turquier, 1968). Hatch (1975) postulated that the primary mechanism for dissolution of $CaCO_3$ by *C. celata* involves a shift in the carbonate solubility product in the microenvironment of etching cells of the sponge. This shift is mediated through activity of carbonic anhydrase, producing hydrogen ions by hydration of $C0_2$ within etching cells and possibly providing an optimal pH for breakdown of the organic matrix of shell. Silén (1956) observed that quantitative analysis of dry shell invaded by the shell-burrowing bryozoan *Penetrantia densa* shows a higher content of phosphate ions than uninvaded shell. Finding no other differences, he concluded that phosphoric acid is used in shell penetration by this species. It is not clear whether account was taken of phosphorus of bryozoan soft tissues.

Vertebrate Osteoclasts. Because osteoclasts attack calcareous substrates, it is important to review briefly what is known about the process of bone dissolution by them as a possible aid in explaining shell penetration by gastropod ABOs. Although investigators have shown beyond a reasonable doubt that vertebrate osteoclasts resorb bone, the mode of action is still unproved (Hancox, 1972).

The osteoclast is a large, generally multinucleated single cell possessing a prominent ruffled brush border and many membrane-bound vesicles in the cytoplasm which morphologically resemble lysosomes. Proof that these bodies contain lytic enzymes is still lacking, nor have enzymes been demonstrated in the altered bone near the ruffled border, but it seems likely that they are involved in degradation of the organic matrix. Carbonic anhydrase, cytochrome oxidase, and succinic dehydrogenase, as well as the hydrolases, acid phosphatase, leucine aminopeptidase, b-galactosidase, and b-glucosidase, have been identified in the cells. Acid hydrolases are of the lysosomal type such as might be involved in the breakdown of bone matrix (Hancox, 1972; Minkin & Jennings, 1972).

Substrates. Secretion of the ABO dissolves shell which consists of calcium car-

bonate crystals in an organic matrix of protein (some of it tanned), mucopolysaccharide, lipid, and glycoprotein (Grégoire, 1972; Wilbur, 1972); secretion of the osteoclast solubilizes bone which contains a calcium phosphate complex in an organic matrix of collagen fibers, mucopolysaccharides, and other substances (Pritchard, 1972). Ultrastructural studies suggest that ABO secretion initially dissolves the nonmineralized organic matrix surrounding shell units and then attacks the mineral crystals (Carriker, 1969), whereas osteoclasts appear to remove bone mineral first and dissolve collagen fibers later (Hancox, 1972). Although carbonic anhydrase and aminopeptidase, as well as the respiratory enzymes cytochrome oxidase and succinic dehydrogenase, have been identified in both the ABO and the osteoclast, no mineral acids or chelators have been found in osteoclasts.

DISCUSSION AND CONCLUSIONS

Although still incomplete, information reported in the literature (Table 1) suggests a tentative hypothesis on the chemical mechanism of shell penetration by the ABO of muricid and naticid gastropods. We hypothesize that a combination of enzymes, an inorganic acid, and a chelating agent is employed in a hypertonic secretion to facilitate dissolution of shell and intracellular transport of calcium during the chemical phase of valve penetration. The hypothesis is based on cellular, histochemical, and physiological observations and experimentation. The following discussion explores the shell-penetrating mechanism of boring gastropods in the context of this hypothesis. Our primary aim is to provide a conceptual framework that will stimulate further research toward elucidation of the chemical mechanism(s) of penetration of calcareous substrates by calcibiocavites (Carriker & Smith, 1969).

Secretory Epithelium

Microvilli facilitate exchange of soluble substances between secretory cells and their surroundings, and it is perhaps this purpose which is served by the unusually long microvilli of ABOs (Nylen et al., 1969). The ultrastructural organization of solute-transporting tissues is distinguished by a series of epithelial dead-end channels such as lateral spaces, basal infoldings, or intracellular canaliculi, and an active cation pump which parallels the distribution of mitochondria at the ion exchange site (Hochachka & Somero, 1973). The ABO secretory epithelium is clearly organized into 2 series of dead-end channels (Fig. 1). The hemocyanin-filled interstitial spaces and their terminal distal sinuses compose the somatic dead-end channels. The microvilli compose the dead-end channels on the lumenal side of the secretory disc. This organization, together with the localization of mitochondria adjacent to both the distal sinus and immediately below the microvilli, is morphological evidence for ion exchange as well as secretory capabilities of the ABO epithelium. The presence of glycogen in the secretory epithelium of the resting ABO and its disappearance from active ABO tissue suggests rapid mobilization of glycogen for the synthesis and release of secretory products as well as the formation of adenosine triphosphate (ATP) to fuel ionic exchange pumps at the somatic and lumenal sites discussed above (Chétail & Fournié, 1969; Nylen et al., 1969). Histochemical identification of adenosine triphosphatase (ATPase) adjacent to the interstitial space in active ABOs is therefore a logical sequel to this generalization (Chétail & Fournié, 1970). The 2-fold increase in cytochrome oxidase activity in active, as opposed to resting, ABOs is likewise in agreement with the mobilization of glycogen and appearance of ATPase activity.

Carbonic anhydrase present both within the secretory epithelium of the ABO and in the free secretion of the gland (Chétail & Fournié, 1969; Smarsh et al., 1969; Carriker & Chauncey, 1973) catalyzes the hydration of $C0_2$ to HCO_3^- and H^+. Intracellular carbonic anhydrase furnishes hydrogen ions for secretion and perhaps is also involved in ion transfer (Koefoed-Johnsen & Ussing, 1960; Carter, 1972; Minkin & Jennings, 1972). The intracellular carbonic anhydrase activity possibly also serves as a mechanism for the production of high chloride ion concentrations in the free secretion (Carriker, Williams & Van Zandt, 1978). Bicarbonate ions, their production catalyzed by carbonic anhydrase, perhaps serve as counter ions for the intracellular transport of Cl^- from the blood plasma (Schoffeniels & Gilles, 1972). The extracellular transport of Cl^- could then follow the

TABLE 1. Summary of information on the muricid-naticid accessory boring organ relative to shell dissolution.

Conditions more pronounced in active than in inactive ABOs are indicated by **.

Conditions more pronounced in inactive than in active ABOs are marked *.

Conditions similar in active and inactive ABOs and cases where the distinction is not clear have no markings.

1. Cytology of secretory epithelium:
   - Clusters of long secretory cells heavily supplied with capillaries and nerves
   - Dense brush border of long slender microvilli (0.1 μm in diameter)
   - **Dense populations of mitochondria
   - **Golgi complexes (Derer, 1975; Nylen et al., 1969)
2. Physiology of ABO:
   a. General:
      - Relatively long chemical period compared to short rasping period (Carriker & Van Zandt, 1972b)
      - Excised whole and half ABOs dissolve shell in normal and CO_2-free atmosphere (Carriker & Van Zandt, 1972b)
      - Bulk of secretion released into borehole initially, minor amount secreted thereafter (Carriker, Williams & Van Zandt, 1978)
      - HCl and glucose do not stimulate withdrawal of ABO; distilled water does (Carriker, Williams & Van Zandt, 1978)
   b. Secretory epithelium:
      1) Substances identified within it:
         - **Cytochrome oxidase (Person et al., 1967)
         - Carbonic anhydrase (Chétail & Fournié, 1969; Smarsh et al., 1969)
         - Succinate dehydrogenase (Person et al., 1967)
         - Lactate dehydrogenase (Person et al., 1967)
         - Aminopeptidase (Smarsh, personal communication)
         - Adenosine triphosphatase (Chétail & Fournié, 1970)
         - Alkaline phosphatase (Chétail, Binot & Bensalem, 1968)
         - Lipase (Chétail, Binot & Bensalem, 1968)
         - **Chelator (Smarsh et al., 1969)
         - PAS positive (mucoproteins, neutral mucopolysaccharides) (Bernard & Bagshaw, 1969; Carriker, Van Zandt & Grant, 1972)
         - No acid mucopolysaccharides (Carriker, Van Zandt & Grant, 1972)
         - **Calcium (Chétail & Fournié, 1970; Smarsh et al., 1969)
         - *Glycogen (Chétail, Binot & Bensalem, 1968; Nylen et al., 1969)
      2) Particles observed within it:
         - Secretion granules (0.2 μm average), multivesicular bodies, vesicles (Bernard & Bagshaw, 1969; Derer, 1975; Nylen, et al., 1969)
   c. Secretion released by secretory epithelium:
      1) Characteristics:
         - Viscid, granular, generally insoluble in seawater and in distilled water, highly hygroscopic, about 65% volatile, "crystallizes" after exposure to atmospheric condensate (Carriker, Van Zandt & Grant, 1972; Carriker, Williams & Van Zandt, 1978)
      2) Substances identified in it:
         - Organic matter and NaCl (E. M. Smith, personal communication)
         - Cytochrome oxidase (Person et al., 1967)
         - Carbonic anhydrase (Carriker & Chauncey, 1973)
         - Hydrogen ions (pH 3.8-4.1 with trace seawater; 5.2 pure) (Carriker, Van Zandt & Charlton, 1967; Carriker, Williams & Van Zandt, 1978)
         - Chloride ions (Carriker, Williams & Van Zandt, 1978)
         - Sodium ions (Carriker, Williams & Van Zandt, 1978)
         - Calcium ions, trace (Carriker, Williams & Van Zandt, 1978)
         - Potassium ions, trace (Carriker, Williams & Van Zandt, 1978)
      3) Particles observed in it:
         - Secretion granules (0.17-0.40 μm), SEM (Carriker, Williams & Van Zandt, 1978)
         - Vesicles (0.04-0.06 μm), SEM (Carriker, Williams & Van Zandt, 1978)
         - Red particles, trichrome stain (Carriker, Williams & Van Zandt, 1978)
3. Factors affecting shell dissolution by ABO secretion:
   a. Accelerate:
      - Increasing temperature (Carriker, Van Zandt & Grant, 1972)
      - CO_2, KCl, NaCl (live snails) (Chétail & Fournié, 1969)
   b. Decelerate:
      - Sodium acetazolamide, in culture (Carriker & Chauncey, 1973; Chétail & Fournié, 1969)
   c. Inhibit:
      - Papain (excised ABO) (Carriker, Williams & Van Zandt, 1978)
      - Heat (80°C) (excised ABO) (Carriker, Scott & Martin, 1963; Carriker, Williams & Van Zandt, 1978)
      - Sodium acetazolamide, in shell-ABO preparation (Carriker & Chauncey, 1973)
   d. Uncertain effect:
      - CO_2 (excised ABO) (Carriker, Williams & Van Zandt, 1978)
      - Trypsin (excised ABO) (Carriker, Williams & Van Zandt, 1978)

electrochemical gradient set up by the active extracellular transport of either Na^+ or H^+ (Carriker, Williams & Van Zandt, 1978) or, alternatively, be exchanged for

HCO_3^- in the free secretion (Cotlove & Hogben, 1962; Rehm, 1972). Production of HCl by parietal cells of the gastric mucosa of vertebrates is visualized as being carbonic anhydrase-dependent with separation of hydrogen and hydroxyl ions; the secreted hydrogen ions are linked to chloride ions (Maren, 1967). The extraordinary density of mitochondria and numerous microvilli in parietal cells (Ito & Winchester, 1963) is reminiscent of these features in the gastropod ABO (Nylen et al., 1969). Active transport of chloride and sodium ions also occurs in the aqueous humor of vertebrates where secretion of chloride ions (with or without sodium ions) is also blocked by carbonic anhydrase inhibitors (Garg & Oppelt, 1970).

Secretion

Membrane-bound secretion granules and vesicles in the secretory epithelium of the ABO (Nylen et al., 1969; Bernard & Bagshaw, 1969; Derer, 1975) and in secretion blots (Carriker, Williams & Van Zandt, 1978) are possibly the extracellular carriers of carbonic anhydrase as well as other precursors of the active dissolving agent in extracellular use. This view is consistent with Hatch's (1975) observation that carbonic anhydrase in *Cliona celata* is particle- or membrane-bound, as well as the fact that cross-membrane transport of such a large molecule as carbonic anhydrase (molecular weight, 30,000) or cytochrome oxidase (240,000) is unlikely. Although fusion of the secretion granules with the plasma membrane has been observed (Nylen et al., 1969), identification of their chemical constituents has not yet been accomplished.

The ABO secretion is a complex chemical mixture which is most probably elaborated by several pathways within the secretory epithelium. However, elaboration pathways and routes of extracellular discharge of secretory products are not yet clear. The brush border appears to serve as the surface of release (Nylen et al., 1969; Derer, 1975). The role of the secretory epithelium is complicated in that it is involved in the intracellular transport of calcium as well as in the production of secretion. The process of solubilization of mineral crystals and organic matrix during shell penetration can therefore be best understood by considering the activities of the ABO secretion and the secretory epithelium as being multiphasic interdependent processes. We suggest that a combination of enzymes and chelator(s), together with acidic and saline properties of the secretion, function in shell penetration and calcium transport into the secretory epithelium.

Enzymes

The pure ABO secretion is a slightly acidic (pH = 5), hypertonic (NaCl), enzyme-bearing (carbonic anhydrase) fluid (Carriker, Van Zandt & Grant, 1972). Enzymes or proteinaceous material other than carbonic anhydrase is possibly present in the secretion as well. Cytoplasmic localization of aminopeptidase in the stalk epithelium (Smarsh, personal communication), organic matter in the secretion (Smith, personal communication), and inactivation of shell-dissolving activity of excised ABOs by heat and papain suggest this view (De Duve & Wattiaux, 1966; Scott, 1967; Carriker, Scott & Martin, 1963; Carriker, Williams & Van Zandt, 1978). That HCl alone is not responsible for shell dissolution is indicated by patterns of dissolution: HCl solubilizes the mineral crystals first when applied to a shell surface, whereas the ABO secretion tends to dissolve the organic matrix first (Carriker & Van Zandt, 1972b). Carbonic anhydrase by itself does not etch shell (Carriker & Chauncey, 1973). We postulate, therefore, that an enzyme or a chelator is involved in digestion of the fraction of organic matrix (nacrosclerotin and nacroin, Grégoire, 1972) insoluble in water and EDTA, and possibly in the dissolution of the mineral components of shell.

Enzymes have not yet been identified, but perhaps are conchiolinase-like molecules which catalyze removal of the organic components of shell (conchiolin and periostracum) (Carriker, 1969; Nylen et al., 1969; Travis & Gonsalves, 1969) prior to dissolution of the mineral component. Action of the ABO secretion is directed 1st toward dissolution of the organic matrix that surrounds each shell unit (prisms, lamellae; Taylor et al., 1969) and then to the intraunit organic matrix and its constituent $CaCO_3$ crystals. Hydrolytic enzymes are assumed to function similarly in removal of organic matrix during resorption of bone (Vaes, 1968). Hydrogen ions secreted by the ABO (Carriker, Van Zandt

& Charlton, 1967; Carriker, Williams & Van Zandt, 1978) possibly contribute toward maintenance of optimal pH for enzymatic activity. Acid mucopolysaccharides are probably not involved in dissolution as they have not yet been identified in the ABO (Carriker, Williams & Van Zandt, 1978).

Chelators

A water-soluble, acetone-precipitable chelator present in the microvillar zone of the ABO (Carriker & Smith, 1969; Smarsh et al., 1969; see also Jenkins & Dawes, 1963) perhaps contribute to dissolution of the water-soluble nacrin in the organic matrix of shell as well as the mineral crystals. The insoluble nacrosclerotin and nacroin are presumably attacked by hydrolytic enzymes (Grégoire, 1972). This suggestion is supported by Travis & Gonsalves (1969) who found that the organic matrix within shell units is soluble in water and in dilute concentrations of EDTA. They also demonstrated that organic sheaths of shell units solubilize with prolonged treatment in mild concentrations of EDTA while a 3rd fraction is insoluble in prolonged treatment.

Inorganic Acid

The presence of hydrogen ions (Carriker, Van Zandt & Charlton, 1967; Carriker, Williams & Van Zandt, 1978) and chloride ions (Carriker, Williams & Van Zandt, 1978) in the free secretion, and of the chelator in the microvillar zone of the ABO, suggest that HCl perhaps functions in combination with the chelator to dissolve the $CaCO_3$ in shell and bind calcium as a water-soluble compound for rapid removal. The pH 5 of the secretion corresponds to an H^+ concentration of 10^{-5}M. The Cl^- concentration reached levels as high as 1.71 M, suggesting that if it is secreted as HCl, the H^+ is used up and Cl^- concentration increases with time. This is in agreement with our results (Carriker, Williams & Van Zandt, 1978). Since methods used in measuring in vivo hydrogen and chloride ion concentrations allowed access to the secretion prior to, or independent of, its application to a $CaCO_3$ substrate, one could argue that the disparity in H^+ and Cl^- concentrations was due to independent mechanisms for the release of the ions, or a reaction necessary for activating a component of the secretion, but not directly participatory in the shell-dissolution mechanism. We reiterate that HCl alone is not responsible for dissolution of shell.

Shell Dissolution

Jaccarini et al. (1968) proposed that the burrowing bivalve *Lithophaga lithophaga,* which apparently does not secrete acid, penetrates shell by complexing calcium through secretion of neutral mucoprotein with calcium-binding ability. In the same way mucoprotein in the gastropod ABO (Bernard & Bagshaw, 1969; Carriker, Williams & Van Zandt, 1978) possibly also participates in complexing calcium during hole-boring. In a recent review of calcium-binding proteins Kretsinger (1976) noted that vitamin D-induced calcium-binding proteins appear in high concentrations in the vertebrate gut, kidney, and avian eggshell gland. Also, active accumulation of calcium in mitochondria is apparently driven by an anion and/or H^+ gradient as opposed to the Na^+ gradient that drives the Ca^{+2} pump of axons (Kretsinger, 1976; see also Wasserman & Taylor, 1969). The possibility of calcium-binding glycoproteins has been discounted since goblet (mucous) cell formation in the ABO, at least in muricid gastropods, is not evident.

That calcium ions freed from shell in the borehole do enter the microvilli of active ABOs and pass into the foot of the snail has been demonstrated by Smarsh et al. (1969) and Chétail & Fournié (1970). Calcium localized histochemically by Chétial & Fournié (1970) appeared at the base of the microvilli in the zone of primary branching of the secretory epithelium. The transmembrane flux of calcium is probably aided by carbonic anhydrase (Istin & Kirschner, 1968) and adenosine triphosphatase, and as shown for intestinal mucosal cells, passage of calcium from the secretory cells seems to be dependent on sodium (Birge et al., 1972). A similar active transport of calcium takes place in the calciferous glands of earthworms (Crang et al., 1968), and across the isolated avian shell gland (Ehrenspect et al., 1971). Active ATPase-dependent secretion of hydrogen ions by the ABO could establish an electrochemical gradient, which in turn is counter-balanced by the passive influx of calcium ions (Chétail & Fournié, 1970). Outflux of sodium ions in the free secretion may likewise contribute to

balancing the influx of calcium ions, the transport possibly being coupled to the movement of chloride ions as suggested by Binder & Rawlins (1973) in the intestinal mucosa and by Garg & Oppelt (1970) in the aqueous humor.

If chloride ions from the blood plasma are exchanged for HCO_3^- generated by carbonic anhydrase in the secretory epithelium, then several explanations could account for the appearance of high chloride ion concentrations in the free secretion: 1) chloride ion extrusion follows an electrochemical gradient set up by secretion of hydrogen ions, 2) chloride ion extrusion follows an electrochemical gradient set up by active sodium ion transport, and 3) chloride ions are exchanged for HCO_3^- produced catalytically by carbonic anhydrase in the free secretion. If chloride ion transport is strictly dependent on the hydrogen ion gradient, then, considering the quantities of Cl^- in the free secretion, it would be difficult to account for the simultaneous intracellular transport of calcium. If chloride ions are exchanged for HCO_3^- generated by carbonic anhydrase or if chloride ion extrusion follows an electrochemical gradient set up by active sodium ion transport, then the remaining electrochemical gradient would owe its ion transport potential to the H^+ gradient, a condition that would favor intracellular transport of Ca^{+2}. The substantial quantities of NaCl (Carriker, Williams & Van Zandt, 1978) and carbonic anhydrase (Smarsh et al., 1969) in the free secretion possibly thus represent the involvement of one or both of these processes and suggest a profitable line for further research. Comparable exchange pumps have been described in the gill of teleost fish where the net extrusion rate of sodium is identical to the potassium influx (Maetz, 1969) and in the avian salt gland which is carbonic anhydrase-dependent (Hochachka & Somero, 1973). In the case of the avian salt gland intracellular hydrogen and bicarbonate ions are exchanged for blood sodium and chloride ions. The cellular NaCl is then actively eliminated by a Na-K ATPase pump (Hochachka & Somero, 1973). Problems which must be faced in postulating a calcium chelator in the free secretion are its stability constant in a hypertonic medium, and competing ions at low pH (Moeller & Horwitz, 1960).

An explanation for dissolution of mineral crystals of shell by the ABO secretion following digestion of the organic matrix is suggested by examination of the chemical constituents of the secretion in the context of their combined influence on the apparent (stoichiometric) solubility product of calcium carbonate (Pytkowicz, 1969). The apparent solubility product (ionization product) of $CaCO_3$ is related to the thermodynamic solubility product by the expression

$K'_{sp} = K_{sp}/f_{ca}f_{co_3}$, where,

K'_{sp} = apparent solubility product

K_{sp} = thermodynamic solubility product

f_{ca} = calcium activity coefficient

f_{co_3} = carbonate activity coefficient (Pytkowicz, 1969).

In seawater the solution and precipitation of calcium carbonate are dependent on chlorinity (more precisely ionic strength), carbonate alkalinity, and pH. The apparent solubility product of calcium carbonate is dependent on ionic strength, ion pairing, and pH. Increases in ionic strength and ion pairing, or decreases in pH, cause decreases in the activity coefficients f_{ca} and f_{co_3} with a resultant increase in K'_{sp}, the apparent solubility product. The ABO secretion accomplishes all 3 of these functions. The concentration of Cl^- (up to 1.7 M!) and Na^+, as measured by ion specific electrode and electron dispersive x-ray analysis, respectively, indicate a very high salt content in the secretion. If Na^+ concentration is as high as Cl^- concentration, then the average ionic strength of the secretion based on these ions alone would be 1.1 (1.7 maximally). For purposes of comparison, the ionic strength of seawater is approximately 0.7. Calcium and carbonate activity coefficients would further decrease due to the pH of the secretion and ion pairing of CO_3^{-2} and HCO_3^- with Na^+ (Garrels & Thompson, 1962; Pytkowicz, 1969). Finally, removal of free calcium ions by either chelation or ion transport across the secretory epithelium, would further serve to decrease the activity coefficient of calcium and render an increase in the apparent solubility product of calcium carbonate.

Increases in concentration of NaCl and KCl in seawater, in which living *Nucella lapillus* were boring, accelerated the rate of shell penetration (Chétail & Fournié, 1969). Perhaps these ions when applied

externally also participate in transmembrane ionic fluxes which contribute to shell dissolution (see also Tormey, 1966). However, if ion uptake was generated by either a Na-K ATPase or a Na-H ATPase activated pump, one would not expect, assuming sodium uptake, that the KCl solution would have the observed effect. In view of the chloride ion concentration of the secretion, the observed influence of both KCl and NaCl would be consistent with either a Cl^- uptake coupled to active uptake of Na^+ or a Cl^-/HCO_3^- exchange. Deceleration of the rate of boring by live snails in seawater solutions of sodium acetazolamide (Chétail & Fournié, 1970; Carriker & Chauncey, 1973) can be explained by inhibition of carbonic anhydrase activity and the hydration of CO_2 to HCO_3^- and H^+ (Carter, 1972). Presumably, this inhibition occurs intracellularly and disrupts hydrogen ion secretion (Chétail & Fournié, 1969) and possibly the chloride ion-secreting mechanism (Carriker, Williams & Van Zandt, 1978). The carbon dioxide experiments performed by Chétail & Fournié (1969) and by Carriker, Williams & Van Zandt (1978) seem to have conflicting results. Increased CO_2 in seawater significantly increased the rate of boring by *Nucella lapillus* in Chétail and Fournié's (1969) behavioral experiment. Carriker, Williams & Van Zandt (1978) conducted a shell-etching bioassay in CO_2-free seawater and found no difference between etchings produced by ABOs in CO_2-free seawater and ABOs in untreated seawater. Chétail & Fournié (1969) surmise from the results of their experiment that increased CO_2 levels in seawater caused a corresponding increase in intracellular CO_2 and a subsequent increase in production of hydrogen ions through carbonic anhydrase catalysis. Obviously, Carriker, Williams & Van Zandt (1978) were looking for a diminution in etching activity as a result of substrate limitation of carbonic anhydrase activity. Their results suggest 2 alternative explanations: 1) residual intracellular CO_2 was being utilized and limitation of carbonic anhydrase substrate was not achieved by decreasing environmental CO_2; 2) the activity of carbonic anhydrase in the free secretion does not require a source of CO_2 independent of the $CaCO_3$ substrate. The latter alternative is consistent with the findings that carbonic anhydrase is a necessary component of the secretion which cannot by itself dissolve $CaCO_3$, but which in some way possibly interacts with solubilized $CaCO_3$ and function in its removal (Hatch, 1975; Carriker, Williams & Van Zandt, 1978). Failure of the ABO to withdraw from the borehole when 1 N HCl (a concentration 1000 times higher than that expected at the pH 4 of the secretion but nearly isotonic with respect to the Cl^- concentration of the secretion) was inadvertently spilled on it, whereas it withdrew quickly when distilled water was applied, indicates that the ABO is conditioned to the HCl in its own secretion and was thus not irritated by an additional amount (Carriker, Williams & Van Zandt, 1978). Total inhibition of etching by heat in the excised ABO-polished shell preparation seems to have resulted not only from inactivation of enzymes but also from volatilization of other shell-dissolving substances.

Possible Organic Substances in Secretion

The possibility that organic matter secreted by the ABO includes succinic acid or lactic acid, or both, should not be discounted, even though these acids are generally found in tissues. According to Simpson & Awapara (1966) and Stokes & Awapara (1968), the main product of metabolic glucose degradation in the molluscs examined was succinic acid with small amounts of lactic acid. Crenshaw & Neff (1969) demonstrated that succinic acid dissolved shell at the mantle interface in closed clams (*Mercenaria mercenaria*). These acids, if present in the ABO secretion, could also function in shell dissolution. Person et al. (1967) reported active succinate and lactate dehydrogenases in the ABO, confirming the presence of succinic and lactic acids in the secretory epithelium, but not necessarily in the free secretion.

Vertebrate Osteoclasts

Information on the chemical mechanism of bone dissolution by osteoclasts, like that of shell dissolution by ABOs, is still in the alpha stage, and thus does not help much in explaining shell dissolution by ABOs. However, it is worth noting that, as in all shell-boring and burrowing invertebrates so far described, carbonic anhydrase also appears to be involved in bone dissolution by osteoclasts. Furthermore, observations

suggest that osteoclastic hydrolases participate in degradation of bone matrix (Hancox, 1972), lending support to circumstantial evidence for possible involvement of hydrolases in the breakdown of the insoluble fractions of the organic matrix of shell.

ABO Activity in Borehole

Initial release of the bulk of the secretion by the ABO as it 1st enters the borehole is important in starting dissolution promptly over the entire bottom of the borehole. Continued, though reduced, secretion would appear useful in extending the period of dissolution. What determines the time of withdrawal of the ABO from the borehole at the termination of the chemical period is not known, but retraction could be triggered by exhaustion of the shell-dissolving agent in the secretion. The highly viscid, partially insoluble nature of the secretion probably aids application to, and holding the secretion in, the bottom of the incomplete borehole by the ABO.

Myroslaw Harasewych (personal communication) calculated that for a hypothetical borehole 1.0 mm in diameter and 3.0 mm deep (borehole of average size for an adult *Urosalpinx cinerea*), the volume of shell removed would be 2.35 mm^3. Assuming the shell to be pure calcite (true in the case of the oyster, except for the organic matrix and trace minerals), 6.4 mg (0.064 mmol) of $CaCO_3$ would have to be removed. This would require 4.67 mg of HCl or 7.6 mg of succinic acid, or 0.064 mmol of a divalent chelating agent, and not take into account removal of portions of the chemically weakened shell by radular activity. An enzyme, on the other hand, is capable of catalyzing a reaction at rates of 10^4 to 10^6 (3.6×10^6 in the case of carbonic anhydrase, isozyme C) moles of substrate per mole of enzyme per min when the substrate is in solution or in suspension. At the slower rate, dissolution of the 2.36 mm^3 of shell in 1 minute would require the production of 10^{-8} mole of a hypothetical shell-dissolving enzyme. Rate of shell penetration by *U. cinerea* normally ranges from about 0.3 to 0.5 mm per day (Carriker & Van Zandt, 1972b), and dissolution occurs only at the surface of shell in the borehole. In nature, excavation of a borehole 3 mm in diameter takes about 6 days at the fastest rate of penetration. Harasewych's calculations demonstrate the extreme difference in the quantities of different kinds of secretion required to perform equivalent dissolution of shell. However, since radular activity alternates with chemical activity in hole-boring, and removes 10 to 20% of the surface area of the bottom of the incomplete borehole to a depth of a few μm at each rasping period (Carriker, 1969), smaller quantities of shell solvents possibly are required than would be the case if shell penetration were entirely chemical.

CONCLUSIONS

We hypothesize that a combination of enzymes, an inorganic acid, and chelating agents is employed in a hypertonic secretion to facilitate dissolution of shell and intracellular transport of calcium during the chemical phase of valve penetration by boring gastropods. Secretion granules and vesicles in the secretory epithelium of the ABO and in the released secretion, organic matter in the secretion, and dissolution by the secretion of organic matrix of shell, especially the insoluble fractions, provide circumstantial evidence for the presence of enzymes. Hydrogen, chloride, and sodium ion concentrations demonstrate the hypertonic and acidic characteristics of the released secretion. A chelating agent, though not chemically identified, and a mucoprotein have been found in the secretory epithelium; the latter perhaps functions in chelation.

The physiological-chemical mechanism of shell-penetration could prove a valuable tool to those physiologists and cellular biologists interested in developing models for elucidating mechanisms of ion transport and exchange (hydrogen, chloride, sodium, calcium), acid secretion (HCl), carbonic anhydrase enzymology, dissolution of calcareous substrates (dental caries), and calcium-binding proteins and chelators, within the context of their own disciplines.

It is a curious fact that, with the exception of 1 group of freshwater polychaetes (*Caobangia;* Jones, 1969), all shell-excavating species so far known are inhabitants of estuarine or marine habitats. Calcibiocavitic algae are likewise predominantly marine (Golubic, 1969; Golubic et al., 1975). These facts suggest that seawater and

estuarine water are characterized by factors that have favored the evolutionary development of the shell-penetrating mechanism. A better understanding of the chemical mechanism of shell penetration would be enlightening with respect to these factors and subsequently allow postulation of methods for control of predatory gastropods and other shell-penetrating organisms harmful to shellfish mariculture.

ACKNOWLEDGMENTS

The research was supported in part by a grant from the University of Delaware Research Foundation.

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MALACOLOGIA, 1978, 17(1): 157-162

AN ELECTROPHORETICALLY DETECTED SIBLING SPECIES OF *"GONIOBASIS FLORIDENSIS"* (MESOGASTROPODA: PLEUROCERIDAE)

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ABSTRACT

Electrophoretic analysis of *Goniobasis* from the Ichetucknee River, Columbia Co., Florida, U.S.A., revealed that 2 morphologically very similar species are present at this site. The lack of common electromorphs at 8 of 18 loci argues strongly against gene flow between these sympatric species. One species is *G. floridensis* (Reeve, 1860), known throughout Florida, and the other has affinities to *G. athearni* Clench & Turner, 1956, a species found 280 km distant in the Florida Panhandle. Even though these species are widely divergent at the structural gene level, some individuals cannot be specifically identified without electrophoretic determination.

INTRODUCTION

The value of the techniques of gel electrophoresis of proteins for the detection of sibling species was first demonstrated by Manwell & Baker (1970) in holothurians. Using gel electrophoresis, they discovered that two indistinct "forms" of the sea cucumber *Thyonella gemmata* are actually different species. Grassle & Grassle (1976) used similar methods and found that the marine polychaete worm *Capitella capitata* is actually at least 6 distinct sibling species. In each case, discovery of sibling species was the result of the detection of protein bands, or electromorphs, that had electrophoretic mobilities unique to a given species. This paper is a report on a sibling species differing from *Goniobasis floridensis* (Reeve, 1860), and detected by starch gel electrophoresis.

Goniobasis floridensis is a common snail in the springs and spring-fed rivers and streams of northern Florida and adjacent parts of Georgia and Alabama. During a wider investigation of electrophoretic variation in *Goniobasis* (Chambers, 1977), a site on the Ichetucknee River was selected as the site of a reference population, individuals of which could be run on all gels as a standard for the measurement of electromorph mobilities of proteins of individuals from various populations. This site was chosen because it was close to Gainesville (where the laboratory work was performed), it had a very large population of *Goniobasis* which appeared to be typical *G. floridensis*, and preliminary work showed that these snails were nearly monomorphic with respect to isozyme patterns. After using this population as a reference for comparison with several other *G. floridensis* populations, the wide divergence between the reference and these populations became obvious. Both the reference population and most *G. floridensis* populations have what shall be referred to as the "standard" *G. floridensis* shell sculpture pattern. This pattern is comprised of axial costae and spiral cords that intersect to form nodules. The peripheral cord is usually the largest. The members of the Ichetucknee reference population were noted to differ in having the shell spire more eroded and less sharply sculptured, but this condition was attributed to abrasion against the bare limestone substrate of this rapidly flowing river. The shell morphology seemed well within the range of variation observed statewide in *G. floridensis*.

Using the glutamate oxalacetate transaminase (Got) locus, which was fixed for an electromorph in the Ichetucknee reference population not found in any of the *G. floridensis* samples, *Goniobasis* were sampled from other nearby sites in the Suwannee River system to indicate the extent of the divergent genotype of the Ichetucknee reference population (Chambers, 1977). All of these samples showed

the typical *G. floridensis* electromorph, Got^{93}. Careful study of the morphology of new collections from the Ichetucknee River locality suggested that there might be two species at the site of the reference population, one of which, true *Goniobasis floridensis*, had not been collected earlier. The electrophoretic analysis of samples of these morphologically very similar species is the subject of this investigation.

MATERIALS AND METHODS

All snails in this study were collected from the Ichetucknee River at the Florida Department of Transportation roadside park on U.S. 27, Ichetucknee Springs State Park, Columbia County, Florida. This exceptionally clear river is fed by Ichetucknee Springs. *Goniobasis* snails were collected by hand and by use of a bottom sampling net. They were brought to Gainesville on the day of collection in unaerated jars or plastic boxes and placed in a 24-gallon aquarium that had been prepared in advance. The bottom of the aquarium had been spread with 2-3 cm of fine sand and the water was aerated and filtered by an outside filter.

Snails to be electrophoretically examined were removed from their holding aquaria the night before electrophoresis and kept in a clean bowl of water so that they could not feed. Only adult snails were used. The following day, each animal was extracted from its shell and placed in an individual centrifuge tube with an equal volume of the Tris-EDTA grinding buffer of Selander et al. (1971). They were then homogenized by sonication with a Branson Model W185 sonicater equipped with a micro tip. The samples were centrifuged at 4300 g for 5 minutes. The supernatant was drawn off and the pellet discarded. Fifty-two individuals of each species were examined.

Horizontal starch gel electrophoresis was carried out with the samples applied to gels as Whatman No. 1 chromatography paper tabs soaked in the homogenate supernatant of a single snail and then lightly blotted. Following electrophoresis, gels were sliced and placed individually in stains for 14 different enzymes. These enzymes, with their abbreviations, are acid phosphatase (Acph), aldolase (Aldo), alkaline phosphatase (Aph), esterase (Est), glutamate oxalacetate transaminase (Got), glyceraldehyde-3-phosphate dehydrogenase (G3pd), hexanol dehydrogenase (Hexdh), leucine aminopeptidase (Lap), malic enzyme (Me), 6-phosphogluconate dehydrogenase (6Pgd), phosphoglucose isomerase (Pgi), phosphoglucomutase (Pgm), sorbitol dehydrogenase (Sdh), and tetrazolium oxidase (To). Throughout the remainder of this paper, these enzymes will be referred to by their abbreviations. Staining solutions were prepared according to Bush & Huettel (1972) for Acph, Aph, Est, Hexdh, Lap, 6Pgd, and Pgi; according to Ayala et al. (1973) for G3pd, Me, and Pgm; according to Shaw and Prasad (1970) for Sdh and Aldo; and according to Selander et al. (1971) for Got. Five different gel buffer systems were used to resolve these enzymes. Gels using the Poulik (1957) system and 21 g of Connaught starch, 21 g of Electrostarch, and 20 g of sucrose per 440 ml of gel buffer were used for Est and Aph. The same starch and sucrose mixture was used for the lithium hydroxide buffer system of Selander et al. (1971) for Pgi and Got. The remaining systems used 44 g of electrostarch per 440 ml of gel buffer. The Poulik (1957) system was used for Sdh, Lap, Me, and Pgm. Buffer B of Ayala et al. (1973) with the pH adjusted to 9.1 was used for G3pd, Aldo, Hexdh, and To. The histidine buffer system of Brewer (1970: 90) was used for Aph and 6Pgd with the following modifications: The concentration of the bridge buffer was 0.2 M and the pH of both the bridge and the gel buffers was set at 8.0.

Voucher specimens were deposited in the Florida State Museum (FSM) as follows:

- *G. floridensis* (Reeve, 1860), 24884, Ichetucknee River, Florida.
- *G.* sp. (reference sp.), 24885, Ichetucknee River, Florida.
- *G. albanyensis* Lea, 1864, 24886, Chattahoochee River, Florida.
- *G. athearni* (Clench & Turner, 1956), 24887, Chipola River, Florida.

The last two species listed were studied by Chambers (1977) and used for comparison with the first two listed species.

RESULTS

The results of the electrophoretic analysis are given in Table 1. Six loci (Acph-1,

TABLE 1. Allele (electromorph) frequencies, proportion of loci polymorphic, and average heterozygosities for 12 polymorphic loci in 2 species of *Goniobasis* from the Ichetucknee River.

Locus	Allele	*G. floridensis*	*Goniobasis* sp.
Aph	102	1.00	—
	100	—	1.00
Est-2	100	—	1.00
	95	1.00	—
Est-3	103	.038	.010
	100	.962	.990
Got	100	—	1.00
	96	.029	—
	93	.971	—
G3pd	100	.048	.942
	95	.952	.058
Lap-1	107	.038	—
	102	.952	.067
	101	.010	—
	100	—	.933
Lap-2	102	1.00	—
	100	—	1.00
Me	102	1.00	—
	100	—	1.00
6Pgd	109	1.00	—
	100	—	1.00
Pgi	109	.029	—
	100	.971	1.00
Pgm	101	1.00	—
	100	—	.490
	99	—	.298
	97	—	.212
Sdh	102	—	.087
	100	—	.913
	98	1.00	—
Loci polymorphic*		.000	.222
Loci polymorphic**		.278	.222
Average heterozygosity		.021	.058

*A locus is considered polymorphic if no allele has a frequency greater than or equal to 0.99.
**A locus is considered polymorphic if no allele has a frequency greater than or equal to 0.95.

Acph-2, Aldo, Est-1, Hexdh, and To) were fixed for the same electromorph in both species samples and have been excluded from the table. Scoring of gels followed the criteria of Ayala et al. (1973). Gels were scored as autosomal loci with codominant alleles. The most common allele, or electromorph, in the reference population for each locus was designated with the superscript ^{100}, with the other alleles at the locus designated by adding to 100 the distance in milimeters a band migrates anodal to the standard, or by subtracting from 100 the number of millimeters a band runs cathodal to the standard. Loci were designated for polymorphic loci when the genotype frequencies were found to approximate Hardy-Weinberg equilibrium. Genetic interpretations were further confirmed for Got, G3pd, and Pgi by the presence of heterodimers in the heterozygotes. All bands migrated anodal from the origin.

DISCUSSION

The presence of 2 genetically distinct species, true *G. floridensis* and the reference species, in the Ichetucknee River is confirmed by the data presented in Table 1. The lack of common alleles (Fig. 1) in these 2 samples at the Aph, Est-2, Got, Lap-2, Me, 6Pgd, Pgm, and Sdh loci is strong evidence that there is no gene flow between these sympatric species. No visible taxonomic shell character will consistently separate the 2 species (Fig. 2). The most useful characters of the reference species include the presence of freshwater sponges, the worn shell spire, and habitat preference for rock rather than vegetation. The overall aspect of the shell of the reference species is shorter and costae are not as strongly developed in most individuals, and the outer lip of the aperture is straighter than the more sigmoidly shaped outer lip of the *G. floridensis* aperture. Even so, some individuals, especially if they are abraded, are impossible to identify as one or the other species without electrophoretic determination. For this reason, the two Ichetucknee River species of *Goniobasis* can be considered sibling species.

The degree of similarity between sets of isozyme data from different populations was estimated using the genetic identity (I) method of Nei (1972), which is the normalized probability of identity of alleles. The two Ichetucknee River samples of *Goniobasis* have an I value of 0.468 (calculated for congruence of alleles over 18 loci), which is in the range of values found when comparing different non-sibling species of *Drosophila* (Ayala et al., 1974). The reference species shows high identities with *Goniobasis athearni* (0.911) and *G. albanyensis* (0.862) (Chambers, 1977), 2 species found in the Florida Panhandle. These values are well within the range of those found between conspecific populations of *Drosophila.* The reference population is most similar in shell morphology to *G. athearni*, which it resembles in overall form, differing only in more-developed costae and a less-developed peripheral cord (Fig. 3). The divergence between *G.*

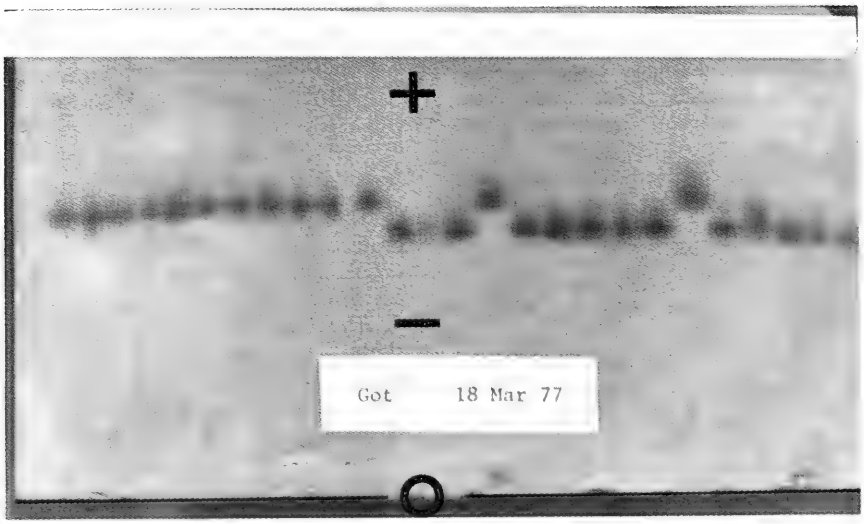

FIG. 1. Gel slice stained for Got. The origin (0), anodal (+), and cathodal (-) ends of the gel are indicated. From left to right patterns 1-11, 15, and 21 are members of the reference species (*Goniobasis* sp.) and indicate the Got^{100} electromorph. Patterns 12-14, 16-20, and 22-26 are of *G. floridensis* and indicate the Got^{93} electromorph. The individual represented by pattern 23 is heterozygous ($Got^{96/93}$). The scale is numbered in cm.

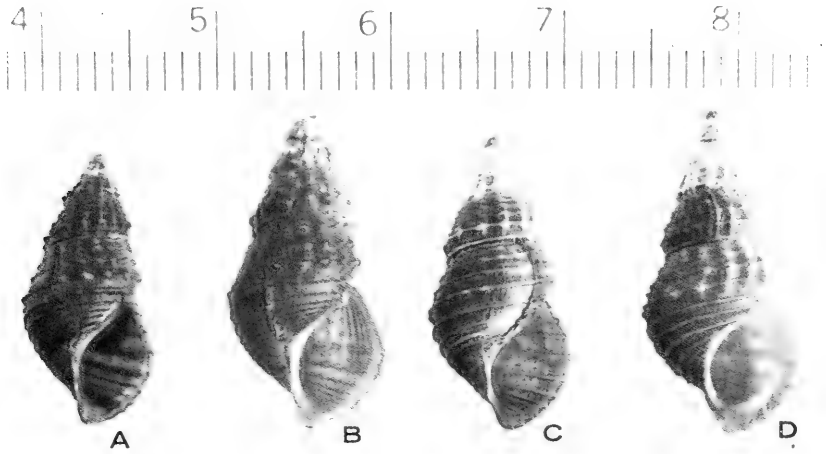

FIG. 2. *Goniobasis* from the Ichetucknee River. A-B, reference species (*Goniobasis* sp.). C-D, *G. floridensis*.

athearni (and the reference species) and *G. floridensis* on the genetic level is in contrast to Clench & Turner's (1956) belief that these 2 species are so similar that it was surprising to find them sympatric in the Chipola River. Morphological and genetic similarities suggest that the reference species would be best considered a population of *G. athearni* until studies of reproductive relationships can be made.

The seemingly disjunct distribution of *G. athearni* in the Chipola River and the reference population 280 km distant in the Ichetucknee River has interesting parallels

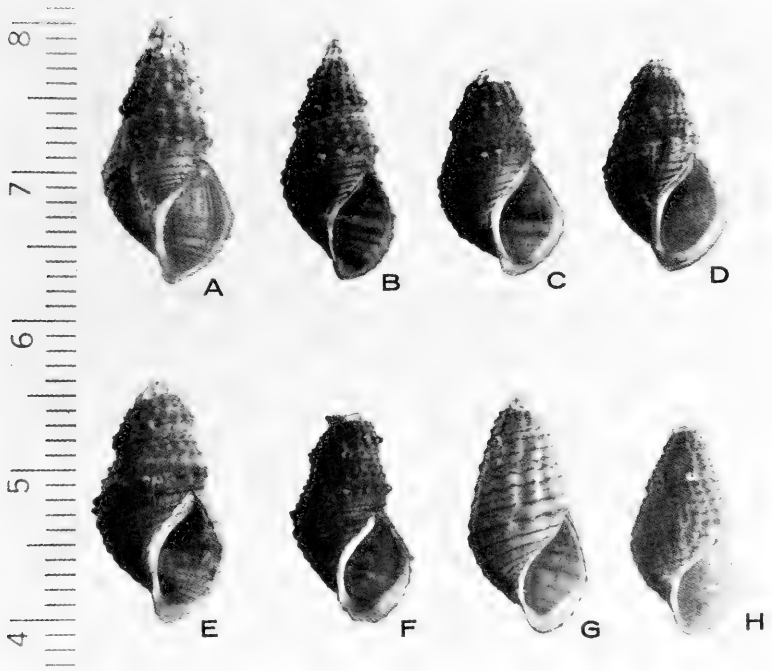

FIG. 3. A-D, reference species (*Goniobasis* sp.). E-F, *G. athearni,* Chipola River. G-H, *G. albanyensis,* Apalachicola River.

in other groups. Jackson (1975) has described the presence of a fossil aquatic turtle of the genus *Graptemys* in the Santa Fe River. *Graptemys barbouri*, to which Jackson has referred these fossils, is found today only in the Apalachicola River system, including the Chipola River. He offers the explanation that the Apalachicola and Suwannee (including the Ichetucknee River) river systems are in close proximity where they drain adjacent river basins in southern Georgia. Similar patterns of distribution appear in other aquatic organisms (Jackson, 1975).

The very low genetic identity between these sibling species of *Goniobasis* differs from the finding of Ayala et al. (1974) that sibling species of *Drosophila* are more closely related to each other than non-sibling species. The identity between the sibling species of *Goniobasis* is closer to the value for non-sibling species of *Drosophila.* This may indicate a fundamental difference between these groups in the process of evolutionary divergence. Mayr's (1963) suggestion that sibling species are no more closely related to one another than any other pair of good species may be true for one group, *Goniobasis*, but not for *Drosophila.* The sibling species of *Goniobasis* may not be sibling species in the same sense as in the study of Ayala, et al. (1974), since all of the species in that study are members of the *Drosophila willistoni* group. The morphological similarity in these 2 species of *Goniobasis* perhaps is due to convergent evolution in shell sculptural characters. The relatively few characters on the shells of these snails would seem to make convergence more probable than in organisms with more complex external morphologies.

Difficulties encountered when using shell sculptural characters for taxonomic determinations are probably not restricted to *Goniobasis.* Further use of electrophoretic techniques will very likely reveal more sibling species among this and other gastropod groups.

ACKNOWLEDGMENTS

Permission to collect *Goniobasis* in Ichetucknee Springs State Park was granted by Maj. Jim Stevenson, Chief Naturalist,

Florida Division of Recreation and Parks. This work was supported by a grant from the Theodore Roosevelt Memorial Fund of the American Museum of Natural History, a Sigma Xi Grant-in-Aid of Research, and a National Science Foundation Grant for Improving Doctoral Dissertation Research in the Field Sciences.

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International Journal of Malacology

AMERICAN MALACOLOGICAL UNION · SYSTEMATICS ASSOCIATION
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12-13 July 1977, Naples, Florida

MALACOLOGIA

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1978

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SYMPOSIUM PROCEEDINGS
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MALACOLOGIA, 1978, 17(2): 163-164

INTRODUCTION

George M. Davis

President of the American Malacological Union, 1976-1977;
Symposium moderator;
Academy of Natural Sciences, Nineteenth and the Parkway,
Philadelphia, Pa. 19103, U.S.A.

Nearly two years before 12 July 1977, Professor A. J. Cain, then president of the Systematics Association (S.A.), and I discussed the possibility of having a joint A.M.U.-S.A. Symposium concerned with molluscan evolution and adaptive radiations. Such a symposium would, for the first time, bring S.A. members together with A.M.U. members in a joint session. We organized the symposium to be held during the 43rd annual meeting of the A.M.U. and thus initiated the first formal international meeting of the A.M.U.

In preparing this Symposium we were interested in current work that has a significant impact on elucidating processes pertaining to evolution, adaptive radiations, and deployment. Accordingly we invited scholars studying aspects of molluscan biology as diverse as shell morphometrics, population ecology, molecular genetics, paleontology, biochemistry, sexuality and reproduction, and functional morphology. We wanted maximum interaction between speakers and audience keeping in mind that many A.M.U. members are amateur conchologists. We hoped that we could present for professional biologists and amateurs alike a broad view of some of the exciting work that is now being done in molluscan studies that transcends the levels of alpha taxonomy and faunistic compilations.

To obtain a strong interaction between the speakers and the audience, the symposium was structured as follows: the 9 speakers served as a panel and were present for all papers. Each panelist was a discussant for one of the papers. Each speaker was given 40 to 45 minutes for the presentation. A presentation was followed by comments and questions by the discussant. Then the paper was open to comments and questions from the panel and subsequently from the audience. The total period of discussion was 15 minutes and sometimes longer. We found this method worked extremely well for stimulating and obtaining audience participation. As the total time allotted to each paper was 60 to 70 minutes, a speaker had time to fully develop a topic. Each level of discussion and questions opened up new avenues for discussion on the part of the audience.

The order of the papers as presented in the symposium is given below along with the name of the discussant for each. In looking at this list and by reading the Proceedings, it is readily apparent that the majority of papers dealt with land snails (five of nine). I feel that this actually reflects the fact that most of the exciting and encompassing work in molluscan evolution over the past 15 years has involved land snails. Three papers are concerned in one way or another with the adaptive significance of shell shape; two of these are with land snail faunas. Three papers are concerned with reproductive modes and the significance of these modes as regards evolution and deployment. The importance of molluscan genetics in assessing changes through time or degree of similarity within or between populations and species is stressed in three papers. The first two papers were presented by paleontologists as there can be no meaningful discussion of evolution without an integration of the fossil record within a framework of geological change. These initial papers underscore the problems of working only with shells in attempting to assess relationships, and the fact that many paleontologists have, over the past decade, turned to studying living mollusks in order to interpret shell morphologies of living and fossil taxa.

The papers presented were:

1. Yochelson, E. L. Contrasting views in present-day literature of phylogeny of the early mollusks. Discussant: Robert M. Linsley (A.M.U.)
2. Linsley, R. M. Shell form and rates of locomotion in gastropods. Discussant: Ellis L. Yochelson (A.M.U.)
3. Cain, A. J. Why is a snail that high and that wide? Discussant: James J. Murray (A.M.U.)
4. Woodruff, David S. Evolution and adaptive radiation of *Cerion*: a remarkably diverse group of West Indian land snails. Discussant: G. S. Oxford (S.A.)
5. Murray, J. and Clarke, B. (S.A.). Changes of gene frequency in *Cepaea nemoralis*: a fifty-year record. Discussant: A. J. Cain (S.A.)
6. Oxford, G. S. The nature and distribution of food-induced esterases in helicid snails. Discussant: D. S. Woodruff (A.M.U.)
7. Runham, N. W. Reproduction and its control in the slug *Agriolimax (Deroceras) reticulatus.* Discussant: P. Calow (S.A.)
8. Calow, P. The evolution of life-cycle strategies in fresh-water gastropods. Discussant: K. E. Hoagland (A.M.U.)
9. Hoagland, K. E. The evolution of protandry and labile sex determination in the Mollusca. Discussant: N. Runham (S.A.)

We have added a tenth paper to the symposium proceedings. This addition by Heatwole & Heatwole on the camaenid land snails of Puerto Rico was made after the paper had been accepted for publication in Malacologia. It very well complements the papers by Woodruff and Cain in that it deals in depth with ecological and biological factors helping to explain the deployment of this Puerto Rican snail group. Such ecological work with land snails is very much needed, a point with which Professor Cain surely would agree, especially as such studies are essential to explain why there are non-random patterns in the height and breadth of shells of land snails not correlated with taxonomic affinity.

The lessons from these contributions are clear. Studies on the evolution and development of mollusks must be based on a firm foundation of systematics and nomenclature. However, sound systematics can no longer be based on an analysis of shells alone, or on the simplistic analysis of a few conveniently studied morphological parts such as penis, radula, operculum. This was exemplified in Dr. Woodruff's paper in which it was argued that 200 nominal species of the land snail genus *Cerion,* with remarkable shell morphological variation, reduced to possibly two species when populations were studied with reference to zoogeography, ecology, multivariate morphometrics, and molecular genetics. Advances in and knowledge of systematics and evolution of molluscan groups will only come from broadly based studies conducted within a framework of population ecology, population genetics, and modern ecological theory. To these essential elements one should add one or more areas of study such as functional morphology, refined quantitative analyses such as multivariate analysis, molecular genetics, and zoogeography in time and space.

MALACOLOGIA, 1978, 17(2): 165-191

AN ALTERNATIVE APPROACH TO THE INTERPRETATION OF THE PHYLOGENY OF ANCIENT MOLLUSKS

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ABSTRACT

Some extinct Paleozoic mollusks have shell shapes which are judged to be distinct enough from those of living mollusks to warrant placement in extinct class level taxa. Recent publications placing several proposed extinct classes in the synonymy of living classes are judged to be based on poor definitions of class limits and excess speculation concerning unpreserved soft parts. More rigorous morphologic definition of the phylum and of the various classes based on the preserved hard parts might reduce some of the differences in interpretation among paleontologists. No clear arrangement of molluscan classes into subphyla is recognized.

The origin of classes may constitute new major morphologic changes which are then exploited rapidly; adaptive radiation could be a pervasive process occurring at all taxonomic levels, including the class level. Much hypothesis and speculation is involved in any attempt to construct a higher classification of extinct forms, and a phylogenetic interpretation must be modified as new facts are obtained. There is no final and authoritarian paleontological viewpoint.

INTRODUCTION

Discussion of phylogeny can be entertaining. Such writings have little impact on biostratigraphy, paleoecology, and any other facets of paleontology, but they can provide some generalizations which help to interpret the evolutionary history of a group of organisms. A discussion of phylogeny includes both facts, subject to change as new fossils are found and old ones redescribed, and opinions, which are subject to even greater modification. Any discussion is useful in pointing out areas of uncertainty and in provoking comment which might lead to greater clarification. It is basic that no discussion of phylogeny be treated as the ultimate word.

My sole earlier attempt to interpret evolution of the phylum Mollusca is a one page paper (Yochelson, 1963) in which I suggested a two-step evolution within the Mollusca. The first step in the early Paleozoic was a time of testing and experimentation by higher categories and ultimately their extinction. The later development of the extant classes of mollusks constituted the second step. My current understanding is that this general pattern is plausible but the notion of steps is far too simplistic. I think there were a number of classes of mollusks which appeared at different times and that some of them are now extinct.

Some neontologists such as Stasek (1972) and Salvini-Plawen (1968, 1972) attempt to interpret molluscan phylogeny with only limited reference to fossils; some paleontologists such as Runnegar & Pojeta (1974), Pojeta & Runnegar (1976) and Starobogatov (1970, 1974) draw extensively on the fossil record and arrive at different conclusions. One can argue that an additional interpretation may be equally valid and so long as speculation is not treated as serious science, no great harm is done. It is human nature to want to classify (Batten, 1960).

My concept of the pattern to be seen in the phylogeny of mollusks is shown in Fig. 1. The question of when a group began is always subject to modification by new interpretation and new discovery. However, I think that the fossil record is fairly good for groups of high systematic rank and the base of range lines is unlikely to change much in the near future, at least. I presume that the first appearance of a fossil is about at the time a group appeared, though some persons like to speculate that ancestral forms appeared far earlier than their first records.

I have not illustrated any of the genera

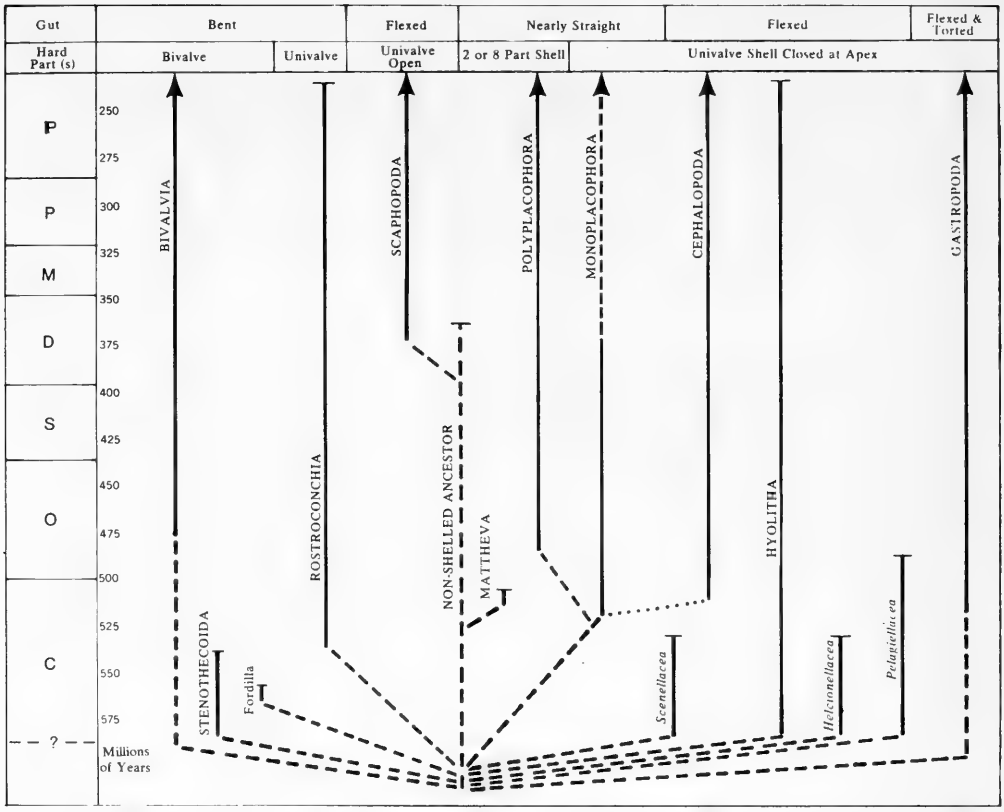

FIG. 1. Ranges of molluscan classes through the Paleozoic. A scale of geologic periods and radioactive dates is given to the left; interpretations of the gut appear at the top, just above a statement on geometry of hard parts. Class names are given in full capitals and their ranges end in the Paleozoic as indicated by a bar or continue to the Recent as indicated by an arrow; groups of probable class rank are indicated by superfamily name and those of possible class rank are indicated by generic name.

discussed herein. It is better to refer to original photographs and original reconstructions than to interpret them through additional diagrammatic illustrations. My writing style is a bit "folksy" but whereas informality in a scientific review may mystify a reader, a generalized sketch of an organism may mislead.

One aspect of my scheme is an ancestral generalized archimollusk which persisted from latest Precambrian through the early part of the Paleozoic; it is not a necessity as major change in larvae is an alternative. For the particular feature of this mythical form I choose to follow Fretter & Graham (1962: 1-6). My view is that as ecologic opportunity or genetic modification presented opportunities, the archimollusk threw off classes as a St. Catherine's wheel throws off sparks. I consider adaptive radiation to be as important in the origin of classes (Hotton, 1971) as it is at other taxonomic levels and I think this type of evolution happened rapidly. However, by mid-Paleozoic opportunities for major adaptive radiation disappeared and competition from other more efficient animals was such that:

> "The Archi-mollusk sought a cleft
> his shame and grief to hide,
> crunched horribly his horny teeth,
> gave up the ghost, and died."
> (Garstang, 1951: 41)

THE TREND IN HIGH-LEVEL SYSTEMATICS

Linnaeus, the father of all systematics, had three kingdoms: Animal, Vegetable, and Mineral. A decade ago, Whittaker had five, not counting the minerals, and

Russian paleontologists have proposed another. The term "taxonomic inflation" was coined by Sabrosky, and it aptly describes the change with increased study in level of elements of the Linnaean hierarchy. Genera become families, superfamilies become orders, classes become phyla, quite apart from increase in the number of species named. The number of taxa recognized at different levels increases at different rates, but they all increase; the two highest levels of taxa, class and phyla, increase at the slowest rate. The number of workers and the interest they generate in high-level classification determines the rate at which changes occur; today few people search for new species of living birds, but many look for additional evidence of the ways families and orders might be related. The living world is not completely known, but the major biological surprises per number of workers is small compared to possible surprises remaining in 600 million years of relatively good fossil record. New major categories are probably going to be based on fossils (Yochelson, 1971a).

Consider the mollusks in this light. They were not even fully separated as a phylum by Linnaeus, most members being within the "Vermes mollusca," though he did recognize a group which is now the Bivalvia. Cuvier in 1797 was able to distinguish the classes Gastropoda and Cephalopoda; the Scaphopoda were recognized by Bronn in 1862. Though de Blainville had recognized the Polyplacophora in 1816, von Ihering in 1876 suggested that the Amphineura was a separate phylum. During the next 75 years, there were some attempts to name additional classes and subphyla, particularly by German zoologists, but by and large, textbooks of zoology discussed five classes of mollusks until 1957. At that time the concept of the class Monoplacophora Wenz (Knight, 1952) came clearly into focus, and the emphasis on phylogeny shifted to paleontology.

Before leaving the Recent Mollusca, one may note that two decades after *Neopilina* was described, six shelled classes were generally accepted. However, there may be seven or eight classes of extant mollusks, depending on whether one wishes to recognize the class Aplacophora or divide this group into the classes Solenogastres and Caudofoveata.[1] Salvini-Plawen has written extensively on this issue and argues that there are eight classes of living mollusks. This is quite a taxonomic inflation over the five accepted in 1957 and inflation has a way of continuing.

WHAT IS A MOLLUSK?

The Mollusca, as conceived by neontologists, is a large (Boss, 1971) and diverse phylum distinguished primarily on the basis of anatomical, as opposed to conchological (shell) features. It is impossible to produce an exclusive definition of the phylum without reference to the soft parts. Hyman (1967: 5) indicated that "Mollusks are one of the most definitely characterized groups of the animal kingdom, having at least two features, mantle and radula, not found elsewhere." However, one of her characteristics is not exclusive, for Brachiopoda possess a mantle; some worms have a feeding structure which shows similarities to a radula, whereas Pelecypoda do not. Neither feature is normally preserved after death of the animal. Likely features of ctenidia are peculiarly molluscan (John Morton, oral communication, 1977), but they, too, are not shown in fossils.

Zoologists do not always study the same features as paleontologists and to some extent this is a division between those who look at soft parts and those who cannot. Too often the neontologist and the paleontologist talk at each other rather than to each other. A way to phrase the question could be: "how may a fossil be assigned, with a high degree of confidence, to the phylum Mollusca?" I suggested (Yochelson, 1961) a number of features which I think are common to many mollusks. These are: shell of calcium carbonate in several layers, prominent growth lines, logarithmic growth, inner structure prismatic, never fibrous and not pierced by canals. Additionally, the shell commonly: lacks an apical foramen or attachment disk; is a univalve, and is bilaterally symmetrical. There may be septa within the shell and there may be an associated operculum.

My aim was to invite discussion on the issue of what is a mollusk, but it was a total failure! After nearly two decades, I

[1]See A. H. Scheltema, 1978. Position of the class Aplacophora in the phylum Mollusca. *Malacologia,* 17: 99-109. ED.

can only say that I do not know of any features which are exclusive to fossil mollusk shells. A plexus of characters must be considered in order to assign a fossil to the Mollusca. The further one goes back into the fossil record, the greater the difference in gross morphology from living descendants.

Most molluscan shells exfoliate from the steinkern when broken from the rock matrix, especially when the matrix is a fine-grained limestone. I suspect this results from diagenetic alteration of incremental shell layers combined with the nacreous laminar structure widespread in members of the various classes. Shells of early forms should have been aragonite which subsequently reverted to calcite; the partially or fully calcitic shell was a later development within the Mollusca and perhaps associated with sedentary habit, though *Pecten,* for example, is a conspicuous exception. Some taxa in the Gastropoda and Pelecypoda have a shell in which the outer layer is calcite and the inner layer is aragonite. This combination of mineralogy seems unique to the phylum, but unfortunately it is not widespread enough in the Mollusca to help characterize them. Thus, those Cephalopoda which have a shell appear to utilize only aragonite. No shells generally regarded as mollusks contain appreciable amounts of calcium phosphate, and in my view, shells primarily phosphatic are immediately excluded from the mollusks; other criteria have exceptions.

The gross morphology of the preserved hard parts alone is not a sufficient criterion to place a fossil in the Mollusca. Tubes, lids, and sclerites, as represented by *Mobergella,* styliolinids, tentaculitids, and tommotiids are not mollusks, even though some mollusk hard parts are similar in shape; they are analogous pieces, rather than homologous. Morphology includes size, and early Paleozoic mollusks in the microfossil size range are uncommon.

A philosophical issue is whether in the high level assignment of a fossil one takes a liberal or a restricted view. I tend towards restriction in that a fossil must fully satisfy me, subjectively, that it is a mollusk before I will accept it into the phylum. A nasty point is that of differentiating remains of fossil "worms" from those of mollusks. There are a limited number of shapes of "worm tubes" today, but who is to say what the past may have witnessed? To take a tiny steinkern—an internal filling which preserves no evidence regarding the original shell—and say that it is a scaphopod because it is curved or a gastropod because it is coiled seems to me to be poor science. Some fossils should not be assigned to phylum level on the basis of current information. I judge it far better to let some organisms remain *incertae sedis* than to assign them to the Mollusca. Keeping strange fossils separate focuses attention on them. Assigning them to the Mollusca presumes more than we know and may lead to spurious data and correspondingly false phylogeny.

Too much philosophy is stultifying. It is better to come to grips with organisms. In the following sections, my aim is to review the high-level taxa of mollusks. In the following treatment of the early history of the second largest animal phylum, generalizations are necessary. It is well known that all generalizations are false, but I plead for mercy because of space limitations. There are some high-level categories for Paleozoic forms which have been proposed, but, have not been generally accepted and have dropped from usage. There seems little point in considering, for example, the Eopteropoda or the Coniconchia (Yochelson, 1961).

CEPHALOPODA

The Cephalopoda have long been recognized as a major molluscan entity. D'Orbigny judged the Bellerophontacea, an extinct order of Paleozoic bilaterally symmetrical gastropods, to be cephalopods without septa. This was the only important piece of systematic confusion, and it was clarified 125 years ago. Most living cephalopods do not have calcareous hard parts, and their ancestors, even though with a phragmocone, show an incomplete fossil record (Donovan, 1977). The chambered *Nautilus* shell serves as a window to the past, but most living cephalopods demonstrate in a dramatic fashion that the present may be an exceedingly poor guide to the past.

One would hope that even if *Nautilus* were extinct, hard parts of *Sepia* and *Spirula* might be sufficient to allow chambered shells of the past to be interpreted as cephalopods. That may be too generous an interpretation of our ability to relate fossils

to living organisms. *Nautilus* has been a mixed blessing because for years paleontologists attempted to interpret fossil cephalopods in the light of what was thought to be known of this organism. Thus, all cephalopods were assumed to be fierce carnivores and we argued fruitlessly whether extinct ones had a single pair or two pairs of gills.

A fossil cephalopod is easy to recognize for the septa are pierced by a siphuncle, though fragments such as a node on the shoulder of a Pennsylvanian nautiloid, or a curved body chamber, are more difficult to assign. The septa/siphuncle combination is the "how" of a cephalopod. Much work over the last two decades has been devoted to gas production and its control within living cephalopods. The theory has been applied, apparently with a high degree of success to fossils, and I believe that we may speak of buoyancy as the "why" of cephalopods; Teichert (1967) has interpreted evolution of the class in terms of buoyancy control.

The record of the cephalopods shows a great deal of diversity in the past, more so than today. Possibly the biomass of living cephalopods is far greater than it was at any one time in the past, even when various extinct lineages are combined. This increase in biomass probably results from most modern cephalopods achieving the acme of molluscan buoyancy by loss of the shell.

In much of the Ordovician and Silurian record of cephalopods, straight, chambered shells predominate. However, in the Late Cambrian a curved shell, *Plectronoceras,* occurs. Yochelson, Flower & Webers (1973) accepted this genus as the earliest cephalopod. They had found a Late Cambrian high, curved, conical shell which contained multiple septa. It was their hypothesis that this form, named *Knightoconus,* was a monoplacophoran and was morphologically similar to the organism which gave rise to the Cephalopoda. Development of a siphuncle, a tube to pierce the septa, would have converted *Knightoconus* into an organism essentially like *Plectronoceras.* Runnegar & Pojeta (1974) have accepted this hypothesis, and attempted to extend the ancestral group downward by placing the Early Cambrian *Tannuella* in the cephalopod precursor group. Curved septate univalves are known in the Middle Cambrian, which might be related to *Knightoconus,* but it is premature to include any Early Cambrian genera in that lineage.

A methodologic point should be emphasized. Presumed relationship of *Plectronoceras* to *Knightoconus* was based on features of hard part morphology. Generalized soft parts were added to each fossil, but arguments based on soft parts were ancillary; paleontologists should deal primarily with facts of hard parts, and only secondarily with assumptions regarding features not preserved.

The presumed relationship of *Knightoconus* to *Plectonoceras* is plausible in part because the nature of the earliest cephalopod was clarified. Previously, Early Cambrian straight-shelled *Salterella* and *Volborthella* were called early cephalopods. Although this notion was repeatedly denied during the 1950's and 1960's, it still lingered in the literature. It is a classic case of organisms being assigned to the Mollusca for the flimsiest of morphologic reasons; their presence in the phylum and class confused interpretations. One can never be totally certain in science, and I can only hope that my decision to establish a new phylum based on *Salterella* (Yochelson, 1977) will stand. If nothing else, that action clearly removes the genus from the Mollusca.

Starobogatov (1974) places most elongate tapering tubed shells in one class of mollusks, ("Solinoconcha"), distinguishing those with an opening at both ends (Scaphopoda) from those which are open only at the aperture. The animal within these simple straight conical shells would as a next step secrete septa perforated by a siphuncle and the resulting animal would be an orthoconic cephalopod; it has been mentioned repeatedly that the earliest known cephalopods are not orthocones. Starobogatov is not clear as to the timing of these events or the selective pressures or other reasons for the dramatic changes. By extending his concepts, Starobogatov also develops a new classification within the Cephalopoda.

I cannot accept Starobogatov's derivation of the Cephalopoda and I am satisfied with the idea presented by Yochelson, Flower, and Webers (1973). There are two general implications to this theory which should be repeated. First, in terms of morphology, Monoplacophora are the closest of all living groups to most published concepts of the ancestral mollusks; in contrast, many physiologists and anatomists judge living cephalopods to be the most advanced of all

mollusks. If one can directly derive the most advanced from the most primitive form of mollusk, this suggests that these terms may actually have little meaning, at least in any evolutionary context. Second, it is generally assumed that most limpet-shaped monoplacophorans had the shell apex anterior, basing this assumption, in part, on morphology of living examples and, in part, on muscle scars of a few of the extinct forms. Acceptance of the relationship of septate monoplacophorans as precursors carries with it the notion that some monoplacophorans had the shell apex directed toward the posterior.

Living Cephalopoda are highly specialized in a few traits but little specialized in other molluscan features (Dr. A. Bidder, oral communication, 1977). In a sense, this view supports a relationship of cephalopods to monoplacophorans.

SCAPHOPODA

My reason for mentioning elephant tusk shells next is that approximately as many persons have seen live *Dentalium* as have seen live *Nautilus.* Scaphopods live partially submerged in mud and hunt with their captacula, a complex of tentacle-like organs; Foraminifera no doubt regard them as fierce predators. They are carnivores, though part of their diet is organic detritus or bacteria in the sediment.

The Scaphopoda and Bivalvia have in common the absence of a head. Whereas the Bivalvia lack a radula, this structure is present in the scaphopods. Accordingly, the pelecypods could not have been ancestors of the scaphopods, because it is unlikely that this complex structure could have been lost and then reacquired. The similarities there may be between the Scaphopoda and some Pelecypoda are probably the result of convergence from a semi-infaunal life habit for both groups. Conchologically, the scaphopods are univalved in having a single hard part, a tapering curved shell open at both ends.

The issue of what is a mollusk clearly applies to scaphopods. If scaphopods were extinct and soft parts were unknown, would they be called mollusks? I think not. Even if details of shell structure were available, they still might not be considered molluscan, for the structure of calcareous tubes constructed by some worms is somewhat similar to that of mollusk shells.

The logarithmic curvature of the scaphopod shell and its general regularity of shape, with little individual variation, is more suggestive of mollusks than of worms, but these are not unique differences. The tube of *Pectinaria* is open at both ends, curved, and fairly scaphopod-like except that it is constructed of agglutinated particles. This worm resembles the scaphopods. The deep-sea quill worms, like *Halcioneia,* live in a curved tube, open at both ends, distinguished from the shell of a scaphopod only because it is a non-calcified organic sheath. In the past, did quill worms ever calcify this sheath? Perhaps I overemphasize the issue of distinguishing worm tubes, for the microstructure seen in recent tubes is quite different from that of mollusks. However, paleontologists seldom find material as well preserved as that just secreted and it is truly difficult to separate these two groups. Even in the Recent, *Ditrupa* has fooled zoologists who have not seen the soft parts of this worm.

The oldest scaphopods that I have seen are Mississippian in age. Haas (1972) reported Devonian ones with convincing molluscan shell structure, though there is a slight chance that he sampled the body chamber of a curved cephalopod rather than a scaphopod. There are no reports of Silurian scaphopods.

Ordovician scaphopods were described by Eichwald more than a century ago from the Ordovician of the Baltic platform, but have not been confirmed by any later investigators. My brief examination in 1962 of several of Eichwald's specimens shows them to be fragments of steinkerns 2-5 cm long. They could be fillings of the body chambers of nautiloids.

Ordovician scaphopods were reported twice in recent years. Bretsky and Bermingham (1970) transferred the Late Ordovician "worm" *Coleolus iowense* James to the scaphopod genus *Plagioglypta.* Specimens have several thin layers which exfoliate singly, quite unlike mollusk shells. Further, Holocene *Dentalium* and Pennsylvanian *Plagioglypta* contain in their shell 0.01% to 0.02% P_2O_5 whereas *C. iowense* contains this compound in concentrations an order of magnitude larger, about 0.1% P_2O_5 (Jaresowich & Yochelson, unpublished).

Runnegar & Pojeta (1974) and Pojeta & Runnegar, (1976: 43) also reported Middle Ordovician scaphopods. These are small

tubes, curved, possibly open at both ends, but replaced by silica; because of replacement, nothing much can be learned of the original shell composition or structure. The cross-section of the tube is not circular and therefore unlike that of known living scaphopods. The highly gregarious occurrence of these small tubes, like the gregarious occurrence of *Coleolus iowense,* would argue more for the feeding of worms on decaying material than for scaphopods hunting prey beneath the surface.

It is likely that the Paleozoic Scaphopoda currently contain some forms which are worm tubes (for an example, see Yochelson, 1971b). It would be wiser to assign to the class only those forms for which typical molluscan shell structures can be demonstrated, rather than assume that all slightly curved tubes are scaphopods.

One can pick the first known fossil scaphopod as Middle Ordovician, Devonian, or Mississippian. Regardless of which period is chosen, the time of origin thus attributed to scaphopods is much later than that of the cephalopods or any other extant class. Bouyancy may be the word for cephalopods but the "essence" of scaphopods escapes me. We need more scaphopod workers to develop new data on this most difficult class.

Starobogatov (1974) has revised the Scaphopoda. Placing them as a subclass of the order named Solinoconcha, he has included within them the Early Cambrian *Volborthella,* a form that I do not consider to be a mollusk (Yochelson, Henningsmoen and Griffin, 1977) and the Middle Ordovician *Polylopia,* which might be a hyolith (Yochelson, 1968a). He also allies the class Xenoconchia Shimanskiy with the reconstituted Solinoconcha so that the class is divided into two groups, those with an elongate tube open at both ends, and those with a tube closed at the apex. As noted, he derived the cephalopods from this latter group. I cannot accept Starobogatov's views, but have not the space to refute them in detail.

PELECYPODA, LAMELLIBRANCHIATA OR BIVALVIA

If there is an essence of "pelecypodness" I believe it to be withdrawal from the epifaunal world by burrowing. *Nucula* and other protobranchs still process mud, moving it inward by labial palps but most kinds of pelecypods filter water through their gills, assiduously pumping gallon after gallon, safe from many predators by hiding in the mud. Of course, there are exceptions to this generalization. A variety of bivalves live on the surface and *Pecten* is certainly active, but students of bivalves seem to be in agreement that pelecypods originally were dwellers within soft sediment and that surface dwelling—free, attached, cemented, or boring—is secondary.

Thanks largely to the work of Pojeta (1971), the major groups of pelecypods can be documented back to the Ordovician. Pelecypods appear in the Early Ordovician and apparently underwent a dramatic radiation so that by the Middle Ordovician most lineages were established and by Late Ordovician even the pectinaceans appeared.

For the animal to dig in, I think several features are needed. Obviously, two valves are required to open and close. This means ligament, teeth and sockets. Valves open only slightly and are rocked back and forth; teeth and sockets guide the valves. Finally, strong muscles are needed. This in turn means strong attachment to the inner surface of the shell on which it leaves a prominent scar inside. If muscles were not strongly attached, the valves could not be closed or rocked back and forth.

There is a persistent myth of competition during the Paleozoic between brachiopods and pelecypods, with brachiopods slowly succumbing to the pressure of competition. To me, this seems most unlikely and Stanley (1968) has indicated that the great flowering of pelecypods occurred in the Jurassic, long after most kinds of brachiopods had become extinct. Paleozoic brachiopods and pelecypods often lived in different habitats; after all, brachiopods do not move, whereas most pelecypods do. In collections of fossils where both occur, nothing suggests competition; apparently there was sufficient food in the water for all filter feeders.

Pelecypods are a fine example of a class which almost from its inception has included a great deal of diversity. In contrast, scaphopods show no diversity today or in the past. Since the Early Ordovician, pelecypods have increased both in diversity and in number of individuals. In terms of biomass, they may be the most successful molluscan class. Pelecypods are also successful in that they live in marine, brackish

and fresh water, whereas cephalopods and scaphopods are limited to marine conditions.

During two centuries many schemes for arranging subdivisions of the class have been propounded. At least one scheme proposed recently by Russian workers (Nevesskaya et al., 1971) has not been critically evaluated by paleontologists in the western world. More new arrangements will result from the 1977 meeting in London on bivalves. There does seem to be consensus that protobranchs form a subclass quite apart from other taxa in the class. The absence of a radula in the clams may be generalized as loss of the front part of the digestive tract in the Scaphopoda and further chopping off of the anterior portion in the Bivalvia (R. Palmer, oral communication, 1977). The necessity to feed by filtration may be an element in the clam success story.

There is one major difficulty with the pelecypod story as recounted here. It concerns the genus *Fordilla,* which occurs in the late Early Cambrian and is widespread in the northern hemisphere. There is agreement that *Fordilla* is restricted stratigraphically and that it is a bivalved molluscan shell.

A troublesome point is the early appearance of *Fordilla* and the lack of authentic records of the Bivalvia until the early Early Ordovician. There is a gap of nearly 75 million years. No acceptable theories aid us in evaluating gaps in the fossil record, but they are frequently associated with rare organisms. The record of the Bivalvia following the dramatic late Early Ordovician radiation is one of steady increase in diversity and biomass. It is hard for me to imagine the Bivalvia originating with *Fordilla,* disappearing, and then reappearing with a great and dramatic diversification. Gaps do occur in the pelecypod record such as between Ordovician *Babinka* and younger lucinoids, but I do not think the apparent absence of Middle Cambrian to early Early Ordovician specimens can be dismissed.

Fordilla has been variously classified. The current prevailing interpretation made repeatedly (Pojeta, Runnegar, and Kříž, 1973; Pojeta & Runnegar, 1974; Pojeta, 1975) is that *Fordilla* is a pelecypod. I disagree and hold that this genus could represent an extinct group of mollusks different enough from pelecypods so that one might assign them a separate class rank. I base my argument on morphology of hard parts; the time gap is only secondary evidence, for the fossil record contains other gaps, though such gaps are usually associated wtih rare groups.

I have reexamined the type lot, as well as those figured by Pojeta, plus additional unillustrated specimens, and have collected a few. Based on this, a reconsideration of the genus is in preparation, but for the moment I can only present statements without much supporting documentation.

Fordilla is a small shell showing closely spaced, fine, but prominent growth lines. Nothing is directly known of the original shell structure. The clean splitting away of shell from steinkern is inferential evidence of molluscan affinity. The shell is far smaller than many of the accepted pelecypod shells of the late Early Ordovician and the size range is near that of large living ostracodes. It does not show a prominent beak nor an obvious straight hinge line.

A significant feature in my view is the lack of hinge teeth and sockets; a number of living pelecypod stocks, such as pholads or pectinaceans, lack teeth or have them highly specialized, but so far as I can determine from the literature, no stock that lacks teeth is considered to be an ancestral group. *Fordilla* specimens from Siberia to be illustrated by Pojeta in a forthcoming paper are slightly asymmetrical near the center of the presumed dorsal edge; a slight overlap of valves at a single point is the most plausible interpretation. The notch in the margin of the steinkern is enigmatic.

Internally, the steinkerns show a number of markings. *Fordilla* is interpreted as a pelecypod because it is reputed to have anterior and posterior adductors and a pallial line extending from the anterior adductor. I do not believe that published illustrations support such an interpretation.

On none of the illustrations is there any clear evidence of a prominent adductor scar at the presumed posterior of the shell. The presumed pallial line is a greatly raised ridge whose proportions and shape are different from those of living shells. I have not examined many tiny clam shells, but in larger ones the pallial line is not a prominent feature. The pallial line on *Fordilla* does not extend from anterior to posterior but only extends halfway across the shell and then disappears. There are living pel-

ecypods which have an interrupted pallial line, but like those which have lost hinge teeth, they are specialized forms. This "pallial" line in at least one specimen is bifurcate (Pojeta, 1975, pl. 1, fig. 3).

Near the middle of the valve, where the "pallial line" ends, there is a broad raised area, which in some individuals appears to be divided (Pojeta, 1975, pl. 1, fig. 1). Even allowing for shell curvature, this feature would appear to encroach further into the shell thickness than the pallial line. It also extends closer to the shell margin than the "pallial" line. Pojeta (1975) considers *Fordilla* a part of the infauna, whereas Tevesz & McCall (1976) judge that it lived on the surface.

Fordilla may be a pelecypod. If so interpreted, the inner features are so unique that, in my view, the genus should be placed in one subclass and all other known pelecypods be placed in a second subclass. That is not a good way of expressing the diversity found within the Pelecypoda.

The discovery a few years ago of a living bivalved opisthobranch should make everyone who speculates about fossil mollusks exceedingly cautious (see Kay, 1968, for a review). Keen & Smith (1961, pl. 5) picture this living snail carrying its bivalved shell; there is some similarity in size and shape of hard parts between *Fordilla* and *Berthelinia*!

I suspect that *Berthelinia* is telling us that Mother Nature has a variety of ways of putting hard parts and soft parts together and that we should not be too fixed in our interpretation of the interrelationship between the two.

STENOTHECOIDA

The genus *Stenothecoides* occurs from mid-Early Cambrian to early Middle Cambrian; allied genera have about the same range. Like *Fordilla* it is widespread in the northern hemisphere. After the discovery of *Neopilina* there was a scramble to find muscle scars in cap-shaped univalves. Horný and Rasseti both illustrated some lateral markings on a slightly asymmetrical valve, plus fainter median markings. Not knowing what else to do with the creatures which were generally accepted as mollusks, Knight & Yochelson (1960) placed them in the Monoplacophora. In retrospect, it would have been better to treat them as Mollusca *incertae sedis.*

Robison (1964) suggested a simple tooth and socket arrangement at the pointed end; anterior and posterior more often than not are misleading terms. Yochelson (1969) eventually published photographs of two specimens which are bivalved, asymmetrical and inequivalved. Akserina (1968) published the Class term Probivalvia, but Yochelson (1968b) also had discussed the concept and the name Stenothecoida in an abstract which had appeared earlier that year. There are no rules of nomenclature at this level, but there seems to be general acceptance of the principal of priority and Stenothecoida is the name used. Multiple independent discovery of a concept in science (Merton, 1961) is not proof that the concept is right, but it does suggest that the concept be examined seriously.

Stenothecoida is an embarrassment to most phylogenetic schemes. The late Henning Lemche (oral communication, 1975) had worked out to his satisfaction a program of evolution for the entire invertebrate world, except that he could not account for *Stenothecoides.* However, Runnegar & Pojeta (1974: 316) have dismissed it out of hand. They wrote "We offer the alternative suggestion that it may have been a bivalve monoplacophoran, with the lower (smaller?) valve formed by the sole of the foot. A few living limpets form a second valve in this way, although in these cases the lower valve is cemented to the rocks." The same view was expressed by Pojeta & Runnegar (1976: 44); both works refer to a paper by Yonge on *Hipponix.*

Even if I were to grant that *Stenothecoides* is a univalve—which I emphatically do not grant—there is no basis for assigning such a univalve to the Monoplacophora. Specimens are asymmetrical and the internal markings are quite unlike muscle scars seen in generally accepted fossil monoplacophorans.

Although other points may be arguable, I believe that it is clear that Runnegar & Pojeta's comparison of *Stenothecoides* with Cretaceous to Holocene *Hipponix* is spurious and can be rejected. The bivalved Class Stenothecoida does exist. I have interpreted these strange little fossils as living on the surface, unattached, but dorsal-ventral like oysters. This may not be correct, but no other bivalved modes of life have been suggested. I point out that if *Stenothecoides* and its relatives can have two valves and

yet not fit in the Bivalvia, perhaps this helps strengthen the case for a bivalved *Fordilla* that is not in the Bivalvia.

About as many genera of Stenothecoida are known as are known of Scaphopoda, but these fossils are rarer than fossil scaphopods.

POLYPLACOPHORA

The Polyplacophora are mollusks which have eight shell valves. The nature of my material forces me to be a paleoconchologist. As a pragmatist, I prefer names which deal with hard parts, not soft tissue. Thus, I think it is high time we dropped the term Amphineura. Likewise, I should use Bivalvia, though the informal term bivalve may cause confusion. The issue of Aplacophora or Caudofoveata and Solenogastres (Salvini-Plawen, 1972) is not mine to be involved with. The few examples of these soft-bodied creatures I have seen look impressively different from the polyplacophorans.

Most living "coat-of-mail" animals cling to rocks. In part, this allows them to live in areas of strong wave action, but perhaps equally important this permits them to fight dessication. *Lepidopleurus* lies on mud in the deep sea and is regarded as more generalized by researchers of polyplacophorans.

The Polyplacophora are more diverse than the Scaphopoda. They are certainly less diverse than the cephalopods, by several orders of magnitude. A point that I have attempted to make repeatedly is that distinctiveness is what separates one taxonomic unit from another at a comparable level. Diversity is a measure of the variety within that level. All molluscan classes are distinctive, but some are diverse and some are not. The Polyplacophora are one that is not.

In the mid-Paleozoic, isolated plates with insertion plates occur. These tiny projections are what allow the plates to overlap, yet retain flexibility. Some of these forms, well preserved in the Mazon Creek Shale, are virtually identical to living genera, including such details as girdle width, pattern of scales on girdle, and marginal spicules. The early Paleozoic forms seem to have been like *Lepidopleurus*; likely they were cryptic, but could not cling so well to hard surfaces. The older genera may have had major overlap of the valves, but the valves might have been set in a long girdle, allowing flexibility of the animal.

The oldest reported polyplacophorans are from the very latest Cambrian strata (Bergenhayn, 1960). The material consists entirely of steinkerns and I am not convinced that these truly are representatives of the group; they may be fragments of other fossils. Authentic earliest Early Ordovician material has been recovered recently (B. Stinchcomb, written communication, 1977). The oldest published fossils of this class that I accept without question occur in the late Early Ordovician (Smith & Toomey, 1964). They are associated with sponges and algae in some local build-ups of hard ground on a limy-bottom shallow sea. I cannot think of any fossil mollusk in the Cambrian which might serve as an ancestor to the polyplacophorans.

Several points can be noted about living polyplacophorans. The mantle cavity is lateral, not posterior. It may well be that the paired lateral position is a result of the crawling and clinging habit of an elongate form. There has been speculation about segmentation of mollusks; the numerous ctenidia of some living forms show that pseudometameric repetition occurs within the mollusks and one need not get too excited about multiple repetition of organs.

Although polyplacophoran plates usually look like the roof of a house and are easy to identify, some isolated fossil echinoderm plates have the same general shape. Murphy's Law has been a dominant theme throughout the history of the mollusks.

MATTHEVA

Mollusks with more than one calcified shell tend to disarticulate after death. The living polyplacophorans provide one model of how several hard parts are used during life, but this need not be the only way more than one piece was assembled. The genus *Matthevia* occurs in the late Late Cambrian. Yochelson (1966) observed that two forms occurred together and interpreted them as front and back pieces with two prominent muscle insertions in each piece. One of the mistakes I made at that time was to be overly generous with alternative hypotheses, for I indicated that there might be small intermediate plates

(Yochelson, 1966, fig. 3B). I did not really believe it then, and am now fully satisfied that these smaller "plates" are only shell fragments.

At the time I wrote, my guess, based on slight evidence, was that *Matthevia* lived on algal heads and used the weight of the anterior and posterior pieces to hold itself in place. Subsequently, I have seen specimens associated with stromatolites and the lithologic evidence of strong water motion in the areas of occurrence is compelling. This would certainly account for the wear of pieces after death and sorting of posterior from anterior pieces. Likely *Matthevia* had some flexibility and this permitted it to move among the algal heads. Mere weight to hold it in place was too simple an answer, but it had a basis in fact.

Runnegar & Pojeta (1974) and Pojeta & Runnegar (1976: 44) have downgraded Mattheva from class status. In their view *Matthevia* is a primitive polyplacophoran. They have introduced a number of intermediate shapes of pieces and restored it to look something like a hedgehog or a stegosaur. I can think of many unacceptable points to their reconstruction, but an obvious one is that this is hardly an adaptive shape for an animal clinging to rocks in turbulent water. Another basis for their taxonomic downgrading is to illustrate a form from the Early Ordovician presumably intermediate to more conventional polyplacophorans. Dr. Runnegar has kindly permitted me to examine specimens of the presumed intermediate (September, 1977), and to me this "missing link" is simply a thick valve of *Chelodes* with the typical single broad and shallow muscle insertion pocket; no polyplacophoran has more than one muscle insertion per plate. It has a clearly differentiated head or tail plate which by its absence of any muscle insertions is unlike a *Matthevia.* In my view the Class Mattheva is well-founded morphologically.

HYOLITHA

The hyoliths are an extinct group. Whereas *Stenothecoides* and *Matthevia* are uncommon fossils and occur only in a narrow time interval, *Hyolithes* and its allies are exceedingly abundant in the Cambrian, provided of course one looks in the proper rock facies; they seem to have preferred a moderately soft bottom. Hyoliths are rare in the Ordovician and exceedingly rare in younger beds, but they do occur in the Permian and thus had a duration of nearly 300 million years. They should not be ignored.

Hyoliths are greatly elongated cones, closed at the apex. They are slightly curved and are bilaterally symmetrical. The tube-like shell has an operculum associated. There has been some confusion with "worm tubes" and with the opercula from such tubes, but the hyoliths stand as a moderately homogeneous group, though a few generic taxa still should be eliminated and moved to "Vermes."

For decades, virtually no generic diversity was recognized, though many species were named. Currently, some dozens of genera are recognized. One might say the diversity approximates that of polyplacophorans, but that the fossil biomass may have been much larger.

Two principal orders are recognized. The Orthothecida have a simple aperture, an operculum which is retracted into the tube, and a cross-section that is smoothly curved and non-angulate; it can be circular, oval, or bean-shaped. The Hyolithida are more complex. Most commonly their cross-section is triangular, with the base of the triangle extending forward to form a semicircular shelf. The operculum covers this shelf, as well as the aperture. Protruding between the lateral edges of aperture and operculum are a pair of curved "whiskers," which have received an inordinate amount of attention. The best guess is that they acted to stabilize the shell, like the outrigger of a Polynesian canoe. It might be mentioned that peculiar as the whiskers are, there are a number of hyoliths—those in the Orthothecida—that did not have them.

I have refrained from mentioning the phylum assignment of the hyoliths. For more than a century they were placed hither and yon, though most commonly at some vague position within the Mollusca. In 1963, Marek finally made a definite statement and placed them as a class of Mollusca.

Not everyone agrees that they are mollusks. Runnegar et al. (1975) judged that they are different enough to form an extinct phylum. Marek & Yochelson (1976) amplified and reinforced Marek's original remarks that these fossils form a class of mollusks. For me, the most convincing argument in favor of Hyolitha being mol-

lusks is the description by Runnegar et al. (1975, fig. 5) of typical molluscan crosslamellar shell structure in a Permian specimen. Runnegar (oral communication, 1977) holds that the ancestral mollusk possessed a dorsal epithelium which subsequently calcified; the reconstruction of soft parts for Hyolitha used by Runnegar et al (1975) indicates that the shell was not dorsal and the group is not molluscan. In proposing an extinct new phylum for consideration, I (Yochelson, 1977) suggested that while the shell of hyoliths does not look much like that of living mollusks, it is not so distinctive as to warrant phylum rank.

There seems to be no room here for compromise. If the hyoliths are mollusks they are different enough to be a class, and if the hyoliths are not mollusks, they are different enough to be a phylum. The question is how different? In my view, Hyolitha belong within the Mollusca, but I cannot add new evidence to what has been presented; each systematist must decide for himself. To a large extent the notion of deposit feeding with palps or tentacles, which might have been present in ancestral mollusks, turns on the question of whether Hyolitha are included or excluded from the phylum.

The Late Paleozoic class Xenoconchia (Shimanskiy, 1963, Shimanskiy and Barskov, 1970) has not been critically reviewed; Runnegar and Pojeta (1974) did not mention it. On the basis of examination of some specimens and discussion with Dr. Shimanskiy (September, 1975) my guess is that class rank may be unwarrented. A few Mississippian specimens (currently in the group) might be steinkerns of a nearly symmetrical *Platyceras (Orthonychia).* However, Permian material is not so readily interpreted, for in some specimens one side is slightly flattened; others are circular in cross section. These shells may be related to the large Permian fossil *Macrotheca.* That genus fits within the Hyolitha but it is so large and expands so rapidly that status as a separate order probably is appropriate. Specimens of Xenoconchia are far rarer than those of Stenothecoida, and like them provoke major problems in interpretation and assignment.

GASTROPODA

In pelecypods there is a reasonably close relationship between the form of soft parts and the enclosing two valves, but in gastropods the relationship of shell to soft parts is far less obvious. Pelecypods can be defined moderately well by reference only to the hard parts, and externally shelled cephalopods can be defined quite well using only hard parts, but the gastropods are another matter.

A gastropod is a mollusk whose soft parts have undergone torsion (twisting) of the nerve commissures and certain other soft parts. Over the years I have asked my neontological colleagues to phrase a class definition without reference to soft parts. The request is treated with amazement, amusement, or both, and I conclude that one cannot assign fossils to Gastropoda with as high a degree of assurance as with other classes of extant mollusks, except perhaps the scaphopods. One might turn this around and suggest that since torsion is unique, and as a consequence the gastropods show the greatest independence of shape between soft and hard parts, Gastropoda is the most noble and most advanced of the molluscan classes. I do not believe it, but I put forth the claim to annoy those who extoll the wonders of living cephalopods.

If one judges by the number of included lower-level taxa, gastropods are the most diverse of all mollusks. It is appropriate to emulate a gastropod and to stick my neck out to make progress. A class in systematics may mean, approximately, a major mode of life. Originally, gastropods were grazing herbivores. The gastropods may have chosen to emphasize the radula, after torsion produced a neck and greater flexibility of movement. This mode of life was so successful that the gastropods were able to proliferate, undergo other anatomical changes, and invade the major niches occupied by other classes. Thus, there are gastropods that float and swim, hunt through the bottom, filter water, and gastropods that cling to rocks. Incidentally, gastropods are the only mollusks to invade the land, and several stocks have done so. Biomass may not be as great as the pelecypods, but gastropod diversity to me illustrates the importance of brain, or at least head, over brawn.

Gastropods were not the oldest herbivores; some "worms" have that distinction (Edhorn, 1977). There is an increase in snail diversity from Late Cambrian through Early Ordovician and speculation that they

inhabited algal stromatolites (Garrett, 1970). If this idea is substantiated, the gastropods may go down in biologic history as the ultimate grazing machine.

Because septa are uncommon and complex suture patterns have not developed where septa are present, the shell of a fossil gastropod is not nearly so complex as that of a fossil cephalopod. However, shell structure can be elaborate and in some groups the shells are aragonite, in others calcite-aragonite, and in still others calcite. Diversification of shell composition, as well as anatomical change, may help explain the success of the class. Some shell shapes occur time after time among fossils and one has difficulty distinguishing convergence from true phyletic lines. However, the main lines are fairly clear, with asymmetrically coiled shells reasonably close to living pleurotomariaceans appearing in the Late Cambrian along with macluritaceans. More advanced, presumed single-gilled forms appeared later. Still later, the neritaceans, and opisthobranchs (Kollmann & Yochelson, 1976) appeared in the mid-Paleozoic. Pulmonates appeared by late Paleozoic time (a review by Solem & Yochelson is in preparation). Among the marine forms, the greatest change seems to have occurred in the Jurassic with a dramatic increase in advanced prosobranchs.

Naturally, the history of gastropods is not that simple, and I can think of at least four grave complications. First is the position of the Macluritacea; Linsley (in press) has considered their possible life-habit and discussion of their high-level systematic position can wait. Second is the position of the Bellerophontacea which will be treated later in the section. Third is the position of *Aldanella,* and fourth is the placement of *Pelagiella* and its allies. These last two may be combined under the general issue of "when did gastropods first appear"?

I have alluded earlier to the issue of worm tubes and I have noted the simplicity of the gastropod shell. *Aldanella* was named from late Early Cambrian rocks in Massachusetts, but first received prominence as a member of the Tommotian fauna, an unusual Siberian assemblage of presumed early Early Cambrian fossils. *Aldanella* is closed at the apex, tubular, and is coiled in three dimensions and therefore fits a superficial conchological concept of a gastropod. I do not agree with that assignment (Yochelson, 1975) for *Aldanella* is based on tiny (1-2 mm) steinkerns, so that no information on the shell is available. The Siberian specimens I have seen show a high degree of individual variation, more so than one would anticipate in gastropods. In addition, some illustrated steinkerns show a straight early portion of the tube not at all like a gastropod protoconch. Finally, in a large collection of steinkerns of comparable size from Ordovician rocks on Spitsbergen (Bockelie & Yochelson, in press), the shape of *Aldanella* is repeated in a population with so much individual variation that its non-molluscan nature is obvious. *Aldanella* is, in my view, a tiny worm tube and adds nothing but confusion to the phylogeny of mollusks.

Pelagiella is another matter, and I do not doubt its molluscan nature. It is asymmetrically coiled, and in that feature is like a gastropod. However, the whorl expands at a rapid rate and the proportions of this small shell are quite unlike those of generally accepted gastropods. Runnegar & Pojeta (1974) consider *Aldanella* a pelagiellid, but I fail to see how the two groups can be considered related, for their shapes are distinct. In my opinion, the position of *Pelagiella* is comparable to that of *Fordilla,* in that both resemble an extant class, but appear early geologically. If it is necessary to bolster distinctiveness in hard part morphology with speculation regarding soft parts, I can, to my satisfaction, reconstruct *Pelagiella* as an asymmetrically coiled non-torted mollusk.

A reconstruction of hypothetical soft parts is not proof. For the moment, no clear-cut morphologic evidence can be derived from the hard parts to distinguish *Pelagiella* from the Gastropoda. There has not been time or inclination to study the Pelagiellacea carefully. In large measure, this is because specimens are uncommon and most are not well preserved. A few good specimens might provide a wealth of data and my guess is that when these are found, they will show that pelagiellids are not gastropods.

It is now appropriate to turn to the Bellerophontacea, even though this essay is not much concerned with taxa below the class level. Bellerophontacea are coiled bilaterally symmetrical shells that occur in the Paleozoic. The classic view is that these shells gave rise to the asymmetrical ones, but I still prefer to consider Bellerophontacea as a specialized offshoot of the Pleurotomariacea (Yochelson, 1967).

During the early 1960's when the concept of Monoplacophora was new, Horný found specimens of the coiled Middle Ordovician *Cyrtolites* which showed several sets of paired muscle scars. Subsequently, a few other coiled forms were found. I have found this to be interesting (Yochelson, 1967), but my conclusion a decade ago is the same as it is today; just because a few presumed monoplacophorans have coiled shells, it does not follow that Bellerophontacea are a branch of the Monoplacophora.

Starobogatov (1970), Runnegar & Pojeta (1974), Pojeta and Runnegar (1976: 32) and Runnegar & Jell (1976) moved all Bellerophontacea into the Monoplacophora mostly because of the superficial similarity of a bilaterally symmetrical shell in *Cyrtolites* and Bellerophontacea. In making this sweeping revision, several points have been ignored. Years ago, there was good evidence of lateral retractor muscles in bellerophonts (Knight, 1947); this has been further reinforced by additional finds. The close resemblance of the slit to that of pleurotomariacean gastropods has been ignored. In addition, the inductura, a secondary deposit seen in many groups of gastropods, has not been emphasized. No undoubted monoplacophorans show such a deposit. I know of no theoretical reconstruction of coiled Monoplacophora which would place a mantle fold in the area where the inductura was deposited.

I do not fully understand how the definition of Monoplacophora came to be expanded to include coiled shells which show no sign whatsoever of dorso-lateral multiple paired muscle scars. Perhaps it is a misunderstanding of exogastric and endogastric coiling. Try as I may to follow the logic of those who state that the extinct Bellerophontacea did not undergo torsion and therefore are Monoplacophora, I cannot. Let me say once again that it is impossible to determine whether an extinct form has undergone torsion. The hard part morphology indicates the Gastropoda as the class for Bellerophontacea. Secondary to this conclusion, reconstructions of presumed bellerophontacean soft parts (Knight, 1952) show those of a gastropod fitting well into this shell form.

Protowenella (Runnegar & Jell, 1976), from the Middle Cambrian of Australia, is a tiny steinkern about 1 mm across, judged by its authors to be the world's oldest bellerophontacean. I am reasonably certain that the paired lateral constrictions in the cross section are similar to those in the cross-section of undoubted worm tubes. It is circular reasoning to place fossils which cannot be assigned with any degree of confidence to any phylum in the Mollusca, draw inferences as to the soft parts, and use this as a basis for classification.

ROSTROCONCHIA

I am not the only one in recent years to have proposed classes of mollusks whose members are entirely extinct. In 1973, Pojeta and others proposed the class Rostroconchia and included specimens ranging from Early Cambrian through Permian age. Pojeta & Runnegar (1976) have elaborated upon their class concept.

The rostroconchs are laterally compressed forms, some of which show a univalve protoconch, and are presumed to be non-torted mollusks. It is judged by the authors of the class that the shell split laterally as a consequence of growth, so that the animal was functionally nearly a bivalve. In one order, Ribeirioidea, a prominent plate occurs at the shortened, presumed anterior edge of the shell, with a gape continuous from anterior to posterior. In my opinion, this hard part morphology is distinct at the class level from that of other mollusks. Two other orders, Ischyrinioida and Conocardioida, emphasize a more symmetrical growth in terms of presumed anterior and posterior, and a prominent posterior siphon, respectively. These are not as distinct from pelecypods as the ribeirioids, and are open to further speculation on their relationship to ribeirioids.

Though I may accept the class, it does not follow that I accept all members of it. The group is certainly well developed in Late Cambrian – Early Ordovician; it is not reported by Pojeta & Runnegar (1976) to occur in the Middle Cambrian. The Early Cambrian record is based on two genera. One of them, *Watsonella* Grabau, comes from eastern Massachusetts in isolated boulders about the same age as those producing *Fordilla.* Others have seen a similarity between the two, and I am quite satisfied that *Watsonella* is a subjective synonym of *Fordilla.*

The other Early Cambrian genus is *Heraultipegma,* known from France and

Siberia. It does not weaken this class concept to note once again that evidence of molluscan affinities should precede assignment to class; phyllocarid crustacean arthropods are only one of the groups which have a shape convergent to that of the Rostroconchia. When *Heraultipegma* was first proposed, it was thought to be an arthropod. More recently, it has again been independently interpreted by two workers (Müller, 1975; Missarzhevskiy, 1976) as an arthropod. However, Runnegar & Pojeta (1974, fig. 4) accept it as a mollusk and as the ancestral rostroconch. They further indicate a series of younger morphological transition forms which lead to the principal subdivisions of the class. Some of these genera in the presumed phyletic lines of the class are coeval, rather than sequential.

I have seen Müller's specimens from France which are steinkerns; in my view they do not yield sufficient information to be assigned to any phylum; it remains to be proven that the class Rostroconchia appeared before the Late Cambrian. The dramatic radiation of the ribeiriids in the Late Cambrian might be the experimentation of a group which has just developed and thereby been able to move into a new ecological niche. I can understand a new group appearing and diversifying rapidly. I cannot understand a new group being rare for 50-75 million years, and then evolving rapidly.

It has been speculated (Yonge, 1953) that the Bivalvia might have been derived from a strongly compressed univalve. In this sense the Rostroconchia appear to be an ideal precursor to the pelecypods. If the pelecypods began in the Early Cambrian with *Fordilla* these two classes are nearly contemporaneous in time of origin, or at least in time of earliest known representative. If the Bivalvia began in the early Early Ordovician, the two classes would be sequential. Nevertheless, the step from univalve to bivalve is abrupt and it seems impossible to derive two centers of calcification from one centre of calcification except in a discontinuous manner. There is a large morphological gap between late Early Ordovician pelecypods and contemporaneous rostroconchs. I cannot accept the concept as presented by Runnegar & Pojeta (1974; Pojeta & Runnegar, 1976) of a series of adult forms in a morphologic transition series linking the two classes.

It is possible as suggested by Runnegar & Pojeta (1974) that the Rostroconchia were directly ancestral to the Scaphopoda. If my interpretation of the earliest scaphopods is correct, they overlap only with the highly specialized conocarditids. Perhaps the two classes were derived from a common ancestor and are not more closely related.

MONOPLACOPHORA

It might have been more logical to begin this review of classes with the Monoplacophora, for it was the acceptance of a sixth class of living mollusks in the late 1950's which began all the current ferment concerning molluscan phylogeny. However, many problems surround this taxon, seemingly with several kinds of mollusks mixed together, so perhaps it is best kept till last.

Malacologists should recall the paper by Knight (1952). He did not invent the concept of the Monoplacophora, as credit for that goes to Wenz (1940), but Knight did extend and clarify it. His conclusion was that the Monoplacophora were not particularly remarkable. Knight divided the class Gastropoda into the subclasses Anisopleura (gastropods in the traditional sense) and Isopleura, which in turn was subdivided into Monoplacophora and Polyplacophora. There the matter stayed until Lemche (1957) described *Neopilina,* a living monoplacophoran. All honor to *Neopilina,* for without it the neontologist would have payed slight attention to the fossil record and the paleoconchologist would probably never have had the courage to propose high-level molluscan taxa, all of whose members are extinct.

One may ask, what is a monoplacophoran? The answer varies, but let me give my opinion for fossils. It is a bilaterally symmetrical, wide molluscan univalve shell, most characteristically limpet-shaped, which shows prominent multiple muscle scars. Wenz was most impressed with paired scars of the Devonian *Cyrtonella,* a strongly arched form; Knight concentrated on *Tryblidium,* a flattened Silurian form. I suppose that it should not have come as any shock that a few monoplacophoran shells grew through several coils. Still, it was a surprise when Horný found Ordovician *Cyrtolites* to have several whorls and multiple paired scars. Some of us do not yet have the concept of coiled Mono-

placophora in perspective. As I mentioned under comments about Bellerophontacea, *Cyrtolites* does not have an inductura, and bellerophontaceans do; there are other morphologic differences between coiled monoplacophorans and bellerophontaceans.

Let us now consider exceptions to my definition as regards shape. Not all monoplacophorans are absolutely bilaterally symmetrical. Yochelson (1958) described Early Ordovician *Cyrtonellopsis* from three specimens, the apexes of which are central, to the right, and to the left, respectively; there are no muscle scars known, so assignment of *Cyrtonellopsis* must carry with it some uncertainty. Even granting that, there should be a limit to the degree of asymmetry. I judge that assignment of *Stenothecoides* as a monoplacophoran—regardless of whether it is a bivalve—to be incorrect, because the asymmetry shown by one valve greatly exceeds the asymmetry shown in any generally accepted monoplacophoran. It seems to me even more preposterous to consider *Pelagiella* as an asymmetrically coiled monoplacophoran.

Muscle scars are not common in fossils. There are a greater proportion of scars in specimens of Monoplacophora than in specimens of other classes, but even so scars are rare. *Patella* and its allies show a scar quite unlike that of typical monoplacophorans but have a similar limpet-like shape. Horný (1961) has described *Damilina,* a Silurian patellid gastropod with a horseshoe-shaped muscle scar, so it is clear that the ranges of these two limpet shell forms overlap. I would be exceedingly cautious in assigning a fossil to the Monoplacophora. I am not satisfied that all taxa assigned to the group by Knight & Yochelson (1960) are correctly placed.

The number of scars in monoplacophorans is not constant. For a brief time, it was nice to think of eight plates in the Polyplacophora and eight scars in *Tryblidium,* but many genera have fewer than eight pairs. One tends to ignore *Archaeopraga* (Horný, 1963) which is the same size, shape, and age as *Tryblidium* and has a single pair of elongate lateral scars.

Bellerophontacean scars are not monoplacophoran scars, both because of their shape and because of their position within the shell; few bellerophontacean steinkerns yield data on musculature. One can argue that some nautiloid cephalopods were monoplacophorans, for the known muscle scars are similar in size, shape, and, to a certain extent, position (Mutvei, 1964). Indeed, if the relationship of *Knightoconus* as an ancestor to cephalopods is correct, that observation makes good sense. An important general feature is that muscle scars are prominent because the muscle is attached to the shell firmly. Weak attachment and non-attachment does not leave any shell scars. As I mentioned in connection with clams burrowing, firm attachment is necessary for some functions of the animals. For clamping against a hard substratum, strong attachment of muscles may be a prerequisite (R. M. Linsley, oral communication, 1977).

Let us consider *Scenella.* This genus occurs in the Early Cambrian and may straggle up to the early Middle Cambrian. One specimen is known at the end of this range which shows muscle scars, paired, but slightly asymmetrical. Further, the external shape is not similar to that of a typical *Scenella,* which has an elaborate radial ornament and periodic development of a frill. I would say that the oldest monoplacophoran known for certain is Late Cambrian for it shows multiple sets of paired scars in a limpet-shaped shell. There is no good evidence of the class in older rocks, though the one early Middle Cambrian specimen is suggestive. Even if this specimen is a slightly asymmetrical monoplacophoran, it may not be correctly assigned to *Scenella* for it has a smooth external shell. The authentic Early Cambrian *Scenella* may be a monoplacophoran or something else.

The Early Cambrian *Aktugaia* (Missarzhevskiy, 1976) has recently been described as a monoplacophoran because it has multiple paired muscles. I have not seen any specimens, but the assignment is suspect to me, for "worm" opercula such as *Mobergella* or *Discinella* also show multiple paired scars. The wedge-shape of *Aktugaia* is not like that of these two genera, nor is it like the shape of a monoplacophoran. *Aktugaia* is tiny, as is *Mobergella.* In my opinion, the oldest undoubted monoplacophoran is still Late Cambrian.

One may gather from the prior discussion that subdivision of the class is in a bit of a mess. Rosov (1975) added a fourth order to the three currently recognized. Runnegar & Jell (1976) placed two orders in synonymy and added the Bellero-

phontida. Anyone who thinks that high level classification is a slow methodical process has not been considering the Monoplacophora.

Biomass for the class was always low, though Early Paleozoic specimens are more common than living *Neopilina.* The number of included genera, if one uses my definition, is slightly greater than that of the Polyplacophora during the comparable time interval; if one uses the class limits of Runnegar & Pojeta, the number of Paleozoic genera is the same order of magnitude as in early Paleozoic gastropods. Presumably fossil monoplacophorans were herbivores; it is not fully clear what *Neopilina* eats. I do not think they were as mobile as gastropods or even some living limpet gastropods, and perhaps this explains in part their rarity relative to fossil gastropods.

Next is the issue of endo- and exogastric coiling. Some fossil monoplacophorans had a larval stage, and if this stage was very long I warrant that the shell curved or even coiled for ease of movement, economy of material, and a host of other reasons. If the apex was strongly anterior, I can see how the larval coil in a wide, low-spired shell might be opposite from that of a living limpet gastropod, such as *Patella.* However, many presumed monoplacophorans are conical, not flattened. In these conical forms the apex is central or subcentral. The assumption has been made by some writers that the apex must curve or coil to the anterior. They gave no clear rationale for that assumption and I tend to reject it. In the absence of differentiated muscle scars, or indeed any muscle scars at all, one cannot determine anterior or posterior and thus judge "endogastric or exogastric" coiling. For example, Pojeta & Runnegar (1976, pl. 15) illustrate a curved protoconch of a species possibly belonging to *Macroscenella.* No muscle scars are known from that genus and this protoconch is squarely in the center of the shell above a smooth regular oval aperture. How does one know which is the right or the left side of the shell?

There is concern about the position of the mantle cavity in coiled monoplacophorans. *Neopilina* has paired lateral mantle cavities, as do the polyplacophorans. If lateral cavities were ancestral and the soft parts were then crowded by increased curvature, the mantle cavity might have moved posteriorly or it might have moved anteriorly. *Knightoconus* was restored with a posterior mantle cavity, but this was to align it with cephalopod soft parts, not for any intrinsic reason.

It is well known that archaeomollusks had a posterior mantle cavity because that is the way Lankester thought of the animal, and that torsion brought it forward. As I have never dissected an archaeomollusk, I do not know this. There is much concern about problems of fouling of the mantle cavity by discharge from the anus. In *Neopilina* the anus is posterior. Does it necessarily follow that anus and mantle cavity were always linked? Perhaps *Cyrtolites* and similar coiled forms had an anterior mantle cavity and a posterior anus discharging over the foot. An anterior mantle cavity need not be a result of torsion but might have been a consequence of the shell broadening anteriorly. Perhaps some difficulties lie in our presumptions about the ancestral form.

I think that monoplacophoran shells coiled through several volutions were carried on the dorsal part of a foot, precisely as gastropods carry their shell. Because there was no torsion, the soft parts were probably less extensible and less flexible than those of gastropods. In particular, the scars are less than one-quarter of a volution inside the body whorl and the organism could not retract deeply into the shell. However, the animal functioned moderately well, for *Cyrtolites* is not rare.

The model of the endogastric monoplacophoran, coiled with the shell balanced over the head, may have been mechanically plausible (Linsley, 1978) though I am still not convinced that the organism ever existed as reconstructed. What evidence is available from the limpet-shaped Late Cambrian monoplacophoran *Kirengella* indicates a subcentral position for an apex which curves toward the posterior. Until better evidence is presented I shall assume that all monoplacophorans which coil through more than one volution follow this same geometry.

I fail to see why non-torsion and coiling over the head must be equated. If one can accept a similarity between *Knightoconus* and *Plectronoceras,* an increase in degree of coiling of a *Knightoconus*-like shell would result in a shell like *Cyrtolites.* Muscle scars in *Cyrtolites* consist of several pairs, above on the dorsum, and a U-shaped ring below ending on the lateral slopes, situated slight-

ly deeper in the whorl. If *Cyrtolites* coiled as do gastropods, the attachment site for the foot retractors could be the U-shaped ring, and the discrete pair of scars on the dorsum near the aperture would be in a position to retract the head (D. Schindel, oral communication, April 1977). This way the head would be last in and first out.

There is a point in homology of muscle scars which could be pursued a bit further. Pojeta & Runnegar (1976) show several members of the Rostroconchia with muscle scars. The general pattern is one or two pairs of discrete scars toward the presumed anterior, and a U-shaped scar posterior to these. If the muscles are in a similar position to those of *Cyrtolites,* Runnegar & Pojeta have one group or the other back to front. It is more likely that the musculature pattern is similar in both groups. I accept their interpretation of anterior in Rostroconchia, and thus have another line of evidence to support my orientation of the coiled monoplacophorans.

An argument has been made that *Cyrtolites* and other coiled monoplacophorans had a posterior mantle cavity. This is based on the assumption of water flowing laterally into the shallow water cavity and out at the dorsum (Linsley, 1978). The morphologic evidence derived from shell shape suggests one orientation and the muscle scars suggest another. Whichever may eventually be judged to be correct, the coiled monoplacophorans occur sporadically beginning only in the Middle Ordovician. Linsley (in press) has argued that their morphology—assuming coiling was over the head—was appropriate to have given rise to the Bellerophontacea, but I feel that they occur geologically too late. As a general principle, I cannot put much faith in presumed morphologic series which do not occur in stratigraphic sequence.

Let me now attack the "*Helcionella* heresy." This genus and its allies are small, bilaterally symmetrical curved shells, very strongly compressed laterally; they characteristically occur in the late Early Cambrian to early Middle Cambrian, but are found in slightly younger and older rocks. Commonly, there are periodic swellings and compressions of the shell, but some genera such as the Early Cambrian *Aldabanella* have smooth sides. The aperture is simple and shows no reentrant. In 1960, Knight, Batten and Yochelson did not know quite what to do with these mollusks, but finally followed Knight (1952) in relating them to the Bellerophontacea on the assumption that the helcionellaceans gave rise to forms which both developed a slit and elaborated the curvature to coiling of several whorls. Specimens are smaller than most bellerophontaceans.

Runnegar & Pojeta (1974), Pojeta & Runnegar (1976), and Runnegar & Jell (1976) regard the Helcionellacea as monoplacophorans. They are judged to be monoplacophorans because it is stated that the curvature is toward the anterior. I disagree both with the interpretation of curvature and with the class assignment.

On the matter of curvature, as noted, it was suggested by Yochelson, Flower & Webers (1973) that one need not have anterior curvature in a high conical shell. Earlier, Knight (1952) schematically restored *Helcionella* with the shell gracefully curving toward the posterior so that the foot is partially covered by a train, as the aperture expands. Restoration with the curvature forward results in an organism very nearly as awkward as the hypothetical forward-coiled monoplacophoran. Some consideration has to be given to the point that these organisms crawled and that they could have crawled far more readily if the shell was carried on the foot, not balanced on the head.

Yochelcionella has been described from the early Middle Cambrian (Runnegar & Pojeta, 1974). It is like *Helcionella,* but midway on the concave surface of the shell is an open tube. This is indicated as an inhalant siphon, partially because the animal as interpreted by Runnegar & Pojeta would have crawled with this tube pointing forward. Because helcionellaceans are both compressed and small, lateral mantle cavities are unlikely. Even assuming that *Yochelcionella* could have moved in an awkward position as suggested, the water flow is completely at odds to the general molluscan pattern. A general rule for water flow is "in below, across the gills, and out above," though in some shell geometries there are modifications. For *Yochelcionella* the flow would be in above and out below. I do not see how this tube could function as an inhalant siphon, advancing through the water. Additional species of *Yochelcionella* with the open tube near the apex (Runnegar & Jell, 1976) make even less sense interpreted as functionally inhalant. The water stream would have to bifurcate

after entering to bathe the gills. However, if interpreted as an exhalant siphon, with the animal crawling away from the spent water, the tube makes a great deal of sense. For helcionellaceans without this tube, the train would have aided movement of water out of the posterior mantle cavity. To recapitulate, I hypothesize that coiled monoplacophorans had an anterior mantle cavity over the head and helcionellaceans a posterior mantle cavity. Water circulation hypotheses were divided from the shape of the hard parts.

To return again to the hard parts of a class, it is important to note that a very large variety of shapes can be made from a univalve. I have mentioned slight asymmetry; let me now touch on lateral compression. Typical Monoplacophora have an elongate wide oval aperture in low shells. In conical shells, the aperture is still widely oval, and even in coiled forms the aperture is relatively wide. The *Helcionella* shell is strongly compressed laterally. Without speculating at all about soft parts, I would judge that the Helcionellacea exceed, by quite a degree, the morphologic variation of hard parts which should be allowed in the class Monoplacophora.

I cannot help but add that nothing whatsoever is known of muscles in Helcionellacea. Nothing suggests that they had multiple paired muscles.

By including Helcionellacea in the Monoplacophora I believe that Runnegar & Pojeta equate bilateral symmetry and a presumption of non-torsion of soft parts with the concept of Monoplacophora. If this is so, the concept of Rostrochonchia has no validity and should be placed in synonymy with Monoplacophora, for these forms are presumed to have been bilaterally symmetrical and non-torted! No matter what arguments might be raised about possible loss of head or specialization of the foot, if non-torsion is equated with Monoplacophora, Rostroconchia is an unnecessary term. Members of that class are closer in hard part morphology to a cap-shaped shell than are the bellerophontaceans which Runnegar & Pojeta transferred to Monoplacophora.

Of course, rostroconchs do have additional features of hard parts which suggest that they exceed the morphologic limits of Monoplacophora by about the same amount as do the Helcionellacea. If Rostroconchia can, on features of hard part morphology, be differentiated as a class, I think any reasonable systematist should be willing to accept the elevation of Helcionellacea to class level.

I do not know what is the proper systematic position of *Scenella,* but would be willing to guess at this time that it might have been a wide helcionellacean, rather than an early monoplacophoran. However, there is a major difference in shape between this genus and the coeval *Aldabanella.* There is a great deal we still do not know. In closing this section, I trust that it will be clear that I do not automatically equate the ancestral hypothetical mollusk with Monoplacophora.

SUPERPHYLA AND SUBPHYLA

Valentine (1973a) is one of a long line of writers to suggest some interrelationships among the various animal phyla. There are a few objectors, but of late the invertebrate divisions of Proterostomia and Deuterostomia seem to have found general acceptance (Carter, 1965) and I judge there is general agreement among those biologists who deal with phylum level phylogeny that there may be some similarities or common ancestry among the mollusks, annelids and arthropods. I do not see what is gained by introducing the level of superphylum (Valentine, 1973b) to encompass one, two, or all three of these phyla, for this gives the impression that our level of knowledge and precision of classification is greater than it really is. Classification certainly does not stop at the phylum level and I concede that discussion of a possible sequence of hypothetical events may provide insight, but to use the category superphylum indicates a precise position in a nested hierarchy. As it has been employed, the superphylum also suggests that changes in morphology and their sequence are known as a far firmer series of events (Valentine & Campbell, 1975) than there is evidence to support.

The question of relationships within the Mollusca is not so easily dismissed. Speculation on grouping of classes has been a topic of discussion for more than a century. I suggest that almost any relationship which might be proposed could be supported by some reference to earlier literature. Nevertheless, the classic notion commonly considered two divisions within the Mollusca,

with the Aculifera (Amphineura or Aplacophora, and in some schemes even the Polyplacophora) giving rise to the Conchifera (univalved and bivalved molluscan groups). There were tacit assumptions within the Conchifera; that the Cephalopoda were in some way higher than other groups; that there was some relationship between the Scaphopoda and Pelecypoda; and that the Gastropoda, because of torsion, were the most aberrant of the Mollusca.

Discovery of *Neopilina* and acceptance of the class Monoplacophora has led to some change in these concepts; modifications have been added to the traditional interpretation of the ancestral protomollusk by Salvini-Plawen (1972) and others. Reexamination of the phylum has led to one rearrangement of the classes into three subphyla (Stasek, 1972). By adding data on the first occurrence of some classes in the fossil record and hypothesizing about the soft parts of extinct forms, Stasek's scheme was then arranged by Runnegar & Pojeta (1974) into: Aculifera for those without a shell; Placophora for the multi-plated chitons; and the old Conchifera broken into the subphyla Diosoma and Cyrtosoma. Obviously, this is not the same scheme as the different subphyla and somewhat different classes of von Ihering (1922) or Johansson (1952) or Harry (1969) or Salvini-Plawen (1972), to cite a few of the other alternatives. Pojeta & Runnegar (1976: 44-45) are the latest authors to consider this topic, but probably they are not the last. These authors recognize eight classes, one of which, the Rostroconchia, is extinct; the classes are rearranged as noted above into four subphyla. It puzzles me as to what tests we might apply to determine which of the schemes of subphyla arrangements is a more accurate summary of the evolutionary history of the phylum.

I have arranged the extant classes and proposed and presumed extinct classes, which I have discussed earlier, into subdivisions based on geometry of the hard parts (Fig. 1 at top). I also included speculations on the geometry of the gut, which are reasonably conservative and closely follow those of most current writers (Fig. 1 at extreme top). These divisions of hard and soft parts do not coincide. If I were to use other features of actual and presumed soft part anatomy, such as presence or absence of a radula, a different grouping could be formed.

Without emphasizing this approach *ad nauseam* I conclude that attention to any particular character would lead to other arrangements. Those who argue for mosaic evolution suggest this phenomenon is a common occurrence with different characters changing at different times. Cladists argue that changes are dichotomous. Neither position is probably entirely true. Theorists do not agree and this further reinforces my pragmatic view that since most of the classes do not appear to be linked in a sequence, perhaps there is no major intra-phylum grouping of Mollusca. At least, we should recognize that to group the classes will be a more subjective exercise than it was thought to be half a century ago in von Ihering's day.

PRECAMBRIAN EVENTS, MOLLUSK LARVAE AND SPECULATION

There are no undoubted Precambrian mollusks.

Glaessner (1969), followed by Runnegar & Pojeta (1974), interprets one Precambrian fossil, *Bunyerichnus,* known only from its trace, as the trail of a mollusk. I do not know what this is, but it is not that of a crawling mollusk. Perhaps it is a frond of some sort. A new picture of the only known specimen (Häntzchel, 1975: W-49) shows how unlike a mollusk trail this was.

Except for a few spicules and a radula, the Aplacophora lack hard parts, and to date this class is unknown in the fossil record, although *Bunyerichnus* has been alluded to as a possible aplacophoran. Salvini-Plawen (1968) judged Aplacophora to be primitive. Because I have not had any experience at all with these modern worm-like forms, my comments are uninformed speculation, but my guess is that these animals are secondarily simplified rather than primitive. First, there is a general trend in many groups of mollusks toward loss of the shell (Morton, 1963). Second, many aplacophorans occur at moderate depths, and shelled deeper water mollusks appear to be mostly derived from organisms moving downslope rather than relics of an early fauna (Clarke, 1962: 4). Thus, I would not include aplacophoran-like mollusks in my speculations concerning the Precambrian.

On the other hand, my notion of the aplacophorans as secondarily naked may be

completely false. As Dr. John Morton put it (oral communication, 1977): "The conclusion would seem irresistible from an ensemble of primitive soft parts (gonadial, pericardial and pallial morphology, as well as central nervous system), that however specialized in present habit and body form the living Aplacophora are, they diverged from the molluscan stem at a very lowly level." Perhaps the ancestral mollusk gave rise to the scaphopods, and lastly gave rise to the aplacophorans. The group does not fit anyone's model of an early mollusk with all its apparently primitive features. The notion of organisms remaining unchanged since the dawn of the Cambrian is quite hard for me to accept, and if the Aplacophora are to be primitive, I prefer that the ancestral stock persisted longer. If this many-fold speculation is true, the relationship between Polyplacophora and Aplacophora need not be close, even if both do possess spicules. But, as noted earlier regarding philosophy, too much speculation can be stultifying. I should at least now change the subject of speculation.

Similarities in the preservation of various fossil mollusks argue for a common organic integument which developed prior to great radiation and diversification of soft part anatomy. Spicules are known in the periostracum of some pelecypods (Carter & Aller, 1975), as well as in the girdle of Polyplacophora (Beedham & Trueman, 1967) and in the Aplacophora (Beedham & Trueman, 1968). A spicular stage might have preceded formation of a true shell as Carter & Aller suggest, with the integument providing the organic matrix to regulate mineral deposition, so that only "molluscan" shell was grown. The sequence of events could have been: first, formation of an organic cover; second, major differentiation; and third, calcification of the integument.

I would follow Fretter & Graham (1962) who, among others, derive premollusks from pre-turbellarians, in part by an increase in size. Suggestions as to development of the coelomic cavity in animals (Vagvolgyi, 1967; Salvini-Plawen, 1968) also lead to the notion of a turbellarian-like ancestor. Consequences of size increase would be the need for better digestion, solved by formation of a complete gut, and the need for more oxygen, solved by gills to provide a greater area for respiration. Development of a cuticle from mucus, and thus a slight protection of the gills, might then have followed.

Runnegar & Jell (1976) suggest that there was a gradual size increase in mollusks, that is, members of the phylum were systematically smaller further back in time. It follows that the prospects of finding Precambrian individuals is exceeding slight. However, specimens of *Dickinsonia* more than 20 mm in length are known, so I cannot subscribe to the doctrine that all Precambrian fossils are necessarily small. The issue of whether mollusks were tiny in the Cambrian and became larger through time depends on how one picks one's data. If *Heraultipegma* is a rostroconch, it is larger than many others. If hyoliths are mollusks, the largest are in the Early Cambrian, *Scenella* is larger than *Cyrtonella,* and so on.

An important point that a paleontologist should bear in mind is the size of material traditionally studied; Tevesz & McCall (1976) have noted the importance of scales when attempting to reconstruct life habits of fossils. It is exceptional in the Paleozoic to break out from a rock a mollusk smaller than 2 mm. By working with residues from acid solutions, one can obtain fossils much smaller, but as I have indicated, it is difficult to establish that these remains are of mollusks. As a result of physiological needs, there is a maximum and minimum size for each kind of organism. Mature living mollusks do occur in the 1 mm range. They are common, but they are atypical of the phylum. In the habitats in which they are found today, preservation as fossils is unlikely.

Even if some tiny organisms should prove to be early growth stages of mollusks, they tell us little. There is a profound difference between the earlier and generally smaller planktonic state and the later and generally larger benthonic stage. Surface tension is significant for small animals but not so much so for larger ones. I do not know what caused torsion, but to look for a mechanical interpretation of this process by suggesting how shells were carried by mature benthonic animals (Runnegar & Pojeta, 1974: 314) is about as logical as suggesting that during the years of burrowing, clams slowly reduced the size of the head to the point where it disappeared.

Levels of integration, loosely related to

size of material, are important, for not all the same rules apply to all sizes of organisms. I think that most dramatic changes in fundamental morphology, the characteristics that distinguish classes, occurred during larval development. It is perhaps easier to evoke in my mind's eye major anatomical changes at that stage than later in development. What appears to us as a major step in evolution may have been minor to the animals. A small change could have had a profound effect. This is why Garstang emphasized the advantages of torsion to the larva.

The ontogenetic stage at which a change first occurs is not important. What is important is that the change be perpetuated.

We do not see missing links between classes in the fossil record. Dr. V. Jaanasson (oral communication, 1977) suggests that for a few generations a small population (dithyrial population of literature) could consist of several morphological forms coexisting until the atypical form became genetically fixed. However, the mechanism for evolution is not so much my concern in this study. I speculate that we do not find missing links because none existed. Such speculation runs counter to the notions of most geneticists who see a gradual accumulation of small changes as the only way organisms evolve. Unfortunately, features such as fusion to form an open tube of the Scaphopoda are hard to envision as a series of small steps. "Hopeful monsters" are not in vogue, but I suspect that any intermediates among classes would be far more monstrous than those ancestral animals which first showed the prime features of the class.

In another vein, new ideas are being proposed as to the choice an organism which developed hard parts had between calcium carbonate and calcium phosphate (Rhodes & Bloxam, 1971). New ideas are being proposed as to the mechanism which caused calcification originally (Rhodes & Morse, 1971) and as to the reason animals with hard parts diversified dramatically at the dawn of the Cambrian (Stanley, 1973). With all this intellectual fare to digest, consideration of the changes from Precambrian to Cambrian cannot be taken without developing some cramps; we are closer to understanding these early events, but we have a long way to go before all is clear and logical.

CRITIQUE

Runnegar & Pojeta (1974; see also Pojeta & Runnegar, 1976) have written extensively on molluscan phylogeny. They do not consider the early mollusks in the same light as I do. It may be unfair of me to recast and simplify the views of these workers. Tabulation is often highly artificial, but I employ it here as the easiest way to summarize differences in our interpretations.

Runnegar & Pojeta	Yochelson
1. Hyolitha an extinct phylum.	1. Hyolitha an extinct class of mollusks.
2. The Rostroconchia the only extinct class of mollusks.	2. At least three extinct classes; other groups which may eventually deserve that rank.
3. *Stenothecoides* an asymmetrical monoplacophoran.	3. *Stenothecoides* and allied genera of extinct bivalves in class Stenothecoida.
4. *Matthevia* a member of class Polyplacophora.	4. *Matthevia* the only member of class Mattheva.
5. Pelecypoda in Early Cambrian.	5. Pelecypoda in Early Ordovician; *Fordilla* possibly a member of an extinct bivalve class.
6. Gastropoda in Early Cambrian.	6. Gastropoda in Late Cambrian; Pelagiellacea possibly in an extinct class. Tiny coiled "worm" tubes resemble gastropod shells.
7. Scaphopoda in Middle Ordovician.	7. Scaphopoda in Mississippian or possibly Devonian; curved "worm" tubes resemble scaphopod shells.
8. Monoplacophora a highly diverse group; many genera without evidence of muscle scars.	8. Monoplacophora restricted to cap-shaped shells plus a few coiled forms; paired muscle scars required for definitive placement in the class. Helcionellacea probably in a distinct class.

Runnegar & Pojeta recognize three of the six shelled extant classes in the Early Cambrian, whereas I judge that all extant classes appeared later. There are other classificatory differences, but these appear to be the principal ones at high taxonomic levels.

There are also several methods in approach which might be noted. Thus, Runnegar & Pojeta make use of series of morphologic intermediates to link classes. I see practically no intermediates between classes. My emphasis is on stratigraphic

occurrences, whereas many of the taxa in their presumed morphological series occur at the same time interval. They apparently view major changes as gradualistic, whereas I consider them to have been abrupt. It is my impression, though perhaps not a correct one, that Runnegar & Pojeta discriminate high level taxa because of assumptions regarding soft parts, whereas I would make a high-level separation on hard part morphology alone, and then try to interpret soft parts.

DISCUSSION AND SUMMARY

There is a reaction in science against spending time on definition, for in some ways it is sterile work. Nevertheless, unless one does consider this aspect, much of the current speculation on phylogeny may be futile. Thus, until there is agreement as to what criteria are needed to place a fossil within the mollusks or remove it from the phylum, controversy regarding position of the Hyolitha will continue. If Hyolitha are mollusks, our concept of the ancestral form will have to be re-evaluated.

The problem of class definition is more complex, for there are more classes than there are phyla. Systematics may be rigorous and totally objective, but, according to one school of thought, systematics is a qualitative art; a species is defined as any group which a competent worker designates as a species. One should not lean on an authority to do one's thinking, yet I cannot help but feel that this unsophisticated definition which calls on practical work as opposed to theory has more to commend it than most of us are willing to admit. Perhaps a class of mollusks should be defined as a group discussed in the earlier sections of this essay.

A class must be distinct from all other classes. For the paleontologist, this means features of hard part morphology. Members of a class have a general morphology which differs from members of other classes. To me, this characteristic morphology implies invasion of a major new ecological niche, so that life habitat enters into the definition of a class. Some adaptations which characterize and indirectly define a class are so broad that they allow the organisms to live in many environments and even to move into the habitats where members of another class more characteristically live. Other adaptations result in a more restricted mode of life. For example, scaphopods live in a very narrow habitat and pelecypods live in a variety of habitats, one of which happens to impinge on that of the scaphopods; I do not mean to imply either competition or a relationship between members of these two classes but only use this phraseology to state that some pelecypods also live as part of the semi-infauna.

"Major," "niche," and "habitat" are all difficult concepts, and obviously I cannot define any of them precisely. I view the history of the mollusks as a series of experiments in morphology to test various habitats. It does not trouble me that some fossil forms are exceedingly rare, for some experiments were good and some were not good. The extant classes are the most successful experiments among the mollusks. They are successful, if one may use that term in the Animal Kingdom, not because they are alive today, but because they have persisted longer than any of the extinct classes. If any of the extinct classes have validity, then the Hyolitha and Rostroconchia were the most successful ones, for they persisted through the Paleozoic. Biomass and diversity are measures of various kinds, but existence or extinction is the most basic measure in evolution.

The term class, as I have employed it, allows a great deal of liberty for the paleontologist. However, liberty is not the same as license. In my opinion, repeated over and over, hard parts should be studied first and then hypothesis about soft parts added. I would also hold that it is far better to state that one has insufficient evidence and to indicate the higher systematic position of an organism as *incertae sedis* than to force it into an accepted and clearly defined class.

To state another truism, no one is fully objective. It has been written that "the eye beholds what the mind perceives." In my mind one of the key features of evolution is adaptive radiation. I view this as essentially instantaneous exploitation of a new feature, be it morphologic, physiologic, behavioral, or otherwise novel. I further think that diversification based on exploitation and refinement of this new feature is a process which occurs at all systematic levels (Hotton, 1971) and which occurs exceed-

ingly rapidly, once the feature has first appeared.

There is no proof as to either the stability of molluscan classes suggested by Runnegar & Pojeta or the more experimental aspect in the phylogeny of the Paleozoic mollusks that I postulated, but I suggest that ancillary data may be instructive in supporting the latter view. In this regard, one of the most dramatic changes in class level systematics during the last two decades is the establishment and general acceptance of a large number of extinct classes of Echinodermata, most of which are found early in the fossil record (for a discussion of some of these see Sprinkle, 1976). In addition, there is emerging from current literature a suggestion that the conventional high level taxonomy among the Arthropoda is undergoing a similar revolution. These two phyla are not related, yet workers independently arrive at the notion of numerous extinct groups of major rank. Runnegar & Pojeta (1974) develop a simple, linear pattern for the evolution of mollusks. My pattern is more like an unpruned bush, but it does resemble the pattern of evolution now postulated for other phyla, whereas the linear pattern does not.

To some people the notion of evolution carries with it the concept of perfect adaptation to the environment. The increase in number of lower level categories through geologic time seems to be generally accepted, and one might argue that a corollary is the relatively greater number of higher level taxa the farther one goes backward. Such philosophical remarks carry little real weight and elaborating them in an attempt to justify my systematic approach would be simply preaching to the converted; this might gain a small, though enthusiastic, response, but it would accomplish little of substance.

However, in spite of my scoffing there may be some major theoretical support coming my direction. "Quantum" evolution is based on the notion that rates of change are not constant. The doctrine of punctuated equilibrium carries with it the acceptance of small, geologically early classes (Gould & Eldredge, 1977) and also the acceptance of non-evolution once a group is securely established in a restricted and stable habitat.

To present the other side of the coin, there are at least three main areas of weakness in my arguments. First, I cannot come up with an unambiguous definition of every extinct class level taxon based on hard part morphology and must call on future work to supply this. I am even willing to suggest that with the best will in the world and the hardest work, we may be puzzled for generations as to the proper class-level placement of a few taxa. Second, with few exceptions I do not see a series of intermediates from one class to another, but insist on dramatic and rapid changes. Hopeful monsters are not in fashion and for those who view evolution as a continuum, the absence of missing links is damning. At the risk of being accused of making a bad pun on systematics, I do think that speciation is trivial, and is not important in the long term history of a group. Third, calling on a non-calcified mollusk which might have persisted from late Precambrian to mid-Paleozoic as the source material for new classes is a piece of special pleading. My only defense is to argue that one might obtain greater variation from the larval stages of an archaic type increasingly under the stress of competition than from larvae of more stabilized lines. I also must acknowledge that changes such as torsion could not have taken place if the mollusk upon which this change was about to be imposed did not already have a shell.

In the face of these uncertainties, I hold that there are a number of classses of extinct mollusks. The variety to be seen within the early mollusks has been sampled, but it has not been fully explored or adequately documented. There may not be as many classes of extinct mollusks as there are of extinct echinoderms, but I would wager that posterity will recognize as many classes of extinct mollusks as there are extant classes. However, no one knows without the shadow of a doubt. Though I have used it before (Yochelson, 1977), I consider any paper of a speculative nature an appropriate place to quote the motto of my mentor, the late J. Brookes Knight: "Say not that this is so, but that this is how it seems to me to be as I now see the things I think I see."

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MALACOLOGIA, 1978, 17(2): 193-206

LOCOMOTION RATES AND SHELL FORM IN THE GASTROPODA

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ABSTRACT

Rate of locomotion in the Gastropoda correlates surprisingly well with the shape of the shell. In general, the fastest snails hold their shells in such a way that the frontal cross-section area is very small, the center of gravity and pressure point are very low, there is little or no torque, and essentially no surface ornamentation. If any of these factors is increased, the net result is a more slowly moving snail. The slowest snails maximize at least one of the parameters (i.e., extremes in ornamentation, very high center of gravity, etc.) and commonly achieve high values in more than one (i.e., snails with high torque also have a high center of gravity and a large frontal cross-sectional area). The very slowest gastropods are the "shell-draggers," those who drag their shell across the substrate rather than balancing it over the cephalopedal mass.

Unlike most other organisms, the size of the snail does not seem to be a major determinant of speed. Possibly this is because "stride" is of less significance in the locomotion of gastropods. It is presumed that the stromboids would exhibit considerable correlation between size and speed. Ciliary movers show the least correlation between size and speed.

Snails typically move at such a sedate pace that there has been no reason to suspect that streamlining would be of any significance to them. Yet a qualitative analysis suggests that the various parameters that reduce shell drag correlate well with locomotion rates in gastropods under laboratory conditions. Factors thought to be significant include total frontal cross-sectional area, height and relative positions of pressure point and center of gravity, surface smoothness and kind of symmetry.

GEOMETRICAL CONSIDERATIONS

Perhaps the problems of motion can best be appreciated if we imagine a preposterous snail carrying an elongate straight shell erect over its dorsal surface (Fig. 1). This shell obviously presents a large cross-sectional area, relative to the volume of the shell, for the fluid medium to act against if the animal tries to move. The center of gravity of the shell is located high above the substrate, and as the organism accelerates there will be a coupling action between the shell and the locomotor organ (the sole of the foot). The pressure point will be located even higher than the center of gravity and equilibrium would be attained only when the pressure point is behind the center of gravity during motion. A great deal of energy would have to be expended to prevent this shell from tipping over during locomotion.

Obviously any coiling will help. Both the center of gravity and pressure point will be lowered and the frontal cross-sectional area will be reduced. These results will enable the shell to be carried more readily through water. However, not all

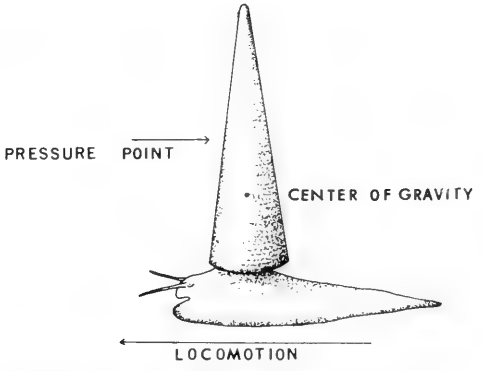

Fig. 1. A preposterous gastropod with an uncoiled shell held erect dorsally. The center of gravity is high, the pressure point even higher. The large frontal cross-sectional area combines with these factors to make forward motion very difficult.

modes of coiling are equal in this regard. The effectiveness of any particular coiling is primarily determined by the way a snail positions its shell while moving (Linsley, 1977). A shell held by a moving snail must be balanced. A limiting factor is that the aperture must be tangential, i.e. in a plane tangential to the ventral portion of the body whorl (Linsley, 1977) and held approximately parallel to the substrate so the shell can be clamped against the substrate.

A bilaterally symmetrical isostrophic shell (with zero translation, Raup, 1966) will of course be balanced, with the plane of symmetry of the shell continuing the plane of symmetry of the foot. It would be balanced whether the animal was untorted (monoplacophoran) or torted (gastropod). The cross-sectional area can be minimized either by making a very compressed shell or by large whorl overlap. However, a large degree of whorl overlap (a doubly anomphalous shell) makes it geometrically difficult to maintain a tangential aperture.

In an asymmetrical (anisostrophic) shell, balance can be obtained by tilting the axis of coiling up (inclination) until the center of gravity of the shell and its contents is over the midline of the foot, or balance can be obtained by rotation of the axis of coiling (regulatory detorsion). Regulatory detorsion will swing the spire forward in hyperstrophic gastropods or backwards in orthostrophic forms. In fact every snail utilizes a combination of these two processes to obtain a balanced shell, and the combination used is that which provides the lowest center of gravity while maintaining a tangential aperture (Fig. 2). However, as Vermeij (1971) has pointed out, the angle that the plane of the aperture makes with the axis of coiling (angle E) is dependent on the shape of the aperture. As the aperture becomes longer, angle E decreases. Since angle E is essentially a measure of the amount of inclination, a snail with an elongate aperture balances its shell primarily by regulatory detorsion (approaching 90°) and little inclination. The relative amounts of detorsion and inclination produce different shell shapes.

To simplify the discussion I begin with an analysis of shells whose generating curves approximate a circle. The major difference among the two forms shown in Fig. 3 is the amount of translation (T), although there are attendant changes in whorl expansion rates (W) and distance (D)

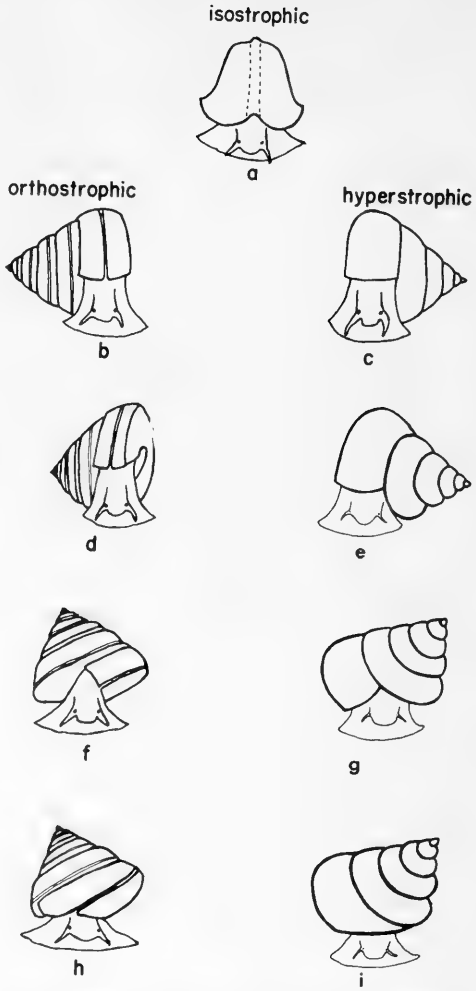

Fig. 2. Shell balancing in Gastropoda. Isostrophic gastropods (a) are assumed to be the ancestral condition, torsion having brought the anus to a position over the head. In right-handed orthostrophic shells (b) the spire projects to the animal's right side, while in hyperstrophic shells (c) the spire projects to the animal's left side. Regulatory detorsion swings the spire backwards in orthostrophic shells (d) but brings it forward in hyperstrophic forms (e). Inclination (f, g) swings the spire up so that the shell is now in a balanced position over the back of the snail. Figures h and i show the shell with tangentially constructed apertures, while all previous diagrams are drawn with radial apertures.

from the axis of coiling. In general, haliotiform, turbiniform and turritelliform shells are distinguished by a progressive increase in T accompanied by a decrease in W and D. Haliotiform shells approximate discoidal (planorbiform) shells in values of T, but discoidal shells have much lower values of

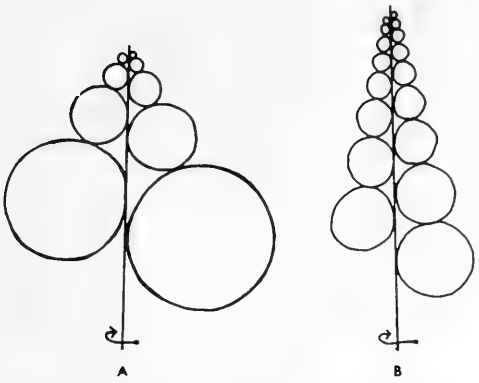

Fig. 3. (A) Longitudinal section of a turbiniform shell. (B) Longitudinal section of a turritelliform shell, each with a circular generating curve. The whorl expansion rate and distance from the axis of coiling are highest in (A) while translation is greatest in (B).

W. In a snail with a discoidal shell, such as *Planorbis,* the shell is held so that the axis of coiling is essentially parallel to the substrate and detorsion is 0°. In contrast, a snail positions a haliotiform shell so that the axis of coiling is almost at right angles to the substrate; regulatory detorsion is still slight (10-20°). Vermeij (1975) suggested that the relative absence of discoidal shells since the Mesozoic is partially due to the appearance of crabs at that time. The discoidal shell is poorly adapted to resist the crushing pressure of crab chelae. While this may be a contributing cause I think that a discoidal shell's high center of gravity and high pressure point suggest that it is poorly adapted to compete in modern oceans. The discoidal form is very common in freshwater pulmonates because the pulmonary cavity acts as a buoyancy chamber and completely changes the analysis of forces acting during locomotion (Linsley, 1977). Thus I suggest that haliotiform shells are the equivalent of discoidal forms among marine prosobranchs. To reduce shell drag they have acquired the lowest center of gravity by positioning the shell primarily by inclination, achieving a proportionately small frontal cross-sectional area, and a pressure point positioned in line with and behind the low center of gravity. In order to construct a tangential aperture the W and D values have to be quite large, unlike pulmonate discoidal shells. Although I have not yet had the opportunity to study haliotiform snails, I expect them to be very fast.

In turbiniform shells with circular apertures the shell is balanced with an inclination of 50-70° and 10-30° regulatory detorsion (Vermeij, 1971). This results in a shell with a large frontal cross-sectional area, a high center of gravity and a high pressure point. Consequently snails with turbiniform shells tend to be fairly sedate in their rates of locomotion. However, because of the position of their retractor muscle (Linsley, in press a), they are well suited for rock-clinging and this seems to be a satisfactory trade-off for their speed limitations. It should be noted that while naticids hold their shells in a similar fashion, they "cheat" by inflating the foot with water to internalize their shell. As a result, an analysis of forces affecting their motion capabilities predicts them to be among the fastest snails, as indeed they are.

As spire height increases through an increase in T, with attendant decreases in W and D, it is possible for angle E to decrease as well. As a result higher-spired shells tend to achieve a lower center of gravity by employing more regulatory detorsion and less inclination. In general this reduces frontal cross-sectional area, lowering the center of gravity and the pressure point, allowing more rapid locomotion. However, as the spire becomes still higher, the center of gravity moves farther from the aperture and the columellar muscle is flexed at an increasingly larger angle between its insertion points in the foot and the shell. Thus as the spire elongates, the snail must work harder to hold the shell up and the muscle becomes positioned in a progressively less efficient attitude. As a result the turritelliform snail does not hold its shell above the foot, but drags the shell along the substrate. "Shell draggers" are among the slowest snails.

Thus there is no way for a gastropod with a circular aperture to solve the geometrical problems in a way that allows it to be a "fast" snail. The only solution is an aperture elongated parallel to the direction of motion. And yet an aperture elongated in this manner is possible only in snails with a single gill. The primitive archaeogastropods with paired gills must maintain a mantle cavity (and aperture) sufficiently commodious for two separate currents through the cavity.

Elongation of the aperture more or less parallel to the axis of coiling reduces inclination while retaining a tangential aperture and necessitates that shell balancing be

accomplished by regulatory detorsion. Any reduction of D simultaneously reduces frontal cross-sectional area, lowers the center of gravity, lowers the pressure point, and in general increases streamlining of the shell. These are all characteristics of the fastest snails.

The preceding analysis seems to be valid for all gastropods supporting their shells above the substrate while moving. However, there are gastropods that are atypical in this regard (Linsley, Yochelson & Rohr, in press). In general they can be divided into three major categories with a number of subdivisions. These artificial groups can be characterized as the "motionless" forms, the "shell draggers" and the "leapers."

The motionless forms never move while adult. They are easily recognized by the presence of an attachment scar (e.g. *Petaloconchus* Lea) where the shell was cemented to the substrate, or by open or disjunct coiling (e.g. *Siliquaria* Bruguière, and *Vermicularia* Lamarck) (Gould, 1969; Yochelson, 1971; Peel, 1975; Rex & Boss, 1976).

The shell draggers include a motley assortment of snails that rest their shells upon the substrate and move by arhythmic motion (Miller, 1974) wherein they extend the foot in front of the shell and then drag the shell up to it. These include high-spired snails (e.g. *Cerithium* Bruguière) whose center of gravity is placed so far behind the aperture that to support the shell over the body would require undue energy. They also include snails whose aperture is off to the side of the shell (e.g. *Conus* Linnaeus). (The placement of the columellar muscle in this group is better situated for lifting the shell than in the high-spired forms, so although these animals typically rest their body whorl on the substrate they hoist it up more frequently than do the high-spired forms.) The final group of shell draggers are those shells with radial apertures (apertures whose plane includes the axis of coiling) (e.g. *Architectonica* Röding). The radial aperture (Linsley, 1977) makes it impossible for these animals to obtain protection by clamping the shell against the substrate. Shells with radial apertures are rare in the modern gastropod fauna.

The last group of atypical snails, the leapers, include genera like *Strombus* Linnaeus, *Aporrhais* da Costa and *Xenophora* Philippi. These animals spend most of their time with the shell resting on the substrate; motion is accomplished by the foot thrusting against the substrate, which lifts the shell up and forward in the characteristic "leap" (Berg, 1972). Unlike the other two groups the leapers at times are active and can cover considerable distances in a short time. However, since their locomotion is so unlike that of the majority of gastropods, I have not included them in the analysis of locomotion.

METHODOLOGY

Time-motion study of gastropods was done with time-lapse movies using a Minolta super 8 mm movie camera. An aquarium was mounted on a stand with a mirror placed below it at a 45° angle. The camera was situated to the side of the aquarium

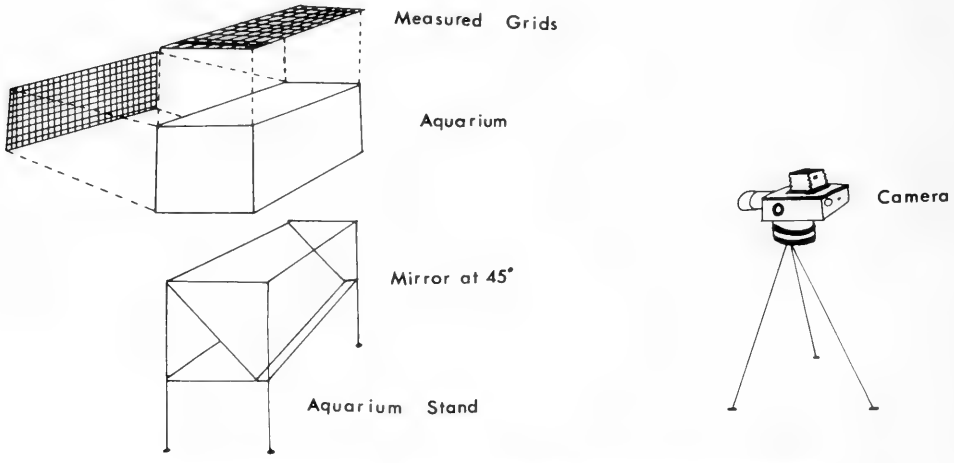

Fig. 4. Camera setup for taking motion picture studies of gastropods.

and the picture was framed so that it included both the mirror and side of the aquarium (Fig. 4). This allowed motion of the snails to be recorded in all three dimensions; measured grids were placed along the top and back of the aquarium. Typically five individuals of one species were placed in the aquarium and their movements were filmed during a two-hour period. Frequently individuals would move actively for some time and then become quiescent. Moving them about the aquarium frequently initiated activity again. I made no attempt to elicit escape behaviors in the snails by introducing potential predators; I thus presume that I am measuring typical locomotion. Some snails vary widely in speed, but the majority are fairly uniform in their speeds. A few browsers have two speeds. One is fairly fast and the animal progresses in a fairly straight line. This I characterize as a "going somewhere" locomotion. The other behavior consists of moving more sedately while the head swings back and forth and the snail progresses more erratically, not in a straight line. This was a pattern found primarily in algae-grazers and I assume it is "feeding" locomotion.

The most obvious objections to this approach are that the snails had light coming at them from all directions, even from below, and they were placed on an artificial substrate. That they moved at all suggests that light below is no deterrent. Observations on a few gastropods in their normal habitats revealed speeds consistent with those observed in the aquarium. It is my supposition that the nervous system of the Gastropoda is simple enough so that only a narrow range of behavioral responses are possible.

In compiling data from the movies, I recorded three kinds of information: (1) fastest recorded speed, (2) average speed, and (3) total motion. The speed was timed from as many straight line "runs" of 2-30 cm as I could obtain on film. "Fastest speed" was the fastest recorded time of all runs. "Average speed" was based on 2-20 runs. "Total motion" was based on total distance covered by each snail (whether it moved or not) and reduced to the average distance for all members of the species in cm/hr. All three of these measurements show positive correlation with shell shape; "average speed" provides the best correlation and "total motion" the poorest.

The first movies were made in the summer of 1975 in the Florida Keys and Sanibel Island. In January 1976 the author and eight students from Colgate University spent one month at the Smithsonian Institution's Marine Biology Station at Carrie Bow Key in Belize, thanks to arrangements made by Dr. Klaus Ruetzler.

Analysis of resistance was made subjectively to determine whether a more sophisticated approach seemed warranted. Shells were evaluated for two characteristics: ornamentation and bilateral symmetry. The amount of ornamentation of the shell was divided into three grades: (1) smooth, (2) raised growth lines or incised sutures and (3) nodes or spines. Bilateral symmetry was used because it correlates with frontal cross-sectional area, height of center of gravity, height of pressure point and the amount of torque on the shell as it is moved through water. The geometrical constraints cause snails with any appreciable spire that position their shells primarily by inclination to experience high levels of resistance. As a result, snails with round apertures and medium spires have the highest level of asymmetry. As regulatory detorsion increases, inclination and asymmetry decrease, as do all of the attendant factors listed above.

Each snail studied was subjectively ranked from 1 to 3 on two categories: asymmetry, where "1" approaches bilateral symmetry and "3" represents the highest level of asymmetry; and ornamentation, where "1" represents smooth shells and "3" the presence of nodes or spines.

The two numbers were then added and all snails were ranked from "2" to "6" with rank "2" being the most streamlined (and presumably the fastest) and rank "6" offering the most resistance (and hence presumably the slowest). In addition a rank "7" was added for the shell draggers, presumably the slowest of all. Data were gathered for 57 species (Table 1). A student t-test was performed to compare the speeds recorded for each rank-group with those of every other rank-group. It was found that for groups 2 through 6 each group was significantly different to at least the 95% confidence level. From inspection of the data in Table 1 it is obvious that significant differences exist. If nothing else, I believe that this cursory and subjective treatment shows that more sophisticated analysis of the problem will be worthwhile.

TABLE 1. A pooled list of gastropod taxa investigated at Carrie Bow Key, Belize, and Key Largo and Sanibel Island, Florida. In ranks 2 through 6 the first figure of the fraction refers to a subjective ranking of asymmetry with rank 1 approximating bilateral symmetry and 3 showing the greatest asymmetry. The second figure of the fraction is a similar ranking of ornamentation. Rank 1 represents smooth shells while rank 3 represents coarse ornamentation. The last number represents average speed.

Rank 2		Rank 3		Rank 4	
Busycon contrarium	1/1 4.0	*Columbella mercatoria*	1/2 0.9	*Cassis flammea*	2/2 1.8
Cyphoma gibbosum	1/1 1.3	*Melongena corona*	1/2 3.6	*Cittarium pica*	2/2 0.9
Cypraea cervus	1/1 2.8	*Melongena melongena*	1/2 2.4	*Crassispira cubana*	1/3 0.4
Cypraea cinerea	1/1 4.3	*Nassarius vibex*	1/2 5.0	*Littorina angulifera*	2/2 2.4
Cypraea spurca acicularis	1/1 3.4	*Trivia maltbiana*	1/2 0.8	*Littorina ziczac*	2/2 0.6
Cypraecassis testiculus	1/1 5.6	*Turbinella angulata*	1/2 0.5	*Murex pomum*	1/3 1.6
Fasciolaria lilium hunteria	1/1 4.6	Average	2.2	*Nerita fulgurans*	2/2 2.4
Fasciolaria tulipa	1/1 6.5			*Nerita versicolor*	2/2 2.6
Hyalina avena	1/1 3.5			*Pisania auritula*	2/2 2.3
Marginella guttata	1/1 3.0			*Planaxis nucleus*	2/2 1.7
Marginella lactea	1/1 1.8			*Tegula fasciata*	2/2 1.8
Marginella pruniosum	1/1 3.4			*Tegula lividomaculata*	2/2 0.9
Marginella sp.	1/1 5.3			*Thais rustica*	2/2 2.4
Mitrella ocellata	1/1 1.9			*Turbo canaliculatus*	2/2 1.5
Nitidella nitida	1/1 2.0			*Vasum muricatum*	1/3 0.3
Oliva sayana	1/1 8.4			Average	1.6
Polinices duplicatus	1/1 5.6				
Polinices lacteus	1/1 3.4				
Tonna maculosa	1/1 3.6				
Average	3.9				

Rank 5		Rank 6		Rank 7 (Shell-draggers)	
Cymatium sp.	2/3 0.6	*Astraea phoebia*	3/3 0.4	*Batillaria minima*	0.6
Leucozonia ocellata	2/3 0.5	*Astraea tecta americana*	3/3 0.3	*Cerithium guinaicum*	0.3
Nodilittorina tuberculata	2/3 0.6	*Astraea tecta tecta*	3/3 0.4	*Cerithium litteratum*	0.1
Ocenebra minirosea	2/3 0.3	Average	0.36	*Conus jaspideus stearnsi*	0.3
Thais deltoidea	2/3 0.6			*Conus mus*	0.8
Vexillum dermestinum	2/3 0.5			*Conus regius*	0.3
Average	0.5			*Muricopsis oxytatus*	0.1
				Polystira albida	0.3
				Average	0.35

One interesting sidelight is that nowhere in the analysis was size considered a relevant factor. In all other studies performed on the rates of locomotion of arthropods, mammals, fish or birds, the factor of "stride" (or its equivalent) had to be considered and thus there is a positive correlation between size and speed. In gastropods dependent upon cilia for their prime locomotor force, the lack of a correlation between size and speed would be understandable, for the effectiveness of an individual cilium probably does not vary appreciably between young and adult. In fact adults may well have proportionately fewer cilia and thus move more slowly than the young. Miller's study (1974) of locomotor types in the Gastropoda suggests that snails moving by muscular contractions show a constant number of waves on the foot within a species. Hence as an individual grows, its "stride" may increase and make an adult somewhat faster. However, between species the kind or number of muscular contractions does not seem to correlate with the speed of the organism.

Although I have not yet assembled all the data, I believe that speed correlates with food preference. It would be nice if we could make simplistic correlations to the effect that "fast snails are carnivores and slow snails are herbivores." Unfortunately the relationship is more complex than that. Although many gastropods are carnivorous, some are carnivores on tube worms, oysters, sea anemones, etc., prey that does not necessitate great speed from the predator. However, I do not think it coincidental that *Natica, Oliva* and *Fasciolaria,* three active carnivores, are also three of the fastest gastropods studied. Certainly it will be possible to make generalizations

from the activity level to the rate of locomotion and thence to shell shape. Although it may be premature, I propose life modes of some Paleozoic gastropods based on my estimates of their speed potential.

INTERPRETATION OF PALEOZOIC BELLEROPHONTACEAN GASTROPODA

Considerable controversy exists over the interpretation of isostrophic shells found in the Paleozoic. Some authors (Wenz, Thiele, Runnegar, Pojeta, Jell, etc.) consider all non-septate isostrophic shells as belonging to the Monoplacophora. Other authors (Batten, Knight, Linsley, Peel, Rollins, Yochelson, etc.) believe that while some of these shells are monoplacophorans, the great majority belong to the Gastropoda. The forms under discussion in this paper are presumed to be gastropods.

Of all Paleozoic gastropods some members of the Bellerophontacea seem the best candidates for fast moving snails. The bellerophontaceans provide us with a wide array of forms which I interpret to be adaptations to a wide variety of life styles. At the risk of oversimplification, I focus on five genera from this superfamily: *Ptomatis, Tropidodiscus, Euphemites, Knightites,* and *Bellerophon.*

The Middle Devonian *Ptomatis* Clarke (Fig. 5) can be taken as typical of a number of taxa which have a large whorl expansion rate with little whorl overlap in the immature conch, and a widely explanate, bell-shaped tangential aperture in the adult stage. The center of gravity and pressure point are both low and, except for the presence of ornamentation in some species, this general form at first seems well adapted to fairly rapid motion. Alternatively, the explanate aperture implies that this was not a particularly active animal. Instead it suggests a form that had a broad foot adapted to life on a firm clayey or silty substrate, possibly as a sluggish grazer or deposit feeder. I would place it in rank 5 for speed.

One enigmatic aspect of *Ptomatis* is the thick, pustulose parietal deposit on mature specimens. In some specimens, presumably the most mature ones, the deposit even projects out into the aperture like a shelf (Knight, 1941, pl. 7, fig. 1f). I believe that this callus can be interpreted as the insertion area for the retractor muscles. In immature forms (before the advent of the flared aperture), the columellar retractor muscles would presumably be situated in a characteristic bellerophontacean position, about one half volution inward from the aperture. This muscle position would allow protection by deep withdrawal. However, with the development of the bell-shaped aperture in adults, the center of gravity of the shell would be rapidly shifted anteriorly. Since clamping presumably would now become more important than deep withdrawal, the columellar muscle insertions would migrate to the parietal lip. The extension of the shelf anteriorly would place the muscle insertions close to or at the center of gravity of the shell and thus maximize the effectiveness of clamping.

Tropidodiscus Meek & Worthen (Fig. 6), from the Lower Ordovician to the Devonian, has a widely umbilicate, compressed shell with a deep slit. Because of the wide umbilicus it is geometrically possible for this form to have a tangential aperture. The shell has a relatively high center of gravity

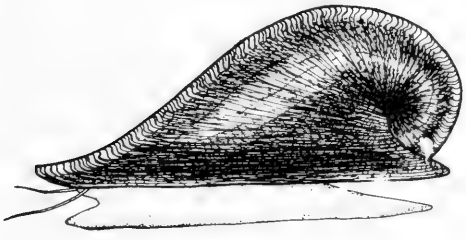

Fig. 5. Reconstruction of *Ptomatis.*

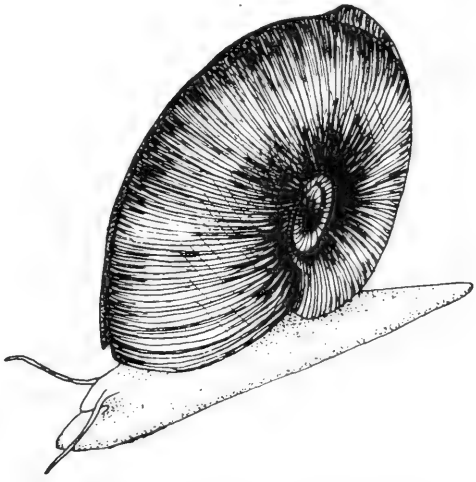

Fig. 6. Reconstruction of *Tropidodiscus.*

and high pressure point, but because of its narrow frontal profile I believe this animal to have been capable of considerable mobility. The curved form of the aperture would not allow it to clamp its shell against a hard substrate. *Tropidodiscus* may have lived on soft substrates. Indeed it is typically found in fine calcilutites associated with reefy limestones such as the Anderdon limestone (Linsley, 1968) and Columbus limestone (Stauffer, 1957). I think that this snail was capable of considerable speed, but because of its high, compressed shell, it would have lived in low current environments like fine muds or lime sands in protected areas in the back reef zone. The shell form of *Tropidodiscus* suggests that it belongs in rank 4 for speed.

The Upper Paleozoic genus *Euphemites* Warthin (Fig. 7) is a "fat" bellerophon with much overlap between successive whorls. One interesting feature of *Euphemites* is the variety of inductural deposits (secondary shell deposits laid down on top of the primary shell) which cover the entire outside of the shell (Moore, 1941). This indicates that various mantle or foot lobes covered the entire shell when it was active, and may explain why *Euphemites* has a tangential aperture rather than a radial aperture. The general form of *Euphemites* is well suited to rapid locomotion and if I were to look for an active predator among the Bellerophontacea this would be a likely candidate. Among modern gastropods, the Naticidae seem to be analogous in mode of life because the shell is also covered by the foot. While I would not expect *Euphemites* to have developed the shell-drilling capabilities of *Natica, Euphemites* may have been similar in that it burrowed into sediment with its shell essentially internalized. I believe speed to have been in the range found in category 2.

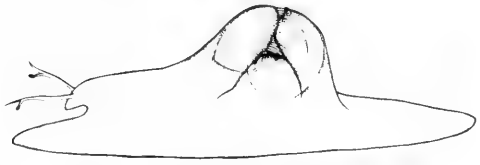

Fig. 7. Reconstruction of *Euphemites.*

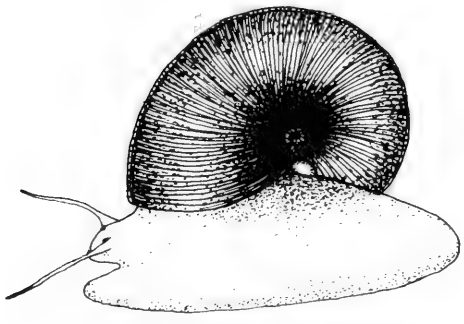

Fig. 8. Reconstruction of *Bellerophon.*

Another Upper Paleozoic form, *Knightites* Moore, is the bellerophontacean on which Knight based his 1952 reconstruction. The shell has small umbilici, a slight flare to the tangential aperture in the adult, but is especially remarkable for periodically constructing paired "horns" or siphonal canal-like re-entrants in the aperture bordering the slit. Since the slit was the site for the exhalant current, it seems reasonable that the "horns" are indeed homologous to the siphonal canal of modern gastropods in function as well as appearance. If this homology is true it makes no sense to reconstruct *Knightites* as a monoplacophoran (Runnegar & Pojeta, 1974; Pojeta & Runnegar, 1976), for in that reconstruction these inhalant currents would be positioned directly to the posterior, something only done by animals that put their heads in the sand, like clams. *Knightites* makes sense only if it is reconstructed as a gastropod, with torsion bringing the inhalant horns to the anterior where they could obtain clean oxygenated water and sample the chemistry of the area directly in front of them. Because of a high development of ornamentation I expect *Knightites* to have been sedate and probably a grazer on algae fitting in category 5 for speed.

The last bellerophontacean to be considered is the long-lived genus *Bellerophon* Montfort (Silurian to Triassic), geometrically the most enigmatic of the group (Fig. 8). The shell has a low profile with many volutions which, as in *Euphemites,* is accomplished by considerable overlap between the whorls of successive volutions. This combination of overlap and a narrowly phaneromphalous or anomphalous condition keeps the shell from having a tangential aperture. This general form is the only one I know of in gastropods that presumably was supported off the substrate in the normal dorsal position and that does not have a tangential aperture. Perhaps it was possible for the form to evolve this

way because of its deep-withdrawal capability. All known isostrophically coiled monoplacophorans that were unable to effect deep withdrawal have tangential apertures. Many specimens of *Bellerophon* show a "waterline" of inductural wash about half way up the shell, suggesting that a great deal of mantle and foot were exposed during locomotion. Possibly these animals were fast enough to avoid most predators and, when faced with the rest, depended on deep withdrawal for protection. We know of no opercula from bellerophontaceans. Perhaps in the absence of an operculum, narrow aperture and deep withdrawal capabilities have strong selective advantage.

Bellerophon typifies the problems faced by bellerophonts in general. In order to be fast, they had to keep the shell low and the only ways to do this were to have either considerable whorl overlap or a rapidly expanding generating curve. The rapidly expanding generating curve makes deep withdrawal impractical and as a consequence, probably limited effective defense to clamping. Clamping, to be effective, requires a large foot and a firm substrate to hold onto; *Ptomatis* seems to have had both. *Bellerophon* used the other alternative, large whorl overlap. In order to maintain a mantle cavity of sufficient breadth for effective separation of two inhalant streams, *Bellerophon* could not have wide umbilici. The geometric problems of a shell with large whorl overlap and no umbilici made it impossible for this form to have a tangential aperture. Because species of *Bellerophon* have varying degrees of ornamentation I would place it either in category 3 or 4 for its speed.

INTERPRETATION OF SELECTED OTHER PALEOZOIC GASTROPODA

While the shell geometry of the Bellerophontacea could have allowed some of them to be among the fastest of the Archaeogastropoda, the geometry of the majority of the members of the Macluritacea suggests that these were among the slowest of the Archaeogastropoda. Within that superfamily are two families, the Macluritidae and the Onychochilidae, each representing distinct adaptations (Linsley, 1977). The Onychochilidae range from the Upper Cambrian to the Lower Devonian and are medium-spired, presumably hyperstrophic gastropods with an elongate aperture tangential to the body whorl. This geometry suggests that the onychochilids were mobile animals carrying the shell dorsally with the spire swung forward over the head (Fig. 9). Shells of this sort would offer a high center of gravity and large frontal cross-sectional area and would therefore be quite slow, speed category 5 or 6. I suspect they were predominantly algae-grazers or possibly deposit feeders, adapted to a hard substrate.

In contrast, the shells of the Ordovician family Macluritidae are very low-spired, verging on discoidal (Fig. 10). All have radial apertures with a sharp angulation on the right side of the shell, which presumably represented the position of the anus (Knight, Batten & Yochelson, 1960). Because there is little translation during coiling in these discoidal shells, they are superficially convergent on the isostrophic form of the bellerophontaceans. But macluritids are distinct in having little or no overlap

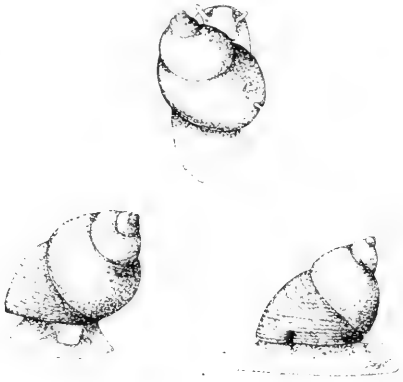

Fig. 9. Reconstruction of *Onychochilus.*

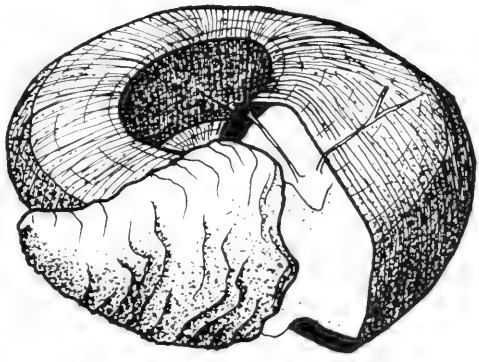

Fig. 10. Reconstruction of *Maclurites.*

between successive whorls and, as well as an asymmetrical whorl profile, a slightly depressed spire and a radial aperture. Many are also associated with a heavy calcareous operculum.

The radial aperture of the macluritids strongly suggests that these animals did not hold their shells over their backs; this has already been suggested for other reasons (Salter, 1859; Banks & Johnson, 1957). It is presumed that they were shell draggers (category 7) that normally rested the flat "base" (left side) of the shell on the substrate with the anal angulation away from the substrate. The heavy shell of these snails is consistent with the idea that they must have only rarely, if ever, moved their shells. For defense they could clamp the thick operculum over their apertures. The almost total immobility I infer for this group suggests they may have been filter feeders. Knight (1952) suggested that macluritids had only a single ctenidium (the left) and thus a unidirectional water current through the mantle cavity, inhalant at the portion of the aperture near the substrate and exhalant past the anal re-entrant.

The Euomphalacea were considered by Knight, Batten & Yochelson (1960) to be closely allied to the Macluritacea and I fully concur, believing euomphalaceans to be derived in the Ordovician as the logical perfection of the macluritid stock. Again the dominant shell form is discoidal, but with variation between hyperstrophic and orthostrophic, even occasionally within the same species (Linsley, in press b). Within this taxon is found a large number of openly coiled shells (Yochelson, 1971; Linsley & Yochelson, 1973), an obvious adaptation that allows the shell to rest flat on the substrate. I interpret the life-mode of the euomphalids to be similar to that inferred for macluritids; essentially motionless (category 7, shell-draggers), filter-feeding forms, adapted to a wide variety of substrates. The open-coiled forms were adapted to soft substrates, while the tightly coiled helical forms were better suited to firmer substrates.

The Pleurotomariacea are the third major group of Paleozoic gastropods, more numerous and diverse than either the Macluritacea and Euomphalacea or Bellerophontacea. They diverged from the bellerophontaceans by extending the spire out to the right where it is repositioned by swinging back and up through regulatory detorsion and inclination (Fig. 11). Most Pleurotomariacea have a tangential aperture and thus almost undoubtedly carried their shells balanced dorsally as they moved about. The geometry of pleurotomariaceans is limited by paired gills. They necessitate two streams of water flowing through the mantle cavity and separation of the two streams can be maintained only in a relatively capacious mantle cavity. As a result, the generating curve cannot depart very far from the circular in Pleurotomariacea. This shape in turn dictates that the shell be positioned with considerable inclination, giving rise to a high center of gravity, large cross-sectional area and high values of torque. Thus the majority of pleurotomariaceans will belong to categories 4, 5 or 6 and will move at a rather sedate pace at best, which leads me to believe they were grazers, deposit feeders or scavengers. If any pleurotomariaceans of the past adopted a carnivorous mode of life, they must have preyed on sessile animals or ones for which they could lie in wait. One group of Paleozoic Pleurotomariacea, the Raphistomatinae, seems to have tried elongating the aperture by introducing a short siphonal canal for the left gill. In at least two genera (*Scalites* Emmons from the Ordovician and *Tylozone* Linsley from the Lower Devonian, Fig. 12) the short siphonal canal combined with a medium spired shell to reduce inclination and to position the shell primarily by detorsion. This lowered the resistance and presumably allowed the snails to be among the most active of the pleurotomariaceans, perhaps in category 3.

Fig. 11. Reconstruction of *Pleurotomaria.*

A second group that may have achieved more mobility than the majority of the superfamily is the Porcelliidae (Fig. 13). They achieved this by converging on the form of the bellerophontaceans so the adult shell approaches the isostrophic con-

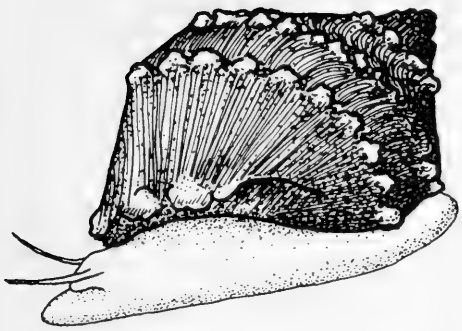

Fig. 12. Reconstruction of *Tylozone.*

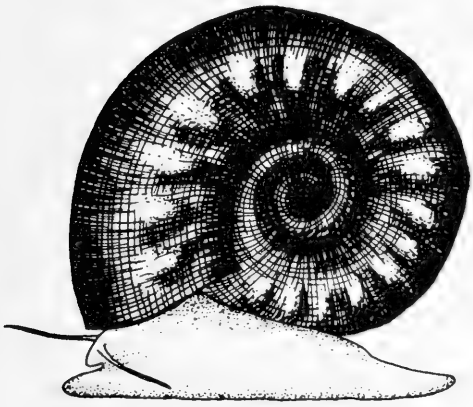

Fig. 13. Reconstruction of *Porcellia.*

dition even though the embryonic shell is orthostrophic. Specifically they converged on the general form of *Tropidodiscus,* doubly phaneromphalous with a tangential aperture and a very deep slit extending over ninety degrees from the aperture. Presumably everything inferred earlier about the mode of life of *Tropidodiscus* applies equally well to *Porcellia.* In fact the stratigraphic record suggests that *Porcellia* replaced *Tropidodiscus* during the Devonian and continued through most of the Upper Paleozoic.

As might be expected, pleurotomariaceans include few shell-draggers. A few possible Paleozoic candidates include *Dirhachopea* Ulrich & Bridge (Upper Cambrian-Lower Ordovician) and *Calaurops* Whitfield (Lower Ordovician), both of which become uncoiled in the adult stage; they are certainly shell draggers, but may not belong in Pleurotomariacea. However, *Eryzone* Koken, *Pagodispira* Horný, *Odontomaria* C. F. Roemer, *Loxoplocus* Fischer and *Mastigospira* LaRocque are all undoubted pleurotomariaceans from Ordovician to Devonian strata and all obtain open coiling. They may be considered either shell-draggers or completely motionless. These pleurotomariaceans achieve open coiling by increasing translation in contrast to the euomphalaceans that accomplish open-coiling by increasing distance or decreasing whorl expansion rate. The Mississippian genus *Rhaphischisma* Knight may also have been a shell dragger because it appears to have a radial aperture. This is an interesting genus for the slit is positioned so close to the upper suture that it is impossible that the right gill was still present. Perhaps it should not be accepted as a pleurotomariacean without further investigation.

Pleurotomariacea are readily recognized by their prominent apertural re-entrant. Other Paleozoic shells possessing a similar circular generating curve and a tangential aperture lack a re-entrant and are interpreted as having a single gill. These are considered members of the Trochina. Like the modern Trochacea and the Pleurotomariacea they must have balanced their shells with much inclination. The Paleozoic forms, like their modern counterparts, were probably very slow and may have lived on a hard substrate, or even on rocks—their shell form is well suited for clamping. However, a few Paleozoic taxa deviate from this general pattern and deserve comment. Perhaps the best known deviants are the coprophagous Platyceratacea (Bowsher, 1955). This large and diverse group took up residence on the calyx of crinoids, apparently subsisting on the fecal matter. Since these snails were sedentary, some of them attained open coiling. The Tubinidae also have been placed in the Trochina and might have resembled the Platyceratacea in that both were relatively immobile. Open coiling is again common in this group, attesting to their lack of mobility. However, the Lower Devonian Tubinidae generally achieve open coiling by increasing D (the distance of the whorls from the axis of coiling) whereas the platyceratids generally become openly coiled by an increase in T (translation rate). In both groups the shell geometry leaves the aperture free of interference from the spire. The last group that departs from the typical trochinid form that is mentioned in the Treatise (Knight, Batten & Yochelson, 1960) as

members of the suborder Trochina, is the Silurian to Devonian Oriostomatidae. These have radial apertures and a heavy, calcareous, multispiral operculum and probably should be transferred to the Euomphalacea (Linsley, in press b).

The Murchisoniacea constitute an enigmatic group of high-spired Paleozoic snails. Knight, Batten & Yochelson (1960) have placed them in the Archaeogastropoda with some question, because although the Murchisoniacea have a slit positioned at or near mid-whorl, presumably indicating paired gills, they are thought to have been on their way towards eliminating the right gill and are considered ancestral to the Loxonematacea, placed by them in the Caenogastropoda. The two families within the Murchisoniacea are the high-spired Murchisoniidae and the moderate-spired Plethospiridae. Plethospirid shells are sufficiently low-spired that their shell could have been carried dorsally. Genera such as Ordovician to Silurian *Plethospira* Ulrich and Silurian to Devonian *Diplozone* Perner show the development of an ill-defined siphonal canal which served to elongate the aperture, thus reducing inclination of the shell. I expect the Plethospiridae to have been more mobile (category 3 or 4) than most of the Pleurotomariacea.

In contrast to the Plethospiridae, the majority of the Murchisoniidae (Fig. 14) are so high-spired according to a "corollary of Linsley's Third Law," they must have been shell-draggers (Linsley, 1977) and thus would have had lower activity levels than most pleurotomariaceans. At least four genera of the Murchisoniidae have made peculiar modifications of their shells that attest to this loss of mobility. *Gasconadia* Ulrich (Ordovician) and *Lodonaria* Dahmer (Devonian) have developed flaring bell-shaped apertures in the adult conch, the plane of which is roughly parallel to the axis of coiling or the ventral margin of the shell. A similar adaptation is made by the modern genera *Cerithium* Bruguière and *Distorsio* Röding. Thus it would have been possible for them to clamp their aperture against the substrate without elevating the spire. A form from the Lower Devonian, *Biangularia* Spitz, has a spire compressed parallel to the axis of coiling, somewhat reminiscent of *Biplex.* This shape would rest stably on the substrate without rolling. However, growth would have to occur in 180°-spurts as it does in the modern genus *Biplex* in order for the forms to be functional. *Brilonella* Kayser (Middle Devonian) is currently assigned to the Murchisoniidae, but may be a pleurotomariacean. It changes the direction of coiling so that the final whorl coils up and backward so the aperture opens near the spire. A conservative interpretation would suggest at least a behavioral change between youth and maturity.

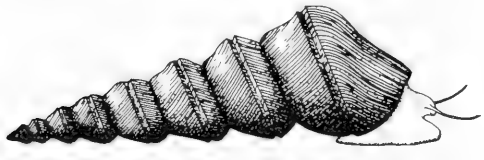

Fig. 14. Reconstruction of *Murchisonia.*

Another group of high-spired Paleozoic gastropods is the Loxonematacea. I concur that they are possibly early Mesogastropoda because the sinus, which is at mid-whorl in Ordovician forms, frequently has migrated towards the upper suture in many subsequent genera. Thus the right gill was presumably lost. The great majority are so high-spired that they must have been shell-draggers, moving slowly and then only rarely. However, a few genera, for example *Loxonema* Phillips (Middle Ordovician to Mississippian) and *Devonozyga* Horný (Middle Devonian) are lower spired and consequently may have been able to hold their shells over their bodies while moving. If this were the case, the spire is high enough so that the shell would be positioned largely by regulatory detorsion with only modest amounts of inclination. Thus if the shell is held erect, these snails would fill the geometric qualifications found in modern moderately fast snails.

However, many specimens of the geometrically similar *Palaeozygopleura* Horný which I have collected from the Middle Devonian of New York have their shells covered with trepostomatous bryozoans, in what may have been a symbiotic relationship. These colonies frequently cover all the shell except the aperture. For a bryozoan to encrust in this manner, the encrustation must have occurred while the snail was not only alive but supporting the shell up off the substrate. On the other hand, the mass that the bryozoan colony would add to the shell certainly does not suggest that *Palaeozygopleura* was a very active gastropod. Even though some modern species of *Thais* are encrusted by red algae

Fig. 15. Reconstruction of *Scalaeotrochus.*

Fig. 16. Reconstruction of *Ceraunocochlis.*

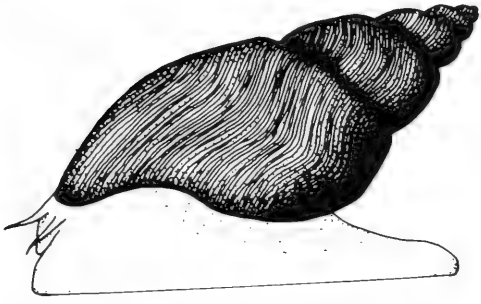

Fig. 17. Reconstruction of *Soleniscus.*

rather than Bryozoa there may be some similarity to these Paleozoic forms. If one can generalize from this example I conclude that the Loxonematacea were rather slow gastropods with activity levels marked by category 7.

Two other small Paleozoic taxa, the Pseudophoridae and Subulitacea, are worthy of comment. The Upper Paleozoic Pseudophoridae (Fig. 15) have a conical shell with a flat base. In most members a frill projects below the level of the base and serves to lift it up off the substrate, much as the frill of the modern xenophorids keeps the base of the shell from contacting the substrate (Linsley & Yochelson, 1973). Linsley, Yochelson & Rohr (in press) have suggested that these extinct animals were immobile through much of their life and possibly had a mode of locomotion similar to the leap of the strombids and xenophorids.

Most Subulitacea, which range from the Ordovician to the Permian, have an elongate aperture with a weakly developed siphonal notch suggesting that they had only a single gill (Knight, Batten & Yochelson, 1960). These authors placed them in the Caenogastropoda. A few genera (*Subulites* Emmons, *Cyrtospira* Ulrich and *Ceraunocochlis* Knight, Fig. 16) are so high-spired that they are best interpreted as having been shell-draggers. In both *Cyrtospira* and *Ceraunocochlis* the final whorl deviates so that its axis of coiling of the final whorl is at an angle to the axis of coiling of the earlier whorls. This change creates something approximating a sickle-shaped shell. This would widen the shell and increase the stability of these otherwise long, cylindrical shells as they rested against the substrate and make them less apt to roll in the presence of currents. However, most of the other subulitids are fusiform (Fig. 17) and geometrically would seem to have been capable of holding the shell erect over the body. Because of the elongated aperture, the shell would be positioned primarily by detorsion and the animal would seem to fulfill all prerequisites of a fast snail. Among the Paleozoic gastropods most Subulitacea along with some Bellerphontacea may be the best candidates for active carnivores.

It has been the classical assumption that because nearly all modern archaeogastropods are grazing herbivores, all Paleozoic archaeogastropods must have inhabited precisely the same ecological niche. Generalizations based on surviving members of a formerly large and diverse taxon should be approached with a measure of caution and skepticism. I believe that there were probably many feed-

ing patterns available to the diverse Paleozoic archaeogastropods. Recent studies of the rhipidoglossate radula (Hickman, 1976) suggest that it is a very versatile scraping organ, readily adaptable to rasping a variety of substrates. Within modern snails it is possible to relate shell form to activity levels. Although gastropods have not been systematically treated yet in regard to relating rates of locomotion to food preference, it seems probable that there is indeed a correlation. Paleozoic gastropods have shell forms that suggest a great diversity of activity levels and I thus infer that dietary preferences were almost as diverse in the Paleozoic gastropods as they are in snails of the modern oceans. The appearance of the Caenogastropoda in the Mesozoic allowed the development of behavioral modes far better adapted than their archaeogastropod counterparts. The relict archaeogastropods, the limpets, turbiniform and haliotiform snails, are taxa where a circular aperture was no great disadvantage.

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MALACOLOGIA, 1978, 17(2): 207-221

THE DEPLOYMENT OF OPERCULATE LAND SNAILS IN RELATION TO SHAPE AND SIZE OF SHELL

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ABSTRACT

Following the discovery of a regular bimodal distribution of shell height *h* versus shell width *d* in taxonomically unrelated terrestrial faunas of Stylommatophora, the land operculate snails of the world are examined. They show the same type of distribution of *h* versus *d* as do the stylommatophorans of the same faunas except in Africa and Madagascar. In the major groups of land operculates, the distribution is clearly different from that of their marine relatives. As with the stylommatophorans, different taxonomic groups of land operculates combine in different ways in different faunas to make up the same type of bimodal distribution. Surprisingly, land archaeogastropods and land mesogastropods seem to make up a single bimodal distribution, not two separate ones. Such reciprocal adjustment of the distributions of subgroups can only be by some type of interference, presumably competition.

Since land operculate and land stylommatophoran distributions of *h* versus *d* overlap widely in most regions, these two major groups are presumably not in competition, although subgroups within them are, but are both subject to the same overall selective pressures determining the typical bimodal distribution. Some consequences of these findings for the interpretation of present-day zoogeographical distributions are pointed out, and the possibility is noted of much more general application of such diagrams (when appropriate measurements have been discovered).

INTRODUCTION

In a previous paper (Cain, 1977) and in current work (Cain, in preparation, a & b), it is shown that the height (*h*) and diameter (*d*) of shells of free-crawling fully retractile gastropods are not distributed at random with respect to each other but show distinct patterns that are characteristic, but by no means invariably so, of major taxonomic groups. In fully terrestrial Stylommatophora a bimodal distribution of *h* versus *d* is normally found, even in faunas taxonomically unrelated, except for certain subfamilies in wet tropical regions (Cain, in preparation, a). Scantily in temperate regions but throughout the tropics, and especially in the Caribbean and in southeast Asia, there exist side by side with the stylommatophorans many species of operculate land snails (prosobranchs), the result of five major and several minor independent invasions of the land. In Europe, within a few feet or inches, species of prosobranchs and stylommatophorans can be found permanently coexisting in what appears to be the same habitat, and apparently in many other regions as well. These prosobranchs form a natural experiment in gastropod terrestrial life, in parallel with the stylommatophorans, and a comparison will show how far they have undergone parallel variation, and how far they retain the characteristic distributions of *h* and *d* of their marine relatives. To a large extent they appear to take on characteristics of the stylommatophorans.

MATERIALS AND METHODS

Measurements of height and diameter were made as described by Cain (1977). They were each made on a single adult shell chosen as representative (when, as usual, choice was possible) of each species currently recognized in the collections of the Academy of Natural Sciences, Philadelphia. Although in groups not recently revised there may be more species than are really warranted, these extensive collections are unlikely to lack any genera or subgenera showing markedly peculiar characters, having been built up over 75 years by H. A. Pilsbry (long the world authority), H. Burrington Baker, and many

others, by collection and by exchange with other authorities all over the world. For the present purposes, the exact numbers of species in particular groups are not important, only the sampling of main shell variations. Indeed, for many parts of the world no existing collection could give authoritative answers on species limits or numbers of existing species. Only a sample can be hoped for in groups so little worked; but as striking variations are readily noticed and collected and as museum material usually over-represents variation within species, it is likely that variation between species has been sampled adequately, if sketchily in some regions.

The choice of a single specimen from a single locality may perhaps reduce the number of symbols on the diagrams, if any wide-ranging species is shown from a single region only. However, the regions taken (southeast Asia, Philippines, Central America, Cuba, etc.) are large enough to have very few species in common, and no perceptible error is likely from this source.

OVERALL VARIATION

The principal land operculates are the archaeogastropod families Helicinidae and Hydrocenidae (superfamily Neritacea), and the mesogastropod superfamily Cyclophoracea and the families Pomatiasidae, and Aciculidae (= Acmidae) (both superfamily Littorinacea). Although the Cyclophoridae have been elevated to a superfamily (Tielecke, 1940) and this rank is accepted by Taylor & Sohl (1962), a number of genera have not been allocated to a family within it. For the purposes of this paper (and with no pronouncement on rank intended), a single family, Cyclophoridae, has been retained within the superfamily. The Chondropomidae are retained as a subfamily of the Pomatiasidae. In various parts of the world particular species or genera have become virtually or wholly terrestrial: for example, the genus *Blanfordia* in Japan and *Pomatiopsis lapidaria* in North America (Rissoacea, Pomatiopsidae; G. M. Davis, personal communication), *Geomelania* in the mountains of the Greater Antilles (Rissoacea, Truncatellidae), and *Cremnoconchus* in the Indian mountain ranges (Littorinacea, Littorinidae). In New Caledonia a few species of neritids are found in very damp places on trees and bushes overhanging water. In the Assimineidae (Rissoacea) the subfamily Omphalotropidinae has terrestrial species from the Mascarene Islands, Japan, and the Marianas to New Zealand. Most of these other invasions of the land are small components of land operculate faunas and are not considered here.

Figs. 1-3 show the overall variation in shell shape and size for each superfamily. The pattern most like any of those previously reported is that of the Cyclophoridae (Fig. 2), which are distributed bimodally very much as is the stylommatophoran fauna of either western Europe or North America (Cain, 1977; figs. 3, 4). The distribution is not at all that seen for mesogastropods in the sea (Cain, 1977: figs. 18, 19, North American species taken as representative) which is a wedge-shaped distribution covering the upper scatter in Fig. 2 but much wider at the higher values of *h* and approaching the bisector (indeed, spilling right across it at values of *d* below

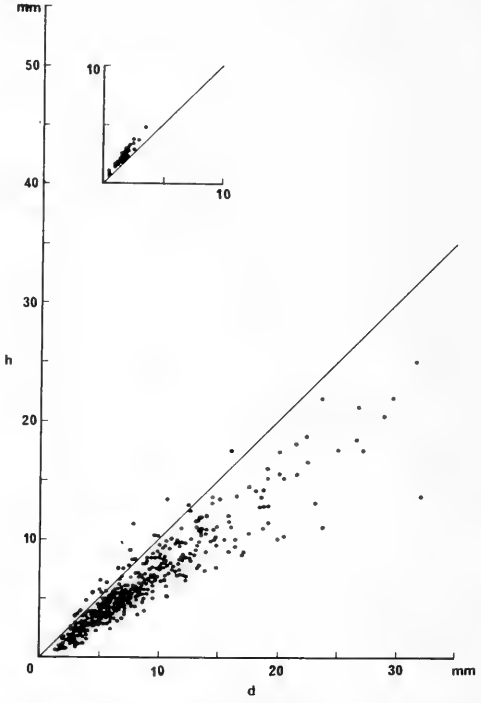

FIG. 1. Variation in shell shape and size in terrestrial archaeogastropods, superfamily Neritacea. Main figure, Helicinidae; inset, Hydrocenidae to same scale. In all figures the vertical axis is the height of the shell *h*, and horizontal, the width of the shell *d*. The line $h = d$ is shown in each to facilitate comparison.

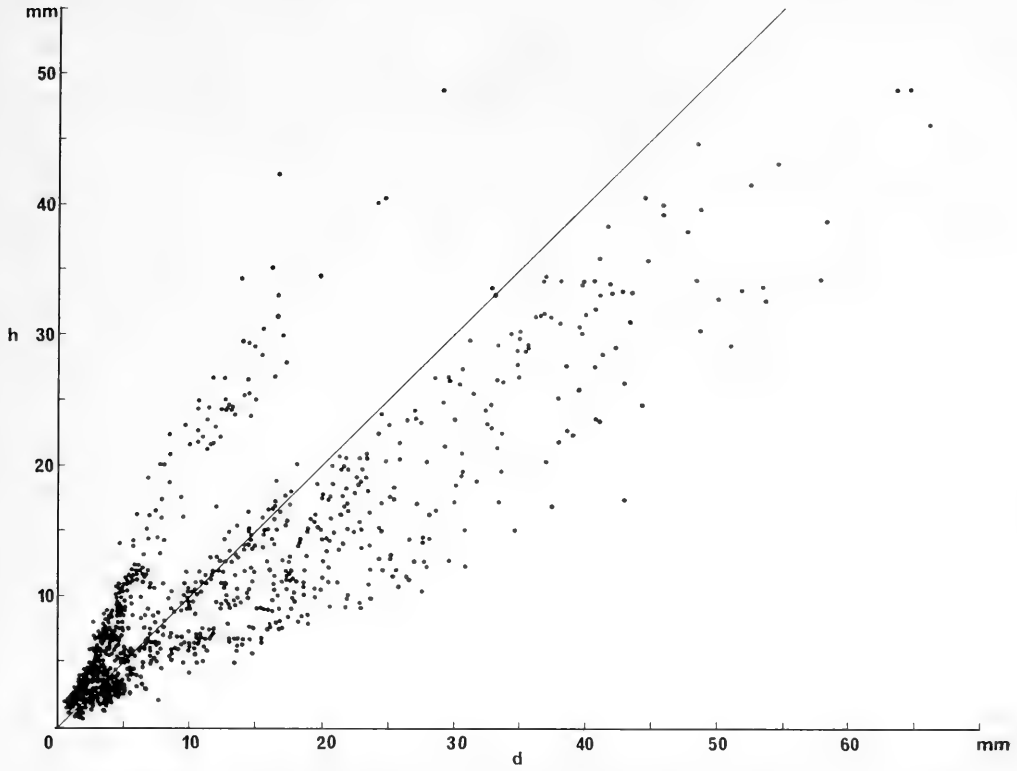

FIG. 2. Variation in shell shape and size in terrestrial mesogastropods, superfamily Cyclophoracea.

20 mm), filling in the gap left in Fig. 2 between the upper scatter and the bisector. Some mesogastropods, therefore, show one type of distribution of *h* and *d* in the sea and a very different type, like that of other terrestrial snails, on land; there is no pattern of distribution characteristic of mesogastropods as such. The archaeogastropod Helicinidae (Fig. 1) correspond to a good part of the lower scatter of the Cyclophoridae; the marine archaeogastropods (Cain, 1977: Fig. 17), however, with very few exceptions, are scattered along the bisector; again, there is a marked difference between the scatters of the marine and the terrestrial representatives of the same major group.

The Pomatiasidae (Fig. 3) show a truly remarkable distribution, reminiscent of the marine mesogastropods, and filling the gap between the upper and lower scatters of the Cyclophoridae; but unlike that of the marine mesogastropods the distribution does not expand upwards, lying instead more or less parallel to the bisector for *d* between 12.5 and 22.5 mm, while at the same values generating a scatter that lies along the bisector and expands more or less equally on either side of it at higher values. This latter is like the marine archaeogastropod distribution (Cain, 1977: fig. 17). The Aciculidae (Fig. 3, inset) fall close to the upper scatter of the Cyclophoridae but, considered by themselves, could be a part of the marine distribution. The Hydrocenidae (Fig. 1, inset), like the Aciculidae, could be considered a part of the marine distribution, or of the terrestrial.

On overall distribution, therefore, in one major family a bimodal distribution highly reminiscent of that of stylommatophorans is found rather than the unimodal distribution of its close relatives in the sea. In two others the distribution is certainly different from that of corresponding marine forms but to a lesser extent, and in two very small and unvarying families no conclusion can be reached.

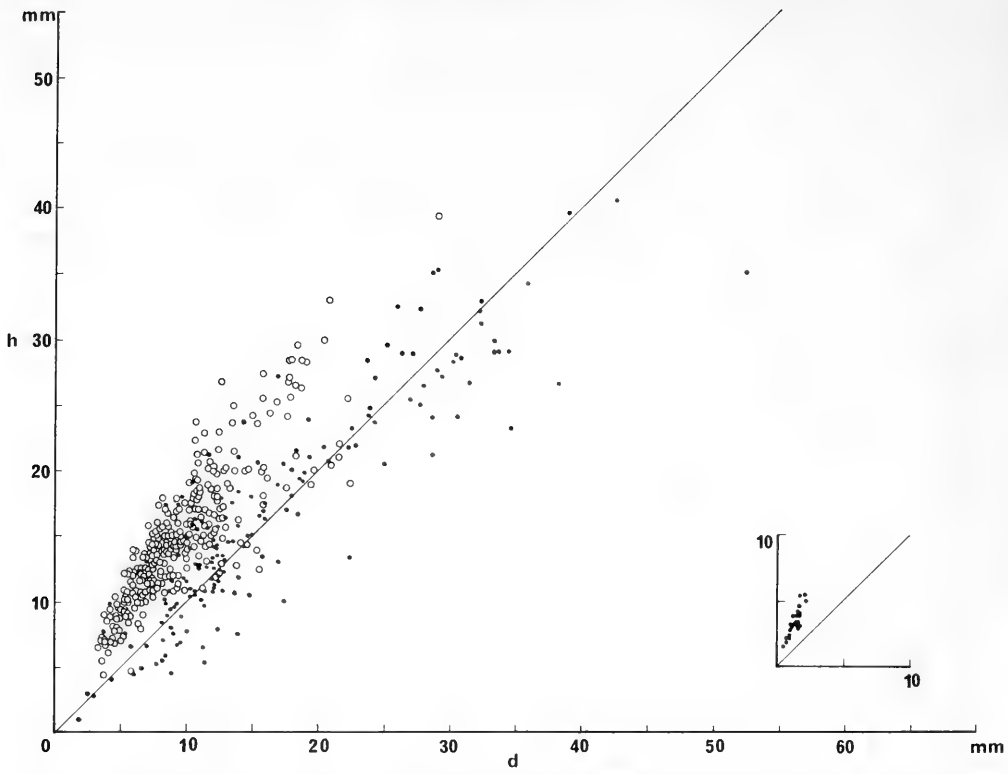

FIG. 3. Variation in shell shape and size in terrestrial mesogastropods, superfamily Littorinacea. Main figure, Pomatiasidae; inset, Aciculidae (= Acmidae) to same scale. Black dots, whole shells; circles, truncated shells, plotted with actual values of *h* after truncation.

GEOGRAPHICAL VARIATION

Deployment of the families

The Helicinidae are found abundantly on the Caribbean islands, less so in the warmer parts of the American continents, and quite separately, often abundantly, in the Philippines, Malay archipelago, Australasia, Polynesia, Micronesia and Hawaii. The Hydrocenidae occur thinly in Dalmatia, some Atlantic islands, South Africa, eastern and southern Asia, islands of the Pacific, and parts of Australasia, including New Zealand.

Of the Cyclophoridae, the Cyclophorinae are widespread from Japan and China through India, southeast Asia, and the Philippines and throughout most of the larger (and many smaller) islands of the Pacific, including New Zealand, with a few species in Africa and islands to the east; one reaches as far westward as the shores of the Caspian Sea. The Hainesiinae comprise a few species in Africa and Madagascar. The Pupininae are also from India, China, and Japan to northern Australia and New Zealand and out into Micronesia and Melanesia. The Alycaeinae reach from India and south China to the Philippines and Greater Sunda isles. The Diplommatinae reach somewhat further east to the Carolines, New Caledonia, and Queensland, but with one genus, *Adelopoma,* in South and Central America. The subfamily Poteriinae is wholly South and Central American and Antillean. Lastly the Craspedopomatinae are confined to Atlantic islands (Azores, Canaries, Madeira—the region sometimes referred to as Macaronesia) and the Cochlostomatinae are southern European and North African, east to the Caucasus.

The Pomatiasidae have one subfamily, Pomatiasinae, predominantly in Africa, Madagascar, Socotra, and the east African islands, with a few species reaching southern and western Europe and the Canaries and east to the Caucasus, and others in

India. The other subfamily, Chondropominae, is exclusively in the warmer parts of the New World, especially in the Antilles. The Aciculidae (= Acmidae, in Thiele, 1931) are wholly Palaearctic, mainly around the Mediterranean and eastward to the Caucasus.

These brief geographical indications show that these families are unequally distributed in different parts of the world. The operculate land fauna varies greatly in taxonomic composition. We must therefore ask what parts of these overall distributions are found together in each major region, and what sorts of distributions are then made up.

Oriental and Australasian

On the Indian subcontinent pomatiasids and helicinids are few; the distribution is made up almost entirely of cyclophorids, and (Fig. 4) both scatters are represented. This distribution corresponds closely with those for western European or North American stylommatophorans (Cain, 1977) and shows the same bimodality. The distribution for North America is similar in all but the degree of extent to high values of the two scatters, the North American upper scatter reaching as high as h = 70 mm. However, the lower scatter seems itself to fall into two groups, a lower departing widely from the bisector and an upper (above about d = 20 mm) running just below it. If such a diagram is made for southeast Asia (Fig. 5), an almost identical picture is obtained, with the same taxonomic arrangement. This again suggests that the lower scatter is itself bipartite, being at medium values of d well below the bisector and at high ones (d more than 20 mm) along and just below it. This bimodality is not apparent in the North American stylommatophoran scatter; there is a hint of it in the western European, which is

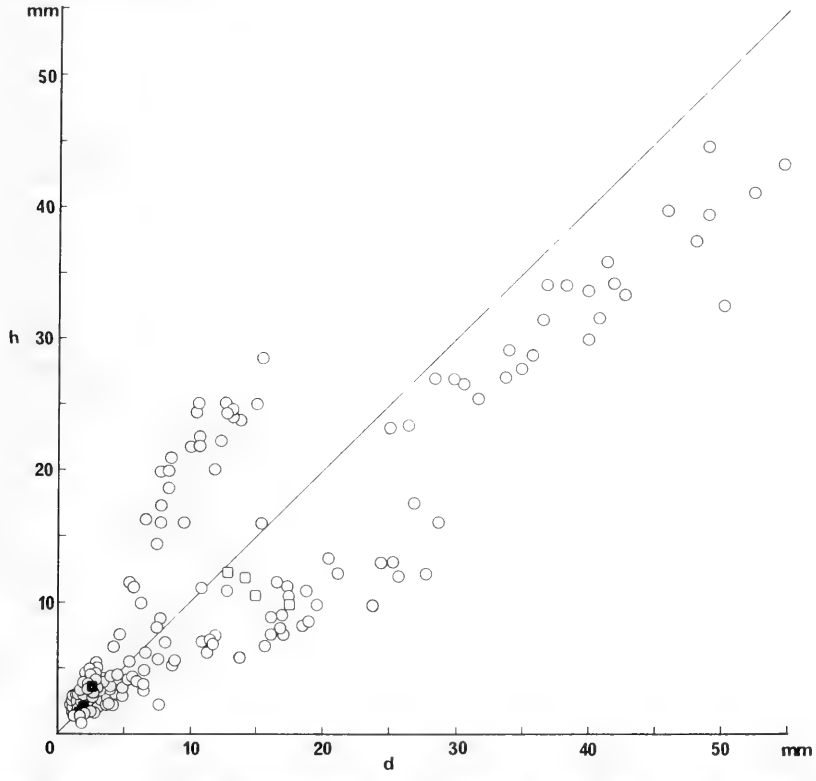

FIG. 4. Land operculates of the Indian subcontinent, including Ceylon and Burma. Symbols on this and following figures: cross, Stylommatophora; stippled triangle, Helicinidae; black square, Hydrocenidae (Old World only); clear circle, Cyclophoracea; clear square, Pomatiasidae; black triangle, Aciculidae (Palaearctic only). In this and subsequent figures many species with small shells (h and d less than 5 mm) are omitted for clarity.

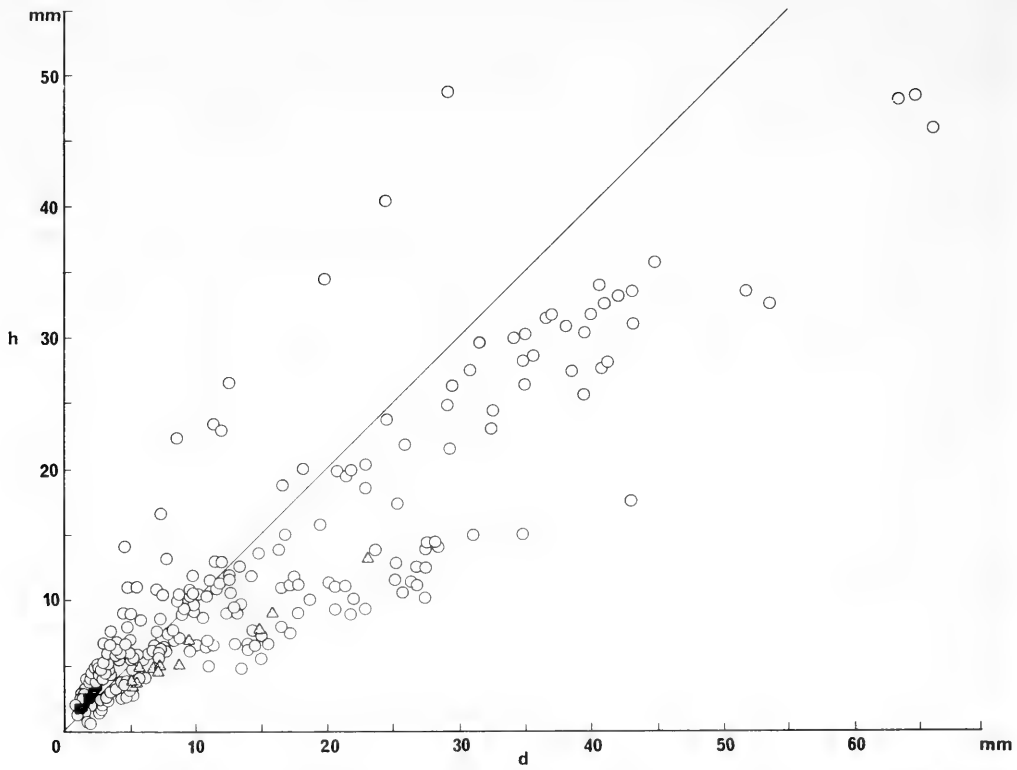

FIG. 5. Land operculates of southeast Asia, including the Malaysian islands, Borneo, Celebes and the Sulu Archipelago, the Andamans and Nicobars, and Hainan. For explanation of symbols, see Fig. 4.

confirmed when the Helicidae of all the Palaearctic are combined (Cain, in preparation, b). The few Indian pomatiasids are in the lower scatter.

In China the upper scatter hardly extends above $h = 15$ and the lower beyond $d = 30$, but in essence they are the same as in the Indian fauna. In the Philippines (Fig. 6) the pattern is again recognizable, but now there are many more helicinids. They tend to occupy the lower part of the lower distribution, which again may be composite. In Australasia (in the wide sense) it is the helicinids almost exclusively which make up the lower distribution below $d = 15$ (Fig. 7). The New Zealand distribution (Fig. 8) has lost the lower scatter altogether, in which it resembles that of Europe (Fig. 14), and consists mainly of cyclophorids.

New World

In striking taxonomic contrast, the land operculates of Cuba (Fig. 9) are mainly pomatiasids and helicinids. Again there are two scatters, but the lower one is mainly helicinid and the upper pomatiasid. The few cyclophorids contribute to the width of the upper part of the upper scatter. A very few helicinids are tall-spired, but they do not move far into the upper scatter. A few very low-spired, almost planorboid pomatiasids are also found. Jamaica shows a similar picture without the cyclophorids. In both, the upper scatter is bent more toward the bisector than in Indian, Philippine, and Australasian faunas, because in the Greater Antilles many species of the upper distribution practice truncation of the shell. If complete specimens of these species could be found, the upper distribution would be like that elsewhere or even steeper. In Central America (Fig. 10) the separation of families is clearer, with the Cyclophoridae at the upper ends of both scatters, Pomatiasidae at the lower end of the upper, and Helicinidae at the lower end of the lower. This separation poses some problems in ecology, which are discussed

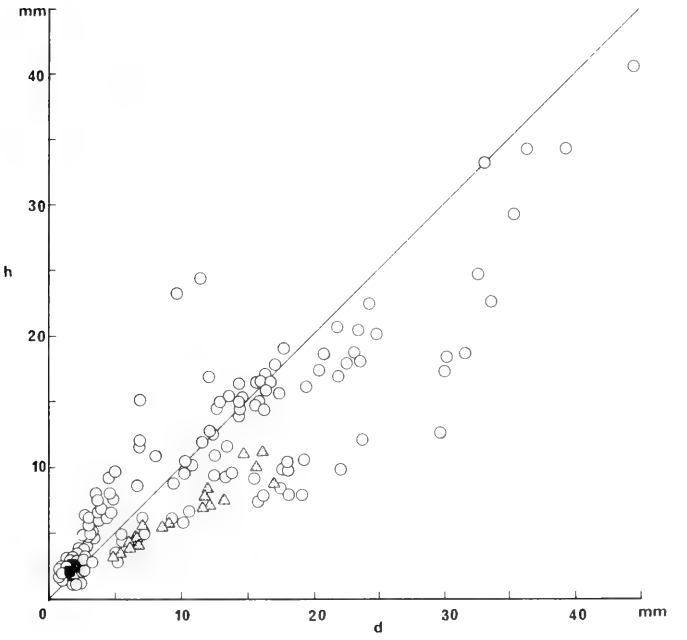

FIG. 6. Land operculates of the Philippines, including Balabac. For explanation of symbols, see Fig. 4.

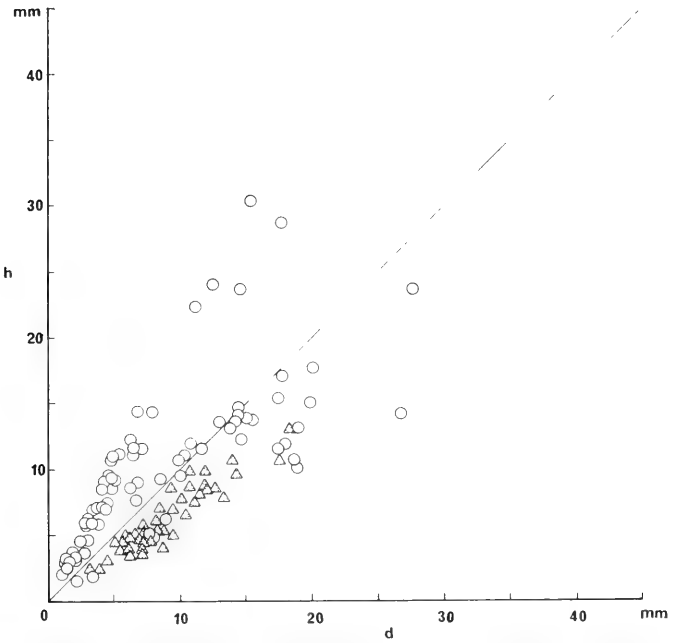

FIG. 7. Land operculates of Australasia (wide sense, excluding the New Caledonian group and New Zealand), from the Moluccas and Bali to Fiji, Samoa, and the New Hebrides. For explanation of symbols, see Fig. 4.

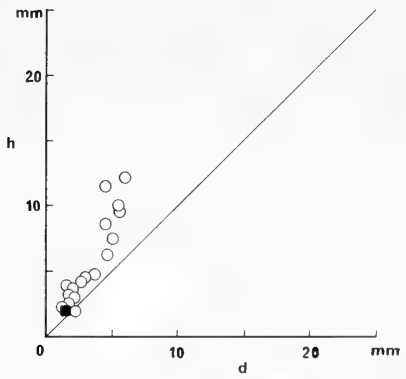

FIG. 8. Land operculates of New Zealand. For explanation of symbols, see Fig. 4.

later. North America has very few land operculates (Cain, 1977: fig. 10); they do not contradict the Antillean distributions. South America agrees well with Central America, with no high-spired pomatiasids.

So far, it appears that land operculates in different parts of the world behave like stylommatophorans in respect to shell size and shape and that much the same two scatters are found anywhere, with the same family filling different parts of the scatters according to its occurrence in different parts of the world. The pomatiasids (Chondropominae) are responsible for most of the upper scatter in the New World, the helicinids for the lower part of the lower scatter; cyclophorids, when present, take the upper part of the lower scatter and occasionally that of the upper. In the Orient and Australasia, helicinids, when present, take the same role as in the New World and cyclophorids fill up the rest, with a few pomatiasids in the lower scatter in India.

Ethiopian region

In Africa (Fig. 11) and Madagascar (Fig. 12) we get a completely different picture. Almost the whole of the pomatiasid distribution that seemed remarkable before (Fig. 3) is concentrated in these regions. Unlike in the rest of the world, there is almost entirely a single scatter, running right up the bisector. If these species were removed from Fig. 3 and notional compensation were made for the truncation of most of the New World species, we would be left with a distribution of pomatiasids very similar to the upper scatter of Indian or Australasian land operculates or of European or North American stylommatophorans but not really like the wide spread

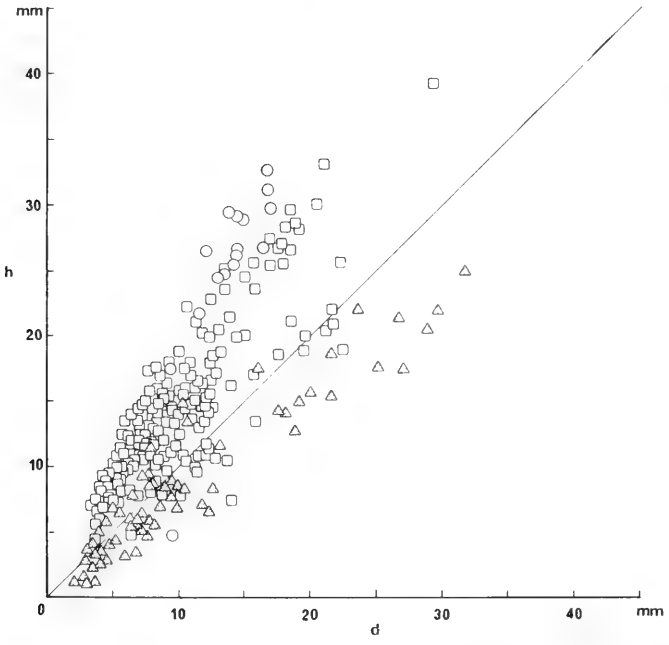

FIG. 9. Land operculates of Cuba and the Isle of Pines. For explanation of symbols, see Fig. 4.

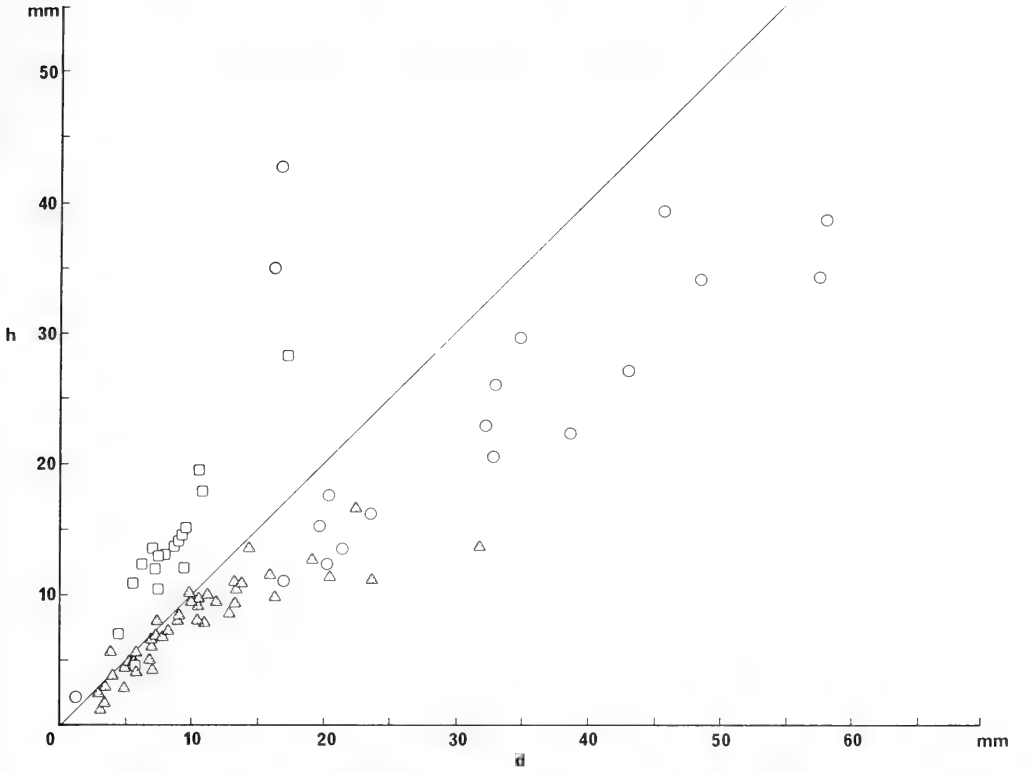

FIG. 10. Land operculates of Central America, from Mexico to Panama, including Swan and Roatan Islands. For explanation of symbols, see Fig. 4.

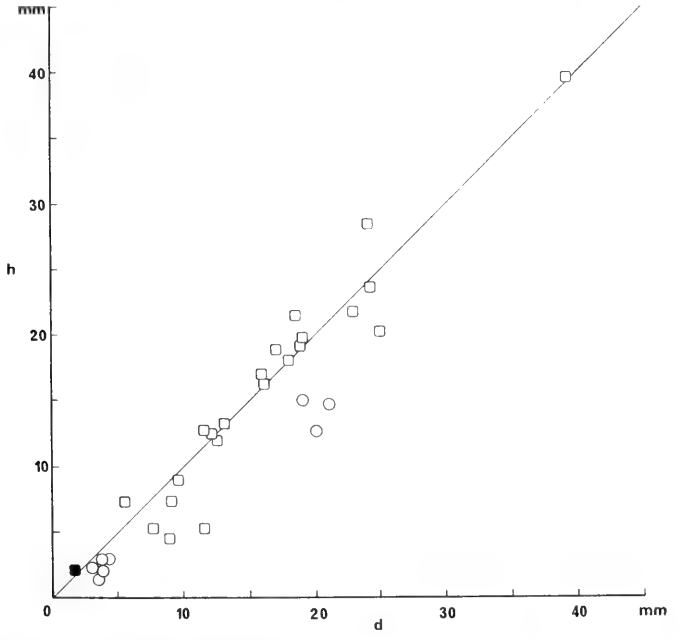

FIG. 11. Land operculates of the Ethiopian region, including Socotra and nearby islands, and the Yemen. For explanation of symbols, see Fig. 4.

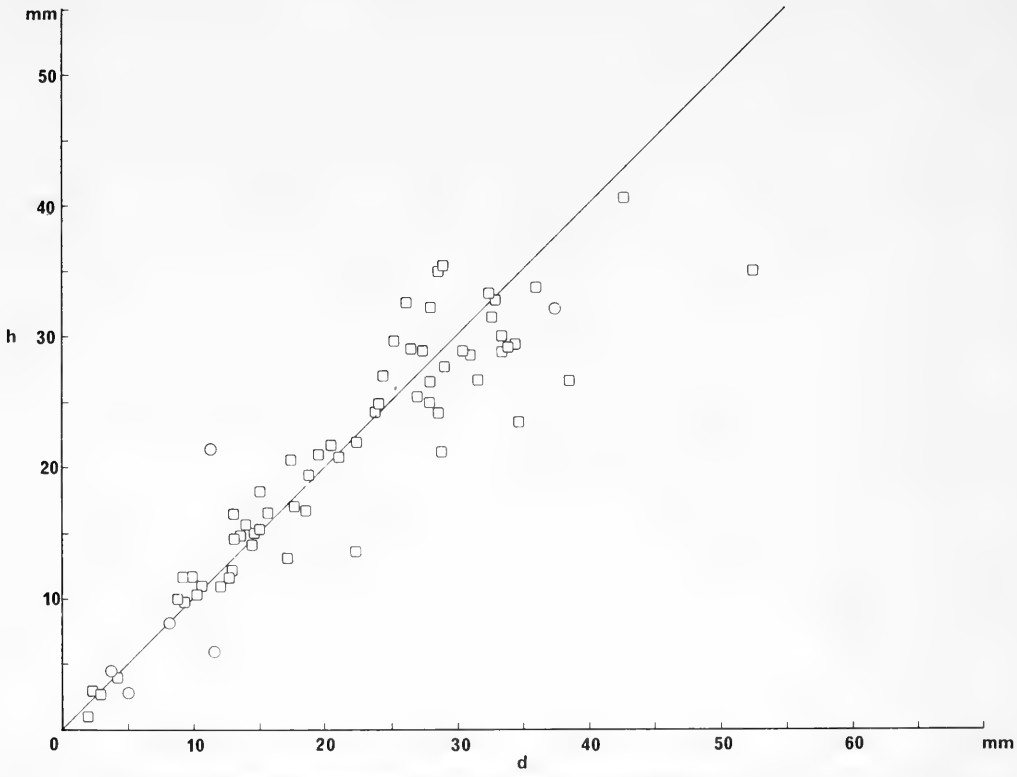

FIG. 12. Land operculates of Madagascar, the Comoros, and the Mascarene Islands. For explanation of symbols, see Fig. 4.

of marine mesogastropods (Cain, 1977: figs. 18, 19). The African and Malagasy distributions resemble, remarkably enough, that of the marine Archaeogastropoda (Cain, 1977: fig. 17). The apparent peculiarities of the Pomatiasidae are therefore partly removed and partly shown to be a phenomenon concentrated in the Ethiopian and related regions. Madagascar appears to have a considerable radiation of pomatiasid types, but unlike in other groups (mammals, birds) it is similar, even generically (Thiele, 1931), to the African and not a special local development. Remarkably enough, a preliminary investigation of the African land stylommatophorans shows no departure from the usual bimodality (Fig. 13).

Palaearctic

In Europe east to the Caucasus and west to the Atlantic islands (Macaronesia), again a different picture is seen (Fig. 14). With one exception (a cyclophorid, *Caspicyclotus,* that reaches northwestward from the main distribution in southeast Asia as far as the Caucasus), all are in the upper scatter. The smallest are the endemic Aciculidae and a few Hydrocenidae. The Craspedopomatinae continue the line of the hydrocenids and are confined to some of the Atlantic islands. On the mainland, the endemic Cochlostomatinae continue upward the line of the Aciculidae. Finally, there is a rather wide scatter of pomatiasids, of genera endemic to the region but related to the African and Madagascan forms. Some of these European Pomatiasinae are rather high-spired, but they are reminiscent of their African and Madagascan relatives. The Cochlostomatinae are remarkably homogeneous in shape and rather high-spired, but they do fit into cyclophorid distributions (upper scatter) in the Old World. The craspedopomatines are close to the bisector, but there are some cyclophorid forms similarly intermediate in position between the two scatters in the Indian and southeast Asian faunas (Figs. 4, 5). Nevertheless, the only picture which corresponds at all well with the European

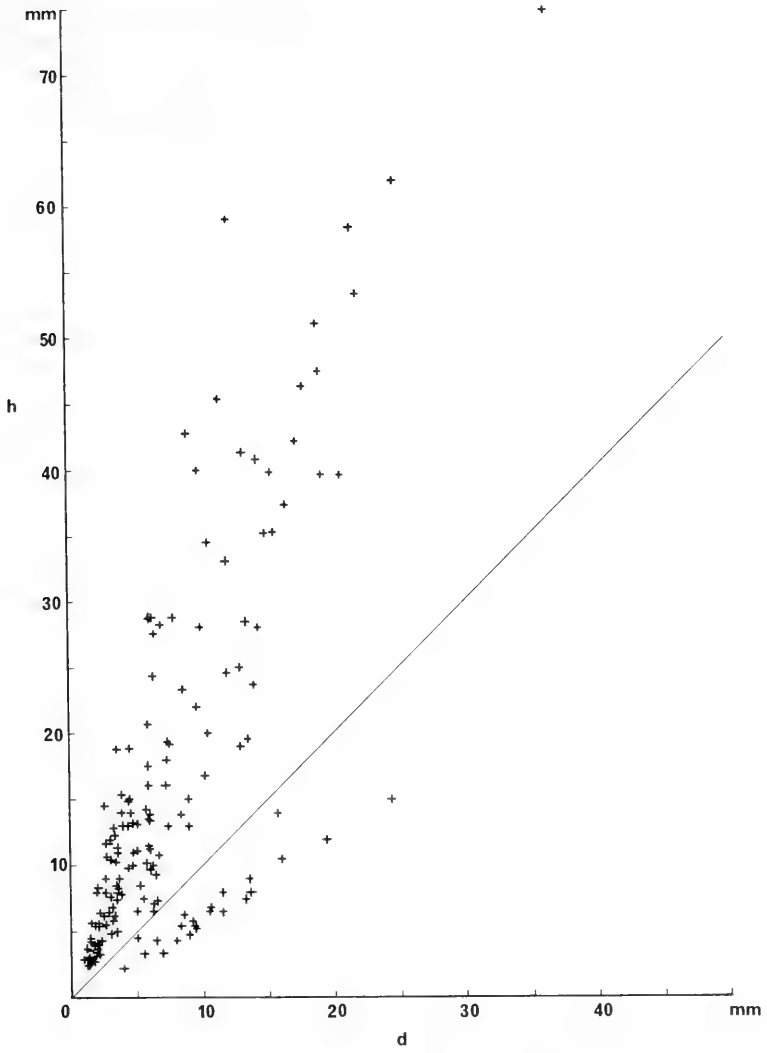

FIG. 13. Land Stylommatophora of the former Belgian Congo (Pilsbry, 1919). Nine forms too large to show on the diagram carry the upper distribution to h = 140, d = 72 mm. For explanation of symbols, see Fig. 4.

is that for New Zealand, the other oceanic-temperate region investigated. The European distribution is certainly in the upper scatter of the Indian, Australasian or (compensated) Antillean faunas, except for the pomatiasines, which are not unlike the African or Madagascan forms but do tend to fill in the gap between the two scatters, although they are not truncated.

Conclusions on distribution

This survey shows that the land operculates, with the possible exception of the Hydrocenidae and Aciculidae and the definite exception of African and Malagasy pomatiasines, conform to patterns of distribution of h versus d that are characteristic of land gastropods generally or of particular regions of the earth, not of their close relatives in the sea. All aciculids and hydrocenids are above the bisector, but wide changes in shape occur in the other three families. Low-spired to planorboid pomatiasids are found in the New World (*Abbottella,* Jamaica; etc.) and Old World (*Cyclotopsis subdiscoidea*, India; *Lithidion forbesianum,* Socotra). High-spired

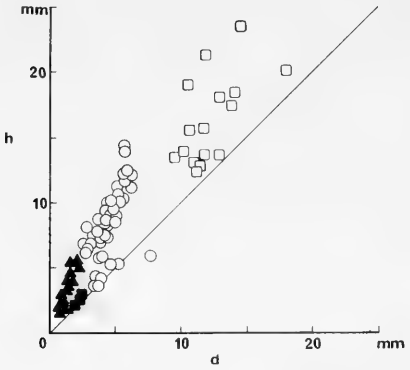

FIG. 14. Land operculates of the Palaearctic region (to the Caucasus) including Macaronesia (the Atlantic Islands). For explanation of symbols, see Fig. 4.

helicinids occur in the New World (*Alcadia (Idesa) charmosyne,* Dominican Republic; *Semitrochatella elongata,* Cuba; *Troschelviana chrysochasma,* Cuba). The tall cyclophorids are poteriines in the New World, cochlostomatines in Europe, various pupinines in southeast Asia and Australasia, hainesiines in Africa, some cyclophorines and local pupinines in New Zealand; depressed ones are cyclophorines and some alycaeines in the Orient and Africa. It is not the case, therefore, that wherever there is occasion for a high-spired (or low-spired) cyclophorid, the same forms taxonomically are found. The filling-in of different areas in the scatters seems to be remarkably ad hoc.

DISCUSSION

Operculate and stylommatophoran scatters

Since in most regions of the earth the operculate land snails and the land stylommatophorans fall into much the same two scatters, filling them up irrespective of taxonomic relationships, it seems that these distributions must be dictated by some very pervasive ecological conditions on land. The largely unimodal distributions shown by the marine archaeogastropods, mesogastropods, and neogastropods are presumably equally dictated by ecological conditions in the sea. It is true that the fully retractile marine euthyneurans (Cain, 1977: fig. 23) show a bimodal distribution very like that of their relatives, the Basommatophora and Stylommatophora, in fresh water and on land. It might be argued that this is an ancestral feature, retained on coming out of the water. But in the first place, species of intermediate characteristics do occur occasionally in various terrestrial stylommatophoran and prosobranch groups, and what is to be explained is not their total absence but their rarity. Secondly, as shown already for the European and North American stylommatophorans (Cain, 1977: figs. 3, 4) and here for land operculates generally, within families, subfamilies, and occasionally even genera, variation between species can occur from the upper to the lower scatter or vice versa. If there is any control from the constitution of the ancestral stock, it is so labile as almost to be a self-contradiction.

Even more important is the way in which both stylommatophoran (Cain, 1977: figs. 6-8, Western European, and fig. 37, a-f, North American) and prosobranch families (this paper) combine to fill up each of their two scatters with little overlapping. The cyclophorids (above, Figs. 4-7, 9, 10) are particularly instructive here. The impression given by the scatter diagrams is that there is a more or less fixed repertoire of both high-spired and medium- to low-spired shapes and sizes allowable in a region and that the available stocks evolve to fill it. Such patterns as these can be brought about only by ecological constraints that determine the extent of available niches and by reciprocal interference, probably competition, between the available stocks that causes them to share out the niches. It would follow, as already suggested (Cain, 1977), that the measurements *h* and *d* estimate properties of the shell which, however indirectly, are themselves estimators of the type of adaptation of the species to filling a particular niche in competition with others. It happens that this can be done for land snails very largely (not completely since there are some overlaps) by a two-dimensional diagram. Raup (1966) has already shown a mutual exclusiveness between major groups of filter-feeding animals with shells, using three dimensions.

Three matters arise from the present diagrams, one predictable on the hypothesis given above, and the other two not. The radulae of land operculates, unlike their shells, do not show much change toward those of stylommatophorans, but resemble

rather those of their marine relatives. If their modes of feeding are different from those of the stylommatophorans, they are probably not in direct competition and can overlap widely with them in *h* and *d*; in competition they would show a mutual exclusiveness. A considerable overlap in *h/d* between the European land prosobranchs, except the pomatiasids and the high-spired stylommatophorans, has already been pointed out. Examination of the Philippine land snails (Fig. 6, and Cain, in preparation, a) shows a strong similarity of operculates and stylommatophorans in the lower scatter, and a partial one at lower values of *h* and *d* in the upper. A rapid check on the Indian and South American land faunas gives similar results. The land operculates, on the whole, are not filling in the gaps between the stylommatophoran scatters but are coinciding with them, as would be expected.

What is unexpected is that the land archaeogastropods should join with the land mesogastropods in filling up their scatters instead of overlapping them. Land archaeogastropod radulae are distinct from those of the mesogastropods. On the same reasoning as that given above, one would not expect effective exclusion in the distributions between these groups. Unfortunately, so little appears to be known of the exact modes of feeding of herbivorous snails in the sea, still less of operculates on land, that only further research can solve the problem.

Equally unexpected is the difference in shape of the overall operculate scatter for Africa and Madagascar. If, as suggested above, this shape defines the repertoire of available niches, then either there is something very different about the Ethiopian region in respect of niches open to land operculates or their relationship with other land snails is different. It is unlikely that a difference in colonization is in question. The African land mass is old, and there has been time in Madagascar for other terrestrial groups to undergo extensive adaptive radiation to occupy available niches. There is no reason to think that the land molluscs have not done so as well. The peculiar distribution of the land operculates on the *h, d* diagram must therefore mean a peculiar distribution of niches. The African land stylommatophorans seem to be orthodoxly bimodal.

A tentative hypothesis

It has been pointed out at various times that tree snails tend to be high-spired, although some that live on large flat leaves may be depressed. Although this idea needs detailed confirmation in regions where there are plenty of tree snails, it may point to a provisional hypothesis of the scatters found. Francis Bacon remarked that we can learn from error, but not from confusion; the disproof of a definite hypothesis may add considerable knowledge. If high-spired shells are usually of species that feed more or less vertically on rock faces and in trees, and low-spired ones are usually of species feeding on the ground or on horizontal surfaces, intermediate-shaped ones are presumably dual-purpose species. Since land prosobranchs and stylommatophorans overlap extensively in their distributions of *h* versus *d* within a fauna, presumably they are living in the same places and so are subject to the same selective pressures with respect to *h* and *d*—on the present hypothesis both groups are feeding on the same variety of surfaces. But they must be doing something different to be able (a) to coexist each with the other major group and (b) to interfere with their relatives within each group in order to fill out the expected distribution. Although many factors must be acting, it seems likely that modes of feeding are the clue to both (a) and (b). The differences in radular teeth and jaws between prosobranchs and stylommatophorans, while permitting a considerable overlap of diet during temporary superabundances of particular foods, may allow the herbivorous stylommatophorans to concentrate more on cutting and less on scraping and the herbivorous prosobranchs, on the contrary, to scrape more than cut. (In particular circumstances, no doubt, there will be convergence, where the ecological situation allows, in this as well.) It might be thought that prosobranchs, considering their general habits on the shore and their direct invasion of the land many times over, might rely principally on fine turves of algae or other plants less relied on by shelled prosobranchs.

Terrestrial algae are world-wide. They have even been isolated from the pavement outside the Marks & Spencer store in Liverpool (Dr. John Eaton, personal communication). But in the British Isles they are

sporadic and evanescent, quite unsuitable for any species to specialize on during the whole of its active season. In warmer and wetter countries they are more abundant and more permanent, as are other epiphytes and surface plants (yeasts, bryophytes, etc.). If there is a feeding distinction between land operculates and land stylommatophorans of the nature suggested, it is reasonable that land operculates should be more abundant in warmer countries. Where it is wet and warm, crops of algae can be more or less permanent or can develop more quickly or persist for longer when the wet season arrives and snails come out of aestivation. The greater abundance of broad-leaved evergreen trees provides additional niches not seen in colder regions. Of the two scatters of *h* versus *d*, the upper one only reaches to high values of *h* in warmer countries. Almost all the large high-spired forms in the North American stylommatophoran fauna (Cain, 1977: fig. 4) are southern; high-spired forms in Europe are predominantly southern in both stylommatophorans and land operculates; and it is the high-spired forms that are cut back as one moves north into China from southeast Asia. In the far north, low-spired stylommatophorans are also cut back to small values in both North America and Eurasia (Cain, in preparation, b). Conversely, in tropical forest in both the Philippines and New Guinea, intermediate stylommatophorans become conspicuous in addition to those of the normal two scatters, as though intermediate habitats (or habits) are opened up (Cain, in preparation, a). It is possible that a large amount of the peculiarities of distribution of present-day snails really reflects the present distribution of meteorological factors in relation to their appropriate food crops.

Interpretation of geographical distribution

If this is so, a further point should be made. The restriction of helicinids virtually to the Caribbean and adjacent countries and the larger islands of the western Pacific is often quoted as a classic example of a relict distribution (e.g. Solem, 1959: 293). So, no doubt, it is; but if these are regions, both highly maritime, with a dampness of air and relative constancy of temperature that allows for better maintenance of standing crops of algae and epiphytes, there may be an excellent ecological reason for helicinids persisting in these disjunct regions, and even an excellent reason for retaining an archaeogastropod radula in those snails with small low-spired shells, as against a mesogastropod one. Similar considerations apply to the cyclophorids and pomatiasids.

We need to distinguish two different meanings of the word primitive. Early members of a particular stock may be primitive both because they are early and because they are attempting to fulfil the same ecological role as their descendants but are not yet specialized for it; if it were possible to put them in competition with their descendants, they would be unsuccessful. But forms may appear early in the fossil record, rapidly adapt as well as possible to their ecological roles, and then remain constant simply because they are still fulfilling the same role and the ecological opportunity for it is still available. They are not then imperfectly specialized for it, although they remain persistently primitive. There are various statements in the literature that, for example, the more primitive endodontid snails (Stylommatophora) have a relict distribution in the mid-Pacific islands, being pushed out elsewhere by more advanced forms. But if they are specialized for the sort of environment now to be found only in such islands, they may be holding their own successfully against invasions by other forms and actually excluding the so-called more advanced forms. In that case, what we have is a peripheral distribution at present of a particular sort of environment and not at all a historical situation, with more primitive forms slowly being eliminated by competition from others more specialized for the same ecological role. Without much further evidence it is dangerous to argue from an observed distribution to historical changes. Of course, one must consider only habitats in equilibrium—the severe damage often caused by man may well allow all sorts of introduced weed species to establish themselves successfully.

One should be very cautious, therefore, in trying to explain distributions like these as purely historical or due to the slowness of evolution (implying that primitive forms are still slowly adapting). The resemblance of the *h* versus *d* scatter diagrams for such different faunas implies that the groups are in some sort of equilibrium within a fauna

and that their characteristics (of shell size and shape) are dictated by present ecological conditions.

Use of diagrams

The use of diagrams such as those shown in this paper can be extended to other groups of animals when one discovers the necessary measurements. The form of the scatter, if repeated in unrelated parts of the world, gives the repertoire of available niches; the mutual exclusion of taxonomically defined scatters shows which taxonomic groups are apparently liable to interaction. Obviously, one could measure height and diameter of elephants and sunbirds, say, and get well-separated scatters on the same diagram; but wide separation does not indicate interaction, only contiguity combined with the sort of role changing within the same framework seen in Figs. 4, 7 and 9. Forms that overlap are not interfering, with respect to the characters measured (or rather, to their consequences and concomitants). Those that exclude each other show the results of interference, probably of competition. In the example worked out in this paper, land operculates and land stylommatophorans appear to be free from interference from each other but members of each group compete with others in respect of characters associated with shell shape and size; at the same time, both groups, being equally terrestrial, come under the same general ecological constraints and show the same overall pattern—except in Africa and Madagascar. When a pattern for a particular group is clearly recognized in several well-known faunas, it may be possible to use it to reconstruct the diversity in a badly known one. The overall scatter should be the same, and some idea of the extent of our ignorance will be given by the area within the expected scatter which is at present vacant. This method could be applied, for example, to the contents of poorly fossiliferous strata or to undercollected or recently devastated faunas.

Rensch (1959: 119) has already pointed to the relative constancy of percentage of small-sized shells in leaf litter land snail faunas of very different taxonomic composition. He thinks, however, that the considerable variation in percentage of medium-sized and large land snails in the same forest faunas shows that "there is hardly any serious competition among the individuals and the species," which is not likely. An examination of detailed diagrams of the sort used in the present paper would be necessary to see whether there is evidence of reciprocal interference in the first place.

ACKNOWLEDGMENTS

I am indebted for criticism and advice to Professors J. D. Currey and M. H. Williamson; Drs. G. M. Davis, J. Eaton, E. Hoagland, and R. Robertson; and Mr. R. W. Cowie.

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MALACOLOGIA, 1978, 17(2): 223-239

EVOLUTION AND ADAPTIVE RADIATION OF *CERION*: A REMARKABLY DIVERSE GROUP OF WEST INDIAN LAND SNAILS[1,2]

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ABSTRACT

The pulmonates of the genus *Cerion* are remarkable for the extreme variability of their shells. The vast array of shell types present in Cuba and the Bahamas, coupled with past typological taxonomic practices, has resulted in the naming of over 600 "species." The complex patterns of geographic variation are so confusing that several workers have despaired of the possibility of ever applying the biological species concept to this group. The allegedly haphazard distribution of fossil and living shell types has been attributed to the vagaries of hurricane dispersal. Stephen Jay Gould, of Harvard University, and I have been collaborating on a holistic study of the geographic variation, ecology, and evolution of these remarkable snails. We have begun to make sense of this extraordinary situation by detailed mapping of geographic variation in the field and by laboratory studies of multivariate morphometrics and biochemical genetics. So far we have found that the 200 "species" of the northern Bahamas reduce to a single pair of contrasting morphotypes: phenetic and genetic variation is continuous rather than capricious. Furthermore, while hurricanes may have played a role in establishing some distribution patterns, their importance appears to have been overestimated. In the northern Bahamas, we can discern an overall biogeographic pattern that appears to have evolved in situ during the late Cenozoic. As a result of these studies and others involving *Cerion* from elsewhere in the Bahamas, Cuba, Hispaniola, Puerto Rico, the Dutch Leeward Islands, and the Florida Keys, we anticipate a downward revision of close to two orders of magnitude in the number of valid species.

Despite the enormous taxonomic literature on *Cerion*, we know remarkably little about its natural history or the adaptive significance of the variation in shell morphology. It was widely held that the snails are obligate halophiles, that they are restricted to a zone close to the shore, and that their abundance (up to 212 per square meter) stems from a lack of predation. Field observation over the last few years has shown that these beliefs are unfounded. Long-term ecological studies in the northern Bahamas have been initiated in an attempt to generate some basic information on population size, dispersion, dispersal, growth rates, predation, and mortality. The activities of over 1,500 individually marked snails have been followed periodically since November, 1973. Laboratory studies on the mechanical strength and thermal properties of the different shell types are also being conducted to try to establish the adaptive significance of the observed morphological variation. The results of these studies will be presented, and, in addition, attention will be drawn to a number of other outstanding problems (including *Cerion*'s ability to estivate for protracted periods) presented by these animals.

In a broader context, *Cerion* will be shown to display one of nature's most impressive displays of morphological variety. While this diversity has been acquired without widespread speciation or extensive genetic differentiation, the group provides prime material for studies of the genesis and maintenance of morphological complexity.

INTRODUCTION

"Those forms which possess in some considerable degree the character of species, but which are so closely similar to some other forms, or are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us." (Darwin, 1859: 47).

"A genus in which many such situations seem to occur is the

[1]This review is No. 10 in a series on The Natural History of *Cerion*. It is respectfully dedicated to William J. Clench and Ruth D. Turner who first introduced the author to *Cerion*.

[2]This research was supported, in part, by grants from the U.S. National Science Foundation to S. J. Gould and the author.

> snail genus *Cerion,* considered "the most difficult genus of pulmonate mollusks to classify" (W. J. Clench, in litt.)" (Mayr & Rosen, 1956: 1).

The West Indian pulmonate *Cerion* is celebrated in the evolutionary literature for its phenetic diversity. Intrapopulation variation is rarely extraordinary in extent, but shells of each local population develop distinctive features, and an astonishing range of forms is found in certain parts of Cuba and the southern Bahamas. Differences in shell size, shape, sculpture and coloration have been used to characterize almost 600 species and the evolutionary importance of these remarkable animals has been buried under an all but impenetrable taxonomic thicket. This is to be regretted as recent studies show *Cerion* to be prime material for the examination of a number of important biological problems including the relationship between growth and form, the maintenance of genetic and phenetic variation, the genodynamics of hybrid zones and the processes of geographic speciation, and the evolutionary biogeography of species distributions.

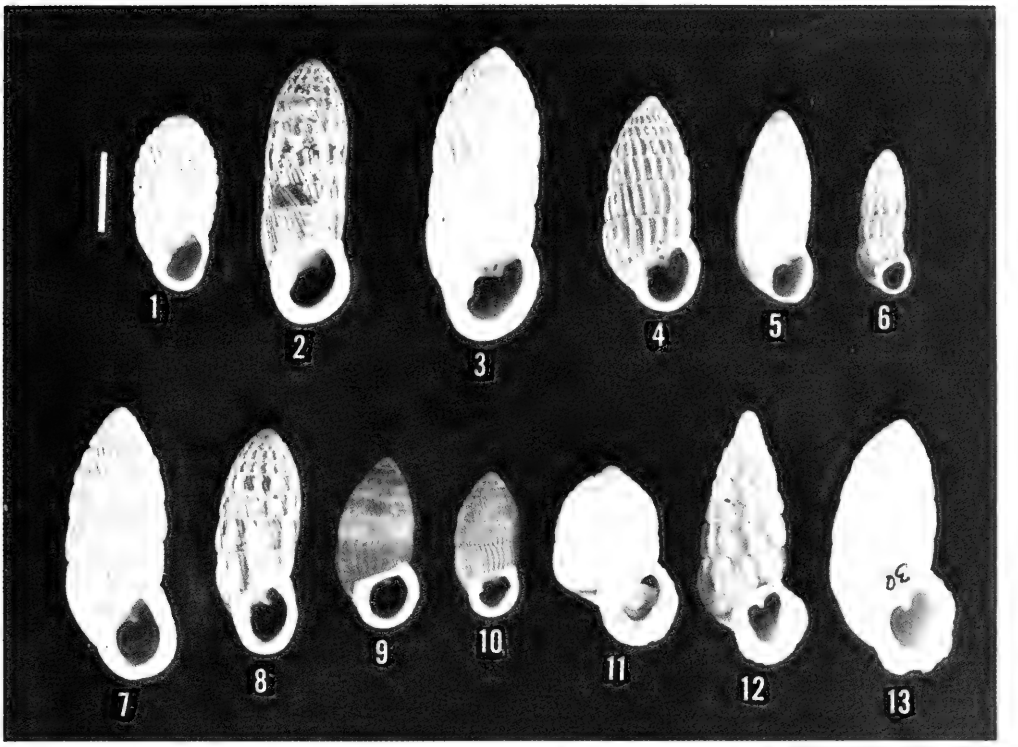
In this paper I review the results of a decade's work on *Cerion* by Stephen Jay Gould and myself. Gould, a paleontologist and biometrist, was attracted by *Cerion*'s basic diversity, by its good fossil record in the Pleistocene dunes, and by the propensity of most individuals to display their entire ontogeny in an accessible shell. In contrast, I initially approached *Cerion* because of its abundance and distribution pattern, characteristics that made it suitable for an experimental study of the relationship between the ecological genetics of natural populations and distribution. Our collaboration quickly transcended these personal interests and many of the studies reported below should be attributed to both workers rather than the author alone. While we have learned a great deal about *Cerion* only a small fraction of our findings have yet been reported. Accordingly, I will describe some of our on-going projects, both to report on our progress (or lack of it) and to indicate problems to which other investigators may hopefully be attracted. This paper, then, constitutes no more than a prolegomenon to the evolution and adaptive radiation of these remarkable animals.

NATURAL HISTORY

Snails of the genus *Cerion* Röding are diverse and conspicuous members of the West Indian land snail fauna. They are found in the Florida Keys, Bahamas, Cuba, Cayman Islands, Hispaniola, Puerto Rico, Virgin Islands and the Dutch Leeward Islands. They are generally restricted to coastal areas where they are typically found along a narrow strip of open vegetation within 100 m of the shore. Cerions are abundant in their preferred habitat, often reaching densities of 10 adults/m^2 and attaining colony sizes of 10^4 individuals. While the vast majority of cerions are typically found hanging in the plants, to which they seal themselves with a thin epiphragm, a few species live in the leaf litter and sand. Very little is known about their ecology and behavior beyond the casual observation that they spend the greater portion of their adult lives in a state of estivation. Inactive during the day, they are thought to emerge and feed at night, when these areas are actively tenanted by mosquitos and sandflies, and malacologists have retired from the scene.

The most remarkable thing about *Cerion* is, of course, the great variation in shell morphology (Figs. 1-13). It has become apparent that nearly every local population has a diagnostic size, shape, sculpture and color. Most of the published work on *Cerion* is devoted to partitioning this variation among nearly 600 species. (Mercifully, Clench (1957) has prepared an authoritative catalogue of this vast taxonomic literature.) Plotting the locality records for these taxa produces a crazy-quilt distribution pattern for many species (see Gould & Woodruff, 1978: fig. 1). There is marked geographic variation with adjacent populations being radically different from one another. Several species found on Long Island in the Bahamas are shown in Figs. 7-13; *C. stevensoni* and *C. fernandina* occur within 200 m of one another. Elsewhere in the Bahamas and Cuba different species replace one another in irregular fashion along the coasts.

This unusual pattern of shell-type (morphotype) distribution is probably real; *Cerion* is an extremely well-collected group. The record does, however, embody two erroneous factors that must be noted at the outset. First, I can substantiate no case of sympatry between any of the species re-



FIGS. 1-13. The phenetic diversity of *Cerion* displayed by selecting average specimens of taxa discussed in the text. The bar represents 1 cm. 1, *C. uva,* Curaçao; 2, *C. bendalli,* Little Abaco, Bahamas; 3, *C. abacoense,* Great Abaco, Bahamas; 4, *C. glans,* New Providence, Bahamas; 5, *C. incanum,* Big Pine Key, Florida; 6, *C. pauli,* Great Exuma, Bahamas. Bottom row, all from Long Island, Bahamas; 7, *C. caerulescens* 8, *C. eximeum*; 9, *C. nudum*; 10, *C.* sp. indet. (? dwarf *caerulescens*); 11, *C. malonei*; 12, *C. (Umbonis) stevensoni*; 13, *C. fernandina.*

ported by earlier workers to coexist. Mayr's (1963: 398) report for eastern Cuba is compromised by the presence of clear intermediates in his sample. Other alleged cases arise from the custom of some earlier conchologists to artificially sort their samples into dissimilar types without regard for the natural intrapopulational variation. Still another source of confusion comes when a sample contains both living and dead specimens. Crabs and various forces of nature carry dead shells vast distances and result in heterogeneous populations of shells. Errors of this type must be borne in mind when examining the literature and older museum collections. The second type of error in the record arises, in part, from the typological systematics practiced by the early *Cerion* specialists. Dissimilar forms were named as separate species without any regard for their interactions in the field. The truth of the matter is that wherever distinct populations come together in nature they appear to be able to interbreed. Thus, while the apparently haphazard distribution of morphotypes in some areas is real, the pattern is the result of a mosaic of contiguously distributed morphotypes rather than the coexistence of different species in different combinations. Until the taxonomic status of these morphotypes is reestablished we can say almost nothing about the specific diversity of different islands or the genus as a whole.

The so-called species of *Cerion* are characterized exclusively on the basis of shell characters. Four subgenera and 15 species groups have been proposed (Pilsbry, 1901-1902) on the basis of features which may or may not have phylogenetic significance. Two subgenera contain single species: *Eostrophia,* for *C. anodonta* from Oligocene deposits in Florida, and *Cerion,* for the type-species *C. uva* from Curaçao.

The name *cerion,* derived from the Greek word for a honey-comb, alludes to the resemblance of *C. uva* to an old-fashioned bee-hive (Fig. 1). The vast majority of living cerions are referred to the subgenera *Strophiops* and *Diacerion* depending on the form of the axial and parietal teeth in the aperture of the shell. However, as Pilsbry himself noted (1901-1902: 179), the distinction between these groups breaks down in some forms. The remaining subgenus, *Umbonis,* is the only species group to have received any recent attention (Clench & Aguayo, 1952). The shells of this group are quite characteristic in their shape, in having spiral sculpture of numerous incised lines, and in agglutinating small sand grains to the outside of the shell. The group includes *C. stevensoni* from the northeast coast of Long Island (Fig. 12) and 13 other species from the Bahamas and Cuba. A dwarf form, *C. turnerae,* occurs on Great Inagua and reaches a length of 15 mm (see Gould et al., 1974: fig. 1).

The anatomy of several species of *Cerion* has been studied; Richter (1926) and Jaenicke (1933) did some careful work on *C. uva* from Curaçao and *C. glans* from New Providence in the Bahamas. Unfortunately, it is difficult to assess the alleged differences in the digestive, nervous and reproductive systems until comparable studies have been made to establish the extent of intraspecific variation due to the age, behavior and physiological state of the snail. Dissections of some other species (including *C. incanum* from Florida) are figured by Bartsch (1920) and Pilsbry (1946), but there are numerous points of disagreement and the work will have to be repeated.

Burch & Kim (1962) have described the karyotype of *C. incanum*: the diploid number is 54. Twenty-seven pairs of chromosomes have also been found recently in *C. fernandina* by Michael Goldman in my laboratory.

Studies of genetic variation of *Cerion* have added a new dimension to our understanding of the evolution and adaptive radiation of these snails (Woodruff, 1975a). Using electrophoretic techniques (Woodruff, 1975b) I have examined variation in more than 20 enzyme systems in several thousand snails from various parts of their range. I have identified more than 30 allozymes or structural gene products and begun to study their distribution in the various morphotypes (Gould et al., 1974; Woodruff, 1975b; Gould & Woodruff, 1978; Woodruff & Gould, in prep.). An analysis of the observed genotype frequencies in each sample revealed that *Cerion,* a facultative hermaphrodite, is apparently outbreeding. *Cerion* populations (large and small, but with one notable exception reported below) studied to date have moderate amounts of genetic variability: mean number of alleles per locus, 1.65-1.70; frequency of loci polymorphic per population, 0.15-0.30; and frequency of heterozygous loci per individual, 0.054-0.128. The systematic and evolutionary significance of these findings will be discussed in the second half of this review.

As noted previously there were virtually no quantitative data available on the ecology of *Cerion.* Accordingly, in November, 1973, I established two study sites on Great Abaco Island in the northern Bahamas. The sites were chosen to characterize high density populations of two morphotypes found throughout the Bahamas. One area (Rocky Point) is inhabited by *C. bendalli* which has a thin shell which is smooth or finely ribbed and mottled with brown and black (Fig. 2). The other area (Shipwreck) is inhabited by *C. abacoense*, a species with a heavy white shell with prominent ribs (Fig. 3). All snails at these study sites have been individually marked, in situ, a process which has a very slight effect on dispersal but no discernible effect on growth rates and survival. The populations have been censused repeatedly over a four year period and data are now in hand for over 1600 snails. As the data analyses are continuing much of what is reported here should be regarded as preliminary.

At the start of these studies snail density at Rocky Point averaged 13 adults per m^2 (range, 0-79). Similar densities occurred at Shipwreck. Typically over 90% of the adults at both sites are found off the ground attached to stems and leaves. They show a strong preference for certain plant species like the lily, *Hymenocallis declinata.* The juveniles (typically 10-20% of the population) are found on the soil surface beneath the shallow leaf litter or at the base of clumps of grass or bushes. Mark-release-multiple recapture studies indicate that the neighborhood area is less than 100 m^2 and that the effective population size (N of Wright, 1946) is approximately 1000 snails.

Cerions appear to spend the greater part of their adult lives attached to the vegetation in a state of estivation. They are typically encountered sealed (aperture up) by a thin epiphragm to the stems and branches of low bushes or the leaves of monocots. How long they remain in estivation in nature is unknown and probably varies between individuals in response to spatial and temporal changes in the microenvironment. In the laboratory I find that the larger species will estivate for over 24 months if undisturbed. In the field, adults are found moving about at night and during the day following rain. They have been observed both moving among the leaves of plants and on the ground surface. Diet is still poorly defined. Contrary to Bartsch's (1920: 6) statement that adults feed on fungal mycelia, other investigators have found vascular plant remains in the feces (Weston, in Mayr & Rosen, 1956; and June Chatfield, National Museum of Wales, *in litt.*). While cerions do not damage the plants with which they are associated they may graze on decaying plant material.

Cerions at Rocky Point and Shipwreck are highly sedentary. Some snails have remained on the same plant for 2-3 years. With the assistance of Annette Adams in my laboratory I have devoted considerable attention to dispersal behavior as it is essential that we be able to estimate the magnitude of gene flow if we are ever to understand the microevolution of these animals. As expected, adult dispersal patterns are leptokurtic. In the first six months (winter) of my study at Rocky Point the mean detected displacement was 107.7 cm (range, 0-1202 cm, N = 109). In the following six-month period (summer) the mean displacement was 215.7 cm (range 0-2200 cm, N = 221). In each census period a significant number of adults (11-16%) were recaptured on the plant where they were first encountered. Using Batschelet's (unpublished) ellipse of inertia technique to compare dispersal in successive census periods I concluded that the adults move more during the wetter summer months than during the winter. Dispersal is not random with respect to direction; it is clearly constrained by the size and shape of the habitat patch and by the distribution of plants within the patch. At Rocky Point, for example, the study site lies about 30 m inland from the high water mark and the snails are distributed more or less continuously in the open vegetation that parallels the beach. The variance of dispersal along an axis parallel with the beach follows a pattern that we might expect for snails making random movements on a large habitat patch. (The variance for the first 12 month period is greater than that for either component 6 month period, although it is not the sum of the two, as would be expected for completely random movements.) The movement of snails with respect to an axis at right angles to the shore shows a completely different pattern: the 1 year variance is actually less than that for either 6 month period. This indicates that the snails perceive the vegetation change along the beach front (and further inland) and move back into the habitat patch.

The statistical interpretation of these data is difficult because several of the parameters are not normally distributed. However, if we make some simplifying (and reasonable) assumptions, it is possible to estimate some very important demographic parameters. If one assumes that generation time at Rocky Point is 5 years (see below) then I estimate the mean distance displaced per generation to be 200 cm (range 100-400 cm). The evolutionarily important gene flow distance may then be estimated as the product of the mean distance travelled in a generation and the square root of the probability of leaving a deme or neighborhood (May et al., 1975). For a high density population of *C. bendalli* at Rocky Point my preliminary estimate for this parameter is 2.8 m. The significance of this low value will be discussed later in the context of the maintenance of clines and narrow hybrid zones.

Given its moderate size, *Cerion*'s abundance is remarkable; in its favored habitats it is far commoner than *Helix, Cepaea* and other land snails that have been subjected to ecological scrutiny. In our field work we have come to designate *Cerion* as being locally rare, uncommon, common and very common at average densities of 0.1, 1.0, 10 and 100 adults per m^2, respectively. It is not unusual to find aggregations of living snails numbering between 100 and 150 individuals on a single bush. In this respect it is not unlike the Mediterranean land snail, *Theba pisana.* Density of dead shells can be even higher (up to 245 per m^2), but this is misleading indicator of natural density and dispersion. The shells accumulate

on the ground surface and are resistant to decomposition. In some areas of the Bahamas these dead shells carpet the ground surface at densities ten times that of the living snails. In their favored habitat cerions are about ten times as abundant as the next most common land snail; usually a member of the genus *Hemitrochus (Cepolis).*

Cerion's abundance has traditionally been attributed to a lack of predation; fires and hurricanes being generally recognized as the main factors determining a colony's success. The common occurrence of areas with vast numbers of dead snails but very few living snails is cited as evidence for the significant role of natural disasters. Undoubtedly, natural and man-made brush fires have a devastating effect on *Cerion.* The impact of a hurricane has yet to be formally established (fortunately my study sites have yet to be perturbed in this manner). Again, the traditional wisdom is that the colony is destroyed and a few fortunate, fertilized individuals survive to colonize new habitats into which they are introduced by the wind or water. *Cerion*'s ability to survive immersion in sea water has been described by Bartsch (1912) and Mayr & Rosen (1956). One case might be cited as evidence that hurricanes may eradicate some colonies: *C. chrysaloides* disappeared from its former habitats on the southwestern coast of Grand Bahama Island following a "direct hit" in the 1930's (Clench, 1938; Gould & Woodruff, 1978). On the other hand, the continued existence of *Cerion* on innumerable low lying cays in the Bahamas suggests that some individuals survive and repopulate these environments.

High recapture rates of adults at Rocky Point and Shipwreck (80% after 6 months and 40% after 48 months) indicate that adult mortality is low. Observations there, and elsewhere, indicate that cerions do in fact experience significant losses to a number of predators. Land crabs (*Gecarcinus lateralis* at Rocky Point) take a number of snails on the ground and may provide part of the selective force that causes the snails to estivate just above ground level. Typically, these crabs will carry the snail back to the entrance of their burrow before breaking the shell into small pieces and removing the animal. Small piles of shell fragments, often with the lip intact, may be found around the edge of the sand pile surrounding an occupied crab hole. Rats also eat *Cerion*; one side of the shell is characteristically sheared off leaving both the lip and protoconch intact. Still another predator, possibly a bird, is implicated by the discovery in the pine forest of Grand Bahama Island of an "anvil" surrounded by irregularly broken shell fragments. Finally, I note that cerions interact with still another organism which breaks a small round hole in the shell. It is not yet known whether the hole is made from within or without; it is subsequently repaired.

As these anecdotes indicate, we still know very little about the ecology and behavior of *Cerion.* The deficiencies are particularly apparent in the area of reproductive behavior where we badly need to establish whether there are specific behavioral traits that could serve as pre-mating isolating mechanisms between the various morphotypes. A knowledge of *Cerion*'s mating system and reproductive strategies is also required if we are to understand the adaptive radiation of these snails. Unfortunately, repeated attempts to induce courtship and mating in the laboratory have failed so we are still limited to some opportunistically gathered observations. I have seen mating once in the field, following an afternoon cloudburst in June on New Providence. Copulation is protracted, taking at least 2 hours, and is not reciprocal. Pairs of snails lie on the ground surface with their apertures adpressed. Several mating pairs were returned to the laboratory where additional matings between some of these pairs were observed. About 3 weeks after the original matings some snails began to excavate small egg-chambers at a depth of 2-4 cm in moist sand. Batches of 4-7 eggs were layed in some of these chambers (details will be presented elsewhere: Woodruff, in preparation). The egg capsule is initially colorless but gradually turns opaque and white, suggesting a calcareous matrix. (Capsular fragments have been sent to Alex Tompa, University of Michigan, for analysis.) Juveniles began to hatch about 20 days later and emerged from the sand about a week after that. Studies of the growth and development of these snails (N = 212) are being conducted by Sarah Burgess in my laboratory. Not only will these observations provide us with information about growth but, as we have the parents of each group of juveniles, we hope to learn something about the heritability of shell traits too.

Additional information about growth rates is coming from studies of marked juveniles at Rocky Point and Shipwreck. Unfortunately, the data on juveniles are not as extensive as that for adults as the former experience quite high mortality rates. Juveniles grow very erratically: in one 6 month period a snail might lay down a single rib while in the next 6 month period, perhaps two whorls. Although I suspect reproduction and hatching occur during the summer the subsequent growth pattern is not strongly seasonal. My preliminary conclusion is that individuals at these localities are typically 3 years old when they secrete the lip characteristic of the adult shell. This conclusion is supported by laboratory growth experiments conducted by Ida Thompson (Princeton University). To calculate the generation time (estimated above to be 5 years) we need to know the age of sexual maturity. On this subject we have no information. However, as some snails live at least a decade after laying down the adult lip I have conservatively assumed that they are reproducing successfully in their second summer of adult life. I tentatively conclude that cerions are outcrossing, iteroparous hermaphrodites with generation times of 4-5 years and a longevity of perhaps twice that duration.

RECENT STUDIES WHICH FOCUS ON THE SYSTEMATIC SIGNIFICANCE OF VARIATION IN *CERION*

Bimini Islands

Ernst Mayr's detailed study of the cerions of the Bimini Islands in the Bahamas constitutes the first notable contribution to the evolution of *Cerion* conducted in a modern framework (Mayr & Rosen, 1956). Their conclusions are worth noting here as they indicate the intractability of the *Cerion* phenomenon and set the stage for our own work. Mayr conducted a very careful survey of the cerions of the six small islands in this group and found geographic variation to be pronounced but irregular. While each colony had its own diagnostic features, three major morphotypes were recognizable. These corresponded to the species *C. lerneri, C. biminiense* and *C. pillsburyi.* Mayr & Rosen stress, however, that even though superficially each morphotype appears to be a separate species, each is allopatric, and adjacent colonies show signs of gene exchange.

Mayr & Rosen argued that these facts were best explained by assuming that the pattern was controlled by two antagonistic tendencies: a high degree of sedentariness and infrequent long-distance dispersal by hurricanes. The checkerboard pattern of morphotype distribution arises as a result of multiple colonizations of each island, extensive hybridization of types secondarily brought into contact with one another, and a steady extermination of colonies as a result of fires and hurricanes. They interpreted the readiness with which the three morphotypes hybridized, in spite of pronounced morphological differentiation, as evidence that reproductive isolation is not easily acquired in this genus. In contrast, they argued that shell characters are highly plastic and evolve rapidly during the early stages of colonization, adapting the snails to their new environment. They wrote "In view of the fact that these snails are hermaphrodites it is possible and probable that many if not most colonies are founded by a single fertilized adult. Two factors tend to promote rapid divergence in such colonies. Comparatively high inbreeding among the early generations derived from the founder individual exposes homozygous genotypes more often to selection than in outbred populations. Also the impact of the new environment may lead to a drastic modification of the contents of the gene pool in the new population and consequently in the phenotype" (Mayr & Rosen, 1956: 43). They emphasize that this great morphological plasticity is an adaptation to the pioneer habitat in which *Cerion* lives: a habitat which changes continuously as a result of eustasy, plant succession, and natrual catastrophes.

On the one hand Mayr & Rosen saw *Cerion* as an actively speciating group and on the other, as an extreme example of reticulate evolution on the intraspecific level. They concluded that the very characteristics (variation in shell morphology) which have adapted these snails so superbly to the habitat in which they live made it impossible to classify them in terms of the conventional categories of species and subspecies.

Dutch Leeward Islands

The type-species, *C. uva,* was described by Linnaeus in 1758. It is restricted to the islands of Curaçao, Aruba and Bonaire which lie off the coast of Venezuela and some 800 km south of the rest of the range of the genus. In marked contrast to the situation in Cuba and the Bahamas only a single variable species was recognized by the early conchologists. Gould resolved to begin his study of the genus with this single, isolated taxon before confronting the more complex situations elsewhere. He was originally attracted to *Cerion* because it provided all the features he could enumerate for an ideal biometrical subject. First, adequate sample sizes are almost never a problem. Second, *Cerion* shows a clear and sharp transition between the protoconch and later accretionary growth. This provides a criterion for the numbering of whorls as a reference for standardized measures at various stages of ontogeny. Finally, and unlike most snails, *Cerion* changes its direction of growth near the end of ontogeny and secretes a single, permanent adult lip. The bugbear of most biometrical studies of molluscs lies in the difficulty (if not impossibility) of sorting ontogenetic from static adult variation. This can be done unambiguously in *Cerion* and we can assess the true standardized variance of adult shells.

C. uva offered one other attraction: it is among the world's best known land snails from a biometrical point of view. It had been the subject of two major studies (Baker, 1924; Hummelinck, 1940; see also De Vries, 1974). Gould began by reanalyzing the published data for a series of 102 samples well distributed over the three islands. In contrast to earlier workers he applied a variety of multivariate statistical techniques to elucidate patterns of intra- and intersample variation (Gould, 1969). He found an excellent correspondence between the data of Baker and Hummelinck on interregional diversity; samples sorted into four groups corresponding to Aruba, Bonaire, eastern and western Curaçao. Diversity patterns did not change significantly during the 16 years between these studies. Next, Gould compared the early samples with a series of 69 modern samples he made himself (see Gould, 1971: unpublished data for monograph in preparation). Again, the patterns do not change over the 50 year period.

Variation within each island or geographic area appears to be influenced by environmental parameters. Surprisingly, the *Cerion* collected from volcanic areas on Curaçao have larger shells than those found on limestone. This anomaly is probably not related to the substrate itself but to the microenvironmental features usually associated with it. On Curaçao the limestone areas are typically very dry, windswept and poorly vegetated; the volcanic areas are more sheltered and have a more mesic vegetation. Baker (1924) was the first to notice an inverse relationship between the number of whorls (and shell height) and the degree of exposure to the dry trade winds. He suggested that the evaporative effect of the wind reduced the snails' activity period and hence its adult size. Gould found additional support for this hypothesis: plotting mean shell height for 15 samples he collected from similar microhabitats on the first terrace along the northern coast of Curaçao he found that shells were largest where the coast runs due east-west and the trade winds do not blow directly on shore. Elsewhere, where the coast runs north-south the shells are smaller (Gould, 1971: fig. 46). Coincidentally, the area where the coast runs east-west is also the area of volcanic soil. Still other evidence for environmental selection for shell size comes from fossil samples from Aruba and Curaçao. Gould (1971) found that shells from three middens on Curaçao dating to about 4000 y BP were larger than any living on this island today. Larger shells were also found in a midden on Aruba dated at 1500 y BP. Gould argued that this suggests that the island's climate was moister during these periods than at present. If these interpretations are correct then the phenotypic variation within this single species is continuous rather than capricious and adaptive rather than adventitious.

Little Bahama Bank

Gould and I began our collaborative field work on the northernmost Bahamian bank: the Little Bahama Bank consisting of Grand Bahama, Little Abaco, Great Abaco and their fringing islands. This area was blessedly spared from visits by Maynard, one of the most exuberant conchological splitters to focus on this genus, and reported diversity was only 12 species. These

names, plotted in the supposed areas of their occurrence, produce the "crazy-quilt" distribution pattern traditionally associated with *Cerion* (Gould & Woodruff, 1978: fig. 1). After several trips to these islands it became clear, however, that the dozen or so names refer to two variable and imperfectly isolated taxa (Gould & Woodruff, 1978). These semispecies, *C. bendalli* (smooth or finely ribbed and mottled shell) and *C. abacoense* (heavily ribbed white shell) are shown in Figs. 2, 3).

This taxonomic simplification was not, however, immediately obvious to us as both species show considerable geographic variation. On our first survey trip, for example, we discovered a local situation where a population of *Cerion* bears a morphology sufficiently distinctive to warrant recognition as a new species by all previous criteria. Our resolution of the taxonomic status of this aberrant population, from Pongo Carpet on the northeast coast of Great Abaco, provided us with an opportunity to test our combination of multivariate morphometric and biochemical genetic techniques (Gould et al., 1974). This population is semi-isolated by mangroves on a narrow coastal strip and is in contact with other populations only in the north. Its morphology is highly distinctive (see Gould et al., 1974: fig. 3) but its patterns of covariation (as revealed by factor analysis) cannot be distinguished from those of *C. bendalli* within whose range it occurs. These patterns of covariation among the 19 variables measured were, however, impressively different from those which characterize *C. abacoense* which is found 70 km away at the southern end of the island. Canonical analysis revealed a multivariate cline extending from Pongo Carpet north towards the area of potential contact with typical *C. bendalli.* Levels of morphological variability did not differ among samples; those of intermediate morphology show no increase (or decrease) in variability or other signs of hybridity. Electrophoresis showed the Pongo Carpet *Cerion* to be genetically indistinguishable from *C. bendalli* at the 18 structural gene loci surveyed. I could find no evidence for any genetic anomaly within the 12 populations (7 from Pongo Carpet) sampled from northern Great Abaco. We concluded that the Pongo Carpet *Cerion* are only a well-marked geographic variant within *C. bendalli.* If this situation is any model for a revision of the genus, we predict that the number of species of *Cerion* may be reduced almost 100-fold.

We have subsequently extended our study to the *Cerion* of the entire bank and have been able to resolve a pattern involving two variable semispecies (Gould & Woodruff, 1978). It turns out that *C. abacoense* is restricted to those present-day coasts which lie close to the edge of the Little Bahama Bank. We will refer to it as being a "bank edge" species. In contrast, *C. bendalli* is a "bank interior" species, found in the interior of the islands and on coasts washed by the very shallow waters covering the bank today. In southern Great Abaco, the geography of the present-day coast relative to the edge of the bank is such that there are several places where the ranges of these 2 morphotypes come into contact and hybridization occurs. To resolve the patterns of variation in each morphotype and to investigate the nature of their interaction we studied variation in 20 shell characters and 28 genetic loci in over 50 samples from across the bank. Morphometrically, the two morphotypes may be sorted unambiguously by factor analysis; 3 factor axes encompass over 96% of the information. All samples from the area where the two taxa meet plot in the intermediate phenetic field: the hybrids have intermediate phenotypes. Near Rocky Point the zone of allopatric hybridization (sensu Woodruff, 1973) is 1 km wide and there is a gradual transition in mean morphology across the zone with no increase in intrasample variability.

My study of allozyme variation for the same snails revealed very little concordance between the genetic and phenetic patterns. All samples of both *C. bendalli* and *C. abacoense* are markedly similar: Nei's genetic identity (I) for 820 pairwise comparisons averaged 0.98. No unique marker gene characterizes any region or morphotype, though characteristic frequencies of certain variable alleles clearly separate Grand Bahamian from Abaconian *Cerion* in a statistical manner. Despite this overall genetic similarity the pattern of allozyme variation supports our decision to treat the Little Bahama Bank *Cerion* as two semispecies. Although hybrids showed no increase in morphometric variability they showed significantly more genetic variability (both within and between populations) than snails collected elsewhere. They

are also polymorphic for alleles not detected in either adjacent "parental" population. I have now resampled this hybrid zone and a more detailed discussion of its significance will be presented elsewhere (Woodruff & Gould, in prep.).

It should be clear from the above discussion that there is no evidence that the various colonies of *Cerion* on the Little Bahama Bank were founded by single hurricane-transported waifs. The very high genetic similarity among the various populations sampled suggest that hurricanes have not played a significant role in the recent evolution of these snails. The situation may be quite different on the tiny offshore cays surrounding the main islands; unfortunately I have not yet been able to study them genetically. I have, however, studied variation in populations of *C. bendalli* from Snake and Tuggy Cays which are adjacent to Great Abaco (Woodruff, 1975b). These populations show the same amount and type of genetic variation as the adjacent populations from the main island. Again, there is no evidence that these populations were derived from single founding individuals. For the Little Bahama Bank *Cerion* we reject the notion that the pattern is a "crazy-quilt" due to hurricane-dispersed morphotypes. Instead we conclude that the pattern is quite coherent and evolved at a time when sea levels were lower and the populations on the various islands were in full genetic contact with each other.

Great Bahama Bank

Following our study of the *Cerion* in the northernmost Bahamian group of islands we turned our attention to the far more complex situation on the Great Bahama Bank. Here, there are a number of large islands (Andros, New Providence, Eleuthera, Cat, Exuma and Long), numerous small cays and several hundred described species. In the last five years Gould and I have undertaken exploratory surveys on all the main islands (collecting both living and fossil *Cerion*) and examined material in the collections of the major U.S. museums. Our studies on three of the large islands have reached the stage where I can announce some of our preliminary findings; detailed reports will be published elsewhere.

New Providence (with the city of Nassau) has been scoured for *Cerion* for over a century and 82 species have been described from this relatively small island (207 km^2). Yet we quickly realized that the situation here was very similar to the pattern we had elucidated on the Little Bahama Bank. There are two imperfectly separated entities: a ribby *abacoense*-like form (called *C. glans* on this island, see Fig. 4) and a mottled *bendalli*-like form (whose correct name we have yet to establish). Again, the ribby morphotype is a "bank edge" species found on the western edge of the island and on the offshore cays. The mottled morphotype inhabits the interior of the island and the eastern and southeastern coasts on the "bank interior" side of the island. The zone of interaction between these morphotypes is, however, different from that described on Abaco. While multivariate clines mark the east-west transition across this island the hybrid zone is characterized by novel phenotypes and by unusual intrapopulation variation in shell size. There is no smooth continuity of phenotype, but rather a host of mixed phenotypes. This is borne out in a Q-mode factor analysis: the hybrid population is characterized by shells which secrete the adult lip abruptly, without undergoing the characteristic changes in the axis of coiling typical of all other cerions. Genetically, the two morphotypes on New Providence are very similar, as they were on Abaco. On New Providence, however, the transitional populations are not distinguished by any genetic anomalies. A paper in which we will subsume 80 taxa and describe the nature of the variation in these two semispecies and the significance of their interaction is now being prepared.

We have made one survey trip to the Exumas and found, once again, that despite the plethora of available names there are basically two contrasting morphotypes present. There are numerous varieties of a *bendalli*-like mottled form (for which *C. eximeum* may be the oldest available name) on Great and Little Exuma and on the innumerable cays in the Exuma chain. There is a ribby *abacoense*-like form on some of the cays which lie close to the edge of the bank. Only two forms depart dramatically from one or other of these morphotypes. One, *C. pauli* (Fig. 6) from Great Exuma, matures at less than half the typical size of the mottled forms but has 10-12 whorls, or 2-3 more than normal. We

have no reason to regard it as anything more than a strongly dwarfed population of the mottled morphotype. It is also atypical in its habits, being found in piles of rotting vegetation near the mangroves on the "bank interior" coast. The other aberrant type is an isolated population of a member of the subgenus *Umbonis* on Great Guana Cay. Museum specimens indicate that this population, which was presumably derived from Cuba, is hybridizing with the local mottled forms.

On Long Island, at the southeastern edge of the Great Bahama Bank, we found that our simplifying generalization about "bank edge" and "bank interior" morphotypes was complicated by the incursion of several additional morphotypes. A few of the species described from Long Island are shown in Figs. 7-13. In the course of 3 field trips to this island we have mapped the distribution of these forms and uncovered by far the most interesting situation that we have yet encountered in *Cerion.* First, we discovered that two basic morphotypes were present: a mottled form, *C. eximeum,* occurs along the western "bank interior" coast and a strongly ribbed form, *C. caerulescens,* occurs on the eastern coast adjacent to the edge of the bank. In addition we found five other distinctive morphotypes along parts of the hilly east coast. These taxa replace one another along the coast and we have now located 12 narrow hybrid zones between various combinations of these forms. Particularly spectacular zones involve the large, white, smooth-shelled *C. fernandina,* the squat, white, smooth-shelled *C. malonei,* and the distinctive member of the subgenus *Umbonis, C. stevensoni* (Fig. 12). Defined on the basis of shell morphology these hybrid zones are quite narrow (some are only 100 m wide), and may or may not be associated with marked changes in abundance or habitat. While the various species involved in these interactions are very similar to each other genetically I found that at least two of the hybrid zones are characterized by significantly greater fluctuations in interpopulation allele frequencies and by the presence of novel genotypes absent from adjacent "parental populations." Michael Goldman, in my laboratory, has carefully checked intersample variation in adjacent populations away from one of these hybrid zones (*fernandina-stevensoni*) to ensure that our preliminary findings are not simply due to more intensive sampling of the intermediate populations. We see the elucidation of the genodynamics of these hybrid zones as important to the understanding of the process of evolution of *Cerion.* In particular we will attempt to establish whether the zones are of primary or secondary origin or whether they are a mixed group. It strikes us that marked geographic differentiation and parapatric speciation are quite possible in a group like *Cerion.* Interestingly, parts of the complex distribution pattern on Long Island are relatively old; fossils indicate that some of these species have been in place for at least 110,000 years.

Florida Keys

A single endemic species, *C. incanum,* occurs in the Florida Keys and north as far as Key Biscayne in Miami. It is a medium-sized, typically smooth-shelled species and has little geographic variation (Fig. 5). I have recently discovered that, unlike all other *Cerion* examined, *C. incanum* is largely invariant genetically. All the populations sampled in the Keys (sensu stricto) to date are monogenic; samples from Key Biscayne were polymorphic at an esterase locus. This contrasts markedly with the situation in *Cerion* from the Bahamas, Puerto Rico and Curaçao which are typically polymorphic at 4-6 loci. In this respect *C. incanum* is more like North American populations of *Rumina decollata* than any other pulmonate gastropod described to date (Selander & Kaufman, 1973, 1975a). We will use this observation to standardize the nomenclature for the allozymes detected in *Cerion* (Woodruff & Burgess, in preparation.) Henceforth, different allozymes will be compared directly to those in *C. incanum* and designated by their quantitative relative mobility.

Gould and I made a second exciting discovery in the Florida Keys. In 1911 Paul Bartsch began a series of transplantation experiments aimed at showing that snails moved to a new environment would rapidly evolve to resemble the local (and presumably optimal) *Cerion* morphotype. He transplanted several samples of Bahamian cerions to the Dry Tortugas and other Keys (Bartsch, 1920). Fires, hurricanes and other setbacks marred the experiments and the transplants bred true to their ancestral phenotype for as many generations as he

could follow. He concluded that phenotypic variation in *Cerion* is not under strict environmental control. As some of his transplants hybridized with the resident *C. incanum,* and as the hybrids were "enormously variable" (a phenomenon Bartsch attributed to mutation), he further concluded that the "crazy-quilt" pattern of geographic variation in this genus was adventitious rather than adaptive (Bartsch, 1949). While Bartsch's experiments were inadequate to assess the process of local adaptation, and while his ideas of "mutating hybrids" as the source of *Cerion*'s diversity, are not supported by modern genetic theory, we are indebted to him for initiating these experiments nearly 60 years ago. We have discovered that the descendents of one of his transplantations still flourish today in a restricted area at the site of the original introduction. The introduced species hybridizes with *C. incanum* and the hybrids continue to show a range of variant phenotypes and a general level of variation far higher than that seen in homospecific populations. Bartsch documented the early stages of his experiments in great detail and it has been possible to re-collect from the original Bahamian source population and confirm their identity genetically. We will describe this interesting experiment and discuss its evolutionary implication in the near future.

Isolated Bahamian banks

We have had limited field experience with the cerions on a number of the smaller island banks: San Salvador, Inagua, Rum and Conception. Rum Cay and Conception Island lie on small, isolated banks to the north and east of Long Island. Each is inhabited by a single, variable species of the ribby morphotype. Kathleen Ligare and I are establishing the genetic and phenetic relationship between these snails and the similar morphotypes on Long Island from which they might have been derived.

Cuba

The north and eastern coasts of Cuba are perhaps the center of *Cerion*'s remarkable diversity. We have begun a revision of this large island's rich fauna; 147 "species and subspecies" according to Clench (1957) and Jaume (1975). We have examined all specimens in the great collection at the Museum of Comparative Zoology and prepared distribution maps of the purported taxa. The resolution of the resulting complex patterns will require additional field work.

In the 1950's Ernst Mayr collected *Cerion* in Cuba and his distribution map of forms on the Banes Peninsula on the northeastern coast has done much to draw attention to the genus and its problems (Mayr, 1963: 398-399, 1969: 17, 1970: 33). In this area Mayr found 7 highly distinct morphotypes replacing each other geographically along a 50 km stretch of coast. Within four zones of contact presenting no ecological barriers to effective gene flow, he found populations which he interpreted as the hybrid products of secondary intergradation. Lynne Galler and Gould (in prep.) have been studying the narrow transition zone between two of the most divergent morphotypes: *C. moralesi* and *C. geophilus.* Their biometrical studies show no increased variability in the geographically intermediate samples. Several univariate and a multivariate cline cover the zone which is less than 1 km wide. They find that the large morphological differences between these taxa may arise from a small alteration in the rate of shell widening during the early phase of post-embryonic growth. The interplay of this simple event with the complex allometries of normal ontogeny produces the large adult differences. We must now confront the fascinating question: can any *Cerion* be transformed into any other by simple heterochronous changes in early ontogeny?

Hispaniola, Puerto Rico and the Virgin Islands

Gould and Paull (1977) completed a study of *Cerion* from the eastern end of its range: Hispaniola, Mona Is., Puerto Rico, and Necker and Anegada in the Virgin Islands. Here 11 names were available for a basic morphology that all students of *Cerion* have recognized as unique to this area (Pilsbry, 1901-1902). They performed a canonical analysis of morphological variation in 23 samples from throughout this area and found that these samples are arranged along the most significant discriminator (the first axis: 59% of all information) in perfect geographic order. The morphological gradient runs from egg-shaped, finely and copiously ribbed shells

with very obtuse apices (Virgin Islands) to more cylindrical, apically-pointed shells with fewer, stronger ribs (Hispaniola). In the light of this clinal pattern they reduce all available names to a single species, *C. striatellum.*

DISCUSSION

Prior to the commencement of our work three major attempts were made to interpret *Cerion*'s complex diversity. Plate (1906, 1907) argued that the variation in shell morphology was adaptive. His interpretation was in a Lamarckian mode, however, and was based on very limited personal experience with the animals and much misinformation about areas he had not seen. Bartsch (1920, 1949) concluded that the pattern was not adaptive and resulted from chance events (colony extinction and long-distance dispersal), evolution in isolation, and subsequent diversification among "mutating hybrids." Mayr (1963; and Mayr & Rosen, 1956) interpreted the variation as adaptive but also argued that stochastic events play an important role in the development of the overall pattern. Mayr regarded each colony as an evolutionary experiment whose ultimate fate is indeterminate. He interpreted the "crazy-quilt" distribution pattern in terms of local extinction and recolonization with subsequent secondary contact between contrasting morphotypes dispersed primarily by storms. He repeatedly cites *Cerion* as the classic example, in animals, of the acquisition of morphological differences without reproductive isolation (Mayr, 1963, 1969, 1970).

In the past few years Gould and I have personally confronted about one third of the alleged diversity in *Cerion.* Our combination of laboratory studies employing multivariate morphometric and biochemical genetic techniques is proving successful. Given time we have every reason to believe that *Cerion*'s taxonomic overburden will be removed and the evolution and adaptive radiation of these snails can be exposed for direct investigation. While it should be clear that our bias is towards an interpretation involving both history and adaptation it is clear that we have not yet reached the point where sweeping generalizations are possible. It turns out that the earlier workers were partly right and partly wrong; the analogy to the situation in *Cepaea*—a problem with too many solutions (Jones et al., 1977)—is striking.

In the interim, how are we to regard these markedly different populations? Are they ecotypic races, morphospecies, or biological species? At present we think *Cerion* may comprise a number of variable and polytypic semispecies. The actual number of these taxa is not yet known but we agree with Clench's (1957) opinion that perhaps only 20% of the described species are valid. I emphasize that we still know nothing about reproductive isolating mechanisms in *Cerion*; statements to the effect that reproductive isolating mechanisms are not easily acquired in this genus are based solely on the observation of shell types that are intermediate between various pairs of morphotypes. Until we identify the potential pre- and post-mating isolating mechanisms and learn more about the reproductive anatomy and behavior of various cerions, we simply cannot comment on the significance of the alleged interspecific differences. For example, we still do not know whether selfing plays any role in *Cerion*'s mating system (as it does in early reproductive life in *Partula taeniata* (Murray & Clarke, 1976)). Working in my laboratory, Daniel Chung has taken on some of these fascinating problems.

The lack of marked genetic differences between the various cerions studied has little bearing on speciation per se. Twelve years of biochemical genetics of many organisms provides little evidence for extensive reorganization of gene pools during speciation (Throckmorton, 1977). There is every indication that changes in a few loci are sufficient for speciation and that geographic variation rather than genetic revolution may be the critical prerequisite.

As I can demonstrate no major differentiation in structural genes among the most widely divergent of *Cerion*'s morphotypes we are forced to look elsewhere for the underlying genetic determinants. Gould (1977) has argued that complex differences in form can often be traced to simple differences in developmental rates expressed during ontogeny. Since these rates are probably controlled by regulatory genes (King & Wilson, 1975) it is not surprising that allozymic (structural gene products) variation is small. The possibility that minor developmental changes will translate into major differences in adult morphology

is strongly enhanced in *Cerion* by the complex allometries that characterize growth: particularly the 3 divergent phases of juvenile triangularity, mid-growth "barrelling," and adult recurvature. Our vision is to reduce all this diversity to a simple system of ontogenetic growth gradients and their quantitative alteration. We have also tried to develop a "dynamic" approach to describing shell variation and choose to work with patterns of covariation rather than static adult morphology. Our initial experiences have been most promising and it is pleasing to note that the raw data presented in our first paper (Gould et al., 1974) has already been used as a basis for two subsequent studies (Sokal, 1976; Schueler & Rising, 1976). To give our enormous data sets even greater validity Kathleen Ligare has recently completed a study of the measurement errors associated with each morphometric trait.

So far I have not discussed the possible adaptive significance of the variation seen in *Cerion.* I proceed into the difficult field of functional morphology with Darwin's remarks (quoted by Cain, 1954) on the danger of believing apparently trivial characters to be of no functional importance and in no way due to natural selection firmly in mind. It is not at all surprising that earlier workers, confronted with the puzzling and apparently random distribution of morphotypes, gave up and decided that the pattern was due to drift and other random processes. It is only from our experience with *Cerion* in nature that we feel emboldened to ask questions about the adaptive significance of variation in the shells and other features.

We have reached the stage where we can speculate about the possible adaptive significance of variation in shell size, shape, sculpturing and color. We proceed by looking for obvious correlations between morphometric traits and environmental parameters. Overall shell size, for example, appears to be correlated with humidity and the degree of shelter from the wind on Curaçao. Unfortunately, this trend does not seem to explain patterns on the Great Bahama Bank where even greater interpopulation variation occurs on single islands. Dwarfing occurs in some populations of most of the main morphotype groups. I note that juvenile and adult shells are quite different in shape and wonder if this might be correlated with differences in microhabitat, shell function, and the mechanics of crawling (see Cain, 1977; and papers by Cain and Linsley in this symposium). In *Cerion* the spire index (height/max. diameter) of juvenile shells is 0.5-0.7; in adults the index is typically above 3.0 and can be as high as 7.0. Juveniles are typically found on the ground surface beneath the leaf litter and are usually oriented apex up. In contrast, the adults are invariably found hanging on plants apex down. Lip size appears to be correlated with habit and habitat: small lipped shells are often associated with grass or leaf litter in humid microhabitats; cerions with large recurved lips are associated with drier areas which are exposed to the prevailing wind and often have rocky substrates. It is possible that the greater surface area of an aperture with a large lip give the shells (which is fastened to the substrate or vegetation by a thin epiphragm) greater stability in windy situations. I can not comment yet on the possible significance of variation in the apertural teeth which are present in most cerions in both juvenile and adult phases. Shell sculpture or ornamentation is another variable character of unknown adaptive significance. While in some areas ribbing appears to be correlated with humidity and with the presence of a calcareous substrate, in other areas this generalization does not hold. John Quensen, in my laboratory, is examining Vermeij's (1975) suggestion that ribbing is an adaptive response to predator pressure in snails since prominent ribs confine the predator's crushing force to the thickest part of the shell. His preliminary results indicate that overall shell size (weight and height) is more important than ribbing in determining a cerion's ability to withstand crushing forces applied generally along the sides of the shell. He is now repeating these experiments using artificial crab chelae to apply forces at right angles to the shell's long axis; a more realistic design in view of our findings at Rocky Point. Quensen has also been considering the possible adaptive significance of shell pigmentation. Mottled shells are initially hard to find as they hang on bush stems and blades of grass in the dappled sunlight and shadow, a clear case of disruptive coloration to our eyes (Gould & Woodruff, 1978: fig. 4). In contrast, white shells are conspicuous in the vegetation (see Bartsch, 1968: fig. 24). Quensen found that when shells are compared be-

neath a heat lamp in the laboratory the interior of a mottled shell averages at least 1°C warmer than the interior of an unpigmented shell. In the Bahamas he found the effect of differential heating to be even greater: up to 4°C differences in November, the actual difference being proportional to the air temperature. As the upper lethal temperature for *Cerion* is about 52.5°C it is quite possible that a pigmented shell exposed to the summer sun risks thermal death. The occurrence of unpigmented shells in exposed coastal situations may be a thermoregulatory adaptation.

We cannot even begin to discuss the possible adaptive significance of variation in characters other than those associated with the shell. Alleged interspecific variation in the radula and reproductive system are not adequately documented. Similarly, we can not yet account for the distribution of the snails themselves. Their absence from Jamaica and the Lesser Antilles is puzzling. Even within the areas where they occur we have no adequate theory to account for *Cerion*'s microdistribution. Even the most basic assertions that cerions are halophiles (Mayr, 1963) or calciphiles (Clench, 1957) are contradicted by the occurrence of snails up to 15 km from the coast on Grand Bahama and in volcanic (non-limestone) areas on Curaçao.

Cerions are remarkably like some variable plants, in which spectacular examples of highly localized, specially adapted ecotypes which replace one another over distances of a few meters are well known (Bradshaw, 1972). I see considerable promise in attempting to reinterpret some of the patterns within the context of a model of parapatric differentiation rather than the allopatric model advocated by Mayr (1963). This is necessary because recent theoretical work on clines (Endler, 1977; Woodruff, 1978) indicates that the traditional criteria used to distinguish between primary and secondary intergradation are inappropriate. We must carefully examine the possibility of explaining some aspects of the *Cerion* pattern in terms of contemporary selection gradients and dispersal patterns. A continuously distributed population of 10^5-10^6 snails cannot be considered as though it were a single random mating population; *Cerion* is apparently broken up into small demes containing approximately 10^3 individuals. Each deme, even those in a more extensive population, occupies an area that is large relative to the gene flow distance and close evolutionary "tracking" of microenvironmental heterogeneity is a real possibility. I am now beginning to look for evidence of this at the study sites on Abaco. The measurement of natural selection operating on various traits in nature is quite difficult; our initial approach involves looking for asymmetry in the various biometric traits. I am also extending my investigation of gene flow to assess its magnitude in low (rather than high) density populations. *Cerion* provides prime material for the experimental investigation of natural clines and hybrid zones.

Land snails are well suited to studies of evolution and adaptive radiation (Clarke et al., in press). The lessons of *Cepaea* (reviewed by Jones et al., 1977), *Theba pisana* (Hickson, 1972; Nevo & Bar, 1976) and *Helix pomatia* (Pollard, 1975; Järvinen et al., 1976) on the role of natural selection and other agents in the microevolution of natural populations are particularly noteworthy. Studies of the variation of *Partula* in the Society Islands resulted in significant contributions to the theory of clines and parapatric differentiation (Clarke, 1968; Clarke & Murray, 1969; Murray, 1972). These studies, and others which focus on other organisms with low vagility, cast increasing doubt on the universality of the allopatric model of speciation (Bush, 1975; Endler, 1977; Woodruff, 1978). Finally, I note that recent work on genic variability and breeding systems in *Helix aspersa* and *Rumina decollata* (Selander & Hudson, 1976; Selander & Kaufman, 1973; 1975a; 1975b) have implications for evolutionary ecology far beyond the confines of malacology. It is our hope that *Cerion* will take its place among this small group of land snails that are of major significance to biology generally.

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MALACOLOGIA, 1978, 17(2): 241-315

ECOLOGY OF THE PUERTO RICAN CAMAENID TREE-SNAILS

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ABSTRACT

This paper compares the ecologies of the Puerto Rican camaenid snails. Interspecific differences in environmental tolerances and responses, and in life history patterns were assessed in relation to geographic distributional patterns. Finally, these aspects were viewed from the standpoint of adaptive strategy.

There are five native species of Camaenidae in Puerto Rico, two of *Caracolus (C. marginella, C. carocollus)* and three of *Polydontes (P. acutangula, P. luquillensis, P. lima).* These occur in species pairs with *C. marginella* and *P. lima* predominating in the lowlands and *C. carocollus* and *P. luquillensis* in the uplands. *P. acutangula* occupies a special upland habitat (leafy parts of vegetation). *C. carocollus* is the most eurytopic and occurs in both upland and lowland habitats, where it has different ecological characteristics.

All five species of camaenid snails in Puerto Rico are primarily nocturnal. Juvenile snails tend to secrete themselves in crevices under objects, whereas adults more often are on tree trunks, or in the case of *Polydontes acutangula,* the leaves of trees or hedges. In wet montane forest a greater proportion of adult *Caracolus carocollus* are on the trunks than is true in the hotter, drier lowland forest. More snails occur on large rather than small trees. There does not seem to be any selection of tree species other than by size; however, trees with scaly or flaking bark are not often used whereas trees with an abundance of shelter sites attract more snails. *C. carocollus* is not common where slopes are steep.

Body temperature of animals in the shade is controlled by heat exchanges with the substrate and to a lesser extent with the air. In direct sunlight, body temperature rises as a result of absorption of radiant energy. As a result, and perhaps because of evaporative cooling and lags in temperature change in the body, air and substrate temperatures are not precise predictors of snail body temperature.

In *Polydontes,* the falling point (temperature at which a snail drops off a vertical substrate) was correlated with the habitat occupied by the species. Both species of *Caracolus* had similar falling points. Neither lethal temperature nor thermal safety margin showed any consistent correlation with taxonomic or ecological groupings. Falling point is not dependent on body size, whereas lethal limit, and hence thermal safety margin are; juveniles have lower lethal limits and safety margins than adults.

Survival of the adult *C. carocollus* deprived of food is more than twice as long at 20°C than at 30°C. In *Caracolus marginella* survival at the two temperatures does not differ greatly and is similar to that of *C. carocollus* at 30°C. Juvenile *C. carocollus* has lower survival than adults at both temperatures tested.

Most *C. carocollus* carry water in the mantle cavity. The amount carried is less when conditions are dry. However, in adults the water content of the body itself remains the same during dry periods and the animals continue to feed normally.

C. carocollus from the lowlands has a high mortality in the very small juveniles with few animals surviving to maturity. Dry periods increase the relative mortality among small juveniles. In the moister uplands, juvenile mortality is less, most individuals surviving until adulthood. Other upland species, including one from a different family have survivorship characteristics similar to those of upland *C. carocollus.* Lowland species tend to be between the extremes represented by upland and lowland populations of *C. carocollus* but have considerable juvenile mortality.

Mating in upland species and in upland populations of *C. carocollus* seems to occur over a prolonged period centering on the dry season whereas in lowland species and lowland populations of *C. carocollus* mating tends to occur in the wetter parts of the year. Individuals stay in breeding condition for at least two months and during that time mate with more than one other snail. A given snail may breed in at least two consecutive seasons.

In *P. acutangula* most egg-laying begins about November and continues till February or March; eggs take at least 1½ months to develop. Sporadic oviposition may occur at other times of year. It deposits its eggs at a variety of epigean sites and on the surface of the ground under objects. Oviposition periods are not known for other species.

After the mating peak, the albumen gland of *C. carocollus* begins to develop and reaches the peak of its cycle in June, after which the reproductive animals probably retreat to secluded refugia where they mature their eggs and oviposit. Embryonic development and hatching were not observed, but the timing of the rest of the cycle suggests that they

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probably occur before the onset of the subsequent late-winter and early-spring "dry" season. At Loiza Aldea, the peak of the albumen gland cycle is about a month later but the general seasonal pattern is similar.

Growth of *C. carocollus* at El Yunque occurs primarily between March and August; the season is somewhat shorter at El Verde. Adulthood is reached in 3-6 years. *P. acutangula* and *P. luquillensis* grow at all seasons and reach maturity after one year and two years respectively. *C. carocollus* lives at least up to ten years and probably longer. Longevity seems to be shorter in *P. acutangula* and *P. luquillensis.*

A variety of foods are eaten by these camaenid species. The diet consists primarily of wood, bark, seeds and leaves of vascular plants, and of diatoms and other unicellular algae. Occasionally animal material and bryophytes are also eaten. There appear to be interspecific differences in diet as well as differences between localities within a given species.

Snails tend to use a restricted area during their inactive periods (home site range [*hsr*]). Juveniles set up *hsr*'s well before maturity and the area of their *hsr*'s does not differ significantly from those of adults from the same region. Size of mean *hsr* varies greatly between different study areas for a given species. A minimum of seven captures is required for accurately ascertaining the size of the *hsr.* Within the *hsr,* particular home sites were favored by individual snails. *Hsr*'s of different individuals overlapped greatly.

C. carocollus maintained a rather stable size-structure throughout the study whereas there were temporal changes in that of *Polydontes luquillensis.*

At El Yunque the population of *C. carocollus* was increasing in density in late 1962 and into mid-1963 after which it declined. Densities were higher around the building than in the forest; both these areas had higher densities than the El Verde region.

INTRODUCTION

Selection can operate upon a population through climatic, edaphic, biotic factors or combinations thereof. There are different ways of responding to such forces, i.e. alternative adaptive modes are available. For example, a population can become adapted to extreme environmental heat by broadening its physiological temperature tolerances (withstanding heat) or behaviorally by selecting only the cooler microhabitats and changing its diel or seasonal pattern of activity (avoiding heat). Which of various alternative modes of adaptation that will occur depends on the genetic background upon which selection is operating, as well as upon purely stochastic processes.

The constancy of selective forces (duration of a particular environmental complex) and the predictability of short term environmental changes can influence the reproductive pattern. For example, a species can have regular seasonal breeding periods controlled by internal rhythms, or reproduction may be opportunistic and triggered by immediate weather conditions. A species may be long-lived and produce only a few young each year, or be short-lived and produce many young at a given reproductive season; these patterns have environmental correlates (Tinkle et al., 1970). Matthews (1976) has reviewed general strategies which could be expected to be successful in different kinds of environments. In ephemeral environments, the most successful strategy is to have good dispersal powers (to reach a new site when the old one changes) and to quickly exploit new areas by a rapid natural increase resulting from high fecundity and short generation time (r strategist). In stable and relatively equable environments an optimum strategy would be to have lower reproductive rates and less mobility, but to develop more effective long term persistence in a given site through better defence against predators, superior competitive ability under prevailing conditions, and food specialization (K strategist). Finally, in predictable but physically harsh environments, species that can survive physical rigors may become adapted through specialization (beyond-K strategist). Ultimately, the mosaic of selective forces over an environmentally diverse area, interacting with the spectrum of possible responses by organisms, leads to adaptive radiations. Such radiations in turn can be expressed in biogeographic and ecological terms.

One way of studying an adaptive radiation then, is to examine the present distribution of a cluster of related taxa, define their niches, habitat selection and biotic interactions, measure their tolerances of physical environmental factors, and compare their demographic and reproductive strategies—in short to conduct a comparative ecological study. One can then begin to assess the factors presently limiting their spatial distribution and evaluate the selective forces operating upon them now and in the past. It is important to study eurytopic species in several different environments in order to ascertain their ecological flexibility.

The need for such a broad comparative approach prompted the present study. Puerto Rico was especially suited as a study area because of the diverse array of habitats, topography and climate which occur in a small area. The island is roughly 160 by 55 km, yet has environments ranging from Montane Rain Forest to xeric scrub. The snail family Camaenidae was selected because (1) it contained a number of large, conspicuous, easily studied species occupying a broad spectrum of local habitats and geographic areas, (2) some species appeared to be highly specialized but others quite eurytopic, and (3) the geographic distributions of the Puerto Rican species had been previously outlined (Van der Schalie, 1948).

The organization of the present paper is unusual and requires some explanation. We have discussed our findings and their significance in six sections. The general reader can grasp the major results and concepts and gain an overall view of the study by reading only these chapters.

Following the text are two appendices. Appendix 1 describes the study areas and Appendix 2 presents the detailed results, methods, tabular and graphic material and statistical treatments. When reference to tables or figures is made in the text, the specialist reader will want to turn to Appendix 2 for documentation of the points being discussed, and may wish to read the description of the result accompanying the illustrative material in the appendix.

THE GENERAL WORLD OF THE CAMAENIDAE

The purpose of this section is to provide the reader with a basis for distinguishing camaenids from other land snails, and to give a general distribution of world-wide camaenids, with notes on general ecological information. Finally, we focus on the five native species of camaenids in Puerto Rico, indicating their probable phylogenetic relationships, describing shell features, species-pairs based on shell features, and the general association of species of different shell morphologies.

The Camaenidae (Pleurodontidae)

The camaenids, like other stylommatophoran pulmonates are hermaphroditic land snails. During copulation each member of the pair inserts its penis into the partner and reciprocal insemination is effected via a spermatophore. Morphologically the family is distinguished by: helices without a dart apparatus; penis continued in an epiphallus and a flagellum (the latter sometimes vestigial or wanting); spermathecal duct not branched; ovotestis a single mass of alveoli buried in, or closely appressed to the inside of the liver (Wurtz, 1955).

There are about 40 genera (94 genera and subgenera), most of which contain only one or a few species. Only about 12 genera can be considered large. Most taxa are known only from the Recent although fossils are known from the Pleistocene of South Australia, the Pliocene and Pleistocene of Caribbean Islands, the Upper Miocene of Florida and a possible member of the family from the Paleocene or Eocene of south central United States (Zilch, 1959-1960).

As one would expect from such a taxonomically diverse family (only three of the 76 families of pulmonate snails have larger numbers of genera and subgenera; Te, 1976) there are a variety of habitats occupied and niches filled. However, there is a prononounced tendency toward arboreality (Burch, personal communication) and large size throughout the family. The distribution of the extant camaenids includes southern and eastern Asia, New Guinea, Australia, Melanesia, Central America, South America and the Caribbean Islands. They do not now occur in Africa, the Palearctic or North America. Te (1976) lists 16 different patterns of distribution of pulmonate families and groups them into major categories of Old World-New World, Old World, New World, Australian Pacific, and Southern. He lists the Camaenidae in the first of these broad categories—with a South and East Asia-Australian Pacific-Middle and South America pattern (pattern IV). This is the only family included in this pattern and thus the distribution of camaenids is unique among the pulmonates.

The Puerto Rican camaenids

There are five native species of camaenid snails inhabiting Puerto Rico; *Caracolus marginella* (Gmelin), *Caracolus carocollus* (Linnaeus), *Polydontes lima* (Férussac), *Polydontes acutangula* (Burrow), and *Polydontes luquillensis* (Shuttleworth) (Van der

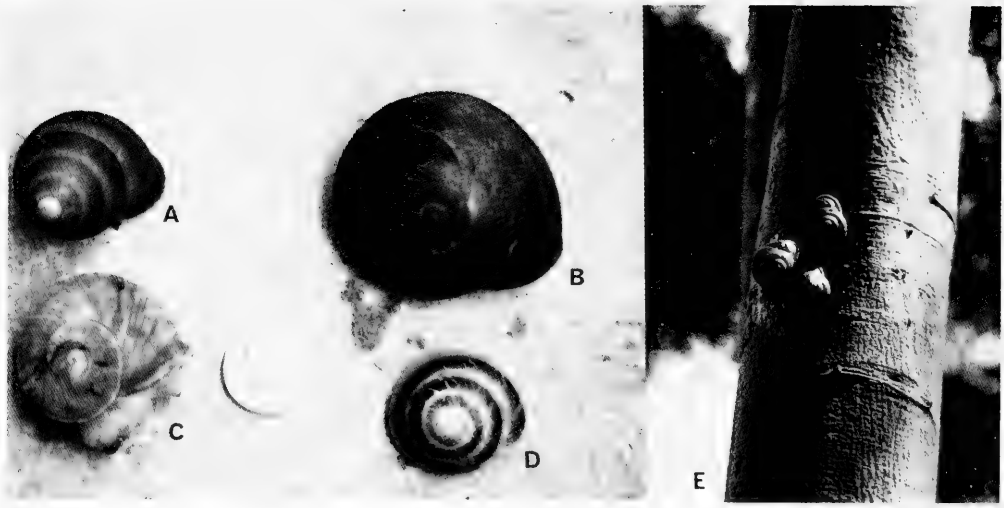

FIG. 1. The Puerto Rican camaenid snails. A. *Polydontes luquillensis*; B, *Caracolus carocollus*; C, *Polydontes acutangula*; D. *Caracolus marginella*; E, *Polydontes lima* on tree trunk near Rio Piedras.

Schalie, 1948; Zilch, 1959-1960; Aguayo, 1966). In addition, the introduced Cuban species *Zachrysia auricoma havanensis* Pilsbry occurs in the dry coastal limestones between Arecibo and Toa Alta (Van der Schalie, 1948); only the native species are considered in the present paper.

Fig. 1 shows the appearance and relative sizes of Puerto Rican camaenids and Table 1 provides data on sizes, color and shell morphology; distribution patterns are portrayed in Fig. 2. The vegetation zones of Fig. 2 are after Little & Wadsworth (1964). They can be ranked in a descending order of wet to dry and cool to hot in the following order: Upper Luquillo Forest, Lower Luquillo Forest, Upper Cordillera Forest, Lower Cordillera Forest, Moist Coastal and Limestone Forests, Dry Coastal and Limestone Forests. In general terms the eastern highlands are the wettest and coolest and the southern coast the hottest and driest.

The Puerto Rican camaenids did not adaptively radiate in situ from a single ancestral stem; rather early members of a number of related lineages probably reached the island independently. Consequently, the pattern of their evolutionary development was not a diverging into various niches and habitats from a single stock, but ecological adjustment through interaction of various taxa to different, though similar genetic entities. Wurtz (1955) recognized four major taxonomic complexes within the American Camaenidae, *Labyrinthus, Caracolus, Pleurodonte,* and *Polydontes* with *Zachrysia.* Bishop (in preparation), on the basis of the presently available anatomical evidence, suggests that *Pleurodonte, Polydontes* and *Zachrysia* form a single monophyletic lineage, while there is an unresolved trichotomy between this group, *Labyrinthus* and *Caracolus. Labyrinthus* is isolated in South America. *Caracolus* has a fossil representative in the Oligocene of Nebraska (Bishop, in preparation), and *Pleurodonte* has a probable rela-

FIG. 2. The distribution of Puerto Rican camaenid snails in relation to climax forest types. Distributional data for snails from Van der Schalie (1948) and the author's collections. Forest types after Little & Wadsworth (1964). Dashed lines = Upper Luquillo Forest; open area = Lower Luquillo Forest; cross-hatched areas = Upper Cordillera Forest; horizontal lines = Lower Cordillera Forest; Hatched from upper right to lower left = Moist Coastal forest; vertical lines = Moist Limestone Forest; hatched upper left to lower right = Dry Coastal Forest; vertically and horizontally hatched = Dry Limestone Forest. A. Location of the study areas. B. Distribution of *C. carocollus* (dots) and *C. marginella* (circles). Localities with both species indicated by half closed dots. C. Distribution of *P. luquillensis* (circles) and *P. lima* (dots). D. Distribution of *P. acutangula* (dots). The boundary lines enclosing the Lower Cordillera and Lower Luquillo forests coincides very closely to the 155 m contour line and the 25°C isotherm.

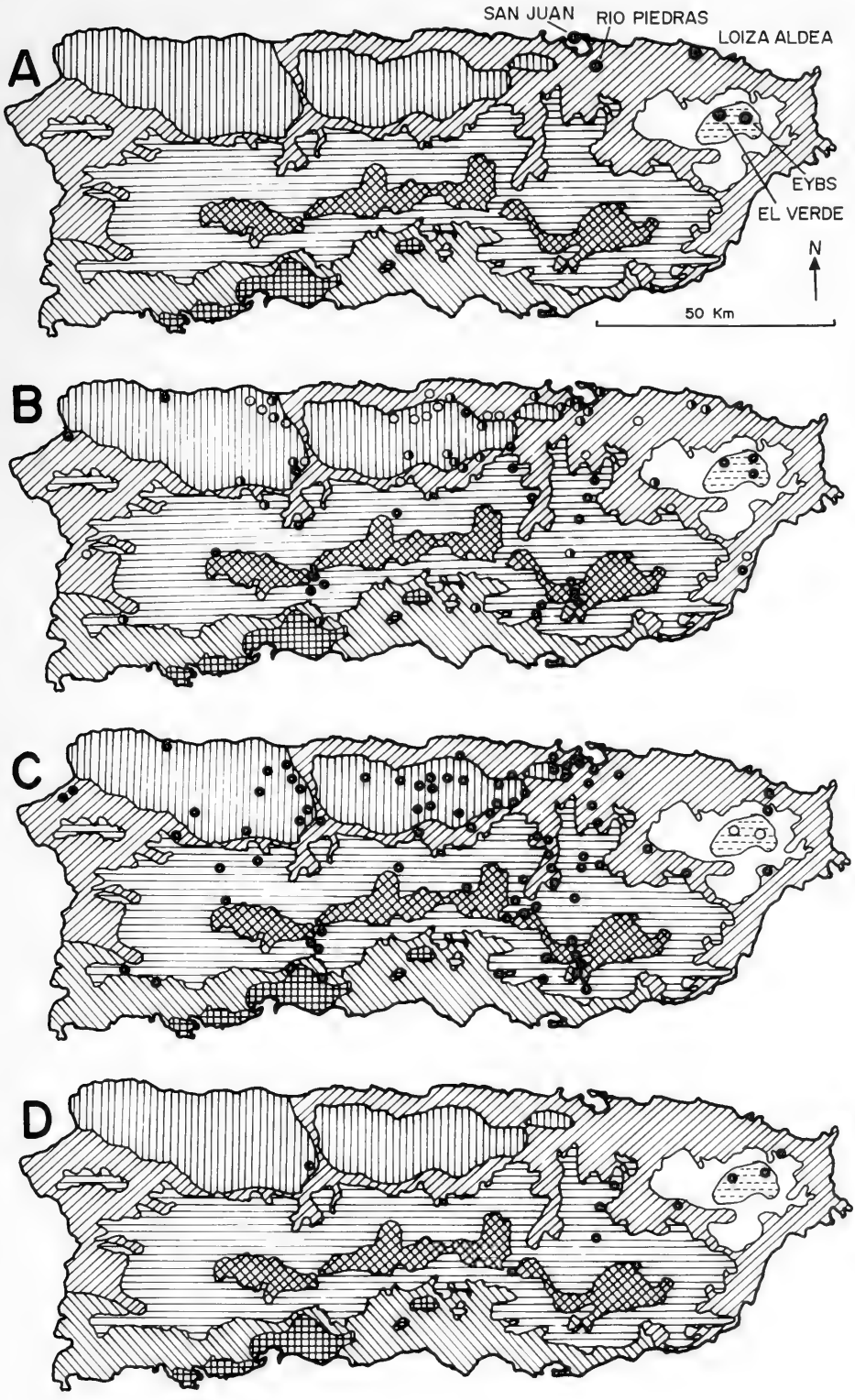
A
SAN JUAN
RIO PIEDRAS
LOIZA ALDEA
EYBS
EL VERDE
N
50 Km
B
C
D

TABLE 1. Shell morphology of adult Puerto Rican camaenid snails.

Species	Form	No. of whorls	Shell diameter (mm) $\bar{x}$	Shell diameter (mm) Range	Shell weight (g) $\bar{x}$	Shell weight (g) Range	Aperture diameter (mm) $\bar{x}$	Aperture diameter (mm) Range	Shell color and texture
Caracolus carocollus	Low spire	5-6	56.9	44.0-65.0[1]	20.8	19.9-26.8	25.7	21.5-27.6	Dark brown, smooth
Caracolus marginella	Low spire	4-5	34.8	31.0-37.4	13.4	11.4-15.0	14.6	13.0-16.8	Pale brown with reddish brown stripe, smooth
Polydontes luquillensis	High spire	4-5	34.9	28.0-38.0[2]	22.7	21.2-24.2	15.2	11.2-17.1	Dark brown, smooth
Polydontes lima	High spire	4-5	28.0	18.5-30.1[3]	16.7	14.1-24.4	12.8	11.5-13.6	Cream to pale brown, usually sculptured
Polydontes acutangula	Flat above, rounded below	2-3	44.6	37.1-49.1[4]	17.5	15.9-19.1	23.9	22.1-25.7	White nearly throughout, smooth

[1]In the size range 44-50 mm, 7.5% were adults; in the size range > 50 mm, 84.1% were adult; the largest juvenile captured was 56 mm. Van der Schalie (1948) on the basis of 313 specimens from various parts of the island gives the range as 42-59 mm with most grouped between 44 and 55 mm. Zilch (1959-60) lists 27-65 mm.
[2]Zilch (1959-60) gives a very different range (35-70 mm) which is probably in error.
[3]Zilch (1959-60) lists 18-30 mm and Van der Schalie (1948) 19-32 mm.
[4]Zilch (1959-60) lists 30-50 mm.

tive, *Pleurodontites* in the Miocene of Florida. Ancestral *Caracolus* and *Pleurodonte* reached the West Indies and *Polydontes* and *Zachrysia* have developed there from antecedents to *Pleurodonte. Pleurodonte* is now restricted to Jamaica and the lesser Antilles and has become extinct on the other Greater Antilles, where an extensive radiation of its derivative, *Polydontes,* has occurred.

A number of distinctive lineages within *Polydontes* can be recognized and have been given subgeneric rank (Wurtz, 1955). The Puerto Rican species are each assigned to a different subgenus, *P. acutangula* to *Parthenia, P. lima* to *Granodomus* and *P. luquillensis* to *Luquilla. Granodomus* and *Luquilla* share an advanced feature of the penial complex, namely that there is a caecum on the flagellum. *Parthenia* is distributed on Hispaniola and Puerto Rico, *Luquilla* is endemic to Puerto Rico, while *Granodomus* extends from Puerto Rico to the Virgin Islands. *Parthenia* is closer to the ancestral stock, *Luquilla* is a montane rainforest derivative, whereas *Granodomus* is better adapted to aridity and has a correspondingly wider distribution.

The biogeographic and ecologic outcome of the interaction of these genetically similar (confamilial) stocks in the varied environments of Puerto Rico is the subject of the following parts of this paper.

Several subspecies have been described for *C. carocollus, C. marginella* and *P. lima* and are listed by Aguayo (1966). However, Van der Schalie (1948) has shown that in *P. lima* at a given locality one encounters the entire size range for the species, and both heavily sculptured and smooth individuals occur together; the proportion of large, coarsely sculptured individuals decreases with increasing altitude, but not in a way conducive to separating out subspecifically named populations. Similarly, he found considerable variation in size in *C. carocollus* but no geographical correlates. We do not have data relevant to polymorphism in size or shell sculpturing at the infraspecific level and cannot make subspecific nomenclatural assessments. Consequently, although we follow the generic and specific nomenclature of the most recent author (Aguayo, 1966), we follow Van der Schalie (1948) in not employing trinomials for any of the Puerto Rican camaenids.

All of the Puerto Rican species are

dextrally coiled and large. All have brown or gray bodies and can pull completely into the shell, except *P. acutangula* which has a bright lemon-yellow body with a brown margin to the foot and cannot completely retract. The species can be additionally distinguished on the basis of shell shape, color and dimensions (Table 1).

Morphologically there are three groups, arranged as two species-pairs (each with a large and a smaller species) and a single species. These groupings are not necessarily indicative of closeness of genetic relationships (see above). One species-pair contains the two flat, round species, *C. carocollus* (large) and *C. marginella* (smaller); the second pair consists of the taller-spired, dome-shaped species, *P. luquillensis* (large) and *P. lima* (smaller). *P. acutangula* with its rather unusual shape is in a group by itself. These species are shown in Fig. 1.

Distribution of the native Puerto Rican species

The distribution from our data and that published by Van der Schalie (1948) is shown in relation to the major vegetation types in Fig. 2. In each of the morphological species-pairs one member tends to inhabit the cooler, more mesic areas and the other the more xeric, warm ones. The geographical separation is most distinct in the *lima-luquillensis* species-pair. *P. luquillensis* has a very restricted distribution, being known only in the wettest, upland rainforests at the eastern end of the island (Fig. 2). By contrast *P. lima* is relatively widespread in the various moist to xeric forests but is absent from the wet, cool forests and from the arid, hot regions. There are altitudinal correlates as well, *P. luquillensis* occurring only in the higher mountains, *P. lima* from moderate elevations down to coastal localities. As far as is known the two species are completely allopatric and thus do not presently compete.

P. acutangula is found in the wettest montane localities in Puerto Rico and at scattered localities in other mesic forests in the eastern third of the island. One locality is an exception (moist limestone forests on the northern coastal plain in the western third of the island; Fig. 2).

C. carocollus and *C. marginella* although largely separated altitudinally and by forest type do show considerable geographic overlap. *C. carocollus* is eurytopic, but primarily from moist areas, particularly in the uplands. It occurs in the wettest montane rain forest, in all of the mesic forest types including coastal ones and even in a few scattered localities from the drier vegetation of the southern coast. *C. marginella* is almost entirely a coastal lowland form, being known only from one (roadside) locality above 150 m. It is most widespread on the northern coastal plain, being known only in a few localities on the more arid southern one. Thus, in the wet upland only *C. carocollus* is represented, but on the coastal plains both species occur and are recorded in the same locality by Van der Schalie (1948). However, the extent of overlap is not as great as would appear. In most lowland localities where *C. carocollus* is abundant, *C. marginella* is relatively uncommon and vice versa. *C. carocollus* inhabits the moister pockets of habitat, and *C. marginella* the drier ones.

The dry southern coast is nearly devoid of camaenids of all species. It is the hottest and most arid part of the island and perhaps exceeds the capacity of adaptation for this group. The few localities in which camaenids are known may represent pockets of unusually moist or cool habitat. It should also be mentioned that the boundaries of the vegetation types are not as precise as a line on the map might suggest; also the superimposition of Van der Schalie's localities on the vegetation map involves a certain amount of plotting error. Consequently, undue significance should not be attached to dots that fall on or very near boundary lines.

In summary, *P. luquillensis* occurs in very wet, cool rain forests and is restricted to the higher altitudes of the Luquillo Mountains. *P. acutangula* is also restricted to the wet and mesic forests of the uplands but has a wider distribution. *C. carocollus* is eurytopic and widespread, but is most common in the cool, moist uplands and in the moister areas of the coastal habitats. *P. lima* is widespread in mesic to moderately dry areas at moderate elevations and down to the coastal plains. *C. marginella* is primarily on the north coastal plain in the drier habitats. The southern coastal plain is probably too hot or dry to support camaenids except in a few localities. In the moist uplands *P. luquillensis, P. acutangula* and *C. carocollus* occur together. In the lowlands *P. lima, C. marginella* and *C. carocollus* occur sympatrically, though the

latter may be partially separated from the others by habitat.

Since for each species-pair the largest member is primarily from the cooler moist uplands and the smaller member from the warmer drier lowlands, it is tempting to speculate that moist, cool conditions select for larger size or that a warm dry environment selects for small size. For the two most eurytopic species, the one that is most successful in the uplands (*C. carocollus*) is large and the one that is most successful in the lowlands is small (*P. lima*). However, the variation within *P. lima* does not support this view, as large-sized individuals are less common at higher than at lower elevations (Van der Schalie, 1948).

The general characteristics of the Puerto Rican camaenids have been outlined above and their distributions described. The next section focuses on the finer details of their environmental relations, i.e. their microdistribution, their use of space and time and their maintenance activities. To accomplish these ends four study areas were selected that collectively included all five species. Details of these areas are given in Appendix 1.

In Table 2 we present the 18 categories of data collected for the five species and the relative quantity of each type of data for each species. We have more data for some species than for others. For example, we have considerable data for 15 or 18 categories for *C. carocollus* and only 6 of 18 for *C. marginella.*

DEFINING THE HABITAT AND NICHE

There are various ways syntopic species can avoid competition. Niche separation can be achieved via temporal or spatial segregation and by use of different resources such as food, shelter, oviposition sites or combinations of the above. Consequently, if one wishes to ascertain the way species have evolutionarily adjusted to other species occupying the same general area, comparisons of activity cycle, habitat selection and use, food habits, and defense against other organisms are essential. Conversely, the niche breadth of a given species can be appreciated by looking at these attributes in the various types of environments occupied by a single species. The purpose of this section is to examine these aspects of the ecology of the Puerto Rican camaenids.

The important points to be made in this section are: (1) The species are primarily nocturnal. (2) The daytime microhabitat occupied by juveniles differs markedly from that inhabited by adults in all species, with the juveniles occupying cooler, wetter and less exposed microhabitats than the adults. (3) These age differences are more pronounced in the Loiza Aldea habitat

TABLE 2. Species and localities used for each of the studies reported in this paper. "X" means that considerable data were obtained. "+" indicates some data but less than was desired for conclusive statements; "—" means no data.

	Caracolus carocollus			*Caracolus marginella*	*Polydontes luquillensis*	*Polydontes acutangula*	*Polydontes lima*
Type of data	El Yunque	El Verde	Loiza Aldea	Rio Piedras	El Yunque	El Yunque	Rio Piedras
Daily activity	X	+	+	X	X	X	+
Habitat selection	X	X	+	X	+	X	+
Home site ranges	X	X	—	+	X	X	+
Food	X	X	—	—	X	X	X
Defensive secretions	—	—	—	—	+	+	—
Mantle water	X	—	X	—	+	—	+
Size at mortality	X	—	X	+	X	+	+
Copulation	X	+	+	+	+	+	+
Eggs	—	—	+	—	—	+	—
Albumen gland cycle	X	—	X	—	—	—	—
Growth	X	X	—	—	X	X	—
Population structure	X	—	—	—	X	—	—
Population density	X	X	—	—	—	—	—
Body temperature		+		+	+	+	—
Temperature tolerance		X		X	X	X	X
Stored reserves		X		X	—	—	—
Evaporative losses		X		X	X	X	X
Tolerance to water loss		X		X	X	X	X

TABLE 3. Effect of weather and time of day on activity of snails. "Highland" observations were made at the El Yunque Biological Station and "lowland" ones at Rio Piedras.

Species	Weather	Activity	Hour beginning 0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800	Total	All weather time
Caracolus carocollus (highland)	Sunny	Active	—	0	1	4	3	1	8	3	0	0	0	20	
		Not active	—	22	104	121	61	86	147	101	14	—	—	656	
		% active	—	0	1.0	3.2	4.7	1.2	5.2	2.9	0	—	—	3.0	
	Cloudy	Active	—	1	6	8	3	1	2	0	3	—	—	24	57
		Not active	—	14	62	89	34	26	23	12	27	—	—	287	1404
		% active	—	6.7	8.8	8.3	8.1	3.7	8.7	0	10.0	—	—	7.7	3.9
	Raining	Active	—	0	3	1	0	3	2	0	0	2	2	13	
		Not active	—	9	74	58	26	90	89	69	28	12	6	461	
		% active	—	0	3.9	1.7	0	3.2	3.3	0	0	14.3	25.0	2.7	
Caracolus marginella (lowland)	All-weather	Active	—	0	2	0	0	0	0	2	0	0	—	—	4
		Not active	—	6	32	18	1	3	10	25	21	15	—	—	131
		% active	—	0	5.9	0	0	0	0	7.4	0	0	—	—	3.0
Polydontes acutangula (highland)	All-weather	Active	0	1	1	0	0	1	4	0	0	3	1	—	11
		Not active	3	6	12	9	11	2	5	13	2	9	2	—	74
		% active	0	14.3	7.7	0	0	33.3	44.4	0	0	25.0	33.3	—	12.9
Polydontes luquillensis (highland)	All-weather	Active	—	—	0	1	0	0	2	1	—	5	—	—	9
		Not active	—	—	2	2	2	1	5	4	—	0	—	—	16
		% active	—	—	0	33.3	0	0	28.6	20.0	—	100.0	—	—	36.0
Polydontes lima (lowland)	All-weather	Active	—	—	0	0	0	—	0	0	0	0	—	—	0
		Not active	—	—	1	6	4	—	2	5	5	5	—	—	28
		% active	—	—	0	0	0	—	0	0	0	0	—	—	0

where more extreme conditions prevail than in the cool, constantly moist El Yunque area. (4) *P. acutangula* differs in daytime habitat from the other upland species. (5) More adult *C. carocollus* are found on large trees than on small ones; they do not use trees with scaly or flaking bark, but otherwise do not seem to show preference for particular tree species, although individual trees may be favored because of an abundance of crevices or other shelter sites. (6) Few snails occur where slopes are very steep.

Activity period

All the species are primarily nocturnal and thus do not have temporal segregation. The 2,969 activity observations that were made are summarized in Tables 3 and 4 by species, time of day, and for *C. carocollus* (for which the most observations were available) by weather conditions. At night, the proportion of snails which were active was high whereas comparable daytime means were low. In general, the lowland species had fewer individuals active by day than did those from montane areas where conditions were not as harsh. During daylight hours, there were no consistent temporal trends in the proportion of snails which were active; rather, activity remained at a similar, low level throughout the day. The few high diurnal values occurred when absolute numbers of snails were very low and can thus be attributed to chance.

Weather conditions affected diurnal activity in *C. carocollus.* On cloudy days there were twice as many snails active as on sunny days. On the other hand, rain inhibited activity, and the activity level was slightly lower than on sunny days. Immediate factors influencing diurnal activity are not evident from the field data, as temperature, humidity and light intensity all differ between cloudy and sunny days. However, humidity may be involved, as snails kept at high humidities in the laboratory seemed to be more active than those in dry containers which were otherwise similar. By contrast, Cameron (1970b) found that temperature affected the activity of several species of helicid snails.

TABLE 4. Nocturnal activity of snails in the montane study areas.

Species	No. active	No. inactive	Total	% active
Caracolus carocollus	551	273	824	66.9
Polydontes acutangula	3	1	4	75.0
Polydontes luquillensis	319	88	407	78.4

The nature of the activity in which snails were engaged did not differ between day and night. Active snails usually foraged over various substrates. A number of copulations were observed during the day as well as at night.

Habitat selection

At night most species roam freely over the forest floor, tree trunks or almost any object within their home range, and with the exception of *P. acutangula* which is most often found on tree leaves, no interspecific difference in foraging habitat was evident. During the day, however, there were interspecific as well as intraspecific differences in the sites selected for spending the inactive period.

On two occasions (31 Dec. 1964 and 30 Jan. 1965) an intensive search was made for *C. marginella* at the Rio Piedras study area, in the two locations where they had been most commonly found, i.e. on tree trunks and under objects (stones, logs or other debris). Both times juveniles were disproportionately represented under objects, whereas the majority of individuals on tree trunks were adults, indicating differences in microhabitat between the two groups (Fig. 3). These data represent only a random sample of the sizes of animals found in the two places; the histograms of Fig. 3 cannot be used to compare relative snail densities in the different microhabitats, as no attempt was made to sample an equal proportion of the total number of tree trunks and objects on the ground.

Juveniles of *C. carocollus* were not abundant in the Loiza Aldea study area. Only on 26 October 1965 were a sufficient number of juveniles found for analysis of habitat selection to be made. All juveniles were under rocks, many of them occupying small cracks or solution pits in the underside of porous limestone boulders. Only three adults were found in the same locations. By contrast, all individuals encountered by carefully excavating plots of leaf litter were adults (Fig. 3). No individuals were seen on tree trunks. The small number of young snails encountered on a number of other occasions collectively supports the conclusion based on the large sample of

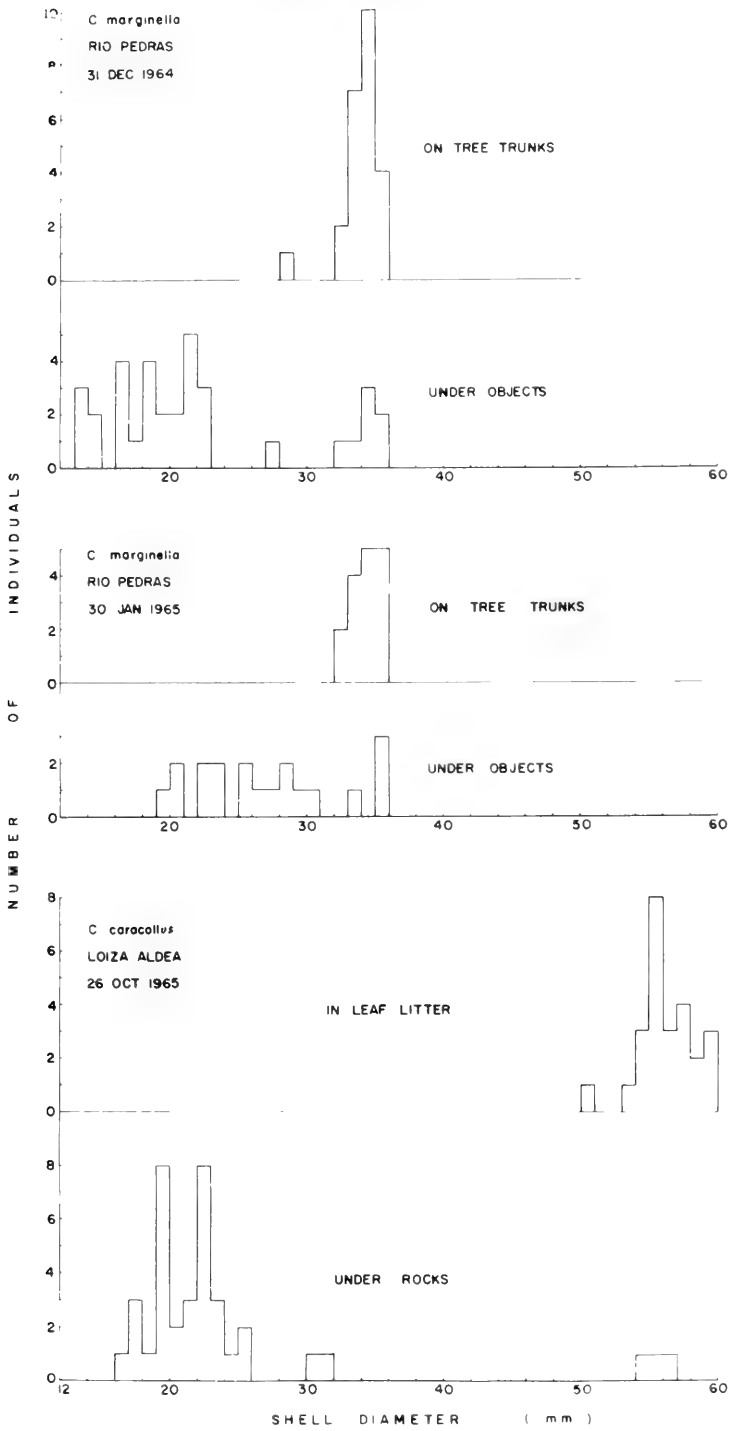

FIG. 3. Frequency of different size classes of *Caracolus marginella* and *Caracolus carocollus* in different microhabitats.

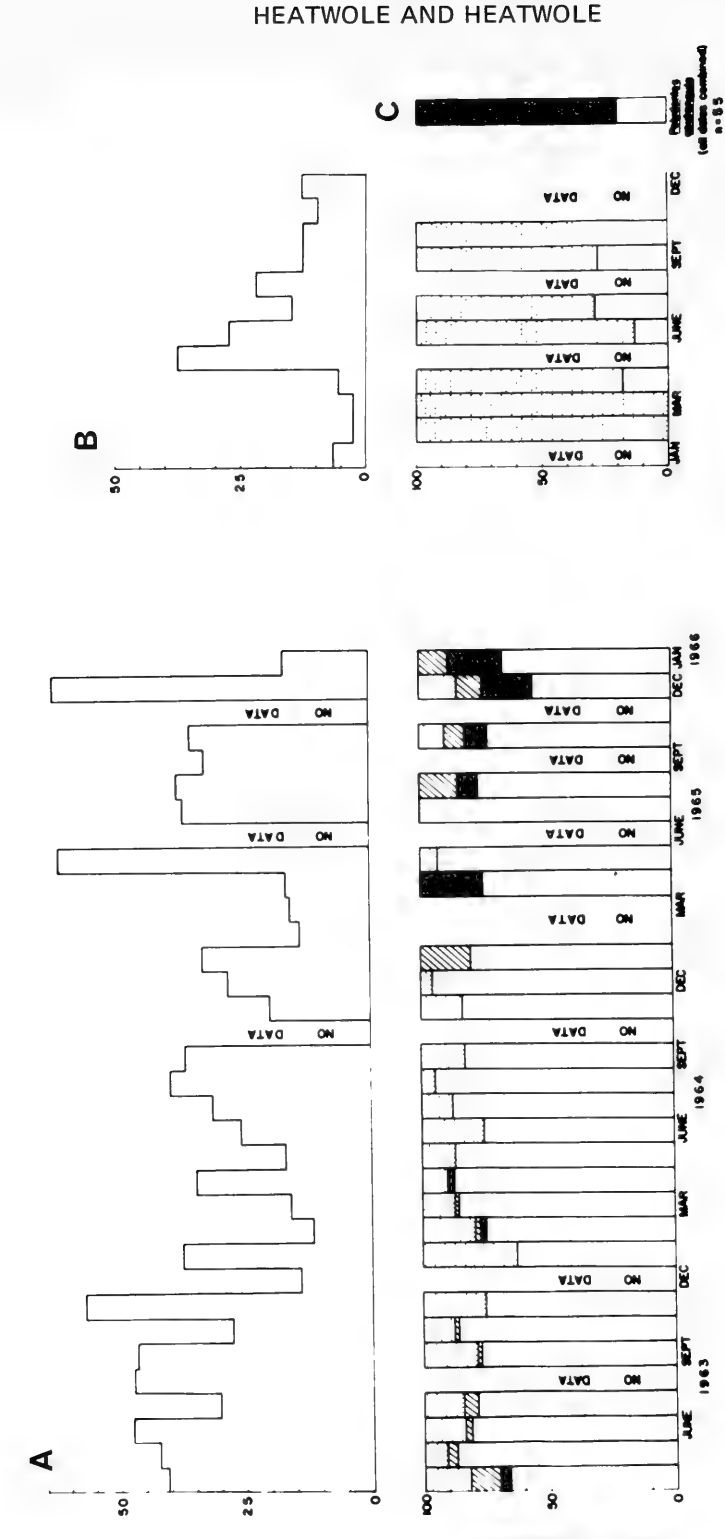

FIG. 4. Distribution of adult snails among various microhabitats in relation to rainfall. A, *Caracolus caracollus* near the El Yunque Biological Station. B, *Caracolus caracollus* at the Loiza Aldea area (composite of the years 1963-66). C, *Polydontes acutangula* from El Yunque. Rainfall data for El Yunque measured at La Mina, that for Loiza Aldea forest at San Juan (Appendix 1, Fig. 38). Open sections of bars indicate snails on tree trunks, cross-hatched ones snails in axils of tree limbs or in tree holes, dotted ones snails on the ground or in leaf litter, and black ones snails on the foliage of trees.

26 October. Of the total of 30 such juveniles found, 24 (80%) were under rocks and 6 (20%) on tree trunks; none were in litter. Adults found during the same observation periods were primarily in the leaf litter, with lesser numbers on tree trunks (see Fig. 4).

C. carocollus shows similar behavior in the El Yunque study area except that juveniles occupy a greater variety of daytime microhabitats. Bromeliads do not occur in the Loiza Aldea forest, but are abundant at El Yunque and are frequently occupied by snails. Bromeliads were carefully examined on October 23-24, 1964, and all snails taken from them were measured. One-fourth (N = 16) were juveniles. A slightly greater proportion (39%, N = 28) of those found under fallen palm petioles on the same date were juveniles, whereas only 15% (N = 13) of those on tree trunks were.

Quantitative data were not obtained for the other species occurring in lower population densities. However, it was noticeable

FIG. 5. Views of snails during their inactive periods at their home sites. A, *Caracolus carocollus* on a banana petiole near the Biological Station. B, *C. carocollus* on the Biological Station building. C, many *C. carocollus* on the trunk of a broadleaf tree in the Downhill Plot. Note that many of the snails are marked. D, *C. carocollus* on the trunk of a Sierra Palm in the Downhill Plot. E, *Polydontes acutangula* on the stem of a hibiscus plant near the Biological Station.

in the Rio Piedras study area that juvenile *P. lima* were usually under rocks and adults were on tree trunks. At El Yunque most juvenile *P. luquillensis* were inside curled-up *Cecropia* leaves (leaves either hanging in vegetation or on the ground), or inside fallen, rolled-up palm petioles. Although adults sometimes occurred in these places, they were usually encountered on tree trunks.

P. acutangula juveniles were almost always in rolled-up leaves (usually above the ground), in bromeliad axils, or the axils of banana trees; adults were more exposed, usually on the leaves of trees.

Such ontogenetic differences in habitat may be common in land snails; Pollard (1975) found a similar situation in *Helix pomatia.*

During searches of the study area for other purposes, notes were made on the exact location of each snail observed. Searches were made under debris and on the ground as well as on trees. Because of the small size of juveniles and their ability to use very small crevices, many were probably overlooked, except when specific searches for them were made. However, the adults are conspicuous enough that many of them sheltering under debris or in leaf litter were found during a routine search. Thus, relative numbers of adults found in

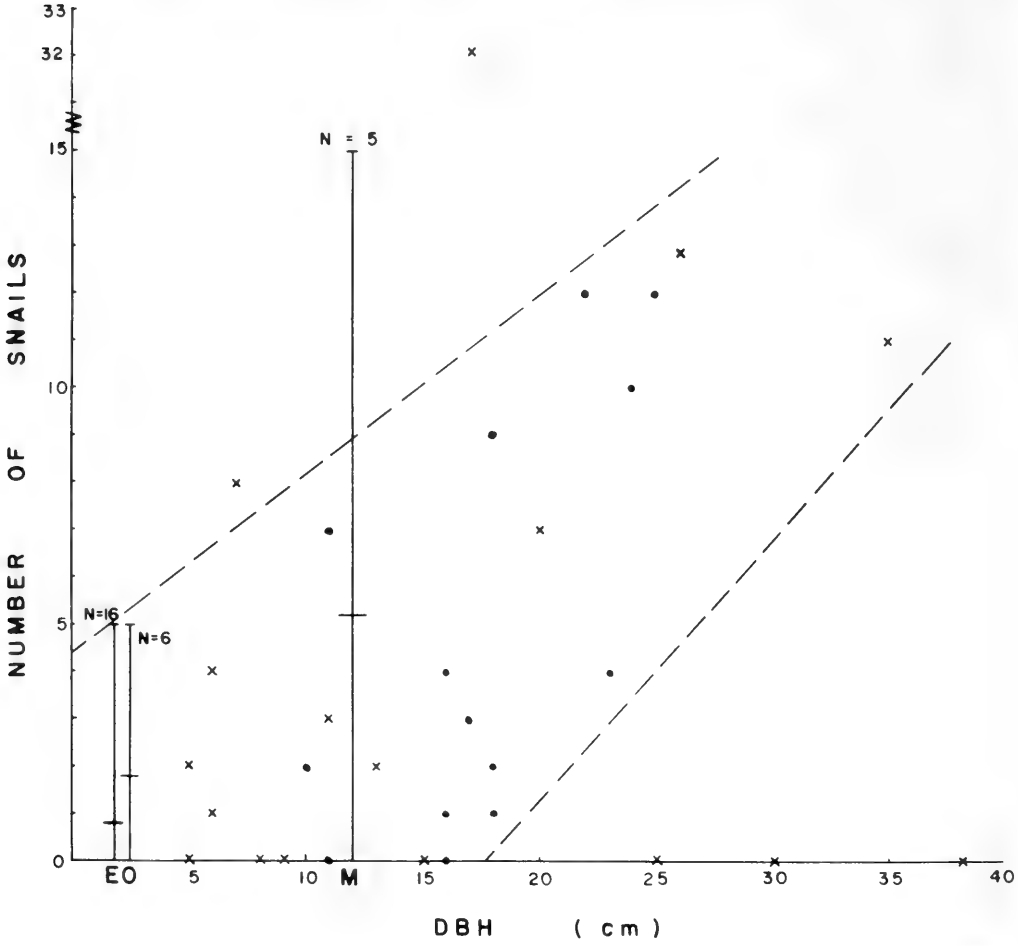

FIG. 6. Relationship of number of resident snails on tree trunks to tree diameter at breast height (*dbh*) in the Downhill Plot at the El Yunque Biological Station. All trees with *dbh* less than 5 cm were grouped as saplings (E represents mean and range for *Euterpe globosa*; O represents mean and range for all other species of saplings). M represents mean and range of banana plants (*Musa cavendishii*) at the level of their mean *dbh*. Dots represent *Euterpe globosa* and X's all other species. Broken lines indicate the limits of values for *E. globosa.*

different microhabitats could be a useful index of species differences or temporal changes in behavior within a species.

One of the most striking differences was between *P. acutangula* and the other species; 80% of the adult *P. acutangula* encountered above ground (during the part of the study when such data were recorded) were on leaves, usually of *Hibiscus,* banana, or palm plants. The remainder were on tree trunks or stems of shrubs. Adults of all the other species in the Montane Rain Forest primarily occupied tree trunks (Fig. 4).

In the El Yunque area, most adult *C. carocollus* spend the day on tree trunks with a relatively small proportion occupying the ground or other more sheltered sites. The reverse was true at Loiza Aldea where most adult snails were in the leaf litter with only a few on tree trunks (Fig. 4). It appears that in less favorable habitats (or at least those with greater fluctuations in temperature and less rainfall) adult *C. carocollus* uses more sheltered (cooler and moister) sites than it does in more equable parts of the range.

It was noticed that certain trees were used as a daytime resting site more often than others; some were observed to almost never have a snail present, whereas others had up to 19 individuals present at one time (Figs. 1, 5). At El Yunque more *C. carocollus* were generally found on large trees than on small ones (Fig. 6), but without differences in number of snails between tree species of the same size (Appendix 2). Thus, size not the species of tree usually influences the number of snails inhabiting trees. Several notable exceptions occurred. Three of the larger trees never had any snails on their trunks. Two of these were tree-ferns (*Cyathea arborea*) which have densely scaly trunks. The third was a Swamp Cyrilla (*Cyrilla racemiflora*) in which the bark splits off in scales or thin plates and becomes spongy at the base of the tree. All the other species have smooth to moderately rough-textured or furrowed bark, but not scaly or flaking.

Two trees of *Cecropia peltata* had numbers of resident snails that fell within the range of *Euterpe globosa*; a third, however, was exceptional in having many more resident snails than expected for its size. Indeed, it had more than twice as many snails as any other tree in the plot. This tree had one unique feature, a rather extensive prop root system at its base under which snails were frequently observed sheltering.

It was apparent in the field that snails were not uniformly distributed throughout the study areas (Fig. 7). *C. carocollus* avoids areas with very steep slopes. This may not be a direct response to topography, but to some factor in turn related to topography, e.g. amount of leaf litter or soil moisture. There is also another possible explanation. Snails were occasionally observed to fall from trees. Should this happen over a very steep slope, the snails would probably roll to the bottom.

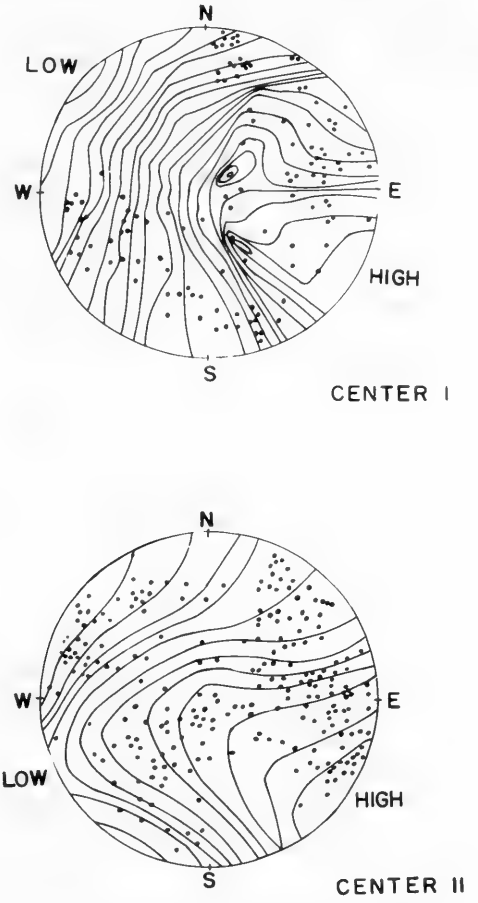

FIG. 7. Distribution of *Caracolus carocollus* in the El Verde study areas. N, E, S, W refer to compass points. High and Low refer to the higher and lower parts of the topographic map to assist in orientation. Contour lines at 1 m intervals. Dots indicate centers of home ranges of snails captured more than once. Center I is the control area not irradiated by gamma radiation and Center II the site that was radiated. Each center is 30 m in diameter.

Home site range

Snails seem to have the sensory perception and neural organization to recognize specific features of their external environment and to use environmental cues in maintaining themselves in, or returning to specific locations. Some are known to restrict their activities to rather small areas (Potts, 1975). The vineyard snail (*Helix pomatia*) tends to return to the same area each year to overwinter and when moved to a new site can accurately home (Edelstam & Palmer, 1950; Pollard, 1975). Similarly, Blinn (1963) found that two terrestrial species (*Mesodon thyroidus, Allogona profunda*) "homed" to overwintering sites in autumn, returning to the vicinity of their previous location every second year.

The home site has been defined as the "location in which a snail passes its inactive period of any given day" and the home site range (*hsr*) as the "area in which the home sites occur over a prolonged period of time" (Heatwole et al., 1970).

In the present study individual camaenid snails had a high fidelity to such sites and returned to them during their diurnal inactive period (Appendix 2, Figs. 8-10, Table 5).

Home site range varied widely among individuals, values ranging from 0.08 m^2 to 43 m^2 for *C. carocollus* at El Yunque; representative ones are shown in Fig. 10.

There was a regional difference in size of *hsr*. Mean areas of the *hsr* of *C. carocollus* at El Verde were 3-8 times as large as for that species at El Yunque (Table 5). At El Verde, mean *hsr* was 21-59 m^2 in the two centers, including periods both before and after irradiating one center.

For a given region, all species seemed to have approximately the same *hsr* areas or distances moved between home sites (Table 5).

Too few data were available for *P. lima* and *C. marginella* to permit accurate calculation of *hsr*. However, both species did show an affinity for particular locations, only one individual of the latter species and none of the former having ever been found at more than one home site (Table 7).

Juveniles do not disperse widely before setting up a home range, or if they do, they do so quickly and establish an *hsr* very early in life, i.e. well before reaching maturity (see Appendix 2).

There did not seem to be exclusion of any individuals from an area by others. On a number of occasions snails hanging on walls or tree trunks were in direct physical

FIG. 8. Home sites (dots) of *Caracolus carocollus* no. 17 recorded between 15 September 1962 and 31 July 1963, and enclosed in a polygon to indicate its home site range on the northwest corner of the El Yunque Biological Station building.

TABLE 5. Numbers of snails captured, characteristics of movements, and home site ranges (*hsr*). Areas are expressed as mean values and ranges. The percentage values refer to the % of the total number of snails marked. El Verde includes data from Heatwole et al. (1970). The radiation area was exposed to gamma radiation, the control area was not (see Heatwole et al., 1970).

	El Verde—*C. carocollus*				El Yunque Biological Station					
	Control		Radiation		*C. carocollus*		*P. acutangula*		*P. luquillensis*	
Number of snails captured only once	154	(58.7%)	158	(38.7%)	97	(26.1%)	60	(65.2%)	23	(71.9%)
Number of snails captured more than once but at only one site	18	(6.9%)	28	(6.9%)	23	(6.2%)	12	(13.0%)	1	(3.1%)
Snails captured twice (two sites)										
Number	40	(15.3%)	38	(9.3%)	21	(5.7%)	10	(10.9%)	5	(15.6%)
Mean distance between capture sites (m)	9.12	(0.5-51.5)	11.92	(0.5-41.6)	5.1	(0.2-14.3)	6.8	(1.2-21.2)	8.5	(3.2-25.0)
Snails captured more than twice but at only two sites										
Number	17	(6.5%)	36	(8.8%)	25	(6.9%)	2	(2.2%)		–
Mean distance between capture sites (m)	12.5	(1.0-55.5)	12.2	(0.8-47.5)	5.2	(0.6-20.6)	5.9	–		–
Snails captured at 3 or more sites (3-6 captures)										
Number	29	(11.1%)	62	(15.2%)	75	(20.2%)	7	(7.6%)	3	(9.4%)
Mean area of home site range (m^2)	44.1	(4.8-496.7)	30.2	(0.5-296.0)	3.2	(0.03-22.1)	4.29	(10.8-18.7)	3.26	(1.2-6.2)
Snails captured at 3 or more sites (7-19 captures)										
Number	4	(1.5%)	34	(8.3%)	66	(17.7%)		–		–
Mean area of home site range (m^2)	20.5	(7.0-43.7)	59.3	(2.0-569.1)	7.2	(0.08-43.4)		–		–
Grand mean for all snails captured at 3 or more sites										
Number of snails	33	(14.5%)	96	(23.5%)	141	(75.8%)		–		–
Mean area	35.8	(4.8-496.7)	21.0	(0.5-569.1)	5.1	(0.03-43.4)		–		–
Snails captured more than once but only on the wall of a building (area of *hsr* not calculated)		–		–	44	(11.8%)		–		–
Snails moving *hsr* away from building after its walls were painted										
Number		–		–	7	(1.9%)		–		–
Mean distance from wall to new *hsr* center		–		–	12.0	(3.0-20.0)		–		–
Snails spontaneously moving *hsr*										
Number	0		1	(0.2%)	14	(3.8%)	1	(1.1%)		–
Mean distance between two *hsr* centers		–		–	14.6	(2.6-28.0)	20			–
Total number of snails marked	262		408		372		92		32	

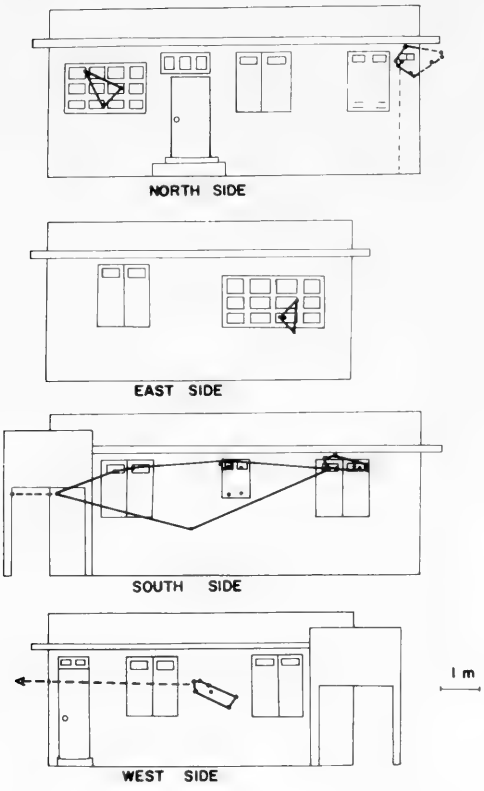

FIG. 9. Home sites of *Caracolus carocollus* on the building of the El Yunque Biological Station. Each dot represents an observed location. Solid lines enclose all home sites for a given individual. Dotted lines indicate home site range around corner of building (upper sketch) or movements to a new area (lower sketches). Arrow indicates a home site off the building. Dates of capture for individual snails occur over periods of 42 days to 8 months and 15 days.

contact with each other, sometimes in large clusters of individuals. Occasionally one snail was attached to the shell of another. Consequently, the *hsr*'s of different individuals broadly overlapped and if all were plotted on Figs. 8-10 one would be unable to distinguish individual outlines.

Most *C. carocollus* favored a particular home site over others. Of those that were observed in only two sites, more than 80% of the observations were in one place and less than 20% in a second place (on some occasions, of course, given snails could not be found); even among snails that were observed occupying a number of different home sites, their use of such places was not evenly distributed, one to three sites being strongly favored and the remaining ones used only occasionally (Table 6).

Data on home range (as opposed to home site range) were very few for any species. Active *C. carocollus* were observed on the forest floor at El Yunque Biological Station as much as 20 m from their known *hsr.* By contrast, 157 marked *P. luquillensis* on a retaining wall near La Mina on the average moved 7 m or less in a horizontal dimension during their nocturnal activity (Table 7).

Food

Food habits of a given species differed in different areas. Eleven *C. carocollus* at El Yunque were observed feeding at night; none were seen feeding by day. The food consumed consisted of a wide variety of items. Three snails were observed eating dead, brown leaves (one leaf was *Hibiscus,* the other two unidentified), two were

TABLE 6. Percentage of observations of *Caracolus carocollus* at different home sites at El Yunque. Only snails for which there were precise data on home site locations are included.

Number of snails	Number of captures	Home sites numbered in order of decreasing use											
		1	2	3	4	5	6	7	8	9	10	11	12
10	72	81.9	18.1										
13	80	60.0	22.6	17.4									
28	159	41.4	21.6	19.0	18.1								
35	245	31.6	21.3	16.1	15.7	15.5							
15	124	29.6	17.3	13.9	13.9	12.7	12.7						
6	68	29.4	20.2	11.4	9.8	9.8	9.8	9.8					
6	80	27.1	17.2	13.1	9.9	8.2	8.2	8.2	8.2				
2	20	14.7	14.7	10.1	10.1	10.1	10.1	10.1	10.1	10.1			
3	42	23.9	14.3	9.4	9.4	7.2	7.2	7.2	7.2	7.2	7.2		
2	31	16.1	16.1	9.4	9.4	9.4	6.7	6.7	6.7	6.7	6.7	6.7	
1	16	18.8	12.5	12.5	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
121 Total	937 Total												

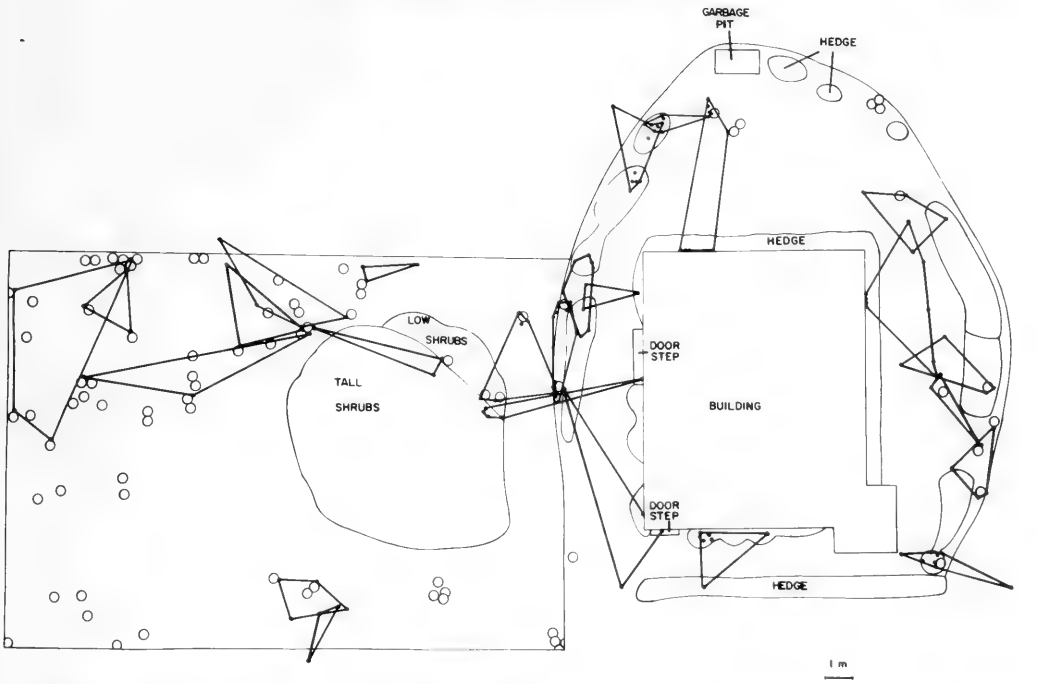

FIG. 10. Some representative home site ranges from the El Yunque Biological Station area. Dots represent home sites, larger circles represent tree trunks.

TABLE 7. Number of snails marked and recaptured at Rio Piedras (October 1963 to May 1965) and on two retaining walls at El Yunque (April 1965 to January 1966). All snails at Rio Piedras were captured at their original sites only. Those on retaining walls were at various vertical positions on the wall but in one horizontal dimension and no *hsr*'s could be calculated.

Species and locality	Number of times captured							Total number of snails
	1	2	3	4	5	6	7	
Retaining wall								
P. luquillensis								
Number of snails	73	30	25	9	29	0	0	157
Distance between two furthest capture points (m)	—	6.7	5.1	6.1	3.6	—	—	
Rio Piedras								
C. marginella								
Number always captured at same site	74	21	7	4	3	3	1	113
Number captured at more than one site (distance between furthest capture point in parentheses)	0	1 (3.2 m)	0	0	0	0	0	1
P. lima[1]								
Number always captured at same site	18	2	2	0	0	0	0	22

[1]No animals recaptured at other than the original site.

eating unidentified live green leaves, two were eating large seeds (one was *Ormosia krugi,* the other unidentified), two fed on wet, discarded paper, and one each on arum roots and on flowers of the tree *Inga vera.* In the laboratory, snails readily fed on *Hibiscus* leaves, raw carrots and paper. Clearly the food preferences of this species are very wide. Only two individuals of other species were observed eating; both were *P. luquillensis* eating dead, yellow *Hibiscus* leaves.

All macroscopically identifiable items in the feces were either parts of leaves, hard fibrous tissues (wood, bark, seeds, etc.) or animals. The last was relatively unimportant and probably represents accidental ingestion of dead animal material (Table 8). The proportion of leafy versus woody material in the feces varied more among localities than among species. All three species analyzed from the El Yunque Biological Station had 73-78% of the items represented by parts of leaves; the values for *C. carocollus* was 73%, whereas in the somewhat drier El Verde area the value for this species was 54%, a greater difference than among species at El Yunque. In the dry lowlands, the only species studied (*P. lima*) had a value similar to El Verde *C. carocollus*. However, the type of leafy material eaten varied among species within one area, some relying more heavily on dicot species, others on monocot ones. This may reflect availability of different plants in the respective microhabitats of these species rather than on actual selection.

There was a greater interspecific difference in the content of fecal material on the microscopic (Table 9) than on the macroscopic level. *C. carocollus* from both areas were similar in having predominantly diatoms represented in the feces, but differed from *P. acutangula* and *P. luquillensis* which had mostly spherocrystals (calcium oxalate), suggesting that they had fed to a relatively larger extent on araceous or other plants which contain these crystals, than

TABLE 8. Macroscopic composition of feces of some camaenid snails of Puerto Rico.

	% of macroscopic items									
Species and locality	Monocot leaf	Dicot leaf	Grass blades	Amorphous leaf tissue	Total leaf material	Thin fibers	Wood	Bark	Seeds	Insect cuticle
El Yunque										
Polydontes acutangula	34	21	6	17	78	8	14	0	0	0
Polydontes luquillensis	26	38	2	12	78	16	6	0	0	0
Caracolus carocollus	2	66	1	4	73	21	1	5	0	0
El Verde										
Caracolus carocollus	30	16	1	7	54	18	14	10	1	3
Rio Piedras										
Polydontes lima	13	37	5	2	57	18	9	14	2	0

TABLE 9. Microscopic composition of feces of some camaenid snails of Puerto Rico. N refers to total number of items.

	% of microscopic items					
Species and locality	Diatoms	Other unicellular algae	Ca oxalate crystals	Plant hairs	Wood cells	Leaf cells
El Yunque						
Polydontes acutangula (N = 410)	5	0	73	0	11	11
Polydontes luquillensis (N = 371)	2	0	65	½	22	11
Caracolus carocollus (N = 348)	51	1	21	0	16	10
El Verde						
Caracolus carocollus (N = 170)	42	1	5	11	34	7
Rio Piedras						
Polydontes lima (N = 509)	0	71	8	2	14	6

did *C. carocollus*. *P. lima* was very different from any other species in that it had mostly non-diatomaceous algae (mostly desmids) represented in its microscopic fecal elements.

Qualitative inspection of the fecal suspension revealed the presence of items in addition to those listed in Tables 8 and 9. *P. lima* feces contained sand or grit (probably accidentally ingested) and those of *C. carocollus* contained a whole mite, several pieces of bryophytes and an entire small invertebrate (either a parasite or a fly larva).

Thus, although there is broad overlap in the food eaten by the various species, some degree of ecological segregation does occur, especially in terms of microscopic food items. Some of these differences are independent of the area investigated but others are area-dependent. The abundance of the types of food eaten would suggest that competition for food does not occur among syntopic species. However, snails may experience a shortage of high quality food of particular kinds even when acceptable food is in abundance (review by Butler, 1976), and more information about the nutrition of these camaenids would be needed to make a complete assessment of interspecific interactions.

Defensive secretions

P. acutangula is unable to completely withdraw into its shell. Furthermore, its shell is relatively thin compared to that of the other species. In the absence of good protection by the shell, other defensive mechanisms might be expected. This species exudes a conspicuous yellow slime over the surface of its body when molested. It is suggested that this secretion is defensive. The only other Puerto Rican camaenid with colored slime is *P. luquillensis*. It sometimes exudes a very pale yellow secretion when molested. On one occasion, two *P. luquillensis* and two each of *P. lima*, *C. carocollus* and *C. marginella* were kept in a desiccator jar at 20°C in a saturated atmosphere with access to food and water. A second similar jar had the same species composition except that it lacked *P. luquillensis*. All the snails except *P. luquillensis* died in the first jar within 15 days, whereas all the snails in the second jar were alive and healthy in appearance at that time. This observation suggests that *P. luquillensis* has a deleterious effect on other species of camaenid snails when in close contact with them. This hypothesis should be tested in a more rigorous way, with testing extended to include the effects of *P. luquillensis* and *P. acutangula* secretions on potential predators.

The above two sections have indicated nearly complete overlap in activity cycle and broad overlap in food, but partial spatial separation among the camaenid snails of Puerto Rico.

The next section investigates how they are affected by and adapted to their physical environments.

RESPONDING TO THE PHYSICAL ENVIRONMENT

As discussed in the introduction, the physical environment has been shown to be an important selective agent in the life of snails generally. This section examines the physical parameters and relates interspecific differences in modes of adaptation to patterns of geographical distribution. Temperature and water were the two parameters selected for study, as they were considered most likely to be of prime importance in regard to interspecific differences in adaptation.

Body temperature

Environmental temperatures are higher in the lowlands than in the uplands. Consequently, it might be expected that coastal species (or populations) would adapt to lowland conditions by (1) developing higher temperature tolerances permitting maintenance of elevated body temperatures and/or (2) modifying heat exchange with the environment by behavioral or physiological means to the extent that body temperature does not rise above the level experienced by upland snails. (Because of potentially different thermal conditions in different parts of the habitats in a given region, species which are sympatric but ecologically segregated by microhabitat might show similar patterns even though perhaps on a reduced scale.) Finally, (3) differences in the habitat occupied by animals of different ages might be reflected in various aspects of thermal adaptation. It was discovered that all of the above situations were present to some degree in the adaptation of the Puerto Rican camaenids.

All species in montane rainforest had similar body temperatures; none exceeded 24°C. All measurements on *C. marginella* (lowland) were above 25°C and in one case reached 35°C (Figs. 12-15).

Although body and environmental temperatures are correlated, the latter are not precise predictors of the former (Figs. 12-14). Body and environmental temperatures were similar for animals in the shade or diffuse sunlight. Body temperature (T_B) correlates more closely with substrate temperature (T_S) than with air temperature (T_A), suggesting that heat conduction via the substrate plays a greater role in determining body temperature than does exchange with the air (Figs. 12, 13).

T_B values lower than either T_A or T_S are perhaps attributable to cooling by evaporation of water from the mantle cavity and/or general body surface. Several *C. carocollus* in the shade had body temperatures much higher than environmental ones (Figs. 12, 13). Some of these were obtained during a day when there were scattered clouds and their positions were such that at times when the clouds were not in front of the sun, the snails would be in or near direct sunlight. The others were obtained just after a brief shower which resulted in sudden lowering of environmental temperatures; presumably the high snail temperature reflected a lag in cooling.

The different morphologies and colors of the various species may be related to thermal adaptation. The lowland species (*C. marginella, P. lima*) are light in color whereas *C. carocollus* (which does extend to high altitudes) and *P. luquillensis* which are restricted to upland rainforest are dark. The only montane species which is light in color is *P. acutangula.* In contrast to other montane species which are tree trunk forms usually occurring in shaded places, it is often found on leaves, frequently in exposed situations.

Animals in sunlight absorbed radiant energy and usually had T_B's above either T_A or T_S (Figs. 12, 13). However, body temperature was usually lower than that of the black bulb thermometer (T_{BB}) placed at the snail's location (Fig. 14), suggesting

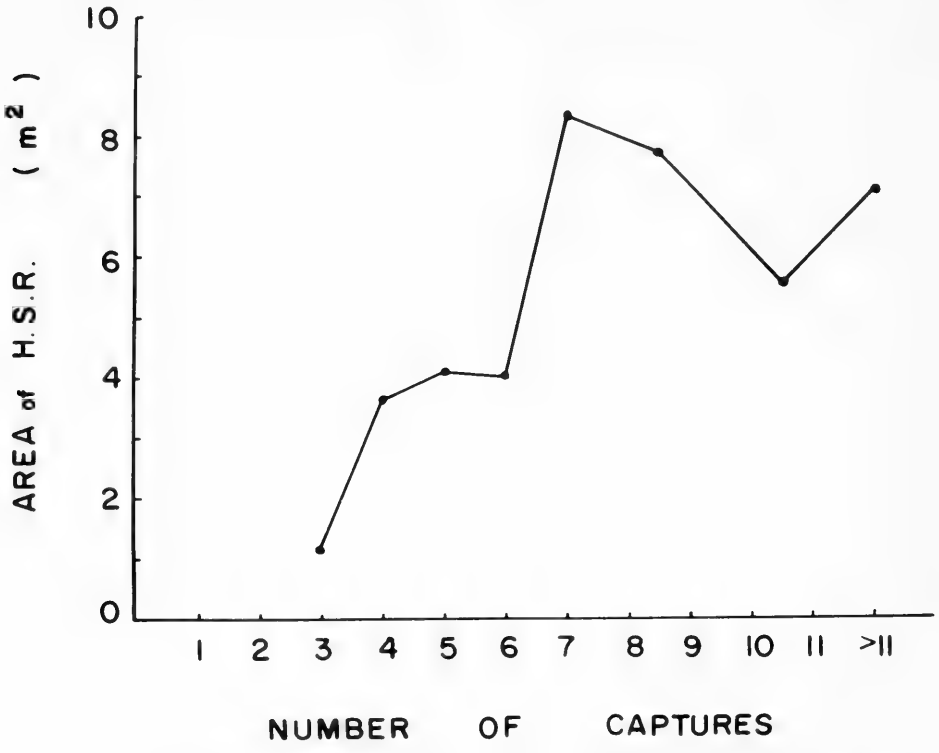

FIG. 11. Relation of estimated *hsr* size to number of recaptures of *Caracolus carocollus.*

either that the snails were less effective absorbers of radiant energy reflected from their surroundings than was the black bulb, or that they were losing heat by evaporative cooling or conduction to the substrate. No marked interspecific differences were observed in the relationship of T_B to T_{BB}. The single snail in direct sun for which a T_{BB} was obtained had a slightly higher temperature than the black bulb. Even so, the light-colored shell of *C. marginella* reflects much of the radiant energy. After measuring the body temperature of two animals, their shells were painted black. Within several minutes the T_B had raised by 4.7° in one and 8.9° in the other, although there had been no change in either air or substrate temperatures (Fig. 13).

It is probable that the reflective light color of *P. acutangula* and the lowland species is an adaptation preventing overheating. *C. carocollus* is dark throughout its range, including the lowlands. However, in coastal localities it tends to occupy leaf litter and other cryptic habitats, rather

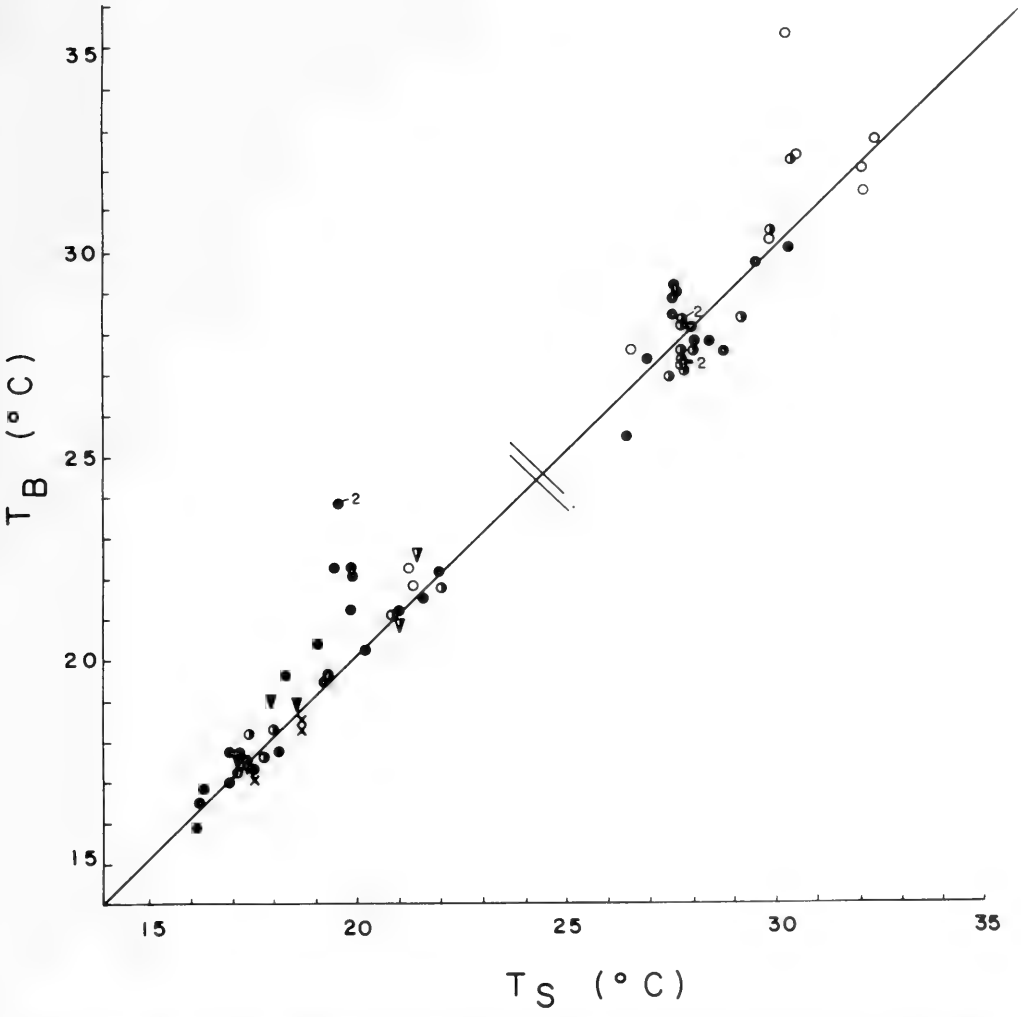

FIG. 12. Relationship of snail body temperature (T_B) to substrate temperature (T_S). Open symbols represent animals in direct sunlight, solid symbols those in the shade, and half solid ones animals in diffuse sunlight. Round symbols in upper part of graph (above 2 short parallel lines) represent *Caracolus marginella,* those in lower part (below short parallel lines) represent *Caracolus carocollus.* Triangular symbols represent *Polydontes acutangula* and X's *Polydontes luquillensis* (all in shade). Diagonal line is Y = X. "2" indicates dots representing two identical values.

than tree trunks; in such places it would be less exposed to direct solar radiation.

Evaporative cooling may also influence heat exchange. *P. acutangula* cannot retract its body completely into the shell and thus would be more subject to evaporative cooling than other species of equivalent size. The occupancy by this species of a more exposed habitat than other sympatric species may be facilitated by a combination of enhanced evaporative cooling and a light shell color.

The relation of shell color and thermal ecology is not restricted to the camaenid snails, nor is it always in the same direction. Jones (1973a, 1973b) suggests that in

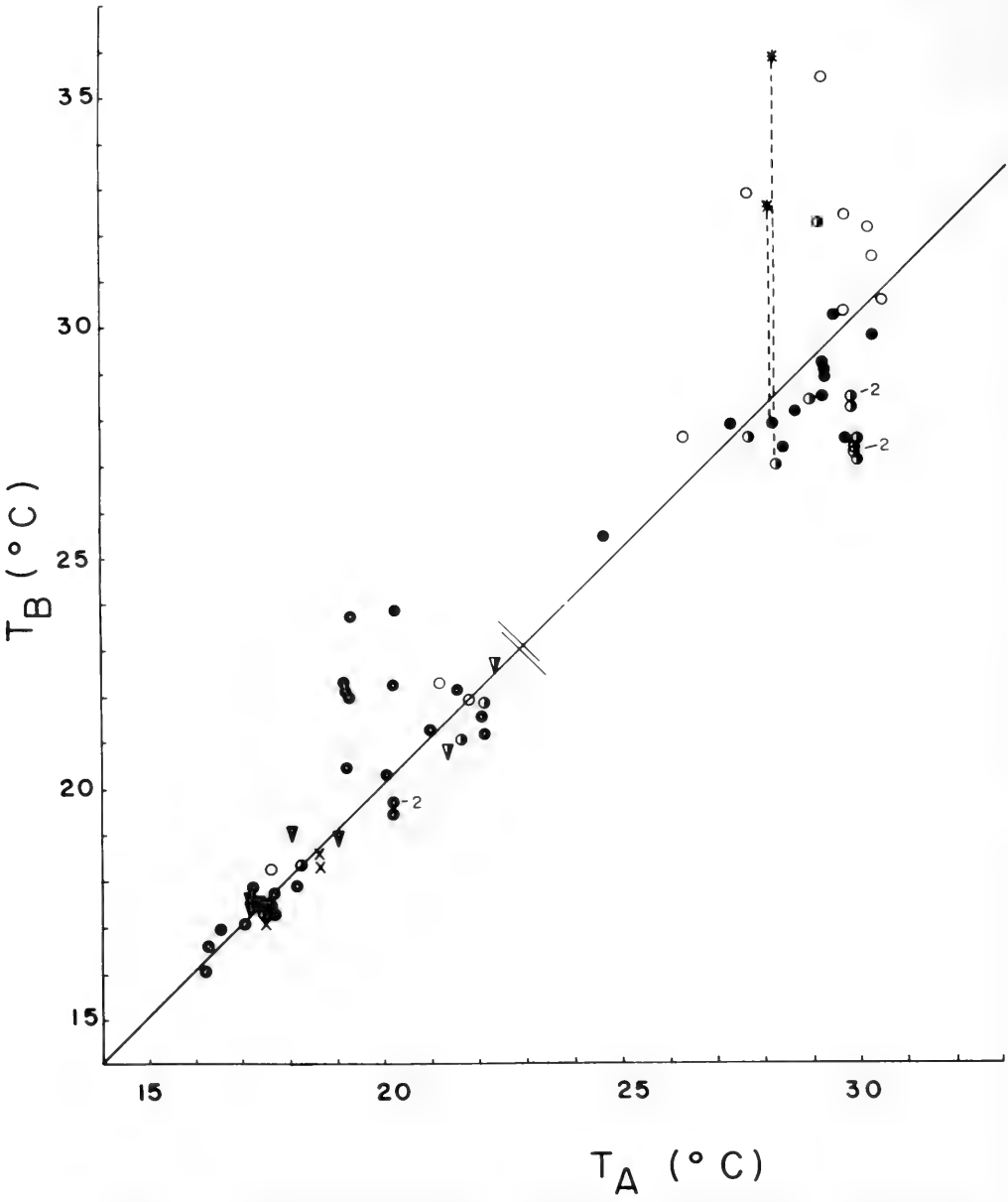

FIG. 13. Relationship of snail body temperature to air temperature. Symbols as in Fig. 12. Dotted lines leading to asterisks indicate increase in body temperature of two *Caracolus marginella* after their shells had been painted black.

warm areas, a light colored shell would be an advantage to snails through reflection of radiant energy, and dark shells an advantage in cold areas because of the enhancement of heating; indeed morph frequencies in species with color polymorphism often show such trends. It has been shown in both *Cepaea hortensis* (Sedlmair, 1956) and *Cepaea nemoralis* (Richardson, 1974) that there is a greater temperature tolerance in bandless (light colored) morphs than in darker ones. However, in the colder limits of its range in Iceland, bandless *C. hortensis* were more frequent in cold rather than in warm areas, i.e. the reverse to previously reported situations. Arnason and Grant (1976) suggest that this reversal near the colder limits is because a dark shell not only absorbs heat more rapidly but also back-radiates more rapidly in the absence of solar input. Thus, a light shell is an advantage in either hot or cold extremes but a disadvantage under intermediate conditions. This conclusion is supported by Arnold's (1968) findings in Spain; the bandless morph of *C. nemoralis* was more frequent under extreme conditions (both cold-dark-humid and hot-dry-open), than under intermediate ones.

Temperature tolerances

Two endpoints of heat tolerances were used, the falling point (temperature at

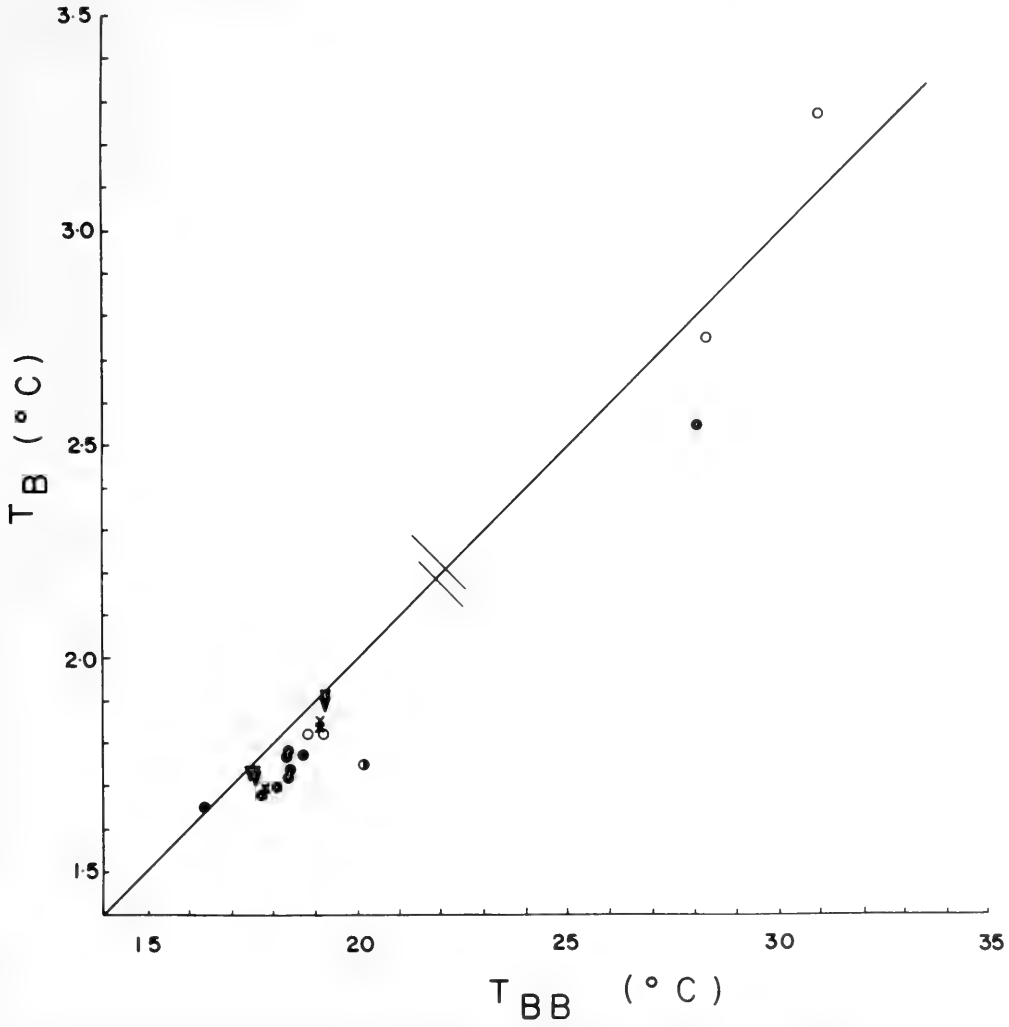

FIG. 14. Relationship of snail body temperature to black bulb temperature. Symbols as in Fig. 12.

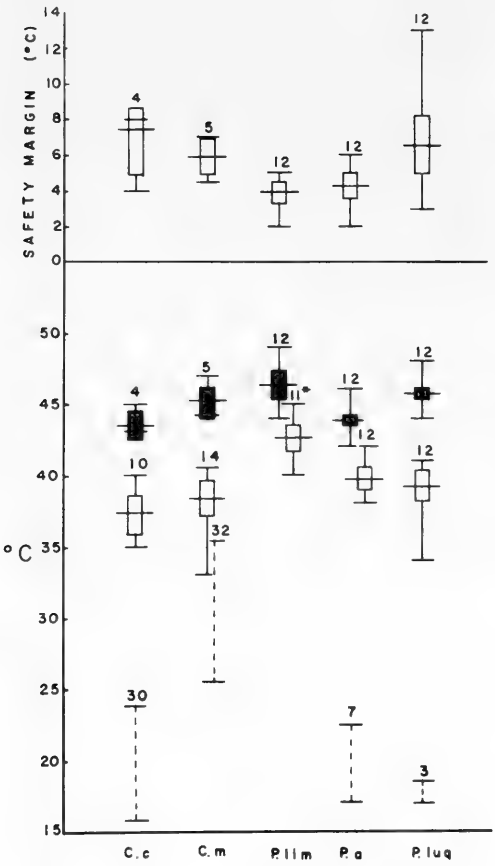

FIG. 15. Comparison of body temperatures (dotted lines), falling points (lower, open figures), lethal points (solid figures) and thermal safety margins (upper, open figures) among five species of tree snails. Numbers above figures represent sample size. The asterisk indicates that one animal was active on the bottom of the container and hence its falling point could not be determined. Sample sizes are different for different measurements on *Caracolus carocollus* (*C.c*) and *Caracolus marginella* (*C.m*) as some include juveniles, others not (see text). Vertical lines represent ranges, horizontal lines means, and rectangles two standard errors either side of the mean. Failure of rectangles to overlap indicates significant differences (approximately 5% rejection level). Safety margin is the temperature difference between the falling and lethal points (see text).

which a snail dropped off a vertical substrate) and lethal point (temperature at which it failed to respond to tactile stimulation); see Appendix 2.

P. lima (lowlands) had a significantly higher falling point than did *P. acutangula* and *P. luquillensis* from the cooler mountains (Fig. 15). The latter two did not differ significantly from each other. The genus *Caracolus,* despite the fact that both its species occur in the lowlands, had generally lower falling points than *Polydontes. C. carocollus* and *C. marginella* did not differ significantly from each other.

The lethal points of *P. lima* (lowland) and *P. luquillensis* (mountains) were not significantly different from each other, but were significantly higher than that of *P. acutangula.* The two species of *Caracolus* were not significantly different from each other or from *P. acutangula,* although the lethal point of *C. marginella* was somewhat higher than that of *C. carocollus.* The latter had a significantly lower lethal point than did *P. lima* or *P. luquillensis* (Fig. 15).

The temperature difference between the falling and lethal points is an indication of the safety margin that dropping from a vertical surface bestows upon an individual. Only adults were used in the analysis of safety margins because of the size-dependence of one of the factors involved in its calculation (lethal point; see Appendix 2).

The mean safety margins of *C. carocollus, C. marginella* and *P. luquillensis* were between 5.9°C and 6.8°C and were not significantly different from each other (Fig. 15). They were significantly higher than those of *P. lima* and *P. acutangula* (3.9°C and 4.3°C, respectively). The latter two species were not significantly different.

Because of the inverse relation of size and lethal limit (but lack of a size effect on falling point) juveniles have lower safety margins than adults (Appendix 2).

Temperature tolerances far exceeded body temperatures experienced in the field for all species except *C. marginella* in which several snails were encountered with a T_B within the lower range of falling points (Fig. 15). In other species the highest T_B was more than 10°C below the lowest falling point measured. However, it should be remembered that body temperatures were not measured in the hottest part of the year, and that all T_B's for *C. carocollus* were from a montane population. Lowland *C. carocollus* would be expected to have higher body temperatures.

In comparison to snails from extreme habitats, the Puerto Rican camaenids have low temperature tolerances. For example, Schmidt-Nielsen et al. (1972) found that some desert species could tolerate temperatures of 55°C for over an hour.

The mechanism of heat death was not ascertained in the present study. However, Grainger (1969, 1975) suggests that in *Patella vulgata, Helix aspersa* and *Arianta arbustorum* death results from an ionic imbalance at high temperatures which leads to failure of neuromuscular transmission.

Effect of temperature on survival of food-deprived animals

Thus far, high rather than low temperatures have been discussed. Although the subtropical climate of Puerto Rico would seem to provide few opportunities for exceeding the cold tolerances of snails, low temperature may influence altitudinal distribution in other ways.

In *C. carocollus,* survival time during food deprivation was size-dependent at both 20°C and 30°C; small animals died more quickly than large ones (Fig. 18). Inasmuch as small snails tend to have higher oxygen consumption per gram of body weight than do large ones (Gromadska & Przybylska, 1960), including *C. carocollus* (Stiven, 1970), size effect on survival may indirectly reflect a metabolic effect. An alternate hypothesis is that large animals have relatively larger stored energy reserves. Regardless of the mechanism involved, the ecological significance is that juvenile animals are less able to survive prolonged unfavorable conditions than are adults. Survival time of all ages was, less than half as long at 30°C as at 20°C. The elevated metabolic rate resulting from the higher temperature would cause faster utilization of energy reserves and an earlier death.

For interspecific comparisons only data from adults were used. Several individuals of *C. carocollus* ate part of an index card that was inadvertently left under the lid of their container on day 102 of the experiment (arrow in Fig. 17). These animals survived much longer than would be expected from extrapolation of the survivor-

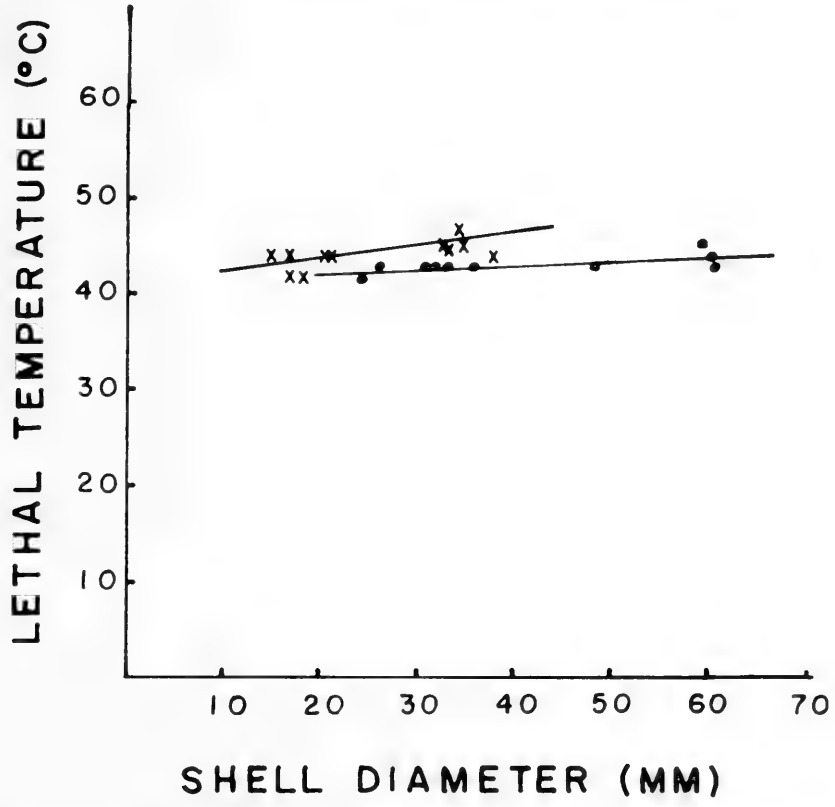

FIG. 16. Relationship of lethal temperature and diameter of shell in *Caracolus carocollus* (dots) and *Caracolus marginella* (X's). Lines calculated by least squares method.

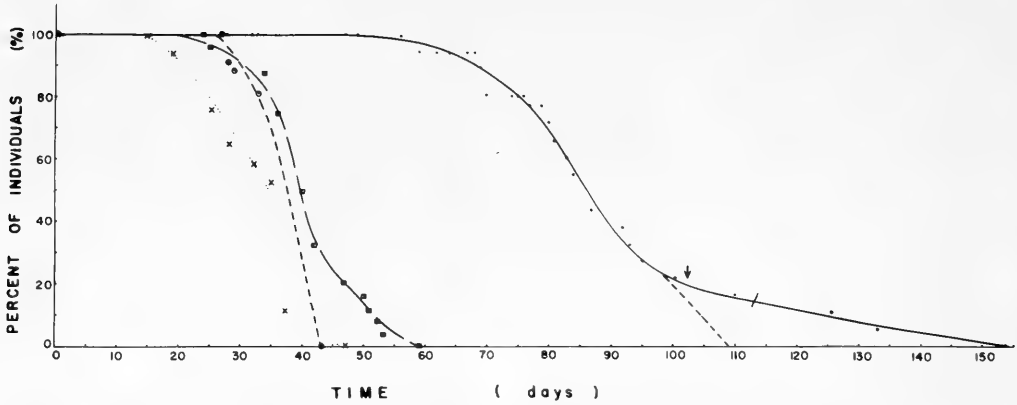

FIG. 17. Survival of 18 *Caracolus carocollus* adults at 20°C (dots) and 17 at 30°C (X's) and of 24 *Caracolus marginella* adults at 20°C (squares) and 44 at 30°C (circles). Lines eye-fitted. Arrow indicates that the surviving *C. carocollus* at 20°C inadvertently received food (see text). Dotted line is an extrapolation of survival curve to abscissa based on data prior to 102 days.

ship curve to the abscissa using data prior to day 102 (dotted line in Fig. 17). They probably have cellulases in their guts as do many terrestrial snails (Mason, 1970), and were able to use the paper as food. The increased survival of these individuals illustrates the importance of even brief opportunities for feeding during generally unfavorable conditions. These animals were excluded from Fig. 18. Excluding them also from the survivorship curves in Fig. 17, maximum survival time of adult *C. carocollus* at 20°C is estimated to be about 108 days, in contrast to 47 days at 30°C, and 57 days for *C. marginella* at 20°C. Although maximum survival of *C. carocollus* at 20°C was more than twice that at 30°C, in *C. marginella* maximum survival at 20°C was less than 1½ times that at 30°C (59 and 43 days respectively). Thus, temperature changes have greater effect on *C. carocollus* than on *C. marginella,* perhaps reflecting different metabolic Q_{10}'s in the two species. However, the survival time of even *C. marginella* was sufficiently long (30-60 days) that it seems unlikely that thermally influenced inanition would often be responsible for differential mortality among species in nature. The mechanism of interspecific differences in rate of depletion of food reserves was not directly ascertained. However, it could arise from (1) interspecific differences in relative amounts or types of energy stored or utilized, e.g. the greater use of polysaccharides by *Planorbis corneus* during starvation as compared to some other species which use primarily lipids (Emerson, 1967), differences among species in assimilation rate responses to (2) temperature as noted for various woodland snails by Mason (1970), or to (3) moisture as in the case of *Biomphalaria* [= *Australorbis*] *glabrata* which lowers metabolic rates under adverse moisture conditions and hence lengthens survival during starvation (von Brand et al., 1957).

Whatever the mechanism of death, it is clear that at the temperatures prevailing at high altitudes *C. carocollus* would use up its stored reserves more slowly than *C. marginella* and could be considered better adapted to such conditions.

Ontogenetic differences in thermal response and tolerance were noted (Appendix 2). Juvenile snails had a lower tolerance to high temperatures (Fig. 16) and at a given temperature survived a shorter period during food deprivation (Fig. 18) than did adults. Thus, it would be advantageous for young snails to avoid high temperatures. Their selection of the less exposed microhabitats probably has adaptive significance in this regard.

Water

Moisture varies tremendously among habitats in Puerto Rico. For a given altitude there is a general decrease in moisture from east to west and from north to south, and at a given longitude from higher to lower elevations. Thus the Luquillo Mountains in the northeastern part of the island

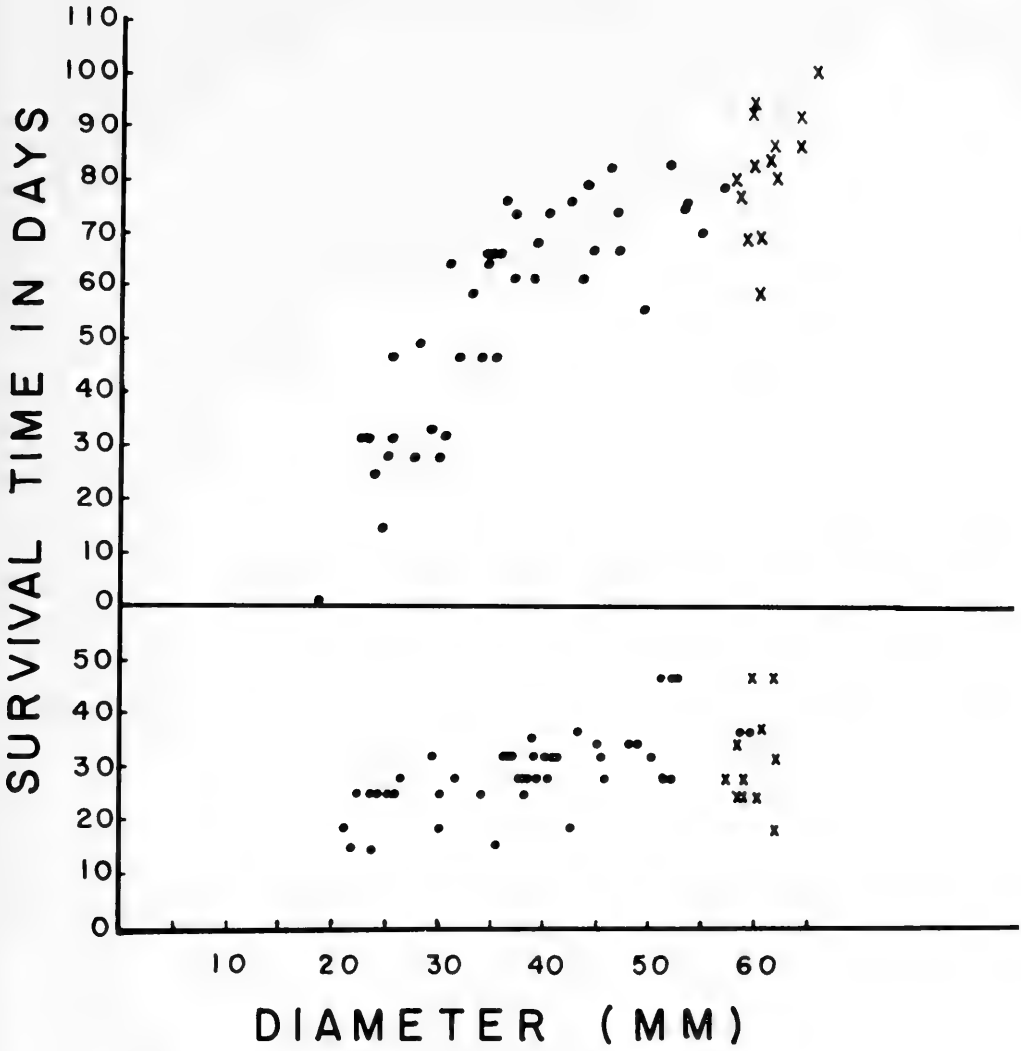

FIG. 18. Relationship of survival of food-deprived *Caracolus carocollus* to shell diameter. Lower: at 20°C. Upper: at 30°C. Dots indicate juveniles, X's adults. Four *C. carocollus* which ate paper during the experiment are excluded.

have the wettest climate and the southern coast (especially the southwestern portion) the driest. Adaptation to unfavorable moisture conditions would be expected to follow strategies similar to those outlined for temperature, e.g. low susceptibility to desiccation (as demonstrated in some snails by Van der Schalie & Getz, 1963) and/or behavioral means of avoiding dry conditions or of regulating water exchange with the environment. In this section we (1) describe the correlation between geographic distribution and tolerance to dry conditions, as expressed in terms of survival time, when access to water is denied, and in terms of evaporative loss, (2) consider the epiphragm and storage of mantle water as possible adaptations prolonging life during dry periods, and (3) evaluate size-effects on drought resistance.

Mantle water and moisture content

During dry periods or in dry localities *C. carocollus* has less water stored in the mantle cavity than under more moist conditions, suggesting that mantle water might be used by the animal for its biological

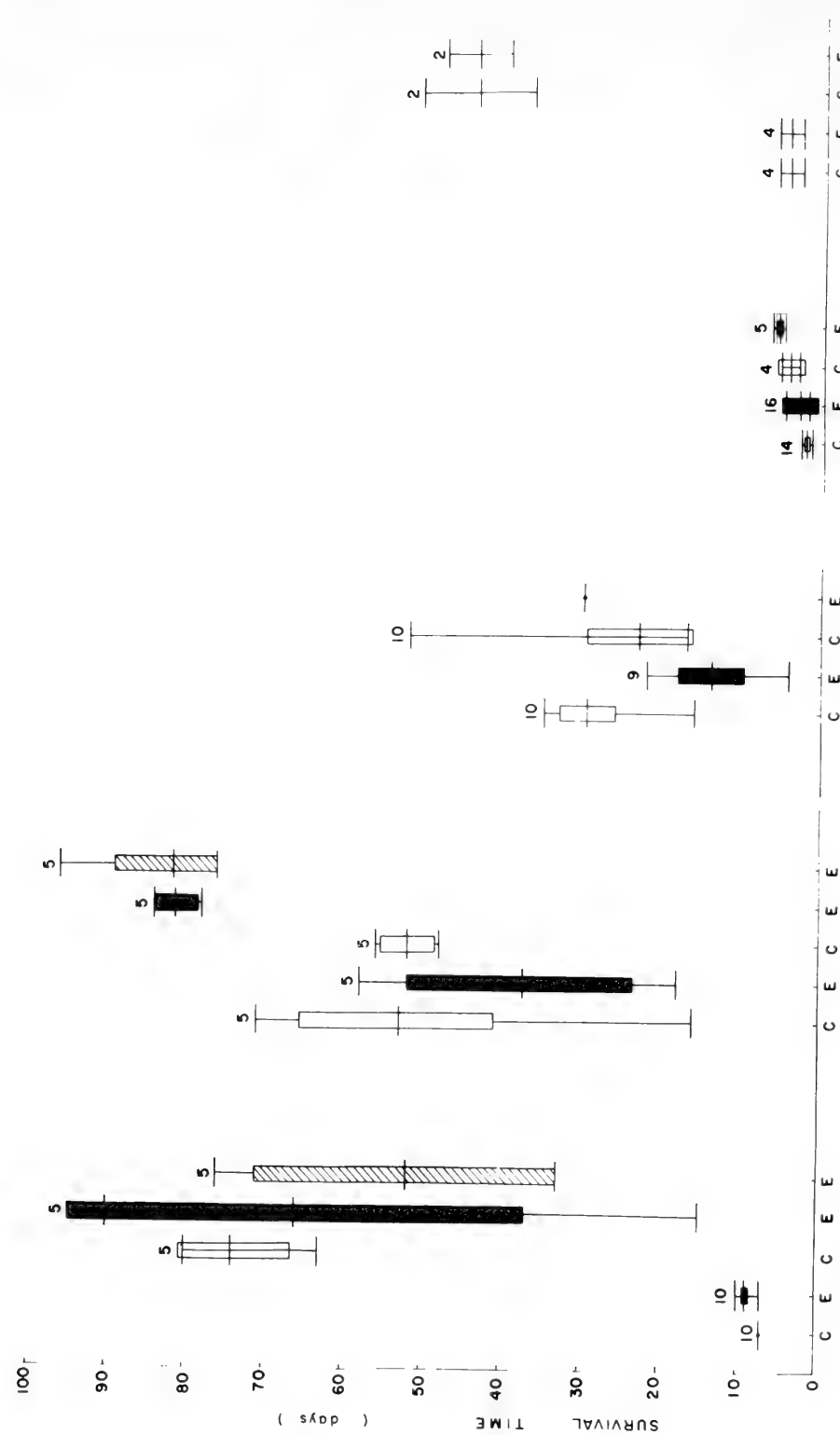

FIG. 19. Survival time of camaenid snails under different experimental conditions. Open figures (C) represent data from animals kept at high humidities, and black figures (E) those that were kept at low humidities. Cross-hatched figures differ from the black ones in that the snails were allowed to retain whatever mantle water they had at the beginning of the experiment. Symbols as in Fig. 15.

needs during periods of moisture stress. However, experimental attempts to establish whether this is true were inconclusive.

The amount of water stored in the mantle cavity showed a lot of individual variation, values ranging from zero to over 7.5 ml. Within a given study area at a particular time, snails from different microhabitats did not show consistent or significant differences in amounts of stored water (Fig. 22). It would appear that snails are mobile enough and move sufficiently often that mantle water is replenished if there is water available in their habitat.

However, there exist seasonal fluctuations in amount of mantle water. From April through December of 1965 when there was considerable rainfall (see Fig. 4), adult *C. carocollus* at both El Yunque and Loiza Aldea had mean volumes of mantle

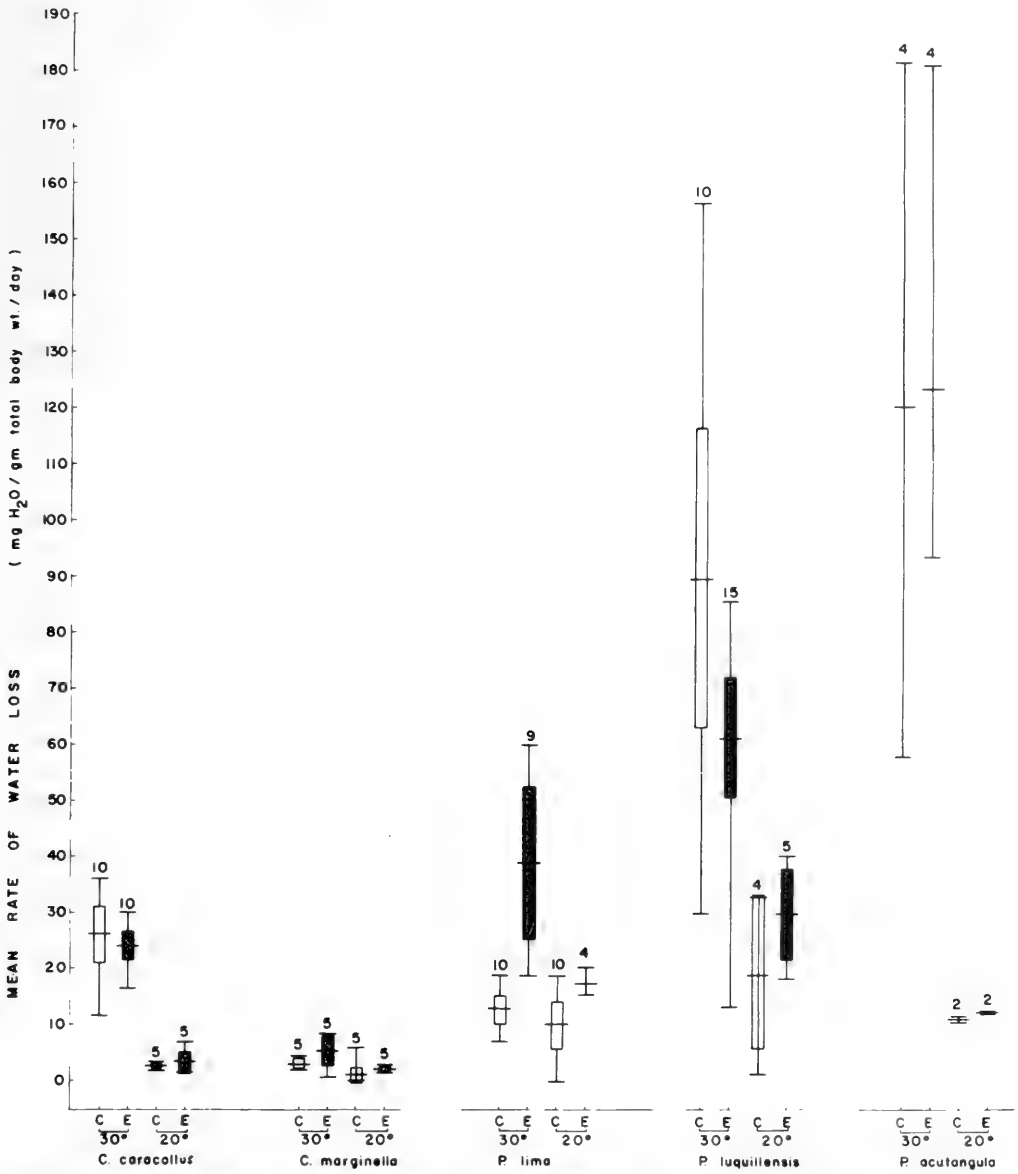

FIG. 20. Rates of water loss from camaenid snails at different temperatures and at high humidities (open figures; C) and at low humidities (black figures; E). All snails lacked mantle water. Symbols as in Fig. 15.

water of about 2.5-3.5 ml. However, during the usually dry period of January through March (Fig. 4), the mean quantity of mantle water was lower in both areas; furthermore the mean March values for the drier Loiza Aldea site were significantly lower than those from El Yunque (Fig. 22). In January and March in Loiza Aldea, some snails were found which contained no mantle water at all, a phenomenon never witnessed at El Yunque. In March at Loiza Aldea the snails contained on the average less than 0.5 ml of mantle water.

Juvenile snails contained mantle water in proportion to their size, no differences occurring during the wet part of the year between the two study areas (Fig. 23).

During the unusually dry conditions of February 1965 the Loiza Aldea snails suffered a slightly reduced body water content compared to that of snails from a wetter area at a time of more abundant rainfall (Appendix 2). The soft body parts had a water content of 86.2% and 89.3% in these two groups, respectively. However, the dry period had not prevented the Loiza Aldea adults from feeding, as they had gut contents of an even larger proportion of the body weight than did El Yunque snails (Table 10).

TABLE 10. Mean weight of various body compartments and of mantle water and gut contents in *C. carocollus*.

			Shell		Body		Body water		Mantle water		Gut contents	
Locality	N	Total wt	$\bar{x}$ wt g	% total wt	$\bar{x}$ wt g	% total wt	$\bar{x}$ wt g	% body wt	$\bar{x}$ wt g	% body wt	$\bar{x}$ wt g	% body wt
El Yunque	25	28.5	12.5	43.9	16.0	56.1	14.3	89.3	1.6	10.0	1.9	11.9
Loiza Aldea	24	23.1	10.7	46.3	12.4	53.7	10.7	86.3	1.4	11.3	2.6	21.0

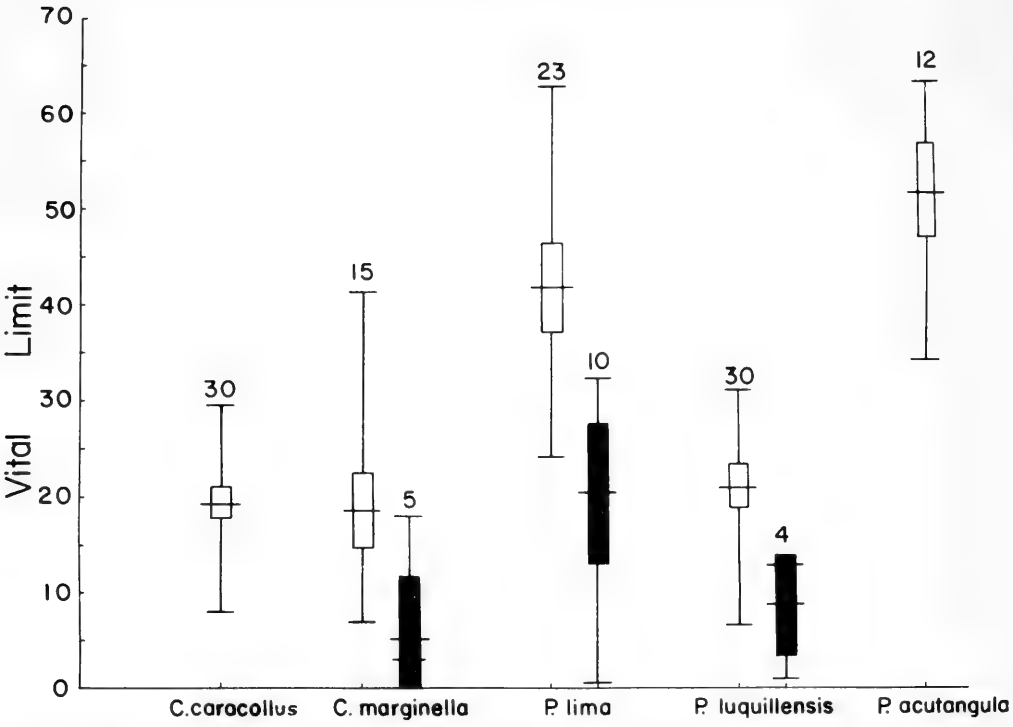

FIG. 21. Vital limit of water loss (percent of hydrated weight lost as water before death occurs) of camaenid snails. Black figures represent data obtained by desiccating snails in moist air at 20°C. Open figures for *C. marginella, P. lima* and *P. luquillensis* represent data from all other treatments. Open figures for all other species represent data from all treatments including humid air at 20°C (see text and Appendix 2). Symbols as in Fig. 15.

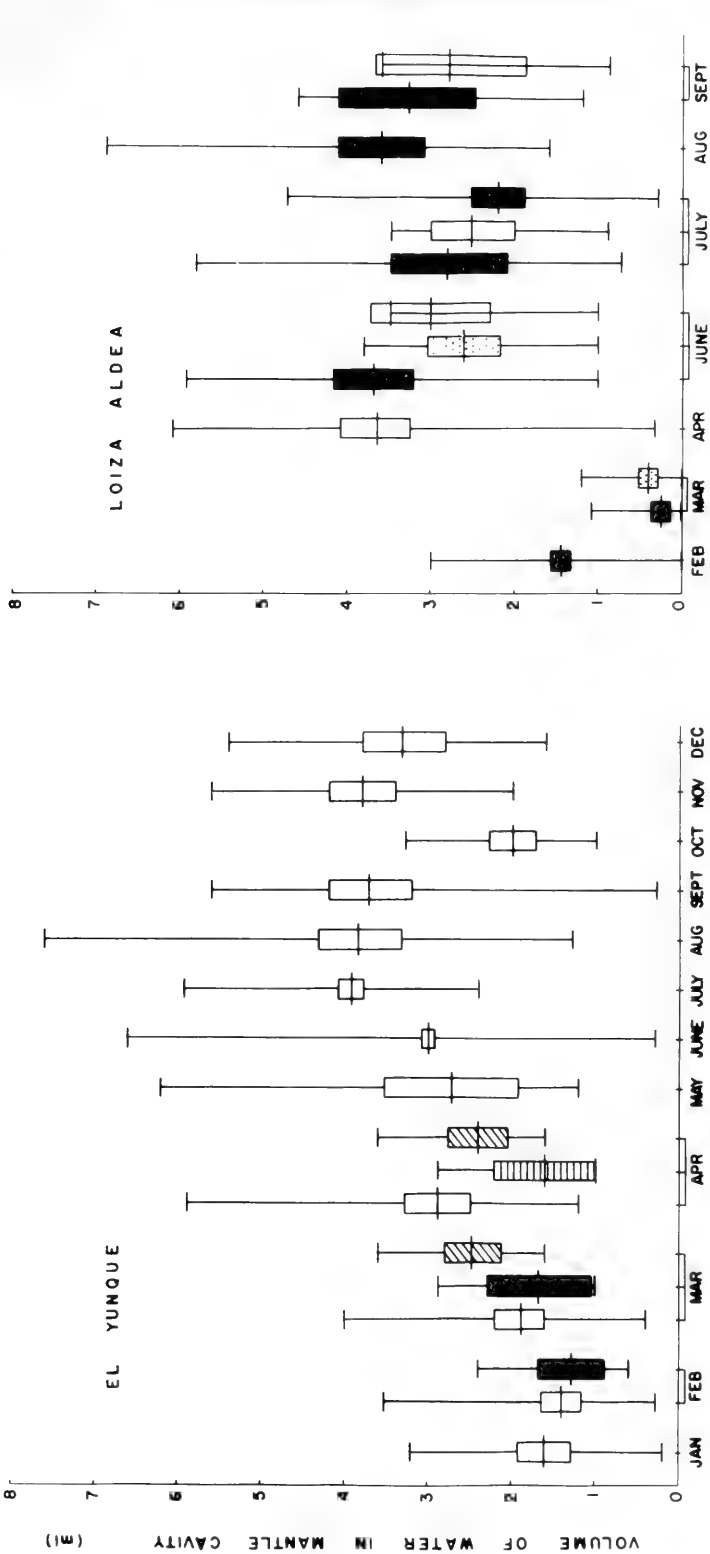

FIG. 22. Seasonal changes in amount of water in the mantle cavity of adult *Caracolus carocollus* in two study areas. Open figures indicate snails on trees, solid figures snails in the leaf litter, diagonally hatched figures snails in bromeliads, transversely hatched figures snails on leaves, and stippled figures snails under rocks. N = 25 for each figure except for the sample of 10 snails from trees at Loiza Aldea on 28 July. Symbols as in Fig. 15.

TABLE 11. Comparison of survival times of adult and juvenile snails under a variety of treatments. Correlation analysis of the relation of survival time to size (diameter of shell) involved a Spearman Rank Correlation test (one-tailed).

Species	Temp	Category	Mantle water	N Juv., Ad.	Survival time in days Mean (range) Juvenile	Adult	Correlation of survival time and size r_s	P
C. carocollus	30	Control	None	10,10	6.9(6-7)	7 (7-7)	0.36	>0.05
	30	Desicc.	None	10,10	8.5(7-10)	8.9(7-10)	0.15	>0.05
	20	Control	None	5,5	65 (61-76)	74 (63-80)	0.75	<0.01
	20	Desicc.	None	5,5	14 (10-25)	66 (15-90)	0.67	<0.05
	20	Desicc.	With	5,5	43 (10-69)	52 (33-76)	0.46	>0.05
C. marginella	30	Control	None	5,5	25 (5-67)	53 (16-71)	0.66	0.05>P>0.01
	30	Desicc.	None	5,5	50 (21-76)	38 (18-58)	−0.14	>>0.05
	20	Control	None	5,5	36 (18-50)	52 (48-56)	0.34	>0.05
	20	Desicc.	None	5,5	69 (62-80)	81 (78-84)	0.88	<0.01
	20	Desicc.	With	5,5	65 (28-76)	82 (76-96)	0.81	<0.01
P. lima	20	Desicc.	None	3,4	25 (19-30)	30 (30-30)	0.66	0.05>P>0.01
P. acutangula	30	Control	None	2,4	1 (1-1)	4.5(3-6)	0.50	>0.05
	30	Desicc.	None	3,4	1 (1-1)	5 (3-6)	0.83	0.05>P>0.01
	20	Control	None	2,2	10.5(9-12)	44 (37-51)	—	—
	20	Desicc.	None	2,2	12 (12-12)	44 (40-48)	—	—

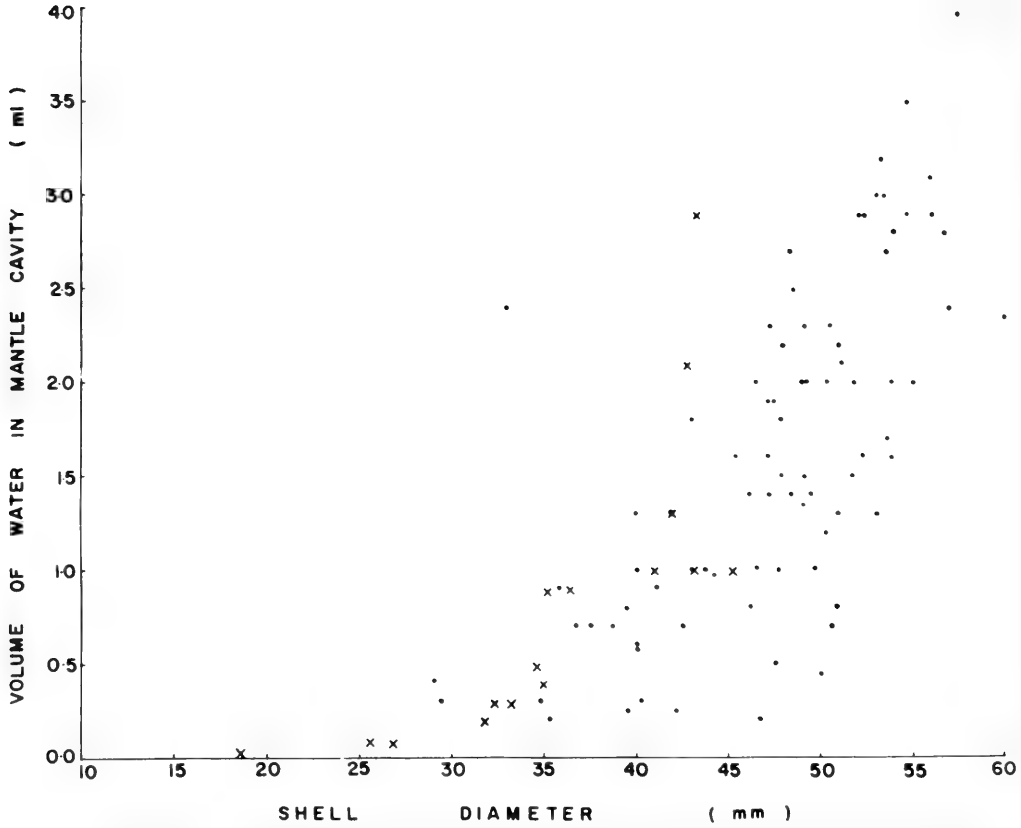

FIG. 23. Relationship of size to amount of mantle water stored by juvenile *Caracolus carocollus* at El Yunque (dots) and Loiza Aldea (X's). Measurements were made during the wet part of 1965.

TABLE 12. Comparison of vital limits of adult and juvenile snails under a variety of treatments. Correlation analyses of the relation of vital limit to size (diameter of shell) involved Spearman Rank Correlation tests (two-tailed). *P. acutangula* not included because raw data for juveniles lost before these correlation analyses were run.

Species	Temp.	Category	Mantle water	N Juv., Ad.	Vital limit Mean (range) Juvenile	Vital limit Mean (range) Adult	Correlation of vital limit and size r_s	P
C. carocollus	30	Control	None	9,10	15.6(8.2-29.1)	18.2(8.1-25.2)	0.28	>>0.10
	30	Desicc.	None	5,10	21.5(17.8-25.6)	21.3(13.1-26.7)	−0.003	>>0.10
	20	Control	None	5,5	11.5(3.97-18.06)	20.1(15.8-28.6)	0.55	>0.10
	20	Desicc.	None	5,5	15.6(9.4-24.3)	17.1(10.7-24.4)	0.14	>>0.10
	20	Desicc.	With	5,5	10.3(5.2-12.5)	17.1(7.4-24.3)	−0.31	>0.10
C. marginella	30	Control	None	2,5	14.2(13.5-14.8)	32.5(7.1-16.9)	0.26	>>0.10
	30	Desicc.	None	5,5	21.6(8.0-32.2)	23.5(12.8-41.7)	−0.07	>>0.10
	20	Control	None	5,5	14.5(8.5-21.3)	3.6(1.3-17.9)	−0.79	<0.02
	20	Desicc.	None	5,5	32.0(15.7-33.0)	18.4(13.1-19.1)	−0.43	>0.10
	20	Desicc.	With	5,5	23.8(19.5-31.6)	20.0(13.9-28.0)	−0.41	>0.10
P. lima	20	Desicc.	None	3,4	44.7(35.7-51.0)	52.1(46.6-61.4)	−0.39	>0.10

TABLE 13. Comparison of mean rates of loss of adult and juvenile snails under a variety of treatments. Correlation analyses of the relation of mean rate of loss to size (diameter of shell) involved Spearman Rank Correlation tests (one-tailed). *P. acutangula* not included because raw data for juveniles lost before these correlation analyses were run.

Species	Temp.	Category	Mantle water	N Juv., Ad.	Mean rate of loss Mean (range) Juvenile	Mean rate of loss Mean (range) Adult	Correlation of mean rate of loss and size r_s	P
C. carocollus	30	Control	None	4,10	24.9 (14.0-41.6)	26.1(11.6-36.1)	0.28	>>0.05
	30	Desicc.	None	5,10	21.9 (13.8-24.6)	24.0(16.6-29.8)	−0.49	0.05>P>0.01
	20	Control	None	5,5	1.65(0.6-3.18)	2.7(1.9-3.6)	0.50	>0.05
	20	Desicc.	None	5,5	10.57(8.6-15.3)	3.4(1.5-7.1)	−0.65	0.05>P>0.01
	20	Desicc.	With	5,5	9.83(5.15-12.46)	4.1(1.0-7.4)	−0.64	0.05>P>0.01
C. marginella	30	Control	None	2,5	6.3(2.1-10.5)	3.1(2.2-5.1)	−0.19	>>0.05
	30	Desicc.	None	5,5	5.0 (1.44-8.24)	6.8(3.65-7.78)	0.26	>>0.05
	20	Control	None	5,5	0.6 (0-3.2)	0.7(0-3.6)	0.17	>>0.10
	20	Desicc.	None	5,5	3.7 (2.49-5.99)	2.3(1.6-2.7)	−0.77	<0.01
	20	Desicc	With	5,5	3.6 (2.52-5.26)	2.4(1.8-3.0)	−0.47	>0.10
P. lima	20	Desicc.	None	3,4	18.3 (17.0-19.0)	17.4(15.5-20.5)	−0.39	>>0.05

Desiccation

Young snails desiccate more rapidly than conspecific adults and survive desiccation a shorter time (Appendix 2, Tables 11, 13). This is in marked contrast to the results of Riddle (1975) who found no effect of body size on rate of evaporative loss in live desert snails, *Rabdotus schiedeanus.*

Adaptation in the Puerto Rican camaenid snails has followed the path of ontogenetic shifts in patterns of response to the environment. Young snails, by selecting more sheltered habitats than adults reduce their water losses and lower the probability of overheating, thereby behaviorally compensating for their high rates of evaporative loss and greater sensitivity to elevated temperatures. That this compensation is not complete is evidenced by the fact that mortality among (and hence selection upon) juveniles is greater than among adults during periods of environmental stress.

There was considerable variation in survival time and rate of water loss among experimental treatments and species (only adults used in comparison). *P. luquillensis* was the only species that had consistently poor survival under all experimental treatments; it survived less than a week in humid conditions at 20°C if it did not have access to free water. Two species, *C. carocollus* and *P. acutangula,* had low survival

at 30°C, both in low and high humidities (10 days or less), but high survival at cooler temperatures (up to 90 and over 50 days respectively) [Fig. 19]. *C. marginella* survived well under all conditions although its survival at 20° was longer than at 30°C. *P. lima* had survival times intermediate between the sets of values previously discussed; differences between temperatures were not great (Fig. 19).

The two groups of snails (*C. carocollus* and *C. marginella*) that were allowed to retain their mantle water did not show significantly greater survival in comparison to similarly treated individuals without mantle water. Thus, either they had retained very little mantle water, or it was quickly lost or otherwise not available for replacing evaporative losses from the body (Fig. 19).

The rates of water loss showed an inverse pattern to that of survival time, species and/or treatments with high rates of water loss showed low survival and vice versa. Thus, *P. luquillensis* had consistently high rates of loss under all treatments (though evaporative rates were significantly lower at 20°C than at 30°C). *P. acutangula* and *C. carocollus* both had low rates of loss at 20°C and high ones at 30°C; *C. marginella* consistently had low rates of water loss and *P. lima* intermediate values. These patterns are almost mirror images of those for survival time.

Of the two physical factors affecting evaporation, temperature exerted a greater effect on the snails' water loss than did humidity (water losses were higher at 30°C than at 20°C in most species). Indeed, the differences in dryness of the air between the control and experimental containers resulted in surprisingly small differences in rate of loss; only in *P. lima* were rates significantly higher in the experimental containers (dry air) than in the control ones (moist air) [Appendix 2, Fig. 19]. Since some snails alter their metabolic rate with changes in relative humidity (von Brand et al., 1957, Riddle, 1975, 1977), the similarity of rates of loss at different humidities may have resulted from the snails lowering their metabolic rate in dry air and consequently reducing water loss below what it would otherwise have been under those conditions.

Rate of evaporative loss in land snails is complex. It is very low in inactive snails, but stimuli such as pressing or pricking the mantle or shaking the snail results in marked increases, resembling those of active snails and perhaps related to mucus production (Machin, 1965). Although shell thickness and area, and aperture size have been suggested as variables influencing evaporative losses (Machin, 1967), it is known that considerable control over evaporation resides in the mantle itself and varies considerably over time and under different conditions (Machin, 1972). Such regulation by the mantle may have reduced water loss at the lower humidities.

Although rates of evaporative loss tended to be higher (and survival time lower) in upland species than in lowland ones (Appendix 2, Figs. 19, 20), the vital limit (the % of total hydrated weight which can be lost as water before death occurs) showed a less clear-cut correlation; *C. carocollus,* one of the upland species (*P. luquillensis*), and one lowland species (*C. marginella*) had similar vital limits (about 20%), whereas values nearly twice that high occurred in lowland *P. lima* and even greater ones in upland *P. acutangula* (Appendix 2, Fig. 21). The high vital limit of the last species may be an adaptation related to the possible necessity to use water frequently for evaporative cooling (see above). Machin (1967) demonstrated temperature lowering of the mantle of the snail *Otala lactea* during evaporative water loss.

Several species of helicids (Cameron, 1970a) showed responses similar to those in camaenids in that differences in survival, rate of water loss and behavior under conditions of low humidity reflected differences in the moisture of the habitats occupied by different species.

Although temperature, water loss, and at the longer survival times perhaps also depletion of energy reserves (see section on temperature), all probably had a synergistic effect on survival under the experimental conditions, the close inverse correlation between rate of water loss and survival time suggests that water loss was the prime cause of mortality in most cases.

Role of membranes

The various species of snails had different behavioral responses to desiccation. Most species became inactive under low humidities and retracted into their shells. *P. acutangula* was an exception in that its body is too large to be enclosed completely

by the shell and it merely retracted as much as it could, reducing the exposed surface as much as possible; often the foot appeared curled and shriveled. The other species, when hanging on the glass walls, would sometimes secrete a clear mucus, which when dry would aid in attaching the snail to the container. It also sealed the opening around the attachment. However, whether this clear membrane retards water loss would depend on its permeability to water vapor. This was tested by comparing the rate of loss of *C. marginella* during periods when the membrane was present with those of the same individuals when they lacked it; no consistent differences were found (Appendix 2). Thus, the clear membrane does not retard water loss from the snails and its function would seem to be the aiding of attachment to a vertical substrate. Similarly von Brand et al. (1957) indicated that the mucus membrane of *Biomphalaria glabrata* did not seem to reduce rate of evaporation.

A different type of membrane, the epiphragm, is secreted across the opercular opening in *C. marginella* and *P. lima*; it does not occur in any of the upland species. It is hard and chalky in appearance, and occurred most commonly in animals kept at 30°C, especially those over the desiccant. Survival was higher among snails which had an epiphragm most of the time than among those which had an epiphragm less often (Appendix 2); this finding suggests that the epiphragm might retard water loss.

It can be concluded that the clear membrane does not influence evaporative rate from snails but that the epiphragm affords partial protection from desiccation and constitutes one of the modes of adaptation. It appears that similar adaptation occurs in other groups of snails as well. Grime & Blythe (1969) have suggested that differences in the local distribution of *Arianta arbustorum* and *Cepaea nemoralis* resulted from the reluctance of the former to secrete an epiphragm and the consequent sensitivity to desiccation which made it unable to colonize south-facing slopes. Machin (1967) found that species of snails from dry habitats tended to form thicker epiphragms than those from wetter ones.

Rokitka & Herreid (1975a, 1975b) observed that *Otala lactea* had a greater tendency to form epiphragms at low humidities than at high ones, but surprisingly, that more snails formed epiphragms at low rather than at high temperatures; the number of epiphragms secreted by a given snail increased with duration of dormancy, and reached values up to 7.

Response to relative humidity

One of the *C. carocollus* subjected to the experimental relative humidity gradient was found to be between 50% and 63% *r.h.* at each of three consecutive daily observations. By the fourth day it had moved to 20% *r.h.* where it remained inactive for nine days before being removed from the chamber. A second snail of this species moved between 48% and 60% *r.h.* during the first day (hourly observations) and then moved to the nearly saturated end of the gradient where it became inactive for three days, after which it was removed. It seems that this species does not have a consistent directional response to a gradient of relative humidity.

Effect of dry periods on survival

In the lowland, mortality in *C. carocollus* is highest among very small juveniles, especially during dry periods; in the uplands where conditions are continually moist, most small snails survive to adulthood and die at large size (Appendix 2). The characteristics of the upland population are paralleled by other upland species, even ones that are in a different family, are much smaller than the camaenids, and have a different ecology [Appendix 2, Fig. 23] (primarily a leaf litter form, Van der Schalie, 1948).

In some studies, other snails from habitats less equable than rainforest, had a large proportion of juvenile mortality attributable to drought (e.g. Wolda & Kreulen, 1973), whereas other factors, like predation, were of greater significance in adults (Wolda, 1972). In other species, mortality by predation assumes greater significance at all stages and drought affects populations by preventing recruitment, rather than greatly affecting mortality (Potts, 1975).

LIFE HISTORY AND POPULATION BIOLOGY

Our intent in this section is to compare and contrast the species in terms of basic

TABLE 14. Seasonal occurrence of copulation and oviposition of some Puerto Rican camaenid snails (1962-1966). A = *P. acutangula,* M = *C. marginella,* Lq = *P. luquillensis,* and Lm = *P. lima.*

	Number of copulations observed				Number of egg clutches found (El Yunque)			
	C. carocollus							
Month	El Yunque	El Verde	Loiza Aldea	Other species	*P. acutangula*	*C. carocollus*	Unidentified	Total
January	2				2		1	3
February	5	1			1			1
March	6			A	2			2
April	12		1	Lq	2	1[1]		3
May	3							
June	2	2						
July	2	4						
August			1	A,M			1	1
September								
October			1					
November				Lm	1			1
December	1						3	3

[1]Data from eggs deposited after captivity by snails from Loiza Aldea.

life history parameters, growth, reproductive cycles, population structure, and factors contributing to population changes.

Reproduction

The mating season of *C. carocollus* is an extended one, copulation having been observed in every month except September and November (Table 14). The pattern seemed to vary geographically. In the uplands the mating season is prolonged and centered on the dry season, but in the drier areas it is more seasonally restricted and tends to occur in the wetter parts of the year. Data are too few for the El Verde and Loiza Aldea sites, however, to permit a precise statement. Also in the Loiza Aldea area the fact that observations were made diurnally may have introduced a bias not present in the El Yunque region in that during the drier part of the year, almost no snails were active by day; they may have copulated at night when observations were not made. The fewer observations in the El Verde and Loiza Aldea area reflect at least in part the fact that fewer years were spent studying those areas than was true for El Yunque and do not necessarily indicate differences in incidence of copulation between areas.

Individual snails remain in breeding condition for at least two months during a given season and during that time copulation occurs more than once and with more than one other individual. Given snails may mate in at least two consecutive years and they remain reproductively active a number of years after achievement of adulthood (Appendix 2).

The wide temporal separation (March and August) of the few copulation records for *P. acutangula* suggest an extended mating season. The only copulation record for *P. luquillensis* occurred in April, the month of highest incidence in *C. carocollus* from the same area. These data are consistent with the view that these two upland species have a similar mating season. By contrast the lowland species were observed to copulate only during the wettest part of the year (*C. marginella* in August and *P. lima* in November) and thus resemble lowland *C. carocollus.*

At El Yunque copulation in *C. carocollus* did not seem to be restricted to any particular type of place; copulating snails were observed on the ground, on stone walls, on tree trunks, beneath leaf litter and within dead, rolled-up *Cecropia* leaves above the ground, in curled-up palm petioles, and in the axils of bromeliads. Both copulations observed at Loiza Aldea took place on the ground (one pair was placed in a plastic bag; spermatophores were produced during the night). The observed copulation of *P. lima* was on the ground, that of *C. marginella* on a tree trunk and those of *P. acutangula* on live tree leaves.

At least in *P. acutangula* most egg-laying begins about November and continues until February or March. Eggs take at least 1½ months to develop. Sporadic oviposition may occur at other times of year (Appendix 2).

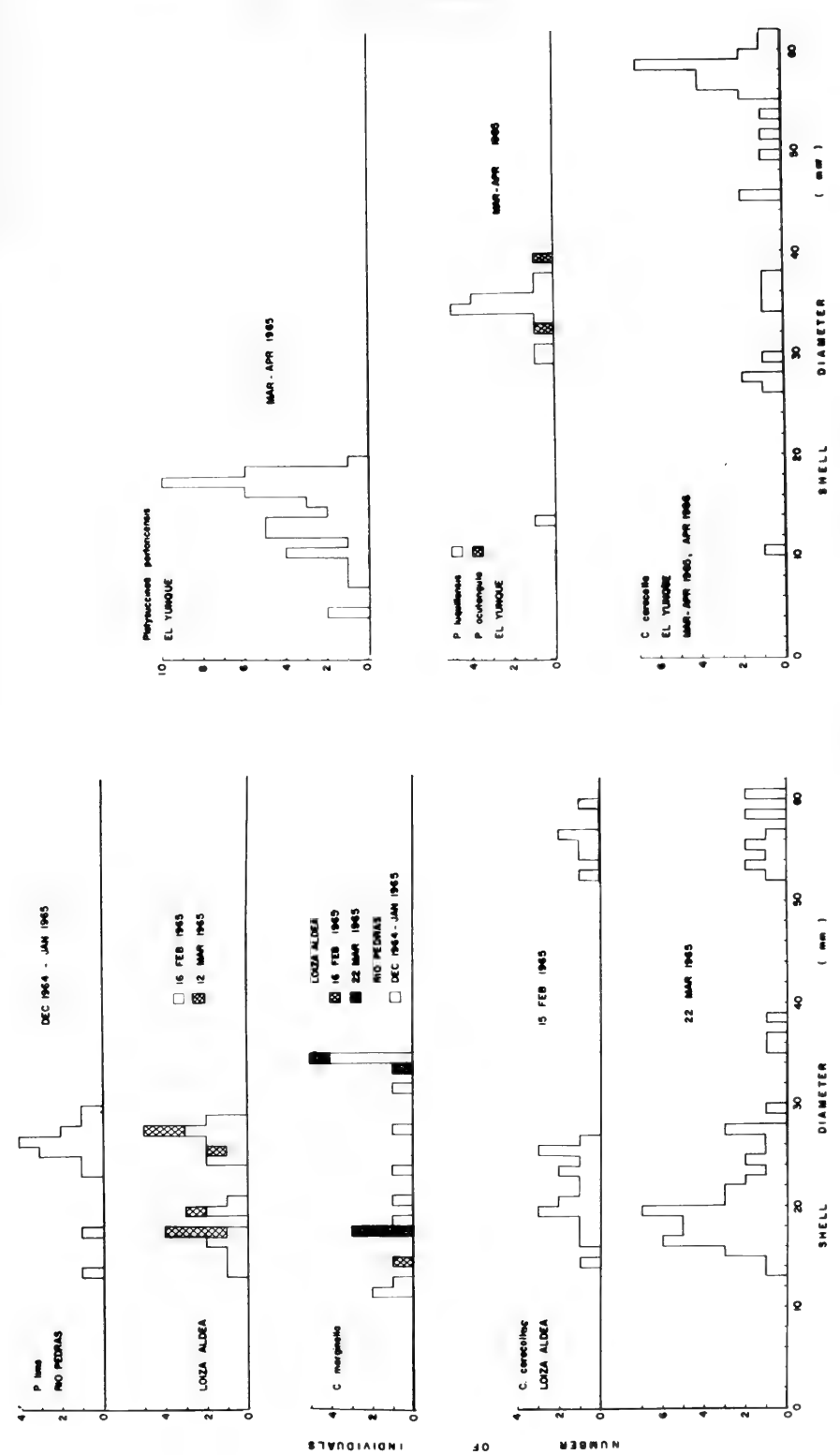

FIG. 24. Sizes of shells of recently dead snails from various areas and dates.

The albumen gland in the El Yunque population usually averaged heavier than at Loiza Aldea, probably a reflection of the generally larger body size in the uplands. The seasonal cycle in the albumen gland also differed between the two localities. At El Yunque the glands averaged between 0.3 g and 0.5 g for most of the year. However, in April average size began to increase and reached a peak of over 1.1 g

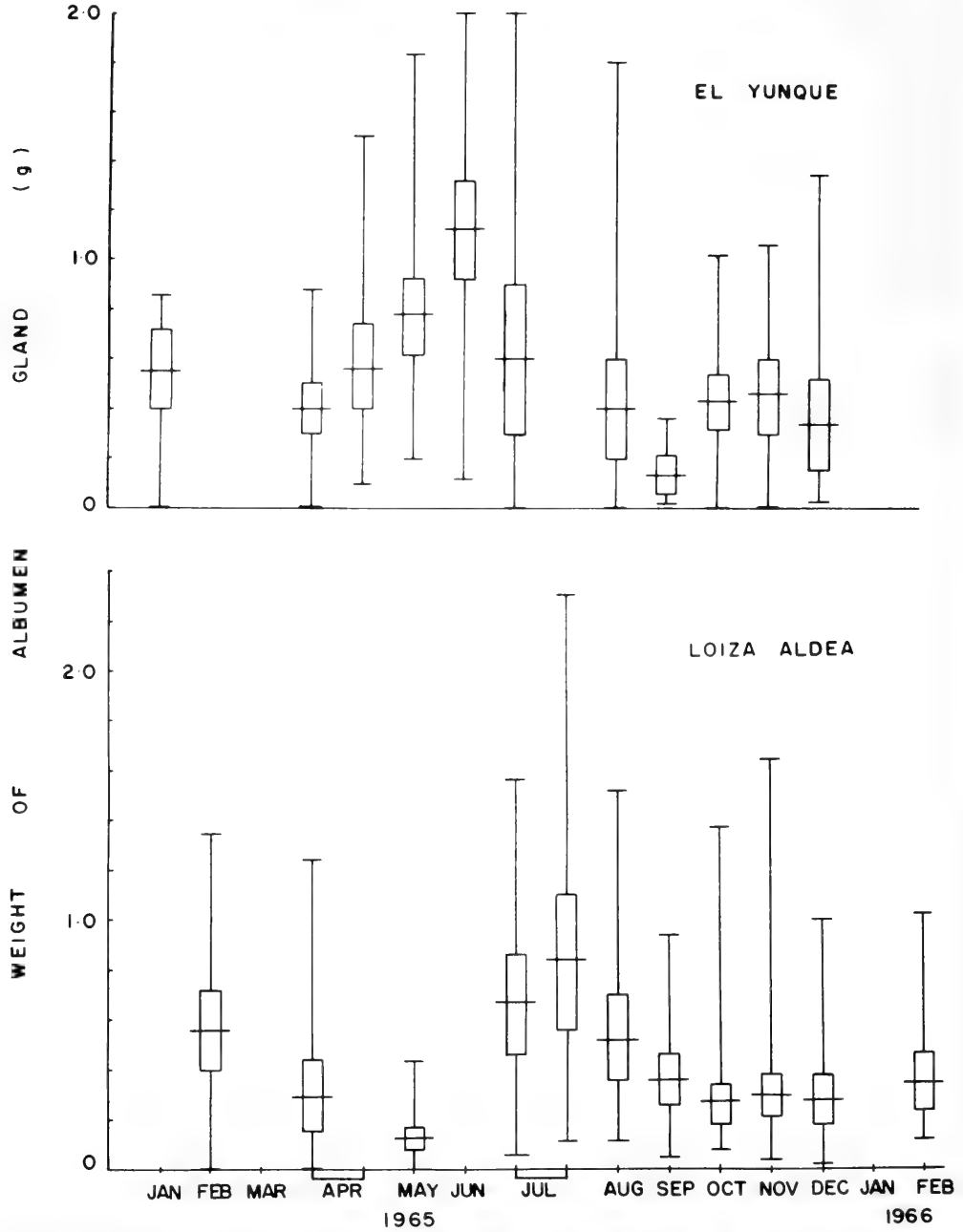

FIG. 25. Temporal changes in weight of albumen gland in adults of two populations of *Caracolus carocollus*. Symbols as in Fig. 15. N = 19-25 per sample for Loiza Aldea and 21-25 per sample for El Yunque.

by June (Fig. 25). The decline to usual levels was reached by August and had dropped to a very low value (slightly over 0.1 g and probably indicating that most glands were inactive) in September. Thus, in September there is little potential for albumen formation, and in June many animals have enlarged glands. Except for September, there were always some animals that had rather large glands (see upper ranges in Fig. 25) and were probably capable of producing albumen. At all seasons there were probably some quiescent individuals, as low albumen weights always occurred (see lower ranges in Fig. 25).

At Loiza Aldea the cycle differed in that maximum albumen weights were attained in late July, i.e., over a month later than at El Yunque. Also the lowest values occurred in May rather than in September. The seasonal pattern obtained with mean gland weights is supported in that the greatest proportion of animals with functional glands is largest at El Yunque in June and lowest in October whereas at Loiza Aldea the peak and low points occurred in late July and May respectively (Fig. 26).

Thus except for very brief periods, at least some individuals of *C. carocollus* in both localities are in "reproductive" condition (capable of producing albumen) but that the number in such a state reaches a maximum in the summer months with lesser numbers at other times of year.

The 1965 albumen cycle did not seem to be closely related to the particular weather conditions of that year. The increase in proportion of the population with functional glands began in late April 1965 but at a time when rainfall was still low—unusually so (compare Figs. 4 and 26); otherwise, however, the albumen gland cycle correlated rather well with rainfall. By contrast at Loiza Aldea, the two months of highest rainfall were at low points of the albumen gland cycle. The cycle does correlate with long term weather averages in that peaks in both areas occur during the warm summer months near the

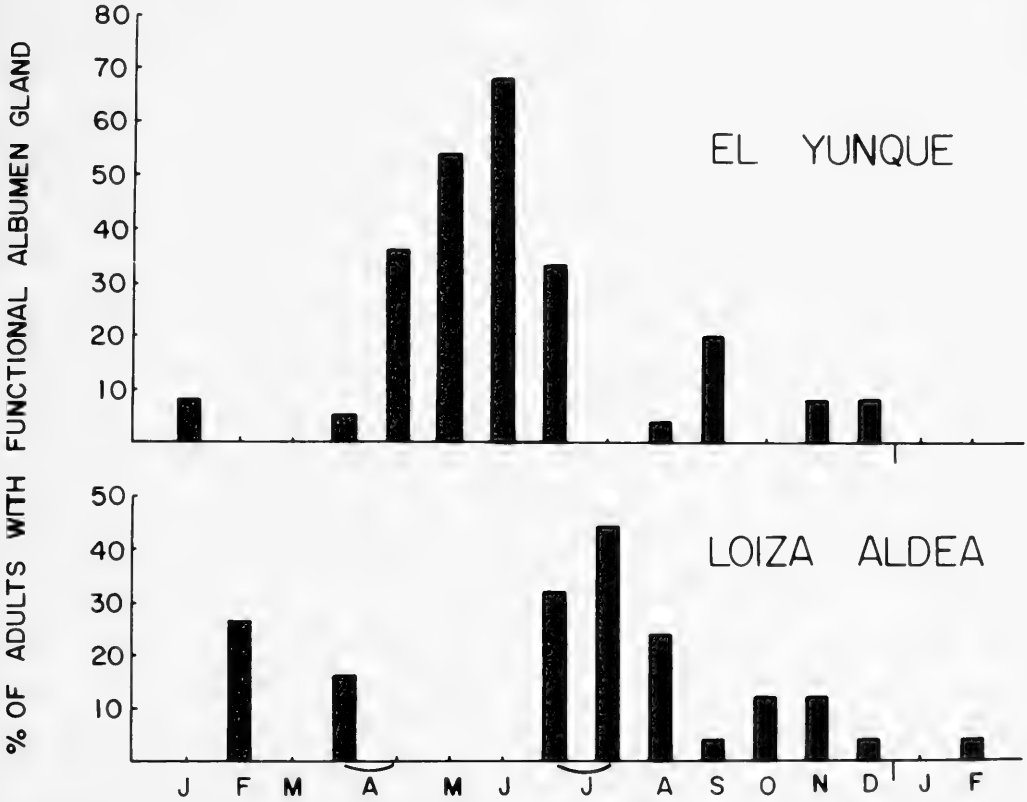

FIG. 26. Temporal change in proportion of adults with functional albumen glands (wt > 0.85 g) in two populations of *Caracolus carocollus.* Sample sizes as in Fig. 25.

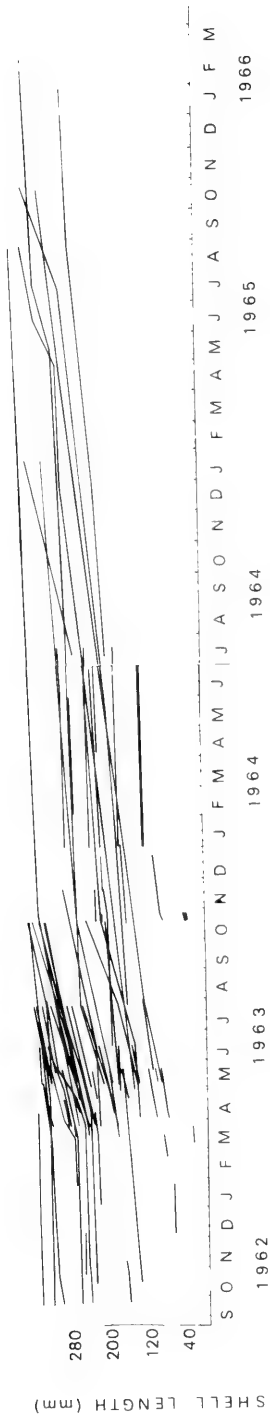

FIG. 27. Growth of *Caracolus carocollus* at the El Yunque Biological Station.

beginning of the wet season (compare Figs. 4 and 26).

The data on copulation and albumen gland cycles in *C. carocollus* all converge to indicate that reproduction occurs throughout most of the year but that there are seasonal peaks of activity; at El Yunque the peak of mating occurs in April, just before major development of the albumen glands. The albumen gland cycle peaks in June, about 2 months after mating. It is probable that development of ova follows immediately after the development of the albumen glands and that oviposition peaks in late summer well in time for egg development to occur before the onset of the dry season in late winter to early spring. The fact that in *C. carocollus* neither animals with ripe ova or shelled eggs inside, nor eggs or even newly hatched snails were ever found suggests that just prior to development of ova (just after development of albumen gland) the animals secrete themselves in places where they are not easily found and deposit their eggs there, the young not emerging until yolk stores are exhausted after hatching. The most likely place for such activities is underground as epigean habitats were extensively and intensively searched without success. Díaz-Piferrer (1962) was similarly unable to find eggs of the Cuban tree snail in the field.

Other species were not studied as extensively as *C. carocollus*. However, *P. acutangula* clearly has a somewhat different life cycle as it oviposits primarily in January to April, at a time when *C. carocollus* is still primarily mating.

Growth and longevity

Most growth of *C. carocollus* took place in the middle of the year but little at other times (Figs. 27, 28).

Growth rates of *C. carocollus* at El Verde have previously been published (Heatwole et al., 1970). In general there was considerable growth from late May to August with little growth throughout the rest of the year. Some juveniles failed to grow at all for a particular growth season, or in some cases even for two consecutive ones.

At the El Yunque site, growth seemed to begin in March and extend to at least August and perhaps several months later

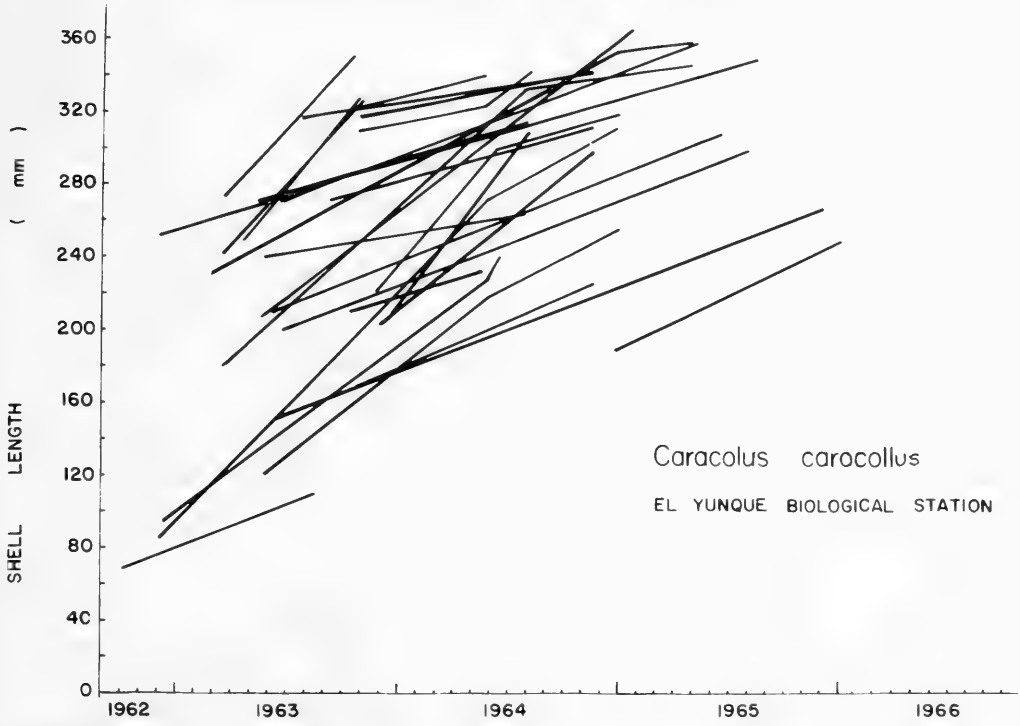

FIG. 28. Growth of *Caracolus carocollus* captured infrequently over long periods at the El Yunque Biological Station.

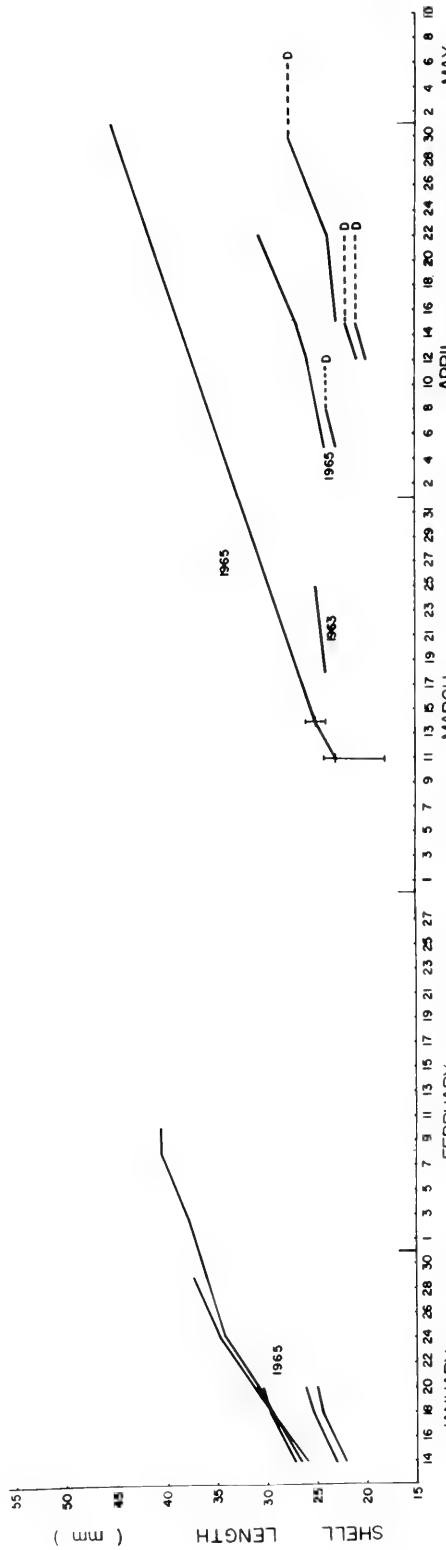

FIG. 29. Growth of newly hatched clutches of *Polydontes acutangula* in the field. D signifies date at which snail was found dead; dotted line connects with last date observed live.

(Fig. 27). Thus, at El Yunque the growing season is somewhat extended beyond that at the drier El Verde site.

Fig. 27 summarizes the growth of different animals than those appearing in Fig. 26 and which were caught infrequently over long time periods and indicates yearly growth but not seasonal patterns. This figure, in conjunction with Fig. 27 and the growth curves from Heatwole et al. (1970), indicate individual variability of growth rates in *C. carocollus.* Slow growers took up to six years to reach maturity whereas fast growers could do so in three years. Such large differences in growth rates among individuals is not without precedence in terrestrial snails; Blinn (1963) found considerable variation in growth in *Mesodon thyroidus* and *Allogona profunda* and Wolda (1970) indicated a polymorphism in growth rates in *Cepaea nemoralis* with fast growers reaching adulthood in one year but slow growers taking three years. Similarly, Pollard (1975) indicates that growth rates among individuals from the same batch of eggs can vary widely, resulting in time of reaching maturity varying from two to five years. Richardson (1975) indicates great variability in growth rates in *Cepaea nemoralis* and Giesel (1969) reports growth rate polymorphism in a marine limpet, *Acmaea digitalis.* In the present study the mechanism of growth rate differences among individuals was not ascertained. In Wolda's study, at least part of such differences were genetically determined.

Seasonal differences in growth rate were less pronounced in *P. acutangula* and in *P. luquillensis.* The growth curves suggest that *P. acutangula* reaches maturity in about one year and *P. luquillensis* in about two years (Appendix 2, Figs. 31, 32).

The most data relevant to evaluation of longevity were obtained from *C. carocollus.* The *C. carocollus* with the greatest known age was marked on 23 Oct. 1962 as an adult and was recovered live on 8 Feb. 1970. The time between first and last capture was thus 7 years and 4 months. Since it was adult when first marked, it must have been at least 3 years older than that (the minimum time required to reach adulthood) for a minimum age of 10 years,

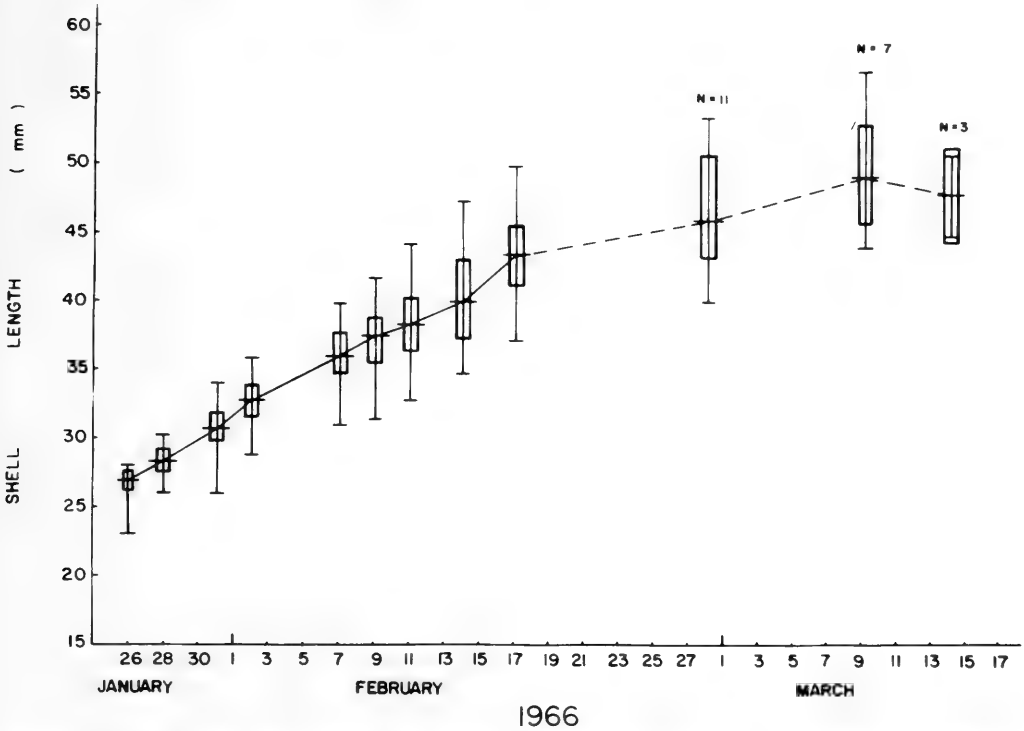

FIG. 30. Growth of 14 newly hatched *Polydontes acutangula* reared at 20°C in the laboratory. Dashed line indicates time after some hatchlings died and N values above figures indicate number of remaining live snails.

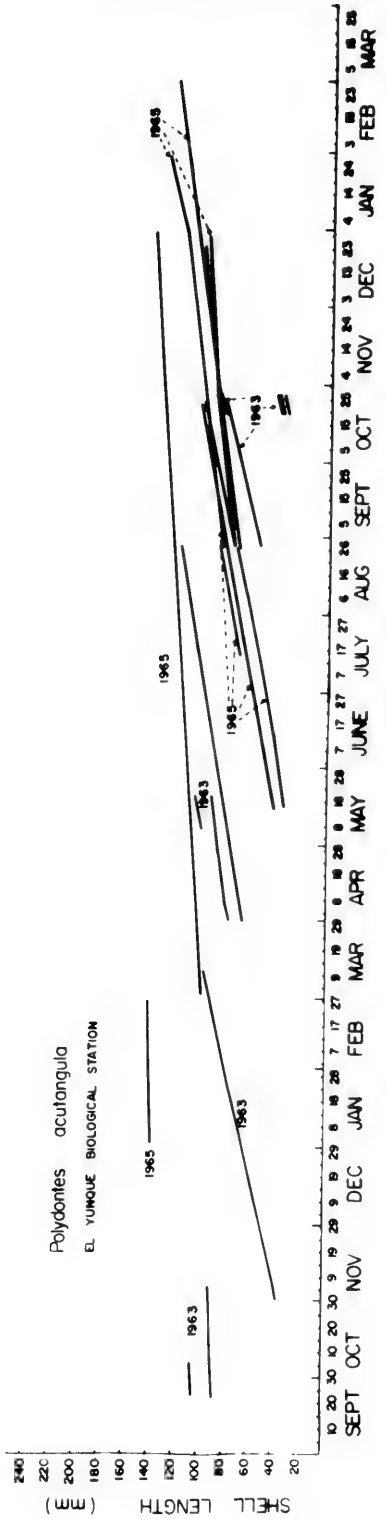

FIG. 31. Growth of *Polydontes acutangula* at the El Yunque Biological Station.

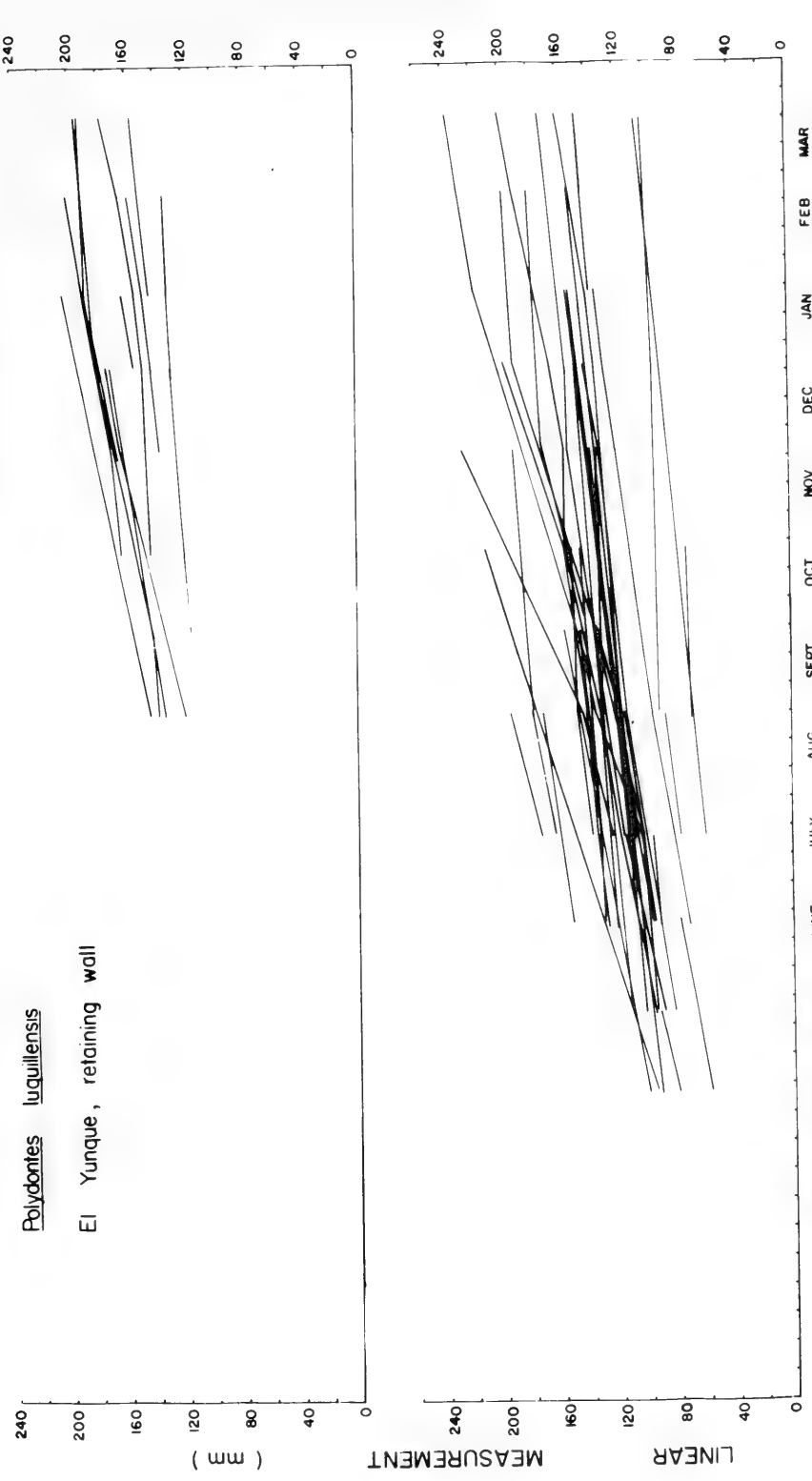

FIG. 32. Growth of *Polydontes luquillensis*. Upper and lower figures separated merely to prevent undue cluttering.

4 months. A second, recently dead shell was found of the same age. In the El Yunque region there were six other snails with known ages of 8 or more years. Their ages were: 8 yrs, 8 yrs, 8 yrs 2 mo., 8 yrs 5 mo., 9 yrs 8 mo., and 9 yrs 9 mo. Snails up to a known age of 5 years were common.

On 8 Feb. 1970 the region of the El Yunque Biological Station was searched for *C. carocollus,* 3 years and 10 months after the last snail had been marked in that area. None of the 18 juveniles had been previously marked, but of the 83 adults found, 19 (23%) had been painted. Thus, almost one-quarter of the adult population were four or more years old. The individual histories of very few of them could be traced as some of the paint had flaked off of some animals and although one could tell they had been painted, their numbers could not be ascertained. Those with numbers still legible were among the group of snails 8 or more years old listed above.

The marked population in the El Verde area was not studied in 1970 and hence longevity data from that area are not available.

It is probable that *P. luquillensis* and *P. acutangula* are not so long-lived as *C. carocollus.* The oldest known individuals of these two species were 3 years 5 months (2 individuals) and 2 years 4 months respectively. There are insufficient data to evaluate longevity for the other species.

Population biology

The size structure of the *C. carocollus* population was remarkably stable with the various size classes rather uniformly distributed (Fig. 33) though there was usually a slight peak in the adult size range and the smallest size-groups of juveniles were poorly represented. With the exception of a large number of very small individuals in Sept. 25, 1965, there was no evidence of temporal peaks of reproductive activity and subsequent seasonal shifts in frequency distribution of size classes. Such a stable size structure with a large number of adults might be expected in a long-lived species with low reproductive rates. *C. carocollus* is definitely long-lived after reaching the adult stage (see growth and longevity section); its reproductive rate is not known except that for a given year up to 68% of the adults at El Yunque have functional albumen glands (presumably heralding reproductive activity) at the peak of the albumen gland cycle. Thus, at least that proportion must reproduce each year.

P. luquillensis had a less stable size-structure than *C. carocollus.* In March 1965, there were 2 peaks in the size-frequency distribution, one consisting of adults and the other of medium-sized juveniles. During subsequent months the juvenile peak shifted progressively toward the adult one (Fig. 34) until they converged in early 1966. That this is not a regular yearly cycle is evidenced by the fact that the size-structure in March 1965 and March 1966 were quite different. Rather, it seems that reproduction had either been inhibited or there had been intense selective mortality among juveniles at a particular period which resulted in the bimodal pattern of March 1965. Subsequent months showed a gradual trend towards re-establishment of the original size-structure.

Thus, although both *P. luquillensis* and *C. carocollus* were studied on the same wall, they showed different stabilities in population size-structure in ways suggesting that the former is more sensitive to immediate environmental influences than is the latter. The wide geographic distribution of the latter and the restricted one of the former would support this view. Also, *P. luquillensis* reaches maturity in much shorter time and the average of many year classes which would tend to obscure year to year variation would be less likely than in the more slowly maturing *C. carocollus.*

Environmental moisture seemed to affect population density. Even in such an equable environment as the montane rain forest at El Yunque, population density of *C. carocollus* showed long term changes. In 1962 and 1963 densities were relatively high but dropped during 1964 which was drier (see Eastern Interior summary of rainfall, U.S. Department of Commerce, 1962-1966). Recovery had not occurred by 1965 or 1966 (both wet years) probably because of the long lag imposed by the long mean generation time of this species. The densities of *C. carocollus* in the El Verde area was about 1/5 or less that of the highest density at the El Yunque forest and about the same as or less than the lower ones. It would appear that the wetter El Yunque forest generally supports higher population densities than does the forest at El Verde; the environs of the station buildings appear to be especially favorable.

Although no quantitative data are available, casual observations suggested that the density of *C. carocollus* at Loiza Aldea is much lower than at either upland site. There were insufficient recaptures for population density estimates of other species.

Capture-recapture methods have been widely used for a long time to estimate population densities of animals. Methods have been continuously refined but even so, the most refined techniques demand either rather specialized attributes of the population sampled and/or very high sampling intensities, and are not strictly applicable to many species.

Parmenter (1976) has recently reviewed the literature on capture-recapture techniques and suggests that even the most refined ones are often grossly inaccurate when applied to the estimation of densities of most natural populations and warns against wholesale acceptance that the basic assumptions of the methods are met by the population under study; the inherent biases are reduced to reasonable levels only by sampling intensities higher than are generally achieved with natural populations and density estimates are often of limited use unless only an order-of-magnitude accuracy is required.

Capture-recapture methods may be especially unsuitable for studies of terrestrial snails. Parr et al. (1968) were unable to get reliable population density estimates of *Helix aspersa* using the Jolly (1965) method because random mixing of marked and unmarked animals did not occur. Snails aestivating on walls were captured frequently whereas those in holes were seldom found. A similar situation was found in the present study. In addition, snails maturing eggs were inaccessible for long periods of time and the assumption of equal catchability was clearly violated. As a result, population estimates close in time varied widely and variances were high. Consequently the estimates should not be considered as precise, but rather as an indication of order of magnitude only. Fig. 35 indicates the number of *C. carocollus* estimated to be present around the El Yunque Biological Station and on the Downhill Plot during the study. The best indicator of general trends would probably be those curves smoothed out to eliminate large fluctuations arising from sampling errors inherent in the method. It appears that the population was increasing in late 1962 and into mid-1963 after which it declined to a nearly constant level in the Downhill Plot but continued to gradually decrease around the Biological Station building. The latter decline may have resulted from disturbance to snails associated with the construction of a new wing onto the building, repeated painting of the walls, and other maintenance.

The estimated number of snails indicated in Fig. 35 represents population densities ranging from 1,285 to 7,070 per hectare for the Downhill Plot and 1,990 to 11,520 per hectare for the station grounds. However, the best estimates for providing a general idea of range in population densities of the station ground would be the average of the estimates for the periods December 1962 to May 1963 (high density) and July 1965 to February 1966 (low density). These values are 8,640 and 2,825 per hectare respectively and are probably representative of the range in population densities during the study. In the Downhill Plot, the average estimate for the period of high population density (March to July 1963) was 5,105 per hectare and that of the period of low density (July 1965 to January 1966) was 1,425 per hectare. The latter values are probably representative of general densities of the forest proper in that region. Thus, the population density of snails on the building grounds was 1.5-2 times that in the forest.

Heatwole et al. (1970) have indicated a wide variation in population estimates from the El Verde region but most fall in the range of 300 to 400 snails in the radiation center (1,060 to 1,415 per hectare); there were about 2/3 that number in the control center (700 to 950 per hectare). There was little consistent temporal change in the estimates.

Life history strategies

When the various topics discussed above are combined, it is evident that the various species have adopted different life history strategies. Timing of reproductive events differs among species in the rain forest. In general, *C. carocollus* has a widely spread reproductive season but tends to avoid the wettest part of the year in the rain forest and the drier periods in the lowland areas.

The proximate factors affecting the reproductive cycle and its spatial variation

were not ascertained. However, the fact that these snails, especially young ones, are moisture sensitive suggests that there may be survival value in adjusting appearance of hatchlings and/or young to coincide with favorably moist but not excessively wet periods. Díaz-Piferrer (1962) noted that reproduction in the Cuban tree snail (*Polymita muscarum*) appeared to be closely related to fluctuations of rainfall and apparently occurred at any season of the year if weather conditions were favorable.

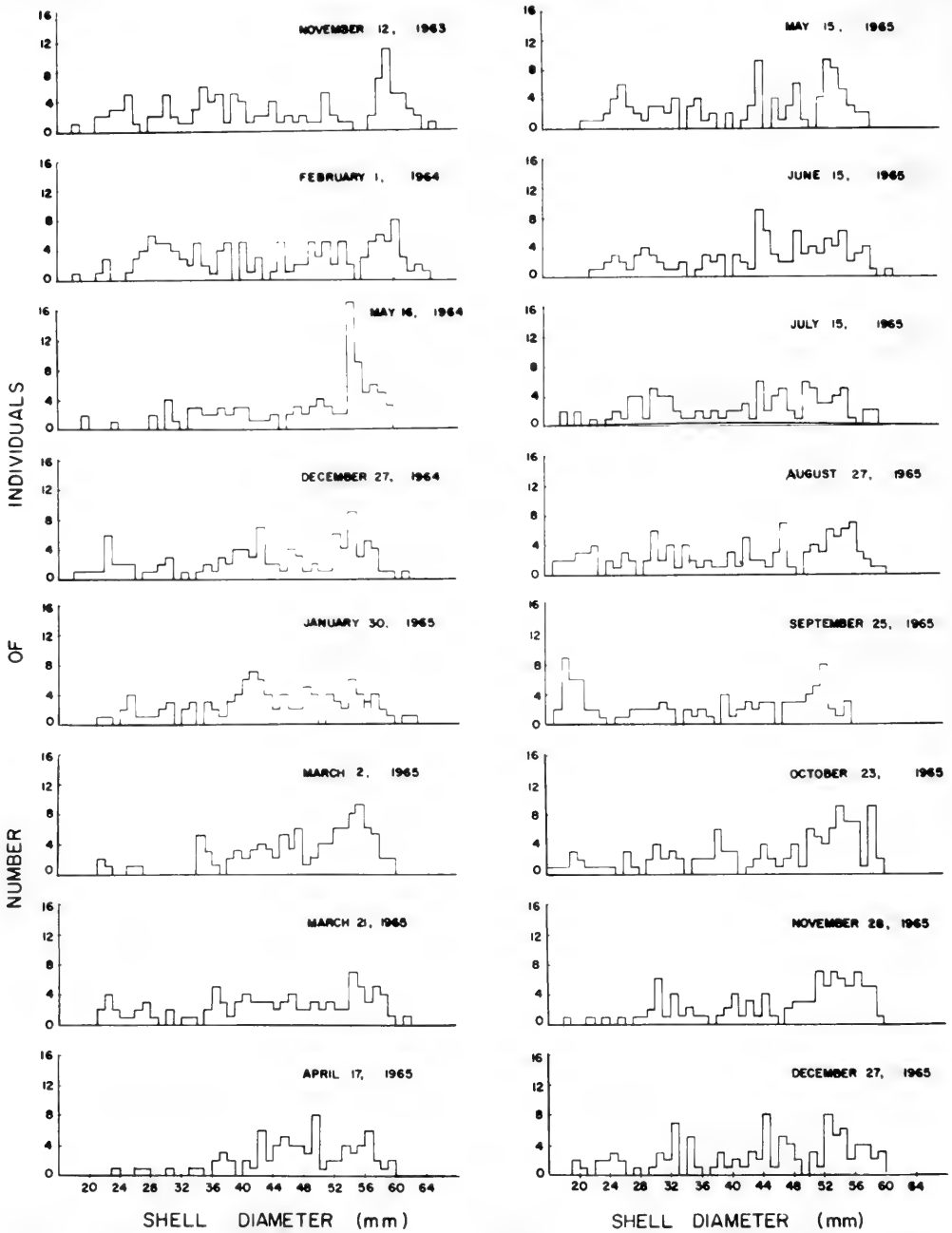

FIG. 33. Size-structure of the *Caracolus carocollus* population on the retaining wall near La Mina on different dates.

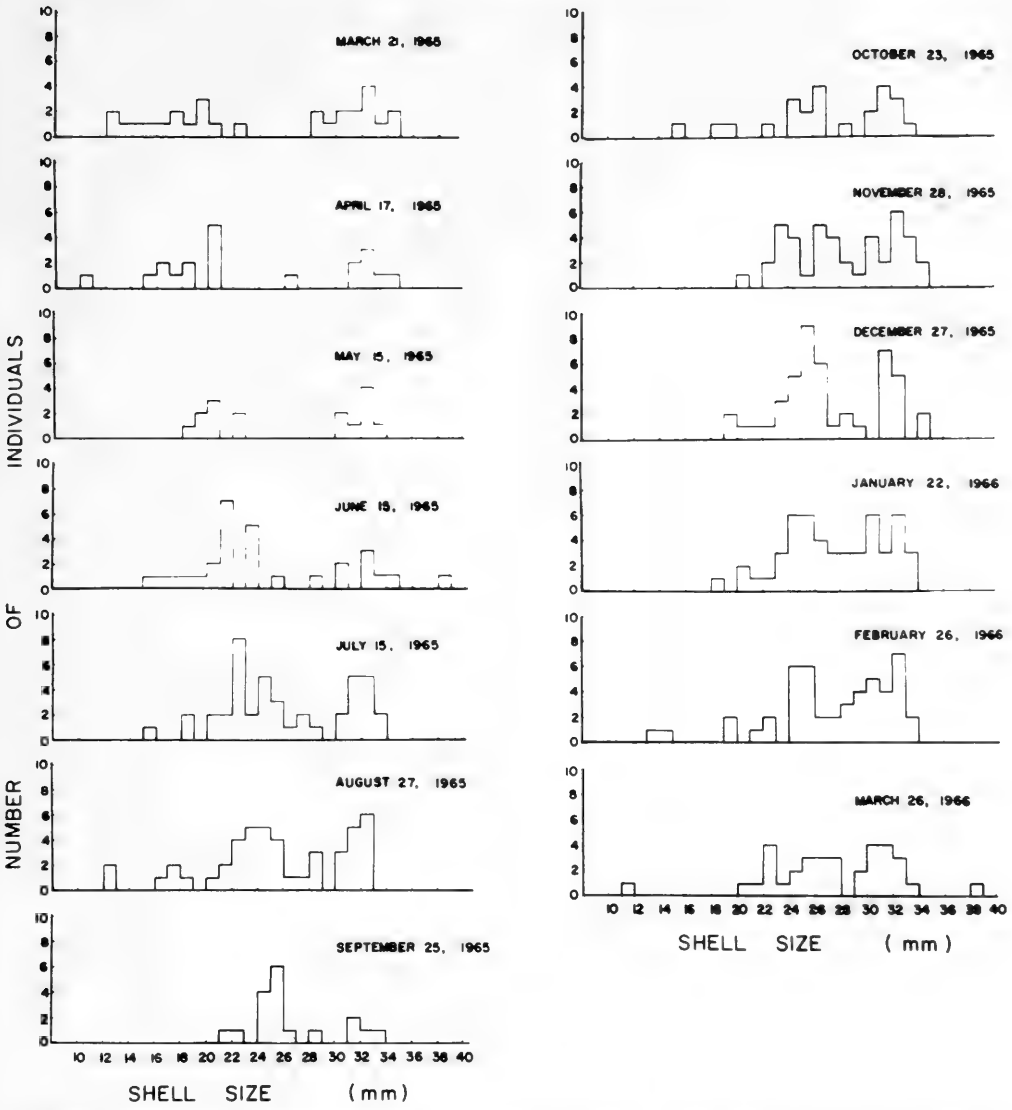

FIG. 34. Size-structure of the *Polydontes luquillensis* population on the retaining wall near La Mina on different dates.

Owen (1964) found that the land snail *Limicolaria martensiana* bred at all months of the year but with two seasonal peaks associated with the two annual wet and dry seasons in such a way that the newly hatched snails appeared during the wettest months.

C. carocollus is a long-lived species with a seasonably stable size-structure whereas *P. luquillensis* and *P. acutangula* from the same locality have much shorter life spans, and *P. luquillensis,* at least, shows seasonal changes in population size-structure. Long life may not be exceptional among large terrestrial snails; for example Dell (1953) found that *Paryphanta busbyi* took 7 years to attain a diameter of 54 mm.

Within a species (*C. carocollus*) survivorship varied between localities, relatively greater mortality occurring among small snails in drier areas. The effect on eggs may have been even greater; no data on egg mortality were obtained.

The various aspects of population and

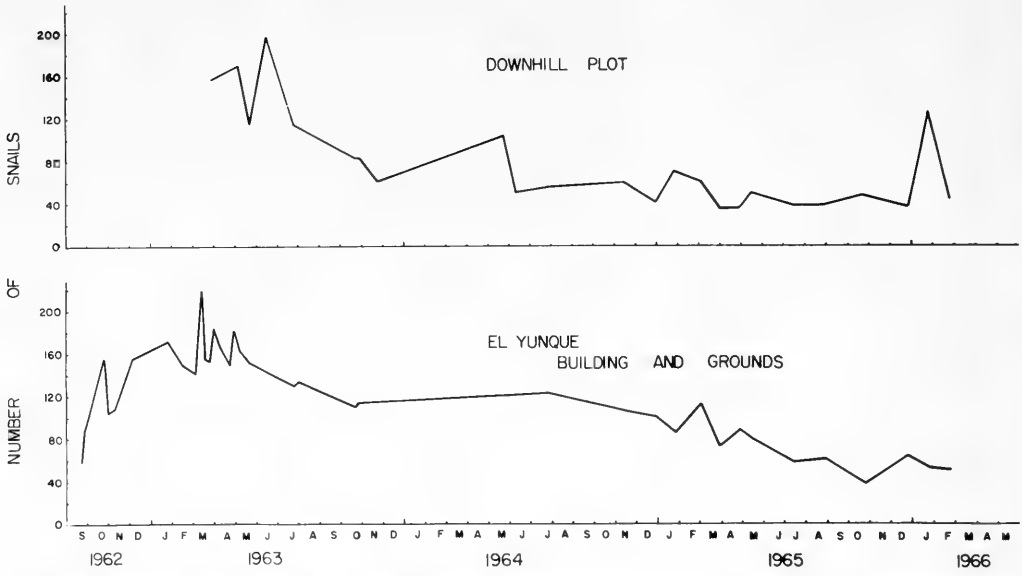

FIG. 35. Change in estimated population density of *Caracolus carocollus* at the El Yunque study area. Estimates made by the method of Jolly (1965).

reproductive biology have consequences for the ways these species can respond to selective influences. These will be discussed in the final section.

SUMMARY AND CONCLUSIONS

In the previous sections the adaptations of camaenid snails to their particular environments were discussed in terms of their behavior, physiology and life history. It now remains to integrate these different aspects into a more complete picture of adaptive patterns.

There are two major ways of viewing adaptation. One is to take a broadly comparative approach and contrast the various modes of adaptation of related taxa occupying different habitats or geographic regions.

The second way is to study the operation of natural selection at the single species level and examine the genetic basis of intraspecific adaptation. This latter approach complements the former in that it provides a background of insight into the mechanics of adaptation against which the comparative aspects can be interpreted.

Study of intraspecific variation (especially color polymorphism) and adaptation at the species level in snails is well advanced and constitutes one of the important pillars of modern population genetics. By contrast, the broadly comparative approach taken in the present study has previously scarcely been attempted for land snails. In this chapter we briefly review the major literature on the population genetics of snails as a background to a discussion of the adaptation of the Puerto Rican camaenids.

The study of ecological genetics and selection in snails has centered around genera which are polymorphic for color and banding, especially *Cepaea*. The genetics of this polymorphism is partly known (Murray, 1963; Murray & Clarke, 1966, Cain et al., 1968) and thus ecological and population data can be interpreted against a certain background of genetic knowledge.

Morph frequencies in nature show "area effects" in that adjacent and apparently similar areas have different morph frequencies but paradoxically, large, ecologically diverse areas may have uniform frequencies (Cain & Currey, 1963a, 1968, Clarke & Murray, 1969). In other cases abundance of certain phenotypes corresponds to particular habitats or conditions which are consistent over a wide geographic area, or which show strong geographic correlates. Much of the research on *Cepaea*

has centered around assessment of the relative role of various factors in influencing morph frequencies. Several categories of factors have been considered.

(1) Drift: Genetic drift was one early suggestion (Lamotte, 1959). It was considered that particular breeding units or demes showed random change in frequencies as a result of stochastic processes operating in small populations. Most later papers have tended to discount the importance of drift relative to that of selection. However, random drift is not completely discounted, and it may operate under particular conditions. Selander & Kauffman (1975) indicated that in *Helix aspersa* in a city, there were demes of only a few (up to 15) individuals which were isolated in part by low mobility. The pattern of variance in allele frequencies suggested that drift was operating. Brussard (1974) found that at some loci in *Cepaea,* stochastic processes in small populations (fewer than 100 individuals) were strong enough to override any heterozygote advantage.

(2) Climatic selection: Another view is that morph frequencies are expressions of the direct effect of selection. Clarke & Murray (1962a, 1962b) and Clarke et al. (1968) collected *Cepaea* in the same localities from which collections had been made several decades earlier and found that there were consistent changes in morph frequencies amounting to a selective advantage or disadvantage of 5.2-6.2 per generation. In other cases, little change occurred and the constancy correlated with degree of habitat change; gene frequencies were more stable in habitats with little change. Some authors have attributed the selective effect to climate or microclimate (Arnold, 1968, 1969, 1971; Jones, 1973b). This appears to be likely in the case of close correlation of local and/or geographic patterns of frequencies with climatic factors if different morphs have different tolerances or responses to environmental factors, as is true in *Cepaea* (see reviews by Lamotte, 1959; Wolda, 1967). Some workers have suggested that climatic effects operate where area effects are evident (Cain & Currey, 1963a, 1963b; Cain, 1968). Arnason & Grant (1976) have pointed to a close correlation of morph frequencies with temperature, shading and humidity in *C. hortensis* near the northern limits of its range in Iceland. Morph frequencies showed constancy over large areas which had sharp discontinuities in habitat. It would appear that habitat and climatic continuities do not necessarily follow the same pattern and that morph frequencies are more closely related to the latter. Cain (1971) has reviewed the work on morph frequencies in subfossil *Cepaea* and concluded that light colored, unbanded individuals were prevalent in hotter times and the darker, banded ones in cooler periods. This also related to the present-day distributional pattern of these morphs (Arnold, 1968, 1969; Cain & Currey, 1963a-c; Carter, 1968; Cain, 1968). Similarly a white-lip seems to be associated with lowered temperature, or more probably, dampness (see review by Cain, 1971). Morph frequencies in *C. hortensis* did not show as clear-cut a temporal pattern as did *C. nemoralis.* The temporal changes in proportions of these 2 species and their present distributions suggest that *C. hortensis* is relatively favored by a cooler climate and *C. nemoralis* by a warmer one (reviewed by Cain, 1971). Finally, Bantock & Price (1975) found that color pattern polymorphism decreased in marginal open areas in ways expected if the effects were related to temperature. The most direct evidence of differential mortality among morphs was obtained by Richardson (1974) who found *C. nemoralis* dying in the field with symptoms of heat death; the morph frequencies of the dead snails departed from that of the population at large. There were fewer than expected of yellow, unbanded snails, a result expected on the basis of geographic and micro-geographic distributions of morph frequencies and on laboratory tests of heat tolerances.

(3) Predator selection: *Cepaea* colonies in woodlands tend to be heavily banded which gives a dark color matching that of the leaf litter, whereas colonies in short grasses have a lower percentage of banding and match the uniformly green background better. This background matching is attributed to "visual selection" such as the selective action of predatory birds against conspicuous color morphs (Cain & Sheppard, 1950, 1954; Currey et al., 1964; Carter, 1968; Cain & Currey, 1968). Two species of *Cepaea* sometimes occur together with rather different morph frequencies. Lamotte (1959) cited this as evidence against visual selection. Clarke (1960), however, points out that different patterns in the two species can appear very similar; one species has a darker background color

whereas the other achieves the same effect by fusion of bands on yellow snails. Bantock & Bayley (1973) found that thrushes could not distinguish between visually similar though structurally different snails of the two species. Richards & Murray (1975) have shown that a population of introduced *Cepaea* into the United States has responded in ways predicted by Clarke's hypothesis. On the other hand, such introduced populations retain genetic characteristics traceable to their site of origin (Brussard, 1975).

Color and pattern polymorphism may become balanced by the tendency of predators to develop search images and take disproportionately higher percentages of common varieties than of rarer ones. Clarke (1962) has called such selection "apostatic selection." The color and pattern polymorphism in the African land snail *Limicolaria martensiana* has been postulated to be influenced by apostatic selection (Owen, 1963); that polymorphism is known to have existed for at least 8,000-10,000 years without loss of any of the types of morphs (Owen, 1966). Thus balanced polymorphism may be relatively stable for long periods.

(4) Stability of co-adapted gene pools: Clarke & Murray (1969) proposed that co-adapted gene pools may account for area effects in species of *Partula* although they were hesitant to suggest that such a mechanism would cause divergence to the point of speciation. Basically the idea is that in species with low mobility, co-adapted gene complexes might arise, and frequencies of shell color genotypes might be determined as much by the genetic environment as by the external environment; there may be evolutionary trends towards steepening of micro-geographic clines and the formation of sharp steps within them which would give rise to regions of comparatively uniform morph frequency, separated from other such regions by various transition zones; these regions need not correspond to discontinuities in the external environment (Clarke, 1966, 1968). However, one would suspect that all successful species would have co-adapted gene pools; a decision as to whether they play any special role in area effects still requires empirical documentation.

Goodhart (1962) has suggested that when large populations are subjected to excessive mortality by disasters (floods in the case of his populations of *Cepaea*) random genetic assortment in the small, isolated populations would operate much as it does in the "founder effect" when a few individuals from a parent population, and carrying only a small part of the variability of the population at large, colonize a new habitat and give rise to a population containing markedly different properties from those of the parent population. Such an effect could account for local populations with different morph frequencies not obviously correlated with environmental features or predation pressures. "Founder" populations might evolve different systems of balanced polymorphism in different areas when exposed to similar selective forces. Upon expansion and meeting of such populations they might maintain their co-adapted genetic integrity.

It is likely that all of these factors, in various combinations in different regions, may be involved. For example, Jones (1973a) has suggested that although shell color may be influenced by the properties related to absorption of solar energy, expression as "area effects" rather than clinal patterns is probably determined by co-adaptation.

A final consideration is that observed morph frequencies may reflect differences in habitat selection by different morphs rather than the categories of effects mentioned above (especially in heterogeneous environments). Different morphs of *Cepaea nemoralis* may select different environments (Sedlmair, 1956). Morph differences may not apply to all aspects of habitat selection, however, as in *Bulimulus,* Heatwole & Clarke (in press) were unable to detect differences among morphs in the selection of height or direction of exposure on tree trunks.

Even in such well-studied groups as *Cepaea* there is not complete agreement as to the relative importance of the various types of selection or of random processes (e.g. Owen & Jones, 1974; Cain & Currey, 1963c versus Goodhart, 1963). Much work remains to be done before the evolutionary dynamics of any snail species can be understood. Perhaps for this reason most previous research has involved detailed study of the ecology of individual species or comparisons of a few aspects among several species. The broadly comparative, synoptic approach has been lacking. The present study is a step in that direction.

Clarke & Murray (1969) have suggested on the basis of work on Moorean *Partula* that tropical islands may provide a favorable environment for sympatric speciation because of their combination of habitat diversity and equable environment and because of a relative lack of resident fauna filling available niches. The camaenids of Puerto Rico, however, do not seem to have followed this path. In contrast to Aegean enids which seem unable to cross water barriers (Heller, 1976) almost every species of Puerto Rican camaenid represents invasion of a different stock from outside, and consequently evolution has centered around interactions of previously reproductively isolated taxa with each other and adjustment to specific climatic regimens rather than divergence in situ from a common stock.

Randolph (1973) obtained experimental data indicating that a species of snail from a more variable physical environment exhibited a wider "tolerance-niche" (temperature and moisture primarily), higher r value, shorter life span, smaller litter size, shorter developmental time, more general behavior relative to its habitat, more general food requirements, and smaller biomass than did a species which occurred in a less fluctuating environment. We have not examined all these aspects for the camaenids; however, some of our results invite comparison. *C. carocollus* has a long life span, a rather long developmental time, a large (but unquantified) biomass, a considerable sensitivity to moisture at high temperatures, and although precise data are lacking it would appear to have a relatively low r value. In these characteristics it would seem to fit the expectation for a species inhabiting a relatively equable environment as indeed it does in part of its range. However, in being a food generalist, having a small clutch size, and being rather temperature-tolerant it resembles more the expectations for a species from a fluctuating environment. When all aspects are considered collectively, it would appear that *C. carocollus* is primarily suited for existence in an equable environment. That it also lives in less equable areas can be attributed to its behavior; in such environments it selects only those micro-environments in which environmental oscillations are damped. This is especially true of the young, and more vulnerable, stages.

Although not studied as well, *P. luquillensis* seems to be more strongly an "equable-environment" type in that it is more sensitive to physical environmental extremes than *C. carocollus,* but less strongly so by virtue of its shorter life span. In reality it occurs only in the most equable environment in Puerto Rico. Both the lowland species, *P. lima* and *C. marginella,* have the expected broad tolerances to the physical environment but insufficient is known of their other characteristics to evaluate whether they fulfil predictions in other regards. The relatively short life span of *P. acutangula* may reflect the fact that it lives in the more exposed parts (and consequently with greater fluctuations) of an otherwise equable environment. However, perhaps it is not realistic to attach too much importance to relative values of life span. Among the species studied, the life span of *P. acutangula* and *P. luquillensis* were short only in comparison to *C. carocollus.* In fact, all of these could be considered as having rather long life spans. Of perhaps more significance is the age-specific differences in survival. Furthermore, a short life span may not always be associated with extreme, fluctuating environments. Maiorana (1976) suggests that uncertain juvenile survival would favor a long adult life. She felt that a benign physical environment imposes no age-specific pattern of mortality on a species and life-history pattern would not be easily predicted. As a species moves into unfavorable environments, two opposite patterns would be expected, depending on whether the adult or juvenile stage were more susceptible to mortality. If the adult stage has a greater probability of survival she suggests that an iteroparous life with delayed maturity and reduced reproductive output might be characteristic whereas if survival of the adult stage were uncertain, early maturity and higher reproductive output may be favored. In the present study in the equable, "benign" rain-forest, survival of all species tested was relatively high at all stages and there was an accumulation of individuals in the adult size classes. In the Loiza Aldea area, however, mortality was high among juveniles of *C. carocollus,* and it has the type of life-history pattern predicted by Maiorana to arise from such conditions.

Taking all aspects into consideration as well as the data of Randolph mentioned above, it would appear that *C. carocollus*

arose under a rather equable environment but that it can persist elsewhere because of its behavioral avoidance of extremes and its pre-adaptation to a life-history strategy suitable under conditions of high juvenile mortality. The other forms are restricted to either upland or lowland areas and their characteristics correspondingly were almost certainly influenced by those environments.

It is appropriate to ask what aspects of the environment were or are most important as selective agents. The physical factors will be considered first. Moisture has been clearly involved as (1) lowland species have a variety of adaptations permitting survival under dry conditions, e.g. secretion of an epiphragm, behavioral avoidance of exposed conditions, and tolerance to desiccation, and (2) mortality in young, lowland *C. carocollus* was observed to be especially high during drought. However, temperature has also played a role as exemplified by the lighter, more reflective color of lowland species and of the upland one that occurs in places exposed to solar radiation. *P. acutangula* is somewhat of an enigma in that it seems to be adapted to prevent excessive radiant heat loads, yet is exposed to higher desiccation because of its inability to withdraw completely into its shell. However, its microhabitat, though often exposed to solar radiation is probably humid. It occurs where there is high rainfall, and humidity is probably often high even at the periphery of the canopy. Certainly it would have access to drinking water in the bases of bromeliads and other locations. The exposed body consequently may not be a detriment and may even enhance temperature resistance (perhaps via evaporative cooling); such a situation would not be unique, as *Mesodon roemeri* and *Helix aspersa* can overcome the effects of high air temperature if sufficient moisture is present (Randolph, 1973; Potts, 1975).

In summarizing the evolutionary responses to the physical environment, it can be said that the Puerto Rican camaenids have the following modes of adaptation. *C. carocollus*: eurytopic, widely distributed, escapes environmental extremes rather than endures them, behaviorally adaptable. *C. marginella*: rather eurytopic, occupies coastal plain, endures environmental extremes rather than escapes them, less adaptable behaviorally. *P. lima*: rather eurytopic, widely distributed except in wetter, cooler areas, endures rather than escapes extremes. *P. acutangula*: stenotopic, restricted in distribution, occupies a rather well-defined habitat, endures rather than escapes extremes. *P. luquillensis*: very stenotopic, very restricted distribution, neither endures nor escapes extremes but rather survives only in an equable habitat.

Biotic interactions may also be involved as the morphologically most similar species tend to be either allopatric or to occupy different habitats. At this stage it is not possible to distinguish whether such habitat differences reflect the habitat preferences and adaptations of the original stocks, or whether competitive interaction among early stocks led to differences in habitat selection and adaptation to the physical environment. It is possible that once certain stocks became established in Puerto Rico and filled particular niches, later immigrant species could not become established because of competition with prior residents. Such a system could result in only mutually compatible or ecologically segregated species becoming established on the island. Whatever the inter-specific interaction, competition for food does not seem to be important as the type of food eaten is abundant and there seem to be few significant dietary differences among syntopic species (but see Butler, 1976). Heavy predation might keep potentially competing populations below competing levels. However, little is known about predation upon Puerto Rican camaenids; no indications of predation were observed during the study. Rats are a probable (recently introduced) predator upon young individuals. The presumably protective slime secreted by *P. acutangula* when disturbed suggests that perhaps in the exposed situations in which it is found avian predators may be a potential problem.

One of the major attributes of snails which has become evident in recent studies is the individual variability in a variety of characteristics. Color polymorphism has long been known in some groups (see above) and has been well studied. However, other more subtle characteristics have also been observed to be polymorphic in the present study. For example, there was a great difference in growth rates among individuals of the same species. Randolph (1973) has shown that a moist substrate and access to drinking water are important for growth of snails. Consequently individual differences in access to moisture

could result in differences in growth rates among individuals. However, in the rainforest such a situation is not likely to arise differentially for different individuals and yet great inter-individual differences in rate of growth were observed. It is more likely that such growth differences were genetically based (see section on growth).

The polymorphism in growth rate may have far-reaching consequences for population dynamics and evolution. Oosterhoff (1977) has shown experimentally that growth rate in *Cepaea* was influenced by various physical environmental factors and by population density. There was also a genetic component. He suggested that changes in growth rate and concomitant changes in size at adulthood would in turn affect juvenile mortality rates and rates of reproduction and thereby contribute to an adjustment of population density. The population data on the Puerto Rican camaenids are not appropriate for testing this model, and more empirical data are required before it can be ascertained whether it is applicable to this group.

There are a number of other attributes of Puerto Rican camaenids which may influence their mode of evolutionary response. For example, they occur in rather dense populations for animals their size, are relatively sedentary and show little migration even as juveniles, are relatively long-lived and breed over a period of years with small clutches at each breeding and have multiple matings among hermaphroditic individuals.

The dense population and the great longevity should result in genetic stability as effects of drift would be reduced and the genetic contribution of given individuals would spread over a number of years. Also, as suggested for *Cepaea* by Williamson et al. (1977) such characteristics mean that adult density may decline for several years with little likelihood of population extinction. The multiple-mating system among hermaphrodites would also tend to minimize the effects of low densities during population fluctuations (Murray, 1964). The combination of low mobility and high population density means that genetic innovations would spread slowly through a population and that stable genetic complexes would develop. One would expect, therefore, that there would be gradual improvement of fitness in respect to the environments occupied but not a marked ability to rapidly exploit new conditions (also low clutch numbers would militate against this). The impression gained is that of a suite of K-selected species.

The different species differ in regard to various of the above attributes and may show varying tendencies in this direction. However, all appear to be generally categorized as above. Their chief differences appear to be in the degree to which they are adapted to wet, cool as opposed to hot, dry habitats, and the amount of behavioral plasticity involved.

ACKNOWLEDGMENTS

This project was carried out under United States Atomic Energy Commission Contract AT-(40-1)-1833, with the Puerto Rico Nuclear Center of the University of Puerto Rico; we are grateful to Dr. Howard T. Odum for his aid and encouragement. The 1970 portion of the study was made possible by sabbatical travel funds from the University of New England. Dr. Hugh Ford read and criticized parts of the manuscript. The following persons aided various parts of the field and/or laboratory work or provided technical assistance: Sheila Blasini Austin, Isabel Colorado, Rita Amadeo, Abel Rossy, Julia Rossy, Zaida Miranda, Ana Vasquez, Sara Armstrong, Carlos Maestri, Frank Torres, Faustino McKenzie, Mary Lou Pressick, Clara Coulsen, Barbara Saylor Done, Joaquin Molinari, Elizabeth Cameron and Harry Wadleigh. Roy Woodbury identified plants from the El Yunque study area and Dr. N. Prakash aided in identification of plant remains in snail feces. Drs. John B. Burch, Phillip Coleman and Carden Wallace provided literature. Viola Watt, Neva Walden, Russell Hobbs and Heather Powell aided in preparation of the manuscript. Dr. M. J. Bishop contributed heavily to the section dealing with the evolution and speciation of the Puerto Rican camaenids.

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APPENDIX 1.
Descriptions of the four study areas

Four study areas were selected that collectively included all five species (Fig. 2). Two areas were in the Luquillo Mountains: (1) the vicinity of the El Yunque Biological Station of the University of Puerto Rico and (2) the El Verde site used as an experimental radiation study area by the Puerto Rican Nuclear Center. *C. carocollus, P. acutangula* and *P. luquillensis* all oc-

curred at both these sites. In addition (3) a lowland, dry forest near Loiza Aldea, in which *C. carocollus* was the principal resident camaenid, was investigated. Small numbers of *P. lima* and *C. marginella* were also present and one dead shell of an adult *P. acutangula* (perhaps transported there by some external agency) was found at this site. The final study area (4) was an open, lowland, park-like area surrounding the faculty residences of the University of Puerto Rico at Rio Piedras. Only *P. lima* and *C. marginella* occurred there. General views of the study areas are shown in Figs. 36, 37 and the climate of each in Fig. 38. Each area will be briefly described.

El Yunque Biological Station

This study area is located in the Luquillo Mountains at the eastern end of the island (elevation 640 m). These mountains receive the highest rainfall of any place on the island. Mean annual precipitation at La Mina, near the Biological Station, is well over 500 cm. Distribution throughout the year is relatively uniform, the wettest month (December) averaging over 50 cm and the driest one (March) about 25 cm. Fog occurs nearly daily. Individual years may depart from this pattern somewhat. For example in 1965, January to April were relatively dry, May to August were wet and the rest of the year intermediate (see Fig. 4).

Air temperature is remarkably uniform, mean monthly values ranging from about 19°C to 22°C (Fig. 38). Over short periods of time, temperatures are quite uniform. For example, air temperatures were continuously recorded by a Friez Hygrothermograph 15 cm above the ground at the Biological Station for 6 weeks in 1963; the range in values for the weeks beginning 15 July, 26 July, 9 September, 27 September, 12 October and 10 November were respectively 18.5-19.8°C, 19.8-21.0°C, 18.8-21.0°C, 21.0-24.0°C, 22.0-24.2°C and 20.2-23.5°C. Relative humidities were continuously recorded for the two weeks beginning 27 September and 10 November. The ranges were 85-98% and 78-97% respectively.

At El Yunque Peak, somewhat higher in elevation than the Biological Station, temperatures at the surface of the soil ranged from 11.5-36.5°C between September 1960 and January 1961 with the difference between maxima and minima for individual months being 9-16.5 centigrade degrees. Corresponding values for 2.5 cm above the soil were 11.5-32.0°C (individual monthly spans 11.5-13.5 centigrade degrees) and for 2.5 cm below ground were 11.5-29.0°C (individual monthly spans 2-9.5 centigrade degrees). These data are presented in more detail in tabular form by Heatwole et al. (1965).

The vegetation consists of a wet forest type variously called Upper Luquillo Forest (Little & Wadsworth, 1964), Montane Rain Forest (Dansereau, 1966) and Subtropical Lower Montane Wet Forest (Holdridge, 1970). It is characterised by most species being evergreen, a closed canopy at 15 m or more, presence of tree ferns and an abundance of epiphytic bromeliads (Fig. 36). In the El Yunque study area, the Sierra Palm, *Euterpe globosa,* is the predominant floral component and the forest there is a separate system or subsystem called Palm Brake by Beard (1944) and Odum (1970). In the study area a large number of broad-leafed trees are also present, including *Croton poecilanthus, Cecropia peltata, Alchornea latifolia, Cyrilla racemiflora, Calycogonium squamulosum, Cordia borinquensis, Guarea ramiflora, Miconia sintenisii, Clusia krugiana, Ocotea leucoxylon* and *Psychotria berteroiana.* The tree fern, *Cyathea arborea,* is also present. The immediate grounds of the Biological Station consisted of low vegetation bordered by a hedge of hibiscus and bananas, beyond which was the forest (Fig. 36). The hedges and the walls of the buildings were occupied by snails and were included in the study. This part of the study area is referred to as the "Station Grounds": that part consisting of the forest itself is referred to as the "Downhill Plot."

El Verde

This area and its vegetation and weather have been described in great detail by Odum & Pigeon (1970). The study sites were located at an elevation of 424-460 m on the western slopes of the Luquillo Mountains; they are in the Upper Luquillo zone of Little & Wadsworth (1964). The forest is Tabonuco Rain Forest (Odum 1970) which corresponds to the Lower Montane Rain Forest of Beard (1944) and Dansereau (1966) and to the Subtropical Wet Forest of Holdridge (1970). The closed

FIG. 36. Views of the El Yunque Biological Station general study area. Upper: Montane Rain Forest near La Mina. Lower: The grounds and building of the El Yunque Biological Station, near La Mina.

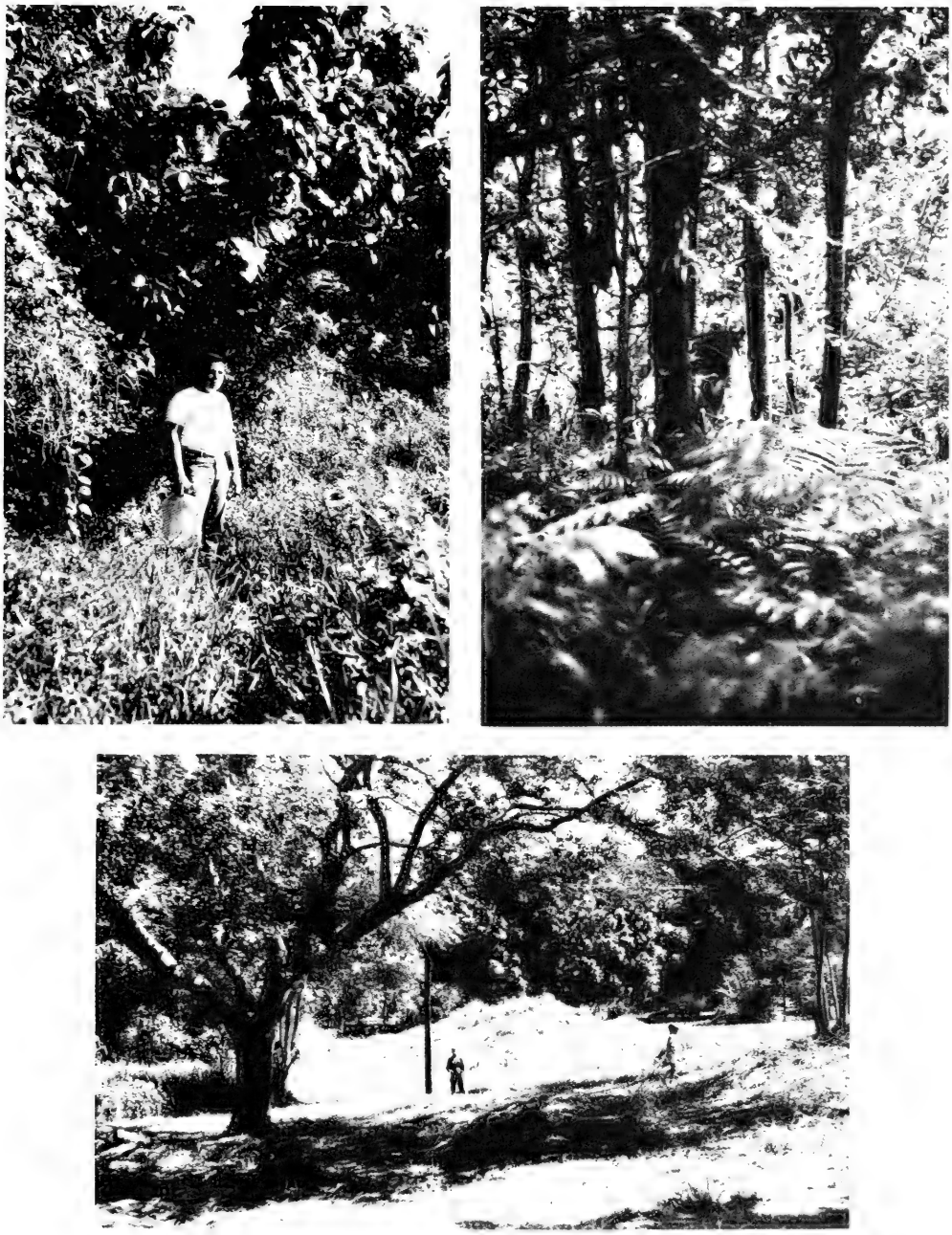

FIG. 37. Views of the El Verde (upper left), Loiza Aldea (upper right) and Rio Piedras (lower) study areas. The photograph of the Loiza Aldea area shows the edge of the forest; underneath the canopy, there is almost no herb cover, only leaf litter and limestone rock.

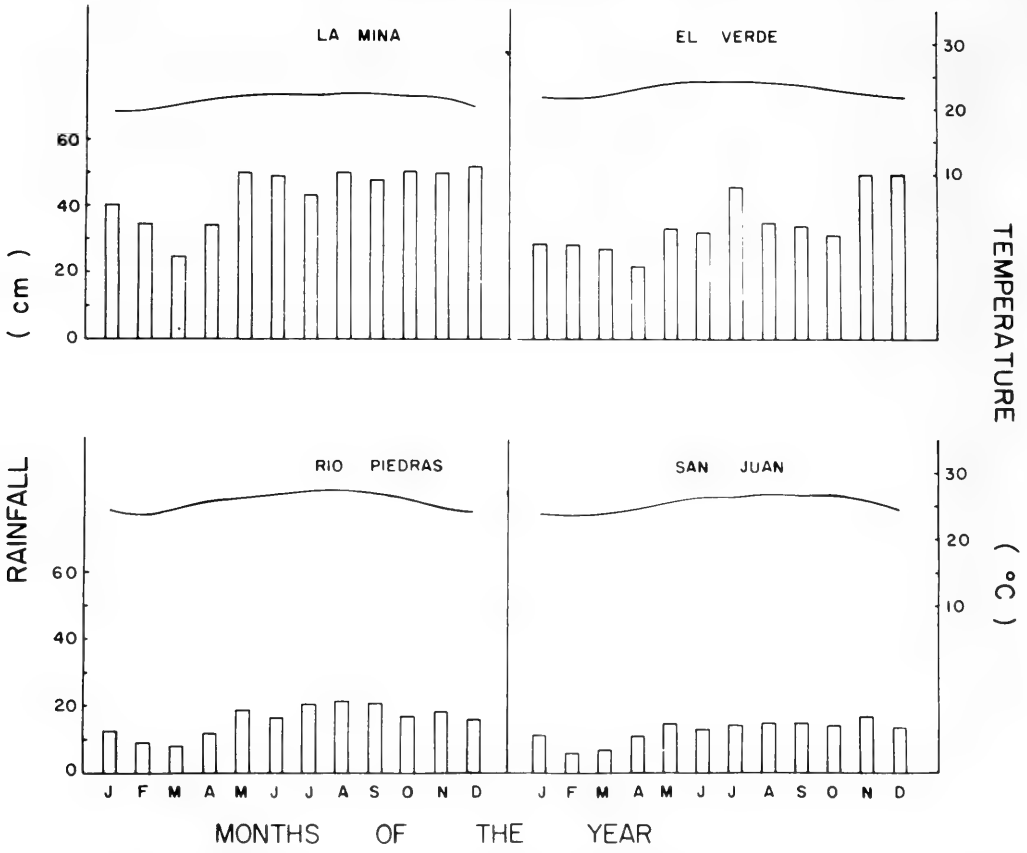

FIG. 38. Mean monthly rainfall and temperature at the study sites. Data not available for Loiza Aldea and consequently data from San Juan are substituted (see Appendix I). Data summarized from Picó (1954), Odum & Pigeon (1970), Desmarais and Helmuth (1970), and U.S. Dept. of Commerce (1966).

canopy extends up to about 25 m; there is an open ground story in deep shade. The vegetation is characterized by buttressed roots, broad thin leaves, bromeliads, lianas, and trunk bark mottled with epiphytic growth (Odum 1970) (Fig. 37). Many of the same tree species occur here as at the Biological Station, including the Sierra Palm. However, at El Verde, the Tabonuco, *Dacryodes excelsa,* is the principal tree (Odum 1970). Rainfall is abundant throughout the year, exceeding 20 cm even in the driest month (April) and reaches 50 cm in the wettest ones (November, December). The seasonal pattern is roughly similar to that at La Mina but is slightly lower in absolute values (Fig. 38). Temperatures are moderate with slightly higher values than at La Mina; mean monthly values range from about 21.5°C to 24°C.

Two subareas were marked out for detailed study in relation to the radiation project carried out there. These are known as Center I and Center II, each a circle of 30 m in radius. Both centers were mapped in detail by the Puerto Rican Nuclear Center (even to locations of individual trees); these maps were available for the present study.

Loiza Aldea

This study area was located near sea level in the vicinity of the coastal town of Loiza Aldea. The substrate was weathered limestone, with large chunks of limestone present on the forest floor.

Little & Wadsworth (1964) have characterized the climax forest in this general area as Moist Coastal Forest (moist in

comparison to the much more xeric forests of the southern coast, not in comparison to the higher altitude forests). However, because of its underlying porous limestone this forest has a more xeric appearance than its name implies. It more closely resembles the Seasonal-Evergreen Forest of the north-western and north central coasts described by Dansereau (1966) who also shows small areas of this forest further east, including the general vicinity of Loiza Aldea. The canopy is nearly closed. Around the edges there is a tangle of vines and other vegetation but inside the forest, the floor is relatively free of low vegetation.

Rainfall in this area is sparser and more seasonal than in the mountains; in the absence of rainfall and temperature data from Loiza Aldea, those for San Juan which has approximately the same weather conditions (Picó, 1954) have been used instead. The driest month (February) receives on the average only 6 cm of rain and the wettest one (November) about 17 cm.

Temperatures are higher than in the mountains, the coolest months (January, February) have a mean value of about 24°C, and the hottest one (August) one of 27°C (Fig. 38).

Rio Piedras

The grounds of the faculty residences (elevation 30 m) consisted of lawns, hedges or shrubs, areas of herbs and low vines bordering a small stream, all interspersed with shade trees (Fig. 37). The mean annual rainfall is 189 cm, with the lowest monthly mean being 8.3 cm for March and the highest one of 21 cm for August (Desmarais & Helmuth, 1970) (see also Fig. 38). Temperature is relatively high with mean monthly values ranging from 23.5°C to 27.5°C (February and August, respectively).

Being a residential area, this study site was exposed to the influence of humans such as trimming of lawns and shrubbery. These influences undoubtedly had some effect on the ecology of the snails. No evidence of overt interference of humans with snails, such as pulling them off trees or stepping on them was ever noticed.

In summary, the study areas can be ranked in order of decreasing moisture and shade and increasing temperature as: La Mina, El Verde, Loiza Aldea, Rio Piedras.

The study was carried out from September 1961 to June 1966, with an additional study period in 1969-70. A preliminary report has appeared (Heatwole, 1965) and results relating to the effects of radiation at El Verde have been published (Heatwole et al., 1970).

APPENDIX 2.
Methods and results

Not all aspects could be studied equally thoroughly for all species in all habitats. Table 2 is presented in order to provide the reader with a synoptic view of the coverage.

Activity cycle

Snails encountered in the field were tallied as either active or inactive. The criterion for activity was that the tentacles were extended. Inactive snails (tentacles retracted) had their bodies completely withdrawn into the shell if on the ground or in a bromeliad axil, or had the foot extended and adhering to the supporting surface if on a tree trunk or wall.

If time were limited, collection of activity data was sometimes omitted for a given day. However, when such data were taken, the activity of all snails encountered on that day was recorded so as to prevent bias.

Tables 3 and 4 indicate the activity of snails captured at different times of day and under different weather conditions.

Habitat selection

Individual trees in the Downhill Plot were categorized as to how many snails used them as their principal daytime resting site and the results tested to see whether trees were selected at random according to either size or species. There were 198 snails, omitting 26 whose principal resting sites were on dead stumps. Expected number of *C. carocollus* (if distributed at random) associated with a given species of tree was calculated from the proportion of the total number of trees of that species represented, grouping the 7 least common species together. The observed values were tested against the expected by a chi-square analysis. Snails were not distributed randomly with respect to tree species ($P << 0.005$). In a similar analysis cate-

gorizing trees in 5 cm intervals of *dbh* instead of by species, snails were not randomly distributed with respect to tree diameter ($P << 0.005$); fewer than expected were associated with small trees and more than expected were found on large ones. Because of the large number of categories required, a contingency test could not be carried out to see whether selection of certain tree species occurred independently of the size effect. However, a graphic analysis permits a rough evaluation to be made (Fig. 6). The Sierra Palm, *Euterpe globosa*, was the most abundant species in the plot (53% of the live trees) and was represented by a wide size range of individuals. A plot of the number of snails associated with individual trees against tree *dbh* shows a direct, though loose relationship (Fig. 6). Values for most of the other species fall within or near the belt defined by *E. globosa.*

In order to ascertain whether distribution was related to topography, the locations of centers of home site ranges (see below) of *C. carocollus* from the El Verde study area were plotted on a topographic map (Fig. 7). In Center I the northwest quadrant was virtually devoid of snails. This section had a very steep slope compared to other parts of the study area. In Center II where slopes were more gentle, snails were found throughout most of the area and were much more evenly distributed.

Home site range

Snails were individually marked (see section on growth). The location of each snail found by day was plotted on a map at each observation period (33 periods for the forest at El Yunque and 45 periods for the area around the building); numbers of recaptures of individual snails reached as high as 19. Some snails were found hanging within a few cm of the same spot on a wall or tree trunk on many different occasions over a period of more than 8 months (Figs. 8, 9). During some observation periods interspersed among the total number, they were found elsewhere, or not seen at all (probably under leaf litter, in bromeliads or other places where they could not be easily observed). The places a snail occupied during the inactive period can be designated its home site, and the polygon whose outline is delimited on the horizontal plane of a map by the most peripheral home sites of an individual snail, as its home site range (*hsr*) (Heatwole et al., 1970). The *hsr* is much smaller than the home range which would also include the area traversed by the active snail at night. It should also be emphasized that the *hsr* should be thought of as a volume, as height on tree trunks adds a vertical component ignored in calculating *hsr*'s in the present study.

Many of the snails were captured only one or two times (Table 5). These probably included individuals that died soon after their first capture, transients moving through the area, juveniles hatched near the end of the study, individuals that had only a small part of their *hsr* included in the study area, or those that had tendencies towards mobility rather than setting up an *hsr* (Lomnicki, 1969). The main distance between capture points for two-site captures were practically identical for snails that were captured only twice and those that were captured more often (Table 5). The difference was not significant (Mann-Whitney U Test, one-tailed, $P > 0.05$).

The greater the number of recaptures of an individual snail, the more accurately would be the outline of its *hsr.* Consequently, those *hsr*'s based on few recaptures might not be reliable indicators of the true *hsr* size. In order to ascertain the minimum number of captures needed to adequately estimate the size of the *hsr,* the mean areas were plotted against the number of captures on which each was based (Fig. 11). Average estimates were relatively low up to six captures but were higher from seven or more captures. Thus seven captures seem to be about the minimum necessary to delimit an individual's *hsr.* Variation among individuals is high and large numbers of individuals are required if the population is to be characterized. For the El Yunque population the mean size of *hsr* of snails captured 7 times or more was 7.2 m^2 (Table 5).

It was thought probable that because of the lower density of trees around the building than in the downhill plot, that snails in the former place might have larger *hsr*'s than those in the latter. The respective mean areas of the *hsr*'s in the two areas were 6.5 and 8.5 m^2 respectively, i.e. the differences were in the opposite direction to those predicted. However, the differences were not significant ($t = 1.18$; $0.4 > P > 0.2$). Many of the snails on the

station grounds were not included in the above analysis as they were found only on the walls of the building and an *hsr* could not be calculated (see Figs. 8, 9).

If juveniles disperse widely it would be expected that they would account for a greater proportion of single captures and captures at two sites than would be true for animals captured more often. Also their *hsr*'s and distance between two capture points would be expected to be larger than those of adults. None of the above predictions proved true.

The percentage of juveniles among single captures and those captured more than three times were quite close (58% and 55% respectively); only 36% of the animals captured twice or more times were juveniles. The mean *hsr* size of juveniles and adults (captured seven or more times) was 7.67 m^2 and 7.18 m^2 respectively; the difference was not significant ($t = 0.29$; $P > 0.50$). Similarly, the distances between capture sites of snails that were captured twice did not differ significantly between adults and juveniles (Mann-Whitney U Test, one-tailed, $P > 0.50$).

Food

In addition to direct observations of feeding the diet of snails was assessed by collecting fresh feces, still clinging to the animals, in the field. The samples were kept either dry or preserved in FPA fixative until analyzed. At the time of analysis, feces from all individuals for a given species and locality were lumped together and soaked in water until disintegrated into component pieces. The suspension was stirred until thoroughly mixed and a subsample taken and the larger fragments sieved out, floated in 70% alcohol in a petri dish and examined under a binocular microscope at ×8 magnification. A grid in 1 cm divisions was placed under the petri dish and the fragment closest to each crosspoint of the grid identified and listed. There were 100 such points examined per species and locality.

Casual inspection under a microscope revealed the presence of many smaller items not identifiable by the above method. Consequently, subsamples were taken from the stirred suspension by a dropper and placed on a glass slide with a drop each of safranine and glycerin, covered with a coverslip and examined at a magnification of ×200. There were 2-5 replicate mounts prepared per species and locality. Microscope fields on these mounts were examined at random and all identifiable items in the field noted. There were 20-40 fields examined per species.

It is important to note that the various percentages indicated in Tables 8 and 9 do not refer to relative weights of different material eaten as the leaf and wood pieces were larger than the unicellular algae; different-sized pieces within a given category were also given equal rank.

Fungal hyphae were abundant and on occasion were noted to have penetrated cells of vascular plants. These fungi may represent molds growing on the dead leaves consumed. However, it is possible that the fungal growth occurred on the feces after it had left the animal (long strings often adhere to the animal for a considerable time after deposition). Because of the uncertainty as to whether mycelia were ingested or not they were excluded from Table 9. They represented 17% (El Verde) to 29% (El Yunque) of the microscopic elements in the feces of *C. carocollus*.

Body temperature

Body temperatures (T_B) were measured in the field for all but the smallest species (*P. lima*) by insertion of a Schultheis quick-registering thermometer, accurate to $\pm 0.1^{\circ}C$, into the opening of the mantle cavity. Air (T_A) and substrate (T_S) temperatures at the site of the snail were then measured. In several instances black bulb temperatures (T_{BB}) were also obtained using a black bulb thermometer constructed by inserting the sensitive end of a Schultheis thermometer into the center of a table-tennis ball, sealing the opening with glue and painting the ball with flat, black paint. Data were collected between 1300 and 1630 h under a variety of weather conditions during the period February-April, except for 3 readings on *C. marginella* in November. Thus, the total daily and seasonal ranges of temperatures are not represented, but rather interspecific comparison is made of afternoon temperatures during the end of the dry season, the period when the highest temperatures were likely to be experienced.

Temperature tolerances

Animals for use in experiments on temperature tolerance were collected in two

localities, *C. carocollus, P. acutangula* and *P. luquillensis* from the El Yunque Biological Station and *C. marginella* and *P. lima* from Rio Piedras. All animals were acclimated at 21° ± 1°C and a 9L15D photo-period for 4-7 days before their tolerances were tested. During acclimation they were kept in containers with screen false bottoms below which a layer of water maintained high humidities. They were supplied with boiled *Hibiscus* leaves daily.

The apparatus used to test tolerances consisted of a glass jar closed by a 2-hole rubber stopper through which passed a thermometer and a glass tube to supply air. A snail was placed in the jar which was then weighted and submerged in a 5,000 ml beaker of water. After the snail had resumed activity inside the jar, the water, initially at 21°C was heated at a rate of 1°C every 5 minutes. An air stone attached to an aquarium pump bubbled the water and promoted uniform distribution of heat within the bath. Two end-points were easily determined. The first was the dropping of the active snail from the wall of the jar to the bottom (the "falling point"). This may have been a behavioral response to high temperatures or perhaps the point at which viscosity of the mucus became too low to permit adhesion to a vertical surface. Its ecological significance is that in nature the snail would drop from a tree trunk to the ground where cooler conditions might be found beneath stones or among litter or vegetation. The second end-point was the cessation of response to tactile stimulation (lethal point). After the falling point had been reached, the jar was raised so that the top protruded above the surface of the water after each 5-minute heating interval (1°C rise in temperature). The stopper was removed and the snail prodded with a needle. The first temperature at which it failed to respond was considered the lethal point.

Large individuals of *C. carocollus* and *C. marginella* had higher temperature tolerances than did small animals, the relationship between lethal point and shell diameter being linear (Fig. 16). The slope of the regression line was 0.041°C/mm diam for *C. carocollus* and 0.104°C/mm diam for *C. marginella*; both were significantly different from zero (t-test; $0.025 > P > 0.010$ and $0.050 > P > 0.025$, respectively). The slope for *C. marginella* was not significantly different from that for *C. carocollus* (t-test; $P > 0.10$) and thus size has the same relative effect on lethal limit in both species.

The falling point was independent of size. Slopes of regression lines of falling point vs shell diameter did not differ significantly from zero (t-test; $P > 0.50$ for *C. carocollus* and $0.20 > P > 0.10$ for *C. marginella*).

Because of the dependency of lethal point on size, juveniles of *C. carocollus* and *C. marginella* were not used in making interspecific comparisons of this end-point; tests of other species involved only adults. Juveniles and adults were grouped, however, for interspecific comparisons of falling point since this showed no dependency on size.

Effect of temperature on food-deprived animals

The following experiment was designed to evaluate thermal effects on depletion of stored energy reserves in *C. carocollus* and *C. marginella.*

C. carocollus was collected at El Yunque and *C. marginella* at Rio Piedras. They were acclimated for 8 days at 20° ± 1°C and a 9L15D photo-period in a closed glass terrarium with about 1 cm of water at the bottom to provide a relative humidity near saturation. During this period it was assumed that all of the snails emptied their digestive tracts completely as no defecation occurred during the post-acclimation period. After acclimation, the *C. carocollus* snails were divided into two groups, each with approximately the same size-distribution. One group was maintained under the acclimation conditions (20°C and near 100% *r.h.*) without food until all were dead. The second group was treated differently only in that they were transferred to a cabinet at 30° ± 1°C. These two temperatures were chosen because they represent typical ones in the field in the uplands and lowlands respectively during the end of the dry season when unfavorable conditions are most likely to occur (see section on body temperature). The lights were usually turned on at 0800 and off at 1700 h. Minor departures from this photo-period were the same for both groups. *C. marginella* was treated identically except that only adults were used.

Mantle water and moisture content

C. carocollus often carried a certain amount of water in the mantle cavity. It

was considered that such water might be a store usable during dry periods and that the quantity might accordingly vary seasonally or spatially depending on immediate environmental availability of water. To test this, at monthly intervals for part of the study, a sample of 25 snails from each of various microhabitats in the Loiza Aldea and El Yunque study areas were forced to yield their mantle water for measurement. All snails were taken well away from mapped areas in which population studies were being carried out. Each snail was repeatedly prodded until it had retracted into its shell as far as it could go. In so doing the mantle water was squeezed out and could be removed from the shell with a hypodermic syringe and the volume measured.

During the driest period of the study, a sample of 25 adult, inactive snails were collected from the El Yunque (23 Jan. 1965) and Loiza Aldea (15 Feb. 1965) study areas for ascertaining whether the drier condition of the lowland site would be reflected in reduced hydration levels of the snails there. The mantle water was first removed and measured and then the animals killed and the shell, soft body parts and gut contents separately weighed. The soft body was then oven dried at 105° C to constant weight for determining moisture content.

The samples of *C. carocollus* taken from El Yunque and from Loiza Aldea differed slightly in water content of the body and the differences were significant ($t = 5.68$, $P < 0.001$; two-tailed). The snails from El Yunque had a significantly greater average absolute body weight than those from Loiza Aldea ($t = 6.23$; $P < 0.001$; two-tailed).

The mantle water of species other than *C. carocollus* was not studied extensively. However, on 22 March 1965, 3 *P. lima* were examined for mantle water; none contained any. On 28 August 1965, a sample of 25 adult *P. luquillensis* snails were obtained at the El Yunque Biological Station. Mean mantle water was 0.86 ml (S.E. = 0.143; Range = 0-2.2 ml).

Desiccation

Standard laboratory desiccator jars with screen separating the upper and lower compartments were used as experimental chambers for ascertaining evaporative loss, survival time and vital limit of water loss under several conditions of temperature and humidity. One group of desiccators was maintained at 30° ± 1°C; some of these contained water in the bottom compartment (atmosphere nearly saturated) and the others contained $CaCl_2$ (dry atmosphere). A second series of desiccators was maintained at 20° ± 1°C, again subdivided into humid and dry groups.

Snails used in the experiment were first kept in glass terraria with access to standing water but not food, for 48 hrs. Their mantle water was then removed and 5 individuals were placed in each desiccator jar. For some species and treatments juveniles and adults were both tested, in others only adults were used. In addition, a separate series of *C. carocollus* and *C. marginella* were treated identically except that the snails were allowed to retain their mantle water. The experiment was carried out at a photoperiod of 9L15D. The snails were not fed during the experiment nor did they have direct access to water.

At intervals of one to three days the animals were removed, checked for presence or absence of either a clear mucus membrane or an epiphragm, weighed, and returned to the chamber. Each terrarium was checked daily for dead animals. These were removed, weighed, and the vital limit of water loss calculated as the amount of weight lost in % of the total fresh weight at the beginning of the experiment (including shell).

Since the experiment lasted a long time, a part of the weight loss was undoubtedly due to metabolic losses of carbon and constituted an unmeasured source of experimental error. Neither the vital limit nor the rate of loss per unit body weight of the snails originally containing mantle water could be calculated as an undetermined part of their original weight resulted from their water load.

Survival time during desiccation depended on animal size; larger animals survived for longer periods than did smaller ones. In all cases except one (*C. marginella* at 30°C in dry air), mean survival of juveniles was lower than for conspecific adults under the same treatment; the correlation of size and survival time was significant in 7 of the 13 testable treatments (Table 11). The means of juveniles can not be used to compare treatments or species as absolute levels of survival times would depend on the relative

proportions of very young to nearly subadult individuals in the juvenile sample. Consequently, only data from adults are used for subsequent analyses. Within the size range of adults, size did not influence survival time (Spearman Rank Correlation Tests; $P > 0.05$ in all treatments for every species, one-tailed).

The vital limit is the percent of hydrated body weight which can be lost as water before death occurs.

In *C. carocollus* and *P. acutangula* the mean vital limits obtained during different treatments were not significantly different (Dice-Leraas graphic test) and consequently for these two species, values from all treatments were combined for interspecific comparisons. In the remaining 3 species the mean vital limit obtained from desiccation at both humidities at 30°C and in dry air at 20°C were not significantly different and were grouped. However, the mean vital limit of snails of these three species subjected to moist air at 20°C was much (and significantly) lower than that for other treatments of the same species. This curious result was probably not a chance phenomenon as it occurred in 3 of the 5 species (Fig. 21). No ready explanation for the function of such a response comes to mind. This result required that data from 20°C in moist air could not be validly combined with those from other treatments and consequently they are presented separately.

Vital limit for a given species and treatment showed no significant correlation to size even in samples including juveniles and adults (Spearman Rank Correlation Test; $P > 0.10$ in all cases except one significant one; Table 12). It can be concluded that there is usually no ontogenetic change in vital limit of water loss in these species and that the difference between adults and juveniles in survival probably reflects differences in rates of loss rather than in vital limits. To test this hypothesis, the rates of loss were analyzed. In 5 of the 11 comparisons of adult and juvenile mean water loss, the observed values were higher in adults than in juveniles but in only one of these cases were the differences significant (Table 13). In the 6 remaining cases, juvenile mean rates of loss were higher than those of adults, the differences being significant in 3 of them (Table 13). However, within the adult size range, there was never a significant correlation between rate of loss and size (Spearman Rank Correlation Test; $P >> 0.05$ in all cases).

Role of membranes

Data on water loss from snails when clear membranes sealed their shell apertures and from periods when such a membrane was absent were available for 6 individuals. In 3 of them rates of loss averaged higher when there was no membrane, in 2 it averaged higher when there was, and in 1 the mean rates were identical for the 2 situations. A Wilcoxon Matched Pairs Signed Rank Test (matching the data by individual animals) indicated no significant difference between water loss values between the two states ($P >> 0.05$, one-tailed).

There were sufficient data from snails with epiphragms to relate survival time of individual snails to the proportion of the time in which they had an epiphragm. A correlation analysis revealed that survival time was positively correlated with proportion of the time an epiphragm was in place (Spearman Rank Correlation Test; $N = 10$, $r_s = 0.76$; $0.05 > P > 0.01$, one-tailed). The line fitted to the data by least squares had the formula:

$$\text{Survival time} = 21.7 + 0.54x$$

where x is the proportion of the time the snail is protected by an epiphragm. A linear relationship between the two variables appeared reasonable from visual inspection. Other groups of *C. marginella* that formed epiphragms did so over too small a range of time to permit correlation analysis.

The desiccation histories of all individual snails of the 30°C group that had days both in which there was an epiphragm and those in which there was no membrane of any kind, were examined on a day by day basis. A Wilcoxon Sums Matched Pairs Signed Ranks test in which the mean rate of loss when an animal had no membrane was matched against that of the same individual when it had an epiphragm, was carried out on both adults and juveniles ($N = 16$). The rate of loss was significantly lower when the epiphragm was present ($0.05 > P > 0.025$; one-tailed). The degree of reduction in water loss afforded by the epiphragm depended on the conditions of desiccation. The reduction was 9.7% in adults at 30°C but not over desiccant and 21.7% for those in the containers with $CaCl_2$ at 30°C.

Response to relative humidity

For testing responses to relative humidity, a gradient (0% *r.h.* to near saturation) employing various saturated salt solutions to maintain proper levels of relative humidity was set up in a chamber at 20° ± 1°C as described by Heatwole (1962) except that small doors permitted introduction of test animals without having to open the lid. Two adult *C. carocollus* snails were tested separately by introducing them into the chamber at the point where relative humidity was 50% and their subsequent location in the gradient periodically observed.

Effect of dry periods on survival

In order to ascertain size-structure of *C. carocollus* at death, the leaf litter at Loiza Aldea was carefully searched on 6 February 1965 for dead shells. Because small shells might disintegrate faster than larger ones and hence cause a bias in the size-structure obtained, only recently dead shells in which the periostracum was still intact were considered. Those which had weathered white or in which part of the periostracum had flaked off were discarded. Following this sample, there was an extended period without rain and the opportunity was taken to ascertain whether size-structure was altered by the drought; correspondingly a second sample was taken on 22 March 1965 for comparison.

The samples of recently dead shells were divided into adults and into two size-classes of juveniles (less than 20 mm in diameter and 20 or more mm in diameter).

If the dry period had caused no differential mortality among size classes then the distribution of numbers in the 3 size classes should be the same in both samples. The pre-drought distribution was thus used as a basis for calculating the expected distribution for the post-drought sample and the observed values compared to it and tested by Chi-square. The two distributions were significantly different ($X^2 = 9.5$; d.f. = 2; $P < 0.01$). The difference resulted from a relative increase in the proportion of animals in the smallest size category (from 29% to 48%) and a corresponding decrease in those in the larger juveniles (46% to 39%) and adults (25% to 19%).

It was difficult to ascertain the size-structure of animals at mortality in the El Yunque area as few shells were found that retained the periostracum; it is probable that shells deteriorate more rapidly under the wetter conditions of the uplands. Despite a prolonged search through leaf litter only a small sample of any camaenid species was obtained. However, shells of the sagdid *Platysuccinea portoricensis* were obtained. The data for all species from both areas are compared in Fig. 24. Despite the small samples of *C. carocollus* from the upland it is clear that at El Yunque the majority of snails dying are large adults, rather than the very small juveniles, i.e. the opposite pattern from that prevailing in the Loiza Aldea region.

P. luquillensis also had highest mortality among adults. This may well be a feature of the montane rain forest as a similar pattern also occurred in the sagdid snail from El Yunque. Only one *P. acutangula* dead shell (adult) was obtained in the El Yunque sample and only 5 *C. marginella* (3 juveniles, 2 adults) and 6 *P. lima* (3 juveniles, 3 adults) shells in the Loiza Aldea area, so these species unfortunately cannot be adequately compared.

Reproduction

At El Yunque copulation occurred from December to July inclusive but no records were obtained from August through November for any year. Inasmuch as (1) field observations were primarily carried out by day when snails tended to be inactive, and (2) most copulations were observed during periods of cloudy weather or light drizzle, evidence of observed copulation might be influenced by weather. However, at El Yunque the largest numbers of copulations were recorded during the 3 driest months and none during the wettest season, the opposite expected on the basis of the above mentioned source of bias. Hence, it appears that seasonal differences in occurrence of copulation are real and that mating occurs chiefly in the driest months (February to April) but extends over an 8-month period. The results from the somewhat drier El Verde site differ in that most copulation occurred later (June and July) when moisture was not at its lowest level. At Loiza Aldea, the driest study area in which *C. carocollus* occurred, 2 of the 3 copulation records obtained were in August and October, both wet months (Fig. 4).

Some information was obtained on the

mating histories of individual *C. carocollus.* Snail no. 12 was known to mate at least twice within a 2-month period (on 22 Jan. 1963 with snail no. 49 and 11 March 1963 with snail no. 166). Snails no. 25 and no. 116 provided similar data, the former mated on 23 March 1963 with snail no. 38 and on 6 April 1963 with snail no. 75; the latter mated with snail no. 94 on 20 April 1963 and with snail no. 322 on 20 May 1963. Snail no. 395 was mating with no. 394 on 9 July 1964 and about 1 year later (25 July 1965) it was seen copulating with an unmarked snail.

Of the 27 snails observed copulating that had been previously marked, 16 were first marked as adults, all but 4 of these had been first marked less than 1 year before they were observed copulating and little information on duration of the reproductively active span was obtained from them. The other 4 were observed copulating the following periods of time after first being marked as adults; 2 years 11 months, 3 years 4 months, 1 year 1 month, 2 years 8 months. In addition two of the snails first marked as juveniles and later observed copulating were captured sufficiently often in between to know approximately when they achieved adulthood. They were known to copulate 1 year 6 months and 2 years 8 months after having become adult. In view of the facts (1) that many of the above snails were already adult when first marked and (2) that the longevity of the species is up to at least 10 years (see section on longevity), the reproductive period is probably much longer than the above figures, which must be considered as minimum values.

Eggs were only infrequently found and all those identified with certainty were of *P. acutangula* except for one clutch of *C. carocollus* eggs; the former species definitely accounted for at least 8 of the 13 clutches found. The other clutches were in early stages of development and could not be identified but were probably *P. acutangula.* The eggs of *P. acutangula* are glossy white, with smooth hard calcareous shells that shatter when broken; they resemble miniature chicken eggs in shape. The unidentified eggs were similar in size, appearance and number per clutch (Table 15). The single clutch of eggs of *C. carocollus* (laid in a plastic bag by a captive snail) were longer and thinner than *P. acutangula* eggs.

TABLE 15. Characteristics of eggs and clutches of camaenid snails at El Yunque. Data on clutch size were omitted for clutches found in leaf litter or on ground which were probably incomplete. The sample size of eggs measured were N = 7 for *P. acutangula* and N = 11 for the unidentified category.

	P. acutangula		Unidentified eggs	
	Mean	Range	Mean	Range
Egg length	9.6	8.3-10.4	8.7	7.9-9.9
Egg width	7.6	6.9-8.2	6.6	6.1-7.3
Clutch size	14.8	10-20	15.7	12-22

Eggs were encountered over a wide span of the year, at least from November through April for *P. acutangula* and perhaps for longer periods if the unidentified clutch in August were in fact of this species (Table 14). However, most eggs were found during the early (dry) part of the year. It is possible that during the wet season eggs of this species are in danger of being flooded. The sites of oviposition include the wettest microhabitats in the forest and the eggs are thus probably protected against desiccation during the dry season. Most *P. acutangula* clutches were found in the axils of bromeliads or of banana trees, both of which tend to collect water in the base. Three clutches were found on the ground, either in leaf litter or under stones. The unidentified clutches were all observed in these same 3 situations.

In the early part of the season most clutches were in early stages of development (and were not identified) whereas from January on, most were found at or near the hatching stage. There were several exceptions. One clutch of 11 *P. acutangula* eggs was found on 29 November 1964 in a bromeliad axil. The entire plant was brought into the laboratory and kept at about 20°C in a beaker of water. On 14 January 1965 (about 1½ months later) most of the snails had just hatched and 1 (8.0 mm in greatest shell diameter) was just leaving the egg. In addition 1 egg contained a half grown embryo (greatest shell diameter 3.4 mm) and one was merely fluid-filled. The other identifiable clutch that was found in an early stage of development was found on 22 January 1966. There were 9 eggs in the axil of a bromeliad accompanied by an adult *P. acutangula.* Several days later there were 16 eggs present but

all subsequently disappeared before completing development.

Very little change in the state of the gonads was observed in *C. carocollus,* the only species dissected in large numbers. No individuals containing shelled eggs were ever dissected. Mean oviduct width of snails in the samples showed little seasonal variation (3.5 mm-6.2 mm) with no consistent seasonal trends. Diameter of the ovotestis (monthly means 2.1 mm to 3.4 mm) similarly showed no consistent seasonal trends. However, if it is assumed that the weight of the albumen gland is an indication of reproductive state an idea of the reproductive cycle can be obtained (Fig. 24).

The appearance of albumen glands differed considerably. The larger, presumably active ones were dark whereas smaller ones were paler. In July 1965 (Loiza Aldea) notes on gland color were made for each snail. The weight of dark glands ranged from 0.76 g to 1.52 g ($\bar{x}$ = 1.21; SE = 0.11; N = 8) whereas pale ones had equivalent values of 0.07 – 0.94 g ($\bar{x}$ = 0.33; SE = 0.07; N = 17). If a weight of 0.85 g (mid-point between smallest dark gland and largest pale gland) is used as the criterion for the albumen gland to be considered functional, the various samples can be divided into animals capable of producing albumen and those not capable of doing so. There was a seasonal pattern in relative proportion of those two types of animals (Fig. 26).

There are few direct data relating albumen gland size to reproductive activity. One copulating pair of *C. carocollus* was captured at Loiza Aldea on 29 April 1965 (when albumen glands were generally low). One snail had an albumen gland of 0.463 g and the other one of 0.069 g, both low values; the latter almost certainly indicates an inactive gland and the former was well below average values at peak season. An individual with a relatively small albumen gland (0.09 g) captured at El Yunque on 28 August 1965 had the duct of the spermatheca filled with sperm and 3 animals with spermatophores on the same date had albumen glands of only 0.18, 0.20 and 0.42 g respectively. All these data suggest that mating may take place well in advance of the time the albumen gland is functional for the season. Sperm are probably stored for a considerable period of time prior to fertilization.

The albumen gland cycle was not studied in detail in other species. However, one sample of 25 *P. luquillensis* was examined on August 28, 1965. Mean albumen gland weight was 0.91 g (R = 0.003-1.62 g; SE = 0.11). Oviduct diameter varied from 2.1-6.5 mm ($\bar{x}$ = 4.2; SE = 0.14). Seven (28%) of the animals had the oviducts convoluted and consequently had oviposited previously. This is a minimum estimate of animals that had previously bred as it is not known whether a convoluted oviduct will return to its former smooth condition between consecutive reproductive periods.

Growth and longevity

Snails were individually marked by painting numbers on their shells with nontoxic plastic paint. Their locations at each time of capture were plotted on a map and data on height above ground, activity, and type of substrate were recorded. Diameter of the shell, defined to be the distance between the peripheral edge of the lip and the opposite side of the shell along an imaginary axis passing through the apex of the whorl was measured with a Vernier calipers. However, it was difficult to make this measurement without dislodging the snails and field measurements of diameter displayed a rather large error. Consequently, it was felt that a more accurate picture of growth could be obtained from the increment in shell length (linear dimension of the shell). In the laboratory a series of snails was used to determine the relationship between diameter and length. Each shell was coated with glue and a string coiled on the upper surface midway between the inner and outer edges of the whorls. When it was in place ink marks were made on the string at each complete revolution. The string was then uncoiled and the linear distances measured and plotted against the corresponding measurements of shell diameter (Fig. 39). When a juvenile snail was first captured its total shell length was determined by measuring the diameter and reading the length from Fig. 39. The edge of the lip was painted and upon recapture increments in length could be directly obtained by measuring the distance from the old lip mark to the new edge of the lip midway between the outer and inner edges of the whorl (Fig. 40). The new lip was then painted. Since snails do not grow after the umbilicus is closed and the lip becomes thickened, these two

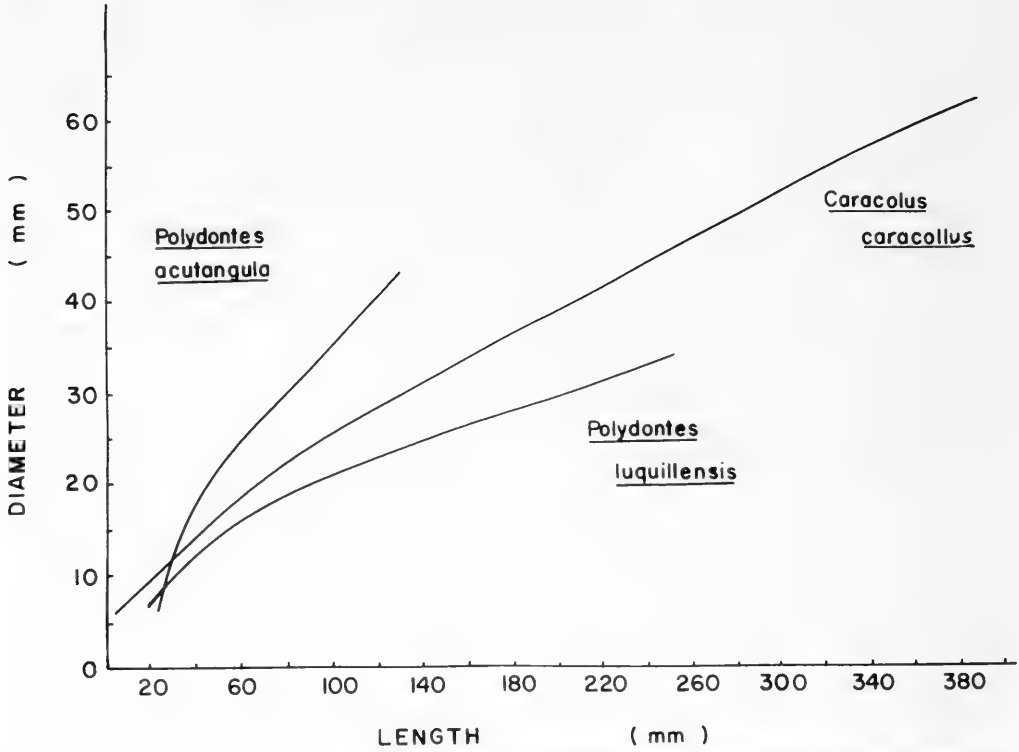

FIG. 39. Relation of shell diameter and shell length in three species of camaenid snails (see Appendix 2 for further explanation).

features were used as the criteria for adulthood.

Cameron & Williamson (1977) found that handling and marking snails increased the dispersal of animals from the study area. We have not evaluated this effect. However, in contrast to their methods (removal of snails from the study area, taking them to the laboratory for 2-4 days, marking them and then scattering them around the study area again), we disturbed our snails very little, and the handling effect should have been minimal.

The shapes of the three species on which growth data were obtained were different. Consequently, unit increase in shell diameter represented different increases in shell length among the various species. The curve relating these two measurements (Fig. 39) was used to obtain the shell length of snails at the time they were initially marked. Thereafter changes in shell length were measured directly.

Growth studies on *P. acutangula* were based on fewer individuals than for *C. carocollus.* However, hatchlings were discovered on several occasions in the former species and the lower end of the growth curve is more complete. Clutches of eggs in the field were observed and young snails marked soon after hatching. Diameter at hatching ranged from 8.0 mm to 10.3 mm ($\bar{x}$ = 9.02, SE = 0.268) or a mean shell length of about 22 mm. Increase in shell length was practically linear, at least during the first month of life. For a clutch first observed on 14 January 1965, growth rate averaged about 0.56 mm per day (Fig. 29). Other, but less complete records gave growth rates ranging from 0.30 to 0.46 mm per day. These rates of growth of hatchlings in the field are lower than the maximum possible. Fourteen hatchlings raised in the laboratory at 20°C and supplied with abundant food (boiled *Hibiscus* leaves) and powdered calcium carbonate grew linearly at an average rate of 0.75 mm per day for the first 22 days after hatching. After that time some died and the rate of growth of the remaining ones decreased (Fig. 30). For the larger juveniles, growth rates seemed to be relatively uniform throughout the year

FIG. 40. Photograph of *Caracolus carocollus* no. 354 showing method of marking. Notice the series of paint marks at the locations of the lip at different recapture periods.

with no apparent seasonal limits as occurred in *C. carocollus.* However, recaptures are too few for most snails for precise details of growth pattern to be evident (Fig. 31).

P. luquillensis marked on a retaining wall near La Mina similarly showed rather linear increases of shell length throughout the year except for a few individuals that showed elevated growth rates in autumn (Fig. 32). It is estimated from these events that it would take about 2 years to reach maturity.

Growth data from other species were insufficient for interpretation.

Population biology

Because of the differences in diurnal microhabitat between juveniles and adults (see Habitat selection) it was difficult to quantitatively sample the population for size structure. Additional problems arose from the fact that juveniles are harder to see than adults. However, a special situation permitted circumvention of these difficulties for 2 species (*C. carocollus* and *P. luquillensis*) and we obtained what we believe to be a reliable estimate of size-structure.

A stone retaining wall 43 m long located near La Mina was periodically censused by flashlight on humid or rainy nights when the wall was wet and when snails of all sizes were actively foraging over it. The relatively smooth surface permitted equal detection of all size classes when a slow and careful search was made. The wall was systematically searched, removing all snails from a given area before proceeding further, thereby eliminating a selective bias of the investigators. Sampling was terminated when about 100 *C. carocollus* had been found or unless weather changes likely to inhibit snail activity (e.g. wind beginning to blow) occurred. *P. luquillensis* was not as abundant and sample sizes are consequently smaller. Occasional *P. acutangula* were encountered but not enough to be of use in estimating size structure. No comparable situation was found in the lowlands and no comparative data on *C. carocollus* there, or on the strictly lowland forms was obtained.

Estimates of population density were made by the Jolly (1965) method using the recapture data from marked snails.

MALACOLOGIA, 1978, 17(2): 317-330

CHANGES OF GENE FREQUENCY IN *CEPAEA NEMORALIS* OVER FIFTY YEARS

James Murray[1] and Bryan Clarke[2]

ABSTRACT

Populations of the land snail *Cepaea nemoralis* inhabiting the coastal sand dunes at Berrow, Somerset, England, have been under observation since 1926. The original survey was carried out by Professor A. E. Boycott and Captain Cyril Diver, who not only collected an extensive series of samples but also produced a careful map of their localities. Thus it is possible for us to make direct comparisons with samples taken in 1959-60, 1963, 1969, and 1975.

Clinal patterns in the distribution of morph frequencies have remained essentially the same over the fifty-year period. However, there have been overall changes in the frequencies of two of the principal morphs. The allele for brown shell color (C^B) has shown a decrease in frequency, while the allele for a single central band (U^3) has shown an overall increase. The direction and consistency of these changes have been investigated by use of the G statistic and the analysis of covariance. These methods show clearly that there are differences in the frequency of both morphs among the localities and over the years. Despite one locality that shows an increase in brown, there is a consistent overall decrease in this morph, representing a mean selective disadvantage of about 3% per generation. With respect to the midbanded form, analysis of covariance indicates that there are real differences among localities in the direction or the amount of change. If we nevertheless calculate the mean selective advantage of this morph, we find it to be about 4% per generation. These differences can be interpreted in terms of changes in the habitats where the snails are found.

INTRODUCTION

Populations of the land snail *Cepaea nemoralis* (Linn.) inhabiting the sand dunes at Berrow in Somerset, England, provide an unusually good opportunity for measuring long-term changes in the frequencies of genotypes. Studies of this sort can be accomplished only in rather special circumstances. First, there must be a set of detailed, accurate, and localized data for some populations in the past. Second, the organism must have genetics sufficiently well understood for the reliable scoring of genotypes. Third, there should be evidence of genetic continuity over the intervening period so that continuous evolution may be distinguished from extinction and recolonization. Finally, some idea of the selective significance of the characters under study is desirable.

It is not surprising, therefore, that well-documented studies of gene frequencies over long periods are not at all common. It is even more unusual for all of the desirable conditions to be realized in a single example. Some of the best-documented cases are the studies of color polymorphisms in the lady beetle *Harmonia* (Komai, 1956; Komai & Chino, 1969) and in the moths *Biston* (Kettlewell, 1965; Ford, 1971) and *Panaxia* (Fisher & Ford, 1947; Sheppard & Cook, 1962; Lees, 1970), and the work on chromosomal polymorphism in *Drosophila pseudoobscura* (Dobzhansky, 1958; Dobzhansky et al., 1964, 1966; Anderson et al., 1975).

There have also been several studies of long-term changes in populations of the polymorphic land snail *Cepaea nemoralis.* Besides our own work based on the collections of Boycott and Diver (Clarke & Murray, 1962a, 1962b; Clarke, Diver & Murray, 1968) there are studies by Goodhart (1956, 1958) and van Heurn (1943, 1945). Some investigations have extended the time scale by comparing present-day and subfossil collections (Diver, 1929; Currey & Cain, 1968; Cain, 1971).

The most extensive and carefully documented series of historical collections of *Cepaea* was taken by Professor A. E. Boycott and Captain Cyril Diver in 1926 on the sand dunes at Berrow. In earlier

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papers (Clarke & Murray, 1962a, 1962b) we compared their data with collections taken in 1959 and 1960 from the same sites. We found that the populations were generally stable, but that there were significant changes in the frequencies of two of the principal morphs. We calculated the selection coefficient associated with a decrease in the frequency of brown shell color to be about 6.2% and that associated with an increase of the single-banded (00300) phenotype to be about 5.2% per generation.

Since the time of our first collections at Berrow, we have visited the area on a number of occasions, most recently in the summer of 1975. We are therefore able to report, in this paper, observations on gene frequencies extending over a total period of about half a century.

METHODS

The sampling area

The sand dunes at Berrow have been described in a previous paper (Clarke & Murray, 1962a; see also Boley, 1944). They comprise an area of coastal dunes bordering the Bristol Channel for about 5 km between the villages of Berrow and Brean. The width varies between 300 and 600 m. The northern half of the area has been extensively disturbed, and our collections have been taken largely from the more stable southern half. Here the beach is bordered by a line of semi-mobile dunes reaching a height of about 5 m. Farther inland there is a higher ridge of stabilized dunes that rises in places to 20 m. Between the two ridges and also on the landward side of the main ridge, the terrain consists of a chain of "slacks" or rolling hollows that are frequently quite damp and occasionally contain standing water. A golf course threads its way along these slacks. Although the greens and fairways obviously represent a serious intrusion into the natural ecology, their net effect has been to resist the encroachment of more disruptive human developments.

The flora of the dunes has been described by Boley (1944). *Cepaea nemoralis* is here particularly associated with clumps of *Iris foetidissima* but is more or less continuously distributed wherever the dunes are covered with herbaceous vegetation.

The road bordering the dunes on the eastern side marks a major change in the ecology. This transition is emphasized by the appearance of *Cepaea hortensis,* a close relative of *C. nemoralis* hardly ever found on sand dunes in England.

Sampling

Fig. 1 is a sketch map of the area showing the localities from which collec-

FIG. 1. Sketch map of the sand dunes at Berrow, Somerset, England. The solid black patches represent collecting areas, drawn to scale. The prefix D indicates a locality sampled by us alone. Double dotted lines represent roads. The continuous line shows the approximate high water mark. CH. = churchyard; P.B. = pillbox; S.P. = sandpit; T.P. = trigonometric point.

tions have been taken. Sites bearing the prefix D are those sampled by Boycott and Diver; sites prefixed B have been sampled only by us.

Boycott and Diver described their sampling localities in detail and marked them on a six-inch Ordinance Survey map of the area. We have resurveyed the dunes with a prismatic compass, fixing the position of our localities with reference to permanent topographic features. Despite some inaccuracies in the Ordinance Survey map, we believe that we have been able to locate Boycott and Diver's sites with a maximum error of about 15 m. During the summer of 1975 we attempted to recollect as many of the localities as possible. Of the 22 samples that we obtained, 14 represent localities originally collected by Boycott and Diver and the remaining 8 repeat our samples of 1959-1960. In addition we are reporting here on two intermediate sets of samples, 9 taken in 1963 and 16 in 1969.

Scoring

The snails were scored for age (mature or immature), condition, color, and banding according to the criteria given by Cain & Sheppard (1950, 1954) and Clarke (1960). The scoring is quite straightforward with the exception of one color class. There is at Berrow an unusual morph that we have designated "pale brown." It is a pale yellowish-brown, dusky, or tawny in color. In unbanded (00000) or middle-banded (00300) shells, this phenotype can usually be identified without difficulty. In fully banded (12345) shells, however, the color is easily confused with pink. Since the error involved in separating these two classes may be as large as 6%, we have not attempted to analyze pinks and pale browns separately.

RESULTS

Table 1 lists the phenotypes of the 5,615 live adult snails collected at Berrow in 1963, 1969, and 1975. The collecting localities are arranged in order from north to south, with successive samples from the same locality listed in chronological order. These data can be compared directly with Table 1 of Clarke & Murray (1962a). The column labeled 00345* includes not only 00345 but also 00045 and 00005. Dark browns are only scored as banded or unbanded because of the effect of this allele in partially suppressing the expression of banding. The numbers of shells with fused bands is given only with respect to the class of shells bearing all five bands. Unusual banding types are listed in a separate column.

The principal findings of our former papers (Clarke & Murray, 1962a, 1962b) were (1) that there was a regular pattern in the distribution of alleles, recognizably similar in 1926 and 1960, and (2) that in frequencies of two phenotypes there had been major changes that were similar in magnitude and direction over the whole area. The consistency of these changes allowed us to calculate the relative selective values of the respective genotypes.

The new data confirm the persistence of the general patterns of distribution over a fifty-year period. The centers of high frequency of all major phenotypes have remained in the same places, and over the years the morph-frequency clines have not shown any tendency to flatten out. These phenomena are apparent in Figs. 2-5, showing the distribution of the principal morphs in 1926, 1959-1960, and 1975.

Fig. 2 shows the percentages of yellow shells. In all three sets of samples the frequency of yellow is highest in the central region, where individual samples range from 90 to 100%. Frequencies decrease clinally in both directions. In the north the main alternative color morph is pink, while to the south there is also a significant proportion of dark brown shells.

Fig. 3 shows the percentages of dark brown shells. All three sets of samples show a center of high frequency in the southern region with a clinal decrease northward. Over the northern half of the area, dark browns occur at such low frequency that chance seems to govern their presence in any particular sample. The decline in the frequency of brown, noted in the 1959-1960 samples (Clarke & Murray, 1962a, 1962b) appears to have continued during the period until 1975 (see below).

Fig. 4 shows the frequencies of the single-banded (00300) morph, expressed as a percentage of the banded yellow, pink, and pale brown shells. Unbanded and dark brown shells were excluded from this calculation because of their epistatic interactions with the gene producing 00300. In

TABLE 1. The composition of samples of *Cepaea nemoralis* collected at Berrow in 1963, 1969, and 1975. The 00345* columns include the numbers of 00045 and 00005 as well as 00345.

	Yellows					Pinks					Pale browns					Dark browns								
Locality	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded	Fusions in 12345	Unusual Banding Types	Total	Comments		
B29	–	13	5	–	55	–	3	3	–	41	–	1	2	–	29	–	10	82	1-PB00000 ? albino banded	163	B. C.,	J. M.	1969
	–	21	10	1	99	–	2	2	1	44	–	–	–	–	–	–	–	28	1-Y12045	181	B. C.,	J. M.	1975
B43	1	7	2	–	32	3	8	2	–	33	–	1	–	1	4	–	1	3		95	B. C.,	J. M.	1975
B44	–	2	–	–	15	–	2	–	–	10	–	–	–	–	4	–	–	3	1-Y12045	34	B. C.,	J. M.	1969
	–	11	4	–	41	–	–	1	–	11	–	–	–	–	–	–	–	6		68	B. C.,	J. M.	1975
D26	–	3	–	1	25	–	–	1	–	17	–	–	–	–	–	–	–	10		47	B. C.,	J. M.	1969
	–	2	–	1	64	–	3	2	–	30	–	–	–	–	–	–	–	28	1-Y12045 1-P12045	104	B. C.,	J. M.	1975
D25	–	2	4	2	51	–	2	1	–	36	–	–	–	–	–	–	–	24		98	B. C.,	J. M.	1975
D29	–	4	–	2	38	–	1	–	–	12	–	–	–	–	–	–	–	14		57	B. C.,	J. M.	1969
	–	7	1	–	98	–	8	–	1	47	–	–	–	–	–	–	4	40	1-Y12045	167	B. C.,	J. M.	1975
D30	–	3	1	–	20	–	2	2	–	26	–	–	–	–	5	–	3	21	1-Y123X45 1-Y12045	64	B. C.,	J. M.	1975
B46	–	22	–	2	57	–	7	1	1	21	–	–	–	–	–	–	–	10	1-Y12045	112	B. C.,	J. M.	1975
D32	–	43	–	2	202	–	–	–	–	12	–	–	–	–	–	–	–	96		259	B. C.,	J. M.	1975
D34	–	12	–	1	41	–	–	–	–	–	–	–	–	–	–	–	–	12		54	J. M.,	E. M.	1963
	–	6	–	–	28	–	–	–	–	1	–	–	–	–	–	–	–	6		35	B. C.,	J. M.	1969
	–	46	–	7	108	–	4	–	–	1	–	–	–	–	–	–	–	30	1-Y12045	167	B. C.,	J. M.	1975
D33	–	7	–	5	91	–	–	–	–	6	–	–	–	–	–	–	–	27		110	J. M.,	E. M.	1963
	–	17	1	6	188	–	–	–	–	16	–	–	–	–	–	–	–	70	1-Y02345 1-Y12045	230	B. C.,	J. M.	1969
	–	25	2	2	141	–	–	–	–	13	–	–	–	–	–	–	–	44	1-Y02345	184	B. C.,	J. M.	1975
D36	–	16	1	4	121	–	–	–	–	12	–	–	–	–	–	–	1	20		155	J. M.,	E. M.	1963
	–	29	5	3	191	–	2	–	2	26	–	–	–	–	–	–	–	48	2-Y12045	260	B. C.,	J. M.	1969
	–	14	–	5	111	–	1	–	1	9	–	–	–	–	–	–	–	28		141	B. C.,	J. M.	1975

B50	–	14	–	–	59	–	–	–	–	–	–	–	–	–	–	–	–	23		73	B. C.,	J. M.	1969
	–	11	–	1	40	–	–	–	–	–	–	–	–	–	–	–	–	9		52	B. C.,	J. M.	1975
B51	–	17	–	–	55	–	–	–	–	2	–	–	–	–	–	–	–	32		74	B. C.,	J. M.	1969
	–	6	–	–	55	–	1	–	–	–	–	–	–	–	–	–	–	25		62	B. C.,	J. M.	1975
D40	–	5	–	2	41	–	–	–	–	7	–	–	–	–	–	–	–	6		55	J. M.,	E. M.	1963
	–	10	–	1	77	–	–	–	–	9	–	–	–	–	–	–	1	29	1-Y albino 12345	99	B. C.,	J. M.	1969
	–	9	–	–	62	–	2	–	–	19	–	–	–	–	–	–	1	15		93	B. C.,	J. M.	1975
D46	–	1	–	2	8	–	–	–	–	–	–	–	–	–	–	–	–	2		11	B. C.,	J. M.	1969
D48	–	9	–	1	27	–	2	–	–	10	–	–	–	–	–	1	2	5		52	J. M.,	E. M.	1963
	–	36	4	3	124	–	7	–	–	16	–	–	–	–	–	3	10	43	1-Y02345	204	B. C.,	J. M.	1969
	1	25	3	1	103	–	3	1	3	19	–	–	–	–	–	1	6	39	1-Y12045	167	B. C.,	J. M.	1975
D50	–	4	2	1	33	–	–	1	–	7	–	–	–	–	–	2	3	8		53	J. M.,	E. M.	1963
	1	26	8	13	170	–	2	–	–	13	–	–	–	–	–	5	15	48		253	B. C.,	J. M.	1969
	–	25	7	6	160	–	5	1	1	15	–	–	–	–	–	7	14	58	2-Y12045	243	B. C.,	J. M.	1975
D52	–	16	4	6	65	–	1	–	2	4	–	–	–	–	–	4	4	27	1-Y12045	107	J. M.,	E. M.	1963
	–	14	3	5	35	–	5	–	–	6	–	–	–	–	–	4	9	6	1-Y albino 12345 1-Y02345	83	B. C.,	J. M.	1969
	–	5	3	3	29	–	3	–	1	2	–	–	–	–	–	3	1	8		50	B. C.,	J. M.	1975
D54	1	55	3	15	80	–	5	–	–	4	–	–	–	–	–	–	4	9	1-Y albino 00300 1-Y12045	169	J. M.,	E. M.	1963
	–	48	6	15	102	–	4	–	2	13	–	–	–	–	–	3	10	23	1-Y12045 1-P12045	205	B. C.,	J. M.	1969
	1	29	2	10	49	1	4	1	2	9	–	–	–	–	–	1	1	15	1-Y albino 12345	111	B. C.,	J. M.	1975
B26	–	14	1	8	87	–	2	–	–	6	–	–	–	–	–	–	17	19	1-Y12045	136	B. C.,	J. M.	1975
B59	–	5	–	–	32	–	–	–	–	–	–	2	–	–	1	–	1	5		41	B. C.,	J. M.	1975
D56	–	20	–	1	77	–	1	–	–	20	–	1	–	–	8	1	8	19	1-Y albino 00300	138	J. M.,	E. M.	1963
	–	17	–	–	111	–	3	–	–	9	–	1	–	–	8	2	16	24	1-Y02345	168	B. C.,	J. M.	1969
	–	15	–	–	75	–	2	–	–	8	–	1	–	–	9	8	13	30		131	B. C.,	J. M.	1975
Total	6	748	82	138	3573	4	97	19	17	622	0	7	2	1	68	45	155	1177	31	5615			

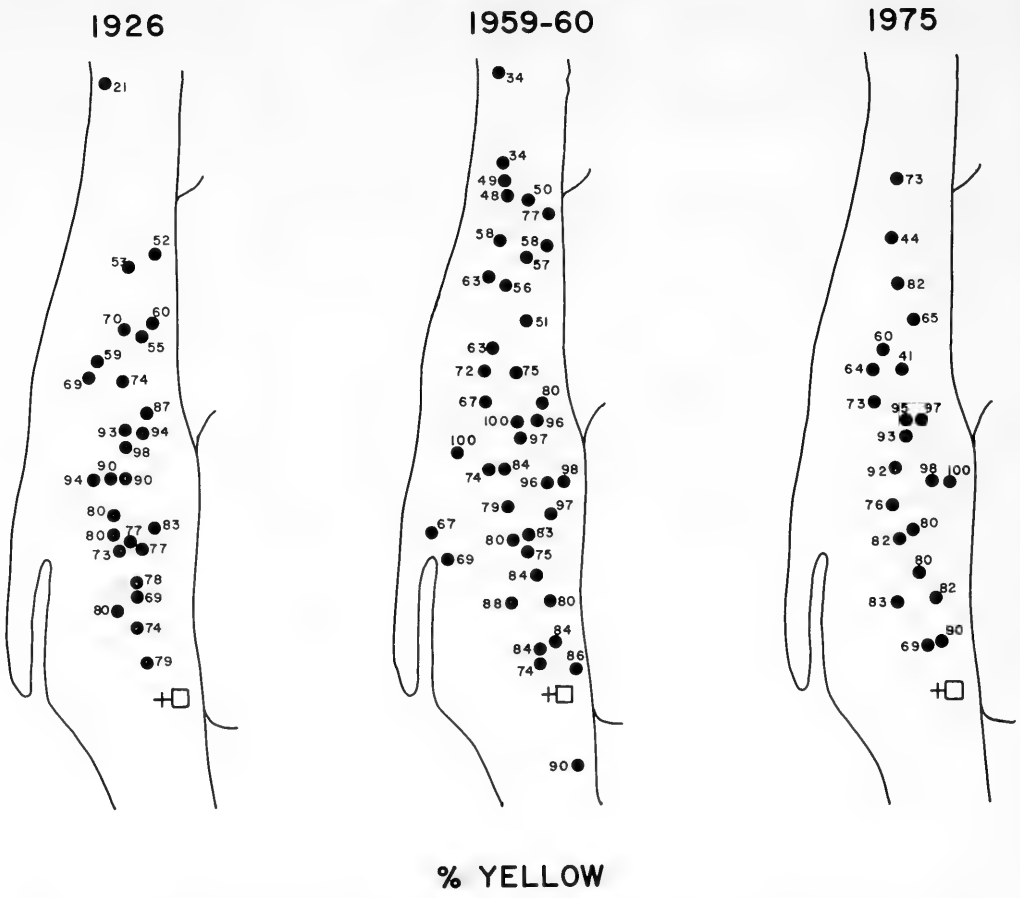

FIG. 2. *Cepaea nemoralis* at Berrow, Somerset, showing the percentages of yellow shells at various localities in successive samples from 1926 to 1975. The proportions are recorded to the nearest per cent, except when they are less than one per cent.

all three sets, the frequency of this morph is higher in the south than in the north, with a local maximum in the center. Overall the frequency of 00300 has increased over time (see below).

Fig. 5 shows the distribution of unbanded (00000) shells, expressed as a percentage of yellows, pinks, and pale browns. Dark browns have been excluded from the calculations to remove the effects of epistatic interactions of this allele with the locus controlling banding. Unbandeds, other than dark browns, are never common. Nevertheless their occurrence seems to follow a pattern since there are two general areas where they appear in all three sets of samples. One of these is in the north, and the other is in the south.

DISCUSSION

These results appear to confirm and extend the conclusions of our previous papers. The populations of *Cepaea nemoralis* on the Berrow dunes have persisted without major disruptions for a period of at least half a century. The persisting patterns of morph frequency demonstrate that there has been no general extinction and recolonization. Therefore the changes that have been observed are the result of gradual evolution of the populations in situ. Moreover, the extent of the inhabited area, the sizes of the populations, and the consistency of the changes suggest that systematic rather than random forces are responsible. In order to investigate this

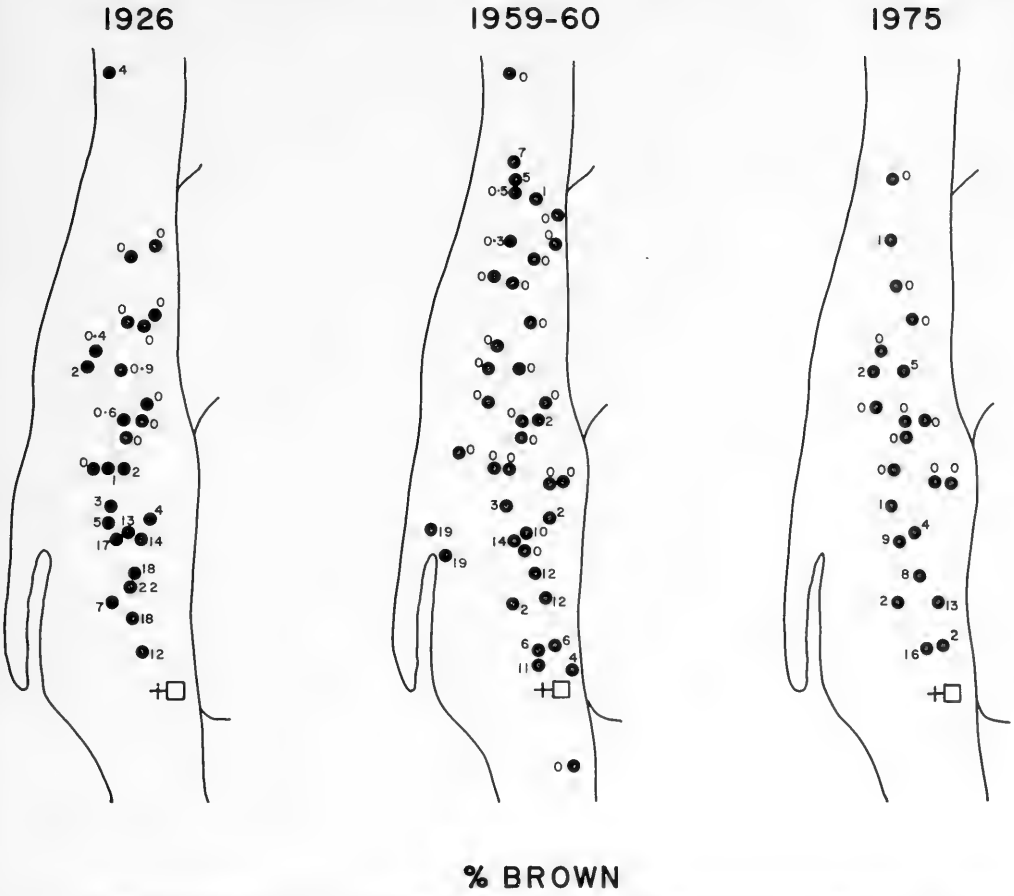

FIG. 3. *Cepaea nemoralis* at Berrow, Somerset, showing the percentages of dark brown shells at various localities.

consistency we have analyzed the frequencies of dark brown and 00300 among the localities and over the years.

The analysis of dark brown is difficult because the decline of this phenotype with time has produced rather low frequencies in many of the recent samples. When they are compared with the 1959-60 collections, the eight samples taken in 1975 from the area of highest frequency (D40-D56) show five cases where the proportion of dark brown has decreased, two where it has remained approximately constant, and one where it has dramatically increased. For six of these localities there are scores for all five years of sampling, and these have been further studied by means of the G statistic and by the analysis of covariance.

Considering first the G analysis of the years since 1959, the results set out in Table 2 show that although there is a strong association between sampling locality and color, the association of color with year of sampling is not significant. (The third association, locality and year, simply reflects variations in sampling conditions, such as weather, that affect sample size differentially.) The final component, the interaction of the three variables, is also significant, reflecting the influence of sample D56, where there has been an increase. When the analysis is extended to include the samples from 1926 (Table 3), this influence is submerged in the general trend toward a reduction in the frequency of the dark brown morph. The interaction is no longer significant, while the two comparisons of interest (locality X color and year X color) are highly significant.

The full impact of these statistics can be

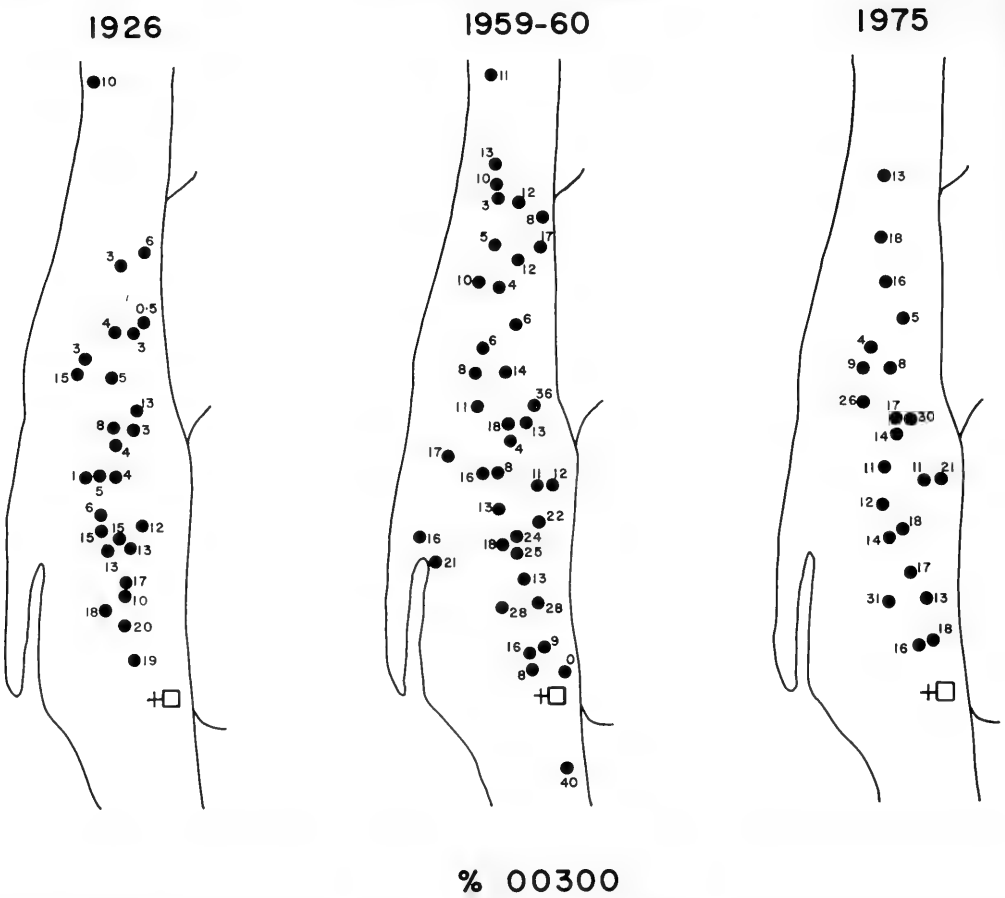

FIG. 4. *Cepaea nemoralis* at Berrow, Somerset, showing the percentages of single-banded (00300) shells at various localities.

TABLE 2. G analysis of the distribution of frequencies of the dark brown morph by locality (D40, D48, D50, D52, D54, D56) and by year for the period 1959 through 1975 (1959-60, 1963, 1969, 1975).

G		Degrees of freedom	Probability
G_{total}	= 779.17	38	$<< 0.001$
$G_{locality \times year}$	= 659.81	15	$<< 0.001$
$G_{locality \times color}$	= 83.81	5	$<< 0.001$
$G_{color \times year}$	= 4.59	3	$0.3 > P > 0.2$
$G_{interaction}$	= 30.96	15	< 0.01

TABLE 3. G analysis of the distribution of frequencies of the dark brown morph, as in Table 2 but with the addition of scores for 1926.

G		Degrees of freedom	Probability
G_{total}	= 1400.91	49	$<< 0.001$
$G_{locality \times year}$	= 1155.31	20	$<< 0.001$
$G_{locality \times color}$	= 155.33	5	$<< 0.001$
$G_{color \times year}$	= 70.70	4	$<< 0.001$
$G_{interaction}$	= 19.56	20	$0.5 > P > 0.3$

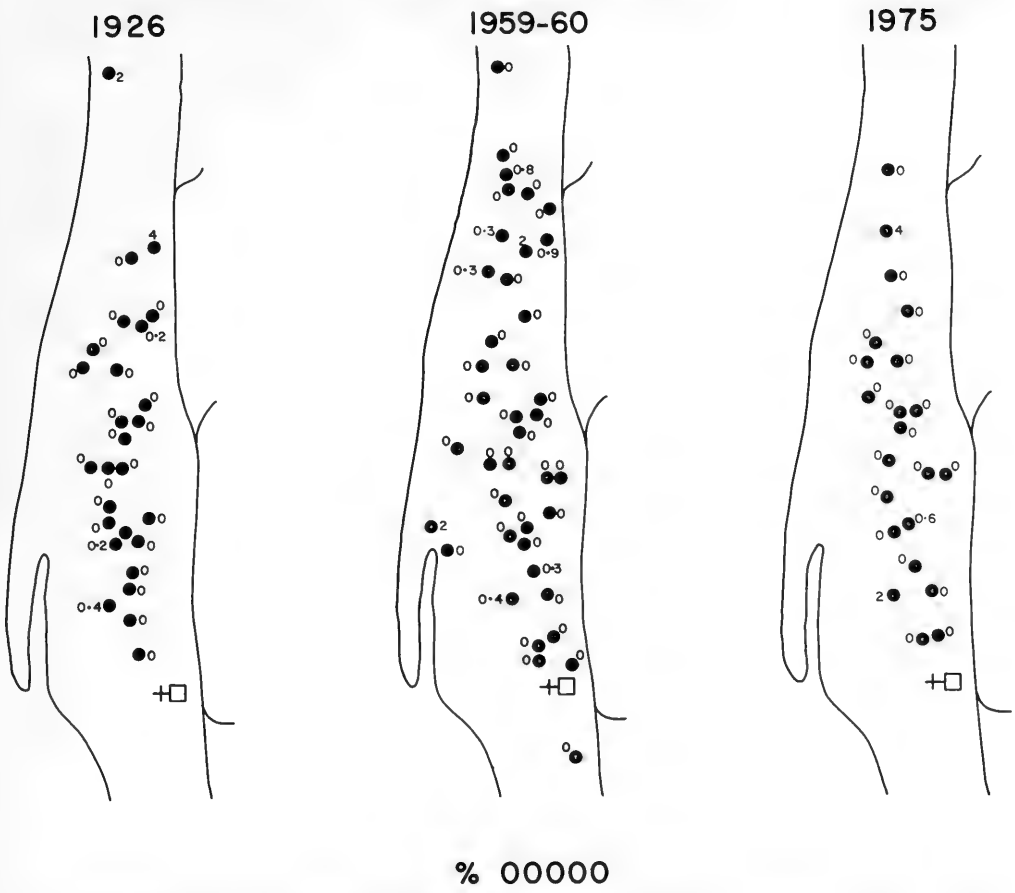

FIG. 5. *Cepaea nemoralis* at Berrow, Somerset, showing the percentages of unbanded (00000) shells at various localities.

TABLE 4. Analysis of covariance of the frequencies of the dark brown morph with time, for the period 1926 through 1975.

	SS dev. from reg.	Deg. of freedom	Mean square	F	P
Within	208.97	18	11.61		
Differences among regression coefficients	41.79	5	8.36	0.72	n.s.
Common	250.76	23	10.90		
Differences among adjusted means	790.84	5	158.17	14.51	< 0.001
Total	1041.60	28			

seen from the analysis of covariance set out in Table 4. For these six localities over the whole period, the means of the frequencies of brown at the various localities are highly significantly different from one another, but the regressions of the frequencies of brown on elapsed time are not different from locality to locality. Hence we can with some justification consider the changes in the frequency of dark brown to be homogeneous and the populations to be subject to the same regime of selection. Using Bulmer's method, outlined in Clarke & Murray (1962b), we can calculate the mean value of the selective coefficient associated with the gene for dark brown (C^B) over the period 1926 to 1975 as 3.5% with a standard error of 1.1%. This figure

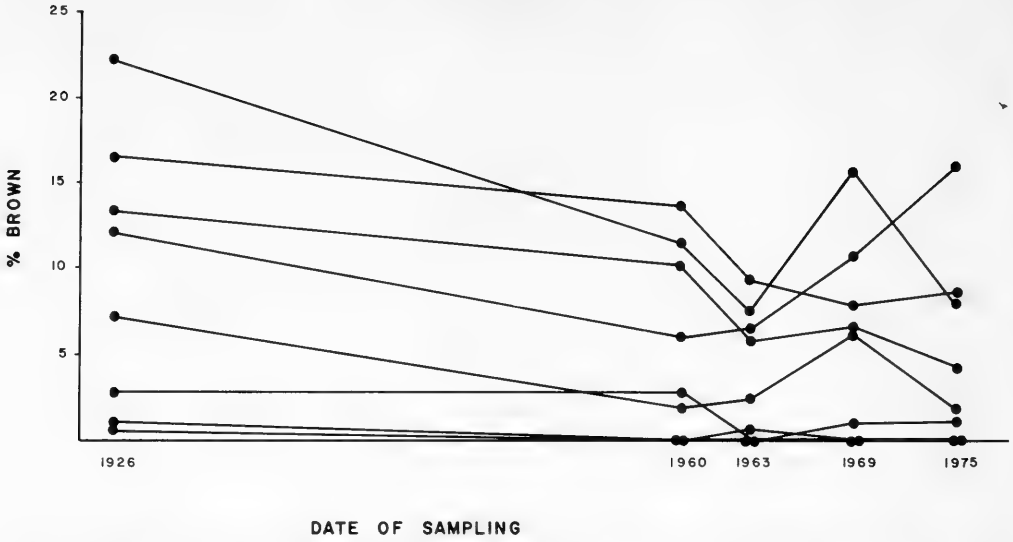

FIG. 6. Changes in the frequencies of dark brown shells in populations of *Cepaea nemoralis* at various localities at Berrow, Somerset.

is not significantly different from that obtained in 1959-60. The changes are shown graphically in Fig. 6.

In calculating coefficients of selection we have used a generation time of three years for *Cepaea nemoralis* (Clarke & Murray, 1962b). This estimate is based on the assumptions that an individual reaches maturity during its second autumn or third spring, when it is eighteen months or two years old, and that the adult population suffers a mortality rate of about 50% per year. Cain & Currey (1968) have suggested, from the size of laboratory bred snails and from the size distribution of young snails in nature, that juveniles do not reach maturity until they are at least two years old and that reproduction may not begin until the third year. Thus generation time would be increased to at least four years. However, they have assigned the size classes in wild-caught samples to year classes on the basis of laboratory bred individuals that grew to a maximum size of 7 mm in a season. Comparable broods in our laboratory are more successful. At 20 weeks a typical brood attained a mean size of 10 mm with the largest animal reaching 15 mm ($N = 74$; $\bar{x} = 10.02$ mm; $s_{\bar{x}} = 0.23$). Thus it seems likely that the size classes reported by Cain & Currey are in fact one year younger than they have suggested, with animals maturing in their second year.

Also, in our experience, individuals in good condition are capable of fertile mating as soon as the lip is formed. The delays reported by Wolda (1963) probably reflect the artificially high densities on his experimental plots. Hence, we believe that three years is a reasonable estimate of the generation time. Indeed, it results in conservative estimates of selective coefficients since the size of the coefficient of selection is directly proportional to the generation time (Clarke & Murray, 1962b).

With respect to the middle-banded morph (00300), sample sizes are less of a problem, because most frequencies are in an intermediate range and increasing. All 22 samples taken in 1975 contain enough 00300 individuals for comparison with the 1959-60 series. There are twelve increases in frequency, three samples with essentially no changes and seven decreases. Since complete scores for all years are available for only nine localities, the 1963 and 1969 samples were excluded from the G analysis of the 00300 morph. Table 5 summarizes the comparison of 1975 with 1959-60. Both the main effects of interest are highly significant, but there is also a highly significant interaction component. It is clear, therefore, that although there are changes in the frequency of 00300 from place to place and from time to time, the changes are not consistent with one another.

Extending the analysis to include the

TABLE 5. G analysis of the distribution of frequencies of the 00300 morph by locality (all those sampled in 1975) and by year (1959-60 and 1975).

G		Degrees of freedom	Probability
G_{total}	= 1641.31	64	<< 0.001
$G_{locality \times year}$	= 1335.21	21	<< 0.001
$G_{locality \times 00300}$	= 243.40	21	<< 0.001
$G_{00300 \times year}$	= 8.62	1	< 0.01
$G_{interaction}$	= 54.09	21	< 0.001

TABLE 6. G analysis of the distribution of the frequencies of the 00300 morph, as in Table 5 but with the addition of scores for 1926. Fourteen samples are included for each of the years 1926, 1959-60, and 1975.

G		Degrees of freedom	Probability
G_{total}	= 2413.34	67	<< 0.001
$G_{locality \times year}$	= 1992.03	26	<< 0.001
$G_{locality \times 00300}$	= 290.03	13	<< 0.001
$G_{00300 \times year}$	= 90.02	2	<< 0.001
$G_{interaction}$	= 41.26	26	< 0.05

1926 samples, there are fourteen localities with scores available from 1926, 1959-60, and 1975. The G analysis (Table 6) shows the same patterns as before, although in this instance the interaction component is only marginally significant. These results suggest that a formerly consistent trend toward the increase of 00300 no longer obtains, and that in recent years the frequencies of 00300 have been changing at different rates in different localities.

The analysis of covariance can be performed for nine localities for which data are available in all five sets of samples. Unfortunately the result is not particularly enlightening. There is greater variability among the individual regression coefficients from 1959 to 1975 than from 1926 to 1975, but for statistical reasons it is only over the longer time span that one can detect significant differences. We can only say with confidence, therefore, that for some portion of the time the localities differ in the direction and magnitude of changes in the frequency of 00300.

Under these circumstances it is doubtful whether one should speak of a mean value of the selective coefficient associated with the gene for middle-banded. The "mean" in this case is an average of disparate values rather than a statistical mean determined by a number of estimates of the same parameter. Nevertheless, we have calculated the average value of the selective coefficient against the gene for unmodified banding (M^-) as 4.0% with a standard error of 0.6%. The changes in the frequencies of 00300 are shown in Fig. 7.

Finally, what can be said about the relation of these gene frequencies to the local ecology? In our previous paper (Clarke & Murray, 1962a) we noted two principal changes that had occurred at Berrow since the visit of Boycott and Diver. The first of these was the spread of sea buckthorn *(Hippophaë rhamnoides),* a woody shrub that has become well established on the main ridge of the dunes. The primary effect of this plant is to reduce the area habitable by *Cepaea,* since the snails do not live in dense thickets. A probable secondary effect is to increase selective predation on *Cepaea.* The shrubs provide cover for the most important avian predator, the song thrush *(Turdus ericetorum).* The "thrush anvils" used for breaking snail shells are characteristically associated with shrubby cover. The second change apparent from our earlier work was a general stabilization of the dunes, especially the sea ridge, with marram grass *(Ammophila arenaria)* giving way to short, rabbit-grazed turf. According to the work of Cain & Sheppard (1950, 1954), both of these changes might be expected to favor yellow shells over browns and effectively unbanded shells (such as 00300) over more heavily banded morphs.

From our 1975 field notes, three trends are apparent over the last fifteen years. First, the spread of sea buckthorn has continued. One of our sampling areas has

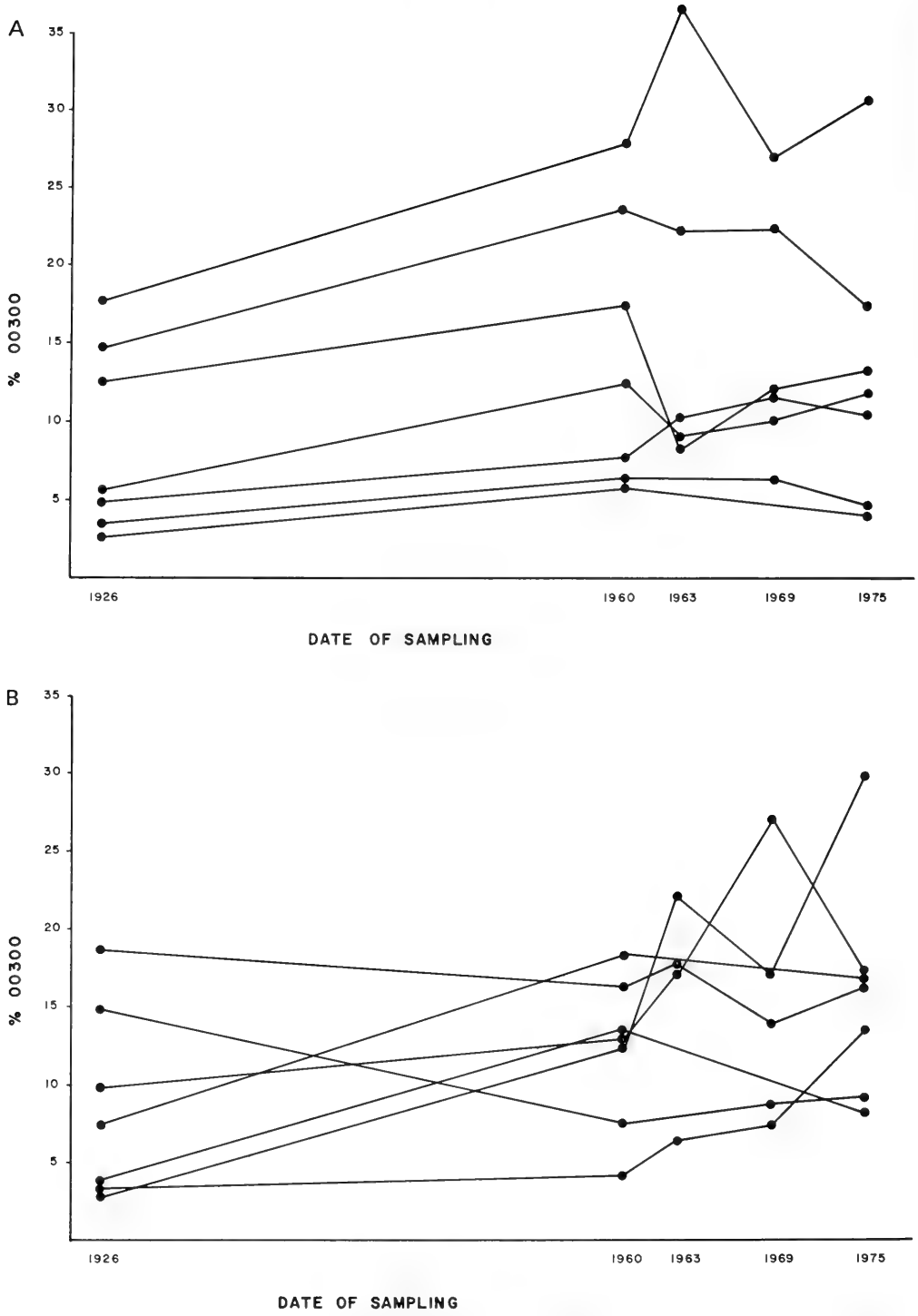

FIG. 7a and b. Changes in the frequencies of single-banded (00300) shells in populations of *Cepaea nemoralis* at various localities at Berrow, Somerset.

been completely lost to it, and several others show some degree of invasion. Second, there has been a partial reversal of the trend toward stabilization of the dunes. Foot traffic has increased dramatically with a resulting deterioration of the ground cover. Some of the dunes are again in motion, and two localities have been engulfed by sand. Third, the short turf is diminishing. Rabbits seem to be far less common, the grasses are longer and coarser, and there are more herbaceous weeds such as umbellifers, *Ranunculus,* and *Rubus.*

Under these conditions it is still to be expected that yellow shells would be favored over pinks and browns, but the more disruptively patterned shells should gain in fitness over effectively unbanded ones. The shifting trends in the frequency of 00300 may reflect this changing regime of selection. If so, and if the current ecological changes continue, then we may expect to see in the future a continuing decline in the frequency of dark browns but a reversal of past increases in 00300, with rising frequencies of the more fully banded types.

A rather different interpretation of the distribution of morph frequencies on sand dunes has been advanced by Cain (1968) based on an extensive survey of dune populations of *Cepaea nemoralis* around the British Isles. While generally recognizing the importance of visual selection by predators in these populations, he has also noted an association between the structural complexity of dune systems and the occurrence of the dark brown morph, and has suggested that selection in favor of browns results from the ponding of cold air in the hollows of the dunes. In addition he has pointed out that the "effectively unbanded" 00300 morph is characteristic of warm, southwest trending dune slopes (Cain, 1968). Thus if climate were a direct selective factor, a change toward a warmer climate at Berrow could be responsible for both the decline of dark brown and the increase in 00300 (Prof. A. J. Cain, personal communication).

Future observations at Berrow may indeed provide an opportunity to test the relative importance of climatic selection and visual selection in determining morph frequencies in *Cepaea.* Too often predictions based on the two hypotheses are confounded, as in the case where dark, uniform habitats tend to be associated with cold climate. At Berrow, however, the present trends in vegetation may allow us to separate these effects. A continuing negative association between the change of frequencies of dark brown and 00300 would be compatible with the hypothesis of climatic control. However if the decline in dark brown continues while the frequencies of 00300 stop increasing, begin decreasing, or show local responses to changes in the character of the vegetation, then the hypothesis of control by visual selection would be supported. For this reason we intend to continue monitoring morph frequencies on the Berrow dunes.

SUMMARY

1. Populations of the polymorphic land snail *Cepaea nemoralis* have been sampled at Berrow, Somerset, England over the past half century.

2. Patterns of distribution of the phenotypes have remained essentially the same over the entire period, with centers of high frequency of the principal morphs located in the same places.

3. There has been a consistent overall decrease in the frequency of the dark brown morph from 1926 to 1975 corresponding to a selective disadvantage of the allele for dark brown (C^B) of 3.5 ± 1.1% per generation.

4. There has been an overall increase in the frequency of the middle-banded (00300) morph, but there is evidence for differences in the magnitude and direction of the changes at different localities.

5. These changes can be interpreted as responses to changing ecological conditions on the Berrow dunes.

ACKNOWLEDGEMENTS

Once again we should like to express our debt to Professor A. E. Boycott, F.R.S., and Captain Cyril Diver, C. B., C.B.E., for their meticulous pioneering field work and for the opportunity of using their unpublished observations. We should like to thank Mrs. Elizabeth Murray, Dr. Ann Clarke, Dr. and Mrs. Aldo Herrera de Araucho, and Mr. J. J. Murray, III for their help with the collections. The 1975 field work was carried out while one of us (J. M.) held a Sesquicentennial Fellowship

from the University of Virginia and was a guest of the Departments of Zoology and Genetics of Liverpool University. We should like to express our appreciation of the hospitality of Profesor A. J. Cain and the late Professor P. M. Sheppard, F.R.S.; and we are grateful to the Science Research Council for financial support. We dedicate this paper to the memory of Professor Sheppard.

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MALACOLOGIA, 1978, 17(2): 331-339

THE NATURE AND DISTRIBUTION OF FOOD-INDUCED ESTERASES IN HELICID SNAILS

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ABSTRACT

The variation, as revealed by gel electrophoresis, in primary and food-induced (secondary) esterases coded for by locus *Es. 1* in the snail *Cepaea nemoralis* is described. Secondary modifications arise as a result of the ingestion of nettle *(Urtica dioica)* and other naturally occurring foods and can be eliminated by feeding snails on a diet of carrot and lettuce. The types of variation encountered demand at least three variable sites on the basic esterase molecule. Attempts at mimicking secondary esterases in vitro with chemical modifying agents have not been successful, although iodoacetamide appears to modify a site that distinguishes primary from secondary zones. Of five other helicid snails investigated, only *Cepaea hortensis* showed food-induced modifications to primary esterase zones. Longer-term studies on the other four species are needed. Consideration of the time-course of secondary esterase induction and loss has led to the hypothesis that a bacterial component of the gut flora is selected by 'inducing' foods. This bacterial component, it is postulated, produces an enzyme which modifies the basic esterase molecule.

INTRODUCTION

Electrophoresis has been increasingly used over the last two decades to measure genetic variability within and between populations of a species and between different species. The great attraction of the method is the relative ease with which genetic information can be extracted from a gel phenotype. However, in most electrophoretic surveys of natural populations little heed is given to the possibility that some enzymes may undergo post-transcriptional modifications that can lead to erroneous genetic interpretations of gels. This may be especially misleading when the modifications do not always occur in all individuals. For example, if two electrophoretically distinct isozymes are occasionally produced from a single primary product, naïve interpretation of the zymogram may lead to (a) an overestimate of the number of loci revealed and/or (b) an overestimate of the number of alleles at a locus and/or (c) a false estimate of the heterozygosity at the locus. Confusion is compounded when the modified zones mimic zones that are primary products of other alleles at the same locus or even alleles at completely different loci.

Fisher & Harris (1972), for instance, describe the production of secondary isozymes at a phosphoglucomutase locus (PGM_3) in man. Some of the secondary isozymes can mimic the primary products of other PGM_3 alleles; if these had not been recognized, three alleles would have been postulated (instead of two) and, of course, allele frequencies and heterozygosity would have been incorrectly estimated. The production of secondary isozymes of PGM_3 in vivo appears to be a function of protein age.

A similar situation has been reported by Oxford (1975) at an esterase locus in the snail *Cepaea nemoralis* (L.). In this case, not only is it often impossible to score allele frequencies in snails assayed straight from the wild, but the multiplicity of esterase zones (both primary and secondary) which can exist in such animals led initially to a gross overestimate of the number of loci involved (Oxford, 1973a).

From the point of view of surveys designed to assess the levels of polymorphism in organisms, it is obviously important to determine the conditions under which secondary isozymes are produced and to take steps to ensure that they do not occur under the electrophoretic procedures employed. Secondary isozymes are also of considerable interest in their

own right. For example, what is the biochemical nature of these secondary isozymes, especially in relation to the biochemical differences in the primary products of other alleles which they might mimic? Also, what is the distribution of secondary isozymes in related organisms and what selective advantage (if any) could be associated with the ability to generate such modified enzymes? In this paper, I will discuss these questions in relation to the secondary esterases found in *Cepaea nemoralis*.

THE PATTERNS AND INDUCTION OF SECONDARY ISOZYMES IN ESTERASES OF *CEPAEA NEMORALIS*

Digestive gland esterases of *C. nemoralis* that exhibit secondary modifications migrate to the cathodal end of polyacrylamide disc gels during electrophoresis. The general methods of electrophoresis and the subsequent staining of esterases have been described elsewhere (Oxford, 1973a). The locus involved is *Es. 1,* at which six alleles have been identified (Oxford, 1973a, 1975, and unpublished results). These 'alleles' are electrophoretically defined and may contain more than one true allele, i.e., coding for different amino acid sequences, within a mobility class. The alleles code for primary products that fall into two distinct series on electrophoresis. The products of alleles $Es.\ 1^1$, $Es.\ 1^2$, and $Es.\ 1^3$ form one series, and alleles $Es.\ 1^{2a}$, $Es.\ 1^{3a}$, and $Es.\ 1^{4a}$ form the other (Fig. 1). Within a series the allelic products are equidistant on a gel, and the distance between adjacent zones is the same in both series. The series of Est. 2a, Est. 3a, and Est. 4a (coded for by alleles $Es.\ 1^{2a}$, $Es.\ 1^{3a}$, and $Es.\ 1^{4a}$, respectively) is shifted slightly anodally compared to the zones in the other series (Fig. 1).

The post-transcriptional (and almost certainly post-translational) modifications to these primary zones involve anodal shifts in units equivalent to the distance between primary zones within a series. The major modification involves just one shift of position so that, for example, a modified Est. 1 has exactly the same electrophoretic mobility as the primary product Est. 2 (Fig. 1). As each primary zone can shift down one unit, it follows that Est. 1 and Est. 2a cannot be mimicked, whereas Est. 4 and Est. 5 can only be the products of modification. Esterase zones at the positions of Est. 2, Est. 3, Est. 3a, and Est. 4a can be either primary products, secondary products, or a mixture of the two. The modification may go to completion so that there is no trace of the primary product, or it may be partial, yielding two zones in homozygotes.

Further modifications are also possible. For example, electrophoresis of an extract from a snail homozygous for Est. 1 might reveal not only Est. 1 and Est. 2 but also very faint traces of Est. 3 and Est. 4. This suggests additional modifications, which again produce unit shifts in electrophoretic mobility.

Obviously it is impossible to score allele frequencies at this locus while there is the possibility of confusing primary and secondary zones. It was the presence of four zones in some individuals, e. g., a snail of the genotype $Es.\ 1^1/Es.\ 1^3$ with both primary and secondary esterases, which led to the idea that five closely linked loci were involved (Oxford, 1973a). Each locus was thought to control enzyme activity at one level in the gel and, at the time, five zones were recognized: Est. 1, Est. 2, Est. 3, Est. 4, and Est. 5.

The conditions that induce secondary modifications have been identified (Oxford, 1975). Ingestion of nettle (*Urtica dioica* L.) produces secondary zones within a snail, whereas ingestion of carrot and lettuce suppresses them. Within an individual these processes are completely reversible. Collections of snails from habitats that do not contain nettle indicate that other foods can induce secondary zones as well. In all samples taken so far, at least some individuals have been shown to possess modified esterases. If a survey of allele frequencies at locus *Es. 1* is required, it is necessary to maintain snails on a carrot and lettuce diet for at least five to six months before extraction and electrophoresis (see below).

PRIMARY AND SECONDARY VARIATION AND THE ESTERASE MOLECULE

A consideration of the variation, both primary and secondary, in electrophoretic mobility of the esterase components described above demands at least three and

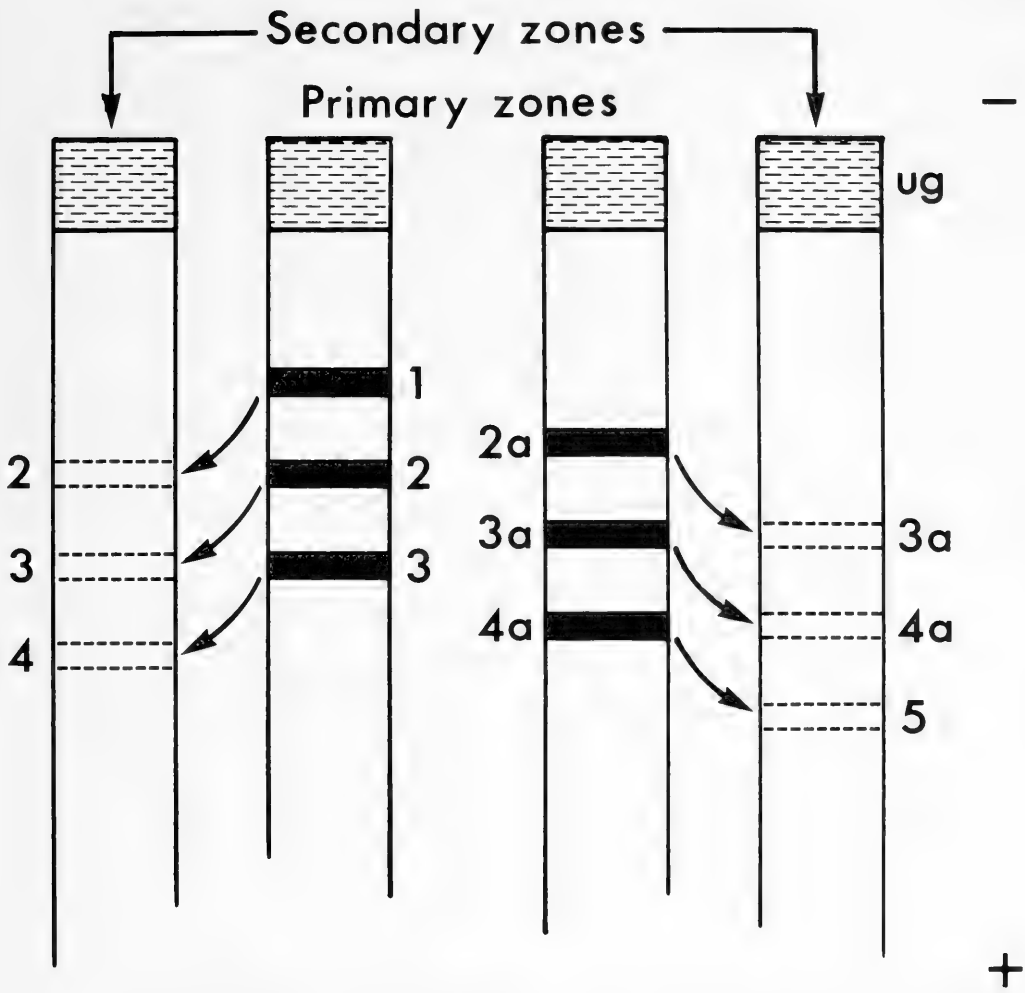


FIG. 1. The primary and major secondary esterase zones in *C. nemoralis,* showing the relationship between the two allelic series, the relative mobilities of allelic zones within a series, and the phenocopying of primary zones by some of the secondary zones. ug = upper gel. Migration is from cathode to anode.

probably more variable sites in the basic esterase molecule.

1. The first and second variable sites on the esterase molecule are those which determine the mobility of an esterase zone within an allelic series. Presumably the difference in mobility between adjacent zones depends on the substitution of one amino acid for another such as to give the molecule an extra negative or positive charge, depending on whether one goes from Est. 1 to Est. 3 or in the reverse direction. The generation of three zones within each series demands a similar change at yet another site on the molecule. It is assumed that the differences in electrophoretic mobility between zones within a series are due to unit charge changes (Oxford, 1975).

2. The shift observed between the two mobility series of allelic zones may be produced by the substitution at a third site of a negatively charged amino acid (under the electrophoretic conditions employed) for a neutral one (e.g., to convert Est. 1 to Est. 2a) or a positively charged amino acid for a neutral one (e.g., to convert Est. 2a to Est. 1). Henning & Yanofsky (1963) have shown that the charge expressed by an amino acid may vary according to the

nature of adjacent amino acids in the polypeptide. It is therefore possible to observe different electrophoretic mobilities with changes involving the same amino acid at different positions in the protein. Also, the substitution of one amino acid for another may slightly change the conformation of the molecule, thus exposing different charged groups. Mechanisms such as these may explain the non-unit mobility shift observed between the two series of allelic zones. Hypotheses based on a carbohydrate moiety (Karn et al., 1973), or more specifically sialic acid (Law, 1967), added or not added to a basic esterase molecule by another locus are doubtful because an individual can be heterozygous for alleles in different series.

The major secondary modification of all the primary esterases probably occurs at yet another site. How secondary isozymes are generated from primary ones is not clear. I have suggested before (Oxford, 1975) that they may be a result of deamination of a vulnerable glutamine or asparagine residue (charge change, 0 to −ive) or the blocking of an amino group by acetylation (charge change, +ive to 0). Other mechanisms are also possible, e.g., the addition of small, charged side groups to the primary products. Sialic acid is not involved since incubation of primary and secondary esterases with neuraminidase does not alter electrophoretic patterns (Oxford, 1973a). However the increase in net negative charge is effected, it must exactly match the increase in negative charge displayed by allelic zones within a series.

It was mentioned earlier that, besides the major modification that causes a unit shift toward the anode, some molecules undergo additional shifts of two and three units. These further modifications may occur at the same site as the major modification or at different sites on the molecule.

Schwartz et al. (1965) have described a very similar esterase system in maize, consisting of seven alleles in two series. As in *Cepaea*, the charge difference between members of a series is constant but the two series, of three and four alleles, are shifted in mobility with respect to one another.

Schwartz (1967) argued on the basis of experiments involving chemical modification of the esterases that electrophoretic differences between the isozymes within a series are unlikely to be due to differences in the net charge of amino acids making up the enzyme. He also suggested that different numbers of charged side groups conjugated to the protein would not explain his findings. The hypothesis finally suggested was that the allelic isozymes within a series had the same primary structure but differed in conformation and that the differences in electrophoretic mobility resulted from differential masking of charged groups. If this interpretation is correct, it is difficult to see how 'allelic' zones with different mobilities can be found within a single plant. In other words, how can different conformational forms be stabilized sufficiently to appear allelic in breeding experiments? In *Cepaea* there is, of course, the added complication of the food-induced modifications of primary esterase zones.

EXPERIMENTAL MODIFICATION OF ESTERASE ZONES

Theoretically, it should be possible to identify the group or groups responsible for the differences between primary and secondary products by treating the esterases with chemicals known to modify particular molecular features. A chemical which converts primary zones into secondary zones but which does not alter the mobility of existing secondary zones may be presumed to affect the molecular feature responsible for the primary-to-secondary modification. Unfortunately, this approach has had only limited success so far. The only chemical agents found that result in a shift in the mobility of zones are formaldehyde, glyceraldehyde, and iodoacetamide.

Treatment of extracts with 0.2M formaldehyde for 3 hr at 37°C prior to electrophoresis results in a shift of both primary and secondary esterases of exactly two units toward the anode. There is a progressive increase, in unit steps, as the concentration is increased from 0.05M to 0.2M. After 0.2M no further change is produced. Identical results were achieved with glyceraldehyde, but acetaldehyde had no effect (Fig. 2). Since both primary and secondary zones are equally affected, this reaction gives no clue to the biochemical differences between them.

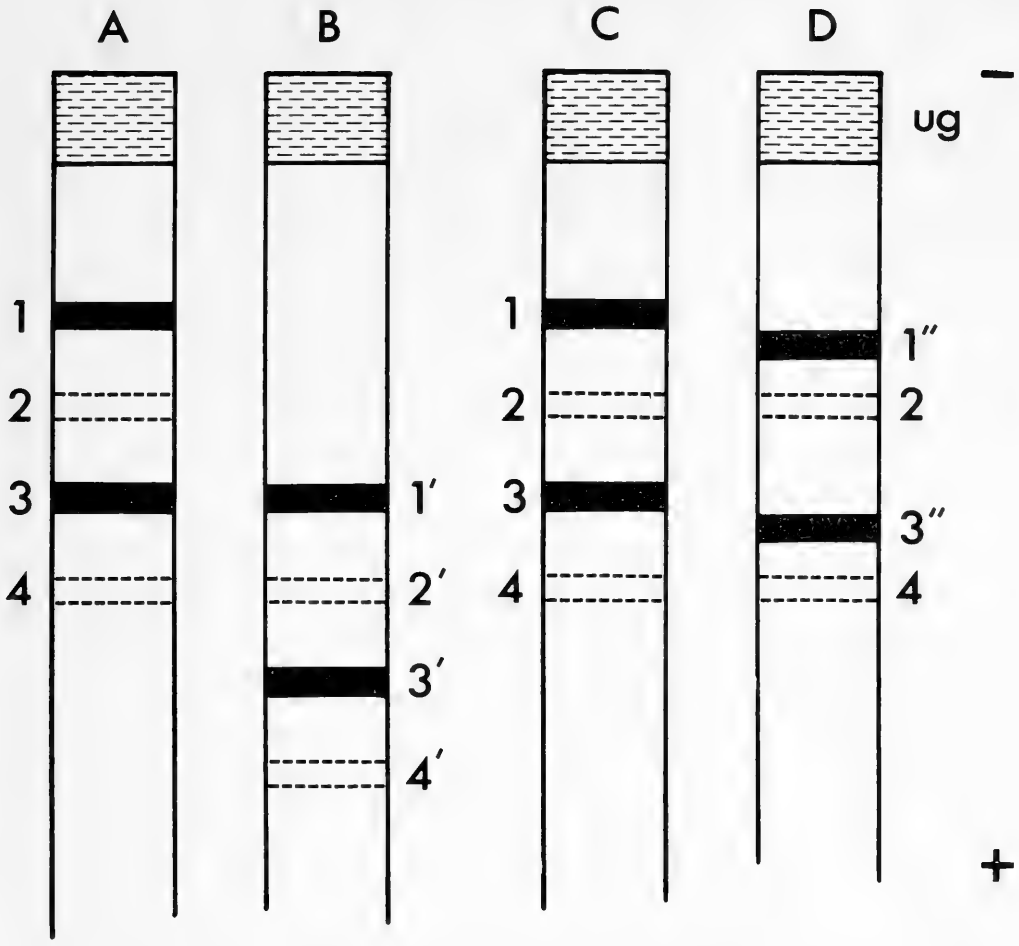


FIG. 2. A, Extract plus an equal volume of distilled water incubated for 3 hr at 37°C before electrophoresis. Primary zones are black; secondary zones are dashed. B, Extract plus an equal volume of 0.4M formaldehyde or glyceraldehyde incubated under identical conditions to A before electrophoresis. Note that all zones move two positions towards the anode. C, Control; conditions of incubation as for A. D, Extract plus an equal volume of 0.2M iodoacetamide incubated under identical conditions to A before electrophoresis. Note that only the primary zones show an increase in electrophoretic mobility. ug = upper gel.

On the other hand, incubation of extracts with 0.1M iodoacetamide for 3 hr at 37°C results in a slight anodal shift in the mobility of primary but not secondary zones (Fig. 2). If one assumes that the shift between one primary zone and the next in a series is equivalent to a unit charge difference, then iodoacetamide produces a shift of about 0.35 unit charge toward the anode. An increase in net negative charge is unexpected since most of the groups that iodoacetamide reacts with e.g., sulphydryl and amino, are probably negatively charged under conditions of electrophoresis. Iodoacetamide should neutralize these groups, thus producing a net increase in *positive* charge. Whatever the anomalies of this situation, it is clear that the iodoacetamide is reacting with a group which distinguishes primary and secondary esterases. The increase in electrophoretic mobility produced by iodoacetamide was not sufficient to mimic the shift that characterizes the two series of allelic products. Clearly, further work along these lines is necessary to clarify the situation.

THE DISTRIBUTION OF FOOD-INDUCED ESTERASES IN OTHER SPECIES OF SNAIL

Five other species of helicid snail, *Cepaea hortensis* (Müller), *Helix aspersa* Müller, *Arianta arbustorum* (L.), *Monacha cantiana* (Montagu), and *Hygromia striolata* (C. Pfeiffer), have been screened for their ability to produce food-induced esterases.

A comparison of esterase patterns found on gels of digestive gland and kidney extracts and of crop juice has identified esterases possibly homologous to those produced by the *Es. 1* locus in *C. nemoralis*. These esterases are present in the digestive gland but not in the kidney, and they are actively secreted into the crop juice where they function as extracellular digestive enzymes (Oxford, 1977, and Fig. 3). In all six species studied, a group of cathodal esterases conforms to this pattern; it was within these enzymes that food-induced modifications were sought.

The experimental method used for the larger snails (*C. hortensis, H. aspersa* and *A. arbustorum*) was to feed the animals on carrot and lettuce for a period, hopefully to eliminate any food-induced zones generated in the wild. A sample of digestive gland was taken and electrophoresed (Oxford, 1975), and the animals were put onto nettle for another period before resampling the digestive gland, usually by killing the snail.

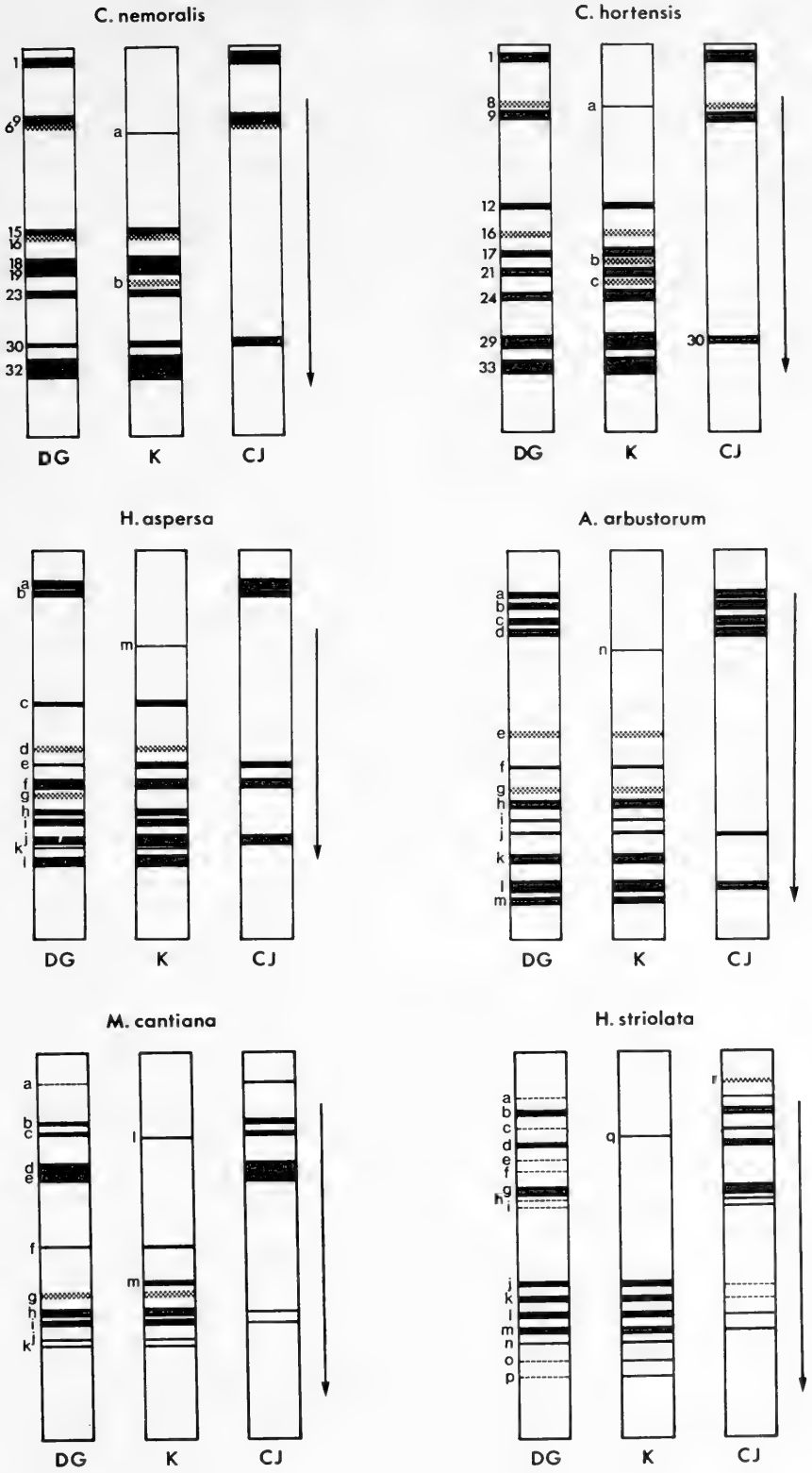
Of these three species, food-induced zones have only been detected in *C. hortensis*. Here only one allele *(Es. 1^1)* is present at what is certainly the homologous locus to that found in *C. nemoralis*. The secondary modifications are identical to those described for *C. nemoralis;* indeed the paucity of alleles makes *C. hortensis* much more amenable for studies on induced zones. With only one allele, Est. 1 must be a primary product and Est. 2 a secondary modification of it. The weaker secondary zones Est. 3 and Est. 4 are particularly clear in *C. hortensis*.

Similar modifications have not been found in *Arianta* or *Helix*. Both species had been maintained on carrot and lettuce for only one month before the initial digestive gland sample was taken; in retrospect, this time may not have been long enough to eliminate any food-induced zones still retained from the wild (see below).

The smaller species, *Monacha cantiana* and *Hygromia striolata,* were investigated for food-induced esterases by dividing a sample taken from the wild into two, half being fed on carrots and lettuce and half on nettle. Samples from both groups were extracted and electrophoresed after one month. No differences were observed between the *H. striolata* subgroups, but in *M. cantiana* a new zone (Est. a, Fig. 3) was present in all the snails fed on nettle but in none of the snails fed on carrot and lettuce. This presumably represents the induction of another esterase rather than the modification of a previous zone. No other esterase zone was affected. A similar phenomenon has been observed in the esterases of the freshwater snail *Potamopyrgus jenkinsi* (Smith) by B. R. Johnson (personal communication). Compared with snails fed on dried lettuce or detritus, those fed on dead leaves develop an extra, powerful esterase zone after 14 days.

With the exception of the apparent induction of a new zone in *M. cantiana,* the two *Cepaea* species seem to be the only ones of those tested which show food-induced modifications. However, longer term studies may show that modifications do occur in other species as well. It will be particularly interesting to examine the esterases of *Cepaea sylvatica* and *C. vindobonensis* from the point of view of modifiable zones. The test described above assumes that nettle can induce modifications in all species (if modifiable esterases exist) and that carrot and lettuce produce no modifications. One or both of these assumptions may not be true for species other than *Cepaea.*

FIG. 3. Tissue distribution of esterase zones in extracts of digestive gland (DG) and kidney (K) and in crop juice (CJ) of six species of helicid snails. Black and dashed zones preferentially hydrolyse α-naphthyl acetate (stain black/purple) and dotted zones preferentially hydrolyse β-naphthyl acetate (stain red) when mixed substrates are used. Relative intensities of esterases are indicated by the width of the bands, with faint esterases shown as dashed or dotted lines. The direction of migration is indicated by the arrows.


C. nemoralis
1
6 9
15
16
18
19
23
30
32
a
b
DG
K
CJ
C. hortensis
1
8
9
12
16
17
21
24
29
33
a
b
c
30
DG
K
CJ
H. aspersa
a
b
c
d
e
f
g
h
i
j
k
l
m
DG
K
CJ
A. arbustorum
a
b
c
d
e
f
g
h
i
j
k
l
m
n
DG
K
CJ
M. cantiana
a
b
c
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f
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DG
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H. striolata
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CJ

THE LINK BETWEEN FOOD AND THE PRODUCTION OF SECONDARY ESTERASES IN *CEPAEA*

Several hypotheses could be suggested to explain the link between nettle ingestion and the generation of modified esterase zones.

Perhaps the most obvious explanation is that some component of ingested nettle acts directly on the esterase molecule, increasing its net negative charge. However, two reasons suggest that this is not the mechanism involved in this instance: (1) incubation of esterase extracts with aqueous nettle extracts results in unaltered zones, and (2) the induction of secondary zones lags behind the ingestion of nettle by a week or so and continues for many weeks after nettle has been removed (see below).

A second hypothesis is that nettle ingestion induces the production of a modifying substance, perhaps an enzyme, by the snail itself. The lag between nettle ingestion and the appearance of secondary esterases could thus be explained by the time needed to induce the intermediate substance. If this was the case, however, removal of nettle should stop the production of the intermediate substance and lead to the loss of secondary esterases. Assuming a rapid turnover rate for the primary esterase, the loss of secondary zones should take about the same time as their initial induction. A fast turnover rate for these esterases is very likely, considering the fact that they are actively secreted into the crop juice (Oxford, 1977).

An examination of results from a number of samples indicates that the induction of secondary esterases on a nettle diet is very much faster than their subsequent loss on a diet of carrot and lettuce (Table 1). In one experiment specifically designed to examine the rate of secondary esterase production, a batch of *C. hortensis* without secondary esterases was fed on nettle and samples were analyzed after various periods of time. Over half the snails had developed secondary esterases by day 13, and all possessed them by day 28. On the other hand, a sample of the same species collected from a colony in which 90-100% of snails were known to exhibit secondary esterases still had 97% of individuals with secondary zones after 86 days on a carrot and lettuce diet. Other observations on both *C. nemoralis* and *C. hortensis* broadly agree with this pattern (Table 1).

TABLE 1. Percentage of snails with secondary esterases under different food regimes.

Species	Day	%	No. analyzed
(a) Carrot/Lettuce → Nettle			
1. *C. hortensis*	0	0	12
	13	58	12
	21	83	12
	28	100	12
2. *C. hortensis*	0	0	15
	40	71	7
3. *C. nemoralis*	0	0	24
	86	61	18
(b) Wild → Carrot/Lettuce			
4. *C. hortensis*	0	c90-100	—
	86	97	32
5. *C. nemoralis*	0	24	25
	19	31	13
	97	0	14
6. *C. nemoralis*	0	100	40
	59	100	2
	203	0	34

This marked asymmetry in the time for induction and loss of secondary esterases has led to a third hypothesis. The intestinal tract of snails, as in most animals, contains a resident population of microorganisms. The suitability of the gut environment for particular microorganisms must depend to some extent on the nature of the ingested food. Certain diets may select for some bacteria, for example, but against others. If ingested nettle imposed powerful selection for a bacterial component of the gut flora which produced and secreted an enzyme capable of modifying the *Es. 1* esterases, then the temporal relationship between the ingestion of nettle and the appearance of secondary esterases would be explained. Carrot and lettuce as foods may impose *mild* selection *against* this bacterial component, reducing its importance over an extended period. This hypothesis would account for the asymmetry of response observed in the time for induction and loss of secondary esterases. As yet there is no evidence from *Cepaea* to support or refute it.

A situation that parallels this hypothesis has been described for salivary amylase enzymes in man (Karn et al., 1973). The bacterial flora of the mouth produce an enzyme that can remove the carbohydrate moiety from the amylase molecule after

secretion, thus increasing its relative electrophoretic mobility.

To be more speculative, if this *is* the mechanism responsible for food-induced esterases in *Cepaea,* then it raises the question of whether it is of any selective advantage for secondary enzymes to be present if nettle (and other inducing foods) are present in the gut. Although the gross biochemical and physical properties of primary and secondary esterases appear the same (Oxford, 1971, 1973b, 1973c), we have no information about their relative hydrolytic efficiencies on natural substrates. If modified esterases *are* more efficient at the hydrolysis of natural esters present in nettle, then we have an intriguing evolutionary situation in which the phenotype of an organism is adaptively changed, not as a result of the environmental stimulus itself but via an environmental effect on an intermediate organism.

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MALACOLOGIA, 1978, 17(2): 341-350

REPRODUCTION AND ITS CONTROL IN *DEROCERAS RETICULATUM*

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ABSTRACT

The basic anatomy and histology of the gonad, hermaphrodite duct, carrefour, albumen gland, common duct, vas deferens, free oviduct, penial mass and bursa copulatrix of the reproductive system of *Deroceras reticulatum* are reviewed and related to their physiology. Evidence for nervous and endocrine integration of reproduction in this protandric hermaphrodite species is discussed.

INTRODUCTION

Deroceras reticulatum (Müller) is an important horticultural and agricultural pest in many countries. In Bangor it can normally be collected throughout the year; most easily at night, although the numbers are very dependent on weather conditions. Over the last ten years, together with a number of good research students, I have studied reproduction and the factors that control it in this animal. There are still many areas, however, where our understanding is far from complete, and it is these areas that I emphasise in this paper.

THE REPRODUCTIVE SYSTEM

Like other pulmonates, this slug is hermaphrodite and the arrangement of the reproductive organs is typical of the stylommatophorans (Fig. 1).

Although *D. reticulatum* is classified as a simultaneous hermaphrodite, with both sperm and ova in the gonad, in fact the sperm and those parts of the reproductive system that produce the spermatophore mature first. The maturation of oocytes and those parts of the tract that form the egg is completed later. This species is therefore said to be protandric. Although the degree of separation of the male and female phases is very clear in many pulmonates, in *D. reticulatum* there is considerable overlap. Reproduction can occur at any time of year but in the United Kingdom is most common in spring and autumn (Runham & Laryea, 1968).

Growth of the animal after hatching is at first slow but then increases considerably in parallel with the growth and maturation of the reproductive tract. At the end of this phase the growth rate decreases, and copulation and egg laying take place, quickly followed by death of the animal. *D. reticulatum* only lives for 6-12 months in the United Kingdom.

Maintenance of the animals in the laboratory is easy as long as they are kept damp and cleaned regularly. They will eat a wide range of food materials. Long-term culture in this laboratory has proved to be much more difficult as there are often outbreaks of infection with *Tetrahymena* which, as they are transmitted in the egg, are impossible to eradicate.

THE GONAD

The gonad or hermaphrodite gland consists of acini connected via small collecting ducts to the main hermaphrodite duct. At hatching one to three acini are present, and these are very small swellings at the tips of outgrowths from the duct. Further outgrowths from the duct increase the number of acini to about 100. At first they are rather globular, but as they increase in size they become more variably shaped: some are rather H-shaped, others irregularly lobed (Runham & Hogg, Ms).

Examination of sections of the gonad reveals that four cell types are present: the male and female gametes and their associated cells, the sertoli and follicle cells, respectively. The relative proportion of

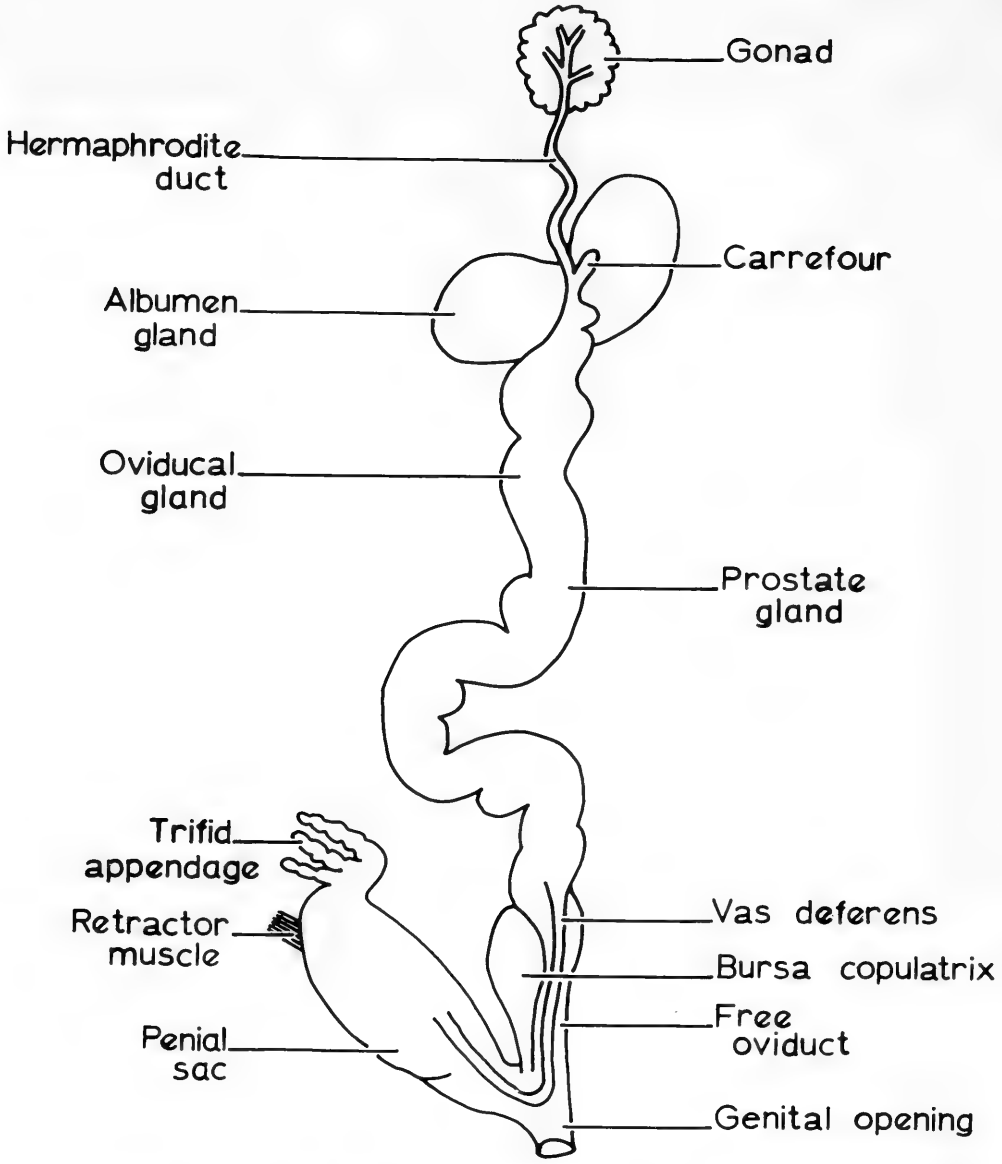

FIG. 1. Diagram of the reproductive system of *Deroceras reticulatum* (adapted from Bayne, 1966).

these gametes and their state of maturity varies throughout the life cycle of the animal. Although these changes are continuous, it is convenient to distinguish eight stages (Runham & Laryea, 1968):

A, undifferentiated: at first only a few acini are present containing primary germinal cells; then acini increase to approximately 100.

B, spermatocyte: recognisable spermatocytes and oocytes differentiate.

C, spermatid: spermatogenesis has advanced to the spermatid stage, although many earlier stages are still present.

D, early sperm: sperm are now present, but a thick layer of spermatogenesis stages lines the walls.

E, late sperm: the acini have reached their maximum size, many sperm are present, and there is a clear space at the centre; the layer of cells in spermatogenesis is thinner, and the oocytes, which have been increasing in number since stage B,

have also increased in size, a few reaching the maximum diameter of 90 μm; as this stage appears to be the most common, it probably lasts the longest time.

F, early oocyte: mature oocytes predominate, but all are present in follicles on the acinus wall.

G, late oocyte: mature oocytes are present in the lumina of the acini, which have contracted in size.

H, postreproductive: a columnar epithelium is present at the base of each acinus and can extend over most of the wall; this epithelium appears to separate the other cells from the wall of the acinus; it may be comparable with similar epithelia found in species that have a resting period between breeding seasons (Galangau, 1964).

While apparently chaotic, the contents of the acini can be shown by reconstructions to have in fact a very ordered arrangement (Runham & Hogg, Ms).

On the wall of the acinus around the entry of the collecting duct there is a cubical epithelium—the germinal epithelium (Fig. 2). Around the edges of this are found the smallest oocytes; the oocytes increase in size the farther away they are from the germinal epithelium. The most mature oocytes are found on the wall opposite the collecting duct. Resorbing oocytes, when present, are mixed up with the mature oocytes. At first sight these sequences indicate that gametes and their associated cells are produced continually from the germinal ring and then move around the wall of the acinus, but it is possible that this arrangement is associated

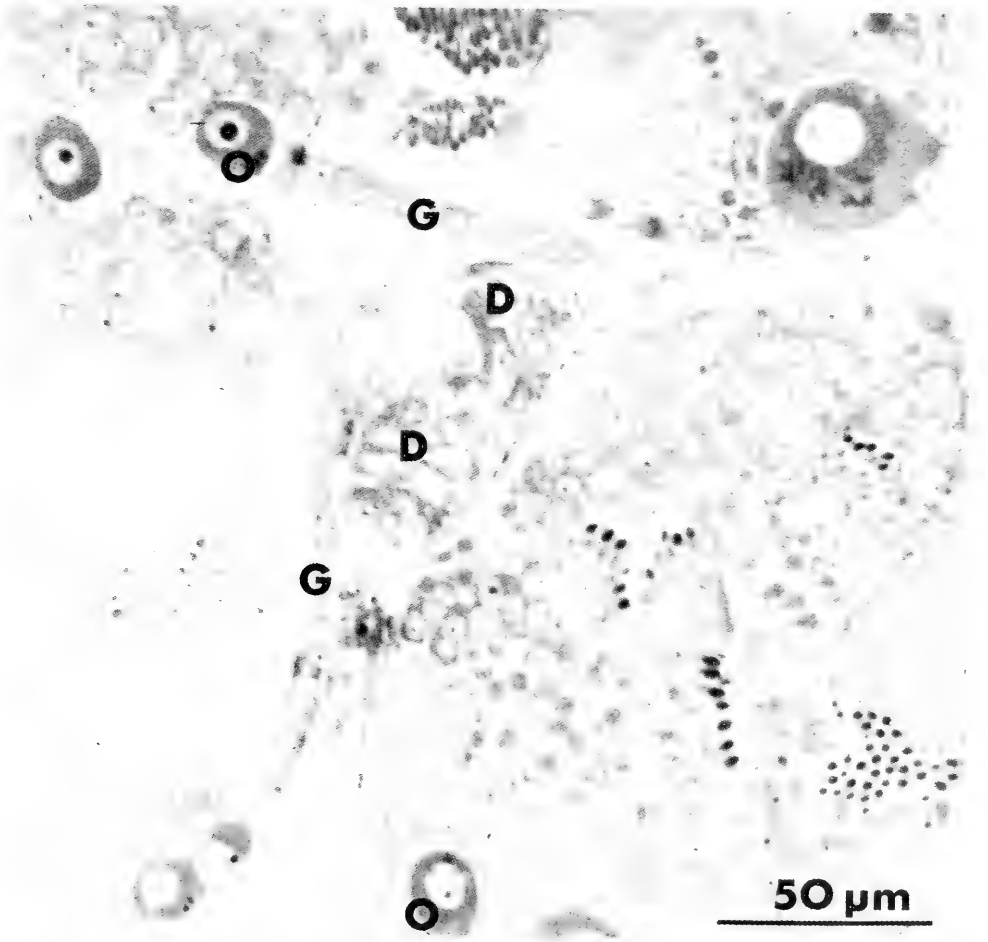

FIG. 2. Transverse section of gonad at late spermatozoon stage. D, hermaphrodite ductule; G, germinal epithelium; O, oöcytes.

with the enlargement of the acinus. Quantitative methods for estimating the rate of production of gametes must be developed to determine which of these views is correct.

Perhaps the greatest problem associated with the gonad is the mechanism by which male and female gametes and their associated cells differentiate from the one cell type present in the germinal epithelium. Recent observations (Runham & Hogg, Ms.) indicate that the germinal epithelium is continuous with a layer of sertoli cells. Cells above this layer differentiate into sperm and those beneath it differentiate into oocytes or follicle cells. There thus appear to be two separate compartments within the acinus, but it is uncertain how the cells come to adopt this distribution.

Castration is a relatively simple operation in this animal as the gonad is superficial and is contained in a blood sinus. Complete regeneration follows castration in stage A and B animals within one month of the operation. Regeneration is rare in stage C animals, and I have only seen one example from an animal at stage D. The complete absence of such regeneration in the later stages has not been explained (Runham, 1976 and in preparation).

HERMAPHRODITE DUCT

At hatching the hermaphrodite duct and the remainder of the reproductive tract are indistinguishable: it consists of a simple tube lined by a simple columnar or cubical epithelium. Within a few days, however, the cells start to fill with glycogen. The development of the carrefour determines the distal limit of the duct, and the proximal end is situated in the gonad. Until stage C the simple epithelium persists, but then the cells differentiate into ciliated and nonciliated. At either end of the duct only ciliated cells are present, but over most of its length these cells form a longitudinal band that occupies approximately one-third of the wall; the remainder is lined with nonciliated cells. During stage D sperm pass into the duct, which swells to accommodate them. Sperm cause the duct to become white in appearance and apparently coiled. Further swelling of the duct is associated with a great extension of the nonciliated cells, which become very thin; the ciliated cells remain columnar until swelling is extreme (Hogg, unpublished data).

The hermaphrodite duct therefore functions as a seminal vesicle. Within some of the nonciliated cells phagocytosed sperm at various stages of breakdown are found, but it is not certain if only abnormal sperm are so ingested. Other nonciliated cells are apparently involved in the passage of fluid material from the duct to the intercellular spaces and hence to the connective tissue. As the sperm are immotile, they must be carried by ciliary action through the collecting ducts into the main duct. It seems likely that the sperm transported in this way are carried in a fluid; but as sperm in the duct are very densely packed, this fluid must be removed, probably by the nonciliated cells. When sperm must pass out of the duct at copulation, it seems necessary to add fluid to the dense sperm mass.

Over the outer surface of the hermaphrodite duct there is a thin layer of circular muscle fibres with an external layer of longitudinal fibres. These layers are thickened at the distal end of the duct where it passes into the carrefour.

THE CARREFOUR

One of the first structures to differentiate from the primary reproductive tract, the carrefour consists of a simple cylindrical process partially surrounded by a larger, flattened diverticulum (Fig. 3). Our observations agree with those on *Helix* (Lind, 1973), where the central process functions as a spermathecal sac for storage of foreign sperm and the larger outer structure is the fertilisation pocket (Nicholas, Ms.) All the epithelia are ciliated, but how the direction of the animal's own sperm and oocytes and foreign sperm are controlled is uncertain. Before it enters the carrefour the hermaphrodite duct narrows and forms a loose coil.

ALBUMEN GLAND

Developing from a pair of processes from the primary reproductive tract distal to the carrefour, the albumen gland enlarges and the walls of the sacs become more and more folded until a compound acinar structure is formed (Fig. 3). The

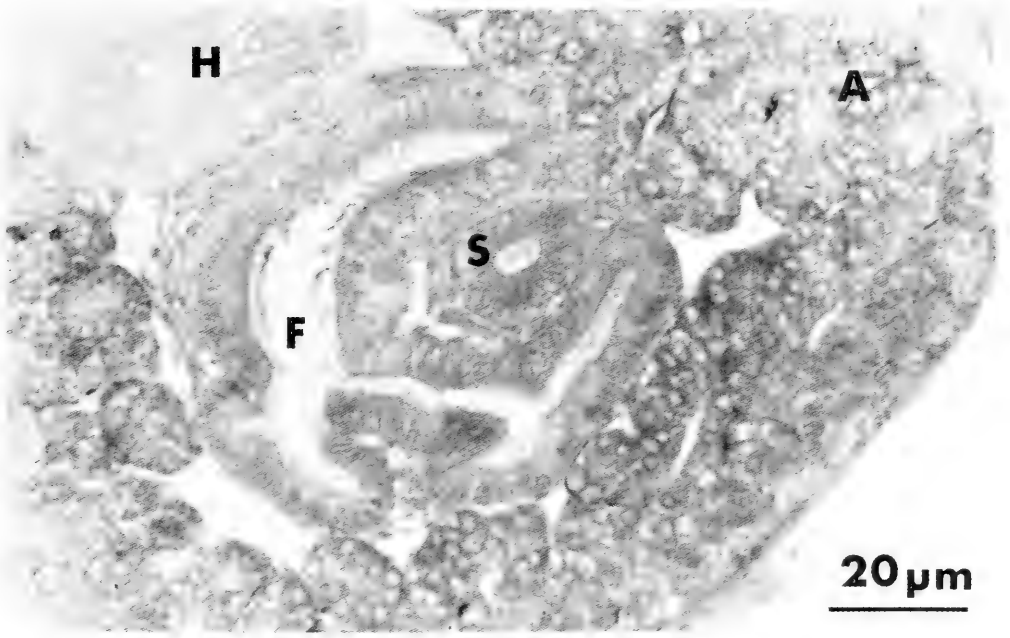

FIG. 3. Transverse section of carrefour. A, albumen gland acini; F, fertilisation pocket; H, hermaphrodite duct; S, spermathecal sac.

epithelium of the primary duct continues into the albumen gland to line the acini. Cells from the connective tissue under the epithelium enlarge and send processes through the epithelium to the lumen. Secretion accumulates in these cells, which swell considerably; the lining epithelium becomes very stretched and thin. The lining cells develop cilia, which presumably help to transport the secretion (Bailey, 1970, 1973). Characteristically this secretion contains large amounts of galactogen; when fixed, this gland is exceedingly difficult to section because of its extreme hardness. Glycoprotein is also present in the secretion (Bayne, 1966).

Fertilised eggs from the carrefour pass through the collecting duct area of the albumen gland, where they receive an aliquot of secretion, the perivitelline fluid that fills the central part of the egg. Since this secretion appears very concentrated, extra water may also be secreted here. Each zygote becomes surrounded by a characteristic amount of this perivitelline fluid so that some metering of the albumen gland secretion appears essential, but it has not been discovered how this is achieved.

COMMON DUCT

Cell multiplication along one side of the primary reproductive tract, distal to the albumen gland, forms many tubular diverticulae, which collectively form the prostate gland. At first only a simple epithelium is present, but cells that accumulate in the connective tissue send out processes that insinuate between the epithelial cells to reach the lumen. The cells differentiate into glandular cells; as these accumulate secretion, they swell and stretch the lining epithelium greatly. At least three types of secretory cell are present in this gland (Bailey, 1970, 1973).

The oviducal gland develops on the duct wall opposite the prostate gland. Epithelial cells line the wall, and again glandular cells develop in the connective tissue beneath the epithelium and push up through it (Fig. 4). Two types of glandular cell appear to be present, and their enormous expansion considerably dilates the gland and attenuates the lining epithelium. The development of the prostate gland is normally complete before that of the oviducal gland starts. Eventually the epithelia lining both

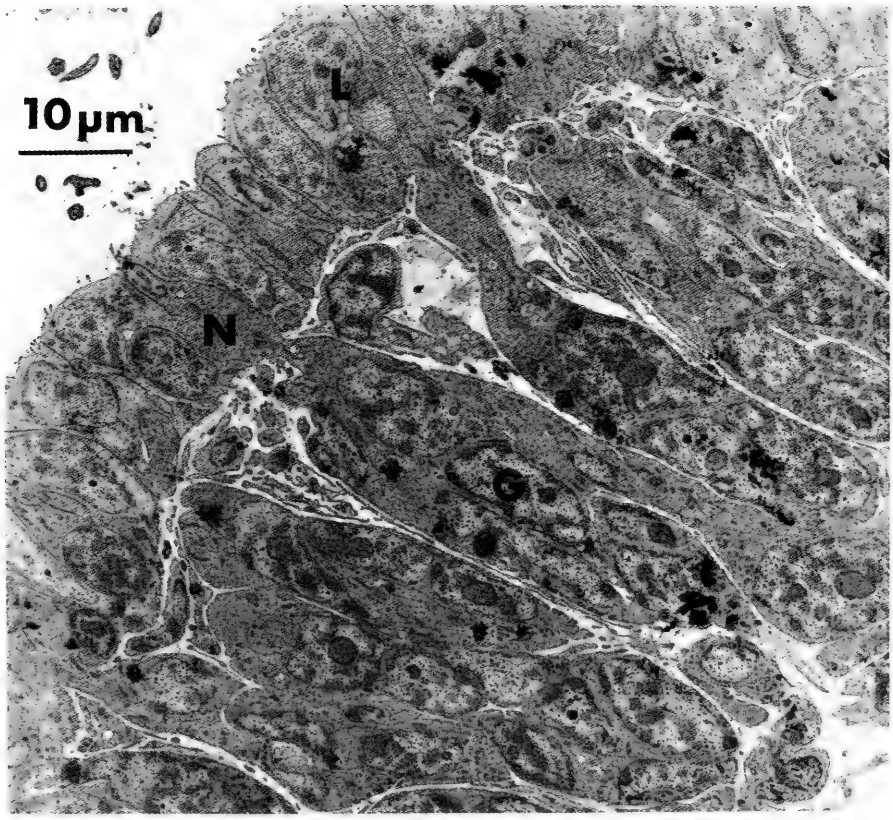

FIG. 4. Developing oviducal gland. G, gland cells; L, lining epithelium; N, necks of gland cells pushing between lining epithelial cells.

glands become ciliated, but the glands remain in open but narrow communication along their length (Fig. 5). Analysis of extracts of the common duct reveal the presence of a complex mixture of materials (Bayne, 1967), but it is not clear which cell type forms which constituent.

At copulation sperm pass from the hermaphrodite duct into the common duct where they are encapsulated in a type of spermatophore known as a jelly mass. The prostate gland forms this jelly mass, but the details are unknown. One function of the rather lengthy courtship in these animals is probably to trigger the processes that form the jelly mass.

Egg shell material is deposited around the perivitelline layer of the egg as it passes down the common duct. The thickness of the shell increases as the egg passes down from the albumen gland, but it is not clear how the secretions produce two apparently distinct layers of egg shell. Calcium granules are present in the egg shell (Tompa, 1976), but their source is uncertain.

At the junction of the albumen gland and the common duct a small gland is present distinct from the rest of the common duct. At the moment its function is unknown, but in this position it could either add water to the albumen gland secretion or form the thin layer that surrounds the perivitelline fluid.

Muscular tissue is present beneath the lining epithelium of the common duct as a thin meshwork. When the glandular cells develop they push through this layer, and as they expand the muscle layer becomes obscured.

Transport of the sperm and jelly mass is probably by ciliary action of the lining epithelium, particularly in the area of the male groove. After copulation some of the partner's sperm must be transported up the

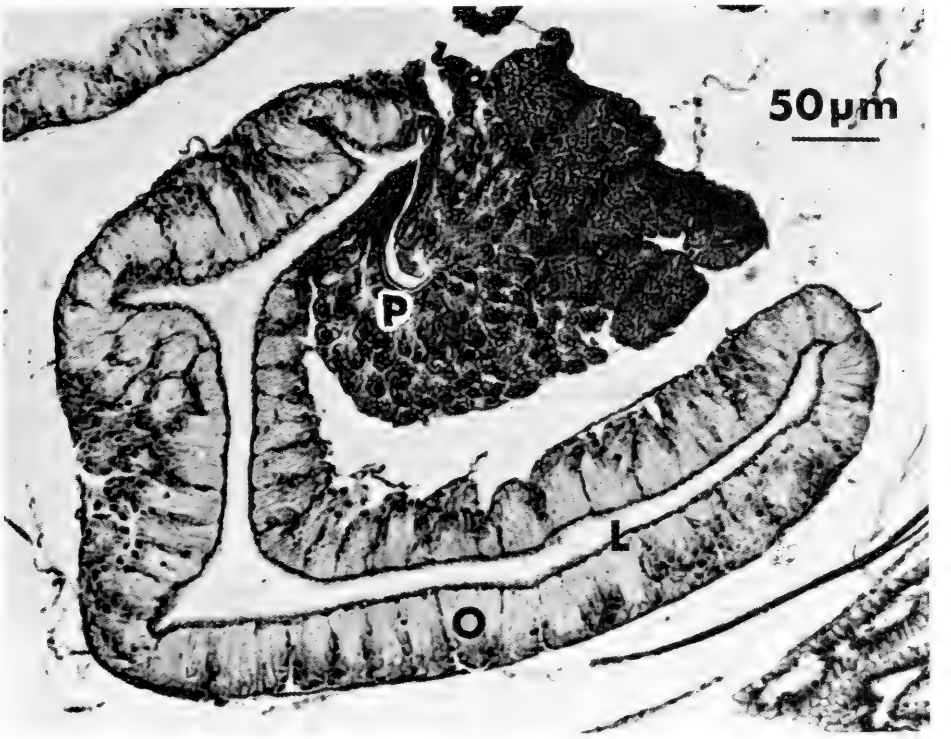

FIG. 5. Transverse section of common duct. L, lining epithelium; O, oviducal gland; P, prostate gland.

common duct to the spermatheca. This requires a reversal of the normal ciliary current, but observations of this process have not been published in this species. In other species it appears to ascend the oviduct and oviducal gland (Lind, 1973). Movement of the completed egg, which must cause considerable distension of the tract, presumably requires muscular action, but again the details are obscure.

VAS DEFERENS AND FREE OVIDUCT

The vas deferens is a small tube with a well-developed muscular layer and a simple epithelial lining. What functions this structure performs, in addition to conducting gametes, are unknown, as is the means by which this narrow tube transports the jelly mass.

Large flask-shaped glandular cells open through the lining epithelium of the oviduct, and these must presumably add material to the outside of the egg. This secretion and its function have not been studied.

THE PENIAL MASS

Very little information has been published on this very complex structure. Anatomically it consists of a large penial sac on the tip of which are found the three processes of the trifid appendage, a distinguishing character of this species. When everted during courtship, the penial mass forms a globular process on the outside of the animal. Surmounting this mass is a large conical process, the sarcobellum, which is highly mobile and is used to caress the partner. This sarcobellum is attached to the inner wall of the penial mass so that it is not everted, although undoubtedly it is a very erectile structure. The trifid appendage is everted during courtship, but it is often difficult to see because it is small.

When the penis sac is everted, the openings of the oviduct and the bursa copulatrix open close together at the base of the everted mass, some distance from the opening of the vas deferens. Another function of courtship must be to align the slugs to bring the vas deferens openings

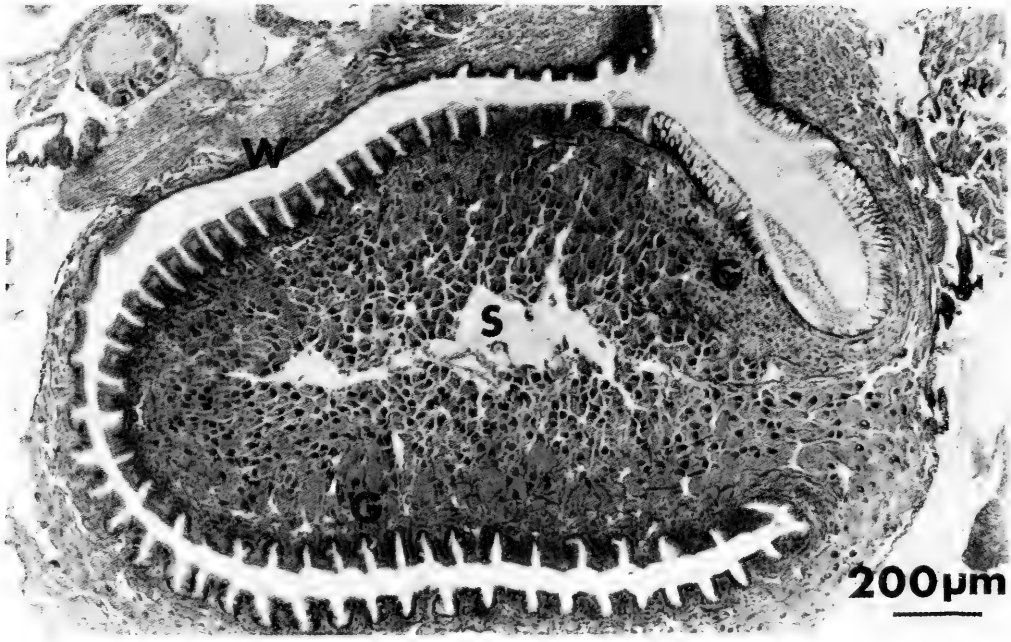

FIG. 6. Longitudinal section of penial mass. G, gland cells; S, sarcobellum; W, wall of penial mass.

opposite the bursa copulatrix openings. Accurate alignment does not appear to be essential, however, as animals fixed immediately after copulation normally have the transferred jelly mass present within the cavity of the retracted penial sac. Eversion of the penial sac is accomplished by relaxation of the genital opening and pressure of blood blowing out the sac. The everted sac is very large and contains a high percentage of the animal's blood together with any organs of the body that push into it. The sac is retracted by the penial retractor muscle attached to the tip of the penis sac.

Histologically the penis sac is shown to be complex (Fig. 6). The sarcobellum is very muscular, but at least three glandular areas are present. The functions of these glands in copulation or courtship are unknown. The trifid appendage processes contain a peculiar granular secretion that coats their surfaces when they are everted.

BURSA COPULATRIX

Often known as the spermatheca, this structure is mainly concerned with the breakdown of genital products rather than their storage; bursa copulatrix is the more accurate term (Bayne, 1973). This gametolytic gland varies considerably in size but is always found closely attached to the free oviduct. It is lined by a tall columnar epithelium and the contents of the lumen vary from sperm and jelly mass to partially digested eggs. In *Helix* the spermatophore is so shaped that when it passes up the duct of the bursa compulatrix by peristalsis, sperm are expressed through a hole in the spermatophore wall; only sperm that escape make their way to the spermatheca, and the rest are digested in the bursa (Lind, 1973). The mechanism by which sperm are released from the jelly mass of *D. reticulatum* is unknown.

NERVOUS CONTROL OF REPRODUCTION

Sections of the reproductive tract examined in the electron microscope reveal the presence of many nerve fibres in the connective tissue in all areas, but distinct nerves are only present around the penial sac area and passing along the hermaphrodite duct to the gonad. It seems likely, however, that the fine nerve fibres are part

of a plexus that exists throughout most of the tract (Minker & Koltai, 1961). Severing the nerves to the reproductive system has so far only been achieved with the intestinal nerve, which sends a branch to the hermaphrodite duct. Eggs laid after this operation are perfectly normal; but if part of the visceral ganglion is also destroyed, the eggs are misshaped and polyembryonic (Button, unpublished data). Much work remains to be done in this area.

ENDOCRINE CONTROL OF REPRODUCTION

Transplants of undifferentiated common ducts into the haemocoel of male-stage and female-stage recipients differentiate differently. The prostate gland became well developed in the male-stage animals and the oviducal gland in the female-stage animal. As the transplants were free in the haemocoel, differentiation and maturation must have resulted from exposure to blood-borne hormones. The source of these hormones was unknown in these early experiments (Runham, Bailey & Laryea, 1973).

Two sources of hormones exist in *Deroceras*—neurosecretory cells in the brain and the glandular dorsal bodies that lie on the surface of the cerebral ganglia and commissure (Fig. 7). Extirpation of the dorsal bodies slowed considerably the development of the oviducal gland and albumen gland, and this effect is reversed by a transplant of dorsal bodies from another animal (Wijdenes & Runham, 1976 and unpublished). This gland is therefore the probable source of a hormone controlling the development of the oviducal and albumen glands. The source of the hormone controlling the prostate gland has not been determined. Recently, transplants of the penial mass indicate that this organ is also under hormonal control.

A study of the maturation of the tract and the changes in the gonad revealed that they are closely related phenomena (Runham & Laryea, 1968). Castration stops or greatly slows tract maturation; this is revealed by cessation of mitotic division in differentiating tracts, by decreased incorporation of radioactively labeled amino acids in more mature tracts, and by their weight. These changes are also associated with a massive increase in the amount of glycogen stored in the tissues of the body (Runham, 1976 and unpublished data). The interactions of the dorsal body and gonad have not yet been studied.

Another function of the dorsal body appears to be the control of oocyte growth. Extirpation leads to reduced numbers of large oöcytes and an accumulation of smaller oöcytes (Wijdenes & Runham, 1976). In *Lymnaea,* oöcyte enlargement is associated with vitellogenesis (Geraerts &

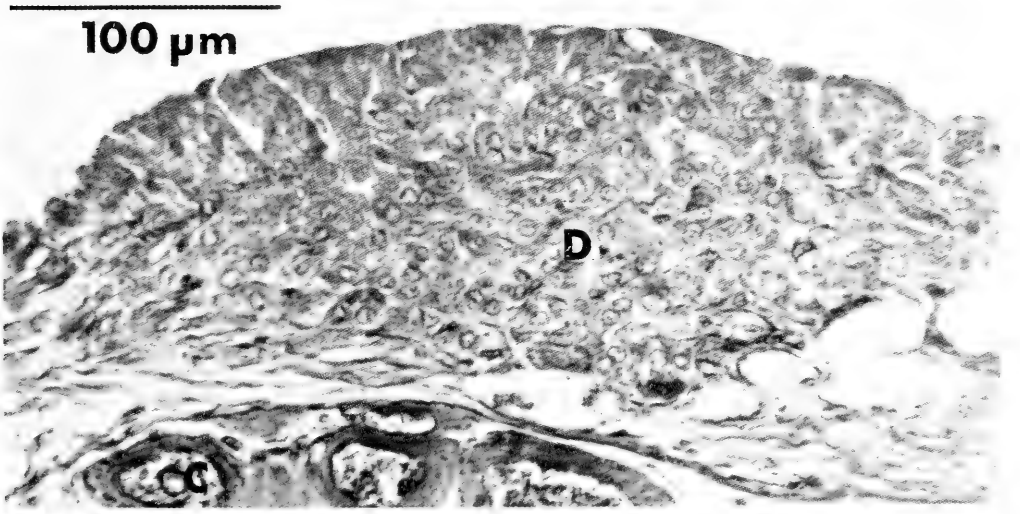

FIG. 7. Longitudinal section through the cerebral commissure and dorsal body. C, cerebral commissure; D, dorsal body.

Joosse, 1975), but this has not been studied in *Deroceras.*

Many workers have attempted to repeat the work of Pelluet (1964) and Pelluet & Lane (1961), which established hormonal control of oögenesis by secretions from the tentacle and cerebral ganglia and control of spermatogenesis by cerebral ganglion secretions. In Bangor we have failed completely to repeat this work, but in *Arion* (Wattez, 1973) it seems likely that the tentacles do produce a secretion that inhibits oögenesis. Full clarification of these relationships really depends on developing simple accurate methods of estimating gamete production.

CONCLUSIONS

As is evident, we have some understanding of reproduction in *D. reticulatum* but there remain many areas where detailed information is lacking. In particular, integration of the reproductive tract during copulation and egg laying and the mechanisms that time maturation of various parts of the reproductive tract in this protandric hermaphrodite are virtually unknown.

Other species of slug and most other pulmonates are even less well known. Environmental factors are known to influence reproduction in temperate species, but again detailed information is lacking. Particularly valuable would be a study of reproduction in species with a wide geographical distribution to discover how climatic factors, expecially at the limits of their distribution, affect the complex process involved.

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THE EVOLUTION OF LIFE-CYCLE STRATEGIES IN FRESH-WATER GASTROPODS

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ABSTRACT

From a review of the available data I examine the adaptive significance of differences in three aspects of the life cycles of fresh-water gastropods: (a) the "choice" between semelparity and iteroparity; (b) the "choice" between egg size and egg numbers; (c) the "choice" between gonochorism and hermaphroditism.

Most temperate, fresh-water gastropods are annual and semelparous. On the other hand, marine and terrestrial species tend, in general, to be perennial and iteroparous. Since fresh-water species must have evolved from marine and possibly terrestrial ancestors, the question arises why iteroparous species should have evolved into a semelparous condition. In the gastropods the semelparous state seems to be associated with reproductive recklessness on the part of the parent, and this strategy has probably evolved in association with adaptations that ensure a greater chance of survival of offspring.

As gastropods have invaded fresh water there has been a trend toward producing larger sized eggs, telescoping developmental stages into the egg, and thus producing larger, more fully developed hatchlings. These adaptations can be explained as a response to the challenge posed by inclement conditions. Some fresh-water gastropods produce larger eggs than others, and these differences can be correlated with differences in ecology. However, since the number of progeny that ultimately reach maturity depends both on the fitness of individual progeny and on their initial density, different strategies (in terms of the choice between egg size and numbers) can feasibly lead to equivalence in parental fitness under the same ecological conditions. This principle, of alternate equifit adaptations to the same environmental challenge, will be illustrated by reference to littoral species.

Most pulmonates are hermaphrodite, but many prosobranchs have separate sexes and are viviparous. These differences are discussed in terms of current theory.

INTRODUCTION

In this review I consider the adaptive significance of various aspects of the life-cycle strategies of fresh-water gastropods. By life-cycle strategy I mean the complex set of adaptations which ensure that the products of reproduction reach a condition whereby they can themselves reproduce. It is assumed that selection will have operated in such a way that these strategies are optimally adapted to maximize fitness—in other words, so that they are matched (or nearly matched; see Rapport & Turner, 1977) to meet the challenge offered by a particular set of environmental conditions.

Two types of study have been directed at life-cycle problems. Firstly, theoretical studies have sought to deduce the optimal strategies to be expected under specified ecological conditions from general evolutionary principles (Stearns, 1976). Secondly, observational studies have sought to correlate particular strategies with particular ecological circumstances and thereby to arrive inductively at the way selection optimizes life cycles under natural conditions (e.g. Tinkle, 1969). Clearly, the deductive and inductive methods ought ultimately to complement each other and to contribute jointly to a deeper understanding of life cycles.

The observational approach is most powerful when carried out on closely related species. Fresh-water gastropods provide a good opportunity for this kind of study since there is already a considerable amount of reliable, descriptive information available in the literature on their life cycles. Here I intend to use these published data to consider what strategies are used by gastropods and when and why. Firstly, I consider what general patterns of life cycle are adopted by fresh-water gastropods; in this context I use Cole's (1954) terminology of **semelparity** and **iteroparity** to

distinguish between different patterns. The semelparous condition is when parents die after reproduction. The iteroparous condition is when parents live on after reproduction to reproduce again. Secondly, I consider to what extent fresh-water gastropods compromise between egg size and egg numbers. Since only a finite amount of energy is available for gamete production all organisms have to "choose" between producing a large number of small eggs or a small number of large eggs. Which strategy is adopted can usually be correlated with the prevailing ecological conditions (Calow, 1977; Wilbur, 1977), and several theoretical attacks have been made on this subject (Pianka, 1970). Thirdly and finally, I consider what methods are involved in reproduction in fresh-water gastropods in terms of gonochorism, hermaphroditism, parthenogenesis, and viviparity. Russell-Hunter (1964; in press) has already made some interesting comment on this subject from the point of view of the fresh-water pulmonates, and a recent theoretical paper by Heath (1977) provides a framework for my discussion here.

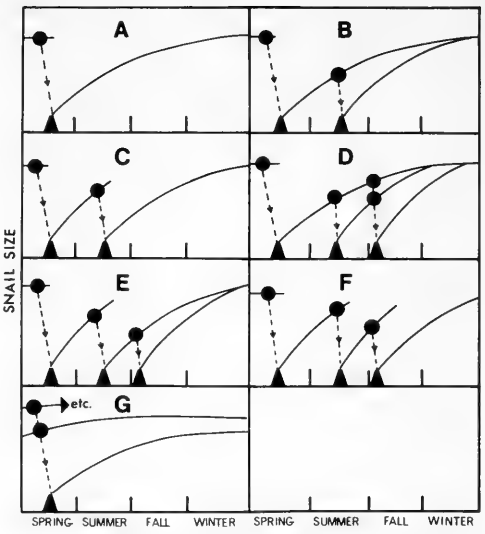

FIG. 1. Patterns of life cycle to be found in fresh-water gastropods. Circles, reproduction begins; triangles, egg capsules appear.

LIFE-CYCLE PATTERNS OF FRESH-WATER GASTROPODS

Little can be added, except in detail, to the reviews of Russell-Hunter (1961a, 1961b, 1964, in press) on the life cycles of fresh-water gastropods. He was able to distinguish between several patterns of life cycle in this group (Fig. 1). The annual condition, with a spring breeding season and complete replacement of one generation by another, predominates (TYPE A); but a second, summer breeding season, either without (TYPE B) or with (TYPE C) a replacement, is common. A third breeding season in autumn, again either without (TYPE D) or with (TYPES E & F) replacement, has been reported for several populations. Finally, a true perennial (sometimes just biennial) condition (TYPE G), though less common, can occur. Life-cycle types A, C, and F are semelparous; life-cycle types B, D, and E are quasi-iteroparous, in that they occur in special circumstances in species which are usually semelparous; life-cycle type G is the true iteroparous condition.

Table 1 catalogues the data available on the life-cycle patterns of temperate gastropods using the above typology, and Fig. 2 gives a summary. Considering the distribution of species between life-cycle types, a number of important points emerge: (1)

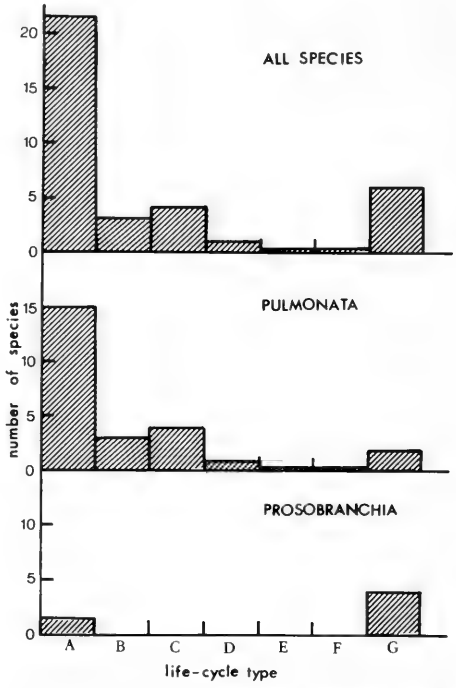

FIG. 2. Distribution of species between life-cycle types.

TABLE 1. Review of life-cycle type and fecundity characteristics in 37 species of fresh-water gastropods.

Species	Life cycle type	Egg			Authority
		Size, mm	No./ capsule	No./ ind./ season	
PULMONATA					
Lymnaeidae					
Acella haldemani	A	1 × 0.6	3-12	?	Morrison, 1932
Lymnaea elodes	B	?	20-47	?	Eisenberg, 1966
L. humilis	C	?	?	?	Van Cleave, 1935
	A	?	6-17	?	McCraw, 1961
L. palustris	?	0.84 × 0.58	10-40	?	Bondesen, 1950
	G	?	?	?	McCraw, 1970
	A	?	?	?	Eckblad, 1973
	G	?	20	250-370	Hunter, 1975
	B	?	—	—	Hunter, 1975
L. peregra	?	1.09 × 0.79	200	?	Bondesen, 1950
	A	?	12-20	300-1100	Russell-Hunter, 1961a
	C	?	—	—	Russell-Hunter, 1961a
	A	?	?	?	Young, 1975
L. stagnalis	?	1.37 × 1.00	100	?	Bondesen, 1950
	G	?	?	?	Frömming, 1956
	G	?	100-150	1000-1500	Berrie, 1965, 1966
	G	?	?	?	De Coster & Persoone, 1970
L. truncatula	F	?	7-9	?	Walton & Jones, 1926
	?	0.78 × 0.53	15	?	Bondesen, 1950
	C	?	?	?	Heppleston, 1972
	C	?	?	?	Bruce et al., 1973
Physidae					
Aplexa hypnorum	?	1.21 × 0.93	20	?	Bondesen, 1950
	A	?	?	?	Hartog & de Wolf, 1962
	A	?	?	?	Vlasblom, 1971
Physa acuta	?	1 × 0.5	?	?	Bondesen, 1950
	A	?	?	?	Duncan, 1959
P. fontinalis	?	1 × 0.8	20	?	Bondesen, 1950
	C	?	6-18	180	Wit, 1955
	A	?	5-11	?	Duncan, 1959
	A	?	15	174	Russell-Hunter, 1961a & b
	B	?	—	—	Russell-Hunter, 1961a & b
	A	?	?	?	Girod, 1969
P. gyrina	?	?	100-200	?	Bondesen, 1950
	—	0.79 × 0.72	13	272	DeWitt, 1954
	A	—	—	—	DeWitt, 1955
	B	—	—	—	DeWitt, 1955
	C	?	?	700	Clampitt, 1970
P. integra	B	?	?	1000	Clampitt, 1970
	A	?	?	?	Eckblad, 1973
P. virgata	D	?	11-17	?	McMahon, 1975
Planorbidae					
Anisus rotundatus	?	?	8	?	Bondesen, 1950
	G	?	?	?	Marazanoff, 1970
Helisoma trivolvis	A	?	20	1600	Eversole, 1974
	G	?	—	—	Eversole, 1974
Planorbis albus	?	0.73 × 0.50	5	?	Bondesen, 1950
	A	?	?	?	Russell-Hunter, 1961a & b
P. carinatus	A	?	?	?	Young, 1975
P. contortus	?	0.70 × 0.54	?	?	Bondesen, 1950
	A	1 × 0.50	3.5	34	Calow, 1972a
	C	?	?	?	De Coster & Persoone, 1970
P. corneus	?	1.68 × 1.50	30-50	?	Bondesen, 1950
	A	?	?	?	Berrie, 1963
P. planorbis	?	0.78 × 0.68	30	?	Bondesen, 1950
	A	?	?	—	De Coster & Persoone, 1970
P. vortex	?	0.76 × 0.60	20	?	Bondesen, 1950
	A	?	?	?	De Coster & Persoone, 1970
Ancylidae					
Ancylus fluviatilis	?	1.42 × 1.20	2-3	?	Bondesen, 1950
	A	1.3	4	46	Russell-Hunter, 1953
	A	1.30 × 1.23	4	50	Geldiay, 1956
	A	1.30 × 1.20	3	31	Calow, 1972a

TABLE 1 (Continued).

Species	Life cycle type	Egg: Size, mm	Egg: No./ capsule	Egg: No./ ind./ season	Authority
A. lacustris	?	0.80 × 0.47	8-10	?	Bondesen, 1950
	A	?	?	?	Russell-Hunter, 1953
Ferrissia rivularis	A	?	1-7	9	Burky, 1971
	C	?	—	—	Burky, 1971
	B	?	?	?	Nickerson, 1972
Hebetancylus excentricus	C	1.82	3-5	?	McMahon, 1976
Laevapex fuscus	A	2.10	3-5	42	McMahon, 1976
	D	—	—	—	McMahon, 1976
	E	—	—	—	McMahon, 1976
PROSOBRANCHIA					
Hydrobiidae					
Amnicola limosa	A	?	?	?	Pinel-Alloul & Magnin, 1973
Bithynia tentaculata	G	0.1	10	25-100	Lilly, 1953
	G	?	?	?	Schäffer, 1953
	G	?	?	?	Pinel-Alloul & Magnin, 1971
Potamopyrgus jenkinsi	G	P	P	P	Robson, 1923
Valvatidae					
Valvata humeralis	A	?	?	?	Gillespie, 1969
V. piscinalis	A	0.05	9-18	?	Cleland, 1954
	A	?	?	?	Gillespie, 1969
Viviparidae					
Campeloma rufum	G	VP	VP	VP	Van Cleave & Altringer, 1937
Campeloma spp.*	G	V	V	V	Medcof, 1940
Viviparus contectoides	G	V	V	V	Van Cleave & Lederer, 1932
V. fasciatus	G	V	V	V	Stańczykowska, 1959
V. malleatus	G	V	V	V	Stańczykowska et al., 1971
V. viviparus	G	V	V	V	Miroshnitshenko, 1958

P = parthenogenetic; V = viviparous; * described as new spp. and different from *C. rufum*

most species, particularly pulmonates, are semelparous and annual; (2) there may be intraspecific variations in life cycles—for example, Type A may transform to B, C, D, and E in productive habitats (McMahon et al., 1974)—a point considered of particular importance by Russell-Hunter (in press); (3) more of the type-G gastropods are exclusively iteroparous and are prosobranchs. However a few species with iteroparous populations also include populations showing other patterns.

Also listed in Table 1 are records on the size and numbers of eggs produced per parent. Fresh-water gastropods produce in the order of 10^2-10^3 eggs per breeding season with diameters of about 1 mm. This contrasts with marine gastropods, where eggs are usually 10^2-10^3 μm in diameter and where fecundity per breeding season may be as high as 10^6 eggs per parent (Fretter & Graham, 1964). In common with these marine prosobranchs, *Melampus bidentatus,* a primitive, saltmarsh pulmonate, produces approximately 33,000 very small eggs per individual per breeding season (Russell-Hunter & Apley, 1966; Apley et al., 1967; Apley, 1970; Russell-Hunter et al., 1972). Hence, the invasion of fresh waters by the gastropods, particularly the pulmonates, has been accompanied by a switch from emphasis on egg numbers to emphasis on egg size. This is a well-documented trend (Bondesen, 1950), which has been ascribed to the more stressful nature of the fresh-water habitat. The "dilute" conditions present osmotic problems, and there can be violent fluctuations in many of the physicochemical aspects of freshwater ecosystems. This has necessitated suppression of the sensitive planktonic stage and the telescoping of development into the egg (Fioroni & Schmekel, 1975). As a result, the egg must carry a more extensive yolk store and provide space for extended development. Hence, the emphasis must be on egg size rather than numbers.

EVOLUTION OF SEMELPARITY

It seems very likely that the predominant semelparous condition of fresh-water gastropods has evolved out of a primitive, iteroparous condition. Most marine prosobranchs are iteroparous (Fretter & Graham, 1964), and the primitive saltmarsh pulmonate *Melampus bidentatus* is iteroparous (Russell-Hunter & Apley, 1966; Apley et al., 1967; Apley, 1970; Russell-Hunter et al., 1972). The question therefore arises why the invasion of fresh waters from marine and perhaps terrestrial environments is associated with a shift from iteroparity to semelparity. Cole (1954), in his classic paper on life cycles, raised the opposite question: what is the advantage of a semelparous species becoming iteroparous? He was puzzled because a shift from semelparity to iteroparity makes little direct contribution to progeny production and hence fitness. However, Cole carried out his analysis on "ideal populations" in which there was no mortality between breeding seasons, and this is clearly a very special case. Whenever, on the other hand, there is a high probability of juvenile mortality, it can be shown theoretically that it is worthwhile preserving the parent as an insurance strategy even if this might lead to slightly reduced fecundity (Stearns, 1976). The advantage of iteroparity comes not from the direct contribution it might make to the production of progeny but because it gives the same parent several chances at replicating and spreading its own genes. Given no apparent disadvantage of iteroparity but the obvious advantage of an "insurance strategy," the next question is why all species are not iteroparous or, more specifically, why iteroparous species should ever return to semelparity? This might be referred to as the "gastropod paradox" since it is the question raised most obviously by a comparative treatment of gastropod life cycles.

There are several possible solutions to the "gastropod paradox." There may be a direct advantage in truncating the life-span of the parent; for example, as Weisman (1882) believed, it may provide more food and space for the potentially more virile progeny. It is difficult to see, however, how such altruism could have evolved when the progeny of different parents apparently intermix completely and freely. Intermixing would mean that all progeny, not just those carrying the gene for the altruistic character, would be advantaged by the death of the parent. Hence, selection would be impotent in favoring the gene(s) for parental death.

Another possibility is that semelparity may have evolved indirectly from the active selection of another, correlated character. Several theories on the evolution of life cycles are based crucially on the assumption that a high reproductive effort leads automatically to a reduced adult life-span (Cody, 1966; Williams, 1966a, 1966b; Gadgil & Bossert, 1970; Charnov & Krebs, 1974; León, 1976). Whenever the gains (in terms of viable progeny) accruing from an increased reproductive effort are greater than the potential loss due to weakening of the parent, then the increased effort will be selectively favoured and will bring with it a shorter adult life-span. On this theory, there should be a negative correlation between reproductive effort and life-span.

A favoured measure of reproductive effort is the proportion of available resources (ingested or absorbed energy) used in gamete formation (Tinkle & Hadley, 1975), but data of this kind are rare. I have therefore tested the hypothesis in two ways. Where data were available, I have used the ratio

$$\frac{\text{energy output in gametes}}{\text{energy input from food}} \times 100$$

as a direct index of effort (DEI). Otherwise I have used the ratio

$$(E \times EV)/SV = IEI$$

(indirect index of effort)

E = no. eggs produced/breeding season
EV = egg volume (calculated from $4/3\pi r^3$ and assuming that eggs are spherical
SV = parent volume (calculated from $1/3\pi r^2 h$ and assuming that snails approximate to a cone, where r = egg or shell radius and h = shell height)

IEI assumes that energy loss per egg is proportional to egg volume and that the demand egg production makes on the parent is roughly proportional to the relative masses of the adults and the total eggs spawned.

IEI's are calculable from the data given in Table 1, together with additional information (derived in the main from the source references) on the shell dimensions of adults. The results are given in Table 2. The mean IEI for the semelparous species was 2.06; for the iteroparous species it was 0.28. These values were significantly different ($t = 4.42$, $P < 0.05$) and suggest that semelparous species expended about 10 times more effort in reproduction than the iteroparous species.

Data on DEI's are summarized in Table 3, using both the total absorbed energy (TA) and the nonrespired part of TA (NRA) as energy input terms over the reproductive period. Here it has been possible to compare effort indices in populations of the same species showing different life-cycle patterns. In all cases semelparous species-populations had values between 10 and 40% greater than the iteroparous species-populations. As judged by the "t test," average indices for semelparous species were significantly greater than those for iteroparous species ($t = 3$ to 4, $P < 0.05$).

Marine prosobranchs, which are predominantly iteroparous, often produce more eggs than fresh-water gastropods but probably put less effort into reproduction. For example, egg output may be as much as 10^5/female/breeding season in this group, but egg diameter is often less than 0.1 mm. Hence with snails usually as large or larger than *Lymnaea stagnalis,* the IEI is 0.02. Grahame (1973) has calculated an energy budget for the iteroparous *Littorina littorea.* Here the DEI (based on TA) was 10.2, which is less than the figures quoted for fresh-water species in Table 3.

There is good evidence, therefore, for the hypothesis that a negative correlation exists between reproductive effort and adult life-span, and this is almost certainly explicable by a causal link between these two variables. However, there is at least one important exception to the rule. *Melampus bidentatus,* the primitive salt-marsh pulmonate, produces about 33,000 eggs per female per breeding season (Russell-Hunter et al., 1972). It has an IEI of 0.89 (calculated from the data of Apley et al., 1967, using egg and snail weight rather than volume) and a DEI (with NRA as denominator) of 80% (Apley et al., 1967). This key species, though iteroparous, puts considerably more effort into reproduction than many semelparous fresh-

TABLE 2. Indirect indices of reproductive effort (IEI) for several species of gastropod.

SEMELPAROUS SPECIES	
Lymnaea peregra	1.23-4.88 (median = 3.05)
Physa fontinalis	2.17
P. gyrina	2.01
Planorbis contortus	1.18
Ancylus fluviatilis	1.42-2.36 (median = 1.89)
AVERAGE*	2.06
SPECIES WITH ITEROPAROUS POPULATIONS	
Lymnaea stagnalis	0.34-0.51 (median = 0.43)
L. palustris	0.03-0.35 (median = 0.19)
Bithynia tentaculata	0.08-0.33 (median = 0.21)
AVERAGE*	0.28

*based on median values.

TABLE 3. Proportion of input energy (taken over the reproductive period), measured either as total absorbed energy (TA) or as the nonrespired portion of TA (NRA), diverted into gamete production (REP).

SEMELPAROUS SPECIES	REP/TA	REP/NRA	Source
Planorbis contortus	22	79	Calow, 1972a
Ancylus fluviatilis	20	51	Calow, 1972a
Ferrissia rivularis	15-20*	45	Burky, 1971
SPECIES WITH SEMELPAROUS (S) AND ITEROPAROUS (I) POPULATIONS			
Lymnaea palustris	S 20	67	Hunter, 1975
	I 2.3	27	
Bithynia tentaculata	S 28*	55	Mattice, 1972
	I 5	25	Calow, unpubl.
Helisoma trivolvis	S 35*	69	Eversole, 1974
	I 16*	33+	
AVERAGES	S 23.8	61.0	
	I 7.8	28.3	

*Calculated assuming that respiration is approximately equal to NRA.
+From a caged population under conditions roughly equivalent to those that promote iteroparity.

water species. Hence, the relationship between effort and life-span cannot be as simple and as straightforward as was initially anticipated.

An alternative way that the life-span might be indirectly shortened by the selection of another trait is if, under adverse conditions, semelparous species are those which risk effort in reproducing despite a possible adverse effect on the parent, whereas iteroparous species show restraint (Calow, 1973a). Selection here would be for reproductive recklessness or restraint on the part of the parent, and the length of the life-span would follow as an indirect consequence of the prime effect. This seems to be true in fresh-water triclads (Calow & Woollhead, 1977), and several lines of evidence support a similar hypothesis for the fresh-water gastropods. Firstly, because risk is likely to vary from place to place and time to time, it is probable that there will be spatial and temporal variation in the life-cycle strategies of reckless reproducers; this is indeed the case for fresh-water gastropods (Table 1). Secondly, many semelparous gastropods can be kept alive and in a reproductive condition for periods exceeding their 'natural' life-span by maintaining them in good, low-risk conditions in the laboratory (Comfort, 1957; Calow, 1973a).

Thirdly, and finally, it is possible under laboratory conditions to obtain some indication of differential recklessness and restraint by measuring reproductive activities under stress. Table 4, for example, illustrates a graded response to starvation in the reproductive output of several gastropods with different life-cycle patterns. In *Bythinia tentaculata* and *Lymnaea stagnalis* (with iteroparous populations) egg output stops quickly during starvation, whereas *L. peregra* (semelparous) shows a more sluggish response, maintaining capsule production recklessly, for some time after starvation begins. In the iteroparous *Littorina littorea* gonadal activity ceases very promptly after the onset of starvation (Le Breton, 1971). There are associated differences in the response of the gonad itself. This organ disappears rapidly during starvation of *L. littorea*. In *B. tentaculata* the gonad reduces rapidly in size during starvation, but gametogenesis still takes place for some time (Neuhaus, 1949). There was no histological data for *Lymnaea peregra,* but from evidence in Table 4 it seems that gonadal size and activity must be maintained for a considerable time during starvation. It has also been shown that under a reduced food supply (rather than complete starvation) reproductive activity may be constrained in *Helisoma trivolvis,* a species with iteroparous populations (Eversole, 1974), but not in *Planorbis contortus,* a semelparous species (Calow, 1973a). What is urgently needed is more data on the relative partitioning of energy between growth, maintenance, and reproduction in semelparous and iteroparous species (Eversole, 1974). Data on *Melampus bidentatus* would also be interesting, since this is the species that did not seem to fit into the hypothesis that the life-spans of semelparous species were shortened by high reproductive effort per se.

It seems likely from the above that the evolution of semelparity in fresh waters has occurred indirectly from the combined selection for increased reproductive effort and reproductive recklessness. Clearly, these strategies can only be favored when progeny have an equal or better chance of

TABLE 4. Average egg output per week in fully fed and completely starved snails in the laboratory.

		Weeks after initiation of starvation in experimental group						
		0	1	2	3	4	5	Source
Bithynia tentaculata	Fed	*	*	*	*	*	*	Calow, unpubl.
	Starved	*	*					
Lymnaea stagnalis	Fed	20.6	32.6	34.9	22.3	38.3	28.2	Joosse et al., 1968
	Starved	22.4	13.4	0.1	0	0	0	
L. peregra	Fed+	21	30	40	42	40	20	Calow, unpubl.
	Starved+	21	30	10	1	0.1	0	

+data approximate.
*signifies occurrence of capsule production—no quantitative data.

surviving than parents; that is, when high effort and recklessness bring real gains in transmission and multiplication of genes associated with these characters. I suggest that, paradoxically, this shift is a response to the harsh fresh-water conditions. The whole emphasis in the invasion of fresh water has been on the confinement of the sensitive, developmental stages within a protecting, egg-capsule envelope. Hence, larval mortality is likely to be much less here than in species that have a small, poorly developed, planktonic stage, and the adult phase will be "needed" less as an insurance policy. Under these circumstances, it is probably advantageous, in terms of fitness, to invest more energy in progeny at the expense of putting the parent at risk. Given that the parent has a reduced chance of postreproductive survival, it is also possible that "aging genes" (in the sense of Medawar, 1952, and Williams, 1957) may express themselves at this time and thereby fix, in genetic terms, what was originally a flexible, probabilistic response.

Interestingly, species that have retained (or re-evolved) the iteroparous strategy are those which tend to inhabit small closed bodies of fresh water (particularly the prosobranchs). Under such conditions it is likely that there is more intense competition for limited resources (space and food), more density-dependent control, and hence, a greater premium on the survival of a large "experienced" adult (Pianka, 1970; Calow, 1977). Moving from the poles to the tropics this "K selection" (Pianka, 1970) is supposed to become more intense and so iteroparity should become more frequent. However, many tropical fresh-water systems dry up seasonally and presumably demand as much, if not more, opportunism as in the more typical "r situations" of selection. Good studies on the life cycles of tropical gastropods are few, but they do point to opportunism and reproductive recklessness (Olivier & Barbosa, 1955a, 1955b; Pringle & Raybould, 1965; Jobin & Michelson, 1967; Sturrock, 1973).

EGG SIZE AND NUMBERS

In general, fresh-water gastropods invest more energy in reproduction than marine species but they partition it into fewer eggs. Even within the fresh-water species, though, there is some variation in egg size and egg numbers (Table 1). These differences might ultimately be explained by differences in the ecology of each species, and more work on this problem would undoubtedly yield rewards. However, phylogenetic limitations may also be involved in this relationship. For example, those prosobranchs which are not viviparous produce small eggs about 10^3 μm in diameter and less. This could represent an inability of the prosobranch organization to produce larger spawn and may explain why several fresh-water species have adopted a viviparous mode of reproduction (see next section). It is also feasible that with complex adaptations, like life-cycle strategies, there may be more than one way of responding to a particular environmental challenge, and I illustrate that principle in this section.

A most intense environmental challenge to a fresh-water fauna comes from water movements in fast-flowing streams and wave-swept lake shores. In the United Kingdom, two pulmonate gastropods are particularly successful in these habitats; the river limpet, *Ancylus fluviatilis,* and the more globose *Lymnaea peregra.* A smaller ramshorn snail, *Planorbis contortus,* is occasionally found at high densities in these habitats (Calow, 1973b, 1974). All three species are semelparous but show significant differences in the reproductive aspects of their life cycles. *A. fluviatilis* lays fewer eggs than *L. peregra* (Table 1), but both spend about 40 Joules in total egg production (Calow, 1972a). Hence, the number of Joules/egg is about 30-fold greater in *A. fluviatilis* than in *L. peregra. P. contortus* adopts a somewhat intermediate strategy, laying a smaller number of larger eggs than *L. peregra* but a larger number of smaller eggs than *A. fluviatilis* (Table 5).

The reproductive products of the three species also differ in morphology. The capsules of *Lymnaea peregra* are globose and covered with a tertiary membrane (terminology after Bondesen, 1950) produced in the reproductive tract. Alternatively, both *Ancylus fluviatilis* and *Planorbis contortus* produce flattened capsules covered over with an extra secretion, a definite quaternary membrane of foot origin in the former species and a loose mucoid secretion in the latter (Bondesen, 1950). These differences seem to have a marked effect on the survival of egg

capsules. For example, my own observations on marked capsules in both a small tarn and a large British lake suggest that of the capsules laid, approximately 90% survive to hatch in *A. fluviatilis,* 70 to 80% in *P. contortus,* and 40 to 50% in *L. peregra*. The flattened capsules appeared to be much less vulnerable than the globose, and the quaternary membrane in *A. fluviatilis* may have endowed it with survival superiority.

The larger eggs and richer reserves typical of *Ancylus fluviatilis* enable more complete, intracapsular development in this species. At 10°C, *A. fluviatilis* takes 26 days to hatch; on emergence it is roughly 6% the shell-free, dry weight of the adult (Calow, 1972a). *Lymnaea peregra* and *Planorbis contortus* may hatch out in under half that time (Calow, 1972a, and unpublished observation) but are only 1 to 2% of their final adult size by shell-free dry weight. The hatchlings of *A. fluviatilis* are likely to be better equipped than those of the other species to cope with the harsh environment; this is reflected in significant differences in survivorship curves between species (Fig. 3). The data for *P. contortus* and *A. fluviatilis* derive from a different habitat than those for *L. peregra*, so this complicates the comparison. However, assuming the habitat effect is negligible (both are in fact very similar in general ecology), the possible influence of size of hatchlings on survivorship can clearly be seen. The survivorship curve for *A. fluviatilis* conforms to a Deevey type II curve (Deevey, 1947) in which the rate of mortality and the mean life expectancy are constant throughout life. *L. peregra* and *P. contortus* curves conform more closely to a Deevey type III curve, in which mortality mainly affects the young, mean life expectancy increases with age, and median life expectancy is smaller than the mean.

It is possible to combine all the above data by using the fitness-set analysis of Smith & Fretwell (1974). This is a graphic technique in which the abscissa plots both size and number of individual offspring (the relationship of these two variables is determined by assuming a fixed finite amount of energy available for reproduction) and the ordinate plots the fitness of individual offspring. Straight lines through the origin of this graph are fitness functions, or lines of equal fitness for the parent (see also Levins, 1968). Greater slopes of the fitness function correspond to higher parental fitness.

Using the data in Fig. 3 in combination with estimates of capsule survival it is possible to calculate the approximate fitness of the individual offspring as the combined probability of their survival to hatching and then to maturity. Joules/egg and total numbers of eggs produced/parent may be obtained from Table 5. Points representing *Ancylus fluviatilis, Lymnaea*

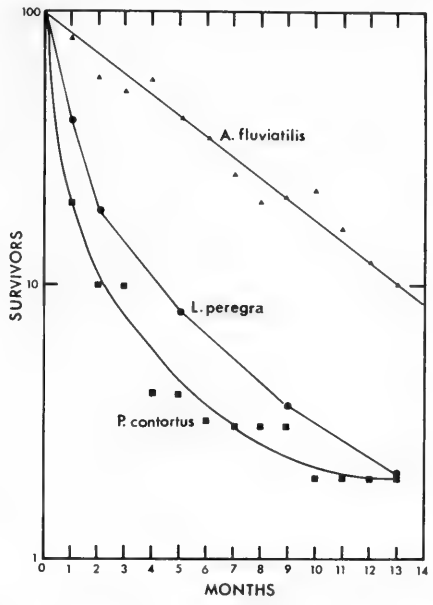

FIG. 3. Logarithmic survivorship curves. Those for *Ancylus fluviatilis* and *Planorbis contortus* are for populations from the littoral region of Malham Tarn (Calow, 1973b) and are based on sequential samples using the technique of Calow (1972b). The curve for *L. peregra* is for a population from the littoral region of Loch Lomond, Scotland, and is based on a sampling technique in which stones of different, predetermined size categories were collected from random sampling points; knowing the relative abundance of stones over the shore (determined from a random sample of 1,000), snail densities per stone of given size were converted, according to the method of weighted averages, to densities per "average stone." In all cases densities were corrected to an initial level of 100.

TABLE 5. Some quantitative data on reproductive output.

	No. eggs/ lifetime/ snail	Joules in eggs/ lifetime/ snail	J/egg
Ancylus fluviatilis	30	*ca.* 40	1.33
Lymnaea peregra	1000	*ca.* 40	0.04
Planorbis contortus	50	*ca.* 40	0.80

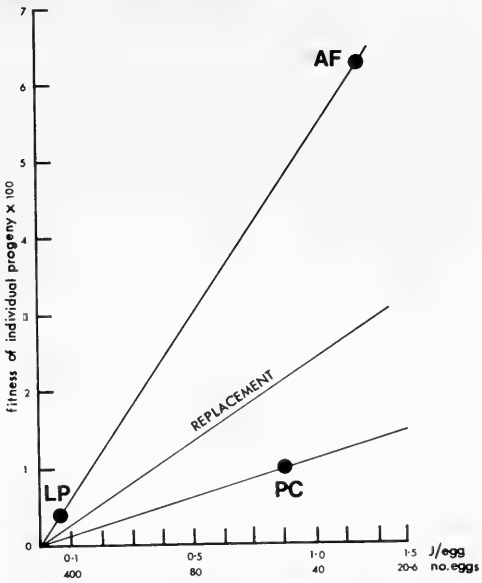

FIG. 4. Fitness-set analysis. See text for further explanation.

peregra and *Planorbis contortus* can be located roughly in the fitness-set space (Fig. 4). Since these points are calculated on the basis of information from specific populations at a specific time, they should not be taken as species-specific constants but rather as indices of the relative ability of the different species to cope with the littoral challenge. To facilitate comparison I have drawn in the fitness set that corresponds to perfect, annual replacement. The points for *A. fluviatilis* and *L. peregra* lie above this set, indicating their ability to cope with the littoral challenge. Furthermore, both points lie close to but at opposite ends of the same set. Hence, the alternative strategies of "egg size" and "egg numbers" may balance to produce equifit strategies under similar environmental conditions.

In comparison with the other results, the point for *Planorbis contortus* lies on a fitness set below that representing replacement. This shows that *P. contortus* is less well adapted for the littoral challenge than the other species. Hence, without continuous replacement it is likely that littoral populations of this species might ultimately become extinct. There is evidence in one lake, however, that littoral populations may be sustained by a process of continuous immigration of *P. contortus* into exposed littoral sites from sheltered refuges (Calow, 1972a, 1974).

METHODS OF REPRODUCTION

Hermaphroditism is the dominant method of reproduction in the Mollusca, and the fresh-water gastropods, particularly the pulmonates, are no exception to this rule. For most phyla it is usually suggested that hermaphroditism is secondarily derived from a gonochoristic condition, but there is strong evidence that in the Mollusca it is the primary state (Morton, 1964). There are two advantages to hermaphroditism. First, the fact that all individuals are both male and female doubles the chance of any one individual meeting a mate. This is important either when population density is low or when individuals in the population are sluggish (Altenburg, 1934; Tomlinson, 1966). Secondly, in principle a population of hermaphrodites can produce twice as many progeny as a population of gonochorists, because there are twice as many females (Maynard Smith, 1971).

In semelparous snail populations where individuals are slow-moving and where densities can be very low during the breeding season, the advantages of hermaphroditism are obvious. It is interesting from this point of view that gonochorism in the Mollusca is only widespread through the more mobile Cephalopoda. There is a price to be paid, of course, for hermaphroditism: the cost of producing and maintaining two sets of reproductive apparatus in one animal is greater than the cost of building and maintaining single sets of apparatus in separate animals (Heath, 1977). This extra cost will become less tolerable as population density and the chances of encountering a mate increase, but it can be offset by any morphological adaptation that allows male and female organs to share common parts (ducts, etc.). In the fresh-water pulmonates, sharing of this sort can be extensive (Fig. 5); here even the gonad (ovotestis) is a shared organ. The latter is usually made up of several to many radiating acini, the lumina of which join at the origin of the hermaphrodite duct. In some species all acini can, at different times, contain stages of both oogenesis and spermatogenesis (Russell-Hunter & McMahon, 1976). The disadvantage of this "sharing strategy"

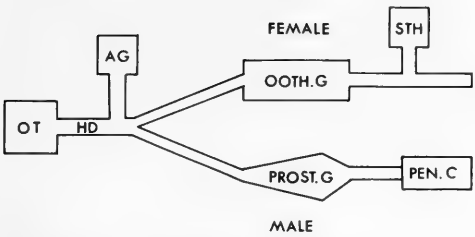

FIG. 5. Hermaphrodite reproductive system of fresh-water pulmonates: OT = ovotestis; HD = hermaphrodite duct; AG = albumen gland; OOTH. G = oothecal gland; STH = spermatheca; PROST. G = prostate gland; PEN. C = penial complex.

comes from the likelihood of self-fertilization. In many groups this leads to impaired vigor in the progeny (Heath, 1977). However, even after twenty years enforced self-fertilization there was no apparent reduction in the viability of *Lymnaea columella* (Colton & Pennypacker, 1934). Anyway, most species of pulmonate are true simultaneous hermaphrodites and only the occasional species is protandric (Russell-Hunter & McMahon, 1976).

In the prosobranchs the methods of reproduction are more diverse. Several species are gonochoristic, and at least two species are parthenogenetic. Of the latter, *Potamopyrgus jenkinsi* is interesting since in Europe over the course of this century it has rapidly invaded fresh-water from brackish habitats (Bondesen & Kaiser, 1949; Hubendick, 1950; Russell-Hunter & Warwick, 1957). This invasion has probably depended crucially on its parthenogenetic habit.

As already noted, the small size of their spawn may have "forced" prosobranchs into viviparity. On the basis of arguments in an earlier section it is surprising that this strategy, which offers protection to young, should invariably be associated with iteroparity (Table 1). There are two possible explanations, which are not exclusive. Viviparity may mitigate recklessness since its success will depend upon the continuing survival of the parent through the brooding period. Also, physical limits to the brood pouch may limit fecundity, thereby preventing any adverse, proximate influence on the parent. It may also prevent the advantage ever shifting completely to progeny production at the expense of the parents.

CONCLUSIONS AND FURTHER STUDIES

In this paper I have advanced a broad theory of gastropod life-cycle strategies which is consistent with extensive but widely scattered malacological data and with the theoretical framework summarized in Stearns (1976). Further progress will require the collection of more quantitative data at the level of both population and individual and from both field and laboratory studies.

At the population level there is already an extensive body of data on life-cycle patterns (Table 1 and Fig. 1). What is urgently needed is more information on the quantitative aspects of the dynamics of molluscan populations. A fundamental need is information on population density at different times of the year, so that life tables and survivorship curves of the sort summarized in Fig. 3 can be constructed. Only when these kinds of data are available will it be possible to measure age-specific survivorship and the relative mortality schedules of iteroparous and semelparous species and to test the predictions that age-specific mortality should be more intense in the juveniles of iteroparous as opposed to semelparous populations. Furthermore, only when we know more about the population dynamics of fresh-water snails will it be possible to test out the consequences of "r" and "K" selection on molluscan life cycles (Stearns, 1976) and to correlate particular life-cycle strategies with the demographic properties of populations occupying particular ecological conditions. Of specific interest, for example, would be comparisons between tropical and temperate populations, lotic and lentic populations (Calow, unpublished data), and littoral and benthic populations. The major difficulty, of course, in these life-cycle studies has been, and will be, the development of methods for obtaining accurate density measures in complex habitats like weed beds and particularly stony shores. Techniques are slowly coming available (Calow, 1972b), and continuing efforts in this area are bound to be fruitful.

At the level of the individual, more quantitative information is required on molluscan metabolism and energetics. In particular, energy budgets should be constructed at different ration levels, for only

in this way will it be possible to obtain a quantitative measure of degrees of recklessness and restraint. The 2 major predictions from this paper are that compared with iteroparous snails, individuals from semelparous populations should (1) invest a greater proportion of their energy flow in reproduction and (2) recklessly maintain reproductive output despite disturbances in food supply. Histological data on gonadal morphology under various trophic conditions would also be useful.

Finally, more attention should be paid to the correlation between the nature of the spawning products, both in size and numbers, and the nature of the ecological challenge experienced by a population, perhaps using the fitness-set analysis developed here. This will depend on the availability of quantitative information on population dynamics.

ACKNOWLEDGEMENTS

I thank Professor W. D. Russell-Hunter for allowing me to see part of his review while it was still in press.

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MALACOLOGIA, 1978, 17(2): 365-391

PROTANDRY AND THE EVOLUTION OF ENVIRONMENTALLY-MEDIATED SEX CHANGE: A STUDY OF THE MOLLUSCA

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ABSTRACT

Many molluscs in several taxonomic groups (Mesogastropoda, Bivalvia) and several ecological settings (parasites, filter-feeders, wood-borers) are protandrous hermaphrodites, with varying degrees of environmental determination of sex. I delineate the selective forces that have led to the establishment of protandry and labile sex determination in some, but not all, molluscs. I first report on a series of experiments on the sexual behavior and sex determination of four species in the mesogastropod genus *Crepidula,* and correlate differences I find with differences in the reproductive patterns of these species. I review the literature on protandry and labile sex determination in molluscs and some other invertebrates in order to test the generality of the conclusions generated from the study of *Crepidula.*

Species of *Crepidula* with planktonic larval development and that are rarely substrate-limited exhibit labile sex determination and have socially influenced sex ratios. Their gregarious behavior and female-induced delay of sex change appear to be mediated by pheromones, possibly through a common mechanism. Species of *Crepidula* lacking planktonic larvae also lack gregarious behavior, and sex change and sex ratio are independent of influence by other members of the species. Therefore, species patterns in control of sex change appear to be correlated with the mode of larval development and dispersal, and with substrate constraints.

Protandry is advantageous—that is, it increases individual fitness—when one sex increases in fertility with age and size faster than the other. This is true in many molluscs where female fecundity is related to large body size, but male fecundity is related to mobility and therefore often to small size. Labile sex change that is influenced by the environment optimizes the size and age at sex change in protandrous species, thus maximizing individual fitness. This is important in species for which the optimal age at sex change varies from place to place and from generation to generation. Sedentary species with planktonic larvae are in this category. Gregariousness, in addition to protandry and labile sex change, ensures that each individual will reproduce. An isolated individual becomes female immediately upon metamorphosis and attracts spat, which become male, providing a mate. The advantage to colonizing populations of sedentary species is that every encounter between individuals is potentially productive of offspring. Molluscs that are largely sedentary as adults but possess dispersal stages and have substrates patchy in time and space have evolved protandry: the Calyptraeidae, oysters, wood-boring bivalves, and the parasitic mesogastropods are outstanding examples.

INTRODUCTION

Malacologists have long recognized the large number of molluscan species that possess some form of hermaphroditism. Several of these hermaphroditic molluscs are sedentary and reproduce via copulation. The pattern of sexuality in a copulating, sedentary organism is interesting to students of evolution because it affects the number of encounters resulting in offspring, hence the chances that an individual will reproduce successfully. The list of molluscs that are proven protandrous hermaphrodites and those that show circumstantial evidence of being protandrous is actively growing, as more and more species are studied biologically. Likewise, environmentally-mediated sex determination (often called labile sex determination) has been demonstrated in a number of molluscs, as well as other invertebrates and numerous

plants (Bacci, 1965; Charnov & Bull, 1977). Many of those with sexual lability and protandry are sedentary as adults.

Environmentally-mediated sex determination in the molluscan family Calyptraeidae is not a new discovery. Gould (1917a, 1917b), Ishiki (1936), and Coe (1944) reported that members of the genus *Crepidula* within the Calyptraeidae are influenced physiologically and behaviorally by others of the same species. In *C. fornicata* and *C. plana,* the protandrous males are not only attracted to females, but attain the male phase earlier and remain as males longer when in contact with females (Coe, 1953).

Protandry and labile sex determination occur in groups of molluscs that are widely separated phylogenetically. These characteristics are often but not always found in the same organism. Thus the interesting question is not so much how these modes of sexuality arose, but rather, why. What are the selective forces that lead to protandry and labile sex determination in certain molluscs (and other organisms)? I attempted to answer this question by using, as a model, species of *Crepidula,* which are all protandrous, low in mobility, and which copulate, but show varying degrees of sexual lability. I compared reproductive characteristics, sexual characteristics, group behavior, and general ecological data. I am able to generalize that substrate requirements plus the type of larval development and dispersal, when superimposed on limited adult mobility, form predictable associations of reproductive and sexual traits in molluscs and perhaps other invertebrates as well.

The type of larval development and dispersal varies within *Crepidula.* Some species disperse as young adults after metamorphosis within a brooded egg sac, while others release planktonic larvae and are nearly sedentary as adults. The starting point for the research described in this report was my hypothesis that, complementing these different reproductive and dispersal mechanisms, there might have evolved differences in mechanisms of sex change and sexual behavior involved in getting the sexes together.

Casual observations of four species of *Crepidula* in the laboratory did reveal striking species-level differences in sexual behavior, including gregariousness, degree of mobility of young males, permanence of mating pairs, and timing of sex change when isolated versus when maintained under crowded conditions. I quantified these differences through a series of experiments in the laboratory and in the field, and correlated them with other aspects of the life histories of the species.

Finally, I reviewed the literature on protandrous Mollusca and other invertebrates so that generalizations could be made on the evolutionary significance of protandry and sexual lability. I have concluded this report with a series of unanswered questions which I hope will stimulate further research and testing of the hypotheses developed in this paper.

MATERIALS AND METHODS

Choice of study organisms and localities

Four species of *Crepidula* that vary in reproductive pattern and population structure were examined. The first two species, *C. fornicata* (Linn.) and *C. onyx* Sow., have planktotrophic larval development preceded by a period of brooded development. They naturally form large clusters or stacks of individuals in muddy bays where hard substrate would otherwise be difficult to find. A third species, *C. plana* Say, is sympatric with *C. fornicata* and has similar larval development, but lives inside empty shells or under stones and never forms stacks. However, several males may live attached to one female. The females of these three species are sedentary, and may remain for months in the same place, although they are capable of limited movement when placed in an unfavorable (e.g., oxygen-poor) environment. Young juveniles and males have limited mobility on hard substrates.

Also sympatric with *C. fornicata* is the fourth species examined, *C. convexa* Say. It broods its young through metamorphosis and forms temporary male-female pairs, but does not form clusters. It is the smallest and most motile of the four species, but during brooding, female movement is virtually nil.

The study sites are described in Appendix A.

Experimental methods

Several laboratory and field experiments were conducted to answer specific ques-

tions generated by the general hypothesis that sex determination and sexual behavior should differ in species with different modes of larval development and dispersal. These experiments are described here, together with the rationale for performing them.

Gregariousness and Sexual Behavior

To quantify possible species differences in gregariousness and sexual behavior, I ran a series of laboratory experiments. Members of the three Atlantic species of *Crepidula* were obtained from Woods Hole, Massachusetts. *C. onyx* was collected in Balboa, California, and transported live to Woods Hole. Individuals of each species were sorted into four sexual categories: juvenile (J), no secondary sex characters present; male (M), presence of a fully developed penis; intermediate (I), presence of a degenerating penis, and female (F), absence of a penis or presence of a stump only, and presence of an oviduct. The reliability of each of these categories was confirmed by examination of the gonads and other internal reproductive organs of animals that were sacrificed. Snails in each sex category were placed in contact with those in every other category, in pairwise tests. Pairs were isolated in finger bowls with running sea water and observed for signs of gregariousness and sexual interaction, such as physical contact, over several intervals (24, 48 hours; 7 days; 1 month). I report the results after 48 hours. Longer intervals included sex changes; the shortest interval was insufficient for stabilized interaction. The sample size was 20 individuals of each category, hence 40 per test.

Field experiments were designed to determine if the patterns of gregarious behavior seen in the laboratory were duplicated under more natural conditions. Six clay pots of equal size were suspended from ropes into 1.5 m of water off docks at the Woods Hole, Massachusetts, Yacht Club. These artificial substrates rested about 20 cm above the water-mud interface. Six snails, consisting of a male-female pair of each of the three local *Crepidula* species, were allowed to attach to each of three of the pots while still in the laboratory. This was to determine if young are attracted to adults of the same species, or merely to any suitable substrate. Newly metamorphosed juveniles settling on the substrates were counted and removed weekly over a four-month period, June through October, 1973, and the ratio of young settling on pots with versus without adults of the same species was calculated.

One further field experiment provided data on attractiveness of adults to juveniles. At several beaches in Woods Hole, *C. fornicata* and *C. convexa* co-occur on discontinuous substrates (small rocks, cobbles, broken glass, and shells amidst sandy mud) in shallow water. Within two of these areas on two occasions in the summer of 1973, the numbers of newly metamorphosed juveniles (shell length less than 3 mm) of each species per substrate unit were counted. A total of 50 substrate units were examined for *C. fornicata,* and 255 for *C. convexa.* The surface area of each substrate unit was calculated, thereby deriving the density of young snails on each substrate. Finally, the densities of males, females, and juveniles greater than 3 mm long were calculated for the same substrates, in order to determine the relative attractive ability of these sex categories for metamorphosing young.

Sex change and population density

Laboratory experiments were conducted to test the null hypothesis that species with different modes of reproduction and dispersal have the same degree of environmental determination of sex. The experiments also tested whether environmental determination of sex can indirectly affect the sex ratio of a population by altering the age at which an individual changes sex, relative to population density.

I maintained 14 bowls each of *C. fornicata* and *C. convexa* of various initial densities and sex ratios, from June, 1972, to June, 1973, under natural temperatures in independent flowing sea water systems. The food supply to all bowls was the same. No recruitment to these artificial populations was permitted. The final densities and sex ratios were recorded.

In the field, I recorded the total adult density and the percentage of males for four populations of *C. fornicata* and five of *C. convexa* in May and/or August, 1972 and/or 1973. Discontinuous substrates such as stones were picked at random and all adults on them were sexed until N = 50 was reached (N = 200 for one very dense

population from Rhode Island). The data were analysed for correlations between population density and sex ratio, and the results were compared with those obtained via the laboratory test of density and sex ratio. Two of the same field populations of *C fornicata* and *C. convexa* were sampled monthly during winter months and twice a month during summer (April through October) to determine seasonal changes in population density, sex ratio, copulation, and egg-laying activity.

Size and age at sex change

Preliminary observations suggested that the size and possibly the age at sex change vary among populations of *C. fornicata.* To test the null hypothesis that size and age at sex change are constant within species, I amassed data on the size, age, and sex of individuals from five populations of *C. convexa,* four of *C. fornicata,* and one each of *C. plana* and *C. onyx.* If the hypothesis was refuted, I planned to look for relationships between total adult density or female density and the timing of sex change. I also wished to identify the effect of direct contact with females on the timing of sex change. Such relationships imply population interaction in the regulation of major events in the lives of individuals, and if present, require an interpretation in the light of current evolutionary theory.

Over a period of three years, all individuals of the four species of *Crepidula* in the study areas found to be intermediate between male and female were measured for shell length. The dry weights of the animals were estimated from a standard curve of length versus dry weight (body and shell) which was constructed previously (Hoagland, 1975). Time of year affected the number of individuals that were changing sex, but did not affect either the age or size at sex change, so all intermediates from each population were pooled. This was necessary because the sample size of intermediates at any one sampling time was small. A note was made for each intermediate as to its position—whether it was sitting on a female. Also, the age of each intermediate was estimated by counting winter growth lines (Sheldon, 1967), which allow age to be estimated within a range of about six months.

RESULTS

Gregariousness and sexual behavior

I report results of the laboratory tests of gregarious behavior in the four species of *Crepidula* in Table 1. Some snails responded to the test situation by crawling onto the shells of other snails; the former are called "respondents." Those that did nothing, but allowed other individuals to climb onto their shells, are called "releasers." The bottom member of a pair rarely moved from its initial position and is assumed to have attracted the individual which crawled upon it.

Interactions are species-specific. *C. fornicata* and *C. onyx* respond alike and have the highest degree of gregariousness. *C. convexa* is least gregarious. Responses are always by smaller, less mature individuals to sexually advanced and/or larger individuals. Females move toward tank aerators, but are otherwise sedentary.

Crepidula fornicata, C. onyx, and to a lesser extent, *C. plana* males and juveniles cluster among themselves and are strongly attracted to larger individuals, especially females. Long-term observations revealed that pairings between male and female are often stable for as long as one year. However, *C. convexa* juveniles are not gregarious. Males pair only with females, and a pairing usually lasts less than three weeks.

The field experiments using artificial substrates to determine if newly metamorphosed young are attracted to adults of the same species gave the following results. The ratio of young settling on the three pots with adults versus the three without, was: *C. fornicata,* 722:232, and *C. plana,* 249:95. Therefore, for species with planktonic larvae, the presence of adults significantly increases the number of young that metamorphose on the same substrate. *Crepidula convexa,* which has no planktonic larval stage, did not appear on the pots. It does occasionally appear on such suspended experimental materials, probably because it is capable of floating on algae or in the water surface film, as I have observed at field sites in Barnegat Bay, New Jersey.

Calculations of the density of newly metamorphosed *C. fornicata* snails with respect to the presence of older individuals on natural substrates are presented in Table 2. The density of metamorphosing young

TABLE 1. Experiments on gregariousness. A summary of data after 48 hours.

"Releaser"	Respondent	Response: % pairings*				
		fornicata	*onyx*	*plana*	*convexa*	*fornicata-onyx*#
F	J	100	100	30	10	0
	M	100	100	100	65++	0
	I	0	0	0	0	0
	F	0	0	0	0	0
I	J	60	50	25	0	0
	M	75	70	80	20++	0
	I	0	0	0	0	0
	F	5	0	0	0	0
M	J	70	60	25	10++	0
	M	10++	15++	10++	5++	0
	I	0	0	0	0	0
	F	0	0	0	0	0
J	J	65+++	45	30+++	0	0
	M	0	0	0	0	0
	I	0	0	0	0	0
	F	0	0	0	0	0

*Percentage of the twenty individuals of a given sex which formed pairs by crawling on the back of one of the twenty members of the sex being tested as a releaser.
#*C. onyx* as releaser and *C. fornicata* as respondent.
++Pairings are often transient; switching of mates was observed within 48 hours.
+++Aggregations of contiguous indiciduals occur as well as pairings, and are included as positive responses.
F = female
M = male
I = intermediate
J = juvenile

TABLE 2. Density of newly metamorphosed *Crepidula fornicata* young per cm^2 of substrate with respect to the presence or absence of older individuals.

	No other *Crepidula*	Females	Males	Mixed adults & juveniles	Juveniles
n*	10	10	10	10	10
$\bar{x}$	.18	.75	.37	.66	.37
S^2	.03	.20	.04	.25	.06

ANOVA TABLE, LOG-TRANSFORMED DATA

Source of variation	df	ss	ms	F
Among groups	4	.170	.042	5.68
Within groups	45	.336	.007	

F(.05) = 2.61 for 4, 40 df
2.53 for 4, 60 df

Significant individual comparisons (Ability to attract spat):
1. No *Crepidula* < mixed, male, and female
2. No *Crepidula,* juvenile and male < female

*number of cobbles and rocks examined

was not related to the density of adults, but rather, to their presence or absence, probably because the response of larvae to adults is a threshold rather than a graded response.

Because the variances were not homogeneous (in fact, were roughly proportional to the means), I used a log transformation before conducting an analysis of variance. The attraction power may be ranked: females > mixed juveniles and adults > males = juveniles > bare substrate, although the difference between females and mixed juveniles plus adults is not significant ($p > .5$). These data support the laboratory findings that *C. fornicata* juveniles are gregarious.

Table 3 presents results for *C. convexa*

TABLE 3. Density of newly hatched *C. convexa* per *Littorina* shell, with respect to the presence of older individuals on the same *Littorina.*

	Females	Males	Mixed adults & juveniles	Juveniles
n*	128	18	27	82
$\bar{x}$	.23	.78	1.04	1.22
S^2	.21	.42	.50	.53

Test for equality of variances: M = .19; 3 df; .975 < p < .99

ANOVA TABLE

Source of variation	df	ss	ms	F
Among groups	3	54.30	18.10	50.28
Within groups	250	89.57	.36	

F(.05) = 2.60 for 3, ∞ df

Significant individual comparisons (Ability to attract young):
1. Female < male, mixed, and juvenile
2. Male < juvenile

*Number of *Littorina* examined. All *Littorina* were approximately the same size.

TABLE 4. Laboratory data on sex ratio after one year, varying the density and initial sex composition.

Initial composition			*C. fornicata**			*C. convexa*#		
			Final composition			Final composition		
M	F	Percent male	M	F	Percent male	M	F	Percent male
5	5	50	4	5	44	1	6	14
5	5	50	4	4	50	0	6	0
10	0	100	4	5	44	0	6	0
10	0	100	4	4	50	0	6	0
10	5	67	6	6	50	1	11	6
10	5	67	6	7	46	2	10	20
20	20	50	16	20	44	3	29	9
20	20	50	15	20	43	2	32	6
50	0	100	28	18	61	2	38	5
50	0	100	24	20	55	0	32	0
50	25	67	32	21	60	4	48	8
50	25	67	34	28	55	6	51	11
50	50	50	43	41	51	2	78	3
50	50	50	40	36	53	4	71	5

*substrate area: 458 cm^2
#substrate area: 135 cm^2
M = male
F = female

on natural substrates. The attraction power for young less than 2 mm long is ranked: juveniles > mixed adults and juveniles > males > females. Females are significantly less attractive than each of the other three categories (in individual t tests, $p < .05$). Variation in hatching size, and the fact that newly hatched young must begin life on the same substrate as the mother, prevented the scoring of density for newly hatched young on bare substrates. All the densities are low, especially that of juveniles living with females, considering that the young are directly released by the females. The results are nearly the reverse of those for *C. fornicata.*

Furthermore, out of the 255 *Littorina* shells observed with *C. convexa* snails attached, 146 carried adults only, 82 carried juveniles only, and a scant 27 carried both adults and juveniles. Attractive power of females for young appears to be nonexistent in *C. convexa.*

Sex ratio and population density

Table 4 presents data on the sex ratio of several artificial populations of *C. fornicata*

and *C. convexa* maintained for one year in the laboratory. Regardless of initial conditions, most *C. convexa* snails eventually become females. There are differences among individuals in the tendency toward femaleness that possibly are genetic.

Crepidula fornicata approaches and maintains roughly a 50-50 sex ratio regardless of the initial ratios among the initial ratios tested. In dense populations there is a slightly higher percentage of males. A regression of sex ratio (percent male) after one year on density as the independent variable gives $r^2 = .28$; the Spearman rank correlation $r_s = .61$, $t = 2.66$, $n = 14$, and $.02 < p < .05$.

The contrast between the sex ratios in isolated populations of the two species is dramatic. The sexuality of an individual *C. convexa* is not influenced by the biotic environment, while that of a *C. fornicata* clearly is.

The sex ratios of several natural populations of *C. convexa* and *C. fornicata* are plotted against population density in Figs. 1 and 2. For *C. fornicata,* total adult density and the percentage of males are positively correlated, as was true in the laboratory tests. If one omits the newly colonizing populations (1972), which initially had high densities but few females,

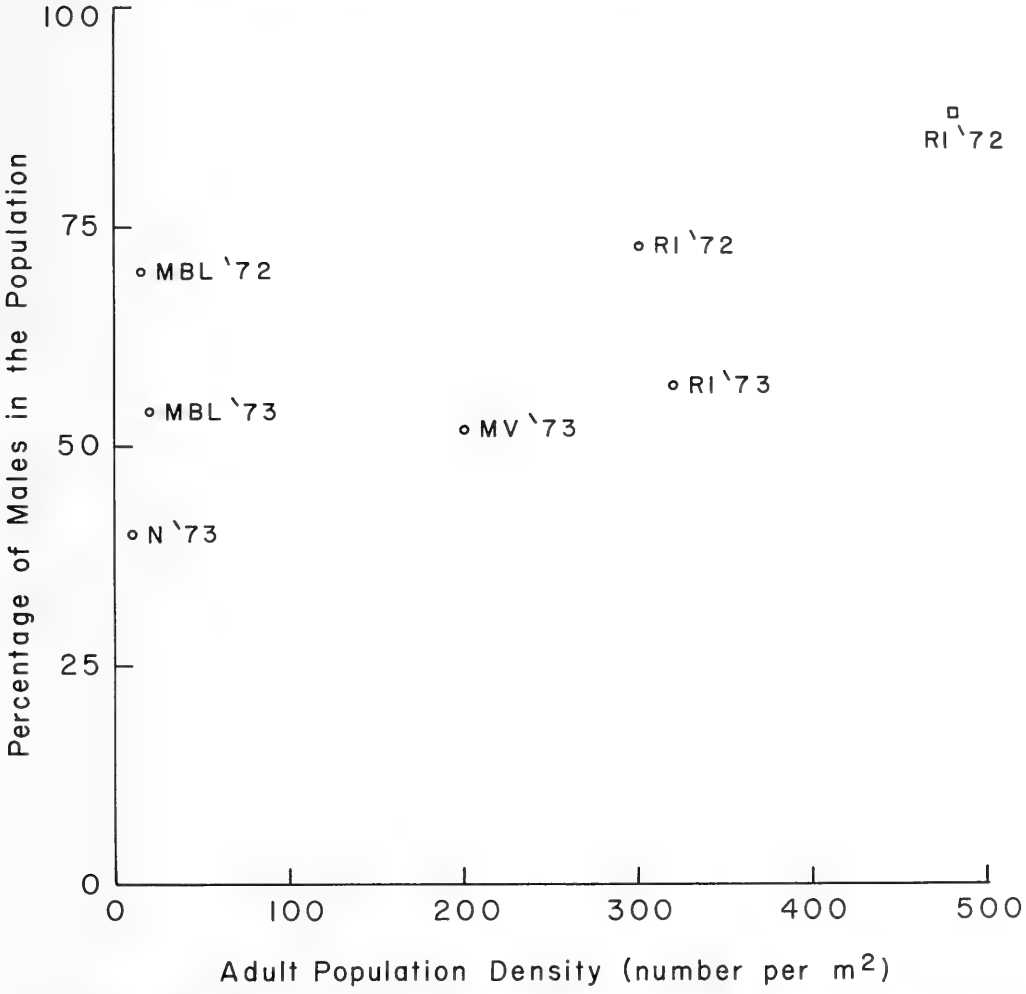

FIG. 1. The adult population density of several *Crepidula fornicata* populations versus the percentage of each population that is male. Circles are data from May collections; the square represents an August collection. Abbreviations as in Appendix A. Adult densities are accurate to ± 10 individuals per m^2. The regression of sex ratio on adult density is: Sex ratio = .0006 (adult density) + .51, $r^2 = .67$

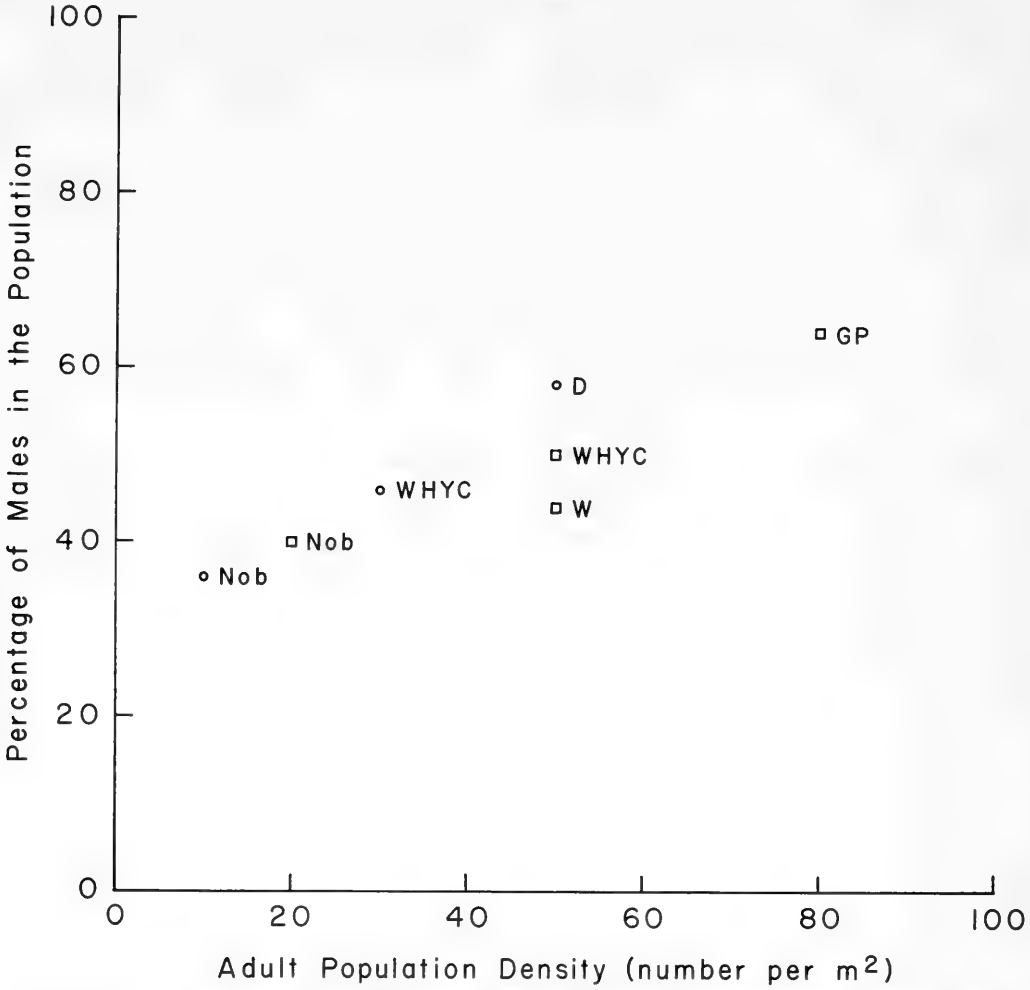

FIG. 2. The adult population density of several *Crepidula convexa* populations versus the percentage of each population that is male. Circles are data taken in May; squares are data taken in August. Abbreviations as in Appendix A. All data were taken in 1973. Adult densities are accurate to ± 5 individuals per m^2. May sex ratio = .006 (May density) + .30, r^2 = .999. August sex ratio = .004 (August density) + .30, r^2 = .933

the proportion of total variation about the mean sex ratio that is explained by the regression on density increases. The sex ratio is initially high in colonizing populations but soon drops (see data points for Rhode Island and MBL Beach, 1972 versus 1973).

The percentage of *C. convexa* males correlates positively with total adult density, unlike the laboratory situation. Comparing field with laboratory data, regular population recruitment is clearly necessary to stabilize the sex ratio in *C. convexa,* but not in *C. fornicata.*

Monthly sampling of the field populations of *C. convexa* and *C. fornicata* in 1972 and 1973 revealed that in all populations of both species, both years, there were similar seasonal changes in sex ratio. The percentage of males is highest in spring when copulation is most intense (representative data are in Figs. 3 and 4). The percentage of females is highest in midsummer when egg production and development are also at a peak (Fig. 5).

The seasonal pattern of sex ratio is accounted for by sex change, mortality, and migration of individuals (particularly juveniles). In late spring and early summer, juveniles enter the population. By the end

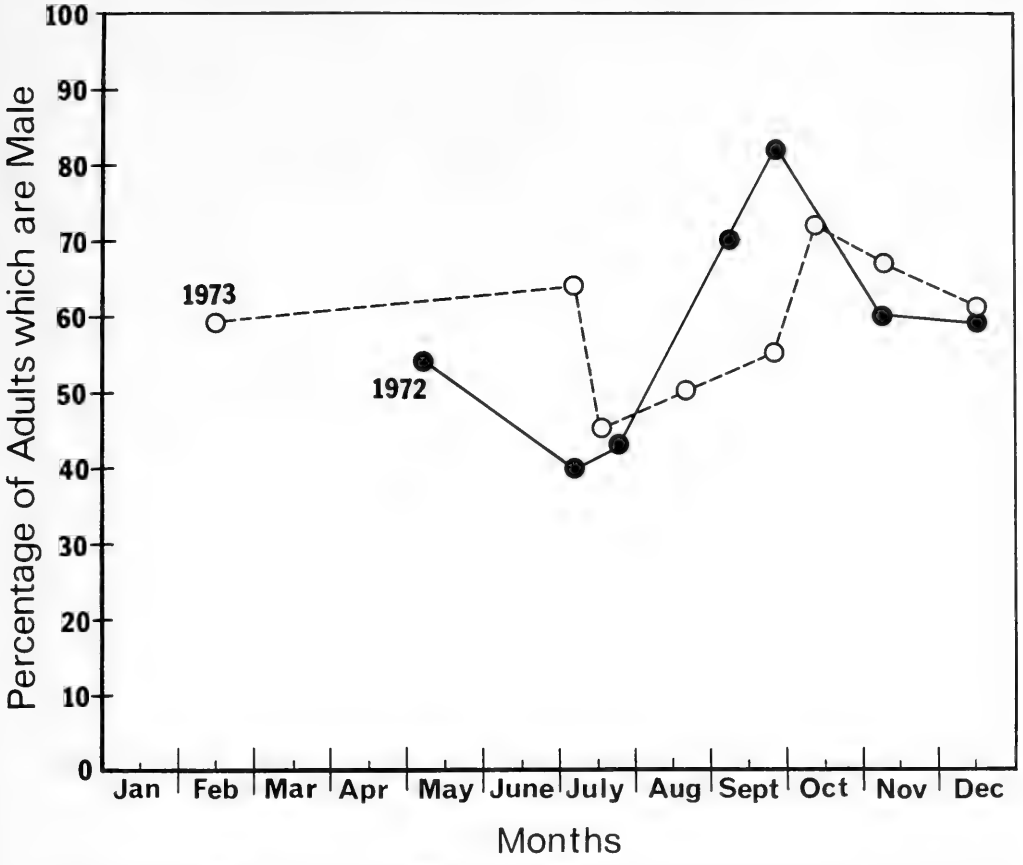

FIG. 3. The percentage of adults in one population of *Crepidula fornicata* which are male, 1972 and 1973. The population is from the Marine Biological Laboratory's Beach, Woods Hole, Massachusetts. The percentage is calculated as: $\frac{\text{(number of males) (100)}}{\text{(number males + females)}}$.

of the summer, many of these are functioning as males. In early fall and early spring, many of the males become females. Over the winter, the population is nearly static. *C. fornicata* is less clear-cut than *C. convexa,* because the proportion of the population that becomes male is highly variable from year to year, as is the time of the year at which sex change occurs.

Size and age at sex change

Tables 5 and 6 and Appendix B summarize the data on size and age at sex change for the several species and populations of *Crepidula.* Of course, size and age increase together, but a regression of size on age gives a high standard error of estimate, due to differences in growth rates temporally and between populations. Therefore, size cannot be used as an estimator of age. Size apart from age is of theoretical interest in the study of sex-change phenomena, because it is possible that an organism changes sex upon reaching some critical mass or energy content. That critical value may not be the same for all individuals. Resolution of age is not good enough to plot useful relationships between age at sex change and other population parameters.

In *C. convexa,* neither size nor age at sex change vary much between populations. However, in *C. fornicata,* both size and age of intermediates vary from population to population and depend on whether the male was mated when sex change began. A standard t test gave a t value for age at sex change, mated versus solitary *C. fornicata,* of 3.87, $p < .02$. The size and age of mated

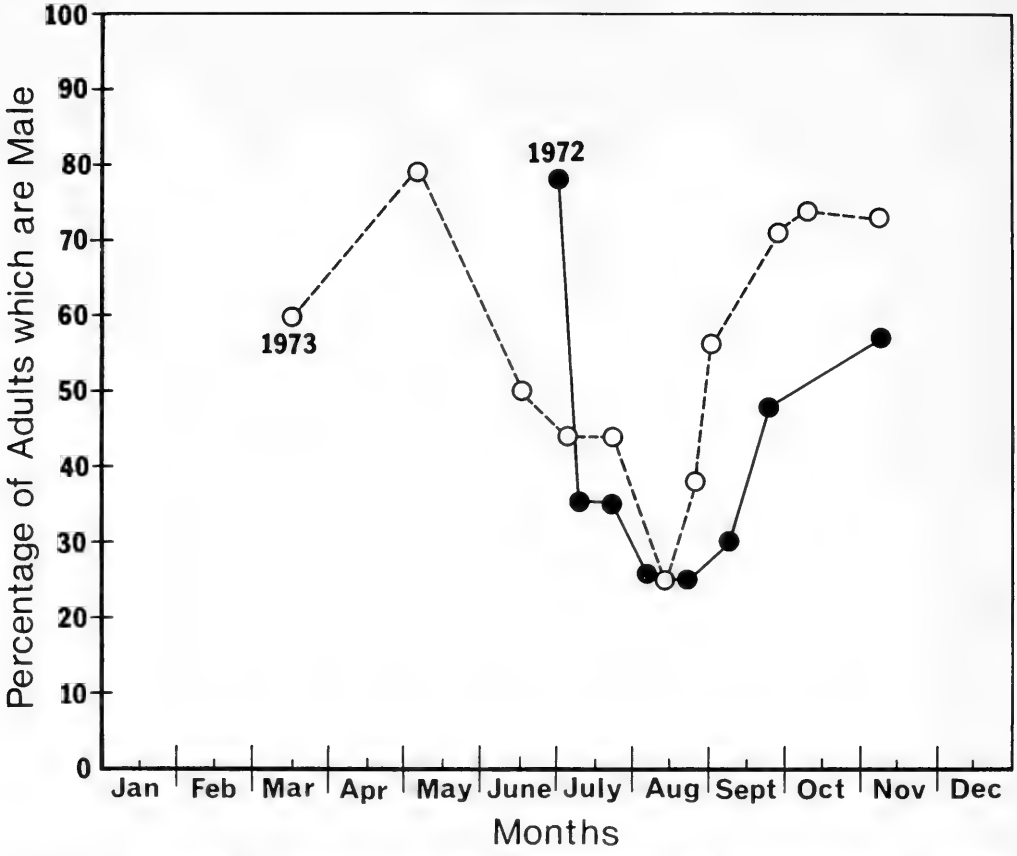

FIG. 4. The percentage of adults which are male in the Woods Hole Yacht Club population of *C. convexa*, 1972 and 1973. The percentage is calculated as in Fig. 3.

TABLE 5. Age and size at sex change for several populations of *C. convexa*.

	WHYC		Duxbury		Gunning Point	
	Solitary		Solitary		Solitary	
Mean age (mo.)	6.9		6.8		6.4	
Variance	8.3		8.7		9.2	
N	82		22		23	
Mean dry weight (g)	0.027		0.026		0.020	
Variance	0.0001		0.0001		0.00002	
N	82		22		23	
	Mated	Solitary	Mated	Solitary	Mated	Solitary
Oldest male (mo.)	12	12	12	—	12	12
Largest male (g)	0.024	0.024	0.026	—	0.014	0.012
N	100	100	50	0	25	10
May % male	46		58		64	
May adult density	30		50		80	

ANOVA TABLE*

Source of variation	df	ss	ms	F	
Among groups	2	1.23	0.62	0.76	(NS)
Within groups	124	100.38	0.81		

F(.05) = 3.07 for 2, 120 df; 3.00 for 2, ∞ df

*Dry weight data, log-transformed to normalize the data and to equalize variances. Age data are not analysed because the means are obviously uniform in the 3 populations.

TABLE 6. Age and size at sex change: planktonic species.

	C. fornicata										*C. onyx*	
	Martha's Vineyard		Waquoit		Nahant		MBL (1972)		R. I. (1972)		Balboa	
	mated	solitary	mated	solitary	mated	solitary	mated	solitary	mated	solitary	mated	solitary
Mean age (mo.)	20.80	6.00	15.00	6.00	13.50	4.50	6.33	3.52	8.00	3.35	–	–
Variance	49.99	12.00	72.00	18.00	40.5	7.71	9.41	2.90	6.00	2.05	–	–
N	60	4	2	2	2	8	18	69	6	34	0	0
Mean dry weight (g.)	4.0566	1.1228	2.6153	1.2132	2.3734	0.4518	0.8216	0.3524	0.2325	0.2442	2.1171	0.9151
Variance	3.7578	0.3593	0.0975	0.1798	3.6085	0.0402	0.1543	0.0401	–	0.0156	0.6271	0.1675
N	63	4	2	2	2	8	18	69	1	32	29	19
Oldest male	27	6	21	12	18	6	18	12	18	12	–	–
Largest male (g. dry weight)	7.702	0.4382	3.6177	1.3430	1.9820	0.3032	1.8203	0.6349	0.9060	0.2714	2.1754	1.1067
N	100	15	30	25	100	25	100	50	100	100	100	64
May sex ratio (% male)	52		76		40		54		73		47	
May adult density	200		200		10		20		300		160	

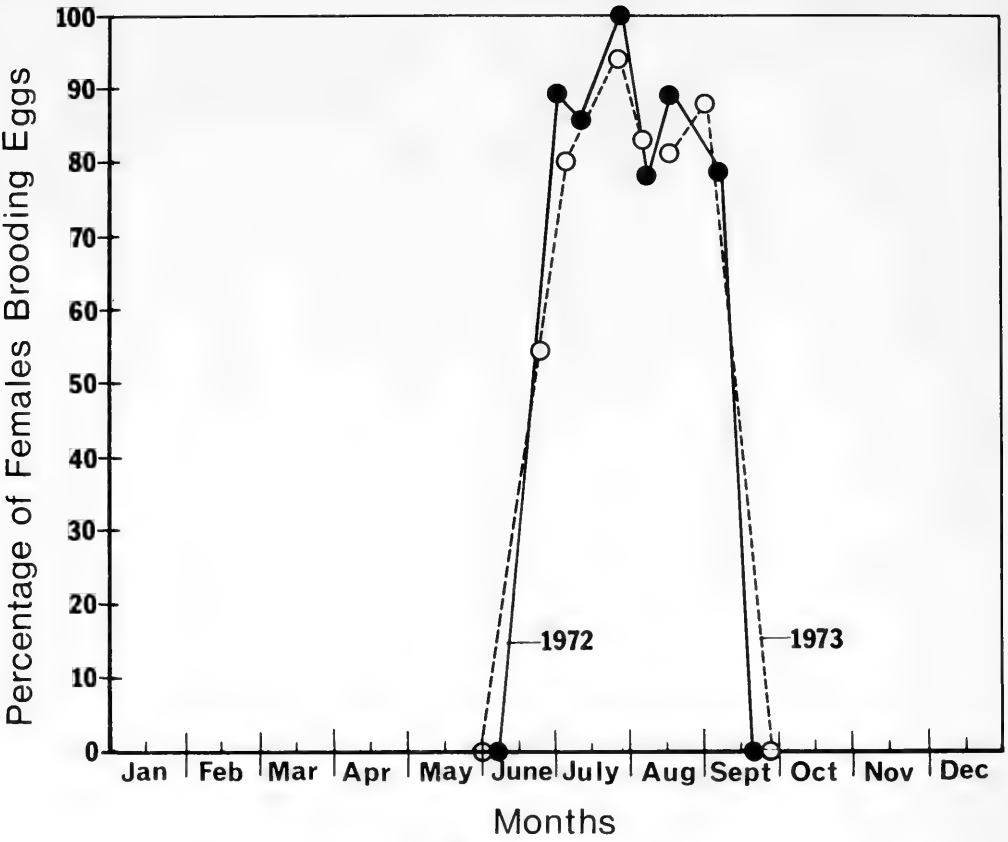

FIG. 5. The percentage of females brooding eggs, *Crepidula convexa* Woods Hole Yacht Club population, 1972 and 1973.

individuals are invariably greater, showing that mated males delay sex change, and that sex change reflects interaction among individuals. Coe (1938a) and Gould (1917b and 1952) obtained similar results. A complete comparison with *C. convexa* is not possible because no mated intermediates were found in that species. All males leave their mates prior to or at the beginning of sex change.

The results concerning age, despite the low precision of the data, are interesting because they demonstrate that the differences in size at sex change in *C. fornicata* are not due solely to differences in growth rates between populations while age at sex change remains the same. If growth rates alone were implicated, a trivial explanation such as differential food availability would be possible. The timing of sex change cannot be explained simply by the presence or absence of energy reserves in an organism, because the explanation does not account for sex change being affected by interaction among individuals of a population.

The relationship between population density and size at sex change is given in Figs. 6-9. For *C. convexa,* size at sex change is inversely related to population density ($r^2 = .92$) and to female density ($r^2 = .94$), but in both cases the regression lines have insignificant slope (Appendix B). Dense, crowded populations such as those living on eel grass *(Zostera)* mature at small size (are stunted), hence the inverse correlation.

In *C. fornicata,* the general trend is for size at sex change to increase with population density. An exception is the colonizing Rhode Island population. My data show that such young, newly colonizing populations have, on the average, earlier sex change than do populations in which older

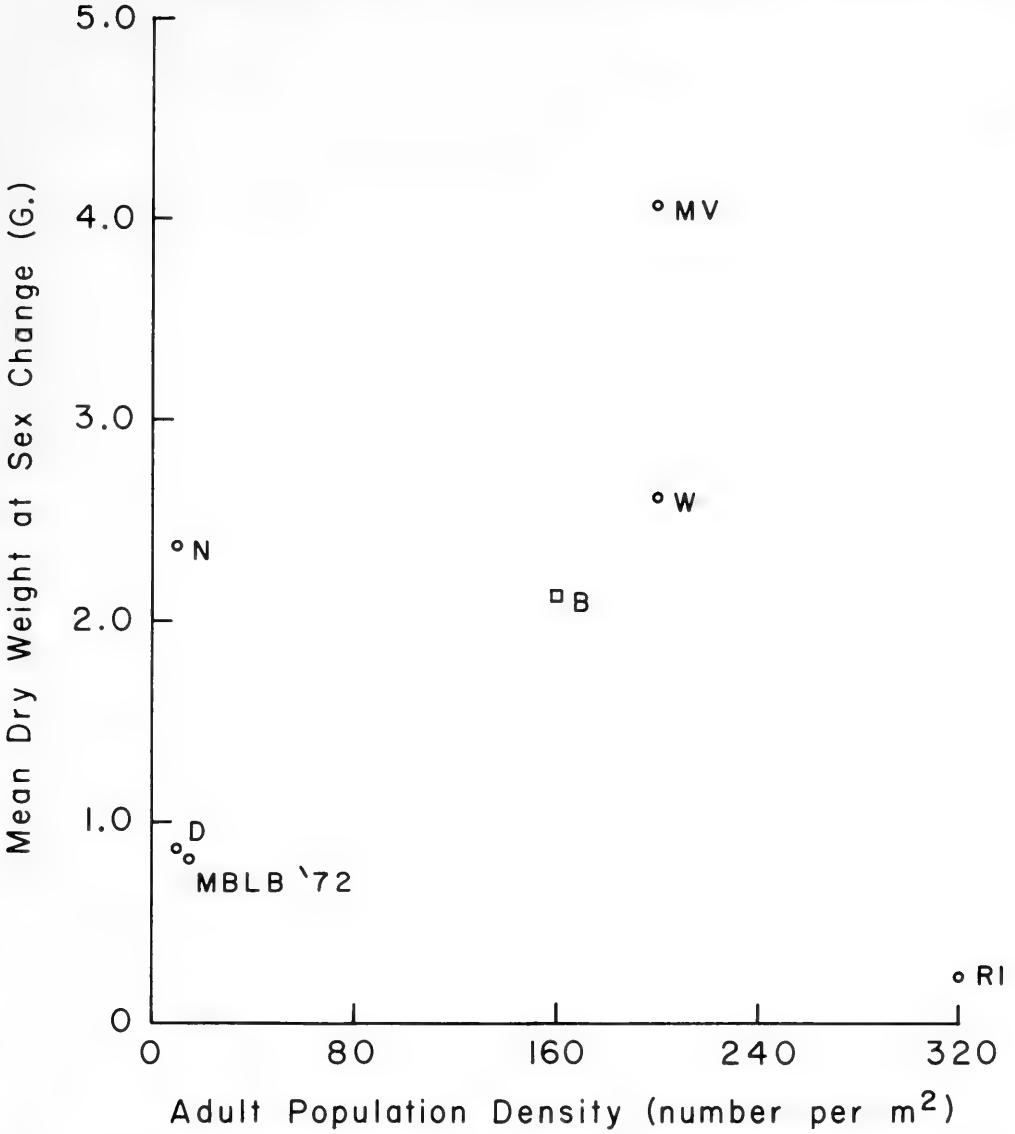

FIG. 6. Size at sex change as a function of adult population density, all species, mated individuals. The adult population density is the May value in each case. Abbreviations of localities are as in Appendix A. There were no mated intermediates found for *Crepidula plana* or *C. convexa.* Key: *C. fornicata* ○, *C. onyx* □

age classes dominate, and in which there are more age classes (e.g., the Nahant population). Such populations as Nahant are more stable, and probably have lower adult mortality (especially density-independent mortality) than newly colonizing populations. Warner (1975) showed a similar trend for protandrous *Pandalus* shrimp; later transformation occurred in populations with a wider range of ages in a population. Charnov (personal communication) states that recruitment of an unusually strong year class to a *Pandalus jordani* population results in a larger than usual percentage of young changing to female without functioning as male.

Crepidula onyx and *C. plana* are included in Figs. 6-9. They fit well into the density versus size curves, reducing r^2 only slightly. The values of r^2 for *C. fornicata*

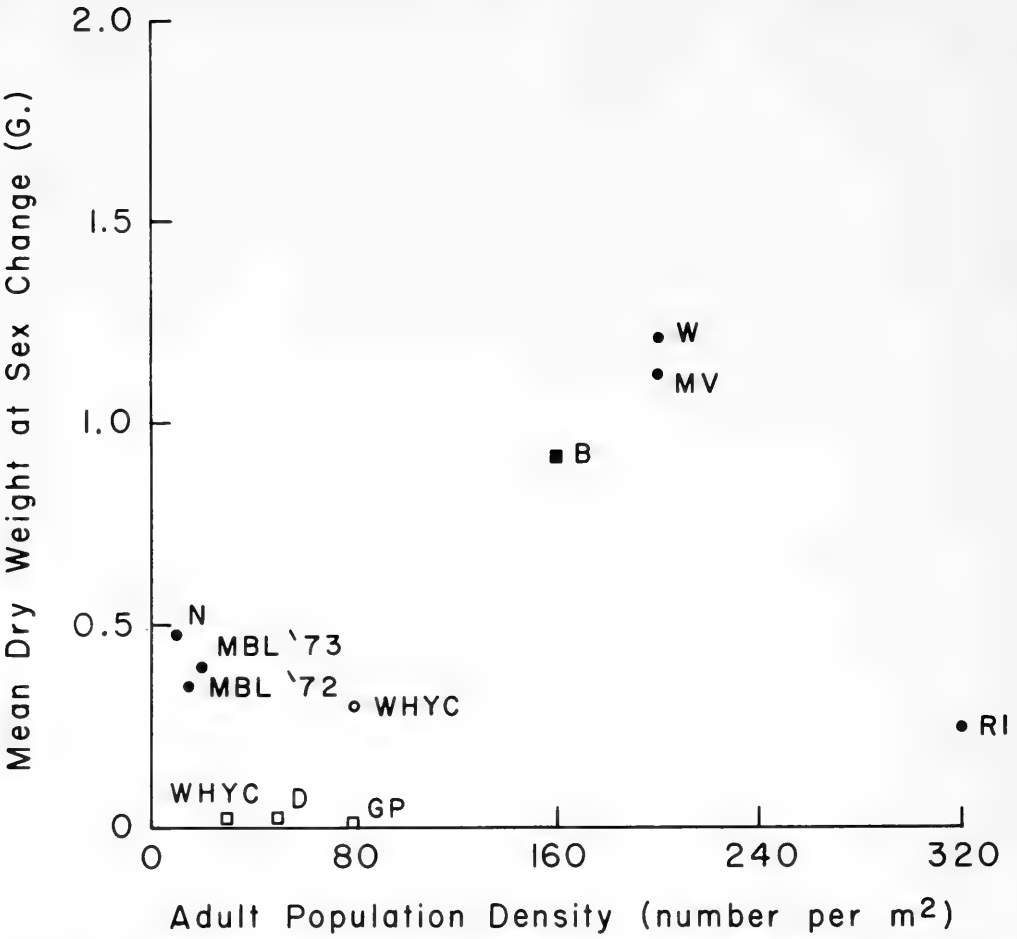

FIG. 7. Size at sex change as a function of adult population density, all species, solitary individuals. The adult population density is the May value in each case. Abbreviations of localities are as in Appendix A. Key: *C. fornicata* ●, *C. onyx* ■, *C. plana* ○, *C. convexa* □

alone improve in the following progression: total density versus size of mated intermediates (poorly correlated) < female density versus size of solitary intermediates < female density versus size of mated intermediates < total density versus size of solitary intermediates.

DISCUSSION

Summary of differences among species of Crepidula

The sexual behavior patterns of *C. convexa* and *C. fornicata* seem to be representative of patterns in non-planktonic and planktonic developers, respectively, of the family Calyptraeidae. In *C. convexa,* which lacks a planktonic dispersal stage, there is no attraction among newly hatched juveniles, nor between adults and juveniles. Coe (1953) studied the sexuality of several species that correspond to *C. convexa* in mode of larval development and dispersal, including *C. norrisiarum* Williamson, *C. adunca* Sow., and *C. williamsi* Coe (= *C. striolata* Menke, Hoagland, 1977b). Like *C. convexa,* matings were temporary, males left females at the time of their sex change thereby precluding stack formation, and protandrous sexual development was not environmentally regulated. *C. williamsi* possesses a two-week period during which newly metamorphosed young are not attracted by females. *C. adunca* young are

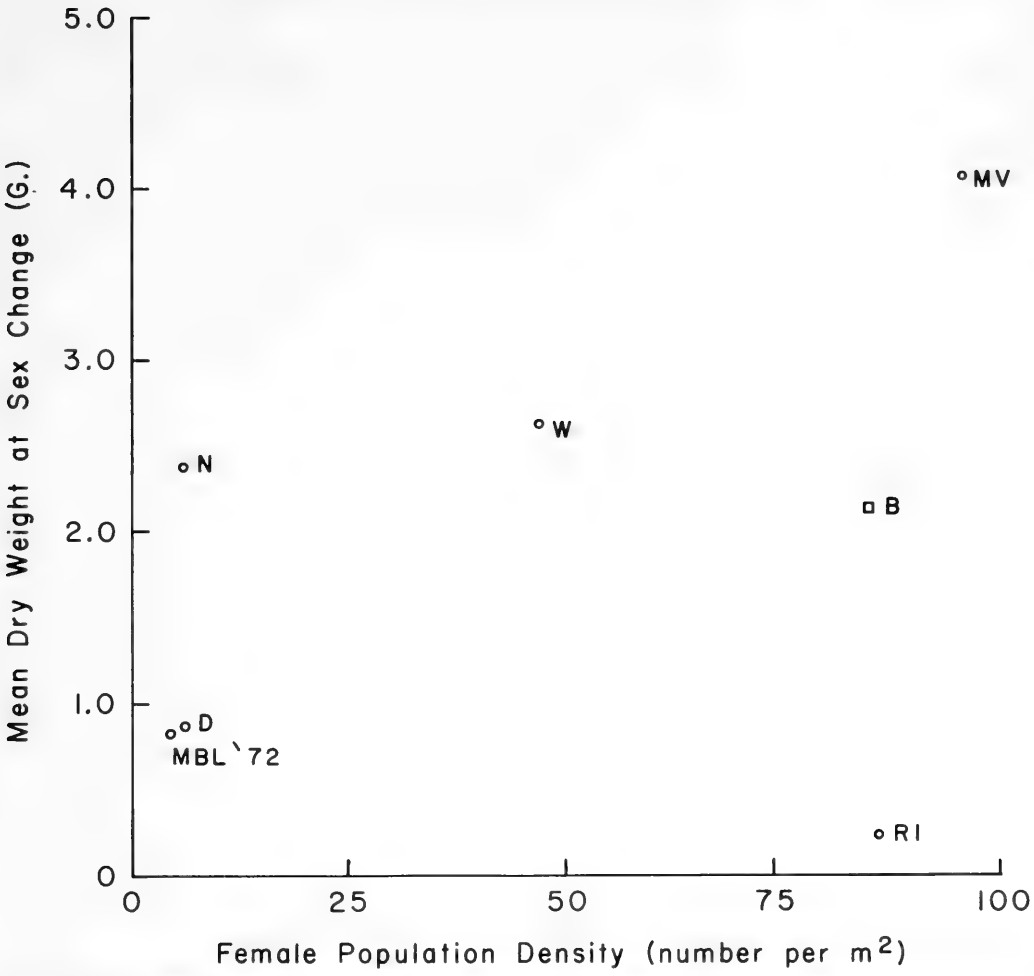

FIG. 8. Size at sex change as a function of female population density, all species, mated individuals. Key: *C. fornicata* ○, *C. onyx* □

not attracted to older individuals (Putnam, 1964). *C. dilatata* Lamarck and an as yet unnamed sibling species from South America possess different modes of larval development, and Gallardo (1977) indicated that the species with indirect development forms permanent stacks, but the species with direct development has temporary matings. At least one *Calyptraea, C. chinensis,* is protandrous and does not have labile sex determination. It has temporary matings, does not form stacks, and is not gregarious. It also broods its young (Wyatt, 1960).

Lack of attraction of juveniles to adults probably aids in juvenile dispersal, and results in increased outbreeding. Outbreeding is further increased by the temporary nature of male-female pairings. Females probably do attract males, but the response is limited to functioning males, for short durations. Timing of sex change is genetically programmed rather than influenced by other members of the population. The result is a sex ratio that is determined by the age structure and genetic composition of a population, rather than by group interaction. A field study (Hoagland, 1975) revealed that, indeed, a *C. convexa* population possessed a stable age structure due to constant recruitment over the three-year study period, whereas *C. fornicata* had variable recruitment and an unstable age structure. Because *C. convexa* young radi-

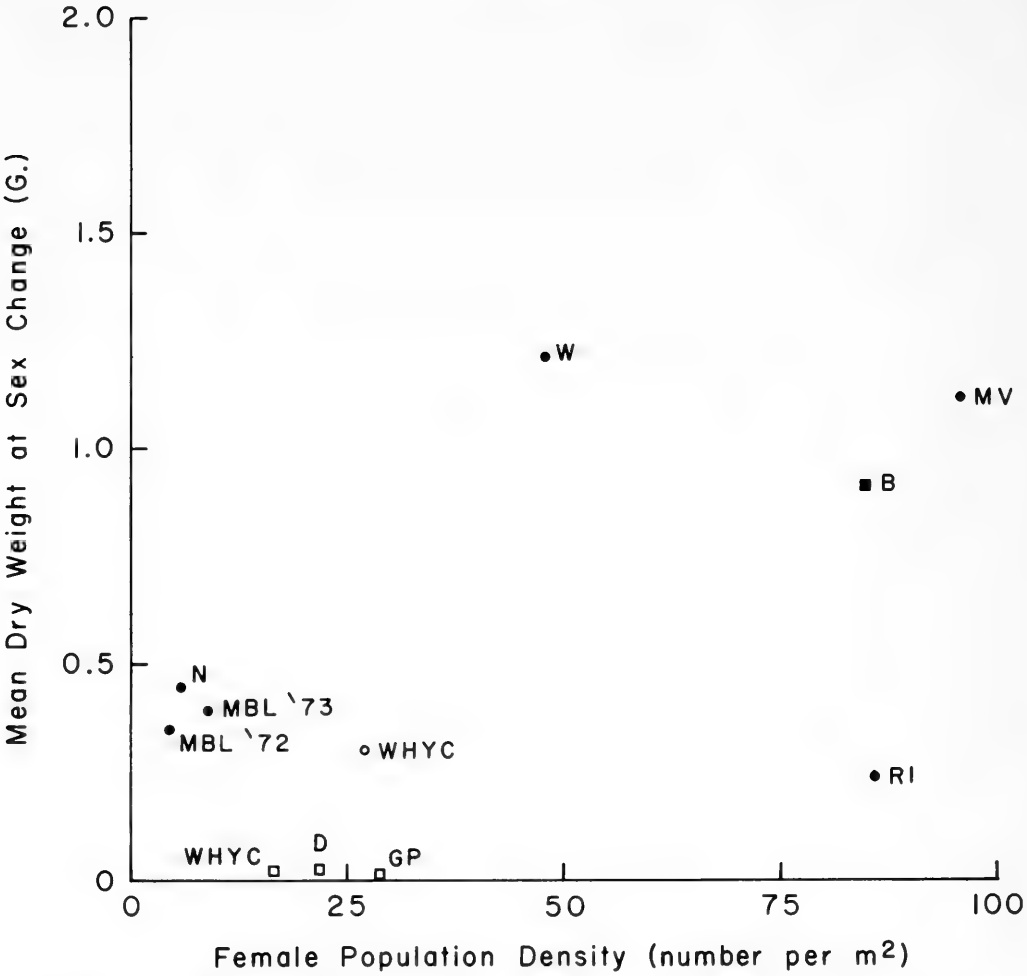

FIG. 9. Size at sex change as a function of female population density, all species, solitary individuals. Key: *C. fornicata* ●, *C. onyx* ■, *C. plana* ○, *C. convexa* □

ate from the parents without the uncertainties of planktonic dispersal, their mode of sexual change and interaction is appropriate.

Under crowded conditions, *C. convexa* snails mature at a smaller-than-usual size, while timing of sex change is not affected. One can only speculate as to the mechanism, but the most obvious possibility is slower growth due to competition for food, or due to physical contact among the individuals.

The behavior and sexuality of *C. fornicata* are highly integrated among individuals of a population. Individuals influence the settling behavior and maturation rates of other individuals. Juveniles are attracted to adults, as is consistent with the planktonic mode of dispersal and the inherent danger of an individual settling and remaining in isolation. Spat which do settle alone rapidly become female and proceed to attract other *C. fornicata* snails. One could assume that outbreeding is accomplished via the planktonic stage. There are no data on degree of outbreeding in *Crepidula*.

The age and size at sex change are factors that influence the demography of a protandrous population, for they determine the female- and male-stage fecundities of the individuals. Size and age at sex change are variable among the *C. fornicata* populations studied. In non-colonizing pop-

ulations, both size and age tend to increase with population density. Data for colonizing populations provide another insight: there are few females already present in a very dense, new settlement of *C. fornicata.* Sex change occurs at a very small size and young age regardless of the high density. On the other hand, an established population with only moderate density but a high proportion of females has a greater size at sex change than could be predicted by total density alone. Hence the density of females is implicated in the timing of sex change in *C. fornicata.* In fact, in the case of mated males, female density explains more variability in size at sex change than does total population density (Appendix B).

Crepidula fornicata responds oppositely from *C. convexa,* in which high density and the resultant crowding bring about a decreased size at sex change. Even very dense populations of *C. fornicata* do not run out of substrate. *C. fornicata* snails may form stacks in an arc, as illustrated in Fig. 10. This capability insures the presence of substrate in this gregarious species. I have observed several males in one stack copulating with the females of the same stack. The length of the penis increases with distance from the females, allowing a small male on the top of a stack to reach a female despite the presence of 3 or 4 males in between. This has the same effect as male mobility in *C. convexa.* Small males of *C. fornicata* also have some mobility. In both species, polyandry is possible. There well could be sperm competition (Parker, 1970). Whether females accept sperm from more than one male is unknown, but they can store sperm for at least one year (Hoagland, 1975). The facts that the male phase is prolonged in *C. fornicata* within the range of a female and that pairs may stay together for over a year imply that a female mates more than once. Otherwise, no advantage (increase in fitness) would accrue to the male by staying with a female, and no advantage would accrue to the female by prolonging the male phase of young *C. fornicata.* One or the other sex (not necessarily both) must be advantaged by the prolonged pairing.

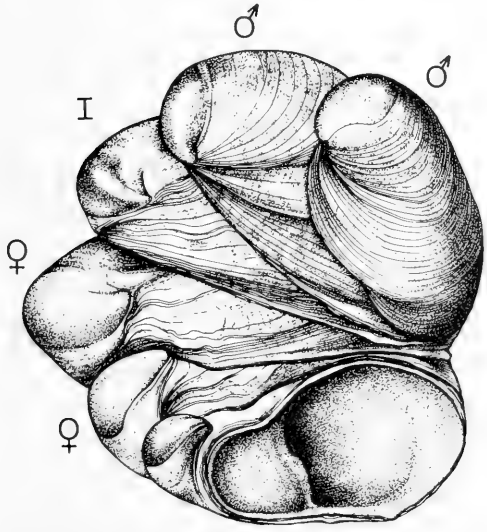

FIG. 10. A stack of *Crepidula fornicata* in life position. Sexes of the individuals are indicated. I = intermediate.

The data available for *C. onyx* indicate that it is similar to *C. fornicata* in essential features of labile sexuality and gregariousness, as well as in reproductive and dispersal modes, and the ability to form stacks. Stacked populations of *C. onyx* and *C. fornicata* are most common in muddy bays, where recruitment and population density are high. The population of *C. fornicata* at Nahant, Massachusetts, did not have stacks greater than three individuals, but animals taken from the area behaved as did other *C. fornicata* in the laboratory.

Organisms expressing labile sex determination are often phenotypically plastic in other ways. For example, *C. fornicata* and *C. onyx* vary their shape and intensity of pigmentation tremendously, depending on environmental conditions. The same factor that leads to sexual lability, the unpredictability of where an organism will settle and what selective regime it will occupy compared to that of its parents, also leads to physical and physiological plasticity. The only difference is that, in the case of sexual labiliy, organisms of the same species are the critical element of the environment. Phenotypic plasticity itself is a character that is affected by natural selection.

Crepidula convexa and other species without planktonic young and labile sex determination also lack much phenotypic plasticity in color and shape. Rather, they possess discrete color morphs, genetically determined, in some populations (Hoagland, 1977a). These species are thus characterized by low interaction between genotype and environment to produce the phenotype. Another way of saying the

same thing is that heritability of traits is high.

A type of sexual behavior and population structure that is intermediate between the *C. fornicata* type and the *C. convexa* type is illustrated by *C. plana.* It produces dwarfed females like *C. convexa,* and is substrate-limited, for it lives underneath objects or inside them, and does not form stacks. With respect to gregariousness, it is intermediate between *C. fornicata* and *C. convexa.* It is quite possible that *C. plana* veligers, being strongly photonegative, settle together more because they are attracted to the same substrate than because they are truly gregarious. Coe (1938b, 1948) found that *C. plana* is also intermediate and highly variable in the time mated pairs stay together when compared with *C. fornicata* (long) and *C. convexa* (short). In reproduction and dispersal, *C. plana* is like *C. fornicata.* It possesses labile sex determination (Gould, 1952). Coe (1953) demonstrated that the presence of a female delays sex change in males of *C. plana* and *C. nivea* C. B. Adams, ecological counterparts in the North American Atlantic and Pacific, respectively. About 90% of the males kept together with females in the laboratory did not change sex, whereas only about 30% of those without females remained male. In nature, 68% of the *C. plana* and 42% of the *C. nivea* remained male over the same length of time. Gould & Hsiao (1948) demonstrated individual differences in growth rates and sexual development in *C. plana.* They presumed these to be congenital. My own experiments indicate that *C. fornicata* siblings also grow and mature at different rates.

It is important that *C. plana* is intermediate in both life history characteristics and in sexual behavior. It substantiates my hypothesis that the mode of reproduction, type of dispersal, and degree of substrate limitation interact to determine the optimal sexual behavior and population structure, in particular the sex ratio and recruitment patterns.

Coe (1935, 1938a and b) claimed that some individuals of every *Crepidula* species are "true" males, incapable of sex change, but evidence is weak. In *C. convexa,* no evidence of "true" males or females, such as juveniles that fail to go through a male phase in the laboratory, has been found. However, *C. fornicata* individuals that appeared not to function as males have been seen in the course of this study. Of the 27% of the individuals in the colonizing Rhode Island population that were reported as females in 1972, most were equal in size to the males of the population. It appeared that these females were less than a year old, and may have passed through the male phase without functioning. From laboratory data and weekly examination of *C. fornicata* juveniles at the M.B.L. Beach locality, also representing colonization, it appeared that some individuals do bypass the functional male phase. The greater component of this capability may be environmental rather than genetic, because an individual selected at will and isolated often fails to develop a complete penis before it begins to resorb.

Warner et al. (1975) found cases in fish where there is male dimorphism. Some members of a population are primary males, with one type of mating system, while others are protogynous males, with a different mating behavior. The presence of "true" males or females in the Calyptraeidae, if they are shown to be genetically distinct from protandrous individuals, would provide an example of dimorphism within a sex in molluscs. Presently, there is no known behavioral difference of genetic origin within males of *Crepidula.* However, the youngest *C. fornicata* males often wander, while larger, older males are permanently attached to a particular stack of females.

Organisms with Comparable Sexuality

Social interaction in sex determination is common among molluscs and other invertebrates, but seems to be most prevalent in sedentary, territorial, and parasitic organisms with dispersive (e.g., planktonic) larval stages. It often accompanies the phenomenon of dwarf males that are parasitic on the females, or the phenomenon of protandry. The parasitic mesogastropod family Entoconchidae (Lützen, 1968) and the sedentary echiuroid genus *Bonellia* (Baltzer, 1931; Gould-Somero, 1975) illustrate labile sex determination in which the sex of a juvenile is determined by whether it settles near or on a female. If it does, the juvenile becomes a dwarf male. However, about 10% of *Bonellia* echiuroids differentiate sexually without regard to their associations with other *Bonellia* individuals; thus there are "true" males and

females (Leutert, 1975). Other genera of echiuroids lack sexual lability; interestingly, these genera are mobile and lack sexual dimorphism.

Socially-mediated sex change has been reported in several fish, such as *Anthias* and *Labroides* (Smith, 1975) and *Thalassoma bifasciatum* (Warner et al., 1975). Labile sex determination of a different sort occurs in the hermaphroditic fish *Rivulus marmoratus.* In this animal, both genetic and physical environmental factors such as temperature control sex determination (Harrington, 1971). Many plants also have environmental sex determination in which physical rather than social factors are important (Charnov & Bull, 1977). In molluscs, the physical environment can play a role in sex determination in protandrous hermaphrodites; for example, starved animals tend to remain male (Coe, 1938a). This is because they do not have enough stored energy to accomplish the drastic anatomical remodeling required by the change from male to female.

The polychaete *Ophryotrocha puerilis* has a sex reversal pattern whereby a female-female contact causes one of the pair to revert to male (Pfannenstiel, 1975). This pattern of labile sex determination does not occur in molluscs. However, sex alteration does occur in some protandrous oysters (Orton, 1927, 1936), in *Teredo navalis* (Coe, 1941), and in *Patella vulgata* (Orton, 1919). It does not seem to be based on social encounters, but its control mechanism is not known.

Bacci (1951, 1955) used the term "unbalanced hermaphrodite" to describe protandrous animals in which the age at sex change varies, he presumed genetically, between populations. In this category he listed species of *Crepidula* and *Patella,* the shipworm genus *Teredo,* the serpulid worm *Pomatoceros triqueter* (see also Føyn, 1950), *Ophryotrocha puerilis,* and the starfish *Asterina gibbosa.* The wide taxonomic range of these examples is noteworthy. It indicates that differences in the timing of sex change are easily evolved in organisms when the proper selective pressures are present. Bacci's claim that the differences in the age at sex change are genetically controlled needs better documentation, in the light of independent evidence that labile sex change exists in many species. The genetic mechanism for sex determination itself has proven to be elusive, but is a critical key in our understanding of the relative importance of genes and environment in sex change.

I review the major references to protandry in molluscs, but I do not attempt an exhaustive list. The important point is that there is a wide taxonomic range of protandrous species, indicating independent lines of evolution. With the exception of Lützen and his co-workers, in the papers cited below, none of the authors commented on the possibility of social regulation of sex determination.

The greatest concentration of protandrous species is in the Mesogastropoda. Lützen (1972) found protandry in *Stilifer linckiae,* a parasite. The male does not change sex in the presence of the female, thereby insuring the presence of both sexes in the starfish host which usually contains a low number of parasites. Protandrous parasitic mesogastropods also include *Robillardia cernica* (Gooding & Lützen, 1973), at least some members of *Echineulima mittrei* (Lützen & Nielsen, 1975), and the ectoparasite *Epitonium albidum* (R. Robertson, personal communication). Protandry occurs in the genus *Trichotropis* (Yonge, 1962) and in the Capulidae (Graham, 1954), Janthinidae and Scalidae (Ankel, 1926). Morton (1958) reported protandry in a gymnosomatous pteropod, the southern "dwarf form" of *Clione limacina.*

Reports of protandry in the archaeogastropods are rare, but Fretter & Graham (1962) claim that protandry is occasionally found in *Acmaea.* Russell-Hunter & McMahon (1976) gave evidence of functional protandry in a fresh-water basommatophoran limpet, *Laevapex fuscus.* In fact, many pulmonate and opisthobranch hermaphrodites approach functional protandry; that is, the male organs mature before the female.

The most surprising report of protandry in the gastropods is that by Smith (1967) on a population of *Lora turricula,* a member of the neogastropod family Turridae. I know of no other protandrous neogastropod, though I expect that more will be discovered. The anatomy of *L. turricula* is closer to the hermaphroditic pulmonates and opisthobranchs than to the protandrous hermaphrodites of the Mesogastropoda, in that there are two separate reproductive systems rather than a single ambisexual gonad. The independent origin

of protandry in this organism is clear. Smith's population of *L. turricula* is insular, providing an advantage for hermaphroditism, but the evolutionary explanation for protandry must be sought in further details of the ecology of the species. Whether all populations of the species are protandrous is unknown.

Turning to the bivalves, all species of the Teredinidae (wood-borers) so far studied are protandrous, alternating, or simultaneous hermaphrodites (Sigerfoos, 1908; Kofoid & Miller, 1927; Yonge, 1926; Coe, 1941; Turner, 1966; Herlin-Houtteville & Lubet, 1975). The adults are completely sedentary; larvae vary from completely planktonic to those released as crawling young. Some teredinids, including members of the genus *Bankia,* have been observed to copulate. Sperm are transferred to the female incurrent siphon via the male's excurrent siphon. Protandrous *Bankia gouldi* shipworms, when living at high densities, mature and spawn when dwarf (R. Turner, personal communication).

Protandrous *Psiloteredo megotara* males release sperm in diaphanous membranes which are actively sucked in by the female incurrent siphon. Turner & Cooley (unpubl.) found several individuals of *P. megotara* isolated in lobster pots. All were males, yet they were all larger than normal females of the species. The implication is that the sex change does not occur in isolated individuals, or at least is not triggered by the organism attaining a certain size, or by its being isolated.

There are a few data that suggest that isolated males of *Bankia gouldi* remain males, while the presence of a second male triggers one to change sex (Culliney, 1969 and personal communication). This would be a reverse form of sexual lability, compared with *Crepidula fornicata.* If more data indicate that this is so, it may be related to the shipworms' facultative copulatory behavior. Isolated males are still able to broadcast sperm, so an isolated male is not reproductively dead. A juvenile attracted to the same substrate at a later date could influence the first individual to change sex via a feminizing compound, if the gonad of *Bankia* is basically male, rather than female as it is in *Crepidula* (Le Gall & Streiff, 1975).

Some unifying hypotheses on patterns of sexuality and their evolution

Table 7 compares the essential population factors and sexual patterns in the *Crepidula* species and some other protandrous and/or sexually labile molluscs. *Bonellia* is added because it is a classic example of labile sex determination, and information about its ecology is available. Can a single hypothesis explain the evolution of sex in all of these organisms?

The organisms of Table 7 have several features in common. The females have internal fertilization and are virtually sedentary. There is a high degree of isolation of breeding units due to discontinuity of suitable substrates, but the species possess effective dispersal stages and may be called opportunistic species (MacArthur, 1962). They frequently colonize new areas, and their preferred substrates are transient. This is particularly true of shipworms, which consume their substrates, but the same effect is achieved with the shallow-water cobble and shell habitats of *Crepidula,* which are transient in geologic time and often shift during a period of several years.

Ghiselin (1969) proposed that simultaneous hermaphroditism is advantageous for individuals living in small, isolated populations (but see Williams, 1975). Ghiselin felt that isolation (small deme size) could not favor protandry, because effective population size would not be increased by protandry. However, effective population size by itself has no relation to individual fitness. The critical factor is that under protandry coupled with sexual lability and/or chemical attraction of larvae (either by the species or by the substrate), each individual minimizes the age at which it first reproduces, increasing its reproductive potential. In *C. fornicata,* for example, isolated individuals rapidly become female and emit species-specific attractants; any second individual contacting the first is necessarily a juvenile at the point of metamorphosis or a male, because only such individuals are highly mobile. The juvenile in such a circumstance is guaranteed to become a functional male, hence immediately to participate in reproduction. As a byproduct, every encounter between individuals can produce offspring.

TABLE 7. Comparison of sex and reproductive patterns in several sedentary invertebrates.

SPECIES	Protandry	Internal fertilization	Planktonic larvae	Substrate patchy?	Substrate consumed?	Substrate may be limited?	Juveniles attracted to adults?	Labile sex?	1st functioning sex of a young isolated individual	Sex of a young individual settling near a female	Females dwarfed under crowding	Postponement of sex change under influence of female	Feeding mode	Sex ratio correlated with population density
Crepidula adunca	+	+	–	+	–	+	–	–	♂	♂	+	–	*	–
convexa	+	+	–	+	–	+	–	–	♂	♂	+	–	*	–
plana	+	+	+	+	–	+	weak	+	♂ or ♀	♂	+	+	*	#
fornicata	+	+	+	+	–	–	+	+	♂ or ♀	♂	–	+	*	+
onyx	+	+	+	+	–	–	+	+	♂ or ♀	♂	–	+	*	+
Calyptraea chinensis	+	+	–	+	–	+	–	–	♂	♂	#	–	*	–
Bankia gouldi	+	facultative	+	+	+	+	to wood (–)	#	♂	♂	+	#	*, wood?	#
Psiloteredo megotara	+	facultative	+	+	+	+	to wood (–)	–	♂	♂	+	#	* wood?	#
Echineulima mittrei	+	+	+	+	+	–	to host	possibly	♂	♂	–	+	parasite	#
Stilifer linckiae	+	+	+	+	+	–	to host	+	♀	♂	–	+	parasite	#
Bonellia spp.	– dwarf ♂	+	+	#	–	#	+	+	♀	♂	#	not applicable	*	#

#insufficient data available.
*sedentary ciliary-mucous net feeder.

Only in the non-planktonic Calyptraeidae and possibly in the shipworms must I invoke another factor to explain the advantages of protandry: small differences in genetic proclivity to change sex. Such genetic differential guarantees the presence of both sexes in any single age cohort, even without the powerful aid of sexual lability. This is necessary to insure the reproductive potential of individuals. This factor, incidentally, is present in the other calyptraeids as well.

Protandry is not the only solution to problems caused by isolation of individuals or small demes. Simultaneous hermaphroditism coupled with facultative self-fertilization theoretically could accomplish the same things (minimization of age at first reproduction, maximization of reproductive events per lifetime, stabilization of the sex ratio) but with the additional cost of inbreeding. Although the sex ratio of protandrous animals is affected by the age at sex change of individuals making up a population, it is not the sex ratio that is under natural selection. Rather, it is the optimal age of individuals at sex change. Apart from providing flexibility in sex ratio, protandry is advantageous to individuals if one sex increases in fertility with age (and size) much more rapidly than the other (Ghiselin, 1969; Warner et al., 1975). This is true of calyptraeids and shipworms, and probably molluscs in general. The female does not discriminate among males on the basis of age or size, hence male fecundity is not strongly correlated with either. Young males of *Crepidula* gain advantage by growing longer copulatory organs, but do not gain advantage from general size increase. In fact, smaller males are more mobile and have a better chance at fertilizing several females than do larger, more sedentary males. I have observed a burst of growth at the time of sex change in many individuals of *C. fornicata,* and Le Gall (1973, 1974) reported that growth is greater in individuals of *C. fornicata* the nearer they are to the base of a chain. Female fecundity is strongly related to age and size, for the number of eggs produced per season rises steeply with body size (Hoagland, 1975). In sum, plots of fecundity versus age or size are different for the two sexes. The differences are just as predicted by models of protandry based on maximization of individual fitness. Lack of male care of offspring and the relationship between size, sex, and fecundity explain why protandry and not protogyny is common in the Mollusca.

Population density has been implicated in the adult sex ratios of several invertebrates with labile sex determination, e.g., two parasitic nematodes (Caullery & Comas, 1928; Christie, 1929), monstrillid copepods (Malaquin, 1901; Anderson, 1961), some rotifers, cladocerans and *Ophryotrocha* (Coe, 1938b). Dense populations tend to have proportionally more males, as described in this paper for *C. fornicata* and as Coe (1944) also demonstrated for *Crepidula* species. In the protandrous species, total egg output per adult tends to decrease as population density increases, because each individual spends a greater proportion of its lifetime as a male. Reduction in egg output is probably counter-balanced by an increase in male fecundity. Unfortunately, there are no data on this critical parameter. However, Warner (1975) found variation in the time of sex change in protogynous fish and could relate it to total (male plus female) fecundity and hence to individual fitness.

Ghiselin (1969) pointed out that brooding of young by the female often accompanies protandry, and hermaphroditism in general. This pattern certainly holds for *Crepidula* and the family Calyptraeidae. Charnov et al. (1976) use a fitness set argument to demonstrate that brooding favors hermaphroditism because female expenditure of reproductive effort occurs later than male expenditure.

The adaptive significance of differences among protandrous organisms

The major distinction in Table 7 is between organisms with and without environmentally-influenced sex change. Those without it are characterized by the possibility of substrate limitation. Most lack a planktonic larval stage. Juveniles are not attracted to adults, and females mature at a small size when crowded. In such organisms, risks of crowding are reduced by the absence of gregariousness; crowding is often associated with rapid maturation and early deaths of adults due to destruction of the substrate.

Labile sex determination is linked to juvenile attraction to adults, and in most cases, lack of substrate limitation. The sex ratio of populations of species with en-

vironmentally mediated sex determination is correlated with population density. The sexually labile species that I have studied all have planktonic larvae, but all species with planktonic larvae are not sexually labile. Environmental influence in the timing of sex change allows the size and age of an organism at sex change to be tailored to suit very localized conditions, even if the genetic constitution of local individuals reflects a much broader gene pool from a broader range of environments. Charnov & Bull (1977) elaborated on this idea for a variety of organisms, including plants.

Crepidula fornicata, C. onyx, C. plana, Bonellia, and even the protogynous fish *Thalassoma bifasciatum* have planktonic development and dispersal coupled with low mobility as adults. In the protandrous calyptraeids without planktonic development, timing of sex change is under direct genetic control. On the basis of this known difference, one could predict that there is sexual lability in *B. gouldi* and other shipworms with planktonic stages, but not in those that hatch at a crawling stage.

Mechanisms of control of sex behavior and sex change

The basic mechanism to control behavior in all the species of *Crepidula* and perhaps the other protandrous invertebrates is likely to be pheromonal regulation, although evidence is circumstantial. In the case of *C. convexa,* pheromonal involvement in sex change and gregarious behavior appears to be minimal, restricted to male-female interaction at the time of mating. In the case of *C. fornicata,* the responses of all individuals to one another are probably pheromone-mediated. Gregariousness and sexual lability probably have a common mechanism in calyptraeids, explaining why they are always coupled.

The most direct evidence for pheromonal behavior control, as opposed to a tactile mechanism, was obtained by Gould (1919, 1952). He suspended males of *C. plana* above females and observed delays in sex change. My finding that the density of females, rather than total population density, affects the mean age at sex change of new members of a population, indicates that a masculinizing pheromone resides in the female. However, discovery that mated males are more affected by female density than unmated males suggests that a tactile or localized component also could be a part of the behavioral mechanism, as suggested first by Coe (1938b). There is no evidence of a feminizing compound produced by males; the female condition is physiologically dominant. That is, an isolated animal eventually becomes female (Le Gall & Streiff, 1975).

QUESTIONS FOR THE FUTURE

To explain the actual path of evolution of protandry in molluscs, it is necessary to know the genetic basis of sex determination, the chemical basis for protandry, and the nature of the suspected pheromone(s) involved in sexual behavior. The chemical basis for protandry is not necessarily the same in all molluscs. Evolutionary relationships in the mesogastropods, particularly in the highly modified parasitic groups, could perhaps be seen through chemical analysis of sexual and behavioral mechanisms. So far, most of the thought in these areas is speculative. The mechanism controlling dwarfing, by which a male matures at a smaller-than-normal size even before it has run out of room to grow, is also unknown.

One reproductive mode barely touched upon here, but which is an extension of protandry, is alternating sexuality within individuals. It should be examined in the light of this paper and the theoretical works of Warner (1975), Warner et al. (1975), Leigh et al. (1976), and Charnov et al. (1976). Species in which some populations but not all have been reported to show protandry also are worthy of further study, particularly to see if sexual lability occurs with protandry.

An examination of all invertebrates exhibiting protandry and/or sexual lability will include many which are not sedentary or substrate-restricted as adults, for example, the pandalid shrimp (Wenner, 1972; Charniaux-Cotton, 1965). How will these fit into the framework developed here? Also, it presently appears that highly mobile carnivores are not protandrous; exceptions should be sought. Finally, are there cases of invertebrates for which we predict protandry on the basis of other life history characteristics, but find it lacking? Answers to such questions will expand both theoretical and intuitive understanding of the evolution of protandry in molluscs and in other organisms as well.

ACKNOWLEDGEMENTS

I thank E. Charnov, G. Davis, and J. Murray for reading and commenting upon the manuscript. This work was supported in part by a Gibbs Fellowship from Harvard University and a contract #AT(49-24)-0347 with the U.S. Nuclear Regulatory Commission.

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APPENDIX A. A description of the populations studied.

	Density*	Description
C. fornicata		
Nahant, Massachusetts (N)	10	Sparse, stable adult population size. Low recruitment. On stones in tidepools. No substrate shortage. Stacks rarely exceed 3. Many individuals 6-8 years old.
Vineyard Haven, Martha's Vineyard, Massachusetts (MV)	200	Dense, stable population, known since before 1900. Numerically constant over time. Large stacks on shells and stones in mud. Subtidal except for some washed onto beach.
Waquoit Bay, Cape Cod, Massachusetts (W)	200	Old, stable population, similar to Martha's Vineyard, but less extensive in area.
Tiverton, Rhode Island (RI)	500	A colonizing population, less than 3 years old, at a new marina. Stacks on *Mytilus edulis,* undersides of docks.
Marine Biological Lab. Beach, Woods Hole, Massachusetts (MBLB)	15	Sparse, unstable population. High physical mortality due to winter ice scour. Low food supply. Substrate of a few rocks and cobbles near stone jetties surrounded by clean sand. Essentially a colonizing population in 1972. Excess substrate. Stacks do not exceed 3. Subtidal.
C. onyx		
Balboa Island, California (B)	160	Old, dense, numerically stable population. First collected *circa* 1900. Stacks on restricted substrate (shells) in muddy sand. Subtidal.
C. plana		
Woods Hole Yacht Club, Woods Hole, Massachusetts (WHYC)	80	Sparse population on concave sides of glass and inside shells. Sandy substrate, shallow subtidal, a few awash in surf attached to shells.
C. convexa		
Gunning Point, Cape Cod, Massachusetts (GP)	80	Small individuals, dense population on eel grass and small *Littorina littorea.* Shallow to intertidal, sheltered bay with mud-sand substrate.
Nobska Beach, Woods Hole, Massachusetts (Nob)	10	Sparse population on rare *L. littorea* and occasionally on stones. Wave-washed rocky intertidal point.
Waquoit Bay, Cape Cod, Massachusetts (W)	50	Small individuals, moderately dense population on eel grass and shells. Shallow subtidal to intertidal, protected bay.
Duxbury, Massachusetts (D)	50	Moderately dense, numerically stable population on shells and stones, sheltered sandy area. Shallow subtidal to intertidal.
Woods Hole Yacht Club, Woods Hole, Massachusetts (WHYC)	30	Sparse, numerically stable population on shells, especially *L. littorea,* stones, glass, and assorted debris. Shallow subtidal to intertidal.

*Number per m^2 of available substrate.

APPENDIX B. Summary of the relationship between population density and the size and age at sex change—four *Crepidula* species.

C. fornicata, C. onyx, and *C. plana:*

1. Population density vs. size (dry weight) at sex change

A. Total density	solitary r^2	solitary eq: y =	mated r^2	mated eq: y =
All planktonic species	.325	.48 + .001 x	.124	1.69 + .001 x
R. I. population removed	.922	.25 + .004 x	.756	1.21 + .009 x
C. fornicata only, R. I. removed	.991	.35 + .004 x	.798	1.24 + .010 x*
B. Female density				
All planktonic species	.500	.39 + .005 x	.405	1.27 + .01 x
R. I. removed	.776	.37 + .008 x	.767	1.24 + .02 x*
C. fornicata only, R. I. removed	.832	.46 + .008 x	.890	1.20 + .03 x*

2. Population density vs. age at sex change, *C. fornicata* only

A. Total density				
With R. I. population	.174	4.42 + .002 x	.191	11.47 + .01 x
Without R. I. population	.938	3.88 + .010 x	.765	9.44 + .04 x*
B. Female density				
With R. I. population	.367	4.15 + .01 x	.507	9.48 + .04 x
Without R. I. population	.832	4.11 + .02 x*	.877	9.34 + .12 x*

C. convexa:

1. Population density vs. size (dry weight) at sex change

A. Total density	.92	.03 – .0002 x
B. Female density	.94	.04 – .0007 x

2. Population density vs. age at sex change: no correlation.

*Slopes significantly different from zero.

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An asterisk (*) denotes a new taxon

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