Quaestiones entomologicae

A periodical record of entomological investigations, published at the Department of Entomology, University of Alberta, Edmonton, Canada

VOLUME IV 1968

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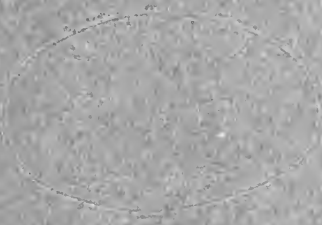

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Editorial - The Trumpet shall Sound

Strictly speaking, insects have no need of a trumpeter, for though they lack both the blow and the lips to perform on this imposing instrument nevertheless some of them seem to produce a similarly regal sound in a related manner. Neither are insects in need of anybody to beat the drum for them, for many are accomplished performers on percussion instruments. But since neither human ears nor hearing aids of any kind are yet attuned to all of the messages thus broadcast, a word of comment may be appropriate.

It is believed that when a queen bee produces the piping sound one so often hears of but so rarely hears, she does so by forcing air out through the thoracic spiracles. These, supposedly, are appropriately tuned or tensioned. By contrast the death's head hawk moth produces a sound sufficiently similar to gain her some, at least, of the privileges of the queen bee by forcing air out through the mouthparts. This is a skill which any trumpeter might covet. It is perhaps no accident that in many places human percussion instruments started from hollow logs, since many insect percussors, from termites to beetles, perform on these instruments. Human ideas in many fields have originated from insect activities, and Pope's advice still applies:

> *Go, from the creatures thy instructions take:*
> *Thy arts of building from the bee receive;*
> *Learn of the mole to plough, the worm to weave;*

Many years ago my duties as an entomologist in Calcutta converged strangely with those of an exorciser of ghosts. I was called in rather early one morning to advise on the possible source of a fine specimen of the cerambycid *Stromatium barbatum* Fabr. When I arrived the beetle was nonchalantly waving its antennae from the mantelshelf at a somewhat less than enthusiastic member of the household of a petty rajah. Across the corner of the room was a recently acquired grand piano. I commented on this and occasioned embarrassment; tactless persistance revealed that the house was haunted and the ghost a pianist - of sorts. Grovelling under under the piano I came upon a tidy pile of rather coarse wood strands

and, dropping a negative plumb line upwards, a rounded rectangular hole in the frame of the piano. Solicitous enquiries of the household in the days that followed confirmed my suspicions that this hole marked the exit of both the beetle and the ghost. The lusty chewing of the larvae, supplemented perhaps by the stridulation of the adult evidently invoked a minor resonance in the strings of the instrument. I put a plug in the hole.

Insects contribute so much that is of interest in life that we should all of us be prepared to put in a plug for them when opportunity offers, for though they need neither trumpeter nor drummer among their own kind, we who are of coarser fibre are too often insensitive to them. They, for their part, too often leave their holes open.

Brian Hocking

EFFECTS OF CROWDING ON WING MORPHOGENESIS IN *MYZUS PERSICAE* SULZ. (APHIDIDAE; HOMOPTERA)

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Adult apterous aphids, Myzus persicae *Sulz., were raised on discs of radish and cabbage leaves to determine the effect of different population densities on the proportion of alate offspring produced. Parents in the higher density treatments produced a greater proportion of alate offspring. It is thought that this was a result of the crowding of the young instars as well as the crowding of the parents. Parents fed on cabbage produced a greater proportion of alate offspring than did those on radish. The fecundity of the adults and the survival of the offspring were reduced when fed on cabbage.*

An effect of crowding was imitated by stroking the dorsal surfaces of the head, thorax, fore and hind abdomen, sides of the abdomen, antennae, and legs of adult apterae. These procedures caused no increase in the proportion of alatae among the offspring. A slight response of questionable significance was obtained to stroking over the general dorsal surface. A few first and second instar larvae were also stroked; none became alate.

Temporarily starving and crowding young adult apterae did not cause them to produce more than a normal number of alate offspring. First instar larvae were crowded. If their parents had not been crowded, most developed into apterae. If their parents had been crowded, most became alatae. In the clone studied larvae remained indeterminate as regards wing development at least until the first moult and possibly until the second.

An association between conditions of crowding and the development of winged forms in aphids has been reported (Wadley 1923 in *Rhopalosiphum prunifolia* Fitch, Bonnemaison. 1951 in *Myzus persicae* Sulz. and *Brevicoryne brassicae* L.). Lees (1959) suppressed alate production by raising *Megoura viciae* Buckton individually. In 1961 Lees reported that individually raised apterae which were producing only apterous offspring, produced alate offspring if they were crowded together in a test tube for 24 hours. Johnson (1965) presented substantial evidence that contact between two adults of *Aphis craccivora* Koch caused them to produce winged offspring. He concluded that mechanical rather than visual or olfactory contact effected the response. The dorsal rather than the ventral surface of the parent aphid was affected by the stimulus. Aphids kept together for periods of time as short as 1 minute produced alate larvae. Most of the parents which responded did so completely, that is, their offspring were all alate. Johnson attempted to effect an artificial mechanical stimulus by stroking adult aptera for 2 minutes with a brush. Aphids so treated produced a percentage of alate offspring intermediate between that of singly reared parents and parents which had been in physical contact with other adults. MacGillivray and Anderson (1958) reported that the development of wings in *Macrosiphum solanifolii* Ashm. was not a response to crowding. *Myzus persicae* Sulz. raised concurrently under the same conditions did not develop winged forms.

My original objective was to produce alate offspring by mechanically stimulating (stroking) their apterous parents. The purpose was to

determine whether the essential, functional component of the "effet de groupe" (Bonnemaison 1951) was physical contact among the crowded adult aphids. Concurrent with efforts to induce the production of alatae mechanically, adult apterae were raised under different degrees of crowding to insure that the particular clone studied responded to crowding by producing alate offspring. The results of the first few experiments forced a broadening of the basis of the general objective, which then became an investigation of the effects of intraspecific interaction on the development of wings in the aphid *Myzus persicae* Sulz.

METHODS AND MATERIALS - GENERAL

A clone of *Myzus persicae* was begun in early December 1964 with a single apterous virginopara taken from a radish plant in a greenhouse. The descendants of this individual were raised in a plywood cabinet, in a constant temperature room at 60 F. From March 1965 a Sherer Gro Lab growth chamber was used (500 foot candles at the leaf surface).

The aphids were reared on discs of radish leaf cut with an 18 mm cork borer. The discs were placed, dorsal surface down, on a column of wet cotton wool in a short glass tube. The aphids were contained on the ventral surface of the disc by means of a small cylindrical glass tube. An elastic band secured the cage and also pressed the disc of radish into the cotton wool and ensured contact between the disc and the water in the cotton. The glass tubes were placed in distilled water, in an aluminum tray. Each cage was 12 mm in diameter inside and 9 mm high, enclosing an area on the leaf of 113 mm^2; the glass tube which supported the cotton wool was 35 mm in diameter and 32 mm high. Circles, cut from a nylon fabric (27 strands per mm), were glued with LePage's Pliobond to the tops of the glass cages (fig. 1). Each cage was identified by a number in wax pencil on the glass tube. At the end of each experiment, both cages and tubes were washed and the tubes were refilled with fresh cotton wool. The discs were cut from radish (*Raphanus sativus* L.) plants, variety Forcing Scarlet Globe, grown under natural lighting conditions during the summer. In the fall and winter supplemental artificial light was added to bring the photoperiod to 14 hours. Only vigorous, growing leaves were used. The photoperiod in the growth chamber was set at a constant 16 hours light per 24 hours for all the experiments. The temperature was set at 72 F for 16 hours and 55 F for the remaining 8 hours. The 16 hours at 72 F began 1 hour after the lights were switched on. The growth chamber malfunctioned twice during the series of experiments. Both times conditions were restored to normal within a few hours.

The leaf discs remained in suitable condition for 4-7 days, depending on the density of aphids feeding on them. Both adults and larvae were handled with a moist camel hair brush. The brush was inserted between the front legs from in front and the aphid was moved. Care was taken to avoid touching the dorsal surface.

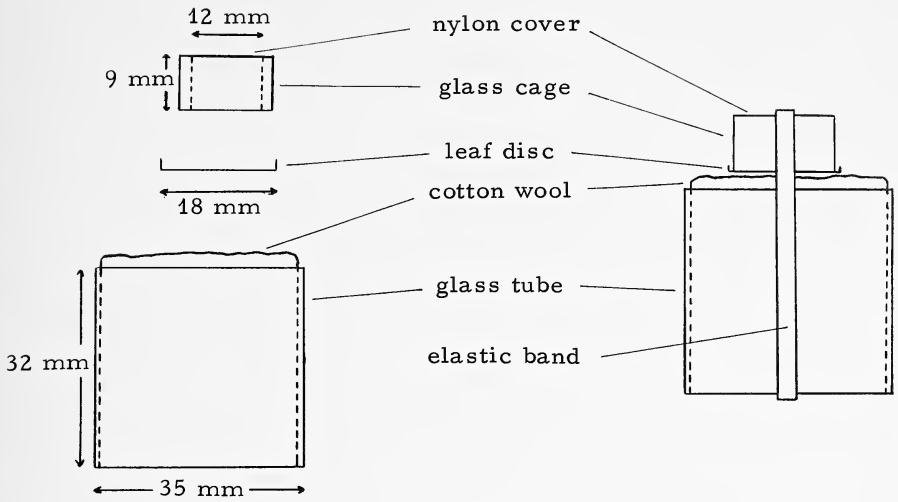

Fig. 1. Rearing cage.

EXPERIMENTS

Twenty five experiments were conducted; 4 on the effect of continuous crowding; 10 on the effect of stroking, 6 with adults and 4 with larvae; 11 on the effects of temporary crowding, 5 of adults from crowded parents, 3 of adults from parents raised singly and 3 of larvae from parents variously raised.

The data from all experiments were analyzed by means of an IBM 7040 computer, program number BMDO2V, an analysis of variance for factorial design. The death of individual aphids resulted in some missing values. Where there was one missing value in an experiment, an empirical value was substituted, calculated by the missing plots method of Goulden (1960). The experiments in which these calculations were made are noted below.

Crowding throughout Reproductive Period

Objective and methods

Adult aphids were subjected to different degrees of crowding during the entire reproductive period in an attempt to confirm that crowded parents produced more alate offspring than parents which were not crowded. Such treatment would also provide information on the relationships, if any, between the age of the parent and the degree of alate production, on the effects of crowding on the fecundity of the parents and on the survival rate of parents and offspring.

Aphids were reared under different population densities for the entire reproductive period. The aphids chosen as parents were selected in the fourth instar from leaf discs on which there were fewer than 10

individuals. When larviposition began, the parents were moved to new discs whenever the number of offspring exceeded 7. An attempt was made to limit the greatest number of offspring per disc to 15. As a result, parents in the greater densities were moved more often than those under less dense conditions. The parents in the lesser densities were picked up and put down when no moving was necessary in order to equalize, across all densities, the number of times parents were disturbed. The offspring were raised to maturity and the form of each, alate or apterous, was noted. A few intermediate forms were born. These were classed as alate.

Four experiments in which parents were crowded during the entire reproductive period were performed. The aphids for the third experiment were selected from among the last offspring of the second experiment and were kept within the density and replication of the parents. In the fourth experiment cabbage as well as radish was used as a host plant.

Results and Discussion

The results of the first experiment (table 1) confirmed the findings of Bonnemaison (1951). The increased proportion of alatae was a result of both an increase in the number of alatae born and a decrease in the number of aptera in the higher densities. The increase in alate offspring did not manifest itself statistically until after the first third of the reproductive period, although the tendency appeared earlier. The length of the reproductive period was much the same at all densities, averaging 21 days. The fecundity of the parents was unaffected by the increased population density, according to the statistical analysis ($F < 1$). However, the number 3 replicate of the solitary group was not a normal individual and perhaps should not have been included in the analysis. It produced 21 offspring, the average of the solitary aphids was 61.2. Aside from this value, there was no overlap between densities. The total offspring per parent per day did not show a statistical difference between densities, but a trend toward decreasing numbers of offspring with increasing parent population density was apparent.

In the first experiment, it seemed that a population density of 4 parents was sufficient to produce the desired effect, therefore, in the next experiment the 8 parent density was eliminated and a 2 parent density begun. The same trend in the proportion of the total offspring which were winged, was observed. In the intermediate density the proportion of alatae was closer to the control than to the highest density (table 2). Unlike the 1, 4, 8 experiment the increase of alatae was greatest in the first third of the reproductive period. The mean total number of offspring (56) was close to that of the 1, 4, 8 experiment (54). However, in the 1, 2, 4 experiment the single parents were the least fecund.

The additional data collected on the mean number of offspring per cage provided two interesting observations. First, although the number born per parent was highly variable (range 41-90), the per cent survival was high (grand mean 87%). The second observation was the highly significant difference between treatments in the mean number of offspring per cage for the first third of the reproductive period and for the total period. In both these parameters the higher density parent

treatments had more offspring per cage (table 2). The third experiment used the second generation from the second experiment at the same densities (1, 2, 4). Two of the solitary parents died soon after the experiment was begun. Analyses of variance were done using a desk calculator. In response to the observation made in the first generation experiment on the mean number of offspring per cage, great care was taken to keep the size of the offspring groups fairly small; otherwise conditions were similar. The results of the second generation 1, 2, 4 experiment are given in table 3. The total number born per parent was even more variable than in experiment 2 (range 12-86); the average was 52. The per cent survival was high (88) and showed little variation (range 82-95%), as in the first generation. However, the per cent alatae differed from both earlier experiments and did not increase with increased parent population density. There was no statistical difference at P = 0.5 between the single parent treatment (10%) and the 4 parent treatment (12%). Very few more alate offspring were born to the crowded parents than to the parents raised singly. Two explanations of the contradiction in the results of the proportions of alatae are possible. The results of the second generation 1, 2, 4 experiment might be due to a "generation" effect. It is possible that the environment experienced by the grandparent (parents in experiment 2) affected the progeny of experiment 3. The phenomenon which makes such a situation possible is the telescoping of generations characteristic of aphids. Very often, the more mature embryos in a parent have within themselves developing embryos. Lees (1959) has shown that such a "generation" effect is possible in the production of sexuals. However, I do not think this is a satisfactory explanation here. If the stimulus which diverts an embryo toward becoming apterous also diverts embryos which it contains, then a first generation of aptera would have to be followed by a second generation of aptera. This is not so. Also, although the conditions under which the grandparents were kept as adults differed, the parents were raised to maturity under very similar conditions, i.e. between 5 and 10 individuals per radish disc.

A better explanation is the one suggested earlier from the results of the first 1, 2, 4 experiment. The size of the offspring group might have an effect on the number of larvae within that offspring group which developed wings. Very few of the offspring groups in experiment 3 exceeded 15. A χ^2 test (Goulden 1960) was performed testing the independence of size class of offspring groups (< 10, 10-15, > 15), and the number of alatae and apterae in these groups. In experiment 2 there was a marked association between a large offspring group and alatae, and a corresponding association between a small offspring group and apterae $\chi^2 = 123.9^{**}$ There were no such associations in experiment 3 ($\chi^2 = 2.46$).

If the association between a high proportion of alatae and a high larval population density is valid, then perhaps the initial premise that high parent population density is the principal cause of alate production is incorrect. That is, the postnatal environment rather than the prenatal environment with respect to crowding may be more or at least equally important. The lack of association between a high percentage of alates and a high larval population density in the second generation 1, 2, 4 ex-

periment could be interpreted as a threshold response. It may take more than 10-15 larvae to make a crowd.

TABLE 1. First crowding experiment. Mean numbers of alate and apterous offspring born to apterous parents raised singly or in groups of 4 or 8.

	Number of offspring per parent			F	Grand mean
	1 parent	4 parents	8 parents		
Segment of reproductive period					
1st third					
alate	4.0	10.2	9.2	4.07	7.8
apterous	16.5	8.0	3.0	5.63*	9.2
2nd third					
alate	3.0	17.5	14.2	30.55**	11.6
apterous	27.5	9.2	5.2	11.31**	14.0
Last third					
alate	1.2	4.2	7.8	15.24**	4.4
apterous	9.0	7.8	5.5	< 1	7.4
Total					
alate	8.2	32.0	31.2	64.46**	23.8
apterous	53.0	24.8	13.2	7.38*	30.3
Reproductive period in days	20.0	21.8	21.2	< 1	21.0
Total offspring:					
per day	3.1	2.6	2.1	< 1	2.6
maturing	61.2	56.8	44.5	< 1	54.2
% alate	16.4	56.6	70.0	101.16**	47.7

* = $P < .05$; ** = $P < .01$

Bonnemaison reported that *Myzus persicae* raised on radish produced more alatae than *M. persicae* raised on cabbage. If the hypothesis that alatae are a result of mechanical stimulation is correct, then there is an implication that aphids raised on radish are more restless than aphids raised on cabbage, i. e. that on cabbage there is less intraspecific interaction. Another implication is that singly raised aphids, whether on radish or cabbage, should give birth to few alate offspring. A fourth experiment was performed with these considerations in mind. The results (Awram 1966, table 5) were quite variable; however these observations could be made. On radish the pattern of results was similar to the first two experiments. The greater the population density of the parents, the greater was the proportion of winged offspring (22% for singly kept parents, 30% for parents kept in pairs, 43% for parents kept in groups of 4). Again the increased proportion of alatae was a result

of both an increase in alatae and a decrease in apterae in the high densities. On cabbage results were similar except that the mean proportion of alatae in the intermediate density exceeded that in the high density (34% alatae for singly kept parents, 58% for paired parents, 49% for parents kept in groups of 4).

TABLE 2. Second crowding experiment. Mean numbers of offspring born to apterous parents raised singly or in groups of 2 or 4.

	Number of Offspring			F	Grand mean
Segment of reproductive period	1 parent	2 parents	4 parents		
1st third					
alate	0.8	6.2	12.8	13.25**	6.6
apterous	15.8	16.2	11.2	3.00	14.1
mean per cage	11.2	13.8	24.0	34.78**	16.3
2nd third					
alate	2.5	9.2	10.0	1.90	7.2
apterous	12.2	11.2	9.8	< 1	11.1
mean per cage	16.5	16.2	21.0	3.66	17.9
Last third					
alate	3.0	3.5	3.8	< 1	3.4
apterous	14.5	15.5	11.5	1.59	13.8
mean per cage	12.8	12.5	13.2	< 1	12.8
Total					
alate	6.2	18.5	26.5	6.37*	17.1
apterous	42.5	42.8	32.5	2.83	39.2
mean per cage(av.)	13.2	14.2	19.0	31.72**	15.5
Total offspring reaching maturity	48.8	61.0	58.5	1.93	56.1
% survival	86.0	86.8	88.3	< 1	87.0
% alate	14.9	27.3	46.2	8.13*	29.4

* = $P < .05$; ** = $P < .01$

Bonnemaison's observations with respect to host plant were not confirmed; in fact, the opposite result was obtained. Both the 2 and 4 parent densities on cabbage (58%, 49%, respectively) had a greater proportion of alatae than the corresponding radish densities (30%, 43% respectively). The hypothesis that intraspecific interaction maintains alate

production is, however, not disproved. The effects of radish and cabbage are just reversed. That cabbage was a less desirable host plant could not be doubted. The per cent survival on radish was characteristically high at 91.2 as compared to 71.7 on cabbage. The fecundity of the parents was also affected. The number born was less on cabbage than on radish. Cabbage would presumably result in restless aphids, there would be greater intraspecific interaction and thus a greater percentage of winged individuals.

χ^2 test for independence on the data of the fourth experiment again indicated an association between a high proportion of alatae and a high larval population density ($\chi^2 = 71.36^{**}$).

TABLE 3. Third crowding experiment. Mean numbers of offspring born to apterous parents raised singly or in groups of 2 or 4.

	Number of Offspring			F	Grand mean
	1 parent	2 parents	4 parents		
Segment of reproductive period					
1st third					
alate	1.0	0.5	3.2		1.7
apterous	14.0	13.0	18.8		15.5
mean per cage	10.5	10.2	12.2		11.1
2nd third					
alate	0.5	0.5	2.5		1.3
apterous	11.0	13.2	19.2		15.2
mean per cage	10.0	11.8	15.2		12.8
Last third					
alate	1.5	0.2	0.5		0.6
apterous	11.5	10.5	11.0		10.9
mean per cage	8.5	12.0	11.8		11.2
Total					
alate	4.0	2.0	6.8	4.79	4.1
apterous	36.5	36.6	48.5	< 1	41.4
mean per cage	9.0	10.8	13.2	3.40	11.4
Total offspring reaching maturity	39.5	38.2	55.2	0.73	45.3
% survival	93.8	84.4	87.4		87.5
% alate	9.6	4.2	12.2	2.79	8.5

Stroking

Objective and methods

One of the more obvious effects of crowding is increased physical contact among the individuals crowded. The mechanical stimulation resulting from this increased contact might influence wing morphogenesis. If mechanical stimulation is the principal factor eliciting the production of alatae, then stroking the adults or the early instar larvae or both should produce more winged forms. Aphids were stroked at various frequencies, over the entire dorsum and over particular parts of the dorsum, to determine if alate production could be so induced (or more properly if apterae production could be reduced) and to find out if a particular area was especially sensitive.

Adults - Fourth instar larvae were removed from offspring groups of 10 or fewer individuals and confined alone in separate cages on radish discs. Stroking began after they had moulted into the adult but before they had offspring, or on the first day that offspring were born. They were stroked at different frequencies with either the leg of an adult apterous aphid, a *Drosophila* leg, or a human hair. The direction of stroking was more or less caudad, with the tarsal claws of the legs raked over the area being stroked. The aphid and *Drosophila* legs and the hair were manipulated with fine forceps under X 12 power of a stereo binocular microscope. Some treatments consisted of timed contact with a live adult aphid which had been mounted with paraffin on a pin. The parent aphids were moved when their offspring numbered more than 7, usually every second day. In the first experiment only, parents were moved almost daily.

There were 3 treatments and a control and 4 replicates in each experiment. Missing values calculations were made for 1 replicate each in the third, fourth, and fifth experiments. In the first experiment 100 strokes were applied daily with a hair, a *Drosophila* leg, and the leg of an adult aphid respectively to the entire dorsal surface. In the second experiment one batch spent 2 minutes daily in contact with a live adult aphid, the other two received 300 strokes daily with the leg of an adult aphid, and with a hair respectively, again on the entire dorsal surface. In the third experiment 500 strokes daily were applied with the leg of an adult aphid to the head, the thorax, and the abdomen respectively. In the fourth experiment 400 strokes were applied daily with the leg of an adult aphid to the hind half of the abdomen, the front half of the abdomen, and to the legs respectively. In the fifth experiment 500 strokes were applied daily with the leg of an adult aphid to the antennae and to the sides of the abdomen, and with a hair to the entire dorsal surface. In the final experiment one batch had 1000 strokes applied daily to the entire dorsal surface with the leg of an adult aphid, one had 5 minutes daily contact with a live adult aphid, and the last had a twice daily smearing with cornicle secretion.

Larvae - The association revealed in all crowding experiments except the third between a high proportion of alate offspring and a large offspring group, provided a basis for suspecting that in *M. persicae*, the postnatal environment may be of greater consequence than prenatal conditions. Four experiments were done to test this suspicion. First and

second instar larvae were stroked at different frequencies with a detached aphid leg. Larvae so treated were of 2 types, either from uncrowded (first 2 experiments) or from crowded (last 2 experiments) parents. They were removed from the parent within 8 hours of birth and raised to maturity alone. The first two experiments were the same: from 3 to 5 aphids were in each batch. One batch was stroked 500 times in the first instar, one batch 500 times in first and second instars, a third batch served as an un-stroked control. The last two experiments depended on the results of the first two and are described below under that heading.

Results and discussion

Adults - In the first adult stroking experiment (table 4), parents receiving 100 strokes daily with an aphid leg gave birth to a proportion of alate offspring much greater than others. The higher percentage of alatae was a result more of increased alate births than of decreased apterous births. In the first third of the reproductive period the number of alate offspring was about the same as in the other treatments; by the last third, the increased incidence of alate offspring was of statistical significance. The length of the reproductive period was about the same for all treatments, averaging 12.8 days. The total offspring reaching maturity during the experiment averaged 47.7 per parent but varied from 6 to 78.

TABLE 4. First adult stroking experiment. Mean numbers of offspring born to apterous parents stroked 100 times daily with a hair, a *Drosophila* leg, or an aphid leg.

Segment of reproductive period	100 strokes hair	100 strokes *Drosophila* leg	100 strokes aphid leg	Control	F
1st third					
alate	1.5	1.2	0.2	1.5	< 1
apterous	14.0	11.0	9.2	15.2	1.02
2nd third					
alate	1.5	2.0	7.0	4.8	2.99
apterous	16.0	12.5	7.4	13.2	< 1
Last third					
alate	3.5	1.8	9.5	1.5	5.90*
apterous	15.2	14.0	11.5	15.2	< 1
Total					
alate	6.5	5.0	16.8	7.8	7.93**
apterous	45.2	37.5	28.2	43.8	< 1
reaching maturity	51.8	42.5	45.0	51.5	< 1
% alate	9.8	9.6	41.4	14.3	14.53**

* = $P < .05$; ** = $P < .01$

For the second experiment (table 5) the *Drosophila* leg was dropped as a stroking device because of its poor utility as such an instrument and because it seemed no more effective than the hair. The difference between treatments, with respect to proportion of alatae, narrowly missed statistical significance at $P < 0.05$. The percentage of alatae was greatest from parents which had contact with a live aphid, next from those stroked with an aphid leg, then those stroked with a hair, and least from the un-stroked control. This supports the hypothesis that alatae are determined by intraspecific interaction. The argument is further supported by the numbers of alatae and apterae in each treatment during each third of the reproductive period. The mean size of the offspring groups was greatest from adults stroked with a hair, however these gave the second lowest proportion of alate offspring. The rate of survival was characteristically high with a grand mean of 89%.

TABLE 5. Second adult stroking experiment. Numbers of offspring born to apterous parents stroked daily with an aphid leg or a hair or kept in contact with a live aphid for 2 minutes.

	Treatment Description				
	Control	2 minutes contact with live aphid	300 strokes aphid leg	300 strokes with hair	F
Total number of offspring					
alate	8.0	19.0	12.2	14.2	1.33
apterous	30.0	20.2	20.0	40.0	3.93*
reaching maturity	38.0	39.2	32.2	54.2	1.59
Mean per cage	12.0	12.5	13.0	16.2	10.99**
% survival	82.3	96.0	85.4	90.8	3.85
% alate	17.4	46.1	38.0	26.3	3.70

* = $P < .05$; ** = $P < .01$

The frequency of stroking in this second experiment was 3 times that in the first, yet there was not a corresponding increase in the effect which the stroking was presumed to induce. However, I viewed the results optimistically and reasoned as follows. If mechanical stimulation was the cause of alatae production, then perhaps there was some special area of the body most receptive to such stimulation. The next 3 experiments were designed to explore this. Concentration of stroking in a small area would also have the effect of increasing the intensity of the treatment. I hoped that the dorsal abdominal area would be a sensitive one because the often observed phenomenon of alatae giving birth only to apterae might then be explained; receptors on the abdomen would be

covered by the wings and could not therefore be stimulated.

The results (tables 6, 7 and 8) were disappointing. In the first 2 experiments the controls had the highest proportion of alatae. In the last experiment the controls had the second highest, being slightly exceeded by those given 500 strokes to the antennae. In none of these 3 experiments was there any statistical difference of consequence in alate production. The per cent alatae increased through the 3 experiments. There was a corresponding increase in the average size of the offspring group and in the per cent survival. A χ^2 test was performed to determine if there was an association between the type of progeny and the size of the offspring groups. The χ^2 values were respectively 2.66, 10.01**, 51.08**.

TABLE 6. Third adult stroking experiment. Numbers of offspring born to apterous parents stroked on head, thorax, or abdomen.

	Treatment Description				
	500 strokes head	500 strokes thorax	500 strokes abdomen	Control	F
Total number of offspring					
alate	5.5	1.5	3.2	3.2	1.53
apterous	38.8	28.0	23.8	21.8	2.53
reaching maturity	44.2	29.5	22.0	25.0	2.68
Mean per cage	10.2	9.0	7.8	9.8	2.83
% survival	93.4	77.7	82.5	80.8	< 1
% alate	13.1	6.6	8.7	15.4	1.05

The results of the 3 experiments on regional stroking caused me to doubt my original success with the first 2 stroking experiments. A final experiment was done to confirm or refute this success. The cornicle secretion treatment was introduced to round off the experiment to 4 treatments. Since it is possible that the intraspecific interaction is of a chemical as well as, or rather than, a mechanical nature, I thought it worth trying this most obvious secretion. Cornicle exudate from roughly handled aphids was brushed against the aphids and solidified almost immediately; by the end of the experiment, these parents were coated with the hardened secretion.

The differences (table 9) in the per cent alatae were not statistically significant, but the treatments rank in much the same order as in the first experiments so far as these are comparable. Again the number of alatae and apterae corresponded to what would reasonably be expected. The live aphid and stroking treatments had more alate offspring

and less apterous offspring than the control. The control had the least alate and the most apterous offspring. Statistical significance was reached in the differences in apterous offspring in the second and third batches of offspring. The cornicle secretion treatment was ineffective.

TABLE 7. Fourth adult stroking experiment. Numbers of offspring born to apterous parents stroked daily.

	Treatment Description				
	400 strokes hind abd.	400 strokes fore abd.	400 strokes legs	Control	F
Total number of offspring					
alate	7.0	11.8	7.0	13.2	1.95
apterous	48.0	48.0	32.0	35.0	1.57
reaching maturity	55.0	59.8	39.0	48.2	1.14
Mean per cage	10.5	10.2	9.0	10.8	2.24
% survival	87.5	85.6	78.6	92.0	< 1
% alate	12.0	19.8	12.8	27.1	3.83

TABLE 8. Fifth adult stroking experiment. Numbers of offspring born to apterous parents stroked 500 times daily.

	Treatment Description				
	Control	500 strokes antennae with aphid leg	500 strokes sides of abdomen with aphid leg	500 strokes dorsum with hair	F
Total number of offspring					
alate	23.5	18.2	14.5	20.8	1.11
apterous	46.8	39.8	55.2	62.5	3.57
reaching maturity	70.2	58.0	69.8	83.2	4.60*
Mean per cage	17.2	15.5	15.0	15.2	1.88
% survival	94.3	96.6	91.3	94.9	1.60
% alate	32.9	33.5	21.8	24.0	1.35

* = $P < .05$

TABLE 9. Sixth adult stroking experiment. Numbers of offspring born to apterous parents variously treated daily.

	Treatment Description				
Total number of offspring	1000 strokes dorsum with aphid leg	5 minutes contact live aphid	Cornicle secretion twice daily	Control	F
alate	10.8	16.2	6.0	4.8	2.78
apterous	29.8	27.0	30.2	38.8	2.52
reaching maturity	40.5	43.2	36.2	43.5	< 1
Mean per cage	11.5	13.2	11.2	12.8	2.30
% survival	92.4	90.1	87.0	86.0	< 1
% alate	24.4	37.4	16.8	10.5	2.71

Reasons for the conflicting results obtained in the stroking experiments were not obvious to me. There appeared to be a response to general stroking over the entire dorsal surface. I can offer no explanation why a stroking frequency of only 100 strokes per day was much more effective than 300 or 1000 strokes per day. The lack of response when stroking was restricted to particular areas implies that the sensitive area, if such an area exists, was missed. This seems unlikely because the areas chosen covered most of the dorsal surface.

Larvae - I have followed a general tendency to think in terms of establishing conditions that result in the production of alatae. As Johnson and Birks (1960) have stressed, one should think in terms of conditions that result in the production of apterae. The embryo probably begins its development toward an alate form. Along its developmental path, it encounters conditions which may keep it on the path to becoming alate or "switch" it into an apterous pathway. Such a switch may be irreversible. This means that conditions designed to maintain an embryo or larva on the alate path may be imposed in vain if that embryo or larva has already been switched to the apterous path. The first two larval stroking experiments may have suffered from this oversight since they were done with the progeny of a single parent which had been raised under uncrowded conditions. None became alate.

Two further experiments were therefore done for which the parents were under very crowded conditions (17 per 50 mm^2) for 4 days before the experiments began. The offspring of 3 of these parents were used for 3 aphid leg stroking treatments and a control in the first experiment and 4 treatments and a control in the second. In the first 200 strokes 3 times in the first instar, in the second instar, and in both of these instars were applied. In the second 300 strokes once in the first

instar, 300 strokes twice in the first instar, 300 strokes once in the first and in the second instar, and 300 strokes once in the first and twice in the second instar were applied. None developed into alatae. The offspring, all apterae, were kept and the first 2 batches of their offspring were collected and raised to maturity. No third generation effects could be observed with respect to the proportion of alatae born (Awram 1966, tables 17 and 18).

Temporary Crowding

Objective and methods

The results which Lees (1961, *M. viciae*) and Johnson (1965, *A. craccivora*) reported on the effect of temporary crowding were unequivocal. In *M. viciae*, individuals which had been producing essentially all apterous offspring switched completely to the production of essentially all alate offspring after they had been crowded for 24 hours. In *A. craccivora*, Johnson found that a similar change could occur after as little as 1 or 2 minutes contact between as few as 2 aphids. I tried similar temporary crowding experiments to clarify the confusing results which I obtained in the rearing and stroking experiments.

Adult aphids were crowded at different densities, for different periods of time away from the host plant in an attempt to induce the production of alate forms. It was hoped to learn the smallest amount of "togetherness" which evoked a response. First and second instar larvae were also crowded for short periods to determine whether or not high postnatal population densities could maintain the larvae on the path to the alate condition.

Adults - Adult aphids were crowded in the standard sized cage previously described, and also in a small cage 8 mm in diameter (inside measurement) and 9 mm high, enclosing an area on the leaf of 50 mm^2, and in microtubes 4 mm in diameter and 7 mm long (inside measurements). They were maintained in these containers on moist cotton. No leaf discs were available to them during the period of their confinement. After a set period of time, they were placed on fresh leaf discs and kept individually. Their first 2 batches of offspring were raised to maturity and the form they had taken was noted. All other conditions were similar to those described above under methods and materials - general. The periods of crowding which lasted less than 16 hours were imposed during the day. The 16 hour periods included 8 hours of night. Because of the lack of time and space, not all 16 of the aphids crowded 16 together could be used; 4 were selected to represent that treatment. A detailed description of the temporary crowding experiments follows.

There were 4 replicates in each treatment in each of the five experiments with adults from crowded parents. One estimate for a missing value was calculated in each of the 4 and 16 hour treatments in the second experiment. In the first temporary crowding experiment single aphids were kept in the microtube overnight (about 12 hours), and groups of 4 aphids were kept in the microtube 1 hour, and overnight (about 12 hours) (table 10). In the other four experiments all aphids were crowded and held in the standard sized cage (see materials & methods - general). In the second temporary crowding experiment 1 aphid was kept alone away from the host plant for 1 hour, for 2 hours, for 4 hours, and for 16 hours

(table 11). The third (table 12), fourth (table 13), and fifth (table 14) experiments on temporary crowding were the same except 2, 4, and 16 aphids respectively were crowded away from the host plant for the same time periods.

TABLE 10. First experiment on temporary crowding of adults from crowded parents. Numbers of offspring born to apterous parents kept alone or 4 together in microtubes.

	Treatment Description			
Number of offspring	1 aphid in microtube overnight	4 aphids in microtube 1 hr.	4 aphids in microtube overnight	F
alate				
1st batch	5.8	6.0	2.8	5.66*
total	11.8	23.8	15.8	3.21
apterous				
1st batch	5.5	10.2	10.0	4.92*
total	28.2	42.0	42.5	3.90*
reaching maturity	40.0	65.8	58.2	8.07*
mean per cage				
1st batch	14.5	17.8	13.8	1.70
total	11.0	13.8	13.2	2.25
% survival	74.0	94.3	89.6	7.17
% alate	29.5	36.2	27.1	1.19

* = $P < .05$

TABLE 11. Second experiment on temporary crowding of adults from crowded parents - control, uncrowded. Numbers of offspring born to apterous parents starved alone for 1, 2, 4, or 16 hours.

	Treatment Description			
Number of offspring	alone 1 hr.	alone 2 hr.	alone 4 hr.	alone 16 hr.
alate	2.8	10.0	6.0	9.0
apterous	16.2	19.8	16.7	21.3
reaching maturity	19.0	29.8	22.7	30.3
mean per cage	13.0	16.2	14.7	19.0
% survival	71.3	91.0	80.0	81.5
% alate	16.9	32.4	26.4	28.5

TABLE 12. Third experiment on temporary crowding of adults from crowded parents. Numbers of offspring born to apterous adults crowded 2 together for 1, 2, 4, or 16 hours.

	Treatment Description				
Number of offspring	2 crowded 1 hour	2 crowded 2 hours	2 crowded 4 hours	2 crowded 16 hours	F
alate					
1st batch	2.0	1.0	4.8	8.2	6.66*
2nd batch	1.5	2.5	0.2	2.0	2.03
total	3.5	3.5	5.0	10.2	3.44
apterous					
1st batch	9.2	10.8	11.2	5.2	1.28
2nd batch	6.5	7.0	3.8	19.8	14.36**
total	15.8	17.8	15.0	25.0	1.29
mean per cage					
1st batch	14.0	12.5	20.5	14.8	1.40
2nd batch	8.2	10.0	7.5	25.0	13.14**
total	11.0	12.0	17.5	20.0	2.23
% survival	86.4	90.9	76.2	88.5	1.86
% alate	22.1	16.1	20.8	30.6	1.05

* = $P < .05$; ** = $P < .01$

Three experiments were done on the effect of temporary crowding of adults from parents raised singly. Only a control and the most intense crowding were used. First instar larvae from uncrowded parents were raised to maturity individually. In the first experiment 8 were then maintained separately as controls, 16 were crowded together for 16 hours (16 per 113 mm^2), after which they were again kept separately. The first several batches of offspring were collected and raised to maturity. The second experiment was the same except that only 4 controls were used and of the 16 crowded together, 8 were selected as progenitors. The 16 aphids were crowded in the small cage (50 mm^2). The use of the small cage increased the effective population density by a factor of 2.25 (table 15). The third experiment was identical except that the aphids were crowded in their fourth instar, rather than as newly emerged adults (table 16).

Larvae - In 3 experiments first instar larvae less than 12 to 24 hours old were crowded in the small cage for 12, 16 or 24 hours at densities of 16, 32 or 48 per 50 mm^2 (table 17).

TABLE 13. Fourth experiment on temporary crowding of adults from crowded parents. Numbers of offspring born to apterous parents crowded 4 together for 1, 2, 4, or 16 hours.

	Treatment Description				
Number of offspring	4 crowded 1 hour	4 crowded 2 hours	4 crowded 4 hours	4 crowded 16 hours	F
alate					
1st batch	2.8	3.8	0.6	9.0	7.07**
2nd batch	0.8	3.2	0.0	3.5	3.17
total	3.5	7.0	0.5	12.5	6.70*
apterous					
1st batch	16.5	16.0	11.2	7.2	3.79
2nd batch	12.8	6.8	9.2	22.5	13.92**
total	29.2	22.8	20.5	29.8	1.73
mean per cage					
1st batch	21.2	22.8	17.0	18.0	< 1
2nd batch	14.0	9.8	11.0	27.5	13.34**
total	17.5	16.2	14.2	22.8	1.94
reaching maturity	32.8	29.8	21.0	42.2	3.37
% survival	93.0	93.8	75.3	92.3	2.26
% alate	11.3	20.6	2.7	28.8	11.09**

TABLE 14. Fifth experiment on temporary crowding of adults from crowded parents. Numbers of offspring born to apterous parents crowded 16 together for 1, 2, 4, or 16 hours.

Number of offspring	16 crowded 1 hour	16 crowded 2 hours	16 crowded 4 hours	16 crowded 16 hours	F
alate					
1st batch	3.5	4.0	1.2	4.8	1.40
2nd batch	2.5	4.0	0.5	8.2	1.41
total	6.0	8.0	1.8	13.0	2.10
apterous					
1st batch	10.8	14.8	6.5	9.8	3.25
2nd batch	10.5	9.8	3.2	11.2	1.99
total	21.2	24.5	9.8	21.0	2.62
per cage (av.)					
1st batch	17.0	20.8	12.8	16.2	3.51
2nd batch	14.8	15.0	3.8	22.0	8.52*
total	16.0	17.8	9.2	18.8	6.10*
reaching maturity	27.2	32.5	11.5	34.0	10.52**
% survival	86.0	92.1	59.0	89.6	4.51
% alate	26.0	22.4	16.8	37.6	< 1

* = $P < .05$; ** = $P < .01$

TABLE 15. First and second experiments on temporary crowding of adults from parents raised singly. Numbers of offspring born to apterous parents kept individually or crowded 16 together.

Number of offspring	8 adults kept individually	16 adults 16 hours small cage	16 adults 16 hours large cage	t
alate				
1st exp.	26.3		18.7	0.01
2nd exp.	13.8	6.6		0.36
apterous				
1st exp.	42.6		43.2	0.63
2nd exp.	16.0	20.4		0.27
reaching maturity				
1st exp.	68.9		61.9	0.44
2nd exp.	29.8	27.0		1.29
per cage (av.)				
1st exp.	18.3		17.3	0.29
2nd exp.	16.8	14.9		0.26
% survival				
1st exp.	94.2		93.8	0.15
2nd exp.	89.8		91.4	2.40
% alate				
1st exp.	38.0		30.0	1.02
2nd exp.	45.4	24.2		2.54

TABLE 16. Third experiment on temporary crowding of adults from parents raised singly. Numbers of offspring born to apterous parents crowded 16 together for 4 hours in their fourth instars.

Number of offspring	Control	16 crowded 4 hours	t
alate			
1st batch	1.8	3.2	0.32
2nd batch	0	6.2	2.28
total	1.8	9.5	1.62
apterous			
1st batch	15.2	13.5	0.65
2nd batch	17.8	13.8	1.54
total	33.0	27.2	0.36
per cage (av.)			
1st batch	18.5	18.1	0.73
2nd batch	20.0	22.8	2.68*
total	19.5	20.4	0.56
% survival	90.4	90.4	0.85
% alate	4.9	25.0	2.96*

* = $P < .05$

TABLE 17. χ^2 for independence between crowded and uncrowded larvae, and development into the apterous or alate state. Results of temporary crowding of larvae from uncrowded parents (above) and from crowded parents (below).

	Apterous		Alate		
	Observed	Expected	Observed	Expected	
Parents uncrowded					
Control - raised singly	3	2.25	2	2.75	
Treated - 16/50 mm^2	6	6.75	9	8.25	
					= 0.61
Control	6	4.29	0	1.71	
Treated - 32/50 mm^2	9	10.71	6	4.29	
					= 3.34
Parents crowded					
Control	6	3.3	0	2.70	
Treated - 48/50 mm^2	10	12.7	13	10.30	
					= 6.19*

* = $P < .05$

Results and discussion

Adults - In the first temporary crowding experiment (table 10) no difference in the proportion of alate offspring among the treatments could be observed. The per cent alatae was almost identical in the aphids kept individually and in those crowded together in a microtube. Four crowded for 1 hour had the greatest proportion of alatae but the difference was not significant. The significantly high F values obtained for the first batch apterous, total offspring, and total apterous were, I think, a result of the unusually small per cent survival of those kept singly (74%).

From the second experiment (table 11) with single aphids off the host for different times, many results were missing so that this could not be analyzed by the IBM program. Only the means are given. This was essentially a test of the effect of different periods of temporary starvation on the production of offspring. No pattern could be observed in any of the measurements taken. The number of alate offspring, the number of apterous offspring, the total number of offspring, the proportion of offspring which were alate, the total offspring born and the per cent survival were not noticeably affected by 1, 2, 4, or 16 hours of starvation.

The results of the third experiment (table 12) were similar to those of the second temporary crowding experiment. There appeared to be no association between the proportion of alatae produced and the length of time that 2 parent aphids were in the same cage with one another. The exception to this general observation was the number of alate offspring in the first batch. Significantly more alate (8.2) were born to individuals caged together for 16 hours than to those caged for 1 and 2 hours. An intermediate number (4.8) was born to the individuals in the intermediate treatment (4 hours). The significant F values for the second batch of

apterae was probably a result of the zero values for replicates 1 and 2 in this treatment. These would probably be best viewed as missing values. The significant F value for the size of the offspring group of the second batch also reflected the zero of replicate 2 in this treatment. The large number of offspring per cage from those crowded for 16 hours might be suspected as the cause of the high per cent alatae of this treatment. However, the second batch here was almost all apterous.

A number of the parameters in the experiment with groups of 4 off the host (table 13) displayed statistically significant differences among the treatments. Most important of these was the total per cent alatae. The proportion of winged offspring was greatest after 16 hours crowding. The percentage after 1 hour crowding was considerably less and that after 2 hours crowding was intermediate. However the pattern was broken by the 4 hours crowding which gave the lowest per cent alatae. The situation was the same for alate offspring in the first batch, the 4 hour treatment falling outside the pattern. The difference between treatments shown by the number of apterous offspring in the second batch was a manifestation of the unusually high fecundity of the individuals crowded for 16 hours, which gave a much higher average number of offspring per cage in the second batch than the other treatments. The large size of the offspring group was not associated with a corresponding increase in alatae.

The experiment with groups of 16 (table 14) completed the symmetry of the experimental design of the crowding experiments. There was no difference between treatments in the number of alatae or apterae produced or in the per cent alatae. The significance attained with respect to total offspring, average, and second day size of offspring group and the total born was caused by the partial sterility and low survival rate of the individuals crowded for 4 hours.

The data obtained from the latter four experiments on temporary crowding of adults from crowded parents differed from some of the results obtained by Lees and Johnson. Several explanations are possible. Both these workers had raised the parent aphids used in their experiments separately. Mine were taken from groups of 10 or fewer individuals. The results I obtained may have been confounded by this previous association. Another possible explanation is that the density of parents was not great enough. It is possible that the 16 aphids would not encounter one another often enough to produce a response. A third explanation might be that the 4 individuals selected to represent the 16 of the high density treatments had never been walked upon, i. e. had themselves been the most active. Still another possible explanation is that I did not use enough replicates. The last three experiments were performed to correct these possible defects. Because there were only 2 treatments in each of these experiments, t rather than F values were calculated.

In the first experiment one of the control parents and 2 of the treated parents did not survive long enough to produce offspring. There was little difference (table 15) between the 2 treatments in any of the measurements taken in either of the first two experiments. Both control groups had the higher proportion of alatae, 38% against 30% in the first and 45% to 24% in the second experiment. As in the first experi-

ment with adults from parents raised singly, none of the measurements taken showed a significant difference between the controls and the treated individuals (table 15). Again, the controls had a greater per cent alatae than the treated aphids.

The results of the last experiment, in which aphids were crowded in their fourth instar, rather than as newly emerged adults are given in table 16. There is a significant difference in the proportion of alatae between the controls and the treatment parents, the treated having a greater per cent alatae. The greater proportion of alatae was a consequence of both more alatae in the treated and more apterae in the untreated group. The other significant factor was the size of the offspring group in the second batch. It was larger for the treated group and therefore postnatal effects could not be ruled out as a cause for the increased alate production.

Larvae - Crowding first instar larvae of comparatively uncrowded parents (raised in groups numbering less than 10) at a density of 16 per 50 mm^2 did not result in proportionately more becoming alate than among the uncrowded control individuals ($\chi^2 = 0.61$). Where the parents were raised individually for most of their lives, the difference in the proportion of alatae between crowded and uncrowded larvae appeared to be more definite, perhaps because the crowding was twice as severe as in the first experiment ($\chi^2 = 3.34$). The more intensive crowding still of the treated individuals in the last experiment resulted in a still larger proportion becoming alate ($\chi^2 = 6.19*$). The effects of heavy parental crowding were apparently reversed in the control individuals.

GENERAL DISCUSSION

A few observations that transcended the 3 general types of experiments were made and are discussed here.

Fig. 2 is a plot of the per cent alate of control treatments against time. The variation was considerable and there was no discernible long term trend. Bonnemaison (1951) found that over a 6 month period during which he experimented with *M. persicae*, there was a general tendency for increased alate production with increased age of the clone. This was not so in my experiments over the period June through December 1965.

No matter how careful I was to keep a parent isolated from other adults during both its infancy and adulthood, I was never able to prevent completely the occurrence of some alatae among its offspring. Fig. 2 illustrates the point. Never were the offspring of control parents entirely apterous. This implies that the production of a small proportion of alatae may be obligatory in the clone studied.

The rate of survival of offspring was high (average 88.1 per cent) in all of the experiments. An exception was the larvae which were stroked during their first and second instars, many of which died before reaching maturity. The survival rate of the parents was also high. Only 14 of 421 died before leaving 7 or more offspring.

The results I have obtained differ from Bonnemaison's, Johnson's, and Lees' on several points. Rearing aphids under crowded conditions

temporary crowding of apterous parents resulted in an increased proportion of alate offspring. Mechanical stimulation of apterous parents by stroking did not result in a predictable increase in alate offspring. When a parent aphid seemingly responded to a treatment by producing alate offspring, it did not necessarily continue to produce alate offspring. A few examples, fig. 3, have been selected to illustrate this characteristic.

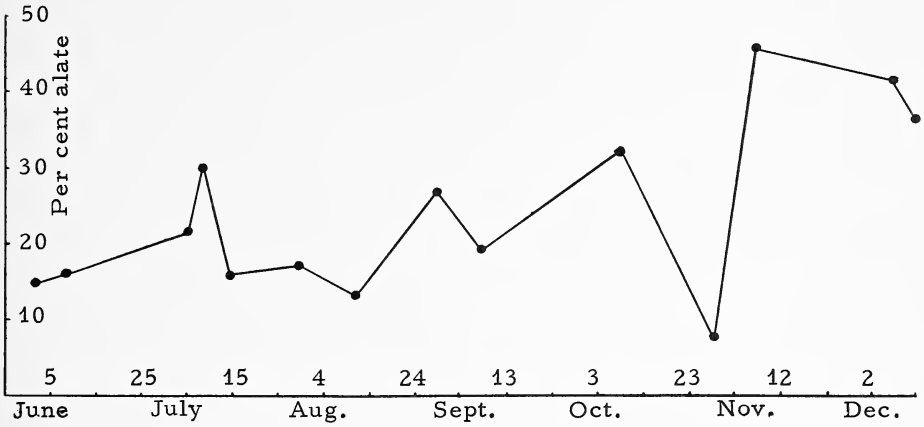

Fig. 2. Per cent alate in control treatments for June to December 1965.

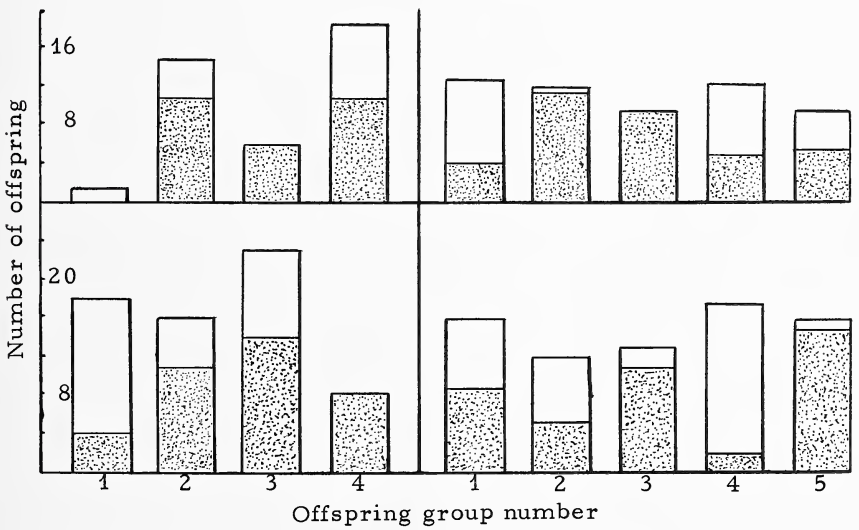

Fig. 3. Numbers and the ratio of alate to apterous offspring in consecutive batches born to individual parents of *M. persicae* ; □ alate, ▨ apterous.

All of these contradictory results can be resolved if it is assumed that in the clone studied, the postnatal effects of population density were at least as important as the prenatal effects. The data obtained gave much support to this hypothesis. In almost all cases where a significant difference in per cent alatae between treatments was obtained, there was a corresponding association between a large offspring group size and a high proportion of alatae. Under the conditions of the experiments, about 15 larvae constituted a large enough population to produce a response.

If the hypothesis that postnatal crowding is at least as important as prenatal crowding is correct, then the ontogeny of an individual would follow one of the following pathways (Johnson and Birks 1960): if the parent is under crowded conditions, the embryo remains on the path toward becoming alate. After its birth, the larva may encounter crowded or uncrowded conditions; if crowded, it continues development toward becoming alate; if uncrowded, it is sidetracked and becomes apterous. If the parent is not crowded, then the embryo is sidetracked and irreversibly set on a developmental path leading to the apterous state, and is insensitive to conditions it encounters in its early instars. Alate offspring are obtained when parents and offspring (first and second instars) are subjected to crowding or conditions simulating crowding. The results of all experiments can be fitted into one or other of these hypothetical pathways. Table 18 illustrates the interaction of parent and offspring crowding and the type of offspring which would be formed if the pathways hypothesized exist. All the treatments are listed according to the parent offspring regime each encountered. An asterisk indicates a statistical significance between the treatment marked and the corresponding control treatment in respect of per cent alatae. All but one of the controls fall into the parent uncrowded-offspring uncrowded quadrant. All but one of the treatments which showed a significantly high proportion of alatae fall into the parent crowded-offspring crowded quadrant. Reasons for regarding this one exception (100 strokes daily with aphid leg) as anomalous have already been given. The data fit the hypothetical pathways.

The data and the hypothesis can be examined in the light of the characteristics of the life history of *M. persicae*. *M. persicae* is a diecious aphid, that is, it has primary and secondary host plants between which it must travel to successfully complete its life history. Wings, twice a year, are necessary. It has the widest range of secondary plant hosts of any aphid. Therefore, winged forms of *M. persicae* are more likely than those of any other aphid to alight on suitable host plants. The percentage of seemingly obligate alatae which I observed, might thus be explained. *Aphis craccivora* and *Megoura viciae* the species used by Johnson and by Lees, are monecious and much more host specific than *M. persicae*.

There are adaptive advantages to maintaining the option of becoming alate or apterous until the first or second instars. A first or second instar larva that is still on the path toward the alate state, might, after a heavy rainstorm, find itself in uncrowded conditions. It would be to its reproductive advantage to be switched to the apterous state. Or an adult aptera might walk away from a crowded condition and deposit her

presumptive alate offspring in uncrowded circumstances. These could take advantage of such circumstances by being shunted to the path leading to the apterous state. This last instance is supported by one of the characteristics of *M. persicae*. Bonnemaison (1951) found it to be a relatively "antisocial" aphid. *M. persicae* individuals tended to space themselves over the available surface of leaf, whereas *Brevicoryne brassicae* L., a more sociable type, remained in family groups.

CONCLUSION

The higher the population density of the *parents*, the greater was the proportion of alate offspring born to apterous *Myzus persicae* Sulz. But this general tendency can be overcome by preventing the size of the *offspring* groups from becoming too great. Apterae raised on cabbage produced a greater proportion of alate offspring than apterae raised on radish. The per cent survival of offspring reared on radish was high, near 90; on cabbage was lower, near 70. The fecundity of parents fed on cabbage was less than that of parents fed on radish. The fecundity and longevity of singly reared parents did not differ from that of parents reared in groups. No association between the physiological age of the parents and the tendency to produce alatae was observed.

An attempt was made to imitate physical contact among aphids by stroking individuals. Stroking adult, apterous aphids on the dorsal surface with a *Drosophila* leg or a human hair, at intensities of 100 or 300 strokes per day was ineffective in causing the aphids to produce alate offspring. Stroking particular parts of the body, with an aphid leg, at intensities of 400 or 500 strokes per day was also ineffective. Areas stroked included the head, thorax, fore and hind parts of the abdomen, the sides of the abdomen, the antennae, and the legs. Stroking applied generally to the entire dorsal surface at 300 or 1000 strokes per day may have caused a slight increase in alate production. One hundred strokes daily applied generally over the dorsal surface elicited a highly significant increase in the number of alate offspring. Two or 5 minutes daily contact of adult apterae with other adults mounted live on a pin, caused a slight increase in the proportion of alate offspring born to them. There was an association, across the treatments of the stroking experiments, between a large number of offspring per cage and a large proportion of alate offspring.

Stroking first and second instar larvae at intensities of 200, 300 or 500 strokes once or twice per instar did not cause these larvae to develop into alatae. Offspring of these larvae were not disproportionately alate.

Starving young, adult apterae for short periods (1, 2, 4 or 16 hours), did not affect the number of alate offspring produced. Crowding young, adult apterae at population densities of 1, 2, 4 or 16 per 50 mm^2 for 1, 2, 4 or 16 hours did not result in an increased number of alate offspring. Crowding first instar larvae whose parents had been crowded, resulted in an increased number of them becoming alate.

In the clone studied, it was necessary for the first instar larvae

as well as their parents to be crowded before there was an increase in the number of alate individuals produced. The capacity to be channelled into the developmental pathway leading to the apterous state was retained beyond the embryo stage and well into the first and possibly second instars.

TABLE 18. A summary of the results, sorted into parents crowded or uncrowded and offspring crowded or uncrowded categories.

Offspring	Parents: Crowded	Parents: Uncrowded
Crowded	Continuous, 4*, 8*; (1 / 8)** 2*, 4* radish, 2*, 4* cabbage; (2 / 9) Temporary, 2 for 1, 2, 4, 16 hr; (12/19) 4th instar 16 for 4 hr*; (16/21) 1st instar 48 for 24 hr*; (17/22)	Simulated***, 1st & 2nd instar 3 x 200; (- /16) 1st & 2nd instar 300, 1st & 2nd instar 2 x 300; (- /17) 1st & 2nd instar 500; (- /12) Temporary, 2 for 1, 2, 4, & 16 hr; (12/19) 16/50 mm^2, 16 hr; (14/20)
Uncrowded	Continuous, 4; (3 /10) Simulated, 100 with hair, *Drosophila* leg, & aphid leg*; (4 /12) 300 with aphid leg, & hair; (5 /13) 500, aphid leg on head, thorax, abdomen; (6 /14) 400, aphid leg on hind abdomen, fore abdomen, & legs; (7 /15) 500, aphid leg on antennae, sides of abdomen, & entire dorsum; (8 /15) 1000, aphid leg on entire dorsum, cor- nicle secretion; (9 /16) Temporary, 2 min with live aphid; (5 /13) 5 min with live aphid; (9 /16) 5 together 1 hr, 4 overnight; (10/18)	Controls: (1 / 8) (2 / 9) (3 /10) (4 /12) (- /14) (- /16) (- /17) (15/21) (16/21) (- /24) 4 together 1, 2, 4, & 16 hr; (13/20) 16 together 1, 2, 4 & 16 hr; (14/20) 16 for 16 hr large cage, small cage; (15/21) 17/50 mm^2 for 4 days; (- /16)

* alate production significantly different from the control
** (table no. / page no.). ($P < 0.05$).
*** crowding simulated by stroking.

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I am grateful to Drs. W. G. Evans and B. Hocking for help given me in the execution of this work.

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THE CLARIFICATION OF A DISCREPANCY IN DESCRIPTIONS OF MAXILLARY MUSCULATURE IN LARVAL SIMULIIDAE

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The first definitive account of the morphology of larval Simuliidae was provided by Puri (1925). In his description of maxillary musculature he states that the maxillary palp is provided with a pair of small adductor and abductor muscles. Cook's (1949) description of the maxillary musculature of larval simuliids agrees with that given by Puri, with the exception that Cook makes no mention of palpal muscles.

To resolve this discrepancy the maxillary musculature of simuliid larvae was re-examined.

Large, recently moulted larvae were examined to provide a clear view of maxillary structures. These were fixed in Bouin's fixative and the head capsule stained in Mayer's carmalum. The maxillae and associated muscles, as well as the adjacent regions of the head capsule, were dissected from the head and differentiated in acid alcohol. The maxillae were then mounted in Canada Balsam and examined and photographed with a Leitz Orthomat.

Examination showed no palpal muscles. However, there is a prominent nerve running to the palp with a ganglion at the palp base (fig. 1). It is unlikely that Puri would mistake the palpal nerve for muscle. What then did he observe?

During the investigation a number of mature larvae were fixed, stained, and examined. In these specimens the pharate maxillae of the next instar stained with the same intensity as muscle (fig. 2). Examination under phase-contrast was necessary here to distinguish muscles from other tissue but again no palpal muscles were detected.

Exactly what Puri observed is not known but this investigation indicates that he interpreted a pharate maxillary palp as muscle.

These observations as well as those of Cook (1949) agree with Das (1937), Hinton (1958), and Matsuda (1965), who state that dipterous larvae never have palpal muscles.

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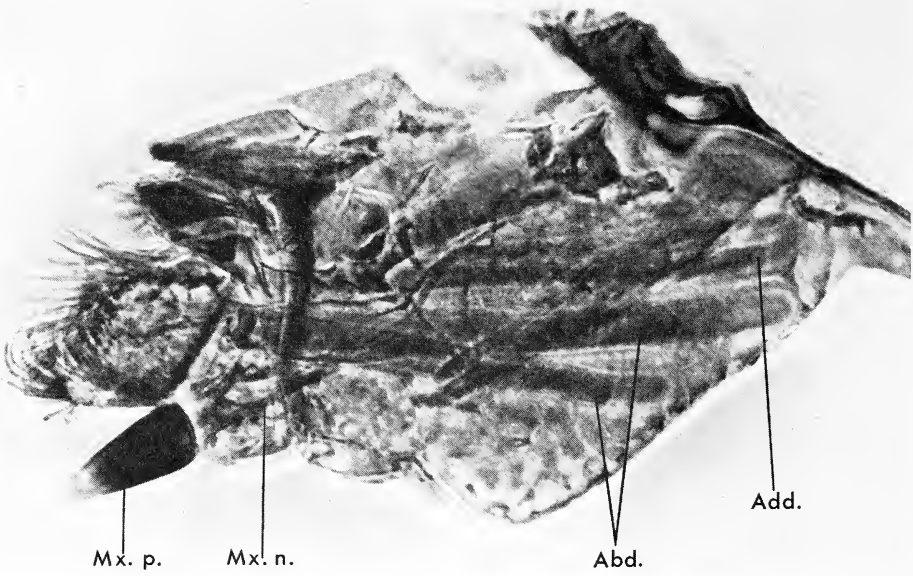

Fig. 1. Aboral view of maxilla from recently moulted larva; Abd. = abductor muscle, Add. = adductor muscle, Mx. n. = maxillary nerve, Mx. p. = maxillary palp, Ph. mx. p. = pharate maxillary palp.

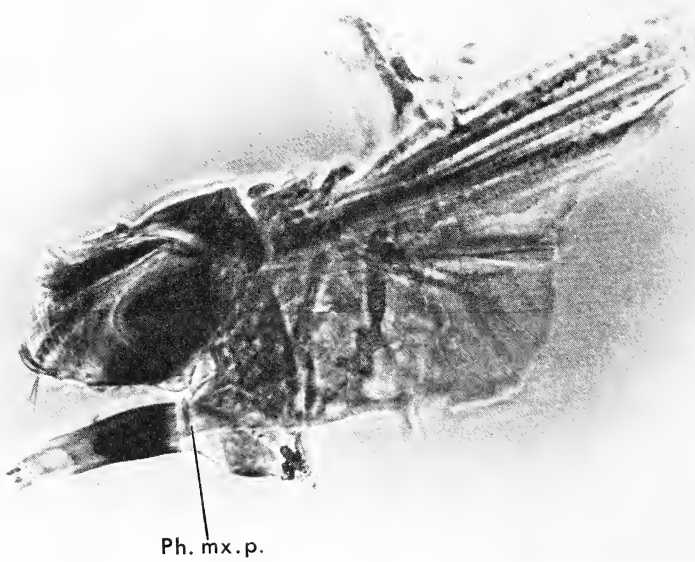

Fig. 2. Aboral view of maxilla from mature larva.

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Editorial - Man and Whose World?

Now that the tumult of a centennial year for Canada is over, it is perhaps no longer picayune or churlish to ask this question in public. Last year man, sponsored by Canadians, raised his standard on a tiny island wrested from the swirling waters and swarming caddis flies of the St. Lawrence River and declared that the world was his. Nobody challenged him; or if they did he never heard. The title "Man and his World" is bad enough; but when this is interpreted as it was: "Man and His own World" when, in other words, man looks upon the world as belonging to him and upon nature as producing *for* him, the voices of biologists must be raised in protest.

Admittedly, I am criticizing from ignorance for I never went to Expo 67. I was sufficiently nauseated by such literature and publicity relating to it as reached me.

If this is man's world, man's own world, then man has a lot to answer for, for ownership carries with it responsibility for control. Man then, must accept the blame for hurricanes and earthquakes, floods and tornadoes, as well as the credit (if any) for atomic bombs and spacecraft, for the Empire State Building and the Pyramids of Giza. Millions of dollars worth of mutual admiration will not help him in this task.

I was told that insects were to find a place among the exhibits in this centennial celebration, but it transpired that all that was meant was insecticides, and valiant efforts were made to ensure that the otherwise ubiquitous caddis flies did not show their genae at the party. They might have reminded man that in his world the waters of the St. Lawrence still swirled; that beneath them the case makers would continue to make a case for themselves as owners of at least this stony substrate.

A centennial, naturally, looks back a hundred years and surely it might at least try to look forward a hundred years too, rather than a mere twenty as on this occasion. Its twenty year vision had its blind spots. Perhaps in another ninety-nine years there will be an opportunity

to focus on some of these, to draw attention to a few of man's mistakes and his many unsolved problems. A second centennial celebration drawing attention to the fact that though God may be in his heaven everything is not all right with man's world would be more accurate, more interesting, and in the long run more profitable. I hope my great grandchildren will visit it. I hope the caddis flies will visit it; even if they have to be trapped on arrival and served as caddisburgers to my great grandchildren and others. Better to build the flesh of men, than give a bellyache to fish.

This is no more man's world than an orange belongs to the coccid whose stylets probe its peel more deeply than man's machines probe the earth's crust. Man and *whose* world then? It is surprising that in a country so rich in religion as Canada, God's spokesmen raised no finger on His behalf; perhaps there was a conflict of interests between Gods. And what about the money men? Was there no banker or billionaire whose claim could rival that of the rest of the human race? Where was the voice of women? And where that of the little green men whose flying objects remain, like so many caddis flies, unidentified?

Brian Hocking

THE LIFE HISTORY OF *COSMOPEPLA BIMACULATA* (THOMAS) (HETEROPTERA : PENTATOMIDAE) IN ALBERTA

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The habits and food plant of Cosmopepla bimaculata *(Thomas) are recorded. A study of the duration of the nymphal instars was made both under fluctuating and constant laboratory conditions. The external genitalia of the fifth nymphal instar are described.*

Cosmopepla bimaculata (Thomas) is a small black and orange pentatomid commonly found on hedge nettle *Stachys palustris* L. in Alberta. It is not considered a serious pest of crops in this province. The species is widespread throughout North America and Mexico.

Habits and Food

The adults were observed feeding on *Stachys* in Edmonton towards the end of June and were freely copulating by the first week in July. Eggs were observed on 13 July and by 22 July first instar nymphs were abundant. The life cycle was completed by early August, but mating continued right through July and first instar nymphs were observed in August. It appears that this insect overwinters in the adult stage, but it is probable that a few overwinter as fourth or fifth instar nymphs.

Cosmopepla was observed and bred on *Stachys palustris* for this series of observations. Blatchley (1926) records many host plants for this species. The insect in all stages feeds on the seed of *Stachys* both unripe seed on the plant and mature seed on the ground. Very little sap sucking occurs and the adults and nymphs can be kept on seed and water alone.

Copulation

Copulation is the same as described for other pentatomids (Poisson 1951). The pair of bugs remain attached on the average one day (S.D. 0.37 days; n = 18).

Egg Laying

The first batch of eggs was laid on the undersurface of a leaf approximately twelve hours after copulation, second and third batches were laid almost immediately afterwards, but occasionally the female would hold her eggs for 24 hours or more between each laying. This is reflected in the wide variation in incubation time for the eggs.

Each female lays from 1-3 batches of eggs (mean 1.9, n = 8) containing 11.5 ± 1.0 eggs (n = 47).

Development and Life History

Eight egg masses were reared through to the adult stage in a laboratory where the temperature was recorded. The average of the maximum and minimum temperatures for the period of development was 23.5 C.

Another series of eight egg masses was transferred on hatching to a growth chamber and reared at a constant temperature and light regime (23 C, day length 14 hours). The intensity of illumination was 2400 foot candles at 30 cm from the light bank. The duration of each instar was recorded when 50% of individuals in each hatch had moulted to the next instar. The two sets of results are given in table 1. Although the mean development times were less for the first three instars at fluctuating temperatures, and for the last two at a constant temperature, the differences in the results are not significant at the 5% level.

Eggs

The eggs (fig. 1) are oval, 0.75 mm long (± 0.04; n = 30) and 0.60 mm in diameter (± 0.04; n = 30) and when laid are pale green. The upper margin of the egg bears 15-23 chorionic processes (mean 18.9 ± 2.0; n = 100) at regular intervals in a circle (fig. 4).

Incubation Period and Hatching

The eggs take from 3-6 days to hatch after laying (mean 4.7 ± 1.0 day; n = 23). The shorter incubation period was due in all cases to the fact that these females retained the fertilized eggs for a longer period before laying them. The vertex of the embryonic head bears an elongate triangular sclerotized egg burster (figs. 3 and 4).

Postembryonic Development

The nymphal instars have been figured and described by DeCoursey and Esselbaugh (1962). The colour of first instar nymphs on hatching is pale yellow-green, the eyes are bright red. This instar is gregarious, clustering around the eggs after hatching. At 23 C the duration of each instar was: 1st 3.2 days, 2nd 4.7 days, 3rd 4.2 days, 4th 2.9 days and the 5th 6.4 days (table 1).

The external female genitalia can readily be distinguished in the 5th instar. Abdominal sternum VIII has a median longitudinal suture (fig. 6) and, apically, two small sclerites, the first gonapophyses (fig. 5, 1 Gp.), one on either side of the suture forming a V.

Abdominal sternum IX has, basally and medianly, a pair of small triangular sclerites; these are probably rudiments of the second gonapophyses (fig. 5, 2 Gp.).

Parasites

Some egg masses collected in the field were found to be parasitised by a small wasp *Telenomus* sp. (Scelionidae).

ACKNOWLEDGEMENTS

I should like to thank Dr. O. Peck of the Entomology Research Institute, Ottawa, for identifying the hymenopterous parasite of the eggs, and Dr. Brian Hocking and Dr. W.G. Evans, Entomology Department, University of Alberta, for reading and editing this manuscript.

TABLE 1. Developmental periods for all instars of *Cosmopepla bimaculata* at constant and fluctuating temperatures.

At 23 C and 14 hours daylight -

Instar	I	II	III	IV	V	Total I - V
Range (days)	2 - 4	3 - 7	2 - 6	2 - 4	6 - 8	19 - 24
Mean (days)	3.2 ± 0.7 (8)	4.7 ± 1.2 (8)	4.2 ± 1.3 (8)	2.9 ± 0.8 (8)	6.4 ± 0.7 (8)	21.4 ± 1.5 (8)

At fluctuating temperatures (mean over 27 days, 23.5 C) -

Range (days)	2 - 3	4 - 5	2 - 5	3 - 5	6 - 12	20 - 27
Mean (days)	2.5 ± 0.5 (8)	4.2 ± 0.5 (8)	3.7 ± 1.3 (8)	3.7 ± 1.0 (8)	8.4 ± 2.0 (8)	22.6 ± 2.2 (8)

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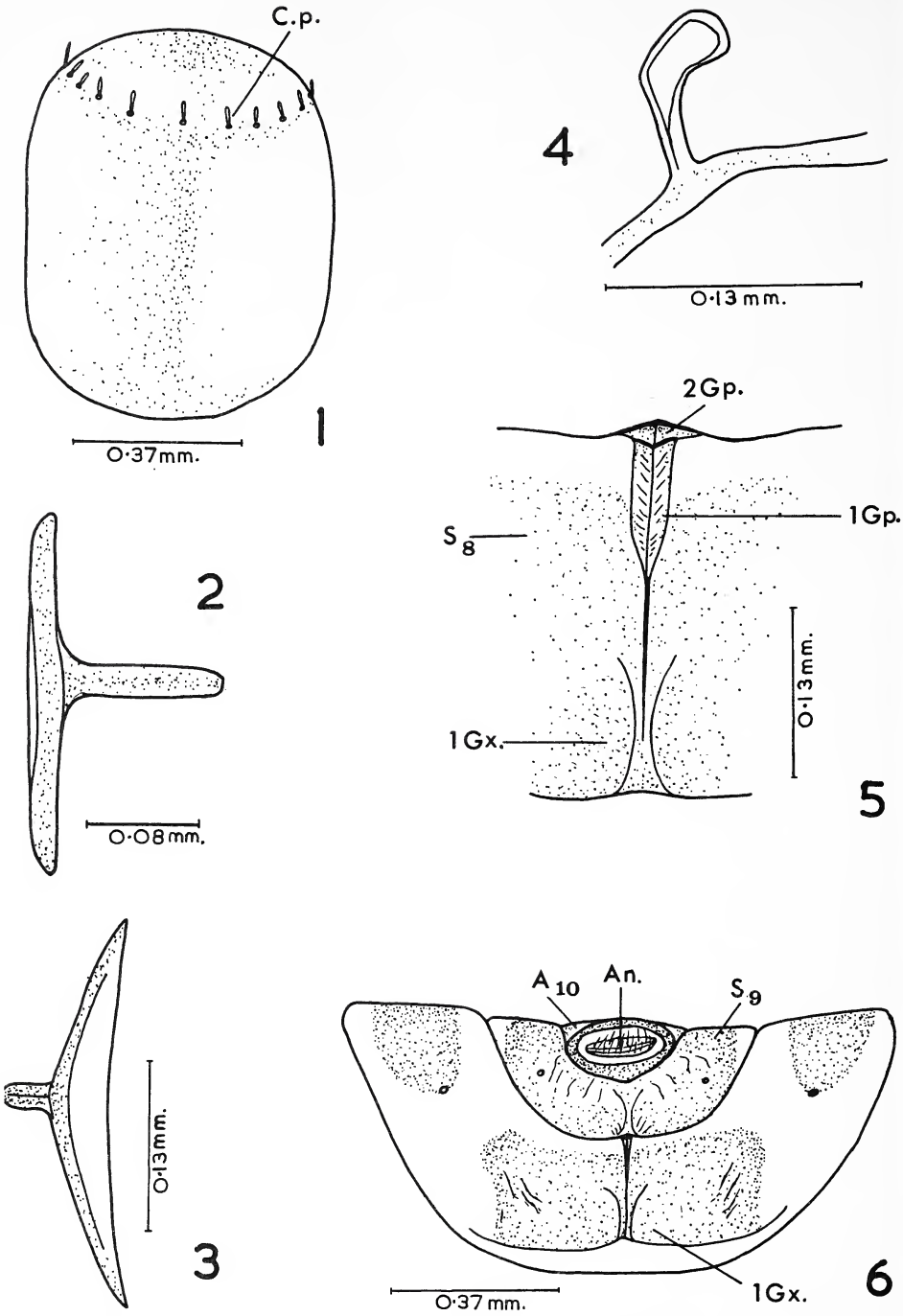

Cosmopepla bimaculata. Fig. 1. Egg. Fig. 2. Egg burster, lateral view. Fig. 3. Egg burster, dorsal view. Fig. 4. Longitudinal section through chorionic process. Fig. 5. Instar V; external female genitalia. Fig. 6. Instar V; abdominal sterna 8-10. A_{10}, abdominal segment 10; An., anus; C. p., chorionic process; 1 Gp., first gonopophysis; 2 Gp., second gonopophysis; 1 Gx., first gonocoxa; S_8, abdominal sternum 8; S_9, abdominal sternum 9.

DISPERSAL OF MOSQUITOES

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This review of the literature shows that mosquitoes may undergo displacement of many miles from the site of eclosion. The dispersal of mosquitoes may be influenced by wind, topographical features, vegetation, and by an illuminated sector of sky. Wind, more than any other environmental factor, influences dispersal. The optomotor responses of mosquitoes flying in a wind are useful in predicting the pattern of dispersal.

Vigorous dispersal is vital to the survival of mosquito species. Although it is frequently wasteful (mosquitoes may perish that venture into deserts, over oceans, or to other inhospitable regions), dispersal prevents extinction of the species because of a fluctuating climate, a fluctuating sparsity or distribution of host species, or the vagaries of human activity. Populations may be totally destroyed in parts of the range, but as soon as these areas again become tolerable, the vigorously dispersing emigrants repopulate them, and the reclaimed area may serve as a refuge when other parts of the range become intolerable. Also, vigorous dispersal causes such rapid gene flow between local populations that the genotypes may be repatterned rapidly to meet the new conditions, i.e., changing climate, vegetation, human settlements, or insecticides.

Some *Aedes* species can fly as far as 30 air miles or remain airborne about 12 hours without feeding (Hocking 1953), and *Culex pipiens berbericus* Roubaud can fly no less than 5 km by using reserves carried over from the pupal stage (Clements 1963). However, the extent to which mosquitoes in nature make use of this ability to travel depends on the species, the vicissitudes of weather, the terrain, and on yet unknown factors.

The tendency to disperse varies greatly between species. Some show a marked tendency to disperse great distances shortly after eclosion and before seeking the first blood meal. The best examples are *Aedes sollicitans* (Walker) (Felt 1904), *Aedes taeniorhynchus* (Wied.) (Provost 1957), *Anopheles sundaicus* (Rodenwaldt), *Anopheles saccharovi* (Favr.), and *Anopheles maculipennis* Meigen (see Eyles 1944), and perhaps *Anopheles pharoensis* Theobald (Kirkpatrick 1925) which regularly disperse in large numbers in excess of 10 miles and sometimes in excess of 100 miles from the site of eclosion. Yet, another species such as *Culex tarsalis* Coquillett show

no marked migratory phase between eclosion and the search for the first blood meal; yet this species may spread 25 miles per generation (Bailey *et al.* 1965), and it and others such as *Anopheles quadrimaculatus* Say (Gartrell and Orgain 1946), *Anopheles freeborni* Aitken (Rosentiel 1948) and *Anopheles maculipennis* (see Eyles 1944) may show a prehibernation migratory phase that takes a portion of the population miles from the site of eclosion. Still other mosquitoes such as the domesticated populations of *Aedes aegypti* (L.) (Morland and Hays 1958), *Anopheles culicifacies* Giles (Russell *et al.* 1944) and *Culex pipiens fatigans* (Lindquist *et al.* 1965) do not fly far from the site of eclosion; nevertheless, the first flight of some individuals of *C. pipiens fatigans* is believed to be truly migrational. Almost nothing is known of dispersal of adults that have overwintered.

Populations appear to be heterogeneous with regard to the drive to disperse. Even in the highly migratory *Aedes taeniorhynchus*, a significant number do not disperse beyond a few hundred meters of the site of eclosion (Bidlingmayer and Schoof 1957). Perhaps these stragglers emerge too late to profit from the stimuli of twilight (see Provost 1957, Pausch and Provost 1965). However, the tendency to disperse also depends on the genotype of the individual: the motility of one laboratory strain of *Anopheles quadrimaculatus* was found to have been selected away through prolonged colonization (Dame *et al.* 1964). Nevertheless, we lack evidence that selection by insecticides has affected the drive to disperse (Sautet 1957), and Wada (1965) believed that crowding during the larval stadia may increase the drive.

With anophelines, males do not always disperse as widely as females (Eyles 1944), and similar observations have been made for *Aedes taeniorhynchus* (Provost 1957) in which mating may occur before the migratory flight (Haeger 1960). However, Klassen and Hocking (1964) did not find any apparent difference between the sexes of *Aedes cataphylla* Dyar; they mated after the initial long-distance flight. Clarke (1943) calculated the average flights of male and female *Culex pipiens fatigans* and *Aedes vexans* (Meigen) to be 9.8, 10.3, 9.4, and 9.1 miles, respectively.

Behavior and Dispersal

Ross (1905) distinguished between two types of flight: relatively long flights from one "breeding ground" to another and "flitters" or trivial flights near the habitat. The long flight is believed to be an example of nonappetential flight (Provost 1952) (nonpurposive spontaneous flight; Nielsen 1958). It starts shortly after sundown in *Aedes vexans* (Clarke 1943), *Aedes taeniorhynchus* (Provost 1957; Bidlingmayer and Schoof 1957; Nielsen 1958), *Aedes cataphylla* (Klassen and Hocking 1964), and *Culex tarsalis* (Bailey *et al.* 1965). The drive to disperse is so pronounced in newly emerged *Aedes fitchii* (Felt and Young) mosquitoes that they will take flight in a wind of 12-14 mph that inhibits the flight of mosquitoes several days old (Klassen and Hocking 1964). In *Aedes taeniorhynchus*, the nonappetential drive to disperse does not persist past the first day of adult life (Provost 1957), but host-seeking females and females searching for resting and oviposition sites do make short appetential (purposive) flights that, though they are markedly influenced by host density, microclimate, and terrain, may add substantially to the ultimate dispersal of

a species (Provost 1957).

Wind and Dispersal

Wind has been shown to be of overriding importance in determining the pattern of dispersal by releases and recaptures of marked mosquitoes and by direct observations of migratory flights (table 1, p. 49). Valuable insights into dispersal may be obtained by a thorough study of the manner in which a mosquito navigates in a wind.

Kennedy (1940, 1951) showed that insects flying in a wind navigate by making compensatory responses to visual stimuli from the relative movement of the ground below them. His optomotor hypothesis of navigation postulates that an insect flying in certain windspeeds and at certain heights above the ground will orient into the wind; at other heights and windspeeds, it will orient downwind. Observations in the field by Steiner (1953), Haeger (1960), Klassen and Hocking (1964), and Bailey *et al.* (1965) support Kennedy's hypothesis.

The manner in which a flying insect controls its track is somewhat similar to the manner in which one controls the path of an automobile. On a straight road with ideal conditions, one prefers to drive so the images pass across the visual field from front to back at, say, 60 mph. If the automobile skids, the movement of the images across the visual field has a transverse component, and the driver immediately reduces this component to zero by reorienting the automobile. On a slippery hill, the automobile may cease to make headway and may slide backwards, a movement detected by the back to front movement of the images; it is prevented either by accelerating or by braking.

The dispersing mosquito has similar reactions; however, its navigation is more complex and can be described mathematically (Klassen and Hocking 1964).

Since the velocity of the images varies as

$$V = w(Z - \lambda) \qquad (1)$$

and the windspeed increases as

$$W = \frac{u}{K} \log \frac{Z}{Zo} \qquad (2)$$

upwind flight is described as

$$W(Z - \lambda) = V - \frac{u}{K} \log \frac{Z}{Zo} \qquad (3)$$

and downwind flight as

$$V + \frac{u}{K} \log \frac{Z}{Zo} = w(Z - \lambda) \qquad (4)$$

when z is the height of the mosquito above ground

λ is the height of the vegetation providing the visual pattern

Z is the height of the mosquito above the visual pattern

V is the airspeed of the mosquito

w is the preferred angular rate of apparent movement of the ground from front to back

W is the windspeed at height Z above the ground

u is the friction or the velocity

K is Karman's constant

Zo is the roughness length.

Thus the velocity of the images varies inversely with increasing height above the background (1) and because windspeed increases logarithmically with the height above the ground (2). In addition, the mosquito prefers a certain airspeed (cruising speed) (Hocking 1953). Thus, the dispersing mosquito compensates for undesirable visual effects by changing its airspeed within certain limits, by constantly correcting its orientation along the direction of the wind so no side slippage occurs, by turning from upwind to downwind or vice-versa, or by settling.

In a gentle wind, the mosquito takes off against the wind, climbs to an altitude at which the images pass by at a preferred rate, and flies near its preferred airspeed. Upwind flight is described by equation 3. If the windspeed at the altitude of flight exceeds the preferred airspeed, the insect can lower its altitude or even settle. Also, the mosquito may turn downwind (fig. 1a). Then the windspeed added to the airspeed will greatly increase movement relative to the background, and the mosquito must gain altitude so the movement of images will be at the preferred rate. Downwind flight is described by equation 4. Figure 1b shows the permissible heights of flight of *Aedes punctor* (Kirby) in relation to windspeed measured at 500 cm above ground. The lines in this figure were calculated from equations 3 and 4 and represent the values at which stimuli calling for change start to be received. Actually, because these stimuli must reach a minimum threshold, the permissible heights of flight are zones whose width is small when the background is well-perceived and when the altitude is low. Figure 1c shows the relationship between windspeed and maximum rate of displacements of *Aedes punctor* and *Aedes aegypti* in upwind and downwind flight. Theoretically, the weak flier, *Aedes aegypti*, should be able to disperse upwind and downwind at the same rate when the windspeed at 500 cm above ground is about 120 cm/sec (2.6 mph). At greater windspeeds, downwind displacement increasingly predominates. Also, theoretically, the moderately strong flier, *Aedes punctor*, should be able to disperse upwind and downwind at the same rate when the windspeed at 500 cm is 160 cm/sec (3.5 mph). However, experience (table 1) indicates that in these cases, downwind dispersal would predominate, probably because at the start of migratory flight, mosquitoes climb to fairly high altitudes at which, according to equation 4, only downwind flight is possible.

Clarke (1943) observed with regard to *Aedes vexans* that "during the period of emergence ... *vexans* rises from the marsh singly and continuously at dusk for a period of approximately one hour. They are observed to rise to a height of 40 feet and fly with the wind." Similarly, *Aedes taeniorhynchus* climbs at an angle of 30-60° to above the mangroves (Nielsen 1958, Haeger 1960, Provost 1957). Also, Bailey *et al.* (1965) observed that "when swarms of *Culex tarsalis* emerged from the rice fields in the early evening they would spiral upward in an irregular manner to heights of 12 to 15 feet, according to the temperatures of the atmospheric layers, and then would level off in the wind current." These authors found that *C. tarsalis* usually flies at altitudes between 1.5 and 15 meters (5 and 50 feet). *Aedes cataphylla* rises to 4 to 8 meters, depending on the speed of the wind (Klassen and Hocking 1964). Somewhat similar behavior was observed for *Culex pipiens fatigans* (Lindquist *et al.* 1965) and for *Anopheles gambiae* Giles (DeMeillon 1937).

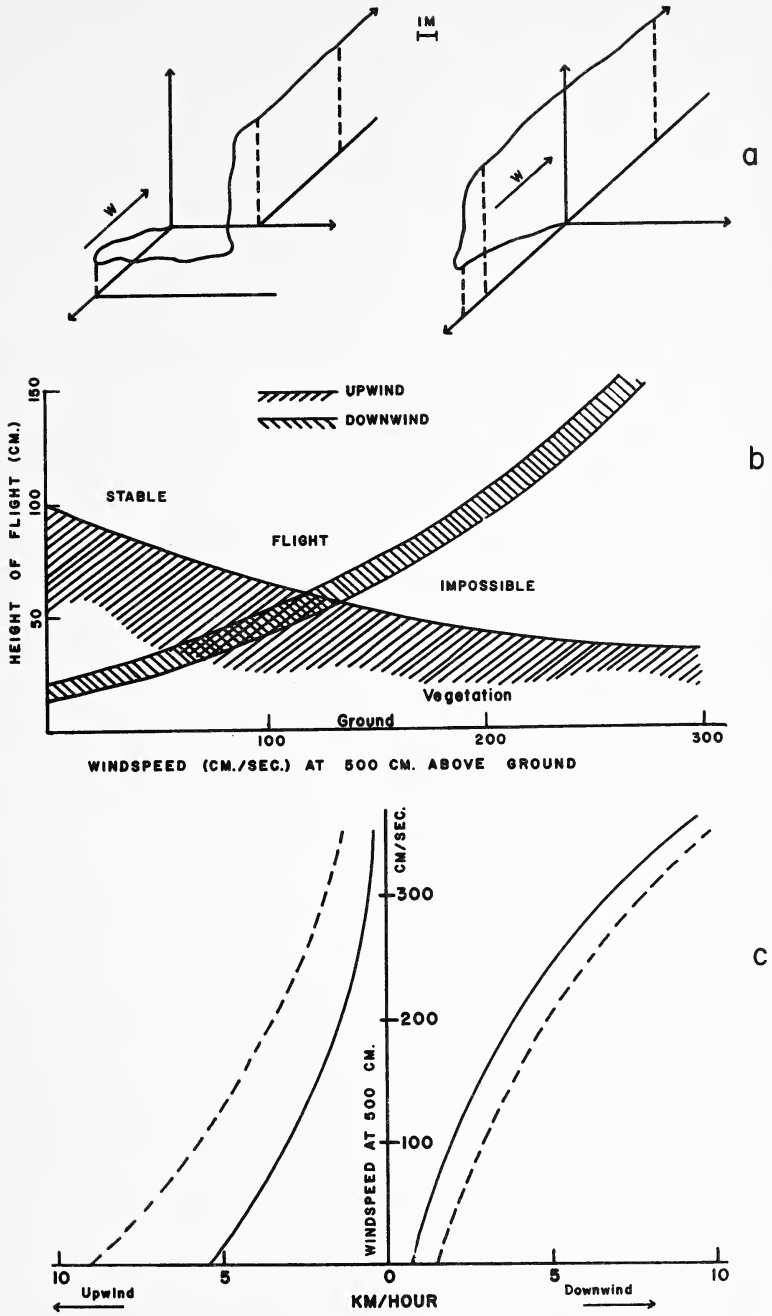

Fig. 1 a, Paths of *Aedes cataphylla* take-off into a wind W, on a plain. b, The permissible heights of flight of *Aedes punctor* in relation to windspeed at 5 m above ground. c, The relationship between windspeed and the max. displacement rate of *A. punctor* (---) and *Aedes aegypti* (——) in upwind and downwind flight. Redrawn from Klassen & Hocking (1964) with permission.

The observations of Bailey *et al.* (1965) on the effect of wind on the dispersal of *Culex tarsalis* agree precisely with the effect predicted by the optomotor hypothesis. They found that:

"1. At low wind velocities, up to 2 mph (94 cm/sec, W.K.), dispersal takes place in all directions. The greatest distance of recapture was 2.75 ... against a wind of 0 to 2.9 mph (136 cm/sec, W.K.).

2. At least 10 per cent of the mosquitoes from any particular release may disperse laterally, i.e., across the direction of the wind.

3. Above a limiting wind velocity of about 4 mph (188 cm/sec, W.K.) the general direction dispersal is downwind. There is only very limited movement against or across a wind as high as 4 mph. No recaptures were made upwind when velocities were 5.4 mph or higher ...".

Moonlight allows the phenomenon of twilight flight to continue (Rees 1945, Ribbands 1945, Provost 1958, Bidlingmayer 1964) because the intensity of the light at full moon approaches that at twilight. The eyes of mosquitoes are adapted for vision in dim light (Sato *et al.* 1957), and their wide visual solid angle is especially suitable for maintaining a track even in dim light. Also, mosquitoes respond to the plane of polarized light (Kalmus 1958), and since polarization of skylight is maximum one hour after sundown, mosquitoes may use it as a navigational aid.

Dispersal and Topographical Features

Topographical features may affect dispersal by their influence on micrometerological conditions, i.e., they may influence the prevailing wind and the creation of local winds, and by presenting visual stimuli to dispersing mosquitoes.

Evidence exists that, in the absence of wind, mosquitoes with a pronounced drive to disperse tend to orient toward the low point of the horizon; this response, then, contributes to the movement of mosquitoes into and along valleys (Klassen and Hocking 1964). Are dispersing mosquitoes attracted to prominent sections (skototaxis) or are they attracted to low points (hypsotaxis) of the landscape? Movement of mosquitoes up the side of mountains has been recorded (Hearle 1926); however, this may have been caused by upwind orientation to the sloping wind.

Also, the assembly of mosquitoes in wooded areas may be directed not only by a taxis but by wind-borne moisture that causes an upwind orientation (see Klassen and Hocking 1964).

On clear evenings, plains radiate their heat and cool the air immediately above them. This cool air sinks into ravines and flows down river channels at ca. 5 mph (measured at Edmonton, Canada; Klassen 1962), and these local winds are not usually affected by the prevailing wind. Perhaps river channels thus accumulate dispersing mosquitoes and then channel their movements up or down a valley (Klassen and Hocking 1964). During dry seasons in the Transvaal, dispersal is restricted to wooded river valleys (DeMeillon 1933 as cited by Horsfall 1965). Similarly, dispersal of *Aedes vexans* and *Aedes aldrichi* occurred mainly along the Columbia River Valley and its tributaries and not on the plain (Stage *et al.* 1937). Causey and Kumm (1948) recaptured most

tagged mosquitoes within a river valley.

In deep valleys (fig. 2), winds blow up their sides and up-valley by day and reverse direction by night (Defant 1951). By flying against the down-valley wind during a single night, female *Anopheles maculipennis* may disperse into human settlements no less than 5 km from the site of eclosion (Ivanova 1962). Moreover, female mosquitoes locate their hosts by flying against the scent-bearing downslope wind (Ivanova 1962).

Near bodies of water, a wind may blow from water to land by day and reverse at night. These winds, too, affect dispersal patterns (Ivanova 1962). Regularly, an early evening wind from the Pacific is channelled along the Sacramento River and affects the up-valley dispersal of *Culex tarsalis* (Bailey *et al.* 1965).

Movement along Lines or Borders

Mosquitoes have been observed to orient and move along lines or borders, irrespective of the wind (Jenkins and Hasset 1951, Snow and Pickard 1957). Recently, Giglioli (1965) observed that *Anopheles melas* (Theobald) traveled 1 to 2 miles from the site of eclosion to a village by following the interface between bushes and cleared land. The mosquitoes formed streams about 20 to 60 feet wide, flew below an altitude of 5 feet, and did not align with the direction of the wind.

Movement with Strata of Vegetation

Horsfall (1955) reported that the movements of mosquitoes may be restricted to the undergrowth. "Females were moving eastward in the undergrowth of a forest area during a sultry afternoon when no wind was blowing. The females flitted from one low plant to another, but always in the same general easterly direction." Such creeping migration seems to occur in Utah also; Rees (1945), too, noted that females followed paths above a low canopy of vegetation and that a flight moved 8 km in 2 to 5 days in this case.

Movement toward Illuminated Sector of the Sky

Horsfall (1955) reported that a mass flight of *Aedes vexans* took place toward "the flowing skyline caused by suburban glowing lights." Similarly, Gunstream and Chew (1964) observed that this species may orient to the strongly illuminated western sky rather than to the wind. Orientation of Coleoptera to a lighted section of the horizon has been demonstrated by Lindroth (1948) and deserves investigation.

MacCreary (1939) operated 5 light traps in Newark, Delaware, and found that the catch in each varied. He felt that the mosquitoes were observed to fly toward areas that had the highest population density of man and cited the opinion of Headlee (1936) that "Both *Aedes vexans* (Meig.) and *Culex pipiens* L. tend to migrate in the direction of great populations regardless of the wind direction." Similarly, Gillies (1961) found the dispersion of *Anopheles gambiae* was related primarily to the distribution of human settlements. Perhaps the lights of human settlements are attractive, or perhaps the mosquitoes simply accumulate near the blood source by klinokinesis. Ivanova (1962) believed that mosquitoes several kilometers distant from a human settlement responded to human odors carried on katabatic winds.

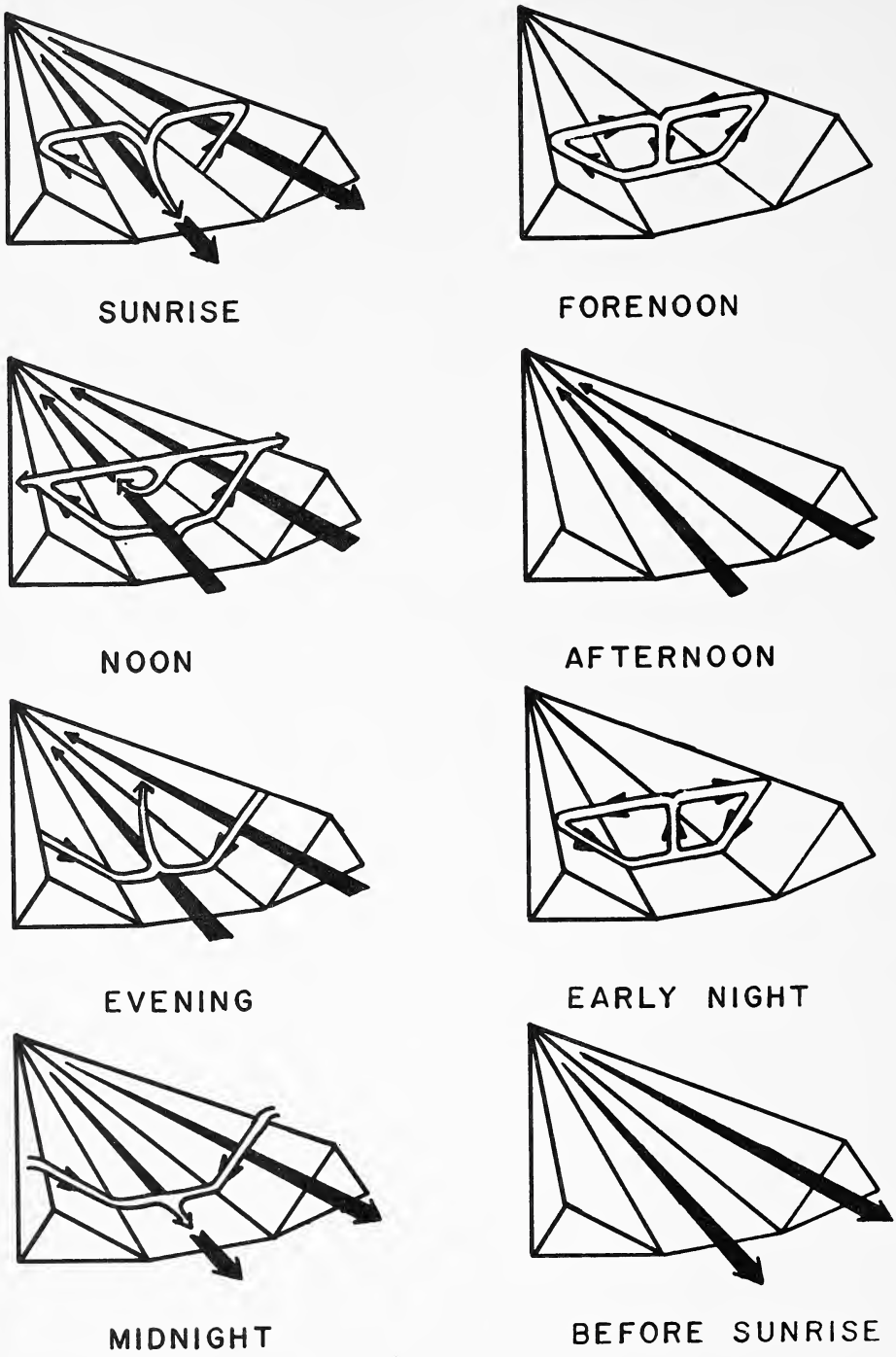

Fig. 2. Air currents within a large valley at various times of day. Based on Defant's (1951) model of the valley-slope wind system.

Passive Transport of Mosquitoes

Movements of mosquitoes other than those near the surface of the earth have been reported. Smith *et al.* (1956) demonstrated with marked mosquitoes that when a cool air mass moved across a semitropical area in California, vast numbers of mosquitoes were picked up and deposited 30 to 60 miles distant from the site of eclosion. Smith (personal communication) explained this transport as follows: "I do not have any air temperature data of the moving air but would assume it would not be much below 10° lower than the valley air ... I do not believe this mass could be considered as passing over in the nature of a frontal movement. Meteorological information indicated that it was a local movement drawn in from the coast over about 60 miles of low hot hills to fill a local barometric low and dissipated once it entered the valley. My belief is that the mosquitoes were not carried on this cool air or any local surface movement it set up, but were stimulated by cool air and carried by thermal currents at higher altitudes ...".

A mass transport of *Aedes vexans* in a cold front was described by Horsfall (1954). Perhaps storm cells of the advancing air mass picked up the mosquitoes and carried them high into the air as is apparently also the case with the spruce budworm moth, *Choristoneura fumiferans* (Clemens) (Greenbank 1956). Some species of mosquitoes were caught by Glick (1939) at 3000 feet and 5000 feet over the central United States, and adult *Culex tarsalis* were taken at 500, 1000, and 2000 feet over Texas (Glick and Noble 1961). Also, Bailey *et al.* (1965) trapped this species at the top of a 1540-foot television tower in California.

Changes in pressure associated with the passage of cold fronts "excite" some Diptera (Wellington 1945, 1946), and Haufe (1954, 1963) demonstrated that *Aedes aegypti* exhibits increased activity with such changes. Moreover, Kennedy (1940) and Kalmus and Hocking (1960) showed that mosquitoes take flight when windspeeds are decreasing. Conceivably, mosquitoes, particularly newly-emerged ones, could be picked up by frontal systems as a result of their flight activity.

Armstrong (1963) felt that observations are needed to determine whether the low-level jet streams that develop above temperature inversions on the Great Plains could transport mosquitoes. The chances seem small that significant numbers of mosquitoes would be entrained into these jets that occur at a height greater than 800 feet above the plain.

Also, mechanical dispersal of eggs or larvae of *Aedes aegypti* in water containers moved along commercial routes has expanded the range of this species (MacDonald 1956).

TABLE 1. Reports on the influence of wind on mosquito dispersal.

Species	Maximum distance flown (miles)		Remarks	Sources
	Upwind	Downwind		
Anopheles:				
albimanus Wiedemann	1		4 mph wind.	LePrince (1912)
funestus Giles	< 0.75	2.8		DeMeillon (1937)
funestus	< 4.5	3.25	Other specimens seen flying with wind.	Adams (1940)
gambiae	< 4.5	3.25	Other specimens seen flying with wind.	Adams (1940)
gambiae		< 2		DeMeillon (1937)
maculipennis		3		Wenyon (1912)
labranchiae atroparvus Van Thiel		< 8.7	Many more downwind than upwind.	Swellengrebel (1929)
minimus flavirostris (Ludlow)	< 0.25	< 1.4	Strong wind.	Russell & Santiago (1934)
pharoensis		5.6		Low (1925)
pseudopunctipennis Theobald	< 3.7	< 3.7	Most flew upwind.	Rickard (1928)
sundaicus			Flew 4 miles, windspeed negligible.	Van Breeman (1920)
culicifacies			251 downwind, 69 upwind, 145 crosswind, max. < 1.75.	Russell *et al.* (1944)
pharonensis		< 18-28	Downwind migrations over desert at full moon "looked like a dust storm."	Garrett-Jones (1950)
		45		Kirkpatrick (1925)
minimus flavirostris			Rough terrain, windspeed and direction variable, max. distance 1.25 miles, flew in all directions.	Ejercito & Urbino (1951)
vagus var. *limosus sollicitans*		40	Experiments of Smith. Flew up to 4 miles, perhaps upwind.	Felt (1904) Elmore & Schoof (1963)

TABLE 1. (cont.)

Species	Maximum distance flown (miles) Upwind	Downwind	Remarks	Sources
Anopheles:				
sollicitans		ca. 10	Regularly flies across Delaware Bay.	MacCreary & Stearns (1937)
cantator (Coquillett)		40	Experiments of Smith	Felt (1904)
		ca. 10	Regularly flies across Delaware Bay.	MacCreary & Stearns (1937)
aldrichi (Dyar & Knab) = *sticticus* (Meigen)		several	Flew along Fraser River valley.	Hearle (1926)
			Flew up and down Columbia River valley & tributaries.	Stage *et al.* (1937)
vexans		several		Rempel (1953)
		several	Flew along Fraser River valley	Hearle (1926)
			Flight mostly restricted to Columbia River valley & tributaries.	Stage *et al.* (1937)
		14	Flight of newly emerged adults has strong downwind tendency, windspeed < 34 mph.	Clarke (1943)
		90-230	Cold front following hot weather.	Horsfall (1954)
Aedes:				
dorsalis (Meigen)	5-8		Slow movement, level plain.	Rees (1935)
albopictus (Skuse)	0, 11			Bonnet & Worcester (1946)
leucocelaenus Dyar & Shannon			Flight has downwind bias.	Causey & Kumm (1948)
communis (DeGeer)			Flew in all directions < 5000 ft, wind variable in speed and direction.	Jenkins & Hassett (1951)
taeniorhynchus		< 20	Downwind bias, terrain may affect orientation.	Provost (1952)
		25	All downwind 4 days after eclosion.	Provost (1957)

TABLE 1. (cont.)

Species	Maximum distance flown (miles)		Remarks	Sources
	Upwind	Downwind		
Aedes:				
taeniorhynchus		30-60	Carried on W or SW wind.	Harden & Chubb (1961)
		20	Most flew upwind; winds 6-9 mph.	Bidlingmayer & Schoof (1957)
			Wind somewhat variable, flew up to 18 miles.	Elmore & Schoof (1963)
spencerii (Theobald)		several		Rempel (1953)
"blacklegged *Aedes*"		several		Rempel (1953)
flavescens (Muller)		6.6	Majority moved with prevailing wind.	Shemanchuk (1955)
nigromaculis (Ludlow)		30-60	Probably caught up in convection of cool air mass.	Smith *et al.* (1956)
aegypti		1.6	In absence of wind dispersal is "homogeneous"	Wolfinsohn & Galun (1953)
		1165	Wind more instrumental in dispersal than flight.	Bugher & Taylor (1949)
cantator		ca. 10	Regularly flies across Delaware Bay.	MacCreary & Stearns (1937)
Culex: spp.		< 18-28	Migrations over desert at full moon.	Garrett-Jones (1950)
tarsalis	2.5	1	Variable wind.	Reeves *et al.* (1948)
		15.75	3-4 mph wind carries mosquitoes 5 miles downwind in 1 night. Some fly upwind. At low windspeeds, mosquitoes fly in all directions.	Bailey *et al.* (1965)
quinquefasciatus			Shifting wind; flew in all directions and up to 3.5 miles.	Fussell (1964)
	1			Reeves *et al.* (1948)
Culex: *pipiens fatigans*			Flew 900 yds, "with a strong wind, dispersal would be increased".	Burton (1965)
		14		Clarke (1943)
salinarius Coquillett		ca. 10	Regularly flies across Delaware Bay	MacCreary & Stearns (1937)

TABLE 1. (cont.)

Species	Maximum distance flown (miles)		Remarks	Sources
	Upwind	Downwind		
Other genera:				
Unknown		22	Several genera; "slow steady breezes brought large numbers over desert".	Davis (1901)
Stegoconops spegazzinii (Brethes)			Flight has downwind bias	Causey & Kumm (1948)
Psorophora sp.		< 6	Strong tendency to move downwind.	Quarterman *et al.* (1955)

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DESCRIPTIONS OF NEW SPECIES OF FLIES OF THE FAMILY AGROMYZIDAE FROM ALBERTA, CANADA (DIPTERA)

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Thirteen new species of flies of the family Agromyzidae (Diptera) described in this paper are: Agromyza albertensis, A. masculina, Ophiomyia monticola, *O. pulicarioides,* Phytobia flavohumeralis, Cerodontha occidentalis, Liriomyza conspicua, L. montana, L. cordillerana, L. septentrionalis, Lemurimyza pallida, Phytomyza lupini *and* P. lupinivora. *Necessary amendments to existing keys are given to include the above species.*

The description of new species in this paper is a partial report on the survey of Agromyzid flies of Alberta, conducted during the years 1966 and 1967. The holotype and allotype of all new species will be deposited in the Canadian National Collection, Ottawa, Canada.

Since the publication of Frick's (1959) keys for the North American Agromyzid flies, many changes have taken place. Nowakowski (1962) suggested a new grouping of the genera based on genitalic structures. While I agree with Nowakowski's classification, the generic arrangements of Frick's keys have not been changed. The new species have been included in the existing framework of keys by giving the necessary amendments. Mr. K. A. Spencer intends to publish shortly a synopsis of the Canadian Agromyzidae and these new species will be included in his new keys.

As will be evident from the discussion of various species, the characters of the male genitalia have been extremely important in distinguishing between closely related species.

Agromyza albertensis new species

Description

Head (fig. 1). Frons slightly narrower than the width of an eye (1 : 0.9), almost equal in length to its width at level of front ocellus. In profile orbits and frons projecting in front of eye margin particularly above antenna. Lunule lower than a semicircle. Two strong *Ors* directed

upwards, two strong *Ori* directed inwards; orbital setulae few, about four, reclinate; lower ocellar bristle reaching the base of lower *Ors*. Eyes bare; ocellar triangle small. Gena about one fourth of the eye height midway between vibrissal and posterior margins, not extending in front of eye in profile. Vibrissal hair short and bent inwards. Antennal bases approximate; third antennal segment rounded anteroventrally, covered with uniform pubescence; arista 1.75 times the total length of antenna, pubescent.

Mesonotum. Two small presutural and four postsutural *dc* increasing in length posteriorly; *acr* in about 5-6 irregular rows.

Leg. Mid-tibia without a bristle medially.

Wing (fig. 2). Length about 2.0 mm in ♂ and 2.6 mm in ♀; costa extending to vein R_{4+5}; costal segments 2-4 in ratio of 1 : 0.36 : 0.31; last segment of M_{3+4} 0.9 times the punultimate segment; r-m crossvein slightly beyond the middle of the discal cell; wing tip between R_{4+5} and M_{1+2}.

Male Genitalia (fig. 3). Hypandrium with pregonites large and flattened; distiphallus elongate and well sclerotized, with a sharp characteristic bend at its base; basiphallus consisting of a pair of broad bent sclerotized strips, relatively short in relation to the long distiphallus. Phallophore weakly sclerotized; postgonites with a small apical lobe as in other members of the *nigripes-ambigua* group (Griffiths 1963). Ejaculatory apodeme broad and fan shaped, bulb small.

Colour. Frons matt black; ocellar triangle weakly shining; maxillary palpi black; mesonotum and scutellum shining black; wing veins dark brown; calypter with margin and fringe yellow; halteres yellow; abdomen black; legs black.

Material Examined

Holotype ♂ (with genitalia preparation) Canada, Alberta, Banff, 28. vi. 1966. Allotype ♀ same data. Paratypes, 1 ♀ Alberta, Blairmore, 26. vi. 1966; 1 ♂ and 1 ♀ Alberta, Banff, 28. vi. 1966 (K.A. Spencer) and 1 ♂ Alberta, Blairmore, 26. vi. 1966 (K.A. Spencer).

Derivation of the Specific Name

The specific name *albertensis* has been derived from the name of the province of Alberta, Canada.

Comparisons

Agromyza albertensis belongs to the *ambigua* group and is very close to *A. ambigua* Fallén. In Frick's (1959) key to the North American species of *Agromyza* Fallén, *A. ambigua* Fallén and *A. niveipennis* Zetterstedt both key out at couplet 18. Spencer (1965a) synonymized *niveipennis* Zett. with *ambigua* Fallén after examination of Zetterstedt's type series in Lund. The aedeagus of *A. ambigua* Fallén has been illustrated by Spencer (1965a). *A. albertensis* may be included in Frick's (1959) key to *Agromyza* species by amending couplets 17 and 18 as below:

17 Gena in height midway between vibrissal and posterior angles about one fourth the eye height 18
Gena in height midway between vibrissal and posterior angles about

one seventh the eye height ..... *barberi*

18 Wings shining milk white (Hendel 1936), aedeagus as figured by Spencer (1965a) ..... *ambigua*

Wings normal. Distiphallus with a characteristic bend at its base, relatively longer and narrower ..... *albertensis*

Biology

Not confirmed, but as far as known all species of this group are grass feeders in the larval stage (Griffiths 1963).

Agromyza masculina new species

Description

Head (fig. 4). Frons 1.2 times longer than broad, slightly narrower than the width of the eye (1 : 0.8) at the level of front ocellus, not projecting above the eye in profile. Lunule lower than a semicircle, slightly sunken below the frons. Two strong *Ors* directed upwards; two *Ori* directed inwards and upwards; orbital setulae 6-8, reclinate. Eyes bare; ocellar triangle small. Gena narrow, one eighth of the eye height in its middle, not projecting in front of the eye margin in profile. Vibrissal hair short and bent inwards. Antennal bases approximate; third antennal segment broad with slightly longer pubescence in front; arista long about twice the total length of the antenna.

Mesonotum . Dorsocentrals 3+1, strongly developed; *acr* in about six irregular rows.

Leg . Mid-tibia without a bristle medially.

Wing (fig. 5). Length about 2.5 mm; costa extending to vein M_{1+2} costal segments 2-4 in the ratio of 1 : 0.2 : 0.2; last segment of M_{3+4} 0.79 times the penultimate segment; r-m slightly before the middle of the discal cell; wing tip between R_{4+5} and M_{1+2}.

Male Genitalia (fig. 6). Hypandrium V-shaped, with small pregonites at the anterior end, basal half of the side arm flattened. Distiphallus elongate and well sclerotized; mesophallus with two sclerotized bars, of which the right continues as a narrow strip to join the basiphallus; basiphallus with weakly sclerotized broad strip on one side and a membranous fold on the other. Ejaculatory apodeme fan shaped, bulb small.

Colour . Frons dull greyish black; ocellar triangle weakly shining black; antennae black; maxillary palpi brownish black; mesonotum, scutellum and abdomen weakly shining black; wing veins dark brown; calypter margin and fringe yellowish brown; halteres yellow; legs with femora brownish black, tibiae and tarsi yellowish brown.

Material Examined

Holotype ♂ (with genitalia preparation) Canada, Alberta, Blairmore, 26. vi. 1966. Allotype ♀ same locality, 27. vi. 1966. Paratypes, 1 ♀, Alberta, Okotoks, 10. vi. 1966 (K. A. Spencer).

Derivation of the Specific Name

Agromyza masculina belongs to the *spiraeae* group. The species in this

group are poorly differentiated in external characters but examination of the male genitalia reveals conspicuous differences among the included species. The name *masculina* was suggested by K.A. Spencer in view of the distinctly larger and sclerotized aedeagus when compared to that of *spiraeae* Kaltenbach.

Comparisons

Agromyza masculina can be included in Frick's (1959) key to North American *Agromyza* species by amending and extending the couplets 19 and 20 as follows:

19 Calypter with margin and fringe brown or yellowish brown 20
Calypter with margin and fringe white or yellow 21
20 Mid-tibia medially with two posterolateral setae ..... *isolata*
Mid-tibia without posterolateral setae 20a
20a Calypter with margin and fringe brown; distiphallus widely separated from basiphallus by a completely membranous section .....
..... *spiraeae*
Calypter with fringe yellowish brown; mesophallus with a pair of sclerites distally; hypandrium with broader arms .....*masculina*

Ophiomyia monticola new species

Description

Head (fig. 7). Frons slightly narrower than the width of the eye (1 : 0.9) at the level of the front ocellus, projecting conspicuously in front of the eye margin in profile. Two equal, strong *Ors* directed upwards; two *Ori*, weaker than *Ors*, directed inwards and upwards; orbital setulae few, 5-6, reclinate. Eyes 1.3 times longer than broad, bare; ocellar triangle small. Gena about one third of the eye height midway between the vibrissal and posterior margins, projecting in front of the eye margin in profile. Vibrissal hair normal. Facial keel broad and distinctly bulbous below the antennal bases. Antennal bases separate; third antennal segment rounded; arista about one and a half times the total length of the antenna; pubescent.

Mesonotum. Two distinct postsutural *dc* ; *acr* numerous, in about ten irregular rows.

Leg. Midtibia without a bristle medially.

Wing (fig. 8). Length about 2.1 mm in ♂ and 2.3 mm in ♀; costa reaching the vein R_{4+5}, costal segments 2-4 in the ratio of 1 : 0.35 : 0.25; last segment of M_{3+4} about three quarters the penultimate; r-m cross vein beyond the middle of the discal cell.

Male Genitalia (fig. 9). Hypandrium with a distinct hypandrial apodeme; aedeagus with a complex distiphallus; basiphallus with two broad sclerotized strips, of which the right is longer and bent at base; phallophore broad and strongly sclerotized; ejaculatory apodeme long, fan shaped with a small gland at the base.

Colour. Completely black species; frons matt black; ocellar triangle weakly shining; mesonotum and scutellum matt black; abdomen weakly shining; wing veins dark brown; calypter with margin and fringe dark

brown; halteres black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Banff, 28.vi.1966. Allotype - ♀ same data. Paratypes - 2 ♀♀ Alberta, Jasper, 16.vi.1966; 2 ♂♂ same locality, 18-19.vi.1966; 1 ♀ Alberta, Cypress Hills, Elkwater, 24.vi.1966.

Mr. K.A. Spencer has reported the following further specimens which are referable to this species:

CANADA, Alberta, Jasper - 1 ♂, 19.vi.1966 (K.A. Spencer); Banff, 2 ♂♂, 2 ♀♀, 28.vi.1966 (K.A. Spencer), 1 ♂, 1 ♀, 8-29.vi.1922 (C.B.D. Garrett); British Columbia, Atlin - 1 ♀, 14.vii.1955 (H. J. Huckel), 1 ♂, 13.vi.1955 (B.A. Gibfarl); 32 miles SW of Terrace - 1♀, 11.vi.1960 (G.E. Shewell); Manitoba - Mile 505, Hudson Bay Railway - 1 ♀, 29.vi. 1952 (J.D. Chillcott); Yukon Territory- FirthRiver, British Mountains - 1 ♂, 1 ♀, 25.vii.1956 (R.E. Leech). ALASKA - Big Delta - 1 ♂, 10.vi. 1951 (J.R. McGillis), 1 ♀, 27.v.1951 (W.R. Mason).

Derivation of the Specific Name

The name *monticola* signifies that the species is mountain inhabiting.

Comparisons

Ophiomyia monticola is very close to *O. nasuta* (Melander) (=*Tylomyza nasuta* (Melander) *sensu* Frick 1959) in not having a distinct vibrissal horn in the male, but differs in having only two dorsocentrals and distinct genitalia. *Ophiomyia monticola* may be included in Frick's (1959) key to North American *Ophiomyia* species as given in the extension of couplet 1 at the end of the description below of *Ophiomyia pulicarioides* .

Ophiomyia pulicarioides new species

Description

Head (fig. 10). Frons narrower than the width of an eye (1 : 0.87) at the level of front ocellus, not projecting in front of the eye margin in profile. Lunule lower than a semicircle. Two strong *Ors* directed upwards; two *Ori* , weaker than *Ors*, directed inwards and upwards; orbital setulae about 12-15, reclinate. Eyes 1.2 times longer than broad, bare; ocellar triangle small. Gena about one fifth of the eye height midway between vibrissal and posterior margins, not extending in front of the eye in profile. Vibrissal hair normal. Facial keel narrow. Antennal bases approximate; third antennal segment rounded; arista about twice the total length of the antenna, pubescent.

Mesonotum . Two distinct postsutural *dc* ; *acr* numerous, in about ten irregular rows.

Leg. Midtibia without a bristle medially.

Wing (fig. 11). Length about 2.0 mm in ♂ and 2.25 mm in ♀; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1 : 0.26 : 0.24; last segment of M_{3+4} about 0.77 times the penultimate; r-m crossvein beyond the middle of the discal cell.

Male Genitalia (fig. 12). Hypandrium typical V-shaped; aedeagus with a complex distiphallus; basiphallus with two broad sclerotized strips, of which the right is larger than the left; phallophore broad and strongly sclerotized.

Colour . Completely black; frons matt black; ocellar triangle weakly shining; mesonotum, scutellum and abdomen shining black; wing veins dark brown; calypter with margin and fringe dark brown; halteres black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Cypress Hills, Elkwater, 24.vi.1966; Allotype - ♀ same data.

Derivation of the Specific Name

The name *O. pulicarioides* indicates that this species belongs to the *pulicaria* group.

Comparisons

Ophiomyia pulicarioides resembles *O. pulicaria* (Meigen) in having no vibrissal horn in the male and no distinct facial keel (both sexes); but the male genitalia are distinct. Like *O. pulicaria* , *O. pulicarioides* can easily be confused with the genus *Melanagromyza* Hendel on the basis of external characters alone, but both possess an aedeagus typical of the genus *Ophiomyia* Braschnikov.

Ophiomyia pulicarioides and *O. monticola* described above may be included in Frick's (1959) key to the North American species of *Ophiomyia* by extending couplet 1 as below:

1 Haltere black 1a
Haltere with a white spot on the knob ..... *punctohalterata*
1a Distinct facial keel separating antennae 1b
Distinct facial keel lacking; male without a vibrissal horn ..... *pulicarioides*
1b Male with a distinct vibrissal horn 2
Male without a distinct vibrissal horn; two strong postsutural *dc*; aedeagus as in fig. 9 ..... *monticola*

Phytobia flavohumeralis new species

Description

Head (fig. 13). Frons almost equal to the width of the eye at the level of the front ocellus, slightly projecting in front of the eye margin in profile. Lunule low, reaching slightly above the base of lower *Ori*. Two strong *Ors* directed upwards; two strong *Ori* directed inwards and upwards; orbital setulae about 8-10, reclinate. Eyes about 1.25 times higher than broad; ocellar triangle small. Gena about one eighth of the eye height midway between vibrissal and posterior margins, not extending in front of the eye margin in profile. Vibrissal hair normal. Antennal bases approximate; facial keel narrow; arista long and pubescent.

Mesonotum . Dorsocentrals 3+1; *acr* numerous, in about 10 irregular rows.

Leg . Midtibia with a couple of conspicuous setae medially.

Wing (fig. 14). Length in males 2.8-3.1 mm; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1.0 : 0.30 : 0.24; last segment of M_{3+4} about 0.9 times the penultimate; r-m cross vein approximately at the centre of the discal cell.

Male Genitalia (fig. 15). Hypandrium U-shaped with darkly sclerotized broad arms; aedeagus tubular and lightly sclerotized structure, the distiphallus complex has a swollen bulb at the base, basiphallus consists of a long tube with a distinct curvature; phallophore darkly sclerotized section at the base; ejaculatory apodeme broad and lightly sclerotized, ejaculatory bulb large.

Colour . Frons, orbits and lunule greyish black; gena slightly yellowish; antennae black; maxillary palpi black; mesonotum dull greyish black; humeral areas with a characteristic yellow ring; pleural region dull black; mesepisternum with a narrow yellow band along the upper margin; legs black with a slight yellow at the tip of the femora; wings normal; calypter with margin and fringe dark brown; halteres yellow; abdomen dull greyish black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, George Lake, from the Malaise trap collection of Peter Graham of the University of Alberta, Edmonton, 18.v.1967; Paratypes - 10 ♂♂ (all with genitalia preparations), same data, 18-23.v.1967; 7 ♂♂, same data, 11.v.1966; 5 ♂♂, same data, 19-20.v.1966. Mr. Spencer has examined the following:

CANADA, British Columbia, Robson - 1 ♂, 14.v.1947 (H.R. Foxlee); Saskatchewan, Saskatoon - 2 ♂♂, 9.v.1949 (A.R. Brooks); Ontario, Ottawa - 1 ♂, 14.v.1925 (C.H. Curran); Bell's Corner - 2 ♂♂, 25.v.1949 and 21.v.1950 (G.E. Shewell).

Derivation of the Specific Name

The name *flavohumeralis* indicates that the species possesses a characteristic yellow on humeral areas.

Comparisons

Phytobia flavohumeralis is very close to *Phytobia (Phytobia) setosa* (Loew); but differs from it in having a black third antennal segment and a characteristic yellow ring around the humeral areas. It may be included in Frick's (1959) key to the subgenus *Phytobia* Lioy by amending and extending the couplets 6 and 7 as follows:

6 Cross vein m-m about its own length from r-m 7
Cross vein m-m not more than six-tenths of its length from r-m.. ..... *waltoni*

7 Head with one upper-orbital reclinate; dorsal margin of lunule semicircular (figs. 57 & 58, Frick 1959); mid-tibia with three posterolateral setae ..... *amelanchieris*
Head with both upper-orbitals reclinate; dorsal margin of lunule flattened; mid-tibia with one or two posterolateral setae 7a

7a Mesonotum black; third antennal segment reddish .....
Mesonotum with a characteristic yellow ring around humeral areas; third antennal segment black; aedeagus as fig. 15 ..... *flavohumeralis*

This species belongs to the genus *Phytobia* Lioy in the restricted sense proposed by Nowakowski (1962). This group was given subgeneric rank (*Phytobia*, subgenus *Phytobia*) in Frick's (1959) classification.

Cerodontha occidentalis new species

Description

Head (fig. 16). Frons broad, about 1.1 times the width of the eye at the level of front ocellus; conspicuously projecting in front of the eye margin in profile, less so at the rear. Lunule higher than a semicircle. Two strong *Ors* directed upwards; one *Ori* directed inwards with a small proclinate hair in front; orbital setulae 2-4, the lower two usually proclinate or bent inwards the upper reclinate. Eyes oval about 1.1 times higher than broad; ocellar triangle small. Lower ocellar bristle long extending below the base of the *Ori*. Gena about one-third of the eye height midway between vibrissal and posterior margins. Vibrissal hair strong. Third antennal segment elongate and produced into a sharp spine at the upper angle; arista long and pubescent.

Mesonotum . 3+1 strong dorsocentrals; first two dorsocentrals of almost equal length and strength, third and fourth increase in size posteriorly; *acr* absent.

Leg . Midtibia without a bristle medially.

Wing (fig. 17). Length 2.2-2.5 mm in ♂ and 2.7-2.9 mm in ♀; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1 : 0.2 : 0.2; last segment of M_{3+4} about 0.9 times the penultimate; r-m cross vein slightly before the middle of the discal cell; M_{1+2} at the wing tip.

Male Genitalia (fig. 18a, b). Hypandrium typical U-shaped; aedeagus conspicuously elongate structure; distiphallus with a pair of long sigmoid tubes with a distinct sclerotized bulb at its tip; the apical bulb at the most twice as long as broad; mesophallus is an elongate tubular structure swollen at the base, it has a small recurved process at the distal and a distinct sclerotized section at its base; hypophallus consists of a pair of recurved processes; basiphallus is a distinct, broad, bent and sclerotized part, its narrow basal end is connected to the distal end of the broad circular and sclerotized phallophore; ejaculatory duct is visible inside the phallophore and basiphallus; ejaculatory bulb broad and has a sclerotized lower wall; ejaculatory apodeme broad, fan shaped and well sclerotized.

Colour . Frons, lunule, facial keel, gena, first and second antennal segments yellow; second antennal segment of antenna sometimes brownish; orbits usually yellow but sometimes slightly darkened along the eye margin in front, in the region of orbital bristles; maxillary palpi and proboscis yellow; mesonotum matt black, humeral callus yellow, with a black spot anteriorly; notopleural areas yellow; scutellum usually matt black sometimes with a light brown central area; mesepisternum and mesepimeron varies from dark brown to black, but always with a yellow band along the upper margins; legs with coxae and femora yellow, tibiae and tarsi brownish yellow to dark brown; wings normal; calypter margin and fringe dark brown; halteres yellow; abdominal terga matt black with a narrow yellow posterior margin.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Canmore nr. Banff, 28.vi.1966, swept on open grass. Paratypes - 20 ♂ (all with

genitalia preparations) and 4 ♀♀, same data; 7 ♂♂ and 2 ♀♀, same data in K.A. Spencer's collection; 1 ♂, Alberta, Blairmore, 27.vi.1966.

Mr. K.A. Spencer has kindly compared the following further specimens referable to this species:

1 ♀ - CANADA, Yukon Territory, Rampart House, 17.vi.1951 (J.E.H. Martin). 1 ♂ - ALASKA, Big Delta, 24.vi.1951 (J.R. McGillis), 1 ♀ - Anchorage, 27.vi.1951 (R.S. Bigelow).

Derivation of the Specific Name

The name *occidentalis* indicates that the species is described from western Canada.

Comparison

Cerodontha occidentalis is very close in its external characters to the only other Nearctic species, *C. dorsalis* (Loew), which has been widely reported from United States and Canada (Frick 1959). This new species like *C. dorsalis* (Loew) shows some colour variation. This colour variation however does not affect the uniformity in the structure of the male genitalia. A detailed examination of a long series of specimens collected mainly at Canmore near Banff, Alberta, shows conspicuous differences from those of typical *C. dorsalis* (Loew). The aedeagus of a typical *C. dorsalis* from the United States (Indiana, Lafayette) collected by J.M. Aldrich is illustrated (fig. 18c) for comparison with that of *C. occidentalis* (figs. 18a, b). The aedeagus in *C. occidentalis* is about one and a half times as long as in *C. dorsalis*. The main distinguishing features are the broader hypophallus, the incurved process at the distal end of the mesophallus, the longer tubes of the distiphallus and comparatively shorter bulb at the tip of the distiphallus.

Other specimens of *C. dorsalis* examined are: Manitoba, Aweme - 1 ♂, 27.viii.1917 (N. Criddle); Alberta, Banff - 1 ♂, 3.ix.1966; Blairmore - 2 ♂♂, 4.ix.1966; Crowsnest - 1 ♂, 5.ix.1966; Medicine Hat - 1 ♀, 16.vi.1928 (F.S. Carr) det. C.H. Curran; British Columbia, Crowsnest - 1 ♂, 26.vii.1926 (A.A. Dennys); Shuswap Lake - 1 ♂, 22.vii.1926 (J.M. Dunnough); Chilliwack - 1 ♂, 14.x.1938 (J.K. Jacob).

Cerodontha occidentalis may be separated from the only other species *Cerodontha dorsalis* (Loew) known in the Nearctic region, by the following key:

1 Scutellum adjoining mesonotum usually with a variable yellow spot; the sigmoid tubes of the distiphallus comparatively short with an elongate apical bulb; the latter at least three times as long as broad (fig. 18c) ..... *dorsalis*

Scutellum and adjoining mesonotum usually with a uniform matt black; the sigmoid tubes of the distiphallus long with a short bulb at the tip; the apical bulb of the distiphallus at the most twice as long as broad (fig. 18a, b) ..... *occidentalis*

Nowakowski (1962) has proposed an enlarged concept of the genus *Cerodontha* Rondani, including a number of subgenera which were transferred from *Phytobia* Lioy. The above new species *Cerodontha occidentalis* like *C. dorsalis* (Loew) belongs to the genus *Cerodontha* in the restricted sense (subgenus *Cerodontha* in Nowakowski's classification).

Liriomyza conspicua new species

Description

Head (fig. 19). Frons narrower than the width of the eye at the level of front ocellus, projecting conspicuously in front of the eye margin in profile. Lunule reaching up to the base of second *Ori*. Two strong *Ors* directed upwards; three *Ori* in male, the two females referable to this species have four *Ori* on one side, all directed inwards and upwards; orbital setulae about 13-18, reclinate. Eyes oval, 1.3 times higher than broad in profile; ocellar triangle small. Gena high about one fourth the eye height midway between vibrissal and posterior margins, not extending in front of eye margin in profile. Vibrissal hair normal. Facial keel narrow. Antennal bases approximate; third antennal segment broad; pubescent.

Mesonotum (fig. 21). Dorsocentrals 3+1; *acr* in about 5 irregular rows.

Leg . Midtibia without a bristle medially.

Wing (fig. 20). Length 2.5 mm in ♂ and 3.0-3.25 mm in ♀; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1 : 0.3 : 0.2; last segment of M_{3+4} about 1.5 times the penultimate; r-m cross vein approximately at the centre of the discal cell.

Male Genitalia (fig. 22). Hypandrium typical U-shaped with broad pregonites. Aedeagus complex; distiphallus consisting of two long narrow processes joined at the base by a thin membrane; mesophallus a long darkly sclerotized spindle shaped structure; hypophallus complex, partly fused with the basiphallus; the two arms of basiphallus are fused at the base, a short sclerotized segment is connected to one side of basiphallus by a thin membrane. Phallophore well sclerotized and continuous with the basiphallus. Aedeagal rod broad at its anterior end. Ejaculatory apodeme broad and fan shaped; ejaculatory bulb large with thin walls.

Colour . Frons, orbits and antennae yellow; ocellar triangle dark brown; gena yellow; black of occiput touching upper posterior margin of the eye; *vte* and *vti* on the margin of dark brown and yellow grounds; maxillary palpi yellow; mesonotum (fig. 21) brownish black with a characteristic yellow before the scutellum; humeral and notopleural areas yellow; humeral areas with a small brown spot anteriorly; scutellum yellow with very small brown areas near its basal corners; mesepisternum dark brown with upper one third yellow; mesepimeron black with a yellow upper margin; legs with coxae distally yellow; femora yellow; tibiae and tarsi brown; wings normal; calypter with margin and fringe dark brown; halteres yellow; abdominal tergites dark brown with a yellow line along posterior margins.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Manitoba, 5 miles S. W. Shilo, Floodplain community nr. Tamarack Bog, open grassy marsh, 2.viii.1958, coll. J.G. Chillcott. Paratypes - 1♀, Saskatchewan, Butland, 19.vii.1940, coll. A.R. Brooks; 1 ♀ - Alberta, Jasper, 16.vi. 1966.

Mr. K.A. Spencer has kindly compared the following further speci-

mens also referable to this species:

Manitoba, Minnedosa - 1 ♂, 7.vi.1926 (R.M. White); 9 miles N of Forrest - 1 ♂ and 3 ♀♀, 29.vi. and 19.vii.1958 (R.B. Madge and R.L. Hurley), 5 miles SW of Shilo, 2 ♀♀, 5.vi. and 16.vi.1958 (R.L. Hurley). Ontario, Ottawa - 1 ♀, 3.vi.1958. Saskatchewan, Saskatoon - 2 ♂♂, 3 ♀♀, 9.v.1949 (A.R. Brooks), 1 ♀, 28.vii.1923 (N.L. Atkinson), 1 ♀, 28.vi.1941 (Arnason); Indian Head - 1 ♂, 3.viii.1939 (C.R. Douglas); Assiniboia - 1 ♀, 27.vi.1955 (J.R. Vockeroth).

Derivation of the Specific Name

The name *conspicua* indicates that this species is very conspicuous in having very bright colouration and characteristic genitalia.

Comparisons

Liriomyza conspicua belongs to the group of species having a characteristic prescutellar yellow. It can be included in Frick's (1959) key to North American *Liriomyza* species by amending and extending the couplet 15 as below:

15 Acrostichal setae in 4-5 rows; prescutellar yellow area subrectangular 15a
Acrostichals five or six in number, in two rows; yellow area triangular ..... *assimilis*

15a Acrostichals about 13 in number, in four rows; all four dorsocentral setae on yellow ground (Frick 1959) ..... *flavonigra*
Acrostichals many in about five irregular rows (fig. 21); all four dorsocentral setae on dark brown ground; aedeagus as illustrated (fig. 22) ..... *conspicua*

Liriomyza montana new species

Description

Head (fig. 23). Frons slightly wider than the width of the eye at the level of front ocellus, slightly projecting in front of the eye margin in profile. Lunule low, reaching to the base of the lower *Ori*. Two *Ors* directed upwards; two to three *Ori* directed inwards and upwards; orbital setulae 4-6, reclinate. Eyes about 1.3 times higher than broad; ocellar triangle small. Gena about one fifth of the eye height midway between vibrissal and posterior margins, not extending in front of the eye margin in profile. Vibrissal hair normal. Facial keel narrow; antennal bases approximate; third antennal segment rounded; arista long; weakly pubescent.

Mesonotum . Dorsocentrals 3+1; *acr* numerous in about 4 irregular rows; humeral callus with 4-5 hairs.

Leg . Midtibia without a bristle medially.

Wing (fig. 24). Length 1.9-2.0 mm in ♂; and 2.0-2.25 mm in ♀; costa extending strongly to the vein M_{1+2}; costal segments 2-4 in the ratio of 1 : 0.3 : 0.24; last segment of M_{3+4} about twice as long as the penultimate; r-m crossvein almost at the centre of the discal cell, sometimes slightly before it; vein M_{1+2} ending at the wing tip.

Male Genitalia (fig. 25). Hypandrium typical U - shaped; pregonites broad; distiphallus elongate with a characteristic bend and sclerotization at its base and has a distinctive small bulb at its tip; basiphallus consisting of two long and narrow sclerites enclosing characteristic sclerotized and swollen ejaculatory duct; hypophallus almost membranous fold; phallophore long and well sclerotized; ejaculatory apodeme fan shaped with a sclerotized bulb at its base.

Colour . Frons, lunule, orbits and gena entirely yellow; third antennal segment yellow, arista brown; black of occiput touching the upper posterior margin of the eye; *vte.* usually on dark brown ground; *vti* on the margin of dark brown and yellow ground; mesonotum shining black with yellow humeral and notopleural areas; humeral area with a small dark spot, humeral seta on yellow ground; scutellum with very small dark area on the basal corners; mesepisternum with upper two thirds yellow and lower one third dark brown; mesepimeron dark brown with a dorsal band of yellow; legs with coxae yellow but have slight brown bases; femora primarily yellow; tibiae and tarsi brown; wings normal; calypter margin and fringe brown; halteres yellow; abdomen shining black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Jasper; 17.vi.1966. Allotype - ♀, same data. Paratypes - 14 ♂♂ and 5 ♀♀, same locality, 17-19.vi.1966; 3 ♂♂, Alberta, Banff, 28.vi.1966. All specimens swept on open grass.

Derivation of the Specific Name

The name *montana* indicates that the species is mountain inhabiting.

Comparison

Liriomyza montana belongs to the *flaveola* group of species on the basis of the male genitalic structures and is very close to European species *L. pedestris* Hendel, but has very distinct genitalia. It is also very close to *L. graminicola* de Meij. in external characters but the male genitalia is very distinct in the shape of the distiphallus.

L. montana resembles *L. richteri* Hering in the general shape of male genitalia, but differs in having yellow femora.

L. montana resembles *L. eupatori* (Kaltenbach) in having yellow femora and four rows of acrostichals but has male genitalia typical of the *flaveola* group. *L. montana* can be included in Frick's (1959) key to the *Liriomyza* species by extending the couplet 29 as below:

29 Crossvein m-m not more than its length from r-m; ultimate section M_{3+4} about three times as long as the penultimate 30
Crossvein m-m more than its own length from r-m; ultimate section of M_{3+4} about two times as long as the penultimate 29a

29a Crossvein m-m 1.5 to two times its length from r-m; humeral callus with 4-9 hairs (Hendel 1936) ..... *eupatori*
Crossvein m-m slightly less than 1.5 times its length from r-m; humeral callus with 4-5 hairs; male genitalia as illustrated (fig. 25) ..... *montana*

Liriomyza cordillerana new species

Description

Head (fig. 26). Frons almost equal to the width of the eye at the level of front ocellus, slightly projecting in front of the eye margin in profile. Lunule low, reaching to the base of lower *Ori*. Two strong *Ors* directed upwards; usually two *Ori*, of which (but one female has lower *Ori* missing on one side) the lower one slightly weaker than the upper; both *Ori* directed upwards and inwards; orbital setulae about 5-6, reclinate. Eyes about 1.3 times higher than broad; ocellar triangle small. Gena about one fifth of the eye height midway between vibrissal and posterior margins, not extending in front of the eye margin in profile. Vibrissal hair normal. Facial keel narrow. Antennal bases approximate; third antennal segment slightly elongate; arista long and weakly pubescent.

Mesonotum . Dorsocentrals 3+1; *acr* numerous in about 4-5 irregular rows.

Leg . Midtibia without a bristle medially.

Wing (fig. 27). Length 2.4-2.8 mm in ♂ and 2.5-3.0 mm in ♀; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1 : 0.2 : 0.2; last segment of M_{3+4} about twice as long as the penultimate; r-m cross-vein approximately at the centre of the discal cell.

Male Genitalia (fig. 28). Hypandrium typical U-shaped with broad pregonites; postgonites characteristically elongate; distiphallus with a characteristic bend at its base and a darkly sclerotized bulb at its apex, within which two strongly sclerotized ducts can be seen; basiphallus consisting of two long narrow sclerites enclosing the sclerotized ejaculatory duct which does not form a swollen bulb before entering the distiphallus; hypophallus consisting of long narrow and bent sclerites; phallophore broad and strongly sclerotized. Ejaculatory apodeme fan shaped with a strongly sclerotized bulb at its base; ejaculatory duct also sclerotized for a short distance.

Colour . Frons usually yellow but sometimes orange or brownish yellow; ocellar triangle shining black; antennae yellow, with the third segment usually darkened distally; black of occiput touching the upper posterior margin of the eye; *vte* on black ground; *vti* usually on the margin of black and yellow; orbits usually darkened along the eye margin; maxillary palpi yellow; mesonotum shining black with yellow humeral and notopleural areas; both humeral and notopleural areas with a black spot; scutellum yellow with dark areas near its basal corners; pleural areas mainly black; mesepisternum with a narrow yellow upper margin; legs with coxae black; femora black with a yellow distal tip; tibiae and tarsi black; wings normal; halteres yellow.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Banff, 3.ix.1966. Allotype - ♀, same data. Paratypes - 9 ♂♂ and 7 ♀♀, same data; 3 ♂♂, Alberta, Jasper, 1.ix.1966; 1 ♂, Alberta, Jasper, Sunwapta Falls, 2.ix.1966; 2 ♂♂ and 2 ♀♀, Alberta, Blairmore, 26-27.vi.1966; 2 ♂♂, same locality, 4.ix.1966; 1 ♂, Alberta, Crowsnest, 5.ix.1966; 13

♂ and 5 ♀, Alberta, Waterton Park, 6-7.ix.1966.

Derivation of the Specific Name

The name *cordillerana* indicates that the species is mainly distributed in the Rockies.

Comparisons

Liriomyza cordillerana belongs to the *flaveola* group of species and is very close to *Liriomyza pedestris* Hendel and *L. richteri* Hering in external appearance but has very distinct genitalia. The male genitalia of *L. richteri* Hering was illustrated by Griffiths (1964), and of *L. pedestris* Hd. by Spencer (1965b).

Liriomyza cordillerana is also very close to *L. septentrionalis*, the new species described below, and can be reliably separated only by detailed examination of the characteristics of its male genitalia. *L. cordillerana* can be included in Frick's (1959) key to North American *Liriomyza* species as shown below at the end of the description of *L. septentrionalis*.

Liriomyza septentrionalis new species

Description

Head (fig. 29). Frons narrower than the width of an eye (1 : 0.8) at the level of front ocellus, not projecting in front of the eye margin in profile. Lunule low, reaching to the base of lower *Ori*. Two strong *Ors*, directed upwards; two slightly weaker *Ori* (three in one specimen), directed inwards and upwards; orbital setulae about 5-8, reclinate. Eyes about 1.2 times longer than broad; ocellar triangle small. Gena about one fifth of the eye height midway between vibrissal and posterior margins, not extending in front of the eye margin in profile. Vibrissal hair normal. Facial keel narrow. Antennal bases approximate; third antennal segment oval; arista long and weakly pubescent.

Mesonotum . Dorsocentrals 3+1; *acr* in about 5 irregular rows.

Leg . Midtibia without a bristle medially.

Wing (fig. 30). Length about 2.5 mm in ♂ and 2.7 mm in ♀; costa extending to vein M_{1+2}, costal segments 2-4 in the ratio of 1 : 0.24 : 0.20; last segment of M_{3+4} about 1.7 times the penultimate; r-m crossvein at the middle of the discal cell.

Male Genitalia (fig. 31). Hypandrium typical U-shaped; pregonites broad; postgonites characteristically elongate; distiphallus with a characteristic bend at the base and weakly sclerotized bulb at its apex, within which a long narrow and weakly sclerotized duct can be seen; basiphallus consisting of two long narrow sclerites enclosing the strongly sclerotized ejaculatory duct which forms a characteristic swollen bulb towards the apex of basiphallus; hypophallus consisting of a pair of short bent sclerites. Ejaculatory apodeme fan shaped; ejaculatory bulb large with strongly sclerotized walls.

Colour . Frons yellow; ocellar triangle dull black; antennae yellow, sometimes third antennal segment slightly orange or brownish; orbits yellow, but may be very slightly darkened; black of occiput touching the

upper posterior margin of the eye; *vte* on black and *vti* on the margin of black and yellow; maxillary palpi brownish yellow; mesonotum shining black with yellow humeral and notopleural areas; both humeral and notopleural areas with a black spot; scutellum yellow, with a black margin near the upper scutellars; pleural areas mainly black; mesepisternum with a narrow yellow upper margin; legs with coxae black; femora black but with a distal yellow; tibiae and tarsi black; wings normal; calypter margin and fringe black; halteres yellow; abdomen black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Banff, 28.vi.1966. Allotype - ♀ same data. Paratypes - 1 ♀, same data; 1 ♂ and 1 ♀, Alberta, Jasper, 17-18.vi.1966; 2 ♂♂, Alberta, Blairmore, 26.vi.1966; 3 ♂♂, Alberta, Waterton National Park; 6-7.ix.1966.

Mr. K.A. Spencer has kindly examined the following specimens referable to this species:

CANADA, Alberta, Frank - 1 ♀, 13.vii.1966 *ex* mine in grass leg. 26.vi.1966 (K.A. Spencer); Jasper - 1 ♂, 25.vii.1926 (J. McDunnough); Banff - 1 ♀, 7.vii.1955 (J.R. Cogles); Elkwater - 1♀, 2.vi.1955 (J.R. Vockeroth); British Columbia, Cultus Lake - 2 ♂♂, 4-10.vii.1948 (H.R. Foxlee); Brilliant - 1 ♀, viii.1947 (H.R. Foxlee).

Derivation of the Specific Name

The name *septentrionalis* indicates that the species is northern in its distribution.

Comparisons

Liriomyza septentrionalis belongs to the *flaveola* group and is very close to *L. flaveola* (Fallén) but has a darker mesepisternum and a distinct aedeagus. It is also very close to European species *L. pedestris* Hendel externally, but has a distinct genitalia. The genitalia of *L. flaveola* (Fallén) and *L. pedestris* Hendel have been illustrated by Spencer (1965a, 1965b).

Liriomyza septentrionalis is also very close to *L. cordillerana*, the species described above, from which it can be reliably differentiated only by a close examination of aedeagus, which has characteristically swollen ejaculatory duct between basiphallus and distiphallus, and a paler distiphallus. It also differs in having usually yellow orbits and third antennal segment.

Liriomyza septentrionalis and *L. cordillerana* can be included in Frick's (1959) key to North American *Liriomyza* species by amending and extending the couplet 23 as below:

23 Femora primarily yellow, usually marked with brown or black streaks or spots 24
Femora black, distal one third yellow 23a

23a Mesepisternum with at least dorsal one third yellow 23b
Mesepisternum approximately with upper half yellow (Frick 1959); aedeagus as illustrated by Spencer (1965a) ..... *flaveola*

23b Orbits usually yellow; aedeagus with ejaculatory duct characteristically swollen between basiphallus and distiphallus (fig. 31) distiphallus lightly sclerotized and as illustrated (fig. 31) .... *septentrionalis*

Orbits usually darkened; ejaculatory duct between basiphallus and distiphallus not swollen, distiphallus darkly sclerotized and as illustrated (fig. 28) ..... *cordillerana*

Lemurimyza pallida new species

Description

Head (fig. 32). Frons almost equal to the width of the eye; slightly projecting in front of the eye margin in profile, particularly so at the base of the antenna. Two strong *Ors* directed upwards; one *Ori* directed inwards; orbital setulae about 8-9. Eyes oval, 1.2 times higher than broad; ocellar triangle small. Gena about one third (1 : 0.3) of the eye height midway between vibrissal and posterior margins, not extending in front of the eye in profile. Antennal bases approximate; third antennal segment elongate; arista long and covered with uniform pubescence.

Mesonotum . Dorsocentrals 3+1; *acr* in two rows.

Wing (fig. 33). Length in ♂ 2.1 mm; costa extending to vein M_{1+2}; costal segments 2-4 in the ratio of 1 : 0.26 : 0.21; last section of M_{3+4} about twice as long as the penultimate; r-m crossvein almost at the centre of the discal cell; wing tip at M_{1+2}.

Male Genitalia (fig. 34). Hypandrium typically U-shaped with narrow side arms and broad fused pregonites. Distiphallus with the characteristic paired tubules bent outwards; mesophallus long and cylindrical, darkly sclerotized; hypophallus consists of sclerotized narrow ventral appendages at the base of mesophallus. Surstyli with sclerotized teeth on their ventral surface. Ejaculatory apodeme broad and fan shaped; ejaculatory bulb large and membranous.

Colour . Frons, orbits, genae and antennae entirely yellow; arista brownish; ocellar triangle brown. Mesonotum matt black; humeral and notopleural areas yellow with a small black spot on both. Legs with femora and tibiae yellow; tarsi brownish. Wings normal; calypter margin and fringe brown; halteres yellow.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Banff, 28. vi. 1966.

Derivation of the Specific Name

The name *pallida* indicates that the species is mostly yellow in colour.

Comparisons

The genus *Lemurimyza* was described by Spencer (1965) and includes four world species. *Lemurimyza pallida* represents the first record of the genus in the Nearctic region. This species is characteristic in having a yellow third antennal segment and characteristic male genitalia.

Phytomyza lupini new species

Description

Head (fig. 35). Frons almost equal to the width of the eye at the level of the front ocellus, conspicuously projecting in front of the eye margin in profile. One *Ors* directed upwards; two *Ori* directed inwards; orbital setulae many, proclinate. Eyes almost circular; ocellar triangle small. Orbits prominantly projecting in front and below the eye margin. Gena about one third (1 : 0.33) of the eye height midway between the vibrissal and posterior margins, becoming higher posteriorly. Antennal bases approximate; third antennal segment elongate; arista long and swollen at the base, weakly pubescent.

Mesonotum . Dorsocentrals 3+1; *acr* in 2-3 irregular rows.

Wing (fig. 36). Length 2.6 mm in ♂, 2.8 mm in ♀, costa extending strongly to vein R_{4+5}; costal segments 2-4 in the ratio of 1 : 0.24 : 0.4; wing tip at the vein M_{1+2}.

Male Genitalia (fig. 37). Hypandrium V-shaped with broad side arms and flattened pregonites; aedeagus complex; distiphallus with a characteristic bent section which has a sclerotized tip; basiphallus consisting of two broad sclerotized arms joined at the base which are produced into broad sclerotized plates distally; phallophore broad and continuous with the basiphallus; aedeagual apodeme very broad; ejaculatory apodeme fan shaped with a slightly sclerotized bulb.

Colour . Frons dominantly yellow or slightly brownish at the base; orbits yellow; black of the occiput touching the posterodorsal margin of the eye; *vte* on black ground and *vti* on the margin of yellow and black ground; ocellar triangle weakly shining black; third antennal segment shining black; first and second segments yellowish black. Mesonotum and scutellum matt black; pleura matt black. Legs: femora with a distal ring of yellow; tibiae and tarsi black. Wings normal; calypter margin and fringe yellowish brown; halteres yellow. Abdomen black.

Material Examined

Holotype - ♂ (with genitalia preparation) CANADA, Alberta, Blairmore, *ex* stem mines on *Lupinus sericeus* Pursh (Leguminosae) collected 6.ix.1966, emerged 11.iii.1967; puparium chilled at 45 F for 12 weeks; Allotype - ♀, same data, emerged 20.iii.1967; Paratypes - 1♂ and 3♀, same data.

Mr. K.A. Spencer has kindly examined the following specimens which are referable to this species:

CANADA, British Columbia, Albion - 2 ♂, 7.viii.1952, ex " crown of *Lupinus* sp." (Y. Ayre).

Derivation of the Specific Name

Phytomyza lupini is named after its larval food plant *Lupinus sericeus* Pursh (Leguminosae).

Comparisons

Phytomyza lupini may be included in Frick's (1957) key to the genus *Phytomyza* Fallén by amending and extending the couplet 17 as below:

17 Genovertical plates darkened ..... *angelicella*
Genovertical plates yellow 17a
17a Acrostichals in 4-5 rows (Frick 1957); intraalar row with 10-12 setulae anterior to and 13-15 posterior to the transverse suture .. .. *aquilegiana*
Acrostichals in 2-3 irregular rows; intraalar row with about 3 setulae anterior to and 2-3 posterior to the transverse suture; male genitalia as illustrated (fig. 37) ..... *lupini*

Biology

The larvae bore inside the stems of *Lupinus sericeus* Pursh (Leguminosae). Pupation takes place inside the stem. The puparia are characteristic in having a distinct horn on the posterior spiracles.

Phytomyza lupinivora new species

Description

Head (fig. 38). Frons narrower than the width of the eye (1 : 0.84) at the level of the front ocellus, not projecting in front of the eye margin in profile. Two strong *Ors* directed upwards; two slightly weaker *Ori* directed inwards; orbital setulae few, about 4, proclinate. Eyes rounded; ocellar triangle small. Gena about two fifths (1 : 0.4) of the eye height midway between the vibrissal and posterior margins, not extending in front of the eye in profile. Antennal bases approximate; third antennal segment rounded; arista small and thickened at the base; weakly pubescent.

Mesonotum . Dorsocentrals 3+1; two small *acr* present between first and second pair of dorsocentrals.

Wing (fig. 39). Length in ♀ 1.75 mm; costa extending strongly to vein R_{4+5}; costal segments 2-4 in the ratio of 1 : 0.24 : 0.75; wing tip at M_{1+2}. The wing of the ♀ holotype is abnormal in having an additional vein near the tip of R_{2+3}.

Colour . Completely black species; frons dull black; ocellar triangle weakly shining; gena, orbits and antennae matt black; mesonotum and scutellum matt black; pleura weakly shining black; legs black; wing veins dark brown; calypter margin and fringe dark brown; halteres yellow; abdomen matt black.

Material Examined

Holotype - ♀ CANADA, Alberta, Blairmore, *ex* leaf mines on *Lupinus sericeus* Pursh (Leguminosae), collected 6.ix.1966, emerged 20.ii.1967; puparium chilled at 45 F for 8 weeks.

Derivation of the Specific Name

Phytomyza lupinivora is named after its larval food plant, *Lupinus sericeus* Pursh (Leguminosae).

Comparisons

Phytomyza lupinivora is very unusual in having a very short second costal

segment and a rather long fourth segment. It can be included in Frick's (1957) key to North American species of the genus *Phytomyza* Fallén by extending the couplet 30 as below:

30 Antenna dark brown or black; tarsi and tibiae black, brown or dark reddish brown 30a
Antenna with first, second, and basal portion of third reddish yellow; tarsi and distal portion of tibiae yellow ..... *agromyzina*

30a Acrostichals in 2-8 rows 31
Acrostichals very few (about two); wings with costal segments 2-4 in the ratio of 1 : 0.24 : 0.75 ..... *lupinivora*

Biology

Larvae feed inside the linear mines in the leaflets of *Lupinus sericeus* Pursh (Leguminosae). Mines are for the most part upper surface, irregular and partly lower surface. The pupation takes place outside the mine.

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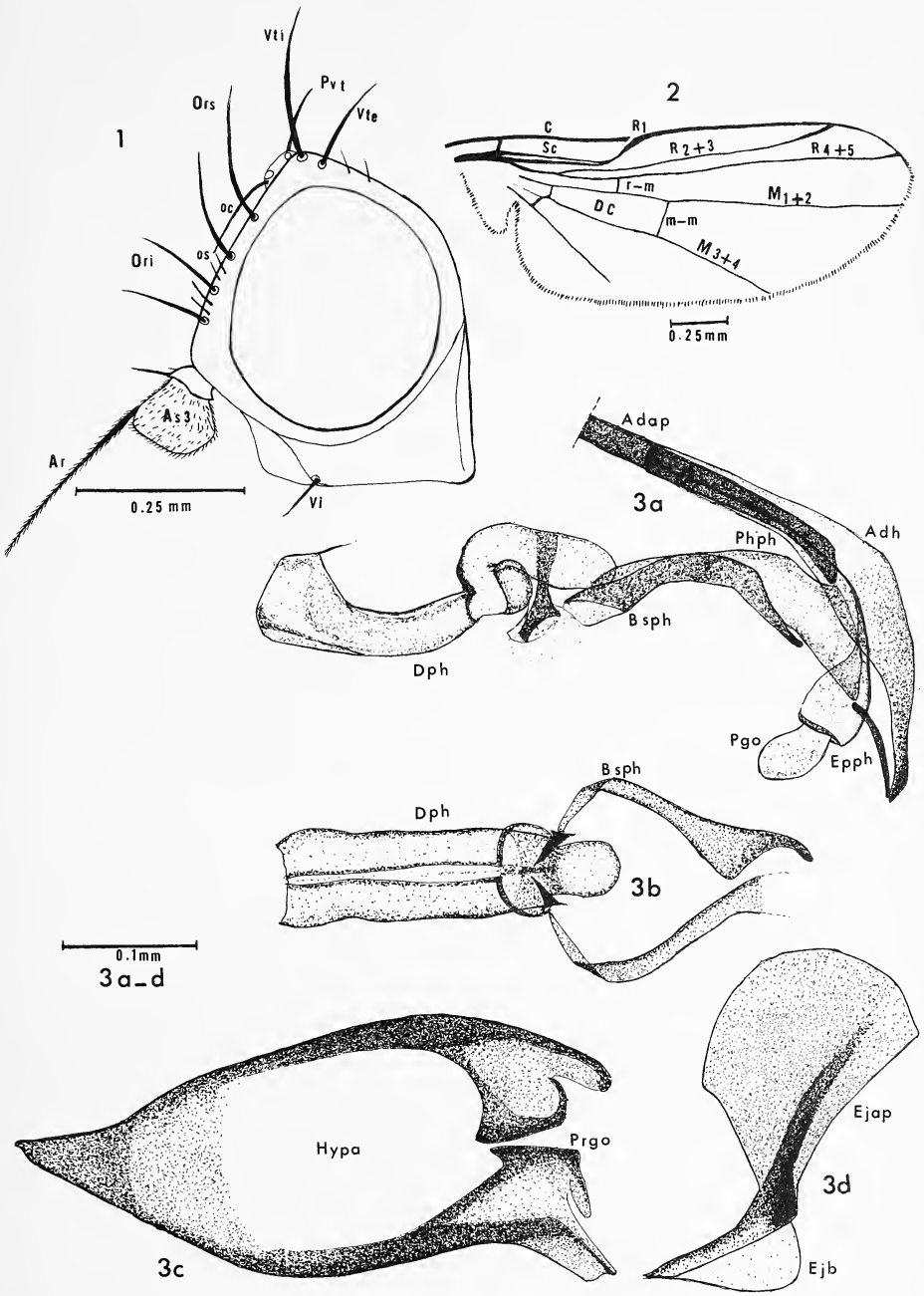
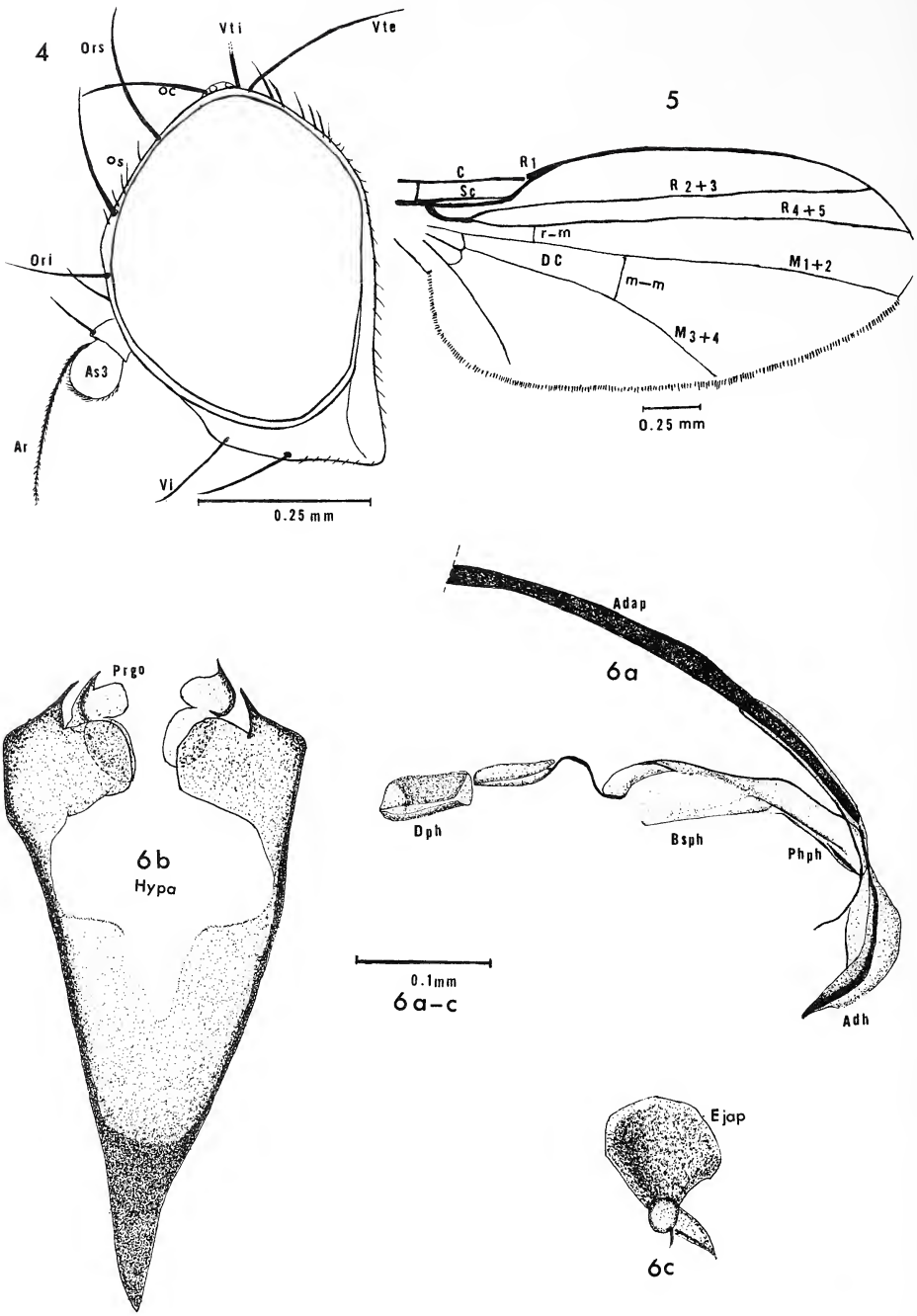
ABBREVIATIONS

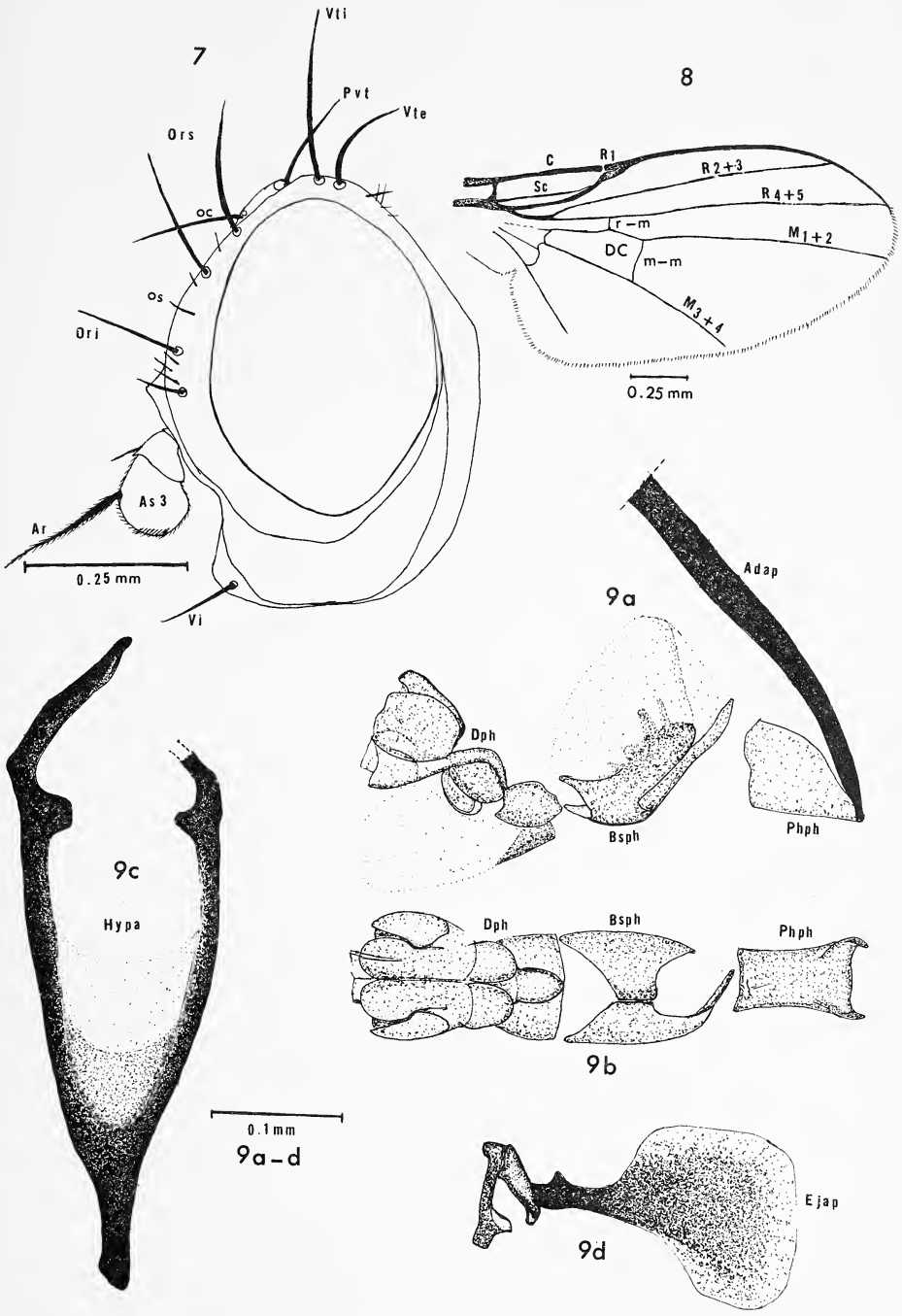
acr, acrostichal hair; Adap, aedeagal apodeme; Adh, aedeagal hood; Ar, arista; As3, third antennal segment; Bsph, basiphallus; C, costa; dc, dorsocentral bristles; DC, discal cell; Dph, distiphallus; Ejap, ejaculatory apodeme; Ejb, ejaculatory bulb; Ejd, ejaculatory duct; Epph, epiphallus; Hph, hypophallus; Hypa, hypandrium; m-m, medial cross vein; M_{1+2} and M_{3+4} median veins; Mph, mesophallus; oc, ocellar bristles; Ori, lower orbital bristles; Ors, upper orbital bristles; os, orbital setulae; Pgo, postgonites; Phph, phallophore; Prgo, pregonites; Pvt, postvertical bristle; R_1, R_{2+3} & R_{4+5}, radial veins; r-m, radiomedial cross vein; Sc, subcostal vein; Vi, vibrissal hair; Vte, outer vertical bristle; Vti, inner vertical bristle.



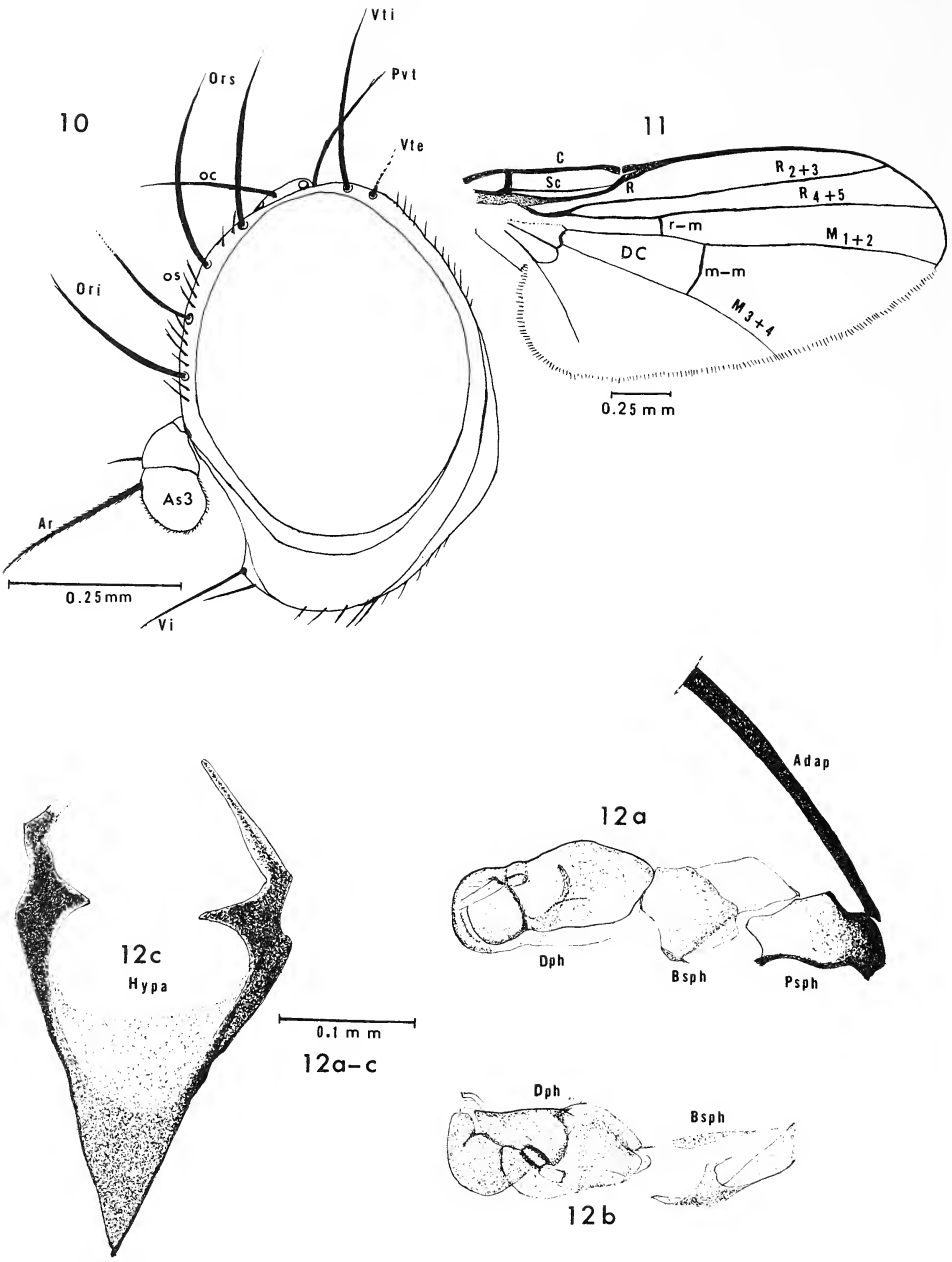
Figs. 1-3. *Agromyza albertensis* new species: 1 - head, lateral view; 2 - wing; 3a - aedeagus, lateral view; 3b - aedeagus, ventral view; 3c - hypandrium, ventral view; 3d - ejaculatory apodeme.



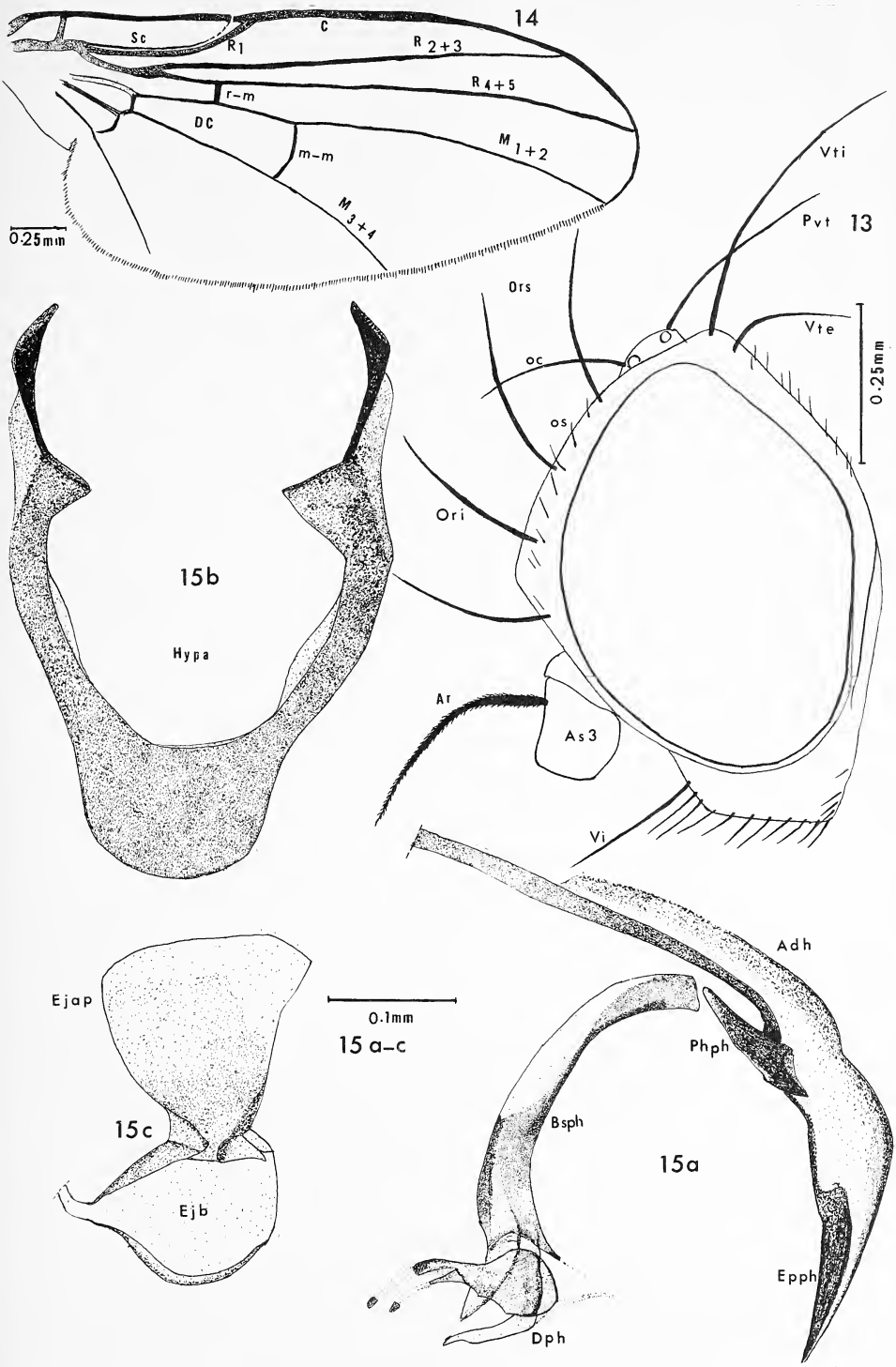
Figs. 4-6. *Agromyza masculina* new species: 4 - head, lateral view; 5 - wing; 6a - aedeagus, lateral view; 6b - hypandrium, ventral view; 6c - ejaculatory apodeme.



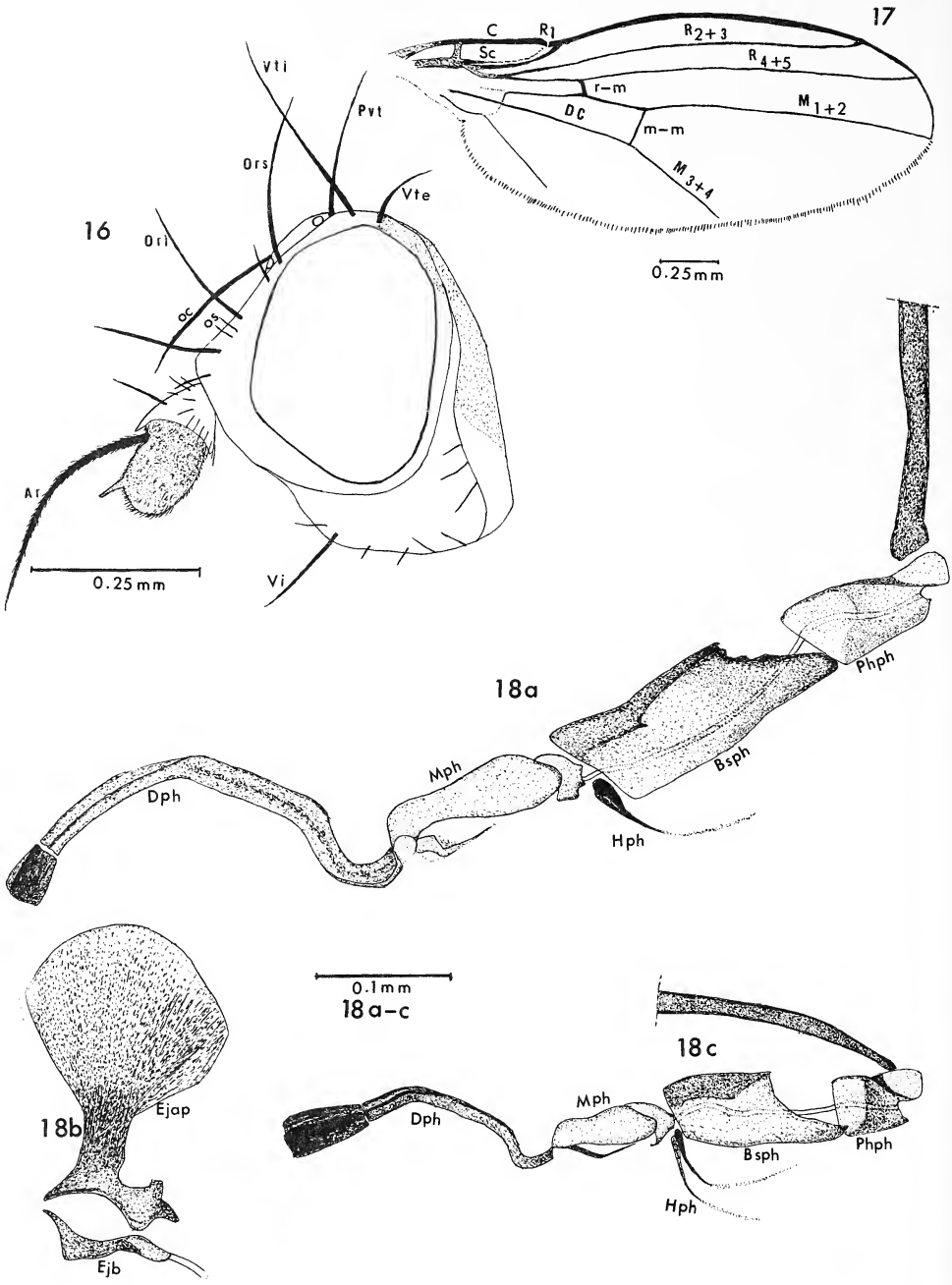
Figs. 7-9. *Ophimomyia monticola* new species: 7 - head, lateral view; 8 - wing; 9a - aedeagus, lateral view; 9b - aedeagus, ventral view; 9c - hypandrium, ventral view; 9d - ejaculatory apodeme.



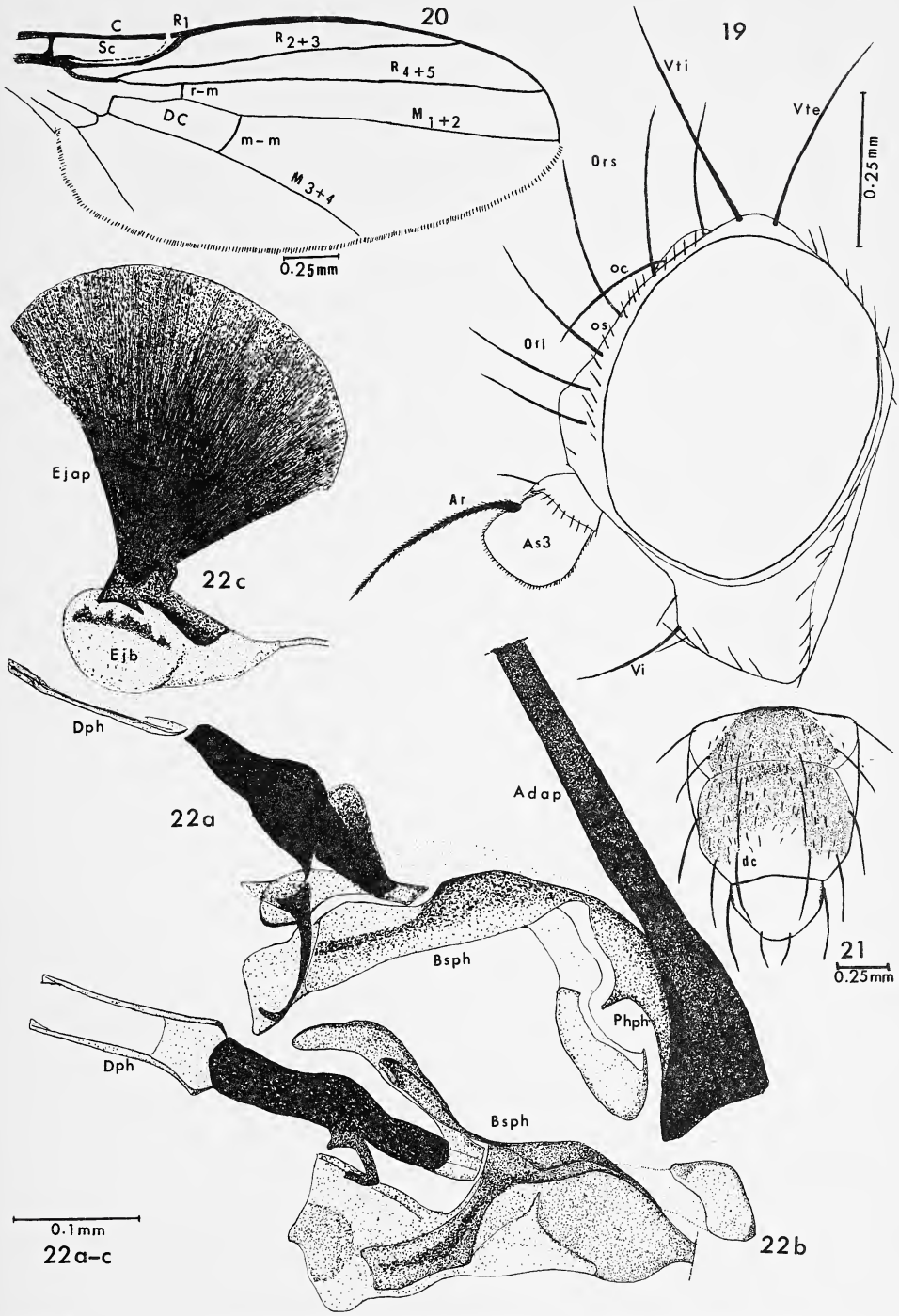
Figs. 10-12. *Ophiomyia pulicarioides* new species: 10 - head, lateral view; 11 - wing; 12a - aedeagus, lateral view; 12b - aedeagus, ventral view; 12c - hypandrium, ventral view.



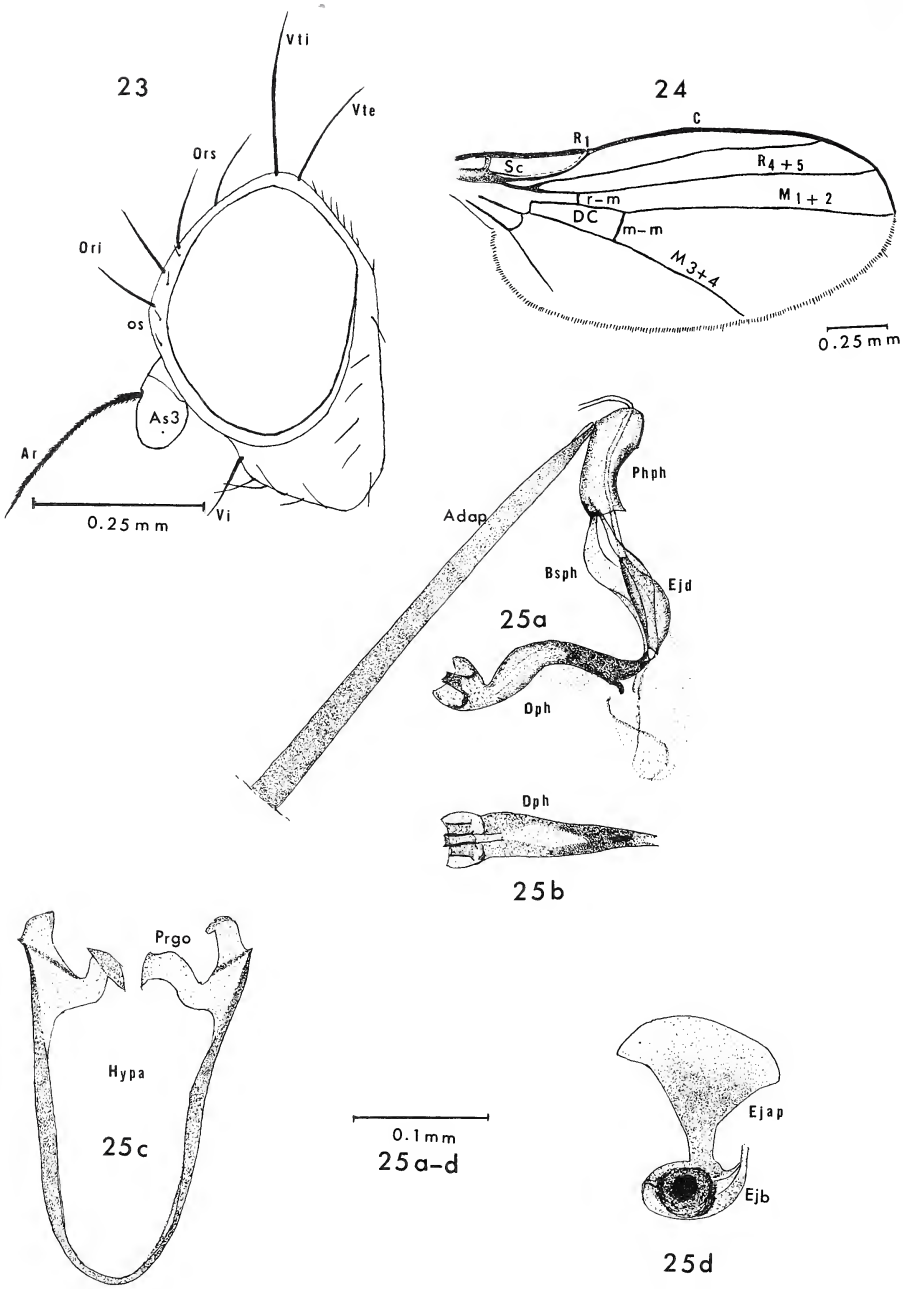
Figs. 13-15. *Phytobia flavohumeralis* new species: 13 - head, lateral view; 14 - wing; 15a - aedeagus, lateral view; 15b - hypandrium, ventral view; 15c - ejaculatory apodeme.



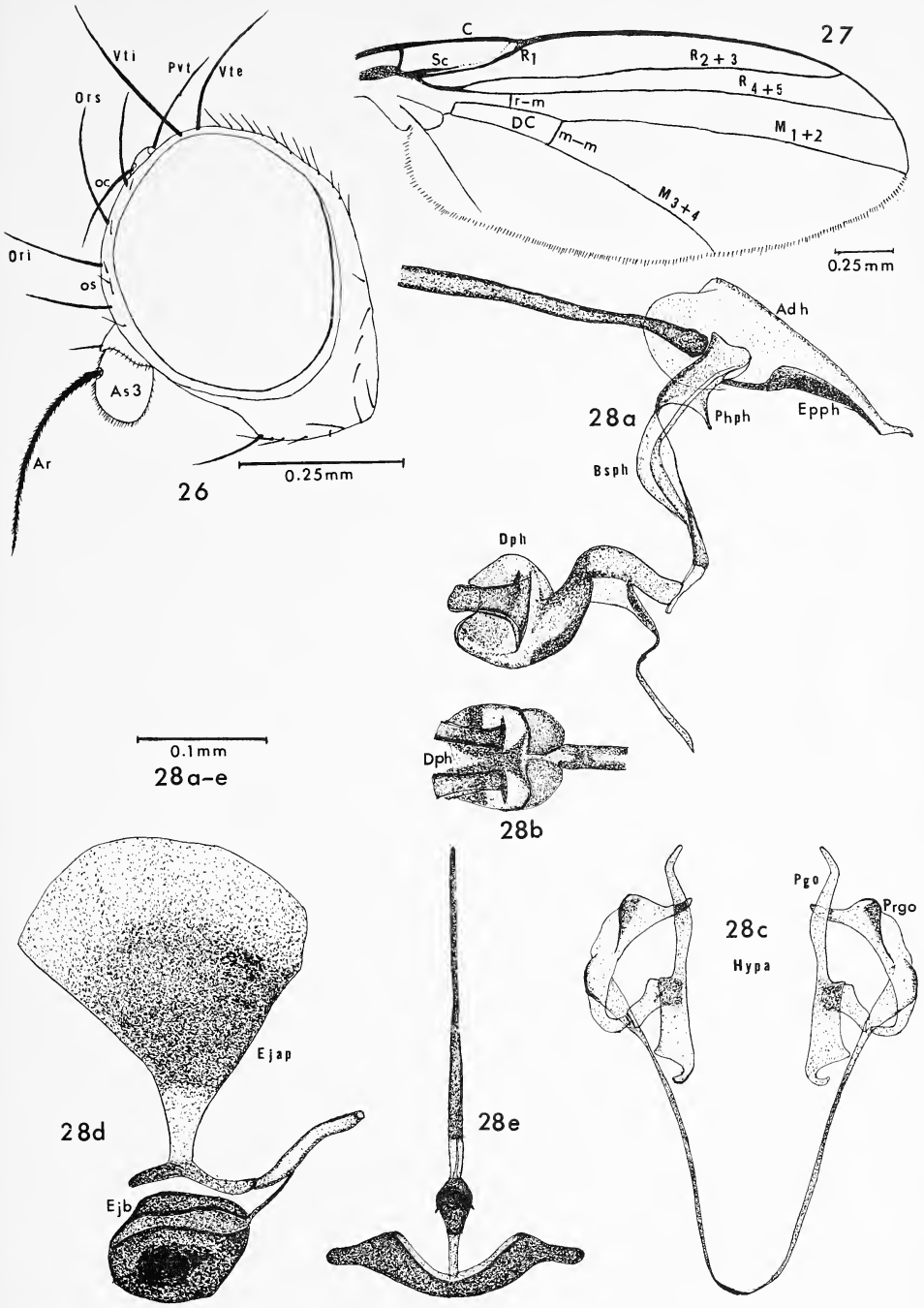
Figs. 16-18. *Cerodontha occidentalis* new species: 16 - head, lateral view; 17 - wing; 18a - aedeagus, lateral view; 18b - ejaculatory apodeme; 18c - *Cerodontha dorsalis* (Loew) - aedeagus, lateral view.



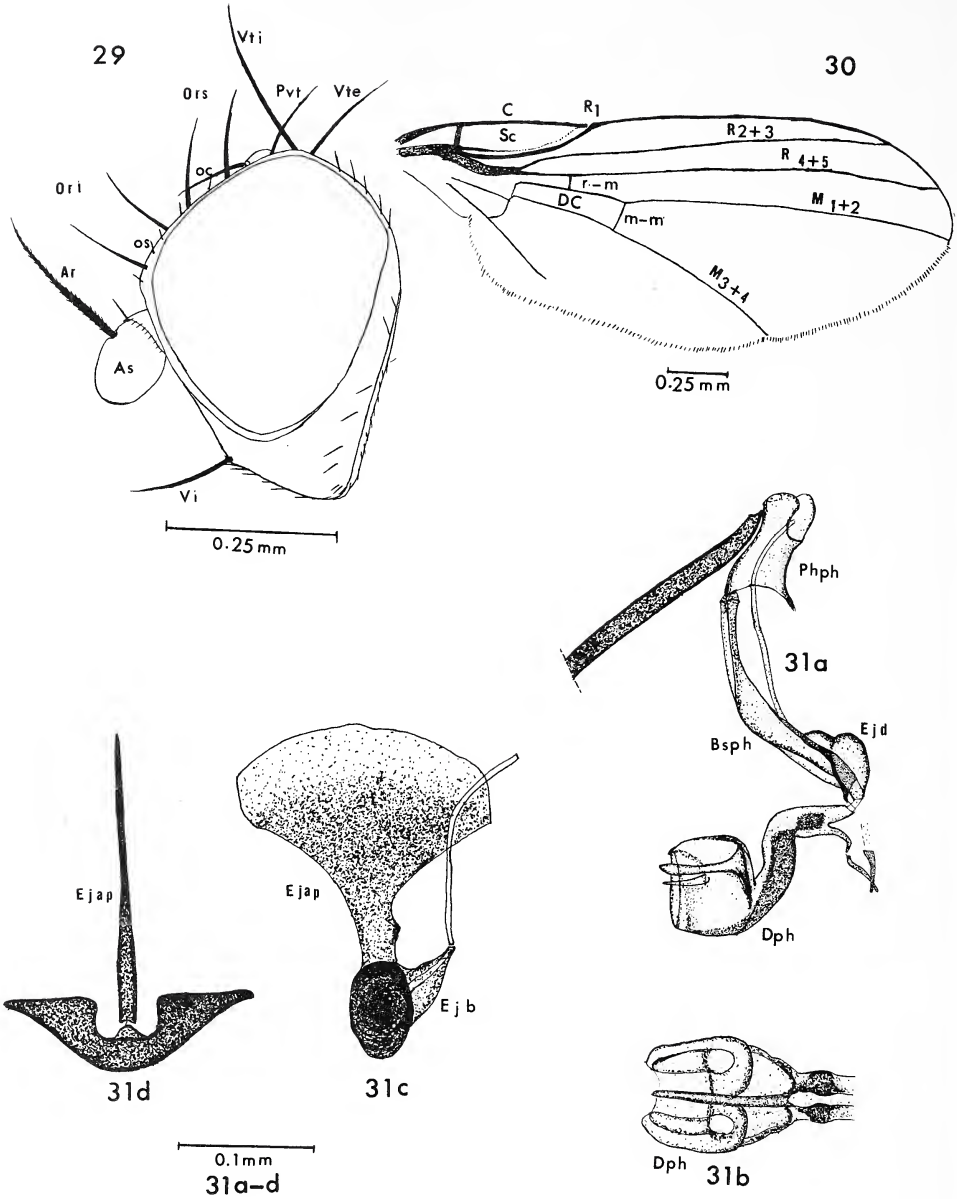
Figs. 19-22. *Liriomyza conspicua* new species: 19 - head, lateral view; 20 - wing; 21 - mesonotum, dorsal view; 22a - aedeagus, lateral view; 22b - aedeagus, ventral view; 22c - ejaculatory apodeme.



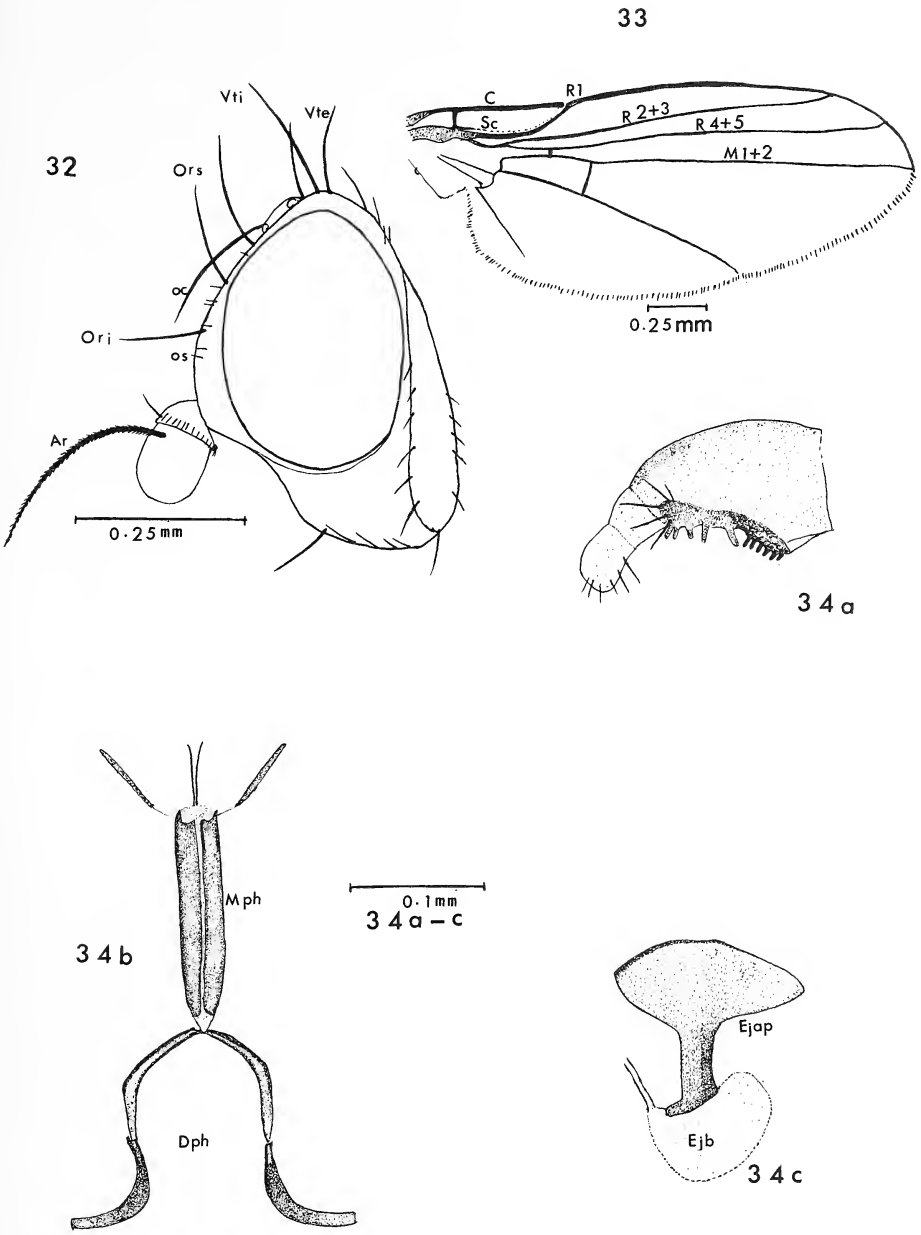
Figs. 23-25. *Liriomyza montana* new species: 23 - head, lateral view; 24 - wing; 25a - aedeagus, lateral view; 26b - distiphallus, ventral view; 25c - hypandrium, ventral view; 25d - ejaculatory apodeme.



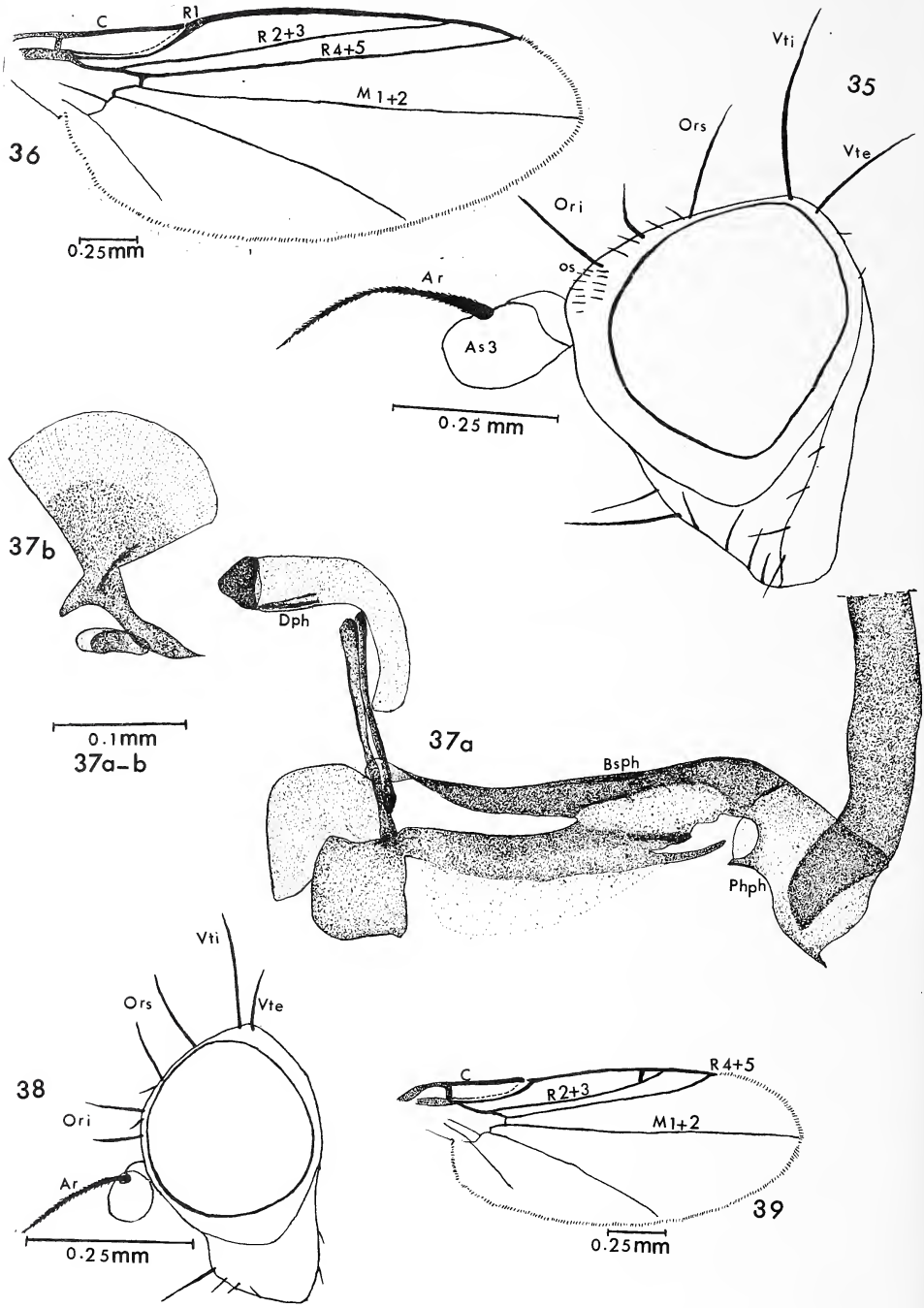
Figs. 26-28. *Liriomyza cordillerana* new species: 26 - head, lateral view; 27 - wing; 28a - aedeagus, lateral view; 28b - distiphallus, ventral view; 28c - hypandrium, ventral view; 28d - ejaculatory apodeme, front view; 28e - ejaculatory apodeme, side view.



Figs. 29-31. *Liriomyza septentrionalis* new species: 29 - head, lateral view; 30 - wing; 31a - aedeagus, lateral view; 31b - distiphallus, ventral view; 31c - ejaculatory apodeme, front view; 31d - ejaculatory apodeme, side view.



Figs. 32-34. *Lemurimyza pallida* new species: 32 - head, lateral view; 33 - wing; 34a - surstylus, ventral view; 34b - distiphallus, ventral view; 34c - ejaculatory apodeme.



Figs. 35-37. *Phytomyza lupini* new species: 35 - head, lateral view; 36 - wing; 37a - aedeagus, lateral view; 37b - ejaculatory apodeme. Figs. 38-39. *Phytomyza lupinivora* new species: 38 - head, lateral view; 39 - wing.

Quaestiones entomologicae

A periodical record of entomological investigations, published at the Department of Entomology, University of Alberta, Edmonton, Canada.

VOLUME IV NUMBER 3 JULY 1968

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Volume 4 Number 3 July 1968

CONTENTS

Book Review

HABU, AKINOBU. 1967. Fauna Japonica. Carabidae, Truncatipennes Group (Insecta: Coleoptera). Tokyo Electrical Engineering College Press, Hakushin-Sha Printing Co., Ltd., Tokyo, Japan. 338 pp. + xiv, 527 figs., XXVII plates, some in color. Cloth bound. $20.00 USA.

This work deals with the heterogeneous assemblage of ground beetles with truncated elytra. In Japan this includes 109 species arranged in 43 genera of the tribes Odacanthini, Hexagoniini, Pentagonicini, Masoreini, Lebiini, Zuphiini, Dryptini, and the subfamily Brachininae.

For each species, Habu gives the common Japanese name, an extensive synonymy, a synoptic description and a statement on geographical distribution. Following the generic descriptions, notes are provided on biology and references to larval descriptions are given.

The text is in English, however, in many places it is confusing and some errors exist. This work seems to have suffered much in trans-- lation perhaps because of poor editing. For example, on page 2 the first sentence after the introduction states "The head is generally wider than the pronotum, but in the Odacanthini and the Dryptini it is narrower than this". In fact, the opposite is true. Lesser mistakes which are certainly the result of mistranslation occur throughout the text.

The illustrations are excellent and are certainly one of the outstanding features of the book. Almost all characters of taxonomic importance have been illustrated with fine line drawings by the author. The colored plates by Mr. T. Sekigvchi and the black and white plates by the author have been superbly drawn and reproduced. The only criticism I have of the illustrations deals with the figures of the male genitalia. The structure of the internal sac of the aedeagus is shown in only its inverted position. In species that lack well-defined sclerotized fields on the internal sac, this is completely satisfactory. However, many species possess characteristic arrangements of spines and sclerites that are often very useful for identification and classification. Madge (Quaest. ent. 1967

(3) : 139-242) made good use of this character in his revision of the North American species of the genus *Lebia*. Habu indicates, through the use of stippling, that such structures are present in the internal sacs of many of the Japanese Truncatipennes. However, as the relative positions of the fields in the internal sac vary with slight differences in inversion, the most satisfactory method of studying this structure in a uniform manner is to completely evert it. In this way the fields are readily observable and their relative sizes and positions can be easily determined.

Keys are provided as an aid to identification and as a summary of characters used in erecting classifications. The keys to the species of the various genera appear to be straightforward; each couplet consists of a pair of rather distinct alternatives. However, the keys to the higher taxa, especially the supra-generic groups, are much more difficult because the alternatives presented are less distinct. These keys, which are apparently a summary of the classification, are to some extent based on variable characters and for this reason present difficulties in defining some higher taxa. An example of this is shown by the classification provided for the subtribes of the Lebiini. Here the principal characters that Habu has used are the structure of the legs and mandibles. The structure of the leg is related to the habitat of the insect. For example, most arboreal carabids possess dilated legs and tarsi, an adaptation that increases the area of contact between the insect and the surface it is climbing and, hence, providing better traction; physical considerations indicate that a slender, longer leg is more suitable for a cursorial animal. Within the Lebiini, the various subtribes tend to be adapted to a particular habitat. For example, the Callidina is primarily an arboreal group and shows adaptations for an arboreal existence in the structure of the legs. On the other hand, the terrestrial genus *Anomotarus* Chaudoir possesses all the characteristics of the more typical members of the Callidina except that its tarsi are slender and lack dense ventral setose pads. Because of this, Habu chooses to separate this genus from the Callidina and places it in a new subtribe. Hubu's Callidina may be homogeneous as regards the structure of the legs and mandibles, but the female genitalia, mouthparts -- especially the ligula and mentum, and general habitus suggest that the subtribe Callidina is a heterogeneous assemblage and should be redefined, perhaps by placing *Anomotarus* Chaudoir and those callidine genera that possess styli with setose apices in the Callidina *s. str.* and by removing those genera which possess other forms of female ovipositor to one or perhaps two groups of subtribal rank. This proposed rearrangement provides more homogeneous groups, and I think each of these groups is likely to be monophyletic, and thus natural in a phylogenetic sense.

Species whose members are varied in their color pattern have been illustrated but aside from this, little account has been taken of variation. Subspecies have been recognized in several instances and while criteria for recognizing subspecies have not been given, the author appears to follow currently recognized practice. What is surprising, however, is his formal recognition of "forms". He has described *Lebia bifenestrata* form *ogurai* new form in addition to recognizing the "typical form" and the

"form *lucescens*". All of these "forms" appear to be sympatric and are included in the normal intraspecific variation found in this species. This is a regrettable regression to typological thinking.

This work forms one of the most thorough treatments accorded a group of ground beetles. The morphology of the insects dealt with appears to have been studied in a most careful and painstaking manner. The female ovipositor, a structure that has frequently been overlooked by previous workers, has received detailed study by Habu, who adequately demonstrates the potential of this organ in further taxonomic work. It is unfortunate that Habu himself has not used the possibilities of the data he presents. Few statements are devoted to a discussion of relationships existing among the higher taxa. The classification is traditional even though certain of Habu's data suggest that a modification is necessary of certain groups such as the subtribes of the Lebiini.

Habu has certainly provided an excellent manual for the identification of the species of the Truncatipennes group of Carabidae of Japan. Unfortunately this work narrowly misses being one of the major contributions to the understanding of the higher taxa of this large and very complex group of insects.

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FEEDING MECHANISMS AND THE FORCES INVOLVED IN SOME BLOOD-SUCKING INSECTS

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Quaestiones entomologicae
4 : 92 - 111 1968

In the stages of Cimex lectularius *L. and* Pediculus humanus *L. and in adult* female Aedes aegypti*(L.) the weight of the blood meal was greater than the body weight. In* C. lectularius *L. and* P. humanus *L. the negative pressure required to draw the blood to the cibarial pump decreased from the first to the second instar and then increases to a maximum in the adult. This pressure may be as high as 5.6 x* 10^5 *dyne/cm*^{2}. *In* A.aegypti *female it is smaller than any instar of* C. lectularius *or* P. humanus. *The muscular tension of the cibarial pump dilators in the species studied ranged from 5.7 to 18.4 x* 10^3 *g/cm*^{2} *and the power output ranged from 2.75 to 13.3 x* 10^3 *g cm/sec/g of muscle. Sensilla were found in the cibarium of C. lectularius* but not of *P. humanus.*

Blood-sucking insects can ingest enormous meals in a short time. In view of the narrowness of the feeding canal and viscosity of vertebrate blood, the rate of feeding, the negative pressure produced in the cibarial pump, and the power required from the cibarial pump dilators are of interest. All the nymphal instars and the adult males and females of *Cimex lectularius* L. and *Pediculus humanus* L., as well as adult females of *Aedes aegypti* (L.) were studied.

The structure and many details in the mechanism of sucking apparatus in Hemiptera have been studied by Weber (1928, 1928a, 1929), Dickerson and Lavoipierre (1959), and Lavoipierre et al. (1959). Kemper (1932) and Snodgrass (1935, 1944) described in detail the feeding apparatus in the bedbug *C. lectularius*.

The anatomy of the mouth parts of some mosquitoes has been described in detail by Vogel (1921), Robinson (1939), Gordon and Lumsden (1939), Christophers (1960), Schiemenz (1957), and Clements (1963). The weight of the blood ingested by the females of different species of mosquitoes and the rates of the ingestion have been reported in many publications. Fulleborn (1908) reported the blood taken by gorged *A. aegypti* averaged 0.75 mg with a minimum and maximum of 0.20 and 0.84 mg respectively. He also found that 53 out of 137 took a blood meal much greater than their own weight. Jeffery (1956) recorded that *Anopheles quadrimaculatus* and *Anopheles albimanus* ingest about 3.46 and 2.58 mg of blood respectively. Christophers (1960) reported that the feeding canal in *Aedes aegypti* is about 2 mm long and has a diameter of 0.03 mm. He added that from 2 to 4 mm^3 of blood pass through this channel in 2 minutes and that the linear rate of flow is from 2 to 4 cm/sec.

The morphology of the piercing organs in Siphunculata has been dealt with by Cholodkowsky (1904), Enderlein (1905, 1905a), Pawlowsky (1906), Harrison (1914), Sikora (1916), Peacock (1918), Florence (1921), Vogel (1921), Fernando (1933), Snodgrass (1944) and Stojanovich (1945). Summarized accounts are also given by Patton and Evans (1929), Metcalf and Flint (1962), and Imms (1960). The relationships between food supply and the biology of *Pediculus humanus* have been described by Nuttall (1917). Buxton (1947), Busvine (1948) and Gooding (1963).

Feeding Apparatus and Feeding Mechanisms

Whole mounts of the head and mouthparts were prepared. The insects were soaked overnight in 5% potassium hydroxide solution, washed thoroughly in water, stained with acid fuchsin if necessary, dehydrated in ethanol, cleared in xylol and mounted in canada balsam. Serial cross sections of the mouthparts and the head, and serial horizontal sections of the head were necessary. Mallory's triple stain was used. Measurements were made with an ocular micrometer.

The mouthparts of the bedbug *C. lectularius* (fig. 1) consist of a labium considerably longer than the head and when not in use turned backwards at its base with its distal part between the forelegs. The labium is four-segmented with the first one mostly concealed by the labrum and the maxillary lobes. The labium is grooved on the lower surface and the sides diverge near the tip of the last segment to leave a small aperture. The maxillae and the mandibles are stylet-like and are held together within the groove of the labium. Between the maxillary stylets is a minute salivary duct and a relatively large food canal. The length of the food canal and its radius vary from one instar to the next (table 2). At the base of the labium the maxillae turn backwards into the head pouches and diverge against the base of the hypopharynx where the food canal opens into the anterior surface of the hypopharyngeal lobe that leads back into the cibarial pump. The hypopharynx is a small lobe and its dorsal surface is continued back into a large and deeply concave sitophore with strong lateral margins, which is the floor of the cibarial pump (fig. 2). The dorsal wall of the cibarial pump is in the form of a diaphragm (d.) which is attached by a rubbery margin (r.m.) along either edge of the sitophore (sit.). The diaphragm when not in action is completely collapsed into the cavity of the sitophore. The measurements of the various parts of the cibarial pump in the different instars are shown in tables 3 and 4. The dilator muscles (m.) consist of two lateral bundles of fibers, arising on nearly the whole clypeus. Measurements of these muscles are shown in tables 3 and 4. The cibarial pump discharges directly into the tubular oesophagus as there is no differentiated pharynx.

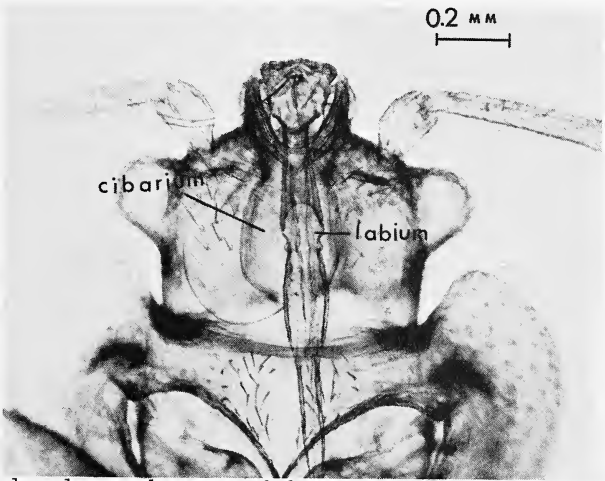

Fig. 1. Head and mouthparts of female *C. lectularius* (ventral view).

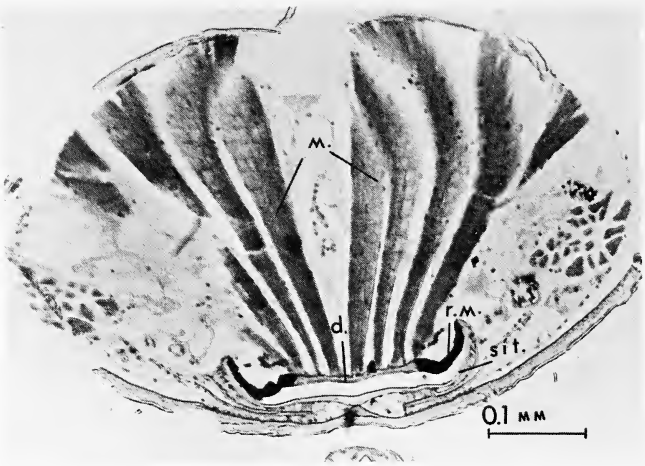

Fig. 2. Cross section through the head of female *C. lectularius*. (d) diaphragm; (rm) rubbery margin; (m) cibarial pump dilators.

When the bedbug feeds the stylets are forced into the skin of the host. The labium helps in lowering the head of the insect near the skin of the host by a backward bending of the third and fourth segments. The mandibular stylets are the effective piercing organs while the two maxillary stylets closely adhere to act as a sucking needle. Contraction of the cibarial pump dilators moves the diaphragm upwards during the filling stroke. Emptying is achieved by the return of the diaphragm under the elastic force of the rubbery margins.

The mouthparts of female *A. aegypti*, like those of *C. lectularius*, are modified for piercing and most of the mouthparts are extended into long and slender stylets. The labium is relatively stout and contains a dorsal groove in which the stylets lie closely beside each other in a fascicle. The labium ends with a pair of labella. The labrum is a broad pointed stylet in the labial groove. It is curved so that its edges meet and thus forms the food canal. Towards the base of the labrum the edges separate and the hypopharynx forms the floor of the food canal. The average length and the radius of the food canal are shown in table 2. The hypopharynx is a very slender stylet with a median rib containing the salivary canal. The mandibles are also stylets which come to a simple point without teeth at the tip. The maxillae, which are the principal piercing organs, each consist of a long flattened stylet with a curved end. The cibarial pump (cb.), fig. 3, is located under the clypeus (cl.) and is trough-shaped. The lower wall of the trough is well-sclerotized and is continuous with the upper surface of the hypopharynx. The upper wall is thinner and is continuous with the inner surface of the labrum. The upper wall is elastic and when the cibarial pump is not in action it lies close to the lower wall. Attached to the upper wall of the cibarial pump are two bundles of muscles, the dilators of the cibarial pump (m.), which have their

origin from the clypeus. Measurements of the cibarial pump and its dilators are shown in tables 3 and 4. For a short distance behind the cibarial pump the cuticle is thin and forms a valve. The cibarial pump is connected to the pharyngeal pump by a narrow canal.

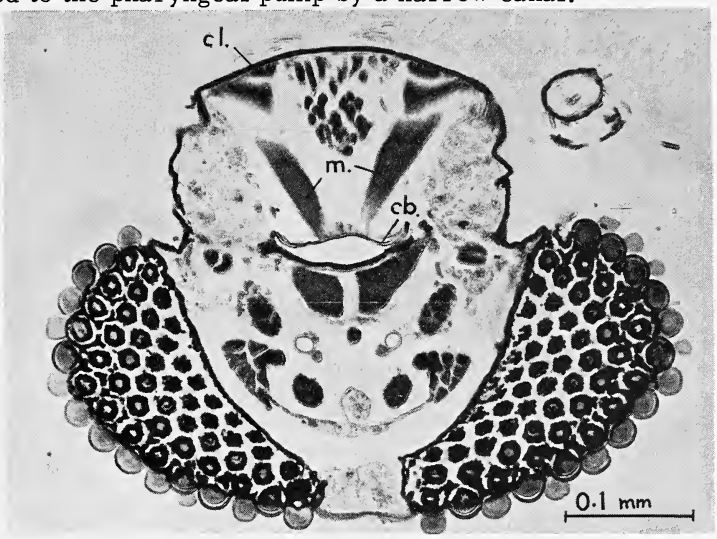

Fig. 3. Cross section through the head of female *A. aegypti*. (cb) cibarial pump; (cl) clypeus; (m) cibarial pump dilators.

The head of *P. humanus* is produced anteriorly into a very small snout-like proboscis probably formed from the labrum and in which is a terminal opening or prestomum. This snout-like proboscis is armed internally with small teeth used to grip the host during feeding. The prestomum opens to a preoral cavity within the head, the upper part of it is continuous with the cibarial pump; the lower part extends to a long sac which contains the stylets and nearly reaches the posterior end of the head. The three stylets lie one above the other, the dorsal and ventral ones forked at their bases. The dorsal stylet is made of two united halves with the edges curved upwards and rolled over each other to form the food canal. The ventral stylet is the effective piercing organ and its apex is armed with teeth. The median stylet is pierced throughout its length by the salivary duct. The homology of these stylets with the mouthparts of biting insects has been a subject of dispute for many years. Scholzel (1937) reinvestigated the development of these mouthparts. He claimed that the ventral stylet is the labium, and he said that the maxillae and mandibles are reduced and merged into the lateral walls of the preoral cavity and that the dorsal and median stylets are both derived from the embryonic hypopharynx. This interpretation was accepted by Snodgrass (1944). During feeding the blood passes through the food canal of the dorsal stylet into a trough that fits into the proximal end of the food canal. This trough, which is closed dorsally by the inner clypeal wall, is connected with the cibarial pump. Fig. 4 shows the action of the cibarial pump during the filling and emptying strokes. The mea-

surements of the feeding canal and the various parts of the cibarial pump are shown in tables 3 and 4.

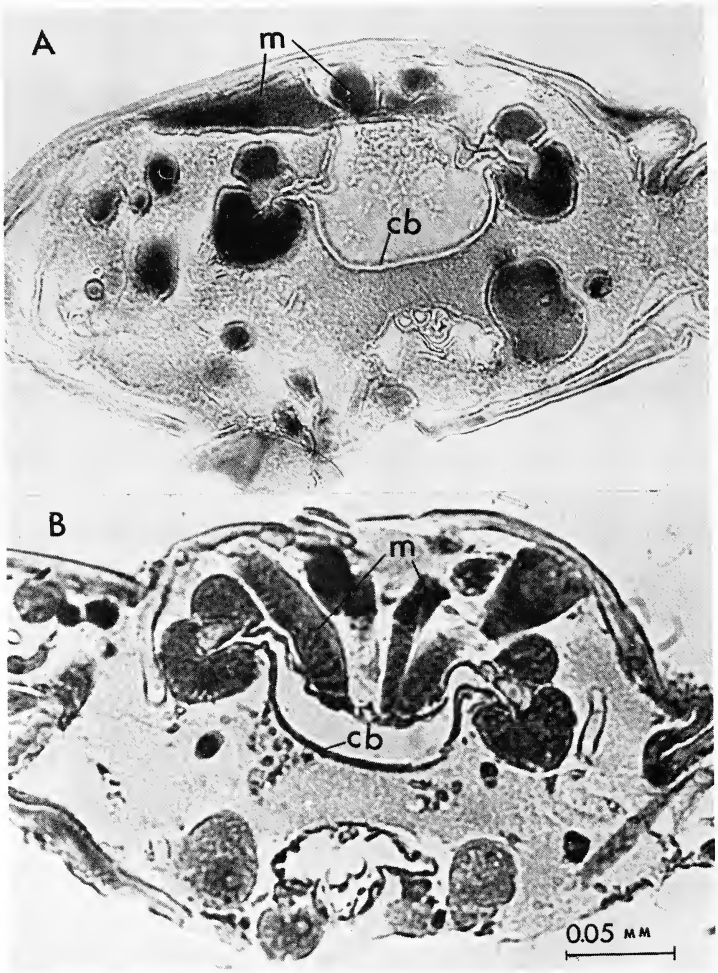

Fig. 4. Cross section through the head of the female *P. humanus*. A. During filling stroke. B. During emptying stroke. (cb) cibarial pump; (m) cibarial pump dilators.

Sense Organs in the Food Canal

Von Gernet and Buerger (1966) studied the labral and cibarial sense organs in some mosquitoes. They suggested that food is detected by the apical labral sense organs and the pumping action is initiated by impulses received by the cibarial muscles from the labral sense organs via the frontal ganglion. They also suggested that the cibarial sense organs control the openings of the stomach and diverticula, thus setting the food-directing mechanism in action.

Similarly, I studied *C. lectularius* and *P. humanus*. No sense organs

could be located on the maxillae of *C. lectularius*. On the other hand, two types of sensilla were found in the cibarium. A dorsal group consists of six sensilla at the anterior end, three on each side of the cibarium (fig. 5A). These are formed from minute hollow spines and innervated by fine dendrites originating from the adjoining sensory cells. These sensory cells seem to be innervated by a branch from the frontal nerve. The second group consists of two campaniform sensilla, one on each side of the sitophore (fig. 4B). Miles (1958) found that *Oncopeltus* and *Dindymus versicolor*, could discriminate between liquids sucked up the feeding canal while any contact receptors were masked.

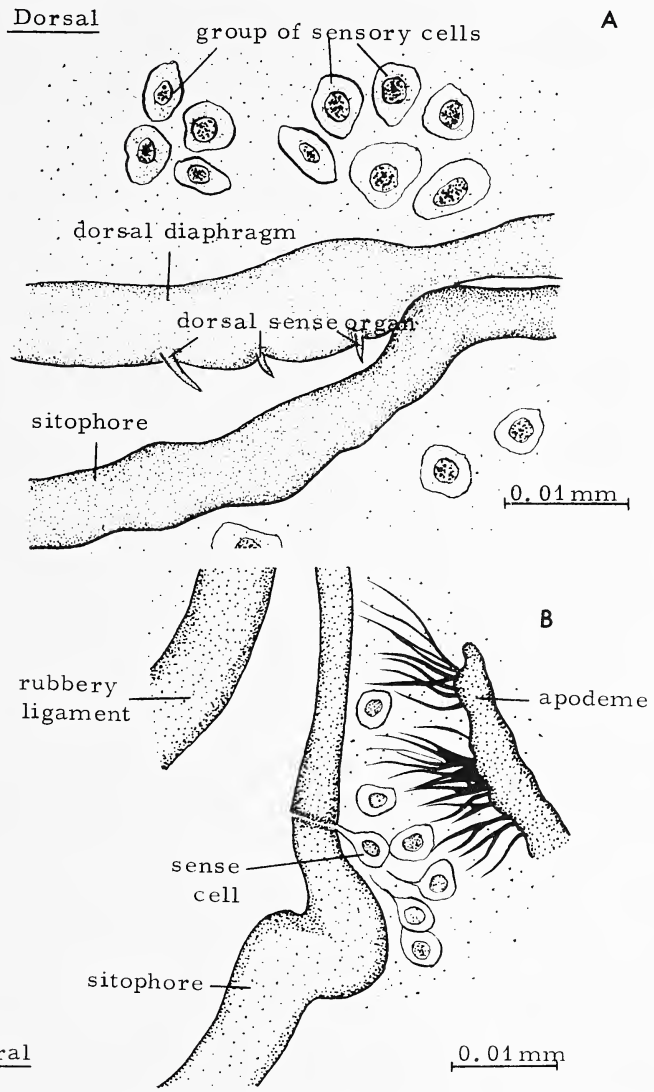

Fig. 5. Sense organs in the cibarium of female *C. lectularius*. A. Dorsal sense organs. B. Sitophore sense organs.

No sensory organs could be located in the food pathway of *P. humanus*.

Rate of Feeding and the Forces Involved

Methods

Feeding was observed under a binocular microscope and the feeding period was timed with a stop watch. The weight of the blood meal was determined by weighing the insects on a torsion balance before and after feeding. In all the experiments the insects were fed naturally on human blood.

Calculation of the pressure difference required to force a liquid along a tube at known rate can be made from Poiseuille's formula:

$$\blacktriangle P = \frac{8\, Q\, l\, \eta}{\pi\, r^4\, t}$$

where $\blacktriangle P$ = pressure difference in dynes/cm^2

Q = volume moved in cm^3, η = viscosity of the liquid in poises, l = length of the tube in cm, r = radius of the tube in cm, t = time in seconds.

Poiseuille's formula was applied for the determination of the negative pressure required to draw the blood to the cibarial pump in these insects, on the assumption that the blood behaves like a simple fluid and the flow is laminar, because no more precise procedure has been developed. These assumptions are questionable.

The volume of the blood (Q cm^3) ingested during feeding for t seconds was determined from the weight, taking the density of human blood as 1.056 g/cm^3 (Spector 1956, p. 51). The viscosity of human blood is approximately 0.025 poises at 38 C (Mitchell 1948, p. 407). The negative pressure in the cibarium was obtained by subtracting from the pressure difference the capillary blood pressure which is 12 mm Hg or 1.6×10^4 dynes/cm^2 (Wright 1952). The muscular force required to provide this negative pressure was calculated by multiplying the corrected pressure difference by the area of the diaphragm.

The force obtained from these calculations was doubled for the estimation of the tension of the cibarial pump dilators on the assumption that half of the time is spent in the filling stroke. The muscular tension per unit cross sectional area of muscles was obtained by dividing the corrected force by the mean cross sectional area of the cibarial pump dilators. The mean cross sectional area was determined from cross sections of the cibarial pump dilators cut at 6 microns.

The power required of the cibarial pump dilators expressed as ergs/sec/g of muscle was found by multiplying the force by the total working travel of the diaphragm in a second and dividing by the weight of the cibarial pump dilators. The total working travel was determined by multiplying the width of the rubbery margin (length in section, fig. 2) by the number of pump cycles per second. The geometry of the cibarium, flat and expanded, suggests that the diaphragm moves a distance approximately equal to the width of the rubbery margin in each stroke. For determining the weight of these muscles the volume was obtained by multiplying the mean cross sectional area by the length of muscles and assuming the specific gravity of insect muscle to be one.

The rates of feeding observed are shown in table 1.

TABLE 1. Feeding period, weight of blood meal, and rate of feeding in *C. lectularius*, *P. humanus*, and *A. aegypti* females.

Species & stage	Time to engorgement in seconds	Weight of meal (mg)	Wt meal / body	Blood taken per sec (μg)	Pump cycles/ second	Blood taken/pump cycles (μg)
C. lectularius						
First	146.5±17.4* (20) 105 - 187	0.26±0.06* (20) 0.13 - 0.44	3.7	1.77±0.045* (20) 1.23 - 2.35	4.0±0.05* (40) 2.9 - 4.6	0.44
Second	156.8±4.2 (20) 129 - 190	0.62±0.06 (20) 0.22 - 0.92	4.5	3.95±0.15 (20) 1.63 - 4.84	3.8±0.04 (36) 3.2 - 4.5	1.03
Third	175.3±4.3 (25) 135 - 282	1.09±0.09 (25) 0.46 - 1.82	3.2	6.20±0.13 (25) 3.41 - 6.62	3.3±0.04 (28) 3.0 - 3.8	1.89
Fourth	234.4±8.8 (20) 135 - 282	2.86±0.09 (25) 1.14 - 3.01	4.4	11.73± 0.14 (25) 8.44 - 12.04	3.3±0.02 (40) 2.9 - 3.6	3.58
Fifth ♀	305.6±4.1 (15) 240 - 422	5.64±0.35 (20) 2.21 - 8.42	4.9	18.46±0.48 (25) 9.21 - 19.98	3.0±0.03 (20) 2.7 - 3.3	6.18
Fifth ♂	290.0±5.9 (15) 240 - 310	5.42±0.31 (15) 2.64 - 8.10	4.8	18.69±0.76 (15) 11.00 - 26.2	3.0±0.03 (20) 2.8 - 3.3	6.29
Adult ♀	268.9±10.3 (19) 213 - 350	6.48±0.41 (15) 5.20 - 11.20	2.8	24.10±0.72 (15) 19.24 - 32.0	2.4±0.02 (30) 2.3 - 2.5	9.88
Adult ♂	286.9±14.6 (20) 195 - 410	6.23±0.41 (15) 3.90 - 11.4	2.8	21.70±0.46 (15) 20.86 - 27.91	2.4±0.02 (30) 2.3 - 2.5	8.89

TABLE 1 (cont.).

Species & stage	Time to engorgement in seconds	Weight of meal (mg)	Wt meal / body	Blood taken per sec (µg)	Pump cycles/ second	Blood taken/pump cycles (µg)
*P. humanus***						
First	504.0±7.7 (15) 445 - 565	0.11±0.005 (15) 0.08 - 0.13	1.8	0.22±0.006 (15) 0.18 - 0.25	2.8±0.23 (15) 2.1 - 3.3	0.08
Second	443.1±9.2 (15) 275 - 720	0.21±0.007 (15) 0.15 - 0.30	1.4	0.48±0.008 (15) 0.40 - 0.53	5.3±0.03 (21) 5.0 - 5.6	0.09
Adult ♀	764.4±45.6 (20) 459 - 1372	1.13±0.078 (18) 0.82 - 1.80	1.2	1.48±0.027 (15) 1.34 - 1.80	4.6±0.001 (38) 3.9 - 5.0	0.32
Adult ♂	782.9±48.4 (15) 430 - 1210	0.95±0.07 (15) 0.50 - 1.40	1.1	1.21±0.016 (15) 1.14 - 1.31	4.2±0.06 (15) 3.7 - 4.5	0.29
A. aegypti						
Adult ♀	255.1±8.8 (15) 210 - 295	1.62±0.18 (15) 0.84 - 2.90	1.2	6.35±0.34 (15) 4.00 - 9.83	4.9±0.20 (15) 4 - 6	1.29

* Mean ± SE
(number of insects used)
Range

** Third nymphal instar of *P. humanus* was not studied because of some difficulties in maintaining the culture.

Rate of Feeding

The feeding period of *C. lectularius* and *A. aegypti* females was taken as the time required for the insect to feed till engorged and of *P. humanus* as the time required for the insect to feed till the blood started to exude from the anus.

The feeding period of *C. lectularius* is a minimum in the first nymphal instar, increases to a maximum in the fifth instar and then decreases in the adult. The feeding period of the adult female is shorter than that of the male.

In *P. humanus* the feeding period of the first instar is slightly longer than that of the second instar and shorter than that of the adult. The feeding period of the female is shorter than that of the male. The feeding period in *P. humanus* is much longer than in *C. lectularius* and in *A. aegypti* female, that to engorgement would be longer still. The duration of the

feeding period can be correlated with the behaviour of these insects and their access to their hosts. The longer feeding period in *P. humanus* may be related to their living on the host while both *C. lectularius* and *A. aegypti* leave their host after feeding.

The feeding period of the female *A. aegypti* is longer than that of each one of the first four instars and shorter than that of the fifth instar and the adult of *C. lectularius*.

When the insects feed till engorgement the weight of the blood meal varies from one instar to the next. In *C. lectularius* and *P. humanus* the minimum value is found in the first nymphal instar and the maximum in the adult stage. The weight of the female's blood meal was greater than the male's in both *C. lectularius* and *P. humanus*. The weight of the blood meal in *C. lectularius* is greater than that in *P. humanus*. The weight of the blood meal in *A. aegypti* is greater than that of any instar in *P. humanus* and that of the first three instars of *C. lectularius*, but smaller than that of the other instars in the latter species.

In all three species the weight of the blood meal is greater than the body weight. The ratio of weight of the blood meal to body weight is greater in *C. lectularius* than in the other two species. In *C. lectularius* and *P. humanus* the ratio is higher in the nymphal instars than in either the female or the male because of the flexibility of the integument which becomes more sclerotized in the adult stage. In *C. lectularius* the number of pump cycles per second decreases gradually from the first instar to the adult stage. In *P. humanus* the number of pump cycles per second increases from the first to the second instar and then decreases slightly in the adult stage.

The rate of feeding in *C. lectularius* and *P. humanus*, expressed as micrograms of blood taken per second or as micrograms of blood taken per pump cycle, increases gradually from the first instar to the adult. The rate of feeding is greater in the adult female than in the male in both species. In the first nymphal instar of *C. lectularius* the rate of feeding is much greater than in any instar of *P. humanus*. The weight of blood taken per second by *A. aegypti* females is greater than that taken by the first three nymphal instars of *C. lectularius* and smaller than that taken by each of the other instars. On the other hand, the weight of blood taken per pump cycle in *A. aegypti* is greater than in the first and second instars of *C. lectularius* and smaller than in the other instars.

Negative Pressure in the Cibarial Pump

Table 2 shows the negative pressure required to draw the blood to the cibarial pump at the observed rates in these insects. In *C. lectularius* the pressure difference decreases from the first to the second nymphal instar and increases from the second to the adult stage. The pressure difference in female *A. aegypti* is smaller than in any instar of *C. lectularius* and than in the first instar and the adult stage of *P. humanus*. In the last species it decreases from the first to the second nymphal instar with a maximum value in the adult. Table 2 also shows the results obtained by Bennet-Clark (1963) for the fifth nymphal instar of *Rhodnius prolixus* (Stahl). He claimed that 1.96×10^6 dynes/cm^2 or about 2 atmospheres, can be taken as the likely minimum, but he ignored the proximal 5 mm of the feeding canal and assumed that the viscosity of the blood is equal to that

of water. He added that if he took these into consideration the pressure required for *Rhodnius* to feed could be as high as 9 atmospheres, i.e. 9.12×10^6 dynes/cm^2.

TABLE 2. Negative pressure (relative to atmospheric) in the cibarial pump in *C. lectularius*, female *A. aegypti*, *P. humanus* and *R. prolixus*

Species & stage	Q/t cm^3/sec	l* = length cm	r* = mean radius cm	▲ P dyne /cm^2	▲ P** corrected dyne/cm^2
C. lectularius					
First	1.7×10^{-6}	0.055 (20) 0.050-0.058	4.4×10^{-4} (5) 4.1×10^{-4}-4.9×10^{-4}	1.6×10^{5}	1.4×10^{5}
Second	3.7×10^{-6}	0.064 (15) 0.060-0.069	6.0×10^{-4} (7) 5.8×10^{-4}-6.4×10^{-4}	1.1×10^{5}	9.4×10^{4}
Third	5.9×10^{-6}	0.079 (15) 0.073-0.082	6.6×10^{-4} (5) 6.5×10^{-4}-6.8×10^{-4}	1.6×10^{5}	1.4×10^{5}
Fourth	1.1×10^{-5}	0.097 (15) 0.090-0.102	6.8×10^{-4} (5) 6.7×10^{-4}-6.9×10^{-4}	3.3×10^{5}	3.1×10^{5}
Fifth	1.8×10^{-5}	0.097 (15) 0.094-0.109	7.0×10^{-4} (5) 6.8×10^{-4}-7.2×10^{-4}	4.6×10^{5}	4.4×10^{5}
♀	2.3×10^{-5}	0.123 (12) 0.119-0.127	8.0×10^{-4} (8) 7.7×10^{-4}-8.3×10^{-4}	4.4×10^{5}	4.2×10^{5}
♂	2.1×10^{-5}	0.100 (10) 0.098-0.108	7.0×10^{-4} (5) 6.8×10^{-4}-7.3×10^{-4}	5.6×10^{5}	5.4×10^{5}
A. aegypti					
♀	6.0×10^{-6}	0.182 (20) 0.200-0.173	1.1×10^{-3} (10) 9.9×10^{-4}-1.4×10^{-3}	4.6×10^{4}	3.0×10^{4}

TABLE 2 (cont.)

Species & stage	Qt cm^3/sec	1* = length cm	r* = mean radius cm	▲ P dyne $/cm^2$	▲ P** corrected dyne/cm^2
P. humanus					
First	$2.1x10^{-7}$	0.027 (10) 0.024-0.031	$2.8x10^{-4}$ (5) $2.5x10^{-4}$-$3.2x10^{-4}$	$6.1x10^4$	$4.5x10^4$
Second	$1.7x10^{-7}$	0.033 (12) 0.032-0.034	$3.1x10^{-4}$ (6) $3.0x10^{-4}$-$3.4x10^{-4}$	$3.9x10^4$	$2.3x10^4$
♀	$1.4x10^{-6}$	0.040 (10) 0.038-0.043	$3.6x10^{-4}$ (5) $3.4x10^{-4}$-$3.9x10^{-4}$	$2.1x10^5$	$1.9x10^5$
♂	$1.1x10^{-6}$	0.040 (12) 0.037-0.042	$3.7x10^{-4}$ (5) $3.3x10^{-4}$-$3.9x10^{-4}$	$1.5x10^5$	$1.3x10^5$
R. prolixus					
Fifth***	$3.3x10^{-4}$	0.020	$5.0x10^{-4}$	$1.96x10^6$ $9.12x10^6$	

* Mean
(number of insects used)
Range

** ▲ P "corrected" = ▲ P - capillary blood pressure.

*** Data from Bennet-Clark (1963, p. 223).

The Muscular Tension and the Power Output of the Cibarial Dilators

In *C. lectularius* the mean tension required of the cibarial pump dilators decreases from the first to the second nymphal instar and then increases to a maximum in the adult (tables 3 and 4). The value for the cibarial pump dilators of the male was larger than for those of the female. In the female *A. aegypti* the muscular tension required per unit sectional area of the cibarial pump dilators is larger than in the first three nymphal instars and smaller than in the other instars of *C. lectularius*. In *P. humanus* the mean muscular tension of the cibarial pump dilators decreases from the first to the second nymphal instar and reaches a maximum in the adult with a higher value in the female than in the male.

From the data published by Bennet-Clark (1963) the mean muscular tension required per cm^2 sectional area of the cibarial pump dilators of the fifth nymphal instar of *R. prolixus* was calculated to be in the range between 1.2 to 5.7 kg/cm^2.

TABLE 3. The mean force and muscular tension of the cibarial pump dilators in *C. lectularius*, female *A. aegypti*, *P. humanus*, and *R. prolixus*.

Species	Area of diaphragm* cm^2	Mean** force dynes	Mean*** muscular tension dynes	Cross sectional* area of muscles cm^2	Muscular tension dyne/cm^2
C. lectularius					
First	8.6×10^{-5} (5) 7.6×10^{-5}-9.4×10^{-5}	12.0	24.0	4.2×10^{-4} (5) 3.7×10^{-4}-4.8×10^{-4}	5.7×10^{4}
Second	1.0×10^{-4} (7) 9.6×10^{-5}-1.4×10^{-4}	11.0	22.0	5.1×10^{-4} (5) 4.7×10^{-4}-5.6×10^{-4}	4.3×10^{4}
Third	1.6×10^{-4} (5) 1.0×10^{-4}-2.1×10^{-4}	22.4	44.8	8.2×10^{-4} (6) 7.9×10^{-4}-8.4×10^{-4}	5.5×10^{4}
Fourth	2.5×10^{-4} (5) 2.2×10^{-4}-2.9×10^{-4}	77.5	155.0	1.2×10^{-3} (6) 1.0×10^{-3}-1.5×10^{-3}	1.3×10^{5}
Fifth	3.2×10^{-4} (5) 2.9×10^{-4}-3.7×10^{-4}	140.8	281.6	1.3×10^{-3} (5) 1.0×10^{-3}-1.8×10^{-3}	2.2×10^{5}
♀	5.7×10^{-4} (8) 4.9×10^{-4}-5.9×10^{-4}	239.4	478.8	2.1×10^{-3} (7) 1.9×10^{-3}-2.5×10^{-3}	2.3×10^{5}
♂	4.1×10^{-4} (5) 3.8×10^{-4}-4.5×10^{-4}	221.4	442.8	1.5×10^{-3} (8) 1.2×10^{-3}-1.9×10^{-3}	2.9×10^{5}
A. aegypti					
♀	1.9×10^{-4} (10) 1.4×10^{-4}-2.3×10^{-4}	5.7	11.4	1.8×10^{-4} (10) 1.3×10^{-4}-2.3×10^{-4}	6.3×10^{4}
P. humanus					
First	2.4×10^{-5} (6) 1.9×10^{-5}-2.7×10^{-5}	1.1	2.2	6.8×10^{-5} (5) 6.1×10^{-5}-7.6×10^{-5}	3.2×10^{4}

TABLE 3 (cont.).

Species	Area of the diaphragm* cm^2	Mean** force dynes	Mean*** muscular tension dynes	Cross sectional* area of muscles cm^2	Muscular tension dyne/cm^2
Second	4.5×10^{-5} (5) 3.9×10^{-5}-5.2×10^{-5}	1.0	2.0	1.1×10^{-4} (5) 9.4×10^{-5}-1.7×10^{-4}	1.8×10^{4}
♀	8.1×10^{-5} (8) 7.3×10^{-5}-9.2×10^{-5}	15.4	30.8	1.9×10^{-4} (8) 1.4×10^{-4}-2.5×10^{-4}	1.6×10^{5}
♂	6.4×10^{-5} (8) 5.8×10^{-5}-7.4×10^{-5}	8.3	16.6	2.0×10^{-4} (8) 1.3×10^{-4}-2.2×10^{-4}	8.3×10^{4}
R. prolixus					
Fifth	9.8×10^{-3} (2) 9.6×10^{-3}-1.0×10^{-2}	1.9×10^{4}+ 8.9×10^{4}	3.8×10^{4}+ 17.8×10^{4}	3.2×10^{-2} (2) 2.8×10^{-2}-3.5×10^{-2}	1.2×10^{6}+ 5.6×10^{6}

* Average
(number of measurements)
Range

** Mean force = ▲ P x area of the diaphragm

*** Mean muscular tension during working stroke = mean force x 2

\+ The two values correspond to the two different values of ▲ P of Bennet-Clark (see table 2).

The muscular tension of the cibarial pump dilators most probably reaches a peak value of twice the estimated mean in table 3. Wigglesworth (1965) reported the maximum load a muscle can raise per square centimeter of cross section in some species and he found that there is no great difference between insects and vertebrates as the value for man is 6-10 kg, for the frog 3 kg, for the mandibular muscles of insects 3.6 -6.9 kg, for the hind leg of *Tettigonia* 4.7 kg and for the flexor tibia of *Decticus* 5.9 kg.

The power output of the cibarial pump dilators expressed as erg/sec/g of muscle and as g cm/sec/g of muscle are shown in table 4. In *C. lectularius* the power developed by these muscles, like the muscular tension, decreases from the first to the second nymphal instar and then increases to a maximum in the adult stage. In the female *A. aegypti* the power output/g of muscle was higher than that of the first three nymphal instars and lower than that of the fifth instar and the adult of *C. lectularius*.

TABLE 4. The power output of the cibarial pump dilators in *C. lectularius*, female *A aegypti*, *P. humanus*, and *R prolixus*.

Species & stage	Travel of* diaphragm cm	Pump cycles/ second	Travel of diaphragm cm/sec	Power** output erg/sec	Length of* muscles cm	Weight of muscles g	Power output in erg/sec/g
C lectarius							
First	2.4×10^{-3} (5) $2.1 \times 10^{-3} - 2.7 \times 10^{-3}$	4.0	9.6×10^{-3}	0.12	0.015 (6) 0.012 - 0.017	6.3×10^{-6}	1.9×10^{4}
Second	2.6×10^{-3} (7) $2.4 \times 10^{-3} - 2.8 \times 10^{-3}$	3.8	9.9×10^{-3}	0.11	0.017 (7) 0.013 - 0.019	8.7×10^{-6}	1.3×10^{4}
Third	3.6×10^{-3} (5) $3.2 \times 10^{-3} - 3.9 \times 10^{-3}$	3.3	1.2×10^{-2}	0.27	0.022 (5) 0.019 - 0.025	1.8×10^{-5}	1.5×10^{4}
Fourth	4.5×10^{-3} (5) $4.1 \times 10^{-3} - 4.9 \times 10^{-3}$	3.3	1.5×10^{-2}	1.20	0.026 (5) 0.021 - 0.031	3.1×10^{-5}	3.9×10^{4}
Fifth	6.2×10^{-3} (5) $5.8 \times 10^{-3} - 6.6 \times 10^{-3}$	3.0	1.9×10^{-2}	2.70	0.028 (8) 0.025 - 0.034	3.6×10^{-5}	7.5×10^{4}
♀	7.5×10^{-3} (8) $6.8 \times 10^{-3} - 7.9 \times 10^{-3}$	2.4	1.8×10^{-2}	4.30	0.033 (8) 0.029 - 0.037	6.9×10^{-5}	6.2×10^{4}

♂	7.5×10^{-3} (5) $6.8 \times 10^{-3} - 7.8 \times 10^{-3}$	2.4	1.8×10^{-2}	3.99	0.030 (5) 0.027 - 0.034	4.5×10^{-5}	8.9×10^{4}
A. aegypti							
♀	3.3×10^{-3} (10) $2.9 \times 10^{-3} - 3.7 \times 10^{-3}$	4.9	1.6×10^{-2}	0.09	0.013 (10) 0.010 - 0.015	2.3×10^{-6}	3.9×10^{4}
P. humanus							
First	$1.0 \times 10^{-3} - 3.9 \times 10^{-3}$ (6) $9.5 \times 10^{-4} - 1.4 \times 10^{-3}$	2.8	3.0×10^{-3}	0.003	3.5×10^{-3} (6) $3.0 \times 10^{-3} - 3.9 \times 10^{-3}$	2.4×10^{-7}	1.3×10^{4}
Second	1.5×10^{-3} (5) $1.3 \times 10^{-3} - 1.7 \times 10^{-3}$	5.3	8.0×10^{-3}	0.008	5.2×10^{-3} (5) $4.9 \times 10^{-3} - 5.5 \times 10^{-3}$	5.7×10^{-7}	1.4×10^{4}
♀	3.0×10^{-3} (7) $2.7 \times 10^{-3} - 3.4 \times 10^{-3}$	4.6	1.4×10^{-2}	0.216	9.2×10^{-3} (7) $8.8 \times 10^{-3} - 9.5 \times 10^{-3}$	1.7×10^{-6}	1.3×10^{5}
♂	3.0×10^{-3} (6) $2.5 \times 10^{-3} - 3.4 \times 10^{-3}$	4.2	1.3×10^{-2}	0.108	8.7×10^{-3} (6) $8.3 \times 10^{-3} - 9.0 \times 10^{-3}$	1.7×10^{-6}	6.3×10^{4}
R. prolixus							
Fifth	0.016	3.0	0.048	912 4.3×10^{3}	0.05	1.6×10^{-3}	5.7×10^{5} 2.7×10^{6}

* Average
(number of insects)
Range

** Power output = mean force x travel of the diaphragm in a second.

TABLE 4. The power output of the cibarial pump dilators in *C. lectularius*, female *A. aegypti*, *P. humanus*, and *R. prolixus*.

Species & stage	Travel of* diaphragm cm	Pump cycles/ second	Travel of diaphragm cm/sec	Power** output erg/sec	Length of* muscles cm	Weight of muscles g	Power output in erg/sec/g
C. lectarius							
First	2.4×10^{-3} (5) $2.1 \times 10^{-3} - 2.7 \times 10^{-3}$	4.0	9.6×10^{-3}	0.12	0.015 (6) 0.012 - 0.017	6.3×10^{-6}	1.9×10^{4}
Second	2.6×10^{-3} (7) $2.4 \times 10^{-3} - 2.8 \times 10^{-3}$	3.8	9.9×10^{-3}	0.11	0.017 (7) 0.013 - 0.019	8.7×10^{-6}	1.3×10^{4}
Third	3.6×10^{-3} (5) $3.2 \times 10^{-3} - 3.9 \times 10^{-3}$	3.3	1.2×10^{-2}	0.27	0.022 (5) 0.019 - 0.025	1.8×10^{-5}	1.5×10^{4}
Fourth	4.5×10^{-3} (5) $4.1 \times 10^{-3} - 4.9 \times 10^{-3}$	3.3	1.5×10^{-2}	1.20	0.026 (5) 0.021 - 0.031	3.1×10^{-5}	3.9×10^{4}
Fifth	6.2×10^{-3} (5) $5.8 \times 10^{-3} - 6.6 \times 10^{-3}$	3.0	1.9×10^{-2}	2.70	0.028 (8) 0.025 - 0.034	3.6×10^{-5}	7.5×10^{4}
♀	7.5×10^{-3} (8) $6.8 \times 10^{-3} - 7.9 \times 10^{-3}$	2.4	1.8×10^{-2}	4.30	0.033 (8) 0.029 - 0.037	6.9×10^{-5}	6.2×10^{4}
♂	7.5×10^{-3} (5) $6.8 \times 10^{-3} - 7.8 \times 10^{-3}$	2.4	1.8×10^{-2}	3.99	0.030 (5) 0.027 - 0.034	4.5×10^{-5}	8.9×10^{4}
A. aegypti							
♀	3.3×10^{-3} (10) $2.9 \times 10^{-3} - 3.7 \times 10^{-3}$	4.9	1.6×10^{-2}	0.09	0.013 (10) 0.010 - 0.015	2.3×10^{-6}	3.9×10^{4}
P. humanus							
First	$1.0 \times 10^{-3} - 3.9 \times 10^{-3}$ (6) $9.5 \times 10^{-4} - 1.4 \times 10^{-3}$	2.8	3.0×10^{-3}	0.003	3.5×10^{-3} (6) $3.0 \times 10^{-3} - 3.9 \times 10^{-3}$	2.4×10^{-7}	1.3×10^{4}
Second	1.5×10^{-3} (5) $1.3 \times 10^{-3} - 1.7 \times 10^{-3}$	5.3	8.0×10^{-3}	0.008	5.2×10^{-3} (5) $4.9 \times 10^{-3} - 5.5 \times 10^{-3}$	5.7×10^{-7}	1.4×10^{4}
♀	3.0×10^{-3} (7) $2.7 \times 10^{-3} - 3.4 \times 10^{-3}$	4.6	1.4×10^{-2}	0.216	9.2×10^{-3} (7) $8.8 \times 10^{-3} - 9.5 \times 10^{-3}$	1.7×10^{-6}	1.3×10^{5}
♂	3.0×10^{-3} (6) $2.5 \times 10^{-3} - 3.4 \times 10^{-3}$	4.2	1.3×10^{-2}	0.108	8.7×10^{-3} (6) $8.3 \times 10^{-3} - 9.0 \times 10^{-3}$	1.7×10^{-6}	6.3×10^{4}
R. prolixus							
Fifth	0.016	3.0	0.048	912 4.3×10^{3}	0.05	1.6×10^{-3}	5.7×10^{5} 2.7×10^{6}

* Average (number of insects) Range

** Power output = mean force x travel of the diaphragm in a second.

It is also higher than that of the first two instars and lower than that of the adult *P. humanus*.

Roeder (1953) reported the power of the flight muscles in some insects. The minimum was 205 g cm/sec/g of muscle for *Vanessa atalanta* L. and the maximum was 558 g cm/sec/g of muscle for *Aeschna mixta* Latr. The cibarial pump dilators in *C. lectularius*, *A. aegypti*, and *P. humanus* are not required to operate at specific power outputs as great as these. The dilators of the cibarial pump of the fifth nymphal instar of *R. prolixus*, on the other hand, would, on the basis of Bennet-Clark's data, be required to work at a greater specific power output than the flight muscles of these insects.

Discussion

The actual laws of blood flow, at least in the range of physiological rates of flow, approximate sufficiently the simple laws (e.g. Poiseuille's law) for these to be applied with caution, using the appropriate value for the effective viscosity rather than the value obtained in viscometers of a large bore. Burton (1965) stated "As long as the diameter of the capillary tube used in the viscometer is more than 1 or 2 mm, the relative viscosity of the blood is the same, whatever the size of the tube used. When, however, tubes of narrower diameter are used, the value for relative viscosity found is less. This is because the absolute viscosity of water is the same however small the diameter of the tube; but that of the blood decreased to less than half the value found when large tubes are used". This has long been known as the Fahraeus-Lindquist effect. From this the viscosity value and consequently the values of $\blacktriangle P$ obtained in my calculations could be in error by a factor of 2. Burton (1965) also discusses the effect of temperature on viscosity of the blood. I should not expect significant variation in the viscosity of the blood through changes in temperature in the quick pumping process of sucking in these insects.

The ability to feed at a relatively high rate through a minute feeding canal is very important for blood-sucking insects. For this purpose they are equipped with very efficient feeding apparatus with a sucking pump which is capable of exerting a high tension on the blood upon which they feed. Differences in the rate of feeding in the different blood-sucking insects can be due to many factors. One of these factors is the negative pressure that can be produced in the cibarial pump. This in turn is dependent upon the tension which can be developed in the cibarial pump dilators, the length of the feeding canal and its diameter as well as the viscosity of the blood and the capillary blood pressure of the host. These experiments suggest that blood viscosity and the capillary blood pressure of the host might be of importance in the evolution of host selection in blood-sucking insects.

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THE BIOLOGY OF SOME BLACK FLIES (DIPTERA : SIMULIIDAE) OF ALBERTA

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The biology of 15 simuliid species in northern Alberta was studied. Simulium vittatum *Zetterstedt and* Simulium venustum *Say were the most abundant;* Prosimulium travisi *Stone and* Prosimulium onychodactylum *Dyar and Shannon were rare. Characterictically, the univoltine species (except* Cnephia mutata *Malloch) were autogenous throughout the area.* C. mutata *was represented by both the triploid (parthenogenetic) and the rare diploid sexual forms. A key for the identification of the 31 species reported from Alberta is given. Regular sampling of simuliid larvae in the rivers and creeks shows that there are several peaks of abundance every year (1963-1965) due to the occurrence of the larvae of more than one species in the breeding localities, and that the dates, numbers, and composition of these differ slightly depending on the date of the ice break-up and the march of temperature during the season. The overwintered larvae of* S. vittatum *were present in the water and the ice. The susceptibility of the larvae to DDT was measured and their migration downstream was investigated by the use of plastic sampling cones. The infection rates of the adults and aquatic stages by nematodes and microsporidian protozoans and an evaluation of both predators and parasites as control agents are given.*

Fredeen (1958) and Fredeen and Shemanchuck (1960) investigated the simuliids of Alberta, Saskatchewan and Manitoba. Strickland (1938, 1946) recorded eight species of black flies in Alberta; the number is now 31.

In the United States additional regional lists of black flies have been published: Western U.S.A., Stains and Knowlton (1940); Minnesota, Nicholson and Mickel (1950); Alaska, Stone (1952) and Sommerman (1953); Utah, Peterson (1955, 1960); New York, Stone and Jamnback (1955).

Ecological and biological studies were included in most of the above papers. The control of black flies using chemicals was started in Canada by Prevost (1947). Hocking, Twinn and McDuffie (1949) investigated various insecticides. Further reports on this subject were published by Arnason et al. (1949), Brown (1952), Brown et al. (1951), Hocking (1950, 1953), Hocking and Richards (1952), Peterson and Wolfe (1958), Peterson and West (1960), and West, Brown and Peterson (1960).

Cytological studies on black flies commenced with the work of Rothfels and Dunbar in 1953. Additional studies were reported by Rothfels (1956), Dunbar (1958), Basrur and Rothfels (1959), Landau (1962) and Pasternak (1964). The following species have been studied cytologically in North America: *Simulium vittatum* Zetterstedt, *Simulium tuberosum* Lundstroem, *Simulium aureum* Fries, *Simulium latipes* Mg., *Cnephia mutata* Malloch, *Prosimulium fontanum* Syme and Davies, *Prosimulium hirtipes* Fries, *Prosimulium frohnei* Sommerman, *Prosimulium fulvum* Coquillett, *Prosimulium travisi* Stone, *Prosimulium formosum* Shewell, *Prosimulium fuscum* Syme and Davies, *Prosimulium mixtum* Syme and Davies.

STUDY AREA

The Flatbush study area is about 100 miles north of Edmonton (54° 15'-50' N, 113° 30'-114° 15' W) and lies within the boreal forest region of central Alberta. The field station was seven miles west of Flatbush village (54° 40' N, 114° 10' W). Smith is 50 miles north of Flatbush, Athabasca town is 40 miles east, and Hinton 200 miles west. All these localities were used as centres during the survey.

The aspen and spruce forest is intact in long stretches and the cultivated land (farms and pastures) is located away from the rivers. There are no large urban centres (Happold 1965a & b).

Hinton (elevation 3265 feet) is about 177 miles west of Edmonton. It lies on the bank of the Athabasca River which has many riffles in which S. *arcticum* and S. *tuberosum* breed.

Muskuta Creek and three other creeks flowing into the Athabasca River above Hinton were surveyed for black fly species present. S. *vittatum*, S. *venustum* and S. *arcticum* were collected. Adults of *Prosimulium travisi* and *P. onychodactylum* were captured in netsweeps.

Athabasca town is 40 miles east of Flatbush, situated on the Athabasca River. In one locality downstream from the town, and many localities above the town, S. *arcticum* was collected but in small numbers. Muskeg Creek and three other creeks yielded large samples of black fly larvae, S. *venustum* being the dominant species.

Climate

The long cold winter, characteristic of the continental climate, commences in November and ends in March. The snow melts in April and the ice breaks in the rivers and creeks in April and May. During April the temperature rises to 40 F by day and drops to 20 F by night. In May the temperature reaches 80 F and drops to 35 F at night. The average maximum and minimum temperatures in June are 78 F and 42 F. July and August are warm and the temperature stays above 40 F. In September low temperatures are recorded.

The relative humidity records indicate an overall average of 61% (40-92%) during the May to September period. The diurnal records show fluctuations of relative humidity especially before sunset and for a short period after sunrise. There seems to be a peak in the morning followed by a drop at noon and a rise in the afternoon which continues well into the night. Temperature (air), relative humidity and rainfall records are given in table 1. The average date of ice break-up in the Pembina River over 10 years was April 17.

Vegetation

The forest plants include besides aspen and spruce, many shrubs such as rose, cranberry, raspberry, and other berries; horsetails, and grasses (Happold 1965a).

The plants come into leaf in May and by the end of September the herbaceous plant life has ended and the leaves have fallen. The insect population of this area is closely associated with the flora. All the species of black flies were collected feeding or resting on plants.

TABLE 1. Temperature, relative humidity and rainfall records in the field station at Flatbush : 1961-1965.

	May		June	
	Temp. F.	R.H. %	Temp. F.	R.H. %
1961				
Average	58.9	63.5	63.1	68.3
Mean max.	66.1	87.4	75.0	89.2
Mean min.	41.7	39.7	51.3	47.5
Rainfall total in.	0.30		4.49	
1962				
Average	50.3	68.2	58.1	67.5
Mean max.	61.8	94.8	69.7	93.5
Mean min.	38.8	41.7	46.5	43.5
Rainfall total in.	0.32		3.99	
1963				
Average	51.2	64.3	63.2	69.4
Mean max.	62.1	92.1	67.7	91.5
Mean min.	40.7	40.5	43.4	47.8
Rainfall total in.	1.37		2.97	
1964				
Average	50.2	65.5	64.2	70.2
Mean max.	62.1	90.0	68.3	93.4
Mean min.	41.2	41.7	44.6	42.8
Rainfall total in.	0.94		3.24	
1965				
Average	51.4	63.5	62.1	71.1
Mean max.	64.5	92.1	70.2	93.4
Mean min.	42.6	40.5	47.1	49.8
Rainfall total in.	1.41		4.89	

TABLE 1 (cont.).

	July		August		September	
	Temp. F.	R.H. %	Temp. F.	R.H. %	Temp. F.	R.H. %
1961						
Average	62.7	69.9	64.3	67.2	58.4	69.9
Mean max.	74.0	89.7	77.6	90.0	66.5	94.4
Mean min.	51.4	39.7	51.1	42.6	40.1	44.8
Rainfall total in.	4.38		0.88		2.15	
1962						
Average	60.0	72.0	60.3	72.7	57.6	74.3
Mean max.	71.0	94.7	71.4	94.8	67.4	97.8
Mean min.	49.0	49.3	49.2	50.9	38.6	57.7
Rainfall total in.	4.08		2.49		2.01	
1963						
Average	64.1	70.6	60.2	71.5	52.4	72.2
Mean max.	70.5	90.8	69.4	90.5	65.1	93.2
Mean min.	46.9	48.9	46.1	46.6	39.2	50.1
Rainfall total in.	3.12		0.94		1.65	
1964						
Average	65.7	71.5	62.2	71.3	59.3	74.1
Mean max.	71.2	91.2	70.4	94.4	69.1	94.8
Mean min.	46.9	46.9	45.5	48.8	39.7	53.3
Rainfall total in.	3.51		1.21		1.98	
1965						
Average	63.9	72.4	62.4	75.5	59.7	77.7
Mean max.	72.1	92.4	70.0	90.8	64.8	95.5
Mean min.	49.8	47.8	48.2	46.5	41.1	54.5
Rainfall total in.	4.74		1.21		2.11	

In the study area in running water very few aquatic plants were encountered. There were distinct differences in the composition and density of the vegetation in the various streams. The vegetation near the water is composed of horsetails, mosses, and algae. The banks of the rivers are steep and devoid of vegetation but further away from the rivers the valleys are forested. The creeks have low banks and the vegetation is dense and in shallow water extends into the creek bed.

The following aquatic plants were found in the simuliid breeding sites: Chlorophyceae: *Stigeodonium, Pediastrum* ; Fragilariaceae: *Asterionella* ; Fontinalaceae: *Fontinalis dalecarlica* Linn. ; Equisetaceae: *Equisetum* sp. ; Typhaceae: *Typha latifolia* Linn. ; Sparganiaceae: *Sparganium hyperboreum* Laestad, *S. multipedunculatum* Morang; Najadaceae: *Potamogeton americanus* C. and S., *P. richardsonii* (Benn.) Rybd. ; Alismaceae: *Sagittaria australis* J. G. Sm. ; Butomaceae: *Elodea canadensis* (Pursh), *Vallisneria spiralis* Linn. ; Pontederiaceae: *Pontederia* (*cordata* Linn. ?); Ceratophyllaceae: *Ceratophyllum* spp., (*C. demersum* Linn. ?); Cruciferae: *Radicula* sp. ; Haloragidaceae: *Myriophyllum spicatum* Linn.

Association with other Aquatic Organisms

Jamnback and Collins (1955) and Jamnback and Eabry (1962) used standard nets to measure quantitatively the stream organisms, in relation to blackfly control. Other workers had listed the groups of organisms encountered (Anderson and Dicke 1960, Hocking 1950 and Hocking et al. 1949).

In the present study a 20 mesh per inch screen (5 x 5 feet) was used to investigate the association of simuliid larvae and other stream organisms. The screen was fixed among the rocks in the breeding sites and supported by diagonal poles in the back. The collector worked downstream from a point about 100 yards up from the screen, disturbing the bottom substrata and turning stones and logs to dislocate the fauna which was trapped on the screen. The following organisms were collected:

Mollusca -	Gastropoda	
	Pulmonata	
Annelida -	Hirudinea	*Helobdella stagnalis* Linn.
		Theromyzon occidentalis Verrill
		Moorebdella ferrida Verrill
Arthropoda -	Crustacea	*Daphnia* sp.
		Gammarus sp.
	Insecta	
	Ephemeroptera (nymphs)	*Heptagenia* sp.
		Ephemerida sp.
	Odonata (nymphs)	*Aeshna* sp.
		Agrion sp.
	Plecoptera (nymphs	*Nemoura* sp.
	Trichoptera	*Limnephilus canadensis* Banks
		Brachycentrus occidentalis Banks
		Helicopsyche borealis Hagen
		Hydropsyche recurvata Banks
		Hydropsyche sp.

	Trichoptera (cont.)	*Leptocella* sp.
		Athripsodes sp.
		Polycentropus sp.
		Mayatrichia sp.
	Diptera (Chironomidae)	
	Coleoptera (Hydrophilidae)	
	(Dytiscidae)	
	Hemiptera (Corixidae)	
Chordata -	Pisces	*Esox lucius* Linn.
		Catostomus commersonii Lacepede
		Moxostoma sp.
		Pimephales promelas Rafinesque

No phoretic association was observed between the simuliid larvae and any other organism. At the start of the season and when the water levels were high the number of organisms was small but the populations built up following the rise in water temperature and decrease in flow which resulted in the formation of side pools in the rivers and areas of shallow flow in the creeks.

The crustaceans and snails were late-comers in each season while the other groups were usually present, in different numbers, in all stations all the season.

Rivers and Creeks

Some of this information was kindly provided by the District Engineer, Water Research Branch, Department of Mines and Technical Surveys (Calgary).

Athabasca River

The drainage area of this river is 29,600 sq. miles. It flows north from the Rocky Mountains and pours into Lake Athabasca. The mean discharge in April is 4,700 cubic feet per second, in May 21,200 ft^3/sec, in June 33,000 ft^3/sec, in July 32,000 ft^3/sec, in August 29,200 and in September 25,300 ft^3/sec. The mean velocity increases from 1.8 ft/sec in April to 2.8 ft/sec in June and is nearly uniform in the period June-September. The effects of the rains and melting ice in the mountains are seen in the study area.

The river bed is sandy with coarse gravel but a mud layer is slowly deposited at the end of the June floods. The main stream is devoid of vegetation except for some algal growth on rocks under the water, and a narrow zone of horsetails and reeds at the edge.

Due to the gentle slope of the land in the study area there are no rapids in the river but a few isolated riffles are present and *S. arcticum* Malloch and *S. tuberosum* were found breeding in these.

Pembina River

The drainage area of this river is 4,550 sq. miles. The Pembina flows north from the Rocky Mountains and conjoins the Athabasca River

10 miles north of Flatbush. From the low discharge of 320 ft^3/sec in April it reaches 2,230 ft^3/sec in May 1,320 ft^3/sec in June and July, 995 ft^3/sec in August and 2,000 ft^3/sec in September. The velocity of the current ranges from less than 1.5 ft/sec in April to more than 2.3 ft/sec in May.

The river banks are steep and the river valley is bare but a fringe of vegetation is present at the edge of the water consisting of horsetails and willows. The stream bed is a mixture of fine sand, clay and gravel. This condition resulted in a better crop of aquatic foliage than in the Athabasca River. The Pembina River has large stretches of riffles which extend at low water level. At low water also algae cover stones and rocks, and their filaments and the stones provide suitable substrates for attachment of black fly larvae. Seven species of simuliids, *S. arcticum* , *S. tuberosum* , *S. luggeri* Nicholson and Mickel, *S. venustum* Say, *S. vittatum* Zetterstedt, *S. decorum* Walker and *S. latipes* , were found breeding in the Pembina River.

Irish Creek

Irish Creek crosses Highway 44 65 miles north of Edmonton. It drains the marshes east of the highway and flows into the Pembina River. The creek is about 10 feet wide with steep banks. The water depth ranges from 1 to 3 feet. It flows in part in the shade of forest. The creek is rich in vegetation and although no overwintering larvae were found in it, the larvae of four species of black fly, *Prosimulium decemarticulatum* (Twinn), *Cnephia dacotensis* (Dyar and Shannon), *Cnephia mutata* (Malloch), and *C. emergens* Stone, were collected in May. *Simulium vittatum* Say, *Simulium vittatum* Zetterstedt, and *Simulium latipes* (Meigen) were the dominant species later in the season.

French Creek

This creek drains Cross Lake and flows west to the Pembina. The creek is up to 18 feet wide and the water is one to two feet deep. The current varies from one to two ft/sec near the junction of the creek and the Pembina River. The creek is rich in vegetation especially reeds. The effluence point and the numerous beaver dams provided suitable breeding sites for black flies. The larvae and eggs of six species of black flies were collected. The overwintering larvae of *S. vittatum* were present in water under the ice in 1963, 1964, 1965.

Cross Lake Creek

This creek flows into Cross Lake draining swamps and small lakes north of Cross Lake. The creek is eight to ten feet wide but the maximum water depth is one foot. It flows through dense growth of vegetation and is therefore shaded thus providing ideal breeding habitat for *S. decorum* .

Flatbush (Andy's) Creek

This creek flows into the Pembina at Flatbush draining the swamps east of the village. The creek channel is choked with vegetation in some places, dammed in two places, but receives many small rills. Some observations on repopulation of the creek by aquatic fauna especially

black flies were made, after it was dammed.

Chisholm Creek

This creek flows into the Athabasca River at Chisholm (25 miles north of Flatbush). It is dammed near the junction with the Athabasca to provide water for the lumber mills in the village. The creek channel is 15 feet wide and the water depth varies from nine inches to 1.5 feet. The overwintering larvae of *S. vittatum* were abundant and the highest rates of infestation by parasitic nematodes were recorded here in three consecutive years.

Water Analysis

The chemical and physical analyses of the water in the two rivers and in the creeks gave closely similar results. The two rivers were slightly cooler than the creeks; Irish and French creeks were cooler than the others. The pH readings ranged from 7.5 to 8.5. It has been reported in the literature that black fly breeding streams are slightly alkaline (Anderson and Dicke (1960), Fredeen and Shemanchuck (1960), Peterson and West (1958) and Sommerman et al. (1955). Albeit Peterson and Wolfe (1960) mentioned that black flies can breed in water with pH 5.8 to 8.5: Anderson and Dicke (1960) recorded pH values as high as 8.15 and 8.95 in Wisconsin. The dissolved oxygen concentration ranged from 8.1 ppm to 10.0 ppm and the calculated per cent of saturation showed fluctuations over 100%. Wu (1931), Petersen (1924) and Radzivilovskaya (1950) strongly upheld the theory that the function of the current is to maintain the oxygen saturation. Rubtzov (1939) and Zahar (1951) came to the conclusion that above a certain saturation there is no necessary current requirement.

TAXONOMIC PROCEDURE

The family is very poor in fossil records (Smart 1945, Stone 1964). Specimens recovered from Oligocene amber (English Purbeck) were described by Westwood (1854) and Handlirsch (1908). Two species belonging to two genera have been named: *Simulium priscum* Westwood 1854 orthotype of *Simulidium* Westwood 1854 and *Simulium humidum* Brodie 1906 orthotype of *Pseudosimulium* Handlirsch 1906.

Black flies are distinct and well separated from the other nematocerous families yet they share the biting habit with the Ceratopogonidae. There are no species in the simuliids with affinities to the latter, nor to the chironomids although Shewell (1958) and Davies, L. (1961) reported on the similarities between the primitive simuliids and chironomids, especially in the larval stages. Grenier and Rageau (1960) suggested that this highly evolved nematocerous family is a precursor of the Brachycera.

Seven subfamilies have been named by Enderlein (1937): Simuliinae, Prosimuliinae, Hellichiinae, Ectemniinae, Cnesiinae, Stegopterninae, and Nevermanniinae. Other authors have accepted 2 or 3:
Edwards (1939), Stone (1964), and Stone and Jamnback (1955); Simuliinae, and Prosimuliinae.

Smart (1945): Simuliinae and Parasimuliinae.
Dumbleton (1963), Grenier and Rageau (1960), and Shewell (1958): Simuliinae, Prosimuliinae and Parasimuliinae.
Rubtzov (1959): Simuliinae and Gymnopaidiinae.

There are 80 generic names and 1300 specific names in the literature. The genera and subgenera accepted in North America are:

Simulium
- *Simulium*
- *Eusimulium*
- *Byssodon*
- *Psilozia (Neosimulium)*
- *Hagenomyia*
- *Gnus*

Cnephia
- *Cnephia*
- *Stegopterna*
- *Ectemnia*

Prosimulium
- *Prosimulium*
- *Helodon*

Parasimulium
- *Parasimulium*

Twinnia

Gymnopais

The family is cosmopolitan; *Simulium* and *Cnephia* are the most widespread genera. There are species common to most of the adjacent zoogeographical regions.

Materials and Methods

Aquatic stages and adults were collected from the field and preserved in 95% ethyl alcohol; some of the adults were pinned or mounted by gluing them on paper points. Laboratory rearing was also used in the taxonomic study. The eggs of some species were collected easily but other species proved difficult as the eggs are scattered on the river bottom and no efficient method was found for their extraction. It was found that by combining the eggs, larvae, and pupal skins with the adult, identification is feasible. The pupae are more diagnostic than any other stage. Dissection of some stages was of value. The linear dimensions of eggs of the species studied have an overlapping range so that eggs of the different species proved difficult to identify positively.

On the other hand, the larval head capsule and its different structures were repeatedly employed in keying the species. The antenna has four articles and their colour and length are useful. The cephalic apotome (frontoclypeus) has head spots which occur where the muscles attach to the dorsal surface of the head capsule. These spots are constant in anterior and posterior median, and anterior and posterior lateral groups. The ventral side of the head capsule has a number of structures widely used in identification of the species. The shape and size of the postgenal cleft (throat, occipital, epicranial) vary from a slight groove extending to less than one-fifth the distance between the occipital pits and the base of the submental teeth, to a large bulbous opening with an apex almost touching the base of the hypostomial teeth. The submentum (hypostomium, mentum) has three sets of teeth: median tooth, lateral teeth (2 or 3) and a single corner tooth on each side of the laterals (fig. 1c, d. The cephalic fans (head fans or brushes) have short stalks (stems) and the number of rays in the mature larva is fairly constant (usually inc-

reases with each instar). Under each primary fan there is a secondary fan. The labrum, mandibles and maxillae are present. The hypopharynx is of no taxonomic value.

The larval proleg has a circlet of hooks at its apex used for locomotion. The lateral sclerite (plate) of the proleg is of subsidiary importance in identification of the species as it varies little from one species to another. The pupal respiratory organ histoblast is of considerable value.

The abdominal features utilized in the study are scales or setae, coloration, ventral papillae (anal tubercles), anal sclerite (anal crosspiece) and the posterior sucker (posterior circlet of hooks).

The pupa is usually surrounded by a cocoon, the shape of which is characteristic. The pupal respiratory organ consists of two to forty filaments arising from a different number of stalks (trunks, petioles). The filaments may be grouped or not. The hooks on the abdominal segments and the terminal spines are specific. The pupa proved to be a reliable tool of the utmost taxonomic value.

The adult black fly is very difficult to identify. The antennal flagellum has 7-9 articles. The maxillary palpus contains a sensory organ in its third segment. The comparative length of vein R (stem vein, the common base of R and Rs) and the distance to wing apex from the base of Rs are widely used. The basal cell may be present in some species (fig. 1e). The presence of setae or hairs on the ventral and dorsal sides of the veins is a good character for keying some species. The first tarsal article of the hind leg (the basitarsus) may be extended posteriorly in a flattened lobe (calcipala) and the second article may be notched dorsally (pedisulcus). The tarsal claws are simple or toothed (forked, bifid) (fig. 1 a, b).

The sexes are dimorphic and the males are holoptic; the upper facets of the eye in the males are larger than the lower ones. The male genitalia consist of dark pigmented sclerites that can be used to separate the species groups. On each side of the tip of the abdomen lies dorsally the basistyle (coxite, basimere) and attached to it is the clasper (dististyle, distimere) which has a single or many teeth on the inner side. Between the basistyle and clasper is found a ventral plate, a median sclerite and a paramere consisting of two arms and a fringe of hooks (phallosome and aedeagus pile respectively). The female genitalia consist of an ovipositor lobe attached to sternite eight, a genital fork under tergite nine, an anal lobe and a cercus.

Key to the Genera of North American Simuliidae

(Adapted in part from: Davies, Peterson, and Wood 1962, and Wood, Peterson, Davies, and Gyorkos 1963).

Adults

1. Costa with fine hairs only (microtrichia) no spiniform setae (macrotrichia); Rs forked apically (fig. 1); no calcipala or pedisulcus. .2
- Costa with macrotrichia intermixed with microtrichia; Rs not forked at apex; or with a small fork at the extreme portion; calcipala and pedisulcus present 5

2. A slight or well developed bulla behind the eye; antennal flagellum with 7-9 articles 3
- No bulla behind the eye; antennal flagellum with 9-11 articles . . . . . *Prosimulium* Roubaud
3. R joins the costa near the middle of wing; submedian fold apparently not branched; Rs fork distinctly ending before the termination of costa at apex of wing; flagellum with 9 articles. . . . . *Parasimulium* Malloch
- R joining costa well beyond middle of wing; submedian fold forked; Rs fork reaching to or beyond termination of costa 4
4. Scutum with stout, erect hairs but no fine recumbent hairs; flagellum with 7-9 articles . . . . . *Gymnopais* Stone
- Scutum with fine recumbent hairs only; 7-9 articles; male clasper with one apical spine; female ovipositor short, not reaching the anal lobes . . . . . *Twinnia* Stone
5. Basal cell usually present; pedisulcus very small; length of R more than one-third the distance from base of Rs to the wing apex, with hairs dorsally . . . . . *Cnephia* Enderlein
- Basal cell absent or very small and incomplete; pedisulcus well developed; R with or without hairs dorsally; its length less than one-third the distance from base of Rs to wing apex . . . . . *Simulium* Latreille

Pupae

The pupa of *Parasimulium* has not been described.

1. Cocoon irregular, shapeless and reduced; abdomen with a pair of large terminal spines 2
- Cocoon well developed with definite anterior opening; abdomen without terminal hooks 6
2. Almost no cocoon; dorsum without hooks; abdomen with ten hooks on sternites four to six in more than one transverse row; respiratory filaments four (subarctic genus) . . . . . *Gymnopais*
- Cocoon covering part of the body; dorsum with hooks on some of the tergites; if present, hooks on the sternites are in transverse rows 3
3. Tergites 6-8 with anterior row of hooklets 4
- Tergites 6-8 without row of hooklets: a) Pupa 4.0 mm long; respiratory organ with three stout trunks branching in 16 filaments. . . . . *Twinnia*
- b) Pupa 2.0-3.0 mm long; respiratory organ with two trunks branching in 15-23 (average 19) pale slender filaments . . . . . *Cnephia abdita* Peterson
4. Respiratory filaments arising from a rounded knob on a short petiole . . . . . *Cnephia*
- Respiratory filaments not arising from a rounded knob on a short petiole 5
5. Respiratory filaments 12 (rarely 14) or less arising from two trunks . . . . . *Cnephia*
- Respiratory filaments more than 12, if less than 12 not arising from two trunks . . . . . *Prosimulium*

6. Cocoon stalked and anterior margin not well developed . . *Cnephia*
- Cocoon not stalked and anterior margin well developed; lateral margin of terminal segments without short, curved hooks, although setae may be present . . . . . .*Simulium*

Larvae

1. Larva without cephalic fans; anal sclerite Y-shaped 2
- Larva with two cephalic fans; anal sclerite X-shaped or absent. .3
2. Labrum normal; antenna extending beyond the short cephalic apotome; mandible with no teeth on the subapical margin; submentum with distinct teeth . . . . . .*Twinnia*
- Labrum enlarged; antenna not extending beyond the narrow and elongated cephalic apotome; mandible with small teeth on outer subapical margin; submentum with no distinct teeth . . . . . .*Gymnopais*
3. Anal sclerite absent . . . . . *Cnephia* (in part)
- Anal sclerite present 4
4. Antenna with articles one and two pale, three and four darkly pigmented; secondary fan filaments form a straight line at their tips when extended; median tooth of submentum tridentate (fig. 1d) anal gills with three simple lobes . . . . . *Prosimulium*
- Antenna with articles one and two yellow to brown and three and four rarely dark brown; secondary fan filaments form an arc; median tooth of submentum not tridentate (fig. 1c);anal gill with three simple or compound lobes 5
5. Submentum with corner and median teeth large and subequal, lateral teeth three and subequal (fig. 1c) 6
- Submentum not as above; anal lobe with three simple lobes . . . . . *Cnephia* (in part)
6. Ventral papillae absent or small; postgenal cleft either pointed apically or suboesophageal ganglion and/or epidermis of postgenal cleft distinctly dark, or both; head spots light or dark; anal gill with three compound lobes (except *Psilozia*) . . . . . .*Simulium*
- Ventral papillae well developed; anal gill with three simple lobes (except *latipes*); postgenal cleft not pointed apically; suboesophageal ganglion and epidermis of postgenal cleft not black; head spots dark . . . . . (*Eusimulium*)

List of Species of Simuliidae Recorded from Alberta

(Publisher's or collector's name and date of publication or collection follow the colon.)

Simulium *(Simulium)*

decorum Walker 1848 : Strickland 1938.
hunteri Malloch 1914 : Strickland 1938.
tuberosum Lunstroem 1911 : Fredeen 1958.
luggeri Nicholson and Mickel 1950:Fredeen 1958.
venustum Say 1823 : Strickland 1938.
verecundum Stone and Jamnback 1955 : Abdelnur.
meridionale Riley 1886 : Fredeen 1958.
malyshevi Dorogostajakij, Rubtzov and

		Vlasov 1934 : Fredeen 1958
		piperi Dyar and Shannon 1927 : Fredeen 1958.
	(Byssodon)	*rugglesi* Nicholson and Mickel 1950 : Fredeen 1958.
		transiens Rubtzov 1949 : Fredeen 1958.
	(Gnus)	*arcticum* Malloch 1914 : Strickland 1938.
		corbis Twinn 1936 : Fredeen 1958.
	(Psilopelemia)	*griseum* Coquillett 1898 : Fredeen 1958.
		bivittatum Malloch 1914 : Fredeen 1958.
	(Psilozia)	*vittatum* Zetterstedt 1838: Strickland 1938.
	(Eusimulium)	*aureum* Fries 1824 : Fredeen 1958.
		latipes (Meigen) 1804 : Fredeen 1958.
		pugetense Dyar and Shannon 1927 : Fredeen 1958.
	(Hagenomyia)	*pictipes* Hagen 1880 : Strickland 1938.
Cnephia	*(Cnephia)*	*dacotensis* Dyar and Shannon 1927 : Abdelnur 1965.
		emergens Stone 1952 : Abdelnur 1965.
		saskatchewana Shewell and Fredeen 1958 : Shewell and Fredeen 1958.
	(Stegopterna)	*mutata* (Malloch) 1914 : Abdelnur 1965.
	(Cnetha)	*saileri* Stone 1952 : Fredeen 1958.
Prosimulium	*(Prosimulium)*	*fulvum* (Coquillett) 1902 : Strickland 1938.
		pleurale Malloch 1914 : Strickland 1938.
		travisi Stone 1952 : Abdelnur 1965.
		decemarticulatum (Twinn) 1936 : Abdelnur 1965.
		onychodactylum Dyar and Shannon 1927: Abdelnur 1965.
Twinnia biclavata	Stone and Jamnback 1955 : D. M. Wood 1964.	

Key to the Species Recorded from Alberta

(Adapted in part from Peterson (1960b), and Davies, Peterson and Wood (1962).

Prosimulium

Adult females

1. Antenna with 11 articles 2
- Antenna with 9 or 10 articles . . . . . *decemarticulatum*
2. Claw with a strong thumb-like basal projection 3
- Claw simple 4
3. Integument yellow or orange, frons narrow, nearly parallel sided. . . . . *onychodactylum*
- Integument black, frons normal . . . . . *pleurale*
4. Integument yellow to orange . . . . . *fulvum*
- Integument brown or black . . . . . *travisi*

Adult males

1. Antenna with 9 or 10 articles, clasper with one spine apically . ..

. . . . . *decemarticulatum*

- Antenna with 11 articles 2

2. Hind femora at least, yellow 3

- Hind femora brown or blackish, antenna black, ventral plate apically with sharp lateral prongs between which lies a two-tined fork . . . . . *pleurale*

3. Integument of thorax orange, clasper with a single apical spine . . . . . *fulvum*

- Integument of thorax dark or brown-black 4

4. Apex of clasper pointed with two terminal spines; ventral plate broad, shallow and V-shaped; basal articles of hind tarsus swollen and thus broader than other articles . . . . . *onychodactylum*

- Apex of clasper rounded, with two apical spines; ventral plate with a narrow and sharply pointed median recurved lip; tarsal articles not swollen . . . . . *travisi*

Pupae

1. Respiratory organ consisting of two stout divergent trunks on a short petiole, from the former arise 12-20 slender filaments . . . . . *onychodactylum*

- Respiratory organ not as above 2

2. Respiratory filaments 9, arranged in a whorl from a short base. . . . . *decemarticulatum*

- Respiratory filaments 16 or more 3

3. Respiratory filaments 21 or more . . . . . *pleurale*

- Respiratory filaments 14 or 16 4

4. Respiratory filaments closely clumped together; dorsum of head and thorax strongly rugose . . . . . *travisi*

- Respiratory filaments 16; not closely clumped together; dorsum of head and thorax not rugose; pupa orange . . . . . *fulvum*

Larvae

1. Submental median tooth distinctly shorter than corner tooth . . 2

- Submental median tooth distinctly longer than corner tooth . . . 3

2. Submental lateral teeth longer than other teeth; antenna longer than cephalic fan stalk, 45 rays in cephalic fan; nine filaments in respiratory histoblast; anal sclerite subrectangular, lateral plate of proleg very narrow . . . . . *decemarticulatum*

- Submental corner tooth longest; antenna longer than cephalic fan stalk, 54 rays in cephalic fan, 21 or more filaments in respiratory histoblast . . . . . *pleurale*

3. Postgenal cleft simple, antenna reaches tip of cephalic fan stalk. 4

- Postgenal cleft biarctate, antenna extending three-fourths length of cephalic fan stalk; respiratory histoblast with many filaments arising from two trunks . . . . . *onychodactylum*

4. Postgenal cleft slight, last lateral tooth on submentum as long as median tooth, head capsule pale, dorsal pattern absent, 17-19 rays in cephalic fan, 16 filaments in respiratory histoblast . . . *fulvum*

- Postgenal cleft pronounced, last lateral tooth on submentum shorter

than median tooth, head capsule pattern consisting of a median broken line and two lateral spots on each side of it with a broad dark area posteriorly . . . . . *travisi*

Cnephia

Adult females

1. Tarsal claws simple 2
- Tarsal claws each with a distinct basal tooth or a large projection. 3
2. Maxilla with retrorse teeth, mandible serrate, calcipala large, broad, rounded . . . . . *mutata*
- Maxilla without teeth, mandible not serrate, calcipala short, pointed . . . . . .*emergens*
3. Tarsal claws with distinct teeth basally, calcipala small, pedisulcus indistinct or absent, scutum brownish with three narrow pale lines . . . . . *dacotensis*
- Tarsal claws with large basal projections 4
4. Scutum with three pale gray vittae, median narrow and straight, lateral broader and sinuous; scutellum with long, erect, white and few black hairs . . . . . .*saskatchewana*
- Scutum gray, clothes with yellow recumbent hairs, scutellum reddish brown with long, erect pale hairs . . . . . *saileri*

Adult males

1. Clasper with one apical tooth 3
- Clasper with two teeth 2
2. Galea of maxilla reduced, shorter than labrum-epipharynx . . . . . *emergens*
- Galea of maxilla normal, as long as labrum-epipharynx . . . . . *mutata*
3. Upper facets not distinctly enlarged . . . . . *dacotensis*
- Upper facets distinctly enlarged 4
4. Clasper apical tooth very small, basistyle large irregular with an inner apodeme . . . . . .*saskatchewana*
- Clasper apical tooth well developed, basistyle stout, subquadrate . . . . . .*saileri*

Pupae

1. Respiratory filaments 12, arising from two main trunks (dorsal 7, ventral 5) . . . . . .*mutata*
- Respiratory filaments 12 or more arising from more than two trunks 2
2. Respiratory filaments 12 arising from 3 main trunks; dorsal with 4, lateral with 3 and ventral with 5 filaments . . . . . .*emergens*
- Respiratory filaments more than 12 3
3. Respiratory filaments 17-19 on very short trunks arising from a bulbous base . . . . . .*saskatchewana*
- Respiratory filaments more than 30 4
4. Respiratory filaments 30-40 in 6 or 7 main groups arising from a

short bulbous base . . . . . *dacotensis*
- Respiratory filaments 35-45 arising near base, no trunks . . *saileri*

Larvae

1. Postgenal cleft reaching base of submentum 2
- Postgenal cleft not reaching base of submentum 3
2. Postgenal cleft reaches beyond base of submentum, 57 rays in fan, 35-45 filaments in respiratory histoblast, submentum teeth very small . . . . . *saileri*
- Postgenal cleft reaches only the base of the submentum, latter with 13 blunt teeth, 17-19 filaments in respiratory histoblast . . . . . *saskatchewana*
3. Antenna shorter than the cephalic fan stalk . . . . . *dacotensis*
- Antenna long extending well beyond cephalic fan stalk 4
4. Head capsule with distinct brown spots on cephalic apotome and posterior region of gena, entire margin of postgenal cleft narrowly pigmented, submentum teeth heavily sclerotized, distal two articles of antenna darker than basal articles, eye spots normal . . *mutata*
- Head capsule with indistinct spots, postgenal cleft with lateral margins heavily pigmented, submentum teeth weakly sclerotized, eye spots reduced . . . . . *emergens*

Simulium

Adult females

1. Vein R with hairs dorsally 2
- Vein R without hairs dorsally 4
2. Postnotum with a patch of yellow hairs (recumbent scales); scape and pedicel pale brown; legs bicolored; tarsal claws bifid . . . . *aureum*
- Postnotum bare; antenna dark; claws bifid 3
3. Legs brown with distal portion of each part dark; basitarsus of fore-leg long and slender; seven to eight times as long as wide; arms of genital fork diverging from stem at a point half way of total length of fork . . . . . *pugetense*
- Legs uniformly brown; basitarsus of fore-leg short and broad: five to six times as long as wide; arms of genital fork diverging from stem at a point two-thirds the total length of fork . . . *latipes*
4. Tarsal claw with a small subbasal tooth or a basal projection . . 5
- Tarsal claw simple 9
5. Claw with a strong basal projection 6
- Claw with a small subbasal tooth 7
6. Frons and terminal abdominal segments shining; fore coxa yellow . . . . . *rugglesi*
. . . . . *transiens*
- Frons and terminal abdominal segments pollinose; fore coxa dark . . . . . *meridionale*
7. Scutum without vittae; hair on stem vein pale . . . . . *arcticum*
. . . . . *corbis*
. . . . . *malyschevi*

- Scutum with distinct dark vittae 8
8. Pale species, fore coxa yellow, tibia with white pollinose, legs bicolor . . . . . *hunteri*
- Dark species, fore coxa dark, no white pollinose on tibia . . *piperi*
9. Abdomen with distinct black and light grey pattern; fore coxa dark; precoxal bridge absent; fore tibia with conspicuous broad white patch anteriorly, extending two-thirds the length of tibia; vittae on dorsum . . . . . *vittatum*
- Abdomen without pattern 10
10. Abdomen blackish or brown 12
- Abdomen greyish-yellow 11
11. Yellowish species; scutum with orange stripes (vittae) or mesonotum with seven stripes of contrasting colors; frons and abdominal segments pollinose . . . . . *bivittatum*
- Yellowish-grey species; no vittae or stripes; frons and terminal abdominal segments pollinose . . . . . *griseum*
12. Frons and terminal abdominal tergites distinctly pollinose; anal lobe large, subquadrate but narrow dorsally and broadening ventrally, anteroventral margin rounded with a short posteroventral projection under cercus 13
- Frons and terminal abdominal tergites shining black; anal lobe not as above 14
13. Fore tibia with a very distinct patch of white pollen . . . . *decorum*
- Fore tibia with no distinct patch of white pollen . . . . . *pictipes*
14. Fore tibia with a narrow greyish-white streak on anterior surface covering not more than one-third the width of tibia; small dark species . . . . . *tuberosum*
- Fore tibia with a bright yellowish-white patch on anterior surface covering more than one-half the width of tibia 15
15. Subcosta without a row of hairs on ventral surface . . . . *luggeri*
- Subcosta with a row of hairs ventrally 16
16. Inner margin of ovipositor lobe straight, anterior margin of lobe not more sclerotized than rest of lobe . . . . . *venustum*
- Inner margin of lobe concave (with an oval space between the two lobes); anterior margin of lobe distinctly more sclerotized than rest of lobe . . . . . *verecundum*

Adult males

1. Vein R with hairs dorsally 2
- Vein R without hairs dorsally 4
2. Postscutum with a patch of appressed yellow hair; legs bicolored; ventral plate with a laterally compressed median keel . . *aureum*
- Postscutum bare; legs uniformly brown; ventral plate with no median keel 3
3. Ventral plate broad with a medial V-shaped depression . . *pugetense*
- Ventral plate broad with no depression . . . . . *latipes*
4. Clasper with 3 or more apical spines . . . . . *vittatum*
- Clasper with 2, 1 or no apical spines 5
5. Clasper with a stout spine or tubercle at base internally 6
- Clasper without spine or tubercle at base 8

6. Base of clasper with a stout spine internally . . . . . *hunteri*
. . . . . *piperi*
- Base of clasper with a distinct rounded tubercle internally . . . 7
7. Basistyle with a number of short stout spines . . . . . *tuberosum*
- Basistyle with hairs only . . . . . *rugglesi*
. . . . . *transiens*
8. Ventral plate compressed, with denticles on margins 9
- Ventral plate broadly rounded, without denticles on margins . . .
. . . . . *meridionale*
. . . . . *pictipes*
9. Ventral plate narrow (compressed), an inverted Y, with a ventral process or keel 11
- Ventral plate broad, without a ventral process or keel 10
10. Toothed (serrated) margins of ventral plate pointing outwards, visible in profile . . . . . *venustum*
- Toothed margins folded inwards, not visible in profile or dorsally.
. . . . . *verecundum*
11. Ventral keel of ventral plate setose, forming an angle before apex of median portion of plate . . . . . *decorum*
- Ventral keel of ventral plate concave in profile, the angle it forms being at apex of plate 12
12. Clasper shorter than basistyle, former flat, quadrate 13
- Clasper longer than basistyle, cylindrical 14
13. Thorax grey with greenish tinge, median area of scutum not orange
. . . . . *griseum*
- Thorax dark brown to black, with two anterior pollinose spots . .
. . . . . *bivittatum*
14. Basal arms of ventral plate each with a prong but parameral hooks are small . . . . . *malyschevi*
. . . . . *luggeri*
- Basal arms of plate without prongs, some parameral hooks large.
. 15
15. Parameral hooks consist of distinct small hooks and much larger ones, intermingled. . . . . *arcticum*
- Parameral hooks gradually lengthening towards center . . *corbis*

Pupae

1. Respiratory filaments 4 2
- Respiratory filaments more than 4 4
2. Anterior margin of cocoon with a long median projection anteriorly
. . . . . *latipes*
- Anterior margin of cocoon without a projection 3
3. Dorsal respiratory filament strongly diverging from other three. .
. . . . . *aureum*
- Dorsal respiratory filament not divergent 4
a) Respiratory filaments paired, distinctly petiolated . . . *pugetense*
b) Petioles very short . . . . . *transiens*
4. Six respiratory filaments . . . . . *tuberosum*
. . . . . *verecundum*
. . . . . *venustum*

- Respiratory filaments more than 6 5
5. Respiratory filaments 8 6
- Respiratory filaments 9 or more 9
6. Cocoon loosely woven anteriorly; respiratory filaments in more than three groups 7
- Cocoon variably thickened anteriorly; respiratory filaments in three groups 8
7. Respiratory filaments thick, in three short-petiolate pairs, plus two singly . . . . . *decorum*
- Respiratory filaments thin, in 4 petiolate pairs . . . . . *rugglesi*
8. Respiratory filaments whitish, long and slender, the dorsal and medial groups on short petioles, the ventral group on a long petiole; anterior margin of cocoon with only slightly thickened, narrow rim . . . . . *griseum*
- Respiratory filaments shorter and thicker, branching fan-like near base of short petioles; anterior margin of cocoon broader and distinctly thickened . . . . . *bivittatum*
9. Respiratory filaments 9, diverging in a semicircle from center; cocoon boot-shaped . . . . . *pictipes*
- Respiratory filaments 10 or more 10
10. Respiratory filaments 10 11
- Respiratory filaments 12 or more 12
11. Cocoon boot-shaped . . . . . *corbis*
- Cocoon with an anterior projection . . . . . *piperi*
12. Respiratory filaments 12 13
- Respiratory filaments 14 or more 14
13. Cocoon with broad collar and many openings anteriorly . *arcticum*
- Cocoon with narrow collar and one large opening anteriorly . . . . . *luggeri*
14. Respiratory filaments 14 or 16; cocoon slipper-shaped . *vittatum*
- Respiratory filaments 16; cocoon boot-shaped . . . . *malyschevi*
- Respiratory filaments more than 16 15
15. Respiratory filaments 22-26 . . . . . *meridionale*
- Respiratory filaments 100 or more . . . . . *hunteri*

Larvae

1. Anal gill with three simple lobes 2
- Anal gill with three compound lobes 4
2. Ventral papillae conspicuous, conical; head spots dark; suboesophageal ganglion and epidermis in postgenal cleft pale; respiratory filaments in histoblast 4 3
- Ventral papillae small or absent; suboesophageal ganglion and/or epidermis in postgenal cleft usually black; respiratory filaments in histoblast variable 4
3. Antenna dark and conspicuous; submentum darker than adjacent area; lateral head spots double; anal gill lobes with secondary bumps . . . . . *pugetense*
- Antenna pale; posterior half of submentum concolorous with adjacent area; dorsal head pattern consisting of longitudinal patches . . . . . *aureum*

4. Submentum with median tooth as long as corner teeth; second antennal article with a single lobed ventral pale spot; anal gill with no accessory lobes; postgenal cleft slight and rounded apically . . . . . *vittatum*
- Submentum with median tooth longer than corner teeth; second antennal article with a bilobed ventral spot; anal gill with numerous accessory lobes; postgenal cleft extending to more than half the distance to the base of submentum . . . . . *pictipes*
5. Pigmented area anteroventral to eye conspicuous, suboesophageal ganglion and epidermis in postgenal cleft pale, second antennal article more than twice as long as third, respiratory histoblast with 4 filaments . . . . . *latipes*
- No pigmented area anteroventral to eye 6
6. Suboesophageal ganglion and epidermis in postgenal cleft pale . . . . . *piperi*
- Suboesophageal ganglion and/or epidermis in postgenal cleft dark 7
7. Spots on cephalic apotome (head spots) dark; antenna shorter than cephalic fan stalk; abdomen pale yellowish-brown; respiratory histoblast with 12 filaments; postgenal cleft bulbous extending one half the distance to base of submentum . . . . . *luggeri*
- Head spots pale 8
8. Antennae longer than the cephalic fan stalk; the entire two distal articles extending beyond apex of stalk of cephalic fan 9
- Antennae shorter than cephalic fan stalk 12
9. Respiratory histoblast with 6 or 10 filaments 10
- Respiratory histoblast with 12 or 16 filaments 11
10. Respiratory histoblast with 6 filaments; postgenal cleft uniformly tapering; 43 rays in cephalic fan . . . . . *tuberosum*
- Respiratory histoblast with 10 filaments; dorsal head pattern lacking isolated spots; postgenal cleft extending to just below the base of submentum, approximately 50 rays in cephalic fan . . . . *corbis*
11. Respiratory histoblast with 12 filaments; submentum teeth equal in length, 50 rays in cephalic fan . . . . . *arcticum*
- Respiratory histoblast with 16 filaments, postgenal cleft extends to base of submentum; the submentum with long, distinct median tooth; 49 rays in cephalic fan . . . . . *malyschevi*
12. Ventral papillae conspicuous . . . . . *rugglesi*
. . . . . *transiens*
- Ventral papillae absent 13
13. Postgenal cleft extending to less than half the distance to base of submentum . . . . . *hunteri*
- Postgenal cleft extending to more than two-thirds the distance to base of submentum 14
14. Infuscation around head spots wide and extending beyond outer edge of anterolateral spots; arms of anal sclerite broadly fused medially 15
- Infuscation around head spots narrow, not extending beyond inner edge of anterolateral spots; arms of genital sclerite narrowly fused medially; respiratory histoblast with 8 filaments . . . . *decorum*

. . . . . griseum
. . . . . bivittatum

15. Lateral plates of proleg lightly sclerotized; cephalic fan with about 52 rays; anal hooks in 66 rows; postgenal cleft not bordered by a fulvous area . . . . . verecundum
Lateral plates of proleg heavily sclerotized; cephalic fan with less than 42 rays; anal hooks in about 70 rows; postgenal cleft bordered by a narrow fulvous band . . . . . venustum

HABITATS, BEHAVIOR AND LIFE HISTORIES

Alberta Simuliids in General

Eggs

Simuliid females lay eggs in running water. There is no record of oviposition in stagnant water. Cameron (1922), Edwards (1920), and O'Kane (1926) suggested that the eggs of some species of black flies (*S. arcticum* , *S. latipes* and *P. hirtipes* respectively) withstand desiccation. They may be subjected to this through water receding or the drying up of intermittent streams. Jobbins-Pomeroy (1916), Smart (1944), and Wu (1931) concluded from field observation and experimental evidence that the eggs are not resistant to desiccation. Fredeen (1959b) devised a method for the extraction, sterilization and low temperature storage of black fly eggs collected from the field. He found that the eggs of arctic and temperate species overwinter and remain viable for longer periods in storage than the eggs of those species which pass the winter as larvae.

I tested batches of eggs of *Simulium venustum* , *S. vittatum* , and *S. decorum* for desiccation resistance as follows: egg batches were obtained from the breeding places and divided into three groups. The first group was used as a control and was firmly anchored or clearly marked in the breeding site. The second group was left on filter paper in the laboratory during the test periods (48, 144, and 264 hr) and then returned to water. The third group of eggs was used as a laboratory control, covered by water in clean, open museum jars. The results are shown in table 2.

The recorded (room) relative humidity was 67-74% and the temperature was 56-69 F. The eggs of these species need water for hatching and show no resistance to desiccation.

Table 3 shows the average number of eggs laid by the females of ten species. The eggs of a batch mature at the same time but there are differences in the numbers of eggs laid within and between species. This was also recorded by Davies and Peterson (1956) for many species, in most genera. In a few species there is usually a decrease in the number of eggs in the second gonotrophic cycle. This results from degeneration of some ovarioles in which the eggs from the previous ovarian cycle were retained.

The simuliid egg is conical in ventral view and sub-triangular in lateral view. There is a bulge on one side of the egg and the side opposite that is the longest in profile.

Linear measurements were taken from the lateral view. The length

was taken as the maximum measurement along the egg axis parallel to the longest side and the width dorsoventrally and perpendicular to this (table 4). Davies (1950) and Davies and Peterson (1956) compared the dimensions of eggs of various species belonging to all the North American simuliid genera. The eggs of *Gymnopais* and *Prosimulium* were found to be larger than these of *Cnephia* and *Simulium* except for *S. pictipes*, also the eggs of *Prosimulium* and *Cnephia* are narrow and eggs of other genera are subtriangular in profile.

The angles, table 5, were measured with an eyepiece goniometer.

TABLE 2. The progress of hatching of the eggs of three simuliid species in relation to drying.

Species	Field control	Lab. control	In air at 67-74% relative humidity
S. vittatum			
48 hr	10% hatch	13% hatch	no hatch
144 hr	96% hatch	80% hatch	no hatch
264 hr	infected	infected	no hatch
S. venustum			
48 hr	48% hatch	35% hatch	no hatch
144 hr	87% hatch	88% hatch	no hatch
264 hr	100% hatch	94% hatch	no hatch
S. decorum			
48 hr	38% hatch	47% hatch	no hatch
144 hr	35% hatch	96% hatch	no hatch
264 hr	infected	infected	no hatch

Larvae

Breeding sites - The simuliid larvae were found only in rivers and creeks. The young larvae of some species aggregate at the exits of creeks from lakes and bogs. The older larvae are more evenly distributed downstream and the mature ones are in the pupation sites which are in slow water and usually near the bank.

The head waters of the Pembina and the Athabasca rivers are at an altitude of about 3800 feet and the altitude is 2000 feet at Flatbush and 1690 feet at Athabasca town.

For about 4 to 7 miles downstream from big villages and towns there was a decreased population of simuliids. I attribute this to the influence of domestic and industrial waste disposal in the rivers. The creeks may be dammed for agricultural or industrial purposes for short or long periods during the season. Although they may be dry for some time, when there is a leak or overflow they get repopulated in the same season. Beaver dams are common in the area, usually giving rise to favorable breeding sites.

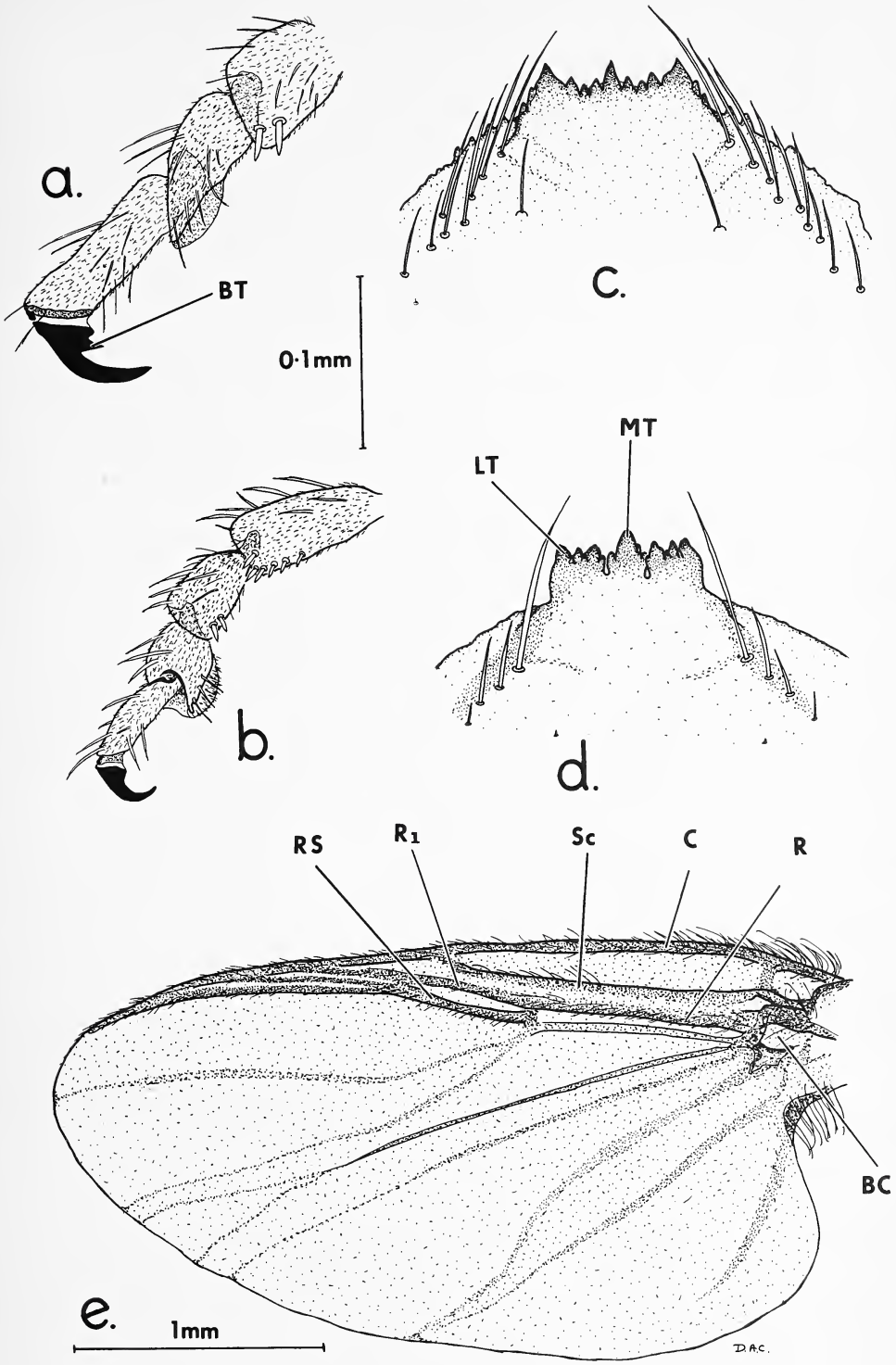

Fig. 1. a. Bifid claw of *Cnephia* sp., BT = basal tooth; b. Simple claw of *Simulium* sp.; c. Submentum of *Simulium* sp.; d. Submentum of *Prosimulium* sp., MT C median tooth, LT = lateral and corner teeth. e. Wing of *Prosimulium* sp. female, BC = basal cell.

TABLE 3. Number of eggs per gonotrophic cycle in some Simuliids, counts and published records, means ± standard deviations, number of counts in brackets, range.

Species	No. of eggs per female				Authority
	Mean	S.D.	No. of counts	Range	
S. arcticum	135	-	-	162-	Peterson 1959a
nulliparous	165	± 27.8	(22)	204-123	Count
parous	139	± 24.0	(20)	177-116	Count
S. aureum	750	-	-	823-	Davies & Peterson 1956
	745	± 13.4	(21)	810-686	Count
S. decorum	475	-	-	580-	Davies & Peterson 1956
nulliparous	550	± 51.0	(18)	594-479	Count
parous	436	± 20.7	(15)	467-411	Count
S. latipes	275	± 26.7	(16)	312-222	Count
S. luggeri	154	± 14.0	(12)	174-135	Count
S. tuberosum	202	-	-	264-	Davies & Peterson 1956
	225	-	-	--	Peterson 1959 a
nulliparous	227	± 7.4	(16)	218-234	Count
parous	196	± 17.0	(14)	212-145	Count
S. venustum	455	-	-	553-	Davies & Peterson 1956
	594	-	-	--	Hocking & Pickering 1954
nulliparous	513	± 28.7	(16)	572-465	Count
parous	481	± 24.8	(15)	511-441	Count
S. vittatum	312	-	-	395-	Davies & Peterson 1956
nulliparous	380	± 12.2	(17)	398-363	Count
parous	285	± 17.2	(16)	311-254	Count
C. dacotensis	287	-	-	348-	Davies & Peterson 1956
	281	± 17.2	(16)	288-276	Count
C. emergens	174	-	-	--	Davies & Peterson
	163	± 7.4	(20)	211-125	Count

TABLE 4. The length and width of mature eggs of some simuliid species.

Species	Length		Width		
	Mean ± S.D.	Range	Mean ± S.D.	Range	No.
S. arcticum	0.38 ± 0.033	0.32-0.44	0.25 ± 0.044	0.23-0.27	16
S. aureum	0.19 ± 0.049	0.16-0.22	0.14 ± 0.048	0.11-0.16	17
S. decorum	0.27 ± 0.052	0.23-0.29	0.16 ± 0.039	0.14-0.17	12
S. latipes	0.22 ± 0.035	0.20-0.24	0.14 ± 0.035	0.12-0.16	8
S. luggeri	0.34 ± 0.059	0.31-0.37	0.23 ± 0.035	0.21-0.255	10
S. tuberosum	0.21 ± 0.040	0.19-0.23	0.12 ± 0.036	0.10-0.14	12
S. venustum	0.25 ± 0.037	0.23-0.27	0.18 ± 0.037	0.14-0.19	19
S. vittatum	0.26 ± 0.040	0.25-0.29	0.16 ± 0.00	0.00-0.00	9
C. dacotensis	0.27 ± 0.0245	0.26-0.29	0.14 ± 0.39	0.12-0.16	15
C. emergens	0.29 ± 0.0	0.00-0.00	0.13 ± 0.0245	0.11-0.14	7

TABLE 5. Egg angles of three simuliid species measured in lateral view using eyepiece goniometer.

	Angles (degrees)					
	Head		Bulge		Third (tail)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
S. venustum	35-55	45.5 ± 5.9 (18)*	89-101	95.6 ± 4.4 (18)	41-55	49.0 ± 5.6 (18)
S. vittatum	35-58	45.3 ± 4.6 (9)	91-116	104.3 ± 9.9 (9)	42-61	51.2 ± 7.9 (9)
S. decorum	34-56	45.2 ± 8.3 (11)	88-102	95.7 ± 5.8 (11)	41-57	49.5 ± 6.8 (11)

* Number of readings, range.

The larvae of *S. arcticum* were limited in their distribution to the two rivers in the study area, albeit they have been collected from streams elsewhere (Fredeen (1958), Fredeen and Shemanchuk (1960) in southern Alberta, Peterson (1956) in Utah, and Sommerman et al. (1955) in Alaska). The current velocity was 1.5 to 5 ft/sec throughout the breeding sites except that mature larvae pupated in slower water near the banks of the rivers. The water was about three feet deep over the young larvae but only 8 to 14 inches above the pupae. Frequently pupae were exposed when the water receded; *Equisetum* stems are preferred pupation sites as the plant grows near the water edge and in the water. The water temperature ranged from 32 to 61 F during occupation by the species collectively.

Closely associated with *S. arcticum* in the Pembina River was *S. luggeri*. The latter was sparsely distributed on the same substrata although stones and rocks were utilized especially for pupation. It was collected also from the lower reaches of French Creek in similar positions.

S. tuberosum larvae aggregated on stones and vegetation exposed to the sun; the preferred current velocity was 0.6 to 3.5 ft/sec and the water depth was 3 to 18 inches. This species bred in the rivers and creeks.

S. verecundum, *S. venustum* and *S. vittatum* bred more in the creeks than in the rivers; the Pembina River usually had a bigger population of aquatic stages per square foot than the Athabasca (fig. 4). The current speed ranged from a trickle in one inch of water to about 5 ft/sec in more than three feet of water. The first two species appear when the temperature rises to 40 F.

S. decorum larvae were found to concentrate on shaded substrata in 3 to 12 inches of water and 0.5 to 1 ft/sec current. During the occupation of this species the temperature was 51 to 72 F.

The larvae of *S. aureum* were encountered only at above 50 F on trailing vegetation in streams with patchy growth in the water. The preferred current velocity was 0.5 to 2 ft/sec in 5 to 12 inches of water. The maximum temperature was 71 F.

The distribution of the larvae of *S. latipes* indicated a discontinuity in the rivers and abundance in the creeks. The larvae were attached to the vegetation, sticks and pebbles in the *S. aureum* breeding sites except that the temperature requirements were more eurythermic (starting from 40 F).

The larvae of *C. dacotensis* and *C. emergens* were collected from vegetation, logs, and bottom stones in 1.5 to 2.5 ft/sec current speed in one to three feet of water. The temperature was 38 to 51 F. The overwintered larvae of *C. mutata* were attached to the bottom pebbles and sticks in the same conditions.

Prosimulium decemarticulatum was well distributed in Irish and Flatbush Creeks. The larval attachment substrata were dead or trailing vegetation, sticks, stones, and logs under 4 to 13 inches of water and 0.5 to 2 ft/sec of current. The water temperature reached 36 F before the larvae appeared.

Population density assessment - Two methods were employed in determining population densities. The first method was the direct counting of the numbers of larvae in a square foot of the river bottom. Two weekly col-

lecting stations were selected in the Pembina River, one in the Athabasca River and one in each creek. Each station was about three miles long. A wooden frame was applied to a selected area (with suitable attachment substrata) delimiting one square foot of the bottom and the substrata were investigated (average 8 ft^2 counts). Bottom pebbles, debris, vegetation, sticks, stones, rocks and logs constituted the substrata (the last two usually encompassing more than one square foot). This method has been used in ecological studies and control assessments. Arnason et al. (1949), Anderson and Dicke (1960), Fredeen et al. (1953), Jamnback and Eabry (1962), Metcalf (1932) and Sommerman et al. (1955), and Wolfe and Peterson (1959) used a five minute stone count in selected areas.

The second method was based on artificial attachment sites. These consisted of hollow, white matt surfaced polystyrene-butadiene rubber ("high impact polystyrene") cones, 0.01 inch final thickness, 20 cm long, with a 10 cm base diameter and 30° apex, vacuum formed from 0.03" sheet by Spencer-Lemaire Plastics of Edmonton. One set of these was freely suspended by wire through the apices to wooden spikes fixed in the collection stations. Depth adjustment was attained by using cork or lead weights. Another set was fixed with the apices pointing upstream by fixing them to pegs through holes in the spikes. A similar method was employed by Peterson and Wolfe (1958), Phelps and DeFoliart (1964) and West et al. (1960).

In every collecting station there were control cones which were not changed during the season; but the other were checked and the larvae removed for counting every week.

Although the projected area of the cone is only approximately 1/9th of a square foot, the number of larvae on a trailing cone was subequal to the number of larvae in a square foot of bottom. This may be due to attraction of simuliid larvae to bright objects or perhaps to the nature or shape or movement of the surfaces. This is supported by the finding that cones painted yellow, brown, red, green or blue yielded less larvae than the white cones (table 6). The relative brightness of the cones to insects was estimated for me by Mr. Peter Kevan who photographed them by diffuse daylight through a quartz lens. Except for yellow, a fairly good direct relationship between brightness and numbers of larvae attaching is seen. Most measurements of spectral sensitivity in insects indicated low sensitivity in the yellow (Dethier 1963). Similar trends were observed by Wolfe and Peterson (1959) using painted parts of a spruce log.

The fixed cones gave very low numbers at all depths, the freely suspended cones had an optimum depth between 2 and 11 inches with a roughly inverse relationship between turbidity and optimum depth.

Peterson and Wolfe (1960) interpreted the population graph obtained in their study as representing three generations of all the species encountered in the period June to July with a small peak in May which resulted from the overwintered larvae and egg hatch (of all species) in spring. In my study area there was overlapping of the generations of one and all species whenever they were associated. This resulted in peaks in the population graphs (figs. 2-7) corresponding to the life histories of more

than one species in any station. For ease of comparison data have been adjusted in such a way as to synchronise the date of ice break-up.

TABLE 6. Numbers of simuliid larvae on plastic cones of different colors at different depths in the Pembina River, weekly collections, July and August 1965.

Depth in inches:-			2	4	6	8	10	12	14	16
	Relative brightness		Average number of larvae per cone/week (5 counts)							
Color	Visual	Visual + UV								
white	50	10	147	181	386	634	943	858	900	154
yellow	30	7	8	14	11	8	-	-	-	-
green	10	5	17	57	48	31	31	60	94	46
brown	9	1	7	11	13	7	-	-	-	-
red	8	3	11	33	21	9	-	-	-	-
blue	7	2	13	34	59	43	76	87	88	97

TABLE 7. Average numbers of simuliid larvae picked up per cone in six hour period (in the Pembina River, July and August 1965).

		Time								
		6 to	12 to	18 to	00 to	Moon			Sun	
Date	Means	12	18	24	6	rise	set	phase	rise	set
July 22	383	31	17	101	234	23:31	13:39	last $\frac{1}{4}$	4:00	20:23
July 25	371	31	17	99	224	0:20	17:52	last $\frac{1}{4}$	4:00	20:23
July 27	340	21	14	84	221	2:02	20:04	none	4:08	20:04
Aug. 2	280	18	13	77	172	10:48	22.17	1st $\frac{1}{4}$	4:16	19:55
Aug. 5	219	13	10	76	120	14:45	23:07	1st $\frac{1}{4}$	4:16	19:55

The spring peak depends on the abundance of the overwintered larvae and eggs and the effect of winter on both stages. The high water of spring flood is of great advantage to the larvae as more substrata and organic drift are provided by the invasion of new areas (Anderson and Dicke (1960), Carlsson (1962 & 1966), Fredeen and Shemanchuk (1960), Peterson and Wolfe (1960), and Phillipson (1956 & 1957). There are adverse

effects of the high water as some of the larvae may be washed away and perish and there may be depositions of silt over the substrata.

The summer peak (or peaks) may result from the combined presence of the first generation larvae of those simuliid species with high temperature requirements, successive ovipositions of some species in the first generation and influx of larvae of the second generation. This may be prolonged until August and overlap the small fall generation.

Anderson and Dicke (1960) recorded 2000 to 4000 larvae/ft^2 (300 to 800 larvae on grass blade 0.5" wide and 6" long). Metcalf (1932) recorded 2880 and 1500 larvae on two rocks and 300 larvae/ft^2. Peterson and Wolfe (1960) reported 2400 larvae/cone.

According to Hocking and Pickering (1954) the current gradient is important in the positioning of larvae on the substrata after the larvae attach. The fact that the cones suited the requirements of larvae is illustrated by the high catch recorded.

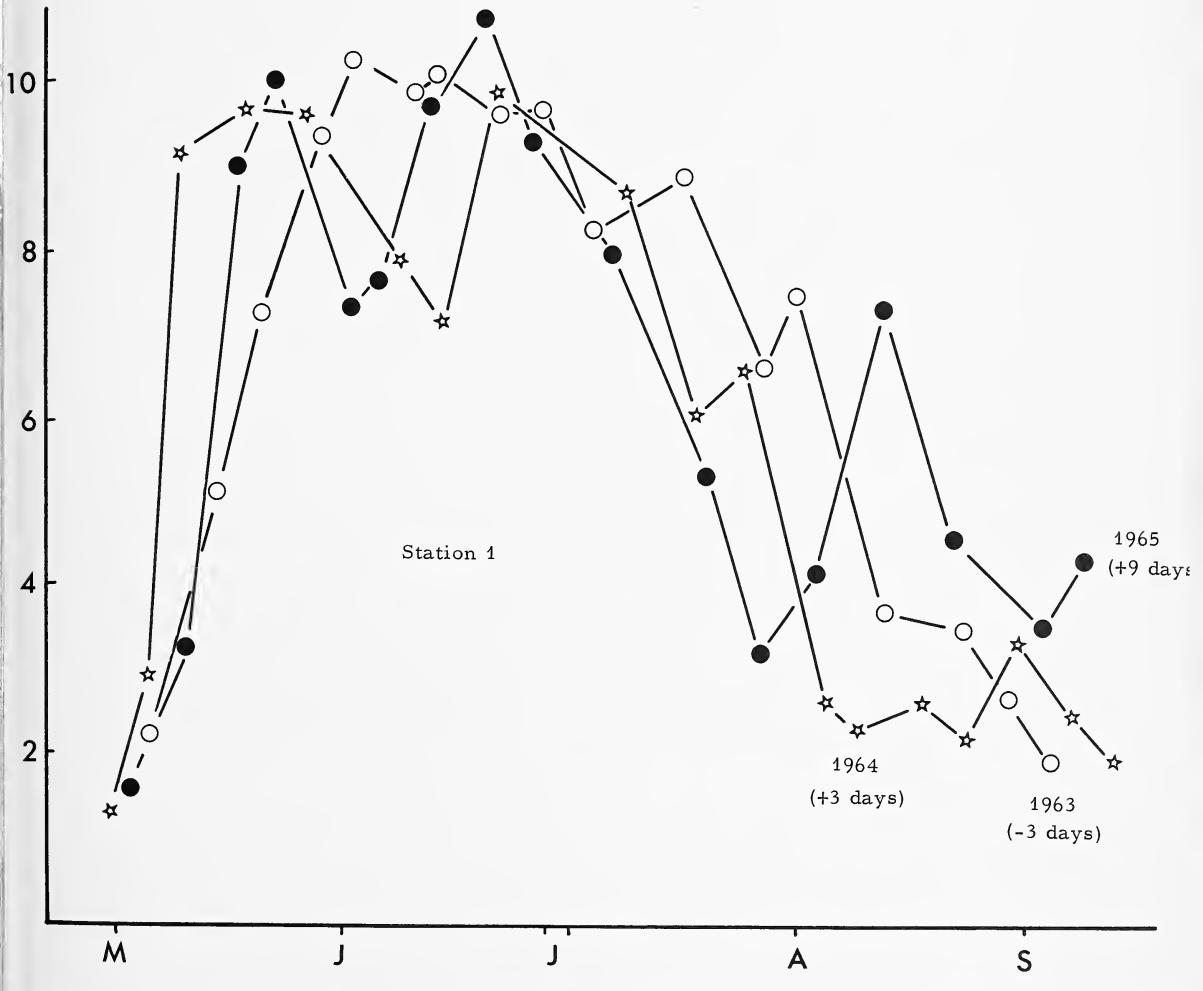

Fig. 2. Total population densities of simuliid larvae in the Pembina River : May to September 1963-1965 (adjusted for dates of ice break-up).

Larval migration - The adult females fly upstream and oviposit, their eggs hatch and the larvae accumulate in these oviposition sites for short periods. They are passively carried downstream by the current. These young larvae were reported by Peterson and Wolfe (1960) to secrete silk threads to suspend themselves in the current. The larvae populate all the favorable breeding sites in the streams and the mature larvae migrate to slower water to pupate.

Rubtzov (1939) concluded from his studies on the migration of simuliid larvae that they migrate downstream continuously during the night and settle on different substrata in the day. He attributed the migration to the decrease in current velocity in one site inducing the larvae to seek higher velocity levels. Radzivilovskaya (1950) reported the larvae migrating during the day and settling during the night and attributed this to lowered oxygen content at the attachment site. Peterson and Wolfe (1960) reported the larvae migrating during the night and settling during the day. Yakuba (1959) suggested that migration may be stimulated by the rapid rise in water level.

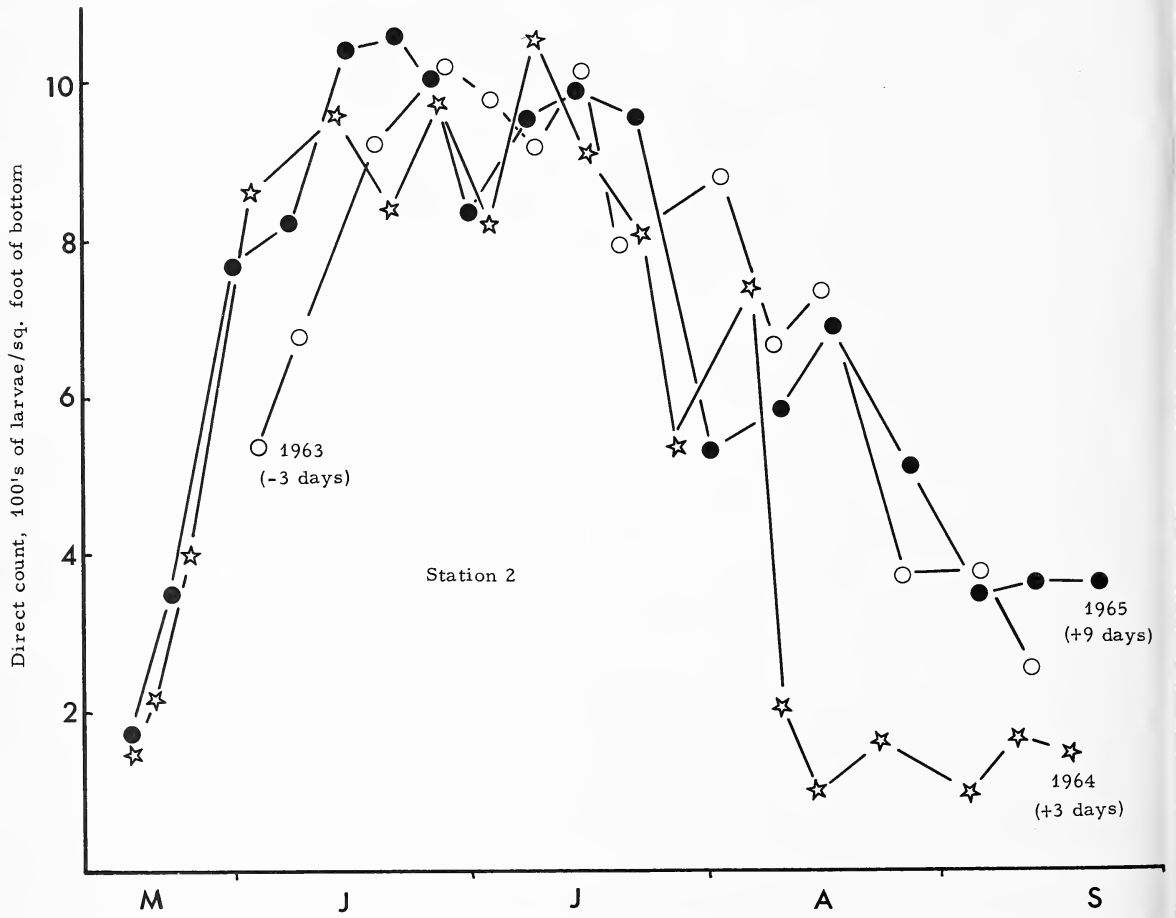

Fig. 3. Total population densities of simuliid larvae in the Pembina River : May to September 1963-1965 (adjusted for dates of ice break-up).

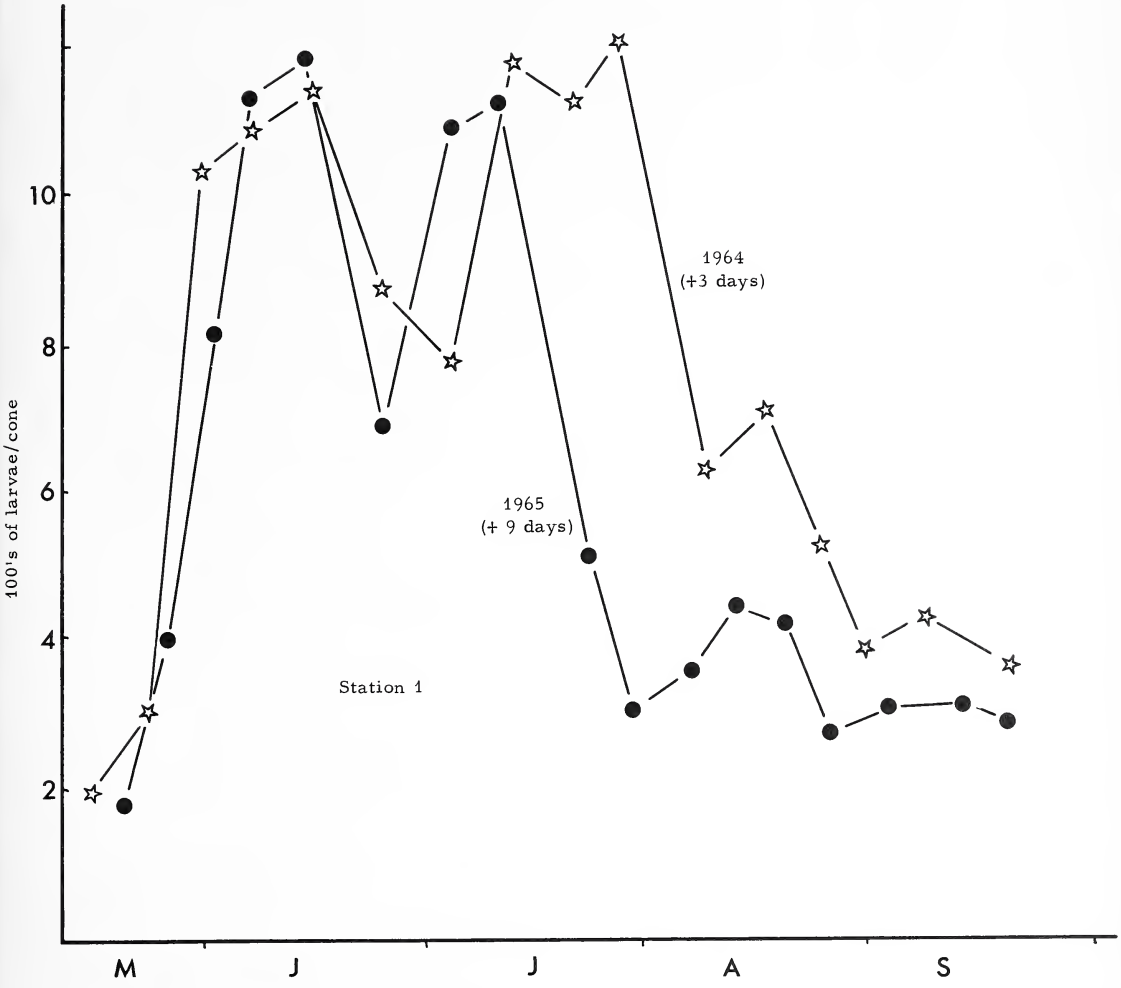

Fig. 4. Total population densities of simuliid larvae in the Pembina River : May to September 1964-1965 (adjusted for dates of ice break-up).

The total numbers attaching decreased with the advancing season in a comparable manner to the total larval density (fig. 2). Attachment, and hence probably numbers migrating downstream seems to be greatest when light intensity is increasing, and to be restricted by the very low light intensity when there is neither moon nor sun. Perhaps decreasing light intensity invokes release, and increasing light, attachment. They may be dislodged by other larvae, drifting objects or as a result of the movement of the attachment object.

Flatbush Creek was dammed in July 1963 and the bottom of the stream below the dam was dry. No eggs were found in June 1964, but the water overflowed the dam in July and continued to flow up to the end of the season. The stream was repopulated by simuliids in July and this was mainly done by females ovipositing below the dam and by larvae migrating from above the dam. In 1965 the simuliid populations in this creek were as high as in other creeks and the species of simuliids were: *C. mutata* , *P. decemarticulatum* , *S. venustum* , *S. vittatum* , *S. latipes* , and *S. aureum* .

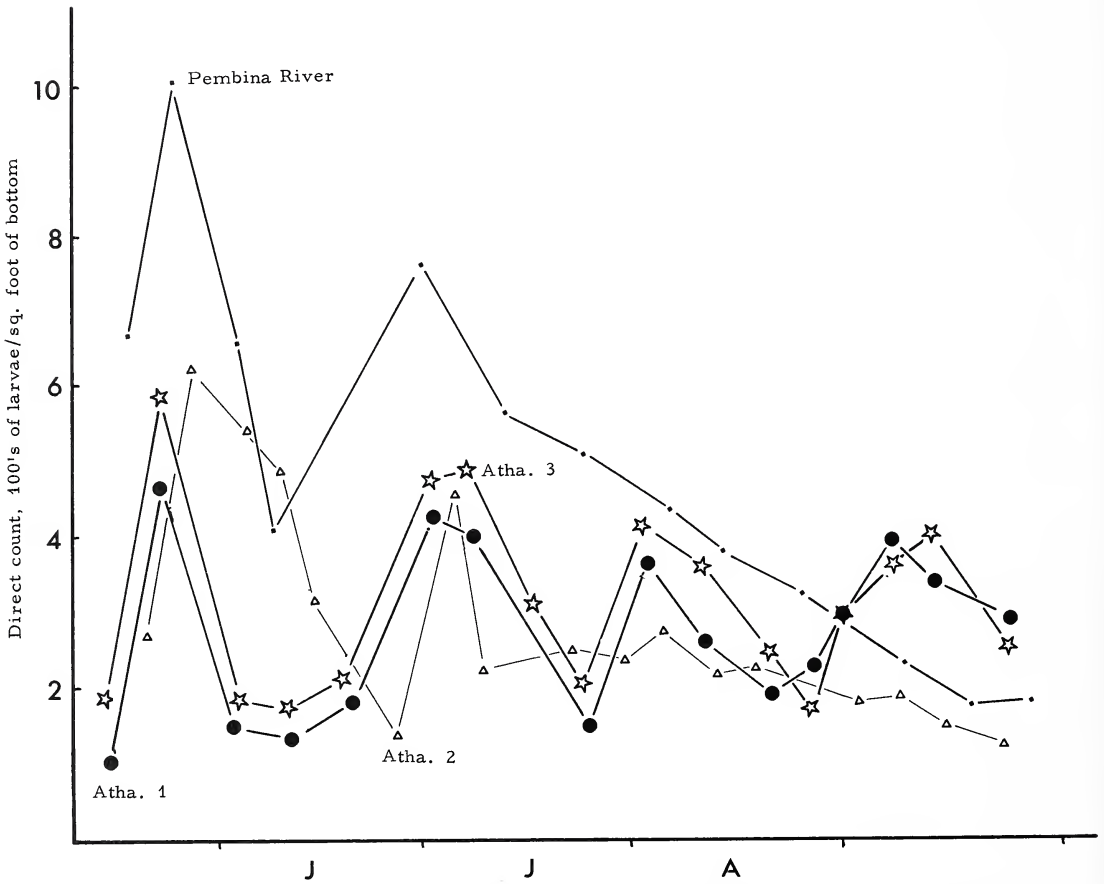

Fig. 5. Total population densities of *S. arcticum* larvae in the Athabasca (3 sites) and Pembina Rivers, 1965.

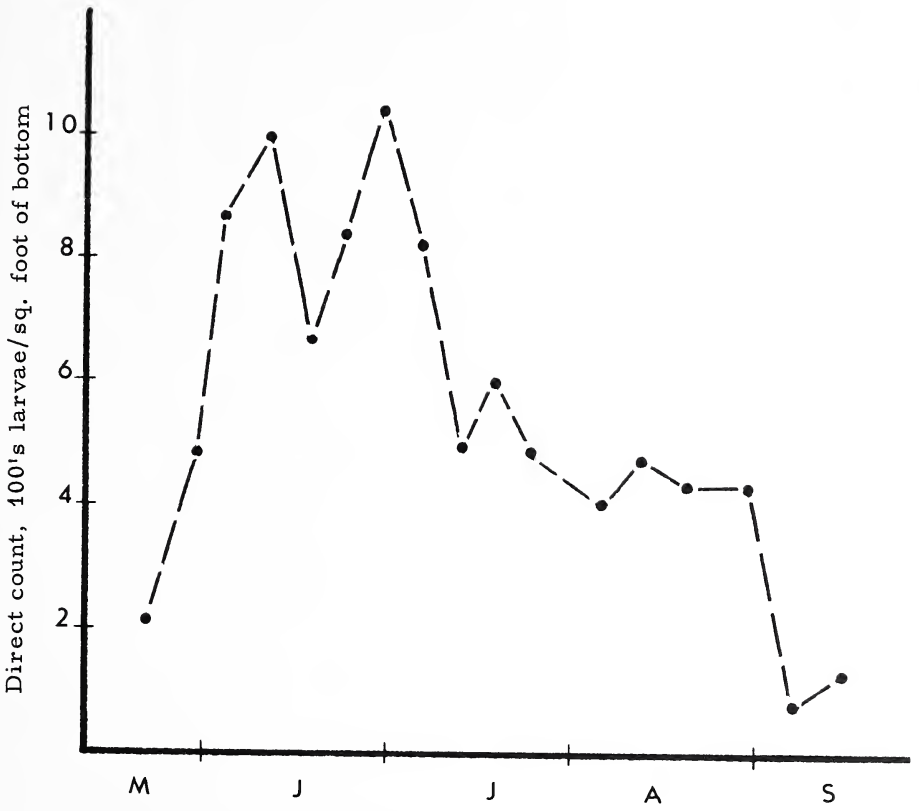

Fig. 6. Total population densities of simuliid larvae in French Creek, 1964.

Laboratory Rearing

Laboratory rearing of simuliid larvae was attempted by Fredeen (1959a), Hocking and Pickering (1954), Mackerras and Mackerras (1948), Puri (1925), Smart (1934), Dalmat (1955), Wood and Davies (1965) and Wu (1931). Only *Boophthora erythrocephala* de Geer has been reared from the pupa through two generations (Wenk 1963).

The methods employed were compressed air to circulate the water in breeding jars, using stone or fritted glass air breakers, and the movement of water: by the shaking or rotation of a platform carrying the breeding jars, by flow through tanks and troughs or by propulsion with propellers. The water used was chlorinated tap water, from aquarium tanks rich in algae or stream water. Rubtzov (1956a) suggested that the maintenance of larvae in the laboratory may be facilitated by rearing them in water rich in micro-organisms. This was attempted by some workers; by supplying bakers' yeast, powdered skim milk and bacteria. At optimum temperature, oxygen saturation and current velocity, food was not an important problem in the laboratory rearing of simuliids.

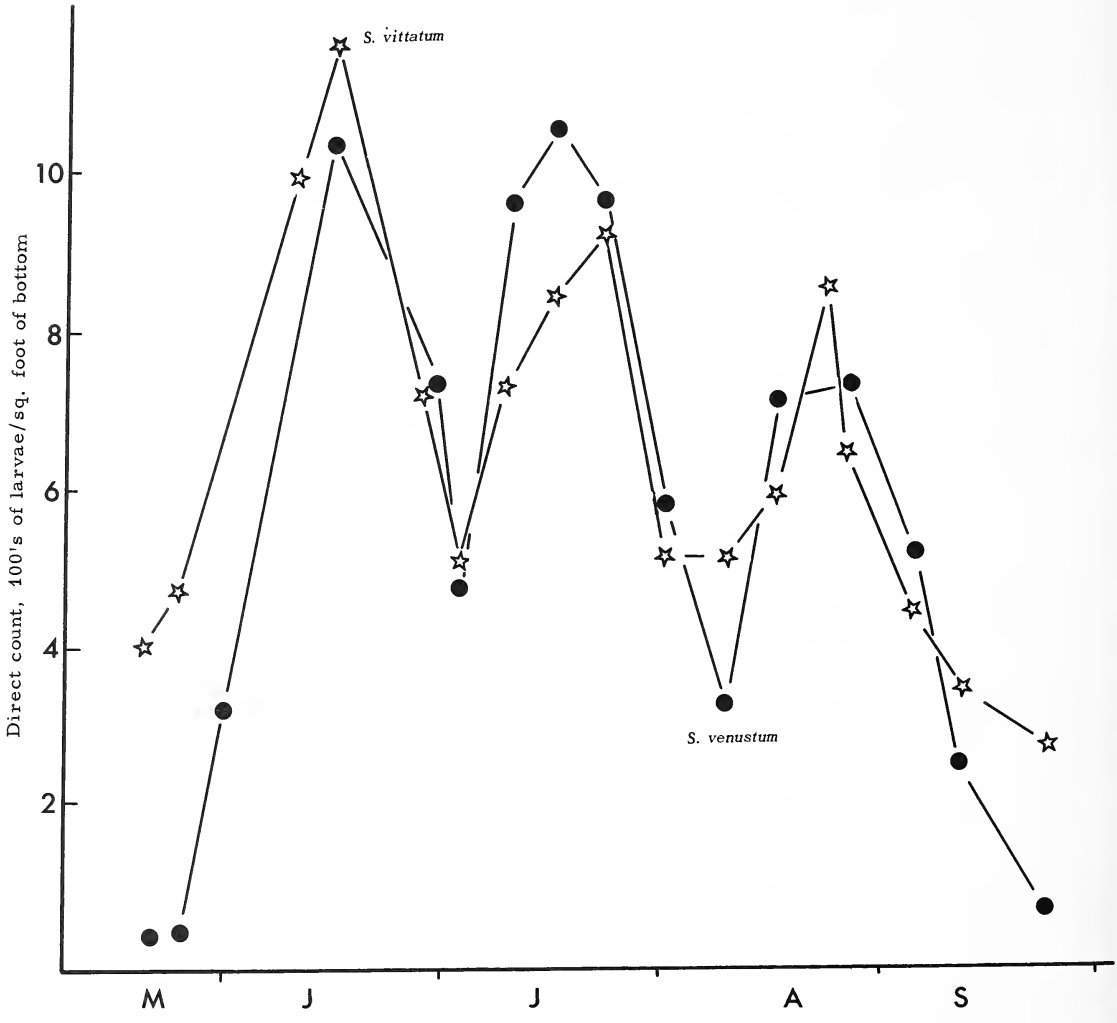

Fig. 7. Total population densities of *S. vittatum* and *S. venustum* larvae in Chisholm Creek, 1964.

In the present study two methods were successfully utilized in the study of life histories and other investigations. Compressed air was bubbled into breeding bowls and museum jars. This method was found satisfactory in raising early stages of *S. venustum*, *S. vittatum*, and *S. decorum* and for insecticide susceptibility tests. Larvae were induced to attach on glass plates facilitating the change of water. As Hocking and Pickering (1954) suggested, autointoxication may be responsible for the mortality observed in jars after four to six days when the water was not changed. 15% to 30% mortality was recorded in 500 cc jars with less than 75 larvae; 40 to 80% in jars with more than 75 larvae. The average used was 250 larvae per 1500 cc bowl with the mortality increasing with increase in the number of larvae.

Two acrylic plastic troughs (described under DDT susceptibility tests) were used in rearing efforts. These simulated breeding conditions in streams and the young larvae from the jars were transferred to the troughs and easily reared to pupae. Water was collected from breeding sites and bakers' yeast was the only food added. The larvae of *S. venustum* were observed to scrape the aggregated yeast cells off the walls of the containers. Algal growth and other microorganisms in the water were not removed.

Field investigations of nutrition were conducted by Anderson and Dicke (1960), Davies and Syme (1958), Hocking and Pickering (1954), Fredeen (1958 and 1964b) and Peterson (1956). The larval gut contents yielded soil particles (sometimes 100%), organic debris : diatoms, algal filaments, spores, pollen grains, pieces of green and decayed vegetation and chitinous pieces of invertebrate body.

In the laboratory the larvae filled their guts with yeast cells, phytoplankton and other inorganic particulate matter. Starved mature larvae emptied their guts in 9 to 17 days; 67% pupated and 12% survived for 21 days without filling their guts again. Larvae of *S. vittatum* without distinct visible histoblasts took 4 to 7 days to empty their guts and survived for 11 to 18 days.

The feeding process was as described by Hocking and Pickering (1954), Peterson (1956) and Osborn (1896). Internal fluid pressure and the current may be responsible for extending the fans. The fans were closed (drawn towards the mouth) two to 23 times in a minute. The secondary fan filaments may be employed to decrease the area between the filaments of the primary fan, thus enabling the larva to strain out smaller particles, e.g., yeast cells.

Puri (1925), Peterson (1956), and Wu (1931) described cocoon spinning. The observed procedure agrees with previous descriptions except that the larvae of *S. venustum*, *S. vittatum*, and *S. decorum* took more than 60 minutes to finish the cocoon in the laboratory.

Pupae

Pupae of *P. decemarticulatum* were recovered mainly from the bottom sand of Irish Creek and in the laboratory the mature larvae pupated in the bottom of the rearing bowls where they spun loose cocoons. Pupal aggregates were encountered in vegetation, rocks, and other substrata located in slow and shallow water. Peterson (1959b) suggested that there

may be a positive thigmotropic response facilitating the pupal development. Stranded pupae of *S. arcticum* , *S. decorum* , *S. venustum* , and *S. vittatum* were observed exposed as a result of a drop in water level. The pupal mass was kept moist by the fine spray from the water splash but when dried the pupae perished.

Carlsson (1966) indicated that each species has a certain pupal optimum temperature but the range between the maximum and minimum developmental temperatures is broad. In the laboratory I noticed that the duration of the pupal stage of *S. vittatum* , *S. venustum* , *S. decorum* , and *S. arcticum* (obtained from the same batch of mature larvae of each species) took from three to eleven days, and that the position of the pupa in the mass did not influence its duration; but as in the field the emerging adult may be trapped under the pupal mass and perish. Organic and inorganic drift sediment piling above the pupal mass may interfere with the emergence of the adult.

Rubtzov (1956b) indicated that there is a correlation between the number of pupal respiratory filaments and the character of the stream in relation to oxygen supply, i. e., pupae in swift water have less filaments than those in slow current. I found that *S. latipes* with four filaments breeds in the same stream as *C. dacotensis* which has numerous filaments, *S. vittatum* with 16 filaments and *S. venustum* with 6 filaments. Pupae kept in vials with moist cotton pads matured in less time than others of the same group left in the breeding troughs.

The adult emerged through a longitudinal split on the dorsal surface of the pupal skin pushing its thorax out first followed by the head and swiftly rising in a bubble of gas to the surface where it took off or was carried by the current to a near support.

Adults

Various methods have been used in studying populations of adult simuliids. Davies (1950), Davies and Syme (1958), Hocking and Richards (1952) and Ide (1940) used emergence traps. Light traps were employed by Davies (1955), Davies and Williams (1962), Fredeen (1961) and Williams (1948). The baited trap was preferred by Anderson and DeFoliart (1961), Bennett (1960) and Fallis (1964). Fredeen (1961) utilized "silhouette traps" which consisted of wooden frames in shape of animals (cow, sheep and horse) and covered by cloth of appropriate color to match the animal it represented. Hocking and Richards (1952) and Davies (1961, 1963) applied sweep-netting and fly-boy-hour counts. The latter method was recommended by the World Health Organization in relation to control.

I used a light trap (ultraviolet) in the period July to September 1963 and June to August 1964, at the field station (4 miles from the river); the total catch was: 91 female *S. venustum* , 49 female *S. decorum* , 36 female and 24 male *S. arcticum* and 18 female *S. latipes*. This represents a low yield compared to some records by the above workers.

Nylon gauze and paper coated with castor oil and sticky traps (tanglefoot and fly paper) were hung near bird nests and on vegetation near the breeding sites. The adults caught were utilized in the life histories studies. Quantitative study of the abundance of adult simuliids was attempted by sweep netting of flying, feeding, and resting flies; the average

number of flies in ten sweeps is plotted in figure 8.

The diurnal activity pattern consisted of a peak of activity two hours after sunrise and a peak starting from two hours before sunset and continuing until after dark. The latter peak was more conspicuous as it consisted of many species and more individuals than the former.

The emergence pattern of the adults was investigated in the laboratory and the field using emergence cages (20 mesh per inch nylon screens). The investigation was on *S. vittatum* in July 1965; approximately 100 pupae were selected from the breeding site for each period (two hour observation); to obtain pupae of similar age dark colored individuals were used. In general agreement with the published data, the observed adult emergence in the field was between 5:30 AM and 1:30 PM. In the laboratory it commenced two hours before sunrise and reached a climax at 9:00 AM but continued throughout the rest of the day in a random pattern (fig. 8).

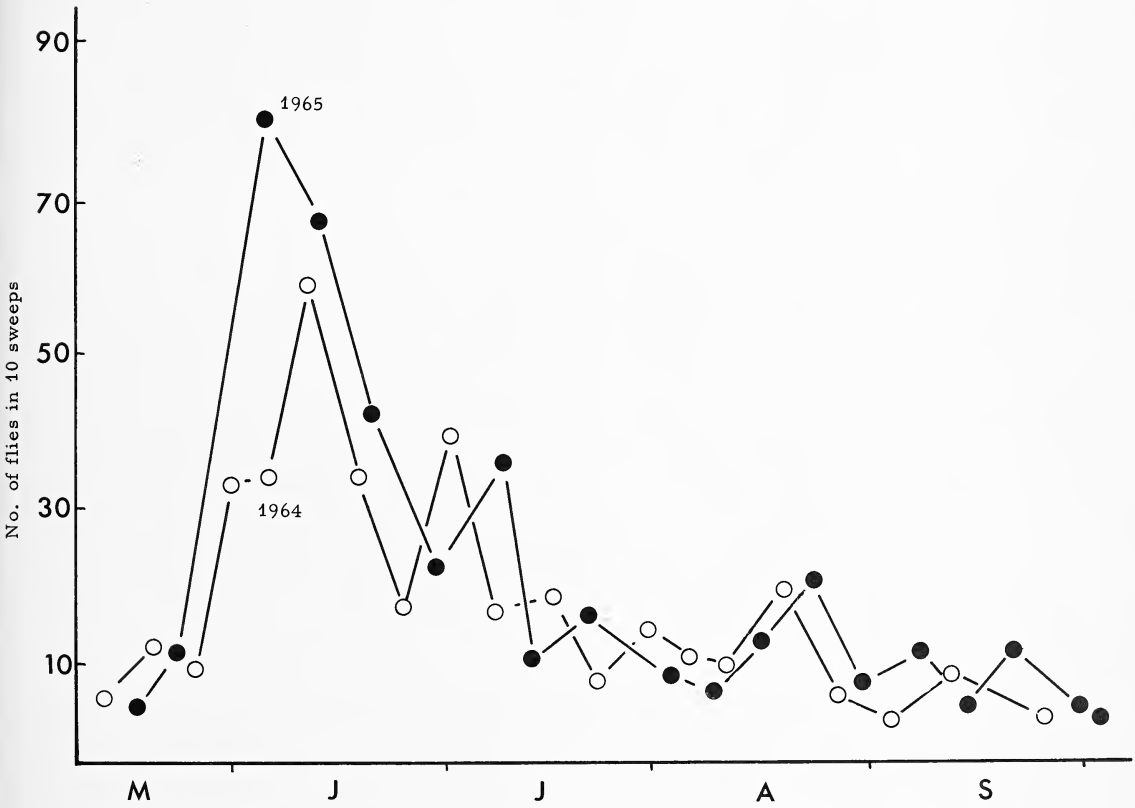

Fig. 8. Populations of adult simuliids, all species (sweep-netting) Flatbush, 1964 and 1965.

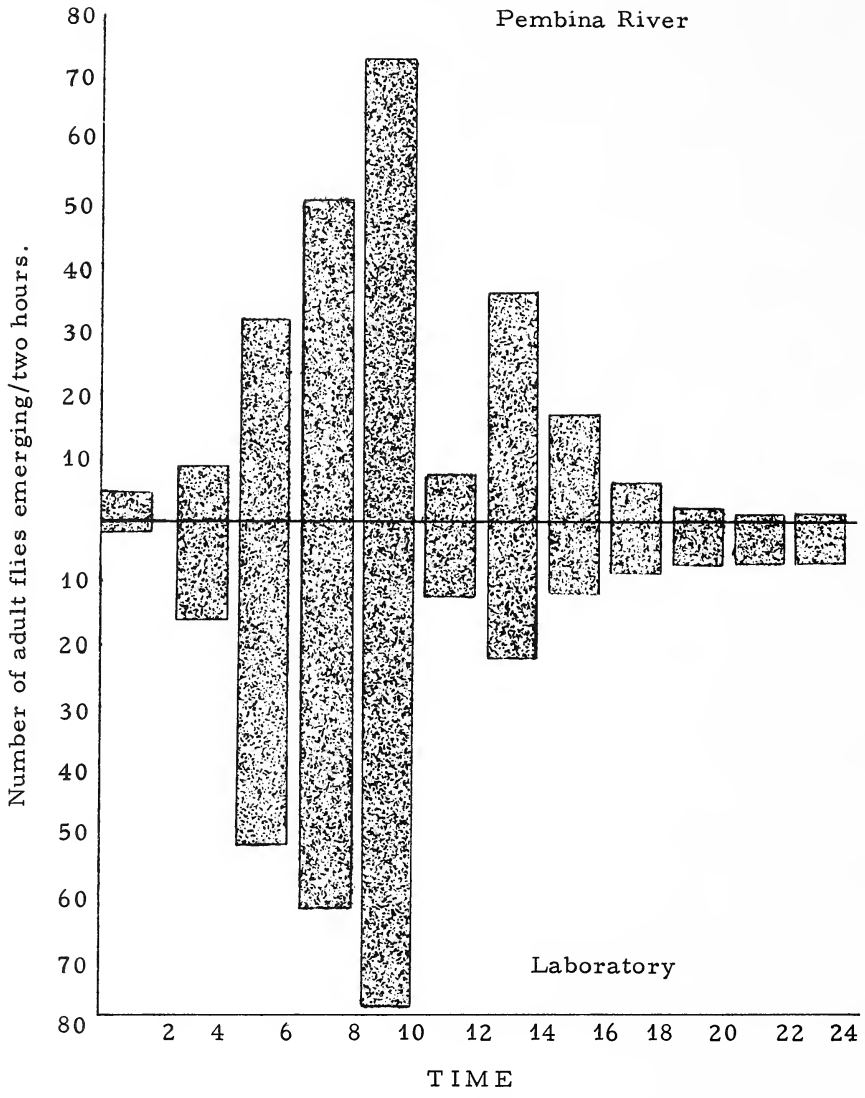

Fig. 9. Adult *S. vittatum* emergence pattern, July 1965.

Species and their Life Histories

Prosimulium (P.) decemarticulatum

This ornithophilic species was collected in 1965 from Irish and Flatbush creeks. The larvae were abundant in May but not before that, indicating that the species passes the winter as eggs. Mature larvae and pupae were taken on June 17. The first adult (a male) emerged in the laboratory on June 24, the females emerged on June 26. In the field a net collection on June 27 yielded only males, later collection yielded both sexes. The females showed no maturation, but were fertilized and most of them had fed on blood. These blood fed females matured their eggs in six days in the laboratory. A few parous females were also collected indicating that the females completed more than one gonotrophic cycle. After July 20 the aquatic stages were encountered singly or in a very random pattern. Similar data were reported by Anderson and Dicke (1960) from Wisconsin. Davies (1950) and Davies et al. (1962) from Ontario, and Sommerman et al. (1955) from Alaska.

Although females did not feed on sparrows in captivity most of the trap collections were from near nests of birds. Females were aspirated from young sparrow chicks in the nests. This is in accordance with the reported host preference for the species (Bennett 1960, Davies and Peterson 1956, and Davies et al. 1962).

Prosimulium (P.) onychodactylum

Three larvae and two pupae of this species were collected in July 1965 and four females and one male were collected on October 2-4, 1964. Except for *P. travisi* this was the rarest species in the study area. Aquatic stages were collected only from the Athabasca River in the Hinton area, above 3000 ft. Both sexes were attracted to the collector but neither landed nor fed on him. Two dissected females contained well-developed ovaries, i.e. the eggs more than half mature.

Sommerman et al. (1955) reported this species to overwinter in the eggs in Alaska and to have one generation annually extending from April to September. Peterson (1959b) collected only larvae in Utah and suggested that the species has one generation a year there.

Prosimulium (P.) travisi

On October 4 in the Hinton area a single male of *P. travisi* was collected in a net sweep from the vegetation on the bank of the Athabasca River, together with six females of *S. arcticum*. Neither aquatic stages nor females of this species were encountered.

Sommerman et al. (1955) collected *P. travisi* from July to September. It was suggested that it has one generation a year with the eggs hatching in June.

Cnephia (S.) mutata

Basrur and Rothfels (1959) discovered that the populations of *C. mutata* in southern Ontario contain bisexual diploid forms and parthenogenetic triploid forms (females only). Davies (1950), Davies and Peterson (1956),

and Davies et al. (1962) found that 90-100% of the individuals collected in Ontario were triploid females.

Basrur and Rothfels (1959) reported the species as univoltine in Peel County; the eggs of the diploid form hatch in January to February and the triploid form then dominates from mid-April to May.

Anderson and Dicke (1960) found that this species passes the winter as eggs in southern Wisconsin and as larvae in the north.

Davies (1950) collected males and females of the diploid form of the species emerging in mid-May and the peak of emergence of the triploid was in late May extending into June (with an odd female collected in August). This attenuated emergence led Basrur and Rothfels to suggest two generations for this species in southern Ontario.

In the study area larvae of *C. mutata* were collected after the ice break-up. They commenced to pupate on May 14 (1965). The females emerged ten to fifteen days later with their ovaries well-developed, containing between 190-250 eggs three-fourths mature. These eggs took five to seven days to mature in the laboratory, without fertilization, the females being fed on water and sugar crystals. In the field unfertilized females were collected feeding on horses and to a lesser extent on cows. These females were examined by me and showed indications of a previous ovarian cycle. There were mature eggs in one or both ovaries intermixed with very immature eggs and plenty of follicular relics.

Fallis (1964) quoted various authors reporting *C. mutata* feeding on deer, hare, cow, and man (ear).

On one occasion an ovipositing female was observed flying low over the surface of water against the current and tapping the water with the tip of its abdomen, it finally fell into the water.

There were no collections of adults or aquatic stages after June.

Cnephia (C.) dacotensis

C. dacotensis is univoltine and fully autogenuous species. It breeds in Irish Creek and was not collected from any other locality. The eggs hatched after the ice break-up and the larvae appeared in May and commenced to pupate in May 17-20. The first male was collected on May 20 and the females two days later. In the laboratory the females raised from pupae showed the eggs almost three-quarters mature. The females fed on water and sugar, matured their eggs in four to six days. In the field females netted from the vegetation on May 24 were all fertilized, their eggs three-fourths mature and the abdomens distended with liquid in the stomach, diverticula and oesophagus. Mating took place on rocks and stones near the water edge occupying from less than a minute to three minutes. No fertilization plugs or spermatophores were detected.

The females oviposited while flying low over the water. The total number of eggs in their ovaries was 276 to 288. Gravid females netted from the vegetation showed indications of partial oviposition, i.e. 20 to 30 ovarioles extended although the eggs were in the same degree of maturation.

The mouthparts of the female were reported as weak, reduced and incapable of feeding (Krafchick 1943, Peterson and Wolfe 1960. Nicholson 1945 and Stone 1964). Twinn (1936), Davies (1950) and Davies and Pet-

erson (1956) compared the size of the male eye with other species and concluded that the eyes are reduced and there is a lack of phototaxis rendering this species incapable of forming a mating swarm. In the present study no feeding or blood engorged females were encountered.

The last date on which the aquatic stages were seen was June 29; although Fredeen (1961 in Davies et al. 1962) reported some eggs hatching in autumn.

My findings are in accordance with the reports of Anderson and Dicke (1960), Davies (1950), Davies and Peterson (1956), Davies et al. (1962) and Nicholson and Mickel (1950), Sommerman et al. (1955), Stone (1964) and Stone and Jamnback (1955).

Cnephia (C.) emergens

C. emergens was taken as larvae and pupae on May 27 and June 10 respectively from Irish and Flatbush creeks. As no overwintered larvae were detected it is considered to pass the winter in the egg. No females were taken in the field but laboratory raised females contained half mature eggs. The eggs ranged from 125 to 211 per female. The females fed on water and sugar crystals but not on a sparrow, a cat or my arm. In describing the species Stone (1952) pointed out the reduction in armament of the mandibles and maxillae which indicated inability to feed on blood. The same condition was reported by Davies and Peterson (1956) and Peterson and Wolfe (1958). Davies et al. (1962) and Sommerman et al. (1955) found a single generation annually, which I confirm.

Simulium (G.) arcticum

This species is the cattle pest of western Canada. Serious outbreaks have been reported since 1912 in Saskatchewan (Arnason et al. 1949, Cameron 1918 and 1922, Curtis 1954, Fredeen 1958 and 1960, Fredeen et al. 1951 and 1953, Hearle 1932 and Rempel and Arnason 1947). The life history and control measures were studied in detail by these authors.

On no occasion were the eggs of this species collected from the breeding sites although presumed ovipositing females were captured in several instances from the Athabasca and less frequently the Pembina rivers. Ovipositing females were seen flying over the breeding sites which were mainly rapids and ripples in the rivers. They lay the eggs singly and they do not dive under water to lay (Cameron 1922 and Fredeen 1958 and 1960). The eggs are scattered in the bottom of the river but my efforts to recover them by brine flotation were not successful. Females captured while ovipositing were kept alive in vials in the laboratory for four days; they did not oviposit and the dissected eggs did not hatch.

Larvae were collected on May 10 1964 and May 11 1965. They accumulate in favorable sites within the breeding localities. They attach to pebbles, stones, rocks and vegetation, although the first three are more abundant in these breeding stations than vegetation it was noticed that there was an aggregation of larvae in the different instars on particular substrates, i.e. up to the third instar larvae are found on smaller pebbles and stones, later instars on stones and rocks, and mature larvae and pupae on rocks and vegetation in slower flowing water. This

indicates a definite migration pattern. Larvae and pupae were collected from the two rivers only although in the southern parts of the province they have been collected from the tributaries of the South Saskatchewan River and from irrigation canals (Fredeen 1958 and 1960).

The data indicate that there are four generations per year with an obvious overlapping of successive generations (as indicated by larval density assessment, fig. 5). The species overwinters in the egg stage as no larvae were collected from under the ice and because the larvae appear after the ice break-up in April. These newly hatched larvae reach maturity at different times in the period from April 20 to May 19 (1964-1965). Pupation of these larvae commences on May 28 and the adult emerges in six to ten days. In the field males were collected two days before the first females were encountered. The overall ratio of females to males was 1 : 4 near the breeding sites and 16 : 1 near grazing cattle and horses; random samples of pupae in the laboratory yielded a 1 : 1 ratio. Average date of pupation for the first complete summer generation is July 1, second generation August 2, the last seasonal generation September 1.

Females emerging from the overwintering eggs have their ovaries well-developed indicating an autogenous condition: thus this species fits into Rubtzov's (1955, 1956a, and 1958) category of "facultative blood suckers" which includes all the species of blood sucking groups that utilize the larval food reserves for the development of the first batch of eggs. Fredeen (1963) and Fredeen et al. (1951) observed this fact and were able to determine the number of previous ovarian cycles using the criterion of the follicular relics. Females that seek a blood meal have mated, oviposited, and usually have fluid in their crops sweet to taste and giving a positive reaction with Benedict solution. In the present study females feeding on cattle and horses were dissected and the ovaries were noticed to be empty except for a few (1-3) mature eggs and in an expanded condition.

Simulium (S.) aureum

Dunbar (1958, 1959) reported seven cytological forms of this species from larvae collected within its range. The two forms studied were:

Form A: Southern and central Ontario (the only form in Algonquin Park, Davies et al. 1962), with two generations extending from May-October (Davies and Peterson 1956) and females feeding on birds on trees about 20 feet above the ground (Davies et al. 1962, Bennett 1960). Form B: southern Ontario; two or more generations a year.

Jobbins-Pomeroy (1916) reported that *S. aureum* has five or six generations annually in southern California; two generations were reported from Illinois (Forbes 1912), Britain (Edwards 1920), France (Pacaud 1942), Ontario (Davies 1950), Davies and Peterson 1956), New York (Stone and Jamnback 1955) and Ontario (Davies et al. 1962). Stone (1964) reported two or more generations annually from Connecticut; Peterson (1956) reported three or four generations a year in Utah and Sommerman et al. (1955) reported one or two generations per annum in Alaska.

This species passes the winter in the egg stage throughout its range. In the study area eggs hatch in late May or early June but adults were

first collected in the period June 17-19 (1963-1965). The second generation pupated from July 11 to 17 and the third pupated from August 9 to 15. Adult collections indicated three peaks corresponding to the observed increases in the aquatic stages.

Females emerged with their ovaries very small and the eggs not developed (stage 1: Christophers' classification 1960). No mating swarms were seen although females collected feeding on cattle and on sticky traps were fertilized. These showed no follicular relics. It may be concluded that *S. aureum* requires a blood meal for each gonotrophic cycle. They were not attracted to humans or to other moving objects.

Ovipositing females were observed and sampled from Irish and Flatbush creeks only, which may explain the deficiency in larval contribution to adult nutrients and the slow maturation of the larvae as these are clear streams with only patchy vegetation.

Simulium (S.) decorum

The species is abundant in the area. The overwintering eggs hatch in May and mature larvae and pupae were encountered in 1964-65 between May 30-June 7. These aquatic stages are found as aggregates on undersurfaces of stones, culvert walls, embankments, beaver dams and vegetation leaves. Three more generations were recorded: the pupation commenced in June 16-20, third July 22-25, and fourth August 11-19 (1963-65). Apparent overlapping is observed between the first and second generations early in the season and third and fourth generations later in the season. Previous records show the species with two or more generations from Alaska southwards (Davies 1950, Sommerman et al. 1955, Stone and Jamnback 1955, Peterson and Wolfe 1960, Anderson and Dicke 1960, and Davies et al. 1962).

Ovipositing females were observed on beaver dams, sticks, stones, logs, cement embankments and vegetation, dipping their abdomens in the water and laying the eggs in mats; up to seven females used the same spots (cumulative ovipositing), 1500 eggs were counted; often dead females were found stuck to these mats.

No mating swarms were encountered but all the females collected were fertilized. Females of the first generation emerged with their ovaries well-developed (eggs in third stage) and the eggs in the laboratory reached maturity without the females mating or feeding; in the later generations there was a decrease in the ovarian development and females failed to develop their eggs without feeding. Fertilized females were collected from feeding swarms of *S. venustum*; attracted to the collector, females started biting on the neck and arm. Females were observed feeding on horses (June 14-17 1964, July 18-21 1965). Up to 70 flies were counted on a single horse at one time.

Simulium (E.) latipes

This is a holarctic species complex. In the palearctic it was reported that the overwintered larvae gave rise to a single generation a year and adults feed on cattle and humans (Davies et al. 1962, Edwards 1920, Smart 1944, and Steward 1931).

In North America the species passes the winter in the egg stage, has

one to three generations a year, feeds on birds, chickens, and man (Anderson 1956, Anderson and DeFoliart 1961, Bennett 1960, Davies 1950, Davies et al. 1962, Davies and Peterson 1956, Fallis 1964, Fallis and Bennett 1958, Hearle 1932, Hocking and Richards 1952, Peterson 1958, Shewell 1958, Sommerman et al. 1955, Stone and Jamnback 1955, and Stone 1964).

In the study area *S. latipes* has two generations per annum. The overwintered eggs hatch in May and mature larvae and pupae appear between June 17 and 20 (1963-65). Commencing on August 17, pupae and mature larvae of the second generation were encountered in increasing numbers.

Females of this species emerged with no ovarian development (eggs in stage II - Christophers' classification 1960). Females induced to feed on chickens and young sparrows failed to develop their eggs beyond stage III and died four days after a blood meal. Fertilized females netted in the field took blood meals from a chicken, a sparrow and the collector's arm, developed their eggs to maturity in 11 days but all died before laying. Davies and Peterson (1956) stated that *S. latipes* developed its eggs in captivity in 5 days at room temperature. Parous females took less than 11 days to develop their eggs in the second ovarian cycle, i.e. it takes less time for each successive cycle due to the fact that the first blood meal is utilized in overall development of the gonads and the eggs. Other likely factors may be the decrease in the number of functional ovarioles and higher temperatures.

Simulium (S.) luggeri

S. luggeri is not a common species in the area. It breeds in the rapids and riffles of the Pembina River. The dates of pupation of the three annual generations are June 13-20, July 21-28 and August 20-26. Nicholson and Mickel (1950) described the species as having three generations in Minnesota, Fredeen collected pupae from mid-June to early July (the first generation) on the Canadian prairies; Hocking and Pickering (1954) collected pupae in northern Manitoba in August; Anderson and Dicke (1960) recorded two generations per annum in Wisconsin; Twinn (1936) as *S. nigroparvum*) described three generations of the species in eastern Canada (Mostly from the Ottawa River, Ontario). *S. luggeri* passes the winter in the egg stage. Adults mated in small swarms near the breeding sites; fertilized females developed their first batch of eggs without blood meals but fed on horses and cattle before the second gonotrophic cycle. After that no females were encountered. Oviposition was not observed and no egg aggregations were detected, it is assumed that, since the known breeding sites yielded no eggs, the females scatter the eggs in the river.

Simulium (S.) tuberosum

Cytologically this Holarctic species was reported by Landau (1962) to consist of "four well-defined breeding units and a likely fifth, all sympatric" in Ontario and with no evidence of hybridization. Davies et al. (1962) suggested the presence in Ontario of two or more undescribed forms of the species which are different from the Palearctic form of *S. tuberosum* .

Previous records revealed two to four generations a year (Smart 1944, Davies 1950, Sommerman et al. 1955, Stone and Jamnback 1955, Anderson and Dicke 1960, Davies et al. 1962). In the study area as elsewhere *S. tuberosum* overwinters as eggs. It has three generations per annum: maturing larvae and pupae were collected on June 14-17, July 19-24 and August 20-24.

Larvae were seen to occur in dense mats on submerged surfaces or rocks and stones exposed to the sun in the Pembina and Athabasca rivers; they were usually well inside the stream and away from the banks.

Females required a blood meal for the first gonotrophic cycle and after mating they attacked cattle and were attracted to the collector in large numbers but only a few fed on my arms and legs and rarely on the neck or face.

Oviposition probably was on the surface of the water when the females were observed to descend from the air and settle on the water; eggs scattered in the water.

Larvae of *S. tuberosum* were closely associated with those of *S. venustum*. At low water larvae of the latter shared most of the substrates previously occupied by *S. arcticum* and *S. tuberosum*.

The last (third generation was more abundant in the southern part of the study area than in the north.

Simulium (S.) verecundum

Separated from *S. venustum* in 1955 (Stone and Jamnback), this species proved difficult to study separately. Stone and Jamnback (1955) suggested two or three generations a year; Davies et al. (1962) found the same in Ontario. In the study area the collection of aquatic stages from the Pembina River revealed a prolonged duration of larvae and pupae from May to August. This suggests three generations per annum. Adults were not attracted to the collector but were captured feeding on cattle.

Simulium (S.) venustum

This Holarctic species was second only to *S. vittatum* in abundance; aquatic stages were discovered in every breeding site and adults were regularly captured in the period May-September.

Stone and Jamnback (1955) questioned the value of the previous biological record of the species as a complex containing *S. verecundum*. They suggested that *S. venustum* has one generation annually (obligatory diapause). The *S. venustum* - *S. verecundum* complex was shown to have more than one generation resulting in a build-up of an almost continuous population of adults in each season (Smart 1944, Davies 1950, Hocking and Richards 1952, Sommerman et al. 1955, Fredeen 1958 and 1960, Anderson and Dicke 1960 and Davies et al. 1962).

In this study three definite peaks were detected in the larval and pupal densities suggesting a three generation pattern for the species: The generation derived from the overwintered eggs is extremely large and reached a peak on June 10-17. The other two generations are smaller but they overlap to cover the rest of the season. Eggs are abundant as they are laid by females in mats sometimes twenty eggs deep and containing more than 1000 eggs. Females dive under water, settle on water,

vegetation, rocks, stones or logs and usually share an oviposition site together. Eggs were recovered easily up to Setpember 20. July 10-15 and August 14-17 were the dates of pupation of the second and third generations respectively.

Females require a blood meal for the first gonotrophic cycle. Mating swarms were encountered and sometimes induced by the presence of the collector or the white top of a car. Females showed a definite preference for humans over cows and for cows over horses. After they land on a cow, a calf or a horse they are difficult to attract, but before landing they were observed to assemble towards a human host in the presence of other hosts. Females were collected feeding on a dog, on young sparrows and on pigs inside a barn.

Simulium (S.) vittatum

S. vittatum was the most abundant species in the study areas. Its aquatic stages were encountered in all the breeding sites examined. In the Athabasca River there was a noticeable decrease in the density of aquatic stages in the Hinton area but a gradual increase downstream (northwards).

Overwintered larvae were detected under the ice in the Pembina River, French, Chisholm and Blackmud (12 miles south of Edmonton) creeks. After the ice break-up the larvae of this species are predominant; they mature and pupate by May 11 to 13. Another peak of mature larvae and pupae was observed on May 28 to 30; this may be an emergence from overwintered eggs. *S. vittatum* was reported to pass the winter in the larval and egg stages in Alaska (Sommerman et al. 1955), British Columbia (Hearle 1932, Saskatchewan (Cameron 1922, 1918), Ontario (Davies 1950), Connecticut (Stone 1964) and Wisconsin (Anderson and Dicke 1960).

Pupation of the second generation commences on June 25 to 28, of the third generation on July 27 to 30 and the last generation on August 24 to 29. Overwintering larvae were common in September. Anderson and Dicke (1960) reported the species to have four to five generations a year in Wisconsin; Davies (1950) reported two generations in Ontario; Davies et al. (1962) recorded *S. vittatum* as multivoltine in Ontario; DeFoliart (1951) assigned three or four generations to *S. vittatum* in the Adirondack Mountains (New York State); Fredeen and Shemanchuck (1960) found the species to pass through four generations in a season; Sommerman et al. (1955) recorded two and three generations of *S. vittatum* depending on the habitat; Stone and Jamnback (1955) reported three to four generations in New York; Stone (1964) reported one to five generations in Connecticut; Twinn (1936) described two to three generations in eastern Canada.

Females developing from overwintered larvae had well-developed ovaries with the eggs almost mature on emergence. Mating swarms were observed from 1800-1900 hours; fertilized females from these were gravid five days later in the laboratory as also were females reared from pupae and not mated. Ovipositing females settled on different substrates mainly well to the middle of the creeks and started depositing eggs in strings. The communal oviposition method was observed with

four to seven females laying on the trailing leaves of vegetation. Females were collected later in the season, including those from overwintered larvae which had oviposited, feeding on horses and cows (mainly on the ear of the latter).

The females of this species were attracted to the collector in large numbers; they crawled inside the front of the shirt thus gaining access to the body. They started biting on the chest and the belly. Those outside the clothing started biting on the back of the neck and the arms. Females still feeding on cattle were carried inside the barns. They accumulated on windows where they were collected dead. Ants and spiders shared the daily crop of flies. Engorged females were seen flying under the barn lights at night. The preferred regions of feeding on cattle seem to be the ears and to a lesser extent the underside of the belly and the inside of the thighs. Only ear-feeding resulted in severe wounds. The swarming females are common; cattle and horses in the pastures are annoyed by these which are attracted to both moving and stationary objects. Females striking against the collector's face are inhaled and taken in the mouth.

Anderson and DeFoliart (1961) in Wisconsin and Wu (1931) in Michigan found the females to feed on cattle and horses but not to bite man although they are attracted to him. Zoophilic and anthropophilic tendencies were reported by Cameron (1922), Davies (1950), Davies et al. (1962), Hearle (1932), Hocking (1953), Jobbins-Pomeroy (1916), Jones (1961), Knowlton (1935), Knowlton and Maddock (1944), Malloch (1914), Sailer (1953), and Stone and Jamnback (1955).

CONTROL

Susceptibility of Larvae to DDT

Fairchild and Barreda (1945) investigated DDT as a larvicide against simuliid larvae in Guatemala and reported the effectiveness of the method. This led to further investigations and resulted in widespread black fly control for medical veterinary and agricultural purposes (Africa: Barnley (1958), Brown (1960 and 1962), Garnham and McMahon (1947), Hitchen and Goiny (1966), McMahon (1957 and et al. 1958), Wanson et al. (1949 and 1950). North America: Arnason et al. (1949), Gjullin et al. (1949 and 1950), Goulding and Deonier (1950), Hocking (1950, 1953), Hocking and Richards (1952), Hocking et al. (1949), Jamnback and Collins (1955), Jamnback and Eabry (1962), Kindler and Regan (1949), Peterson and Wolfe (1960), Peterson and West (1958), Twinn (1950), Travis et al. (1951) and West et al. (1960), and Prevost (1947). Central America: Lea and Dalmat (1954), Vargas (1945). Europe: Petrishcheva and Saf'yanova (1956) and Rubtzov and Vlasov (1934)). Various insecticides and formulations were investigated for the control of both aquatic stages and adults. Albeit eradication was not feasible, spectacular results were obtained.

Laboratory studies were started by Lea and Dalmat in 1954 using screened tubes for containers and circulating river water through them. One and ten parts per million of toxicant in water were used. This method was adopted by the W. H. O. (W. H. O. tech. Rep. ser. 87, 1954, pp. 21-

24). 820 chemicals were investigated in the period Jan. 1952 to Jan. 1953.

Jar tests

Muirhead-Thomson (1957) reported on the reaction of the larvae in the laboratory to DDT and Dieldrin using compressed air to circulate water in test jars.

Jamnback (1962) suggests two methods. The first method involves the use of a pump to circulate water in a reservoir pan to induce the larvae to detach from field substrata, insecticide exposure bags and then employing compressed air to circulate the water in the observation jars. The second method (W. H. O., 1964) employs wooden troughs, the water circulated by a pump from a reservoir tank. This method was modified by Travis and Wilton (1965). They used V-shaped metal troughs for the tests and nylon bags to return the larvae to the stream for the observation period.

I used 500 cc museum jars fitted with glass plates (3.25" x 4"), compressed air, and stream water. The larvae were introduced into the jars and left overnight to attach to the glass plates. After the larvae were selected and the jars were cleaned of excess larvae and substrata, the insecticide was introduced (solutions of DDT in ethanol added to the water to give the required concentration). The exposure time was one hour and after rinsing the jars to remove the insecticide, 500 cc of river water were added to commence the 24 hour observation period. The tests were carried out at room temperature (63 to 67 F); the insecticide was supplied by the W. H. O. in their mosquito larvae test kit (pp' isomer in ethanol). The species composition of the larvae tested was *S. vittatum* 40%, *S. venustum* 30%, *S. tuberosum* 10%, *S. decorum* 10%, and *S. arcticum* 10%. Results are given in table 8.

Trough tests

Two plastic (acrylic) troughs approximately 6 ft long, 7.5 inches wide and 2 inches deep (corrugated transversely; the ridges one inch apart and 3/8 inch deep), were used as simulated breeding sites. The water circulated from a "baby" bathing tub (22 liters capacity) using a 3-gallons per minute discharge pump. One trough and one tub (reservoir) were used in the tests with insecticides, the other was used for the control. It was found necessary to allow more time for attachment of larvae than the overnight period for the jars. It is easier to use the troughs as there is no handling of the larvae (usually a calculated risk). The disadvantage is the amount of water needed to conduct the tests and the large number of troughs required for a set of tests for various concentrations.

Results

Results are given in table 8.

Biotic Control

Predation on adults was not studied. The larvae associated with other stream organisms were accessible for study. In the study it was observed that predators play a minor part in regulating the numbers of

larvae present at any one point. The larvae of Trichoptera, nymphs of Plecoptera, Ephemeroptera, and Odonata were limited in their distribution within a single breeding site and therefore they had access to

TABLE 8. Results of tests of susceptibility of black fly larvae to DDT, Flatbush 1964 and 1965.

Test no.		Concentration of DDT (ppm) 0.002	0.004	0.005	0.010	0.020	control (0)
Jar method							
1)	8.11.1964						
	No. of larvae	50	50	50	60	70	70
	% mortality	40	80	80	100	100	0
2)	8.23.1964						
	No. of larvae	70	80	75	70	65	75
	% mortality	50	100	100	100	100	0
3)	7.18.1965						
	No. of larvae	65	65	65	60	60	65
	% mortality	47	80	100	100	100	0
4)	7.23.1965						
	No. of larvae	55	50	50	50	55	65
	% mortality	40	74	100	100	100	0
5)	8.7.1965						
	No. of larvae	50	55	55	50	60	65
	% mortality	46	71	100	100	100	0
Trough method							
6)	8.8.1965						
	No. of larvae	216	-	-	-	-	207
	% mortality	47	-	-	-	-	0
7)	8.10.1965						
	No. of larvae	-	194	-	-	-	203
	% mortality	-	83.3	-	-	-	0
8)	8.13.1965						
	No. of larvae	-	-	211	-	-	253
	% mortality	-	-	100	-	-	4.4

Percentage mortalities corrected by Abbott's formula

limited populations of simuliid larvae. The above groups were positively recorded as larval predators when their guts on dissection yielded whole larvae or remains of larvae. Other important groups consisted of leeches, birds, and fish. The leeches increased in the creeks, especially late in the season, and the fish and birds were uniformly scarce throughout the season. The two protozoan genera *Thelohania* and *Caudospora* (Protozoa: Microsporidia) are the most common of the simuliid parasites. Davies (1957) recorded *T. bracteata* Strickland and *T. fibrata* Strickland from *Simulium* spp. and *Caudospora* sp. from *Prosimulium*. The infection rates were 4 to 36%. In the present study the infection rate with microsporidians was estimated as 27 to 33% in the creeks and 0 to 45% in the Pembina and Athabasca rivers. It was observed that the infection with these parasites increased in the second generations but decreased rapidly in the middle of August and did not recover again until the end of the season. Adult infection was highest in May-June (2 to 8%).

Nematodes - Mermithid nematodes are parasites of invertebrates and Welch (1963) collected 153 world records of simuliids parasitized by species belonging to the five aquatic genera: *Isomermis* , *Limnomermis* , *Gastromermis* , *Mesomermis* and *Tetradonema* . Rubtzov (1964) reported simuliid parasitism by sphaerularids (Nematoda: Sphaerularidae) in Russia. The overwintered larvae of *S. vittatum* had infection rates of 7 - 47%, mean 26.1% (22 samples from seven localities in 1963 - 1965 seasons). The only species of mermithid parasitizing these larvae was *Gastromermis viridis* Welch. A single record of 79% infection was obtained in a sparse population in Chisholm Creek, in July 22 1964. Other species breeding in the same locality were not infected. *S. venustum* was infected by *Mesomermis flumenalis* Welch. The infection rates were 35 to 64%, mean 45.1% (approximately 89 samples from 7 to 13 sites per season: 1963 to 1965). Sparse and isolated populations of this species, especially in the creeks and the Pembina River reached 94% infection rates. *S. arcticum* , *S. aureum* and *S. tuberosum* were infected at very low rates. Their parasites were *Limnomermis* sp. Pupal and adult infections were estimated as 14 to 23% and 7 to 9% respectively (calculated on the basis of total collections: 1963 - 1965). In the laboratory seven females and four males of *S. vittatum* (raised from infected larvae) emerged in July 1964 with nematode infections. The above data indicate that the parasites are specific, infected larvae were retarded (metathetely), most of them died and pupal and adult infection contributed to the infestation of the upper reaches of the streams. Dr. H.E. Welch kindly helped with the identification of the mermithid nematodes.

DISCUSSION AND CONCLUSIONS

The 15 simuliid species recorded in this study represented the common species in central and central western Alberta. The study area extended from the southern limit of the boreal forest and the northern boundary of the Parkland to the eastern edge of the boreal cordilleran vegetation region (Moss 1955).

The seasonal differences in the dates of ice break-up, river discharge and weather conditions were slight in 1963 and 1964 but 1965 river discharge was higher than the average. This seemed to be without effect on the populations of the aquatic stages.

The systematics of the family are not clear; *Cnephia* overlaps *Prosimulium* and *Eusimulium* (subgenus of *Simulium*); the two new genera, *Paracnephia* Rubtzov and *Crozetia* Davies were erected to accommodate species included in *Cnephia* (in the Ethiopian region). The same problem of *Cnephia* species exists here. The lack of distinctive morphological characteristics at the species level has resulted in the species complexes encountered in the simuliid fauna here. Cytological investigations have revealed distinct forms within the species of many genera in the Arctic simuliids.

S. vittatum underwent no diapause, while the univoltine species *C. dacotensis*, *C. emergens*, *C. mutata*, and *Prosimulium decemarticulatum*, and probably *P. onychodactylum* and *P. travisi* underwent obligatory diapause. The other species were facultative with the eggs only overwintering but there was no indication of summer aestivation.

Mating swarms were not commonly observed but the females attracted to the collector, to other animals, moving objects, and in birds' nests were fertilized. It is suggested that mating precedes blood feeding; this may be the reason why many species failed to feed in the laboratory as they did not mate in captivity. It follows that parthenogenetic species should be easily induced to feed and oviposit. Two species (*Boophthora erythrocephala* DeGeer and *Wilhelmia salopiensis* Edwards) are now known to mate, feed, and oviposit in the laboratory (Wenk 1963, 1965). The oviposition of females in captivity has rarely been reported. *C. mutata* was the only parthenogenetic species in the area. cytological investigations (Basrur and Rothfels 1959) revealed the presence of both the triploid (parthenogenetic) and the diploid bisexual forms in Ontario. Only females were captured in the 1965 season in the study area but a few males were bred out of pupae collected in 1963 and 1964.

Autogeny was exhibited by univoltine species with weak mouthparts which are incapable of piercing the vertebrate skin, e.g., *C. dacotensis* and *C. emergens*; females of the former had their eggs almost mature on emergence, the females of the latter species had much stored nutrients and eggs were only half developed. Other species (*S. arcticum*, *S. vittatum*, and *S. decorum*) were autogenous in the first gonotrophic cycle in the first generation, taking a blood meal for the second ovarian cycle in the first generation and for the first cycle in the subsequent generations. Fredeen (1963) observed that *S. arcticum* females accumulate after oviposition in the first generation and attack in swarms under favorable weather conditions, to obtain a meal for the second gonotrophic cycle.

The third group of females were anautogenous. These were characterized by the large number of eggs in each ovarian cycle, usually laid in masses. These build up large populations of larvae in the breeding sites (*S. venustum*, *S. aureum*, *S. latipes* and *P. decemarticulatum*). This crowding led to competition among the larvae for food and substrata, and might have contributed to the lack of stored nutrients carried over to the adult stage. Lack of stored nutrients could be also inferred from

the quality and quantity of food available to the larvae, the morphology of the cephalic fan (spacing of the filaments) being an important factor. The adult feeding habits of these females indicated no preference. Mammalophilic *S. venustum* fed on 5 different hosts, including a sparrow; the other 3 species fed on different bird hosts. They were at an advantage as they were not exposed to the risks of long flights. Securing a blood meal with such ease contributed to the longevity of the females and ensured repeated ovarian cycles.

The larval development commenced before the growth of vegetation but the species differed in their developmental thresholds of temperature. These differences occurred in all generations of all species in each year. The overwintered larvae have low, and the overwintered egg (embryo) have high temperature requirements.

The seasonal prevalence of the different species indicated by the total population densities of the larvae in the rivers and creeks did not show much fluctuation in the last three years. In June 1965 there was an apparent reduction in the total larval population which could have been due to the effect of adverse weather conditions on the adult population of *S. arcticum* , and other riverine factors.

Larval migration downstream from upstream oviposition sites as well as the influx of migrant females accounted for the repopulation of streams. Predators and parasites would migrate or drift downstream also. There is a possibility that the eggs of those species that lay them singly drift or are washed downstream by the current. It has been reported that inseciticides in the stream induce the larvae to release their grip and be carried downstream where they perish. In the present study, laboratory tests of susceptibility of the larvae to DDT indicated the extreme toxicity of the insecticide to the larvae; calculated LC_{50} was 0.00213 ppm DDT for 1 hour (pp' isomer in ethanol). Similarly, high mortalities resulting from field applications and laboratory tests with low doses of insecticides have been reported.

Laboratory rearing of simuliids ended with the emergence of the adults from the pupae. As Wenk (1965) reported, the problems involved were mating, feeding, and oviposition in the laboratory and these difficulties were overcome with the discovery of two laboratory mating species in Europe. All other species fed on blood developed sterile eggs and oviposited without mating. Eggs dissected out of wild gravid mated females did not hatch. The latter phenomenon suggests that eggs are fertilized in the common oviduct prior to oviposition.

Emergence of aquatic as of many other species follows a diel periodicity, c.f. chironomid pupae (Palmén 1958), gall midges (Barnes 1930) and *Drosophila* (Brett 1955). Davies (1950) studied the factors that affect the emergence of adult simuliids. There was general agreement that light intensity was the main stimulus and that emergence was temperature independent, although the temperature exerted some control on the hourly emergence. I observed that in the laboratory there existed an attenuated emergence between 10 PM and 3 AM (two hours after sunset to about an hour before dawn). In the field the adult yield in the emergence traps dropped considerably after sunset and did not recover except at dawn. The variations in these emergence patterns are likely due

to temperature fluctuating less indoors than in the stream, or to lights being on at night. Temperature may be responsible for initiation of emergence.

Adult activity observations revealed a diurnal periodicity. There were two peaks of flight activity; the first commenced about two hours after dawn, continued for three hours after sunrise and the second occurred irregularly two to three hours before sunset and for sometime in the night. The same pattern of activity was described by Davies (1957) for *S. ornatum* Mg. Davies (1963) and Wolfe and Peterson (1960) reported on studies on the nulliparous and parous females concluding that parous females tended to fly in the late afternoon. Lewis (1958, 1960) observed *S. damnosum* to fly at noon. My studies were on *S. venustum*, *S. vittatum*, *S. arcticum* (mammalophilic species), and *S. latipes* and *S. aureum* (ornithophilic species). Sweep-netting near the breeding sites yielded a large number of nulliparous and a few gravid females and males. The composition of the population of females on the wing was varied. The emergence of the adults of a species of any of the above groups changed the age composition of the flying simuliids. Nulliparous females were dominant at the beginning of the season (late May and early June). The number of flies eventually decreased, and the parous females outnumbered the nulliparous. This pattern continued throughout the season.

Diurnal activity was influenced by the daily weather and meteorological conditions as reported by Dalmat (1954, 1955), Davies (1952), Davies (1957), Wolfe and Peterson (1959, 1960), Wenk (1963, 1965), and Zahar (1951), which indicated a similarity in different regions. In the present study no species exhibited any preference for any set of conditions but there was a uniformity in abundance in both periods of activity with a slight increase in numbers in the afternoon-evening peak. The low light intensity, moderate wind and high relative humidity were the main factors concerned and these were fulfilled in the above periods.

Biotic control agents of simuliids included mermithid nematodes, microsporidians, and predators. The value of Gregarinida (Protozoa: Sporozoa) was not investigated as these parasites were not very common. Microsporidian infections were fatal to the larvae and adults but their importance as biotic control agents of simuliids was not definite. The low incidence of infection with this parasite suggested a secondary value. On the contrary, the mermithid nematodes were efficient parasites reaching 94% in some larval populations (although it may be said here that infected larvae were slow to migrate and to pupate and this led to isolations of these populations which contributed to the high rate of infection observed). Host specificity of the nematodes was significant and was considered a disadvantage from the control viewpoint. The value of the predators (on the aquatic stages) might not exceed that of the microsporidian infection.

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Editorial - On the Life and Death of Information

Statements have appeared in print recently concerning the half-life of biological information which, if they were to be taken seriously, would make a mockery of our best efforts in publishing this periodical and indeed of printing generally. The estimate of this supposed half-life is put at ten years; the author of the statements would be better employed at developing a printing ink which would fade to half its intensity in ten years than in biological research. Think of the forests that could be saved from the hungry pulp mills; and the waterways from their pollution. But think too of the damage to the economy.

Does information die? If so, what constitutes its death -- lack of use? This seems to be analogous to the theory -- moribund, perhaps, but surely not dead -- of use and disuse as a mechanism of evolution. But are theories information anyhow? Or hypotheses? Perhaps they should be regarded as historical information; but we must bear in mind that history repeats itself. The information recorded by Aristotle has considerable influence today, at about 2^{-200} of its original strength; unless perhaps it is only modern information that is so highly mortal. If this be true it may be because of our own inadequate use of preceding information; because, in other words, our information is not really new; not, strictly speaking, information at all.

In the physical sciences, and especially perhaps in chemistry, one hears it said that there is no point in holding back files of periodicals more than ten years. This may be just wishful thinking on the part of overburdened librarians; I cannot believe it to be true. To carry such a proposition over into biology generally, where the very framework of the subject goes back formally to 1758 and informally to the origins of recorded information would, of course, be nonsense. No biologist, surely, would maintain that only 0.0001% of the information produced by Linnaeus is "alive" today.

As we all know, in biological research, ten years is by no means an unusual time lapse between a discovery and its appearance in print. I

once published an account of a piece of work thirty years after doing it -- when it was 87.5% dead. Gregor Mendel's work, though published, was not discovered until it was 95% dead -- what an impact it might have had earlier! Much work, unquestionably, appears before its time, when its viability must increase with age and its immortality must, I think, be conceded. Of course there is also much work that produces results of real interest for a limited time only; new techniques and methods which will in turn by superseded by others, new hyoptheses which ultimately prove untenable. Here, it may be legitimate to speak of a half-life, but preferable to refer to a limited information content; for there are elements in all such work which will endure. Information, as I see it, endures; applications of it, the clothes it wears, may fade. On a human time scale, publications endure well, although sometimes one wishes they wouldn't. If an author feels that his work has a half-life of only ten years; if he feels that publication is a question of now or never, he should probably refrain and content himself with telling his friends and colleagues about it, for it may be half dead before it appears.

B. Hocking

LIPID METABOLISM IN *BLATTELLA GERMANICA* L. : COMPOSITION DURING EMBRYONIC AND POST EMBRYONIC DEVELOPMENT

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Changes in the lipid composition of Blattella germanica *L. during embryonic and post embryonic development were investigated by a combination of column, thin-layer and gas-liquid chromatography. During embryogenesis the loss of dry matter was mainly due to utilization of triglycerides. Hydrocarbon and sterol content increased slightly. Mono- and di-glyceride and free fatty acid content increased substantially. Fatty acid analysis revealed the presence of 17 fatty acids ranging in chain length from C_6 to C_{22}. Oleic acid was the most abundant followed by palmitic acid. Phospholipid content increased during embryonic and post embryonic development. Lecithin, cephalin and sphingomyelin were the major phospholipids. During nymphal development all the lipid fractions increased approximately in proportion to the increase in body weight. Nymphs and adults had similar lipid composition.*

Lipids include neutral fats, phospholipids, cerebrosides, sterols and fat soluble vitamins. Many earlier works on insect lipid were restricted to gross quantitative analysis. The chemistry of insect fats has been reviewed by Timon-David (1930), Scoggin and Tauber (1950) and Hilditch (1956). In the past decade the volume of literature on the lipid composition of insects has increased tremendously as is evidenced by the publication of a number of reviews (Niemierko 1959, Babcock and Rutschky 1961, Gilmour 1961, Kilby 1963, Fast 1964, Gilby 1965, Kinsella 1966b, Gilbert 1967a). The remarkable growth in this field has been principally due to the development of better analytical techniques. The advent of thin-layer and gas-liquid chromatography in particular has enormously facilitated the purification and identification of complex lipids.

Lipids have a high caloric value and their catabolism yields 2.2 and 1.6 times as much energy per gram as carbohydrates and proteins, respectively. They also yield twice as much metabolic water which plays an important role in terrestrial animals. Much of the food eaten by insects during the immature stages is converted into fat and stored in cells of the fat body.

Blattella germanica L. has long been employed as a subject of physiological and toxicological investigations but lipid biochemistry has been neglected. My purpose was to investigate the qualitative and quantitative changes in the lipid composition of *B. germanica* during embryonic and post embryonic development.

REVIEW OF LITERATURE

Embryonic Stages

During embryonic development important qualitative and quantitative changes may occur in the lipid fraction. In insects, neutral glycerides serve as the major energy source. Needham (1931) proposed a theory of a succession of energy sources during ontogeny; the embryo first utilizing carbohydrate primarily, then protein and finally fat.

A number of studies on lipid content of orthopteran eggs indicate that, in general, the lipid content ranges from 2.5 to 14% of the wet weight. The lipid concentration declines during development of the embryo. A large part of the loss appears to be in the triglyceride fraction; lipids are the major source of energy for the developing embryo. The greatest loss of lipids occurs during the later stages of development.

Probably the earliest work on the lipid content of an orthopteroid egg was that of Dubois (1893), who reported that newly laid eggs of the Algerian locust, *Acridium peregrinum* Oliv. contain 4 to 5% fatty material (wet weight basis) and this was considerably reduced during development. No quantitative estimate of the loss was given. Slifer (1930) reported that 9 to 12% of the wet weight of the newly laid eggs of *Melanoplus differentialis* (Thomas) was fatty acid. During embryogenesis 54% of the initial fatty acid reserve was catabolized. Most of the loss occurred in the post diapause period. The iodine value remained constant during embryonic development. This was confirmed by Boell (1935) and Hill (1945). On the basis of respiratory studies, Boell (1935) calculated the loss of fatty acids as 67% of the initial store. Hill (1945) showed that carbohydrate formed the major energy source during the first 5 days of development. Protein and fat were chiefly used in the prediapause and diapause period. During post diapause fat catabolism accounted for 90% of the oxygen consumed.

Carausius (Dixippus) morosus (Br. and L.) used 26% of the lipid reserve during embryonic development and the fat content was reduced from 31% of the dry matter to 23% at the time of hatching (Lafon 1950).

Blackith and Howden (1961) and Allais et al. (1964) recorded loss of lipids during embryogenesis in *Locusta migratoria* (L.). The latter authors observed that lipids accounted for 26% of the dry weight in the newly laid eggs (78.5% triglycerides, 19.5% phospholipids and 2% sterols) and showed a total decrease of 31.2% to form 20.7% at the end of embryonic development (triglycerides 66%, phospholipids 19.5% and sterols 3%). They concluded that this decline in lipid content was due only to catabolism of triglycerides. Phospholipids consisted chiefly of lecithins (70.5%) and cephalins (26.5%) and a small amount of sphingomyelin. The phospholipid content increased by 60% during embryogenesis, but no qualitative changes were observed. Sterol content remained constant and for the most part was in the free form.

Kinsella and Smyth (1966) and Kinsella (1966a, c, d) made an exhaustive study of the lipids of *Periplaneta americana* L. During embryogenesis, total extractable lipids decreased from 39.5% to 23.2% of the dry weight, mainly due to catabolism of the triglyceride fraction. There was an increase in the mono- and di-glyceride fractions (Kinsella and Smyth 1966).

Sterol content remained constant during development. The sterol esters of newly extruded oothecae contained mainly palmitic, stearic, oleic and linoleic acids. Palmitic and stearic acid content decreased during development (Kinsella 1966d). Sphingomyelin, lecithin and cephalin are the major phospholipids. Small amounts of lysolecithin, phosphatidyl inositol and cerebroside were also found. Total phospholipid content increased fourfold during development. Sphingomyelin and cephalin content tripled and lecithin content doubled (Kinsella 1966a). There was close similarity in the fatty acid composition of the total lipid, neutral lipid and triglyceride fractions. Palmitic, stearic, oleic and linoleic acids content accounted for 95% of the total fatty acids. The phospholipid fraction, however, had a greater amount of linolenic acid (Kinsella 1966c).

The lipid picture for the period of embryogenesis in *Leucophaea maderae* (Fabr.) was characterized by a decreasing content of embryonic triglyceride and an increasing proportion of phospholipids (Gilbert 1967b).

Post Embryonic Stages

The lipids of post embryonic stages of orthopteroid insects vary over a wide range (1.7 to 16% of the wet weight). The immature stages, in general, have a higher lipid content.

Tsujimoto (1929) analyzed the fat of *Oxya japonica* (Fabr.). This species contained 3% fat (dry weight basis) and had a saponification number of 175, an iodine value of 122.6 and 15.7% unsaponifiable matter. Palmitic, stearic, oleic and linoleic acids were identified. Seventy-five per cent of the fatty acids were unsaturated. Body lipids of *Acheta mitrata* constituted 2.4% of the fresh weight. Unsaponifiable matter made up 11.3% of this and contained 45.5% sterols.

Sacharov (1930) reported that 3 to 5 day old nymphs of *L. migratoria* contained 2.8% fat. Matthee (1945) investigated some of the biochemical differences between the solitary and gregarious phases of *L. migratoria* and *Locusta pardalina* Walk. The fat content of *L. migratoria solitaria* adult was 11.0% of the dry weight and increased to 14.0% in *L. migratoria gregaria* . Similarly the fat content of the solitary phase adults of *L. pardalina* increased from 12.8 to 14.6% (dry basis) in the migratory phase. Fawzi, Osman and Schmidt (1961) recorded a much higher fat content in the migratory phase of *L. migratoria*; 10.4% of the wet weight in females and 14.6% of the wet weight in the males.

The lipid content of the German cockroach, *B. germanica* was studied by Mellampy and Maynard (1937). The lipid content of the nymphs, females with egg capsules and males was 5.7, 4.8 and 1.7% of the wet weight respectively. The Iodine Number was 69 for the nymphs and 74 for the females and males. In a later study McCay (1938) reported that of the dry weight of adults 15.6 to 17.1% was ether extractable material.

Lipid content of *P. americana* adults has been studied by a number of investigators. Schweet (1941) reported that the lipid content of adult females and males was 28.6 and 25.5% of the dry weight. According to Munson and Gottlieb (1953) the lipid content of the nymphs, adult males and females was 7.7%, 7.1% and 8.9% of the wet weight respectively. Siakotos and Zoller (1960) and Kinsella and Smyth (1966) reported that

30% of the dry matter was lipids. Lofgren and Cutkomp (1956) recorded much lower values, 13.9% and 14.5% of the dry weight in females and males. Kinsella (1966a, c, d) compared the lipid composition of the nymphs and adults with those of the various stages of the embryo. The lipid composition of nymphs and adults was quite similar. Neutral lipid accounted for 75% of the total lipids. Lecithin, cephalin and sphingomyelin were the predominant phospholipids in that order of abundance (Kinsella 1966a). The fatty acid pattern of the various lipid fractions was similar to those found in the embryo (Kinsella 1966c).

On a wet weight basis, the adults of the grasshopper *Melanoplus atlanis* Riley consisted of 0.8% neutral fat and 2.4% fatty acids (Giral, Giral and Giral 1946). The free fatty acids consisted of stearic, palmitic, arachidic, and unsaturated C16, C18, C20 and C22 acids. Linolenic acid was not present but triethenoic acids of the C20-C22 series appeared to be present. The polyunsaturated acids with more than 18 carbons were present in large amounts (46.2% of the total fat).

Components of the body fat of *Taeniopoda auricornis* Walk. was reported by Giral, Giral and Giral (1943). Fatty acids of the females consisted of 35% saturated acids, 6.5% oleic acid and 58.5% linoleic acid. Fatty acids of the males contained 15.5% saturated acids, 24.0% oleic and 60.5% linoleic acid. The lipids of the females contained 5.1% unsaponifiable matter; of the males 6.5%.

Giral (1946) found that the lipids extracted from the adults of *Sphenarium purpurescens* (Charp.) contained a very high proportion of free fatty acids. Glycerides were present in very small amount. The fatty acids consisted of 22.1% saturated acids, 9.6% palmitoleic acid and 35.5% oleic acid. Of the fatty acids, 25.8% had a chain length of more than 18 carbons.

Barlow (1964) found that palmitic, oleic, linoleic and linolenic acids accounted for 95% of the fatty acids in the body fat of *Melanoplus sanguinipes* (Fab.).

MATERIALS AND METHODS

Laboratory Rearing of the Roaches

The roaches were fed rabbit pellets (3.5 - 4.5% lipids) and were kept in battery jars with pieces of folded paper which served as resting and hiding space. A one pound narrow mouthed jar was used as a water reservoir and a cotton wick was provided, the upper end being wrapped with absorbent cotton to make it fit tightly in the mouth of the bottle. Water and food was replenished once every three weeks. The roaches were raised at a temperature of 30 ± 1 C. Two to three hundred individuals were raised in one jar and adults were collected once every three days. Adults collected on the same day from different jars were pooled, maintained under the same conditions as described above, and used for lipid analysis after one week.

Extraction and Purification of Lipids

The procedure of Folch, Lees and Sloane-Stanley (1957) was followed. The insects were placed in a glass vial with 20 volumes of a chloroform:

methanol mixture (2:1 v/v) and homogenized in a Potter-Elvehjem homogenizer for 30 minutes and filtered through a sintered glass funnel into a glass stoppered vial. The filtrate was shaken for 3 to 4 minutes with 0.2 volumes of 0.90% sodium chloride solution. The mixture was centrifuged for 5 minutes at 400 g and the upper methanol:water:salt layer was removed with a fine pipette. To ensure complete removal of the solutes in the upper phase, the interphase was rinsed three times with a small volume chloroform:methanol:saline (3:47:48 v/v/v). The lower phase was evaporated to dryness in a rotary flash evaporator, dissolved in a small volume of chloroform and stored at -10 C under nitrogen until further use.

Separation of Lipid Class Spectrum

The total lipid extract was placed on a 30 g silicic acid:Hyflo super cell column (2:1 w/w; column dimensions 2.2 x 14 cm) and eluted first with 200 ml of chloroform to obtain the neutral lipids. The phospholipids retained on the column were then eluted with 200 ml of chloroform:methanol (1:1 v/v) mixture. The eluates were then evaporated to dryness and weighed. The neutral lipid fraction was then placed on a 30 g de-activated florisil column (2.2 cm x 15 cm) and the lipid classes were eluted according to the method of Carrol (1961). The solvents of the isolated lipid classes were first removed in a flash point evaporator and were then placed in a vacuum oven at 40 C in tared planchets and the solvents removed. The planchets were reweighed to obtain the weights of the individual classes. The efficiency of the separation of these classes of compounds was checked by thin-layer chromatography (Mangold 1961).

Analysis of Fatty Acids by Gas Liquid Chromatography

Aliquots of lipid fractions were transmethylated by refluxing in 5 ml of 5% sulfuric acid in dry methanol (w/v) for 3 hours (Patton, Durdan and McCarthy 1964). An equal volume of distilled water was added and the methyl esters extracted three times with a small volume of redistilled petroleum ether (30-60C). The combined ether extract was dried over anhydrous sodium sulfate and the petroleum ether removed under a stream of nitrogen. The methyl esters were then dissolved in a small volume of spectranalyzed n-hexane and small aliquots were used for separation by gas-liquid chromatography.

Qualitative and quantitative analyses of fatty acid methyl esters were made with a Beckman Model GC 5 Gas Chromatograph equipped with a dual hydrogen flame ionization detector. Copper columns (15' long, 1/8" O D) containing Chromosorb P (60-80 mesh) coated with DEGS (diethylene glycol succinate, 20% by weight of the solid support) were used. Since the column was continuously maintained at 200 C, the amount of liquid phase in time was reduced. This resulted in reduction of retention time. Standard methyl esters of fatty acids for comparative purposes and quantitation were obtained from Mann Research Laboratories. Unknown peaks were tentatively identified by logarithmic plot by the method of James (1959).

Thin Layer Chromatography of Phospholipids

Phospholipid content was determined by silicic acid Hyflo super cell chromatography. The individual phospholipid classes were seperated by thin-layer chromatography on 0.5 mm thick Silica gel G plates according to Wagner, Horhammer and Wolff (1961). The developing solvent was chloroform:methanol:water (65:25:4 v/v). The developing chamber was lined with filter paper saturated with the solvent mixture. Following the chromatographic run, the thin-layer plates were air dried and exposed to iodine vapour for visualization of the phospholipids. Identification of the individual phospholipid was accomplished by comparison of the R_f with those of pure standards obtained from Applied Science Laboratories, State College, U.S.A. and specific color reactions. Quantitation of the major phospholipids was based on the phosphorus content, determined colorimetrically according to the method of Bartlett (1959). Three determinations were made for each stage.

RESULTS

Dry Matter Content

The wet weight, dry weight (obtained by drying at 100 C) and water content of the ootheca, nymphs and adults of the German cockroach are given in table 1. The proportion of water in the egg was initially about 62%. This remained constant during the first five days of embryonic development and increased to about 75% by the 15th day. When calculated on a per egg basis, the wet weight of the egg increased from 1.276 mg to 1.617 mg, while the dry matter decreased from 0.486 mg to 0.399 mg (table 2). There was a slight drop in the per cent water content between the 1st and 3rd nymphal instars. In the subsequent nymphal instars and the adult water content remained more or less constant.

Total Lipids

Table 2 reveals that the total lipid content of the eggs decreased during embryonic development. The newly formed eggs, on an average, contained 0.178 mg of lipids which in 15 days of embryonic development decreased to 0.112 mg, representing a loss of 37% of the original lipid component. During the first 5 days of embryonic development, lipid catabolism accounted for 50% of the loss of dry matter; between the 5th and 10th day 56% of the loss of dry matter was due to lipid loss; nearly all of the dry matter loss between the 10th and 15th day of incubation was due to lipid utilization. This indicates that during the early part of embryonic development, some other component(s) must have been used for fulfilling the energy requirements.

There was a progressive increase in the amount of lipids per individual insect as the development of the nymphs proceded, reaching a maximum in the 6th instar (table 2). However, the lipid content dropped considerably in the adult stage. The females had twice as much lipid as the males.

TABLE 1. Wet weight, dry weight and water content at the various stages of embryonic and post embryonic development of *B. germanica*, means ± S.D. based upon 10 replicates.

Stage of development	Wet wt. (mg)	Dry wt. (mg)	Water content (%)
Ootheca			
0 days	38.29 ± 0.29	14.58 ± 0.25	61.93 ± 0.38
5 days	37.55 ± 0.26	14.29 ± 0.29	61.94 ± 0.31
10 days	43.25 ± 0.27	13.60 ± 0.17	68.55 ± 0.47
15 days	48.50 ± 0.16	11.98 ± 0.27	75.30 ± 1.35
Nymphs			
1st instar	2.25 ± 0.10	0.62 ± 0.07	72.58 ± 0.85
2nd instar	5.09 ± 0.14	1.49 ± 0.07	71.31 ± 1.86
3rd instar	9.92 ± 0.16	2.99 ± 0.09	69.86 ± 0.89
4th instar	20.47 ± 1.42	6.51 ± 0.37	68.19 ± 1.56
5th instar	36.85 ± 1.38	11.59 ± 0.29	68.52 ± 1.21
6th instar	53.48 ± 2.45	16.93 ± 0.94	68.54 ± 1.52
Adults			
♂	47.72 ± 1.14	15.54 ± 0.36	67.41 ± 1.13
♀	64.78 ± 0.98	19.79 ± 1.51	69.44 ± 2.46

Lipid Class Spectrum

At the beginning of embryonic development the neutral lipid constituted 94.6% of the total lipid, but by the time the eggs were 15 days old, it decreased to 86% of the total lipid content (table 2). Embryonic development resulted in a 43% loss of the neutral lipid reserve. During nymphal growth the neutral lipid content per individual increased more or less proportionally to the increase in body weight, reaching a maximum in the 6th instar. The adults have a lower neutral lipid content than the last instar nymphs.

The neutral lipid was fractionated into the following classes: hydrocarbons, free sterols, sterol esters, triglycerides, diglycerides, monoglycerides and free fatty acids. Table 3 is a summary of the analyses conducted at the various stages of development. The values are based on three analyses for each stage. This table shows that the neutral lipid fraction consisted predominantly of triglycerides in all the stages. During embryonic development the level of triglyceride fell from 94% to 76% of the neutral lipid fraction. Gravimetrically there was a 54% loss of the triglyceride content. The loss in the triglyceride content (2.58 mg) was greater than the loss of dry matter (2.51 mg) and neutral lipid (2.16 mg). The proportion of mono- and di-glyceride as well as the free fatty acid content increased considerably during incubation. Quantitatively (table 4) the mono- and di-glyceride content increased 6 and 10 times of the

TABLE 2. Wet and dry weight, total lipid, phospholipid and neutral lipid content per individual during embryonic and post embryonic development of *B. germanica* L.

Stage of development	Wet wt* (mg)	Dry wt* (mg)	Water content (%)*	Lipid content**			Phospholipid**		Neutral lipid**	
				mg	% of wet wt	% of dry wt	mg	Total lipids (%)	mg	Total lipids (%)
Egg										
newly formed	1.276	0.486	61.9	0.178	14.0	36.6	0.010	5.4	0.168	94.6
5 day old	1.252	0.476	61.9	0.173	13.9	36.6	0.011	6.3	0.162	93.7
10 day old	1.442	0.453	68.5	0.160	11.1	35.3	0.014	8.6	0.146	91.4
15 day old	1.617	0.399	75.3	0.112	6.9	28.1	0.016	13.9	0.096	86.1
Nymph										
1st instar	2.25	0.62	72.6	0.123	5.5	19.8	0.021	17.3	0.102	82.7
2nd instar	5.09	1.49	71.3	0.279	5.5	18.7	0.054	19.7	0.225	80.3
3rd instar	9.92	2.99	69.9	0.570	5.8	19.1	0.095	16.7	0.475	83.3
4th instar	20.47	6.51	68.2	1.402	6.9	21.5	0.230	16.4	1.172	83.6
5th instar	36.85	11.59	68.5	2.141	5.8	18.5	0.363	17.4	1.768	82.6
6th instar	53.48	16.93	68.5	3.920	7.3	23.2	0.758	19.3	3.162	80.7
Adult										
♂	47.72	14.54	67.4	1.933	4.1	12.4	0.343	17.8	1.590	82.2
♀	64.78	19.79	69.4	3.699	5.7	28.9	0.658	17.8	3.041	82.2

* *Values presented are means of 10 replicates. These data appear also in table 1.*

** *Values presented are means of 3 replicates.*

initial concentration per individual respectively. The free fatty acid content increased 7 fold.

TABLE 3. Proportion of various lipid classes in the neutral lipid fraction of the German cockroach (% of neutral lipid fraction), means of three replicates for each stage.

Stage	Hydrocarbons	Sterol esters	Triglycerides	Sterols	Diglyceride	Monoglyceride	Free fatty acid
Ootheca							
0 day	1.16	0.89	94.35	2.49	0.24	0.55	0.32
5 day	1.27	1.05	91.84	2.46	1.00	1.52	0.86
10 day	1.62	1.44	88.51	2.17	1.83	2.31	2.12
15 day	2.56	3.21	75.87	3.49	4.29	5.63	4.94
Nymphs							
1st instar	5.90	6.06	70.12	5.33	4.53	4.17	3.89
2nd instar	5.45	5.33	71.21	5.59	4.64	4.04	3.74
3rd instar	5.26	5.64	70.09	5.93	5.63	4.52	2.93
4th instar	4.97	6.03	69.40	5.84	5.71	4.95	3.10
5th instar	7.85	5.78	69.77	5.07	2.83	4.64	4.06
6th instar	5.18	4.40	69.88	7.39	4.52	4.79	3.84
Adults							
♂	6.35	5.70	66.71	7.02	4.97	4.88	4.36
♀	6.40	6.41	71.94	4.48	4.42	3.90	2.45

The relative proportion of the various fractions remained more or less constant in all the nymphal stages. Quantitatively, all the fractions increased in each successive nymphal instar (table 4). Adult females had a neutral lipid class spectrum similar to the nymphs. Adult males had a slightly lower triglyceride level compared to other post embryonic stages.

Sterol content was the same on day 5 as on day 0 of embryonic development. There was a drop between the 5th and 10th day but an increase to a higher amount by the 15th day (table 4). The proportion of esterified sterol progressively increased from 26% in the newly formed oothecae to 48% in the 15 day old oothecae (table 3). Total sterol content increased in successive nymphal instars. The proportion of free and esterified sterol fluctuated. Sterol of females was predominantly in the esterified form while in the males the free forms predominated.

Hydrocarbon content increased gradually at successive developmental stages of the embryo. During nymphal development, the increase in the hydrocarbon content more or less paralleled the increase in the total

TABLE 4. Lipid class spectrum of the neutral lipid fraction during various stages of *B. germanica*, means of three replicates. Weights in mg per ootheca, nymph, and adult.

	Hydro-carbons	Sterol esters	Sterols (free)	Total sterols	Tri-glycerides	Di-glycerides	Mono-glycerides	Total glycerides	Free fatty acids
Ootheca									
0 days	.059	.045	.126	.171	4.775	.012	.028	4.815	.016
5 days	.062	.051	.120	.171	4.481	.049	.074	4.604	.042
10 days	.071	.063	.095	.158	3.876	.080	.010	4.057	.093
15 days	.074	.093	.101	.194	2.195	.124	.163	2.482	.143
Nymphs									
1st instar	.006	.006	.005	.011	0.072	.005	.004	0.081	.004
2nd instar	.013	.012	.013	.025	0.160	.010	.009	0.179	.008
3rd instar	.025	.027	.028	.055	0.333	.027	.021	0.381	.014
4th instar	.058	.071	.068	.139	0.814	.067	.058	0.939	.036
5th instar	.139	.102	.090	.192	1.233	.050	.082	1.365	.072
6th instar	.164	.139	.234	.373	2.210	.143	.151	2.504	.121
Adults									
♂	.100	.091	.112	.203	1.061	.079	.078	1.218	.069
♀	.195	.195	.136	.331	2.188	.134	.118	2.440	.075

lipid content.

Phospholipids

The total phospholipid present in each stage is shown in table 2. The per cent phosphorus content of the major phospholipid fractions (lecithins, cephalins and sphingomyelin) at various stages of development are recorded in table 5. From this the amount of the major phospholipid fractions (in terms of phosphorus content) were calculated and shown in table 5.

During the embryonic development total phospholipid content as well as the three major fractions increased. The proportion of phospholipid at the beginning of embryonic development averaged 6% of the total lipid content. In the 15 days of embryonic development it rose to 13% of the total lipid. Gravimetrically the total phospholipid content increased one and a half times. This indicates a synthesis of phospholipids. Once the nymphs had started feeding, the increment in the phospholipid content was in proportion to the increase in total fat content. During the six subsequent nymphal instars the level of phospholipids remained nearly constant at about 18% of the total lipids.

Phosphatidyl choline (lecithin) and phosphatidyl ethanolamine (cephalin) were the principal phospholipids. In addition to the above two and sphingomyelin, which were quantitated, phosphatidyl serine, phosphatidyl inositol, lysolecithin and phosphatidic acid were detected in trace amounts in all stages studied. During embryonic development the lecithin/cephalin ratio decreased. However, the total increase in lecithin content was greater than the increase in cephalin. The relative proportion of the three major phospholipid fractions remained constant in the nymphal stages. Based on lipid phosphorus, the per cent composition of lecithin, cephalin and sphingomyelin was 57, 33 and 6% respectively. The lecithin/cephalin ratio varied from 1.63 to 1.77. The phospholipid composition of males and females was similar.

Fatty Acid Composition

Figure 1 shows an analysis of fatty acids from an adult female sample. This analysis revealed the presence of 17 fatty acids ranging in carbon chain length from 6 to 22. Of these $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ mixed with $C_{20:0}$ were quantitated. With the column used $C_{18:3}$ and $C_{20:0}$ could not be separated. Table 6 represents a summary of the gas chromatographic analysis of the fatty acids in the total lipid extract at 12 different stages of development.

Oleic acid ($C_{18:1}$) was the largest component in all the stages and accounted for 42 to 48% of the total fatty acid fraction (table 6). Palmitic acid ($C_{16:0}$) was next in order of abundance. About 70% of the fatty acids were unsaturated. Besides oleic acid, linoleic acid ($C_{18:2}$) was present in fair amount (16 to 19%). Of the remaining 30% saturated fatty acids, palmitic acid ($C_{16:0}$) was the most abundant (25 to 30%).

The proportion of myristic acid ($C_{14:0}$) in the nymphs and adults was three times as much as in the embryonic stages. Palmitic acid content in the 0 and 5 day old oothecae was quite similar but it dropped to a lower percentage in the 10 and 15 day old oothecae. The reverse

TABLE 5. Changes in the distribution of lipid phosphorus and phospholipid fractions during embryonic and post embryonic development of *B. germanica*, means of 3 replicates.

Stage	Lipid phosphorus content		Distribution of phosphorus in major fractions							Lecithin/ cephalin ratio
			% distribution				µg/insect			
	%	µg/insect	Sphingo-myelin	Lecithin	Cephalin	Misc.	Sphingo-myelin	Lecithin	Cephalin	
Egg										
0 day	3.68	0.368	5.5	60.0	31.7	2.8	0.020	0.221	0.117	1.89
5 day	3.75	0.413	5.6	59.1	33.7	1.6	0.023	0.244	0.139	1.75
10 day	3.74	0.524	5.8	58.6	34.2	1.4	0.030	0.307	0.179	1.71
15 day	3.88	0.621	5.7	57.4	34.3	2.6	0.035	0.356	0.213	1.67
Nymph										
1st instar	3.97	0.834	5.7	56.4	34.7	3.2	0.048	0.470	0.289	1.63
2nd instar	3.90	2.106	5.8	56.4	33.7	4.1	0.122	1.188	0.710	1.67
3rd instar	3.89	3.696	6.2	56.6	32.9	4.3	0.229	2.092	1.216	1.72
4th instar	4.00	9.200	5.5	58.0	32.7	3.8	0.506	5.336	3.008	1.77
5th instar	3.78	13.731	5.7	55.8	33.8	4.7	0.782	7.656	4.638	1.65
6th instar	4.02	30.472	5.7	56.9	33.4	4.0	1.737	17.339	10.178	1.70
Adult										
♂	3.98	13.651	5.7	56.6	33.9	3.8	0.773	7.676	4.628	1.67
♀	4.00	26.320	5.5	56.5	33.9	4.1	1.448	14.871	8.922	1.67

was the case with oleic acid. The percentage of the remaining major fatty acids were quite similar in all the fatty acid composition between sexes. During nymphal growth, the proportion of palmitic and palmitoleic acid increased, but the proportion of linoleic and linolenic acid decreased.

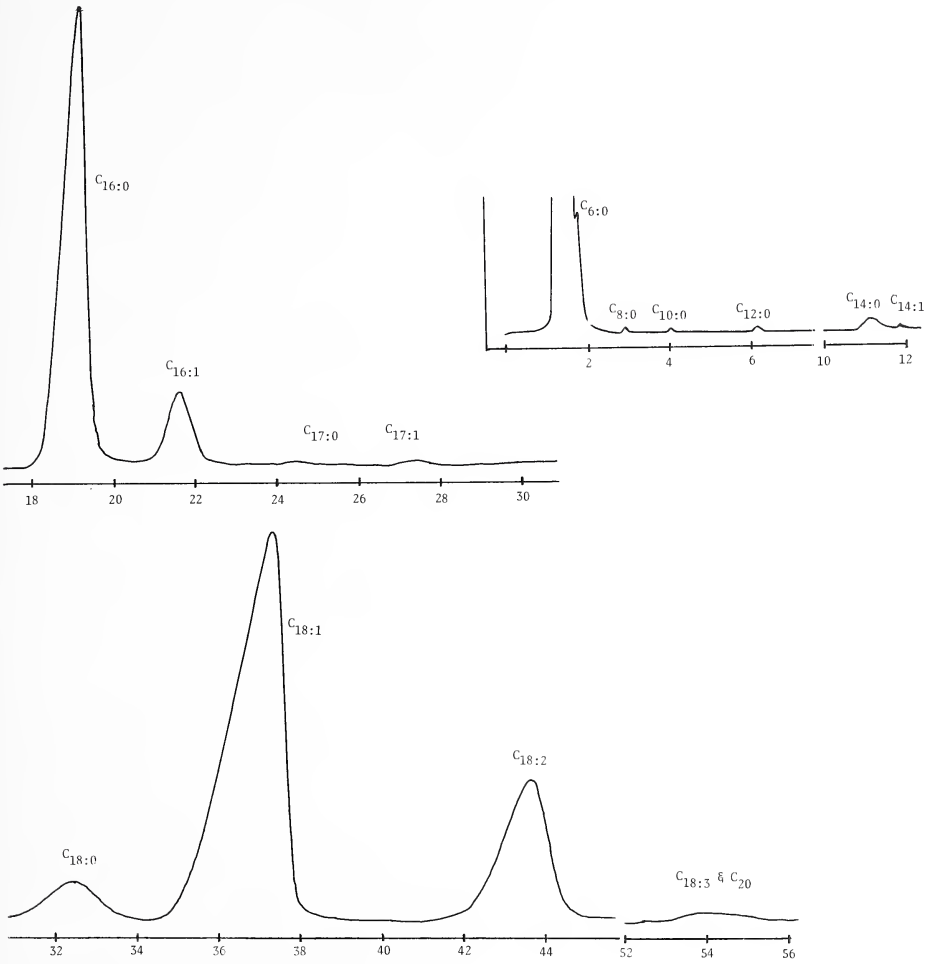

Fig. 1. Gas chromatographic separation and identification of fatty acid methyl esters of total lipids of adult female *B. germanica* . $C_{6:0}$ caproate; $C_{8:0}$ caprylate; $C_{10:0}$ caprate; $C_{12:0}$ laurate; $C_{14:0}$ myristate; $C_{14:1}$ myristoleate; $C_{16:0}$ palmitate; $C_{16:1}$ palmitoleate; $C_{17:0}$ heptadecanoate; $C_{17:1}$ heptadecenoate; $C_{18:0}$ stearate; $C_{18:1}$ oleate; $C_{18:2}$ linoleate; $C_{18:3}$ linolenate; $C_{20:0}$ arachidate. Instrument Settings: Column, 15' x 1/8" OD, 20% diethylene glycol succinate on Chromosorb P; Detector, hydrogen flame ionization; Detector temperature, 240 C; Injection port temperature, 250 C; Column temperature, 200 C; Hydrogen flow rate, 25 ml per minute; Carrier gas and flow rate, helium 40 ml per minute.

TABLE 6. Fatty acid composition of the total lipid extract of *B. germanica* L. at various stages of the life cycle.

Stage	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ & $C_{20:0}$
Ootheca							
Newly formed	0.4 ± 0.1*	29.1 ± 0.9	4.2 ± 0.3	2.3 ± 0.3	44.2 ± 1.9	17.6 ± 2.7	2.2 ± 0.3
5 day old	0.3 ± 0.1	30.7 ± 0.8	4.6 ± 0.3	2.1 ± 0.1	45.5 ± 0.3	15.3 ± 0.4	1.5 ± 0.3
10 day old	0.3 ± 0.1	25.6 ± 0.9	3.6 ± 0.2	3.6 ± 1.1	48.3 ± 0.1	16.5 ± 0.3	2.1 ± 0.9
15 day old	0.4 ± 0.1	25.1 ± 1.3	4.2 ± 0.2	3.3 ± 0.2	47.4 ± 0.7	17.7 ± 0.2	2.0 ± 0.6
Nymph							
1st instar	0.9 ± 0.1	24.2 ± 0.7	3.4 ± 0.4	4.7 ± 0.6	45.5 ± 1.2	19.2 ± 3.1	2.1 ± 0.7
2nd instar	0.9 ± 0.1	26.1 ± 2.1	3.8 ± 1.0	4.4 ± 0.5	44.7 ± 2.2	17.6 ± 0.5	2.5 ± 0.6
3rd instar	1.0 ± 0.0	28.8 ± 0.4	4.9 ± 0.2	3.6 ± 0.1	42.2 ± 0.4	17.0 ± 0.5	2.5 ± 0.6
4th instar	1.0 ± 0.1	28.2 ± 0.6	5.2 ± 0.2	3.7 ± 0.7	43.5 ± 2.5	16.5 ± 1.9	1.9 ± 0.5
5th instar	1.2 ± 0.2	29.4 ± 1.3	6.1 ± 0.6	3.4 ± 0.4	42.2 ± 1.8	15.7 ± 0.7	1.9 ± 0.1
6th instar	1.1 ± 0.1	27.9 ± 2.5	4.8 ± 1.8	3.6 ± 0.8	43.8 ± 1.1	16.7 ± 2.4	2.1 ± 0.3
Adult							
♂	0.9 ± 0.1	24.7 ± 1.0	4.1 ± 0.3	3.5 ± 0.4	45.9 ± 1.6	18.2 ± 1.9	2.7 ± 1.1
♀	0.8 ± 0.1	27.1 ± 1.2	4.6 ± 0.3	3.3 ± 0.1	45.4 ± 1.2	17.0 ± 1.1	1.8 ± 0.3

** Mean of three samples ± standard deviation.*

$C_{14:0}$ myristic acid; $C_{16:0}$ palmitic acid; $C_{16:1}$ palmitoleic acid; $C_{18:0}$ stearic acid; $C_{18:1}$ oleic acid; $C_{18:2}$ linoleic acid; $C_{18:3}$ linolenic acid; $C_{20:0}$ arachidic acid.

Fatty acids contained in the isolated neutral and phospholipid fractions of the 4th instar nymphs, adult males and females were also separated by gas-liquid chromatography. Qualitatively there was no difference between the three types of lipids. Only 8 fatty acids were quantitated. Table 7 shows the relative proportion of these fatty acids. A comparison of the data shows that the proportion of these fatty acids in the neutral lipid fraction approximated the composition in the total lipid extract in all the 3 stages studied. Fatty acid composition of phospholipid fraction showed some differences. The proportion of myristic, palmitic and palmitoleic acids was reduced to half the relative proportion of these acids in total and neutral lipid fractions. The proportion of linoleic acid, on the other hand, was doubled. The phospholipid also contained slightly higher proportion of linolenic and arachidic acids. The fatty acids of the neutral lipid fraction had a higher proportion of saturated fatty acids (40%) than phospholipids (28%).

TABLE 7. Per cent fatty acid composition of the total lipid, and isolated neutral and phospholipid fractions of *B. germanica* .*

Fatty acid	Stage: 4th instar nymph TL	NL	PL	Adult males TL	NL	PL	Adult females TL	NL	PL
$C_{14:0}$	1.0	1.3	0.6	0.9	0.7	0.5	0.8	1.0	0.4
$C_{16:0}$	28.2	30.6	14.5	24.7	28.6	18.3	27.1	31.8	15.0
$C_{16:1}$	5.2	5.6	3.1	4.1	3.8	3.7	4.6	4.5	2.9
$C_{18:0}$	3.7	3.7	6.1	3.5	3.4	3.7	3.3	2.7	4.2
$C_{18:1}$	43.5	41.4	43.3	45.9	45.0	44.9	45.4	44.0	45.9
$C_{18:2}$	16.5	15.3	30.2	18.2	16.4	25.8	17.0	14.3	27.3
$C_{18:3}$ & $C_{20:0}$	1.9	2.1	2.2	2.7	2.1	3.1	1.8	1.7	4.3

** Values presented are the means of 3 replicates. TL - total lipid; NL - neutral lipid; PL - phospholipid.*

$C_{14:0}$ myristic acid; $C_{16:0}$ palmitic acid; $C_{16:1}$ palmitoleic acid; $C_{18:0}$ stearic acid; $C_{18:1}$ oleic acid; $C_{18:2}$ linoleic acid; $C_{18:3}$ linolenic acid; $C_{20:0}$ arachidic acid.

DISCUSSION

Data presented indicate a progressive increase in the wet weight and a decrease in the dry matter content during embryogenesis. The changes reported here agree, in general, with those of Roth and Willis (1955a, b). Ross (1929) and Parker and Campbell (1940) suggested that the wall of the ootheca of *B. germanica* in contact with the female's genital pouch may be permeable to water. Roth and Willis (1955b) demonstrated a difference in permeability between the anterior and posterior end of the ootheca of *B. germanica*. The anterior end, held by the female is lighter in color and less sclerotized, was more permeable. They concluded that the increase in wet weight was due to absorption of water from the female. A similar phenomenon has been observed in *Blattella vaga* Hebard (Roth and Willis 1955b) and *Diploptera dytiscoides* (Serv.) (Roth and Willis 1955c).

The loss of dry matter was accompanied by a loss in the total lipid content. On the average, 75% of the loss of dry matter was accounted for by lipid catabolism. Many workers who studied the embryonic period have found that fat forms the main source of energy of the developing embryo (Tichimirov 1885, Rudolfs 1926, Fink 1925, Slifer 1930, Busnell 1937, Lafon 1959, Rainey 1950, Rothstein 1952, Gilbert and Schneiderman 1961, Kinsella and Smyth 1966, Gilbert 1967b). The only exception was *Tenebrio molitor* L. which utilized glycogen for energy requirements (Ludwig and Ramazzotto 1965). The loss in the initial lipid supply was comparable to the general average of 55% reported by Needham (1931) for various terrestrial animals.

A comparison of the neutral lipid content of newly extruded ootheca and 15 day old ootheca shows a 43% reduction of the initial supply. Of the various lipid classes of the neutral lipids, only triglyceride shows considerable reduction. There is an increase in the mono- and di-glyceride content. These findings agree with those of Slifer (1930), who showed that 54% of the neutral lipids are catabolized by *M. differentialis* during embryonic development. Tichimirov (1885) observed that neutral glyceride fatty acids diminished by 46% during embryonic development in *Bombyx mori*. *Malacosoma americana* (Fab.) utilized 87% of neutral lipids during incubation (Rudolfs 1926). Gilbert and Schneiderman (1961) reported that the moth *Hyalophora cecropia* (L.) catabolized 57% of the initial neutral lipids during incubation. The Japanese beetle, *Popillia japonica* Newman loses 58% of the initial neutral lipid content during incubation. Allais et al. (1964) showed 53% loss of neutral lipid moiety. Kinsella and Smyth (1966) and Gilbert (1967b) observed 55% loss of neutral lipids during embryogenesis in *P. americana* and *L. maderae*.

In the present work it has been shown that the hydrocarbon and sterol content increased only slightly. With the development of the embryo, the proportion of the esterified sterols increases. Tichimirov (1885), Allais et al. (1964) and Gilbert (1967b) reported similar findings during embryonic development of *Bombyx mori* (L.), *L. migratoria* and *L. maderae* respectively, whereas Rudolfs (1926) reported that the cholesterol in the eggs of *M. americana* disappeared by the time of hatching. Kinsella (1966d) and Gilbert (1967b) also observed an increase in the proportion of esteri-

fied sterols during incubation. The more or less constant sterol content ties well with the theory that insects lack the ability to synthesize sterols (Black et al. 1956). However, it is rather difficult to correlate it with the known function of sterols, being a major constituent of cellular membranes. During morphogenesis, it would be expected to increase, unless the sterol stored in the yolk is sufficient to meet the requirements for membrane formation.

During nymphal growth and in the adults the sterol content increases in proportion to the increase of the total fat content. It has been shown that some dietary sterols can be converted into cholesterol (Gilmour 1961).

During nymphal development, all the lipid fractions increase. In order to compare the rate of increase of lipids with that of wet weight, log of wet weight and log of total lipids of the different nymphal instars were plotted and a straight line was drawn. The total amount of fat was related to the wet weight by the equation $y = b\,x^k$, where k is known as the heterauxetic constant. The same formula may also be written in the logarithmic form $\log = \log b + k \log x$. The value of k was close to 1 indicating that the rate of accumulation of fat during nymphal growth occurs more or less at the same rate as that of the total body weight. Similarly, k values were calculated for neutral lipids against total lipids, phospholipids against total lipids, triglycerides against neutral lipids, sterols with neutral lipids and hydrocarbons with neutral lipids (fig. 2a-h). In all the cases the k value was close to unity indicating that all the rate of increase of each fraction paralleled the increase in the other fraction. In holometabolous insects, on the other hand, the k value is greater than unity (Finkel 1948, Fast 1964) indicating that the weight of lipid increases more rapidly during larval growth than the total weight. Accumulation of large amount of fat is advantageous for holometabolous insects, because considerable energy is required for transformation into adult. In addition many adults do not feed and depend upon fat reserves.

The fatty acid composition of *B. germanica* closely resembles that of *P. americana* (Kinsella 1966c). The predominance of oleic acid and palmitic acid is in conformity with other insects (Fast 1964). Only aphids and coccids are peculiar in having a large proportion of myristic acid and low level of oleic acid (Strong 1963, Barlow 1964, Fast 1964). Only trace amounts of fatty acids having carbon chain length more than 20 were found in *B. germanica*. This is in agreement with the observations of most workers. Giral (1946) and Giral, Giral and Giral (1946) reported 25.8% and 46.2% of fatty acids were more than 20 carbon chains long in *S. purpurescens* and *M. atlanis* respectively. Albrecht (1961) reported 72% stearic acid in *Schistocerca gregaria* Forsk. which is a very high value when compared with the iodine number. Usually stearic acid content in insects is lower than 10% (Fast 1964).

The fatty acid composition of all the nymphal instars and the adults are quite similar. These results may be interpreted to mean that there is no selective synthesis or accumulation of any fatty acids during nymphal growth.

The loss in triglyceride is greater than dry matter loss during embryonic development. It is possible that part of the triglycerides are hydrolyzed to 1,2 diglyceride and utilized for the synthesis of phospho-

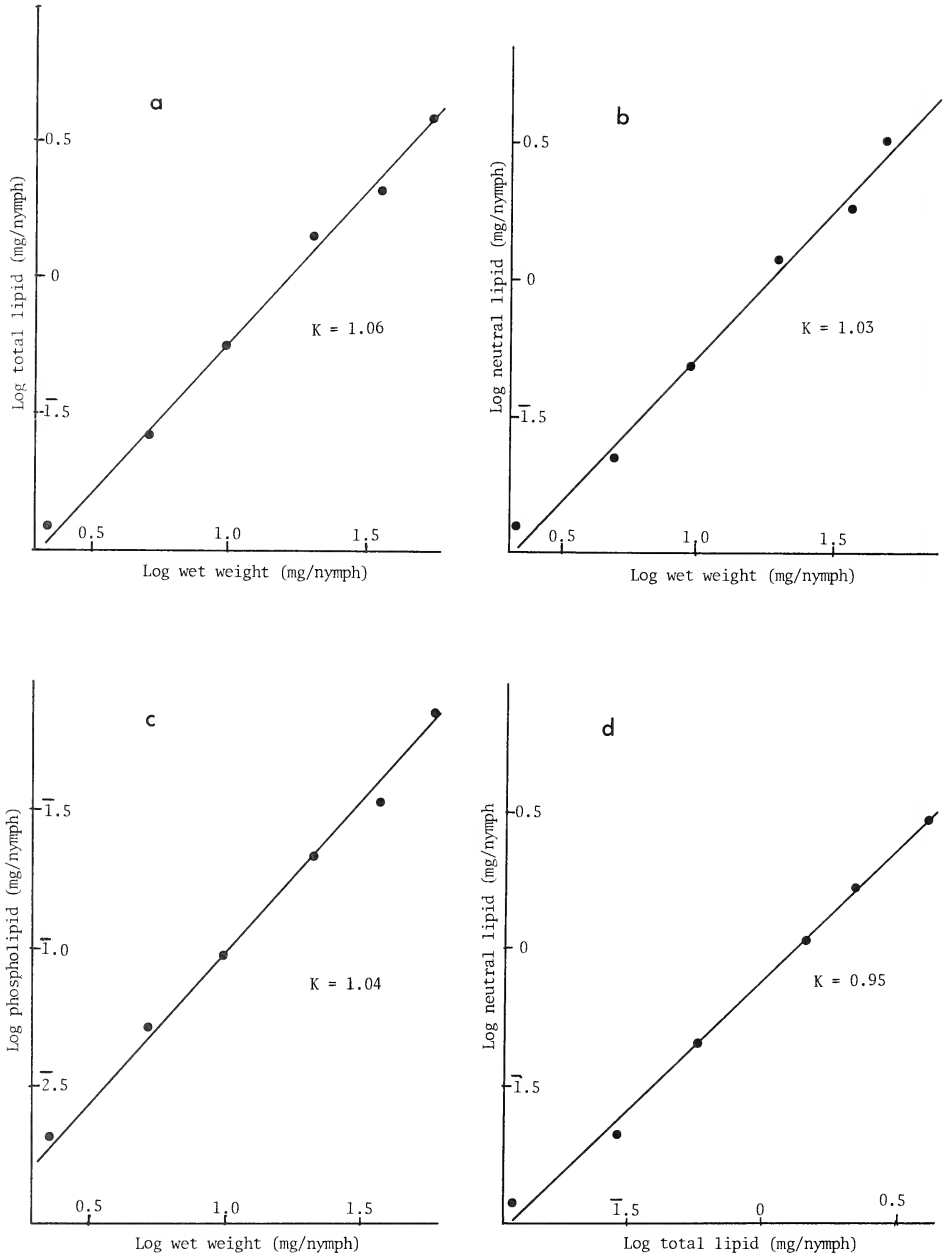

Fig. 2 (a-d). Double-log plot of (a) total lipid against wet weight; (b) neutral lipid against wet weight; (c) phospholipid against wet weight and (d) neutral lipid against total lipid. Graphs are from data in table 2.

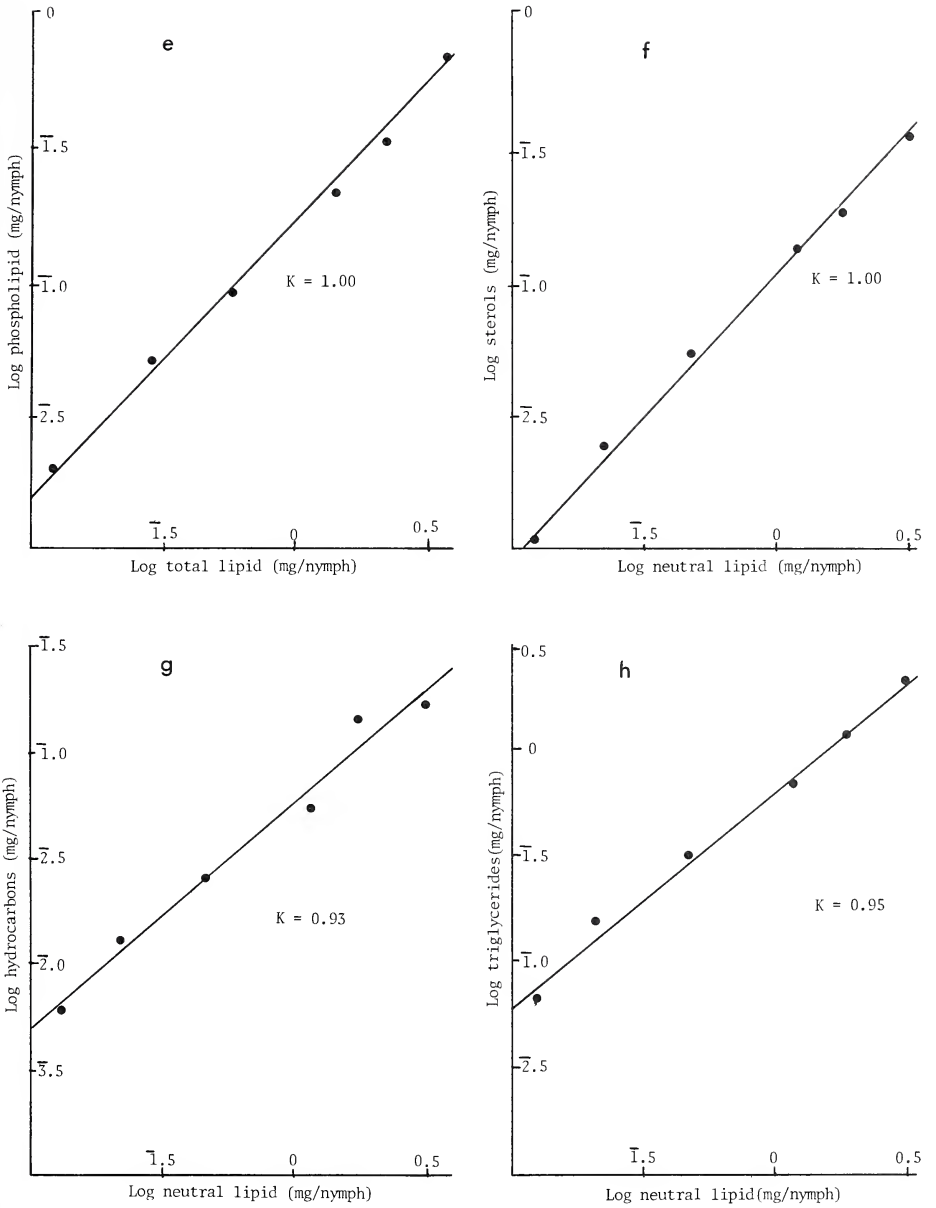

Fig. 2 (e-h). Double-log plot of (e) phospholipid against total lipid; (f) sterols against neutral lipid; (g) hydrocarbons against neutral lipid and (h) triglycerides against neutral lipid. Graphs are from data in table 2 and 4.

lipids via phosphatidic acid. Part of the loss is accounted for by the increase in mono- and di-glyceride content during embryonic development (Kinsella and Smyth 1966, Gilbert 1967b). The fatty acids released from the glycerides may be oxidized completely or utilized for the synthesis of sterol esters.

The phospholipid content increases during embryonic and post embryonic development. During incubation, the increase in the proportion of phospholipids has been shown to be partially due to synthesis and partially due to utilization of triglycerides for energy requirements. Phospholipids are parts of cellular and subcellular membranes (Ansell 1964) and hence will increase during morphogenesis. Similar lipid patterns have been reported by Tichimirov (1885) in *M. americana*; by Pearincott (1960) in *Musca domestica* L.; Bieber et al. (1961) in *Phormia regina* (Meigen); Allais et al. (1964) in *L. migratoria*; Kinsella (1966a) in *P. americana*; and Gilbert (1957b) in *L. maderae*.

The relative proportion of the three major phospholipid classes lecithin, cephalin and sphingomyelin was quite similar to that reported by Allais et al. (1964) in *L. migratoria* and Siakotos and Zoller (1960) and Kinsella (1966a) in *P. americana*. Many dipterans are characterized by the predominance of cephalin over lecithins (Fast 1964).

When calculated on a per individual basis, the lipid phosphorus content increased throughout development as a result of incorporation of non-lipid phosphorus into the phospholipid fraction. Chojnacki (1961) and Chojnacki and Piechowska (1961) studied the mechanism of synthesis in *Celerio euphorbiae* (Fab.) and found it to be similar to biosynthesis in vertebrate liver. Phosphocholine (or phosphoethanolamine) is activated by reaction with cytidenetriphosphate to yield cytidinediphosphate-choline (or ethanol-amine) intermediate which then reacts with a, b diglyceride to yield choline (or ethanolamine) phosphatide.

The three roach species whose lipid metabolism during embryogenesis has been studied, have different oviposition habits. In *P. americana* the ootheca is extruded and carried by the female for only a short period and then deposited. In *B. germanica* the ootheca is extruded and carried by the female until the eggs hatch. In *L. maderae* the ootheca is extruded, then subsequently retracted into a brood sac until or shortly before hatching (Roth and Willis 1954). The incubation period of *P. americana* is twice as long as that of the other two species. The lipid metabolism pattern is, however, similar in all the three species. Thus oviposition or incubation period has very little effect on lipid metabolism.

SUMMARY

During the embryonic development of *B. germanica* there is an increase in the moisture content and a decrease in the dry matter and lipid content. The reduction in the lipid content was due to catabolism of triglycerides. The neutral lipid and triglyceride content decreased by 43% and 54% respectively. The decrease occurs mostly in the second half of development. On the other hand, the mono- and di-glyceride content increased throughout incubation. Phospholipid content increased by 60% during embryo-

genesis. The major components of the phospholipid fraction are phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin. In addition phosphatidyl inositol, lysolecithin and phosphatidic acid were present in small amounts. Hydrocarbon and sterol content showed slight increase. With the progress of embryonic development the proportion of esterified sterols increased. The overall decrease of 75% of the initial lipid store shows that lipids play a dominant role in fulfilling the energy requirements of developing eggs.

During nymphal development, the insect accumulates large amounts of lipid. Increase in the total lipid, neutral lipid and phospholipid content is proportional to the increase in the wet weight of the body. Though the adults had a lower fat content than the last instar nymphs, the lipid patterns are similar. In all the stages studied, triglycerides were the predominant fraction.

Fatty acid analysis of the phospholipid, neutral lipid and total lipid extracts revealed the presence of 17 fatty acids during all stages of the life cycle ranging in carbon chain length from 6 to 22. Palmitic, oleic and linoleic acids were the major fatty acids. Unsaturated fatty acids predominated in the various fractions with oleic acid comprising about 45% of the total fatty acids in all the fractions studied. Palmitic acid was the second most abundant in the neutral lipid fraction, but linolenic was the second most abundant in the phospholipid fraction. Fatty acids of the phospholipids were more unsaturated than the fatty acids of the neutral lipids.

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A PALEOENVIRONMENTAL ANALYSIS OF THREE LATE PLEISTOCENE COLEOPTEROUS ASSEMBLAGES FROM FAIRBANKS, ALASKA

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Fossils of beetles (Order Coleoptera) and other insects are abundant in Pleistocene silts and peats from interior Alaska. Three Wisconsin age silt samples from the Eva Creek exposure near Fairbanks, Alaska were examined for their content of fossil insects. A study of the coleopterous fauna -- and primarily the carabid (ground beetle) portion of the coleopterous fauna -- of each of these samples revealed that at the time of their deposition the environment of Fairbanks, Alaska was similar to alpine tundra at higher elevations or coastal tundra in other parts of the state. This conclusion concerning the paleoenvironment of lowland interior Alaska agrees with conclusions reached by the author and other workers after examination of fossil pollen spectra and fossil mammals from the Eva Creek mining cut and similar exposures near Fairbanks. Minor variations among the three coleopterous assemblages are provisionally related to local environmental differences rather than to changes of the macroclimate of interior Alaska during Wisconsin time.

Like the Quaternary peats and organic silts of Europe, the frozen colluvial silts exposed by placer gold mining in Alaska contain abundant fossils of insects -- predominately beetles (order Coleoptera). In Europe fossil insects have created interest for a long time (see Frey (1964) for a review of the early literature); however, only within the last ten years have such fossils become important for the study of paleoenvironments. Well preserved fossil insects occur in silts and peats in Alaska and Canada yet no paleoenvironmental studies such as those by G. R. Coope in England (Coope 1965) have thus far been published. This paper represents an initial attempt to interpret Pleistocene environments in Alaska using the evidence derived from insect fossils.

Previous Investigations

The success of Pleistocene insect studies in Europe is largely due to the efforts of G. R. Coope and his associates F. E. Shotton and P. J. Osborne at the University of Birmingham, England. This team has gained valuable insight to the glacial, interstadial, and interglacial climates of England since the mid-Pleistocene by comparing the former distributions of fossil Coleoptera with the distributions of their modern counterparts (Coope 1962; Coope 1965, general summary; Coope et al. 1961; Shotton and Osborne 1965). Similar studies have been carried out at Cambridge University by R. Pearson (1962, 1963).

Coope began his studies "blissfully unaware" of the doubts which many entomologists harbored concerning both the geologic longevity of insect species and the possibility of making specific identifications using only single skeletal parts or fragments thereof (Coope 1965). His work was to reveal the fallacy of this skepticism. Nearly all species of Coleoptera have a longevity which greatly exceeds the length of the late Pleistocene and specific identifications often are possible when the morphology of the fossil is compared in detail with modern museum specimens. These two facts form the base for all other studies of fossil insects including this one; however, before identifications of fossil Coleoptera can lead to paleoenvironmental conclusions, two other conditions must be satisfied: the classification of the more important insect groups (in this case certain families of Coleoptera) must be sound, and the ecology or at least the present distribution of the taxa represented by fossils must be known.

Some beetles are of more value than others as indicators of past environments. A fossil of a stenophagous, phytophagous species may be of value in indicating the presence of a certain plant; however, it will yield little direct information about the macro-environment in which the insect originally lived. The family Carabidae (ground beetles) provides the greatest amount of environmental information since its members are predators living in most instances on the surface of the ground and thus under the direct influence of the macro-climate. Some carabid beetles are even restricted to certain types of soils and sediments.

Implied in the use of assemblages of fossil insects to interpret Pleistocene environments is the assumption that the habitat requirements of the species represented by fossils have remained stable. Some workers have considered this assumption to be unacceptable. Greenslade (1965) has cited evidence that certain species of carabid beetles do occupy entirely different habitats in different areas, and he doubts that the ecological requirements of some species can be considered as stable as have been assumed (Greenslade 1965, written communication). Several of the North American carabids, e. g. *Carabus chamissonis* Fisch. and *Nebria nivalis* Payk. possess ecologically disjunct distributions which might be interpreted as being evidence of a great variability in habitat requirements. In such cases, where for example a species common on the tundra is occasionally found in woodland areas, the explanation for the anomaly is best sought by looking for the possibility of accidental dispersal of individuals to areas which only locally fulfill the habitat requirements of the species. Lindroth has invoked this reasoning to explain the occurrence in the coniferous zone of Norway of a colony of the carabid *Amara alpina* Payk., normally an obligate tundra inhabitant (C. H. Lindroth 1965, written communication). Problems of this sort need not prohibit the use of insect fossils as long as the ecology of the species is interpreted in a broad sense and conclusions are based on groups of fossils rather than single specimens. Yet some fossil assemblages still possess anomalous characteristics. For example, studies in England reveal insect assemblages which contain a mixture of now disjunct species (Coope et al. 1961), but in all such cases the assemblages possess an internal consistency which makes the possibility that some species have changed their

ecologic requirements the least likely of several alternative explanations (Coope 1965).

Physical Environment and Biota of Interior Alaska

Fairbanks is located in interior Alaska at the south side of the Yukon-Tanana Upland and adjacent to the Tanana River (fig. 1). The climate is characterized by short, warm summers, cold winters and meager precipitation. The mean annual temperature is -3.28 C (26.1 F) and mean annual precipitation is 29.4 cm (11.6 inches) (Péwé and Paige 1963). Despite a mean annual temperature below 0 C, permafrost in the Fairbanks area has a discontinuous distribution, which has a strong effect on drainage and in turn on local plant communities (Péwé 1966a, Péwé et al. 1965). South facing, well drained slopes support stands of white spruce, birch, and quaking aspen while poorly drained north facing slopes and valley bottoms support more open communities of black spruce, willows, larch (on the poorest drained sites), and sedges (Péwé et al. 1965). Coniferous trees and other upright arboreal species are generally absent above an elevation of 1000 meters.

In Alaska, unlike central Canada, the division between tundra and closed woodland is a complex transition zone. Below the upper limit of conifers in Alaska is an open woodland, usually accompanied by a thick cover of mosses and lichens. This area, is often referred to as the Hudsonian Zone (after Merriam's lifezones) by entomologists (Mason 1956, 1965). Beyond this zone is the tundra, the lower parts of which in interior Alaska are quite shrubby. The tundra in northern Alaska, such as at Barrow (fig. 1), is characterized by a reduced number of plant taxa and a paucity of shrub-like arboreal species (Britton 1966). This type of tundra might be considered equivalent to the "true Arctic tundra" of the Canadian literature or "*regio alpina media*" in Scandinavia (Mason 1965).

A peculiar situation exists in the distribution of part of the Alaskan insect fauna. Whereas in eastern Canada the boundary between forest and tundra is as sharp with respect to insects as plants (Brown 1965), in Alaska members of the Hudsonian insect fauna extend well beyond coniferous treeline. For example, Brown (1965) has described the coleopterous fauna at Umiat (fig. 1) as "primarily Hudsonian", and Mason (1956) considers the insect fauna of all the treeless areas south of the north portion of the Seward Peninsula and including the Aleutian Islands to be Hudsonian in character. Lindroth (1961, 1963b, 1966) in his monographs on the carabid beetles of Canada and Alaska seldom uses Merriam's lifezones. Apparently he considers coniferous treeline to be an important ecologic boundary with the understanding that the distribution of some species seems to be more or less independent of it.

The Origin of the Frozen Silts in the Fairbanks Area

Although interior Alaska was only locally glaciated during the Pleistocene, heavy glaciation in the Alaska Range and the Brooks Range affected the interior indirectly by changing the fluvial regimes of its two major trunk streams, the Tanana and the Yukon Rivers. During periods of marked glacial advance and retreat in the Alaska Range, the Tanana

River at Fairbanks was an outwash stream with a wide, sparsely vegetated, silty flood-plain. Silt removed by wind from the flood-plain was deposited on the hills near Fairbanks as loess, so that today silt partially fills the valleys and mantles the slopes to depths of more than 100 feet (Péwé 1955, 1966a). The loess of the valleys is different from that on the slopes. The valley silt is bedded, perennially frozen, highly organic, and fetid (which explains the miner's term for it -- "muck"). The upland silt facies like the loess of the mid-continent of North America is massive, oxidized, buff brown in color, and compared to the valley bottom facies, quite unfossiliferous. Péwé (1966a, et al. 1965) believes that the "muck" facies is colluvium formed by redeposition of loess from the slopes.

Most exposures of frozen "muck" near Fairbanks have been created by placer gold mining. The muck overlies early Pleistocene gravels that contain gold; consequently, the overburden of silt must be hydraulically removed before actual mining can begin. An operation of this sort creates a mining "cut" with walls of frozen silt. One such mining cut is at Eva Creek, ten miles west of Fairbanks (fig. 1). During the summer of 1964 R. D. Guthrie (University of Alaska) and I made extensive fossil collections at this site.

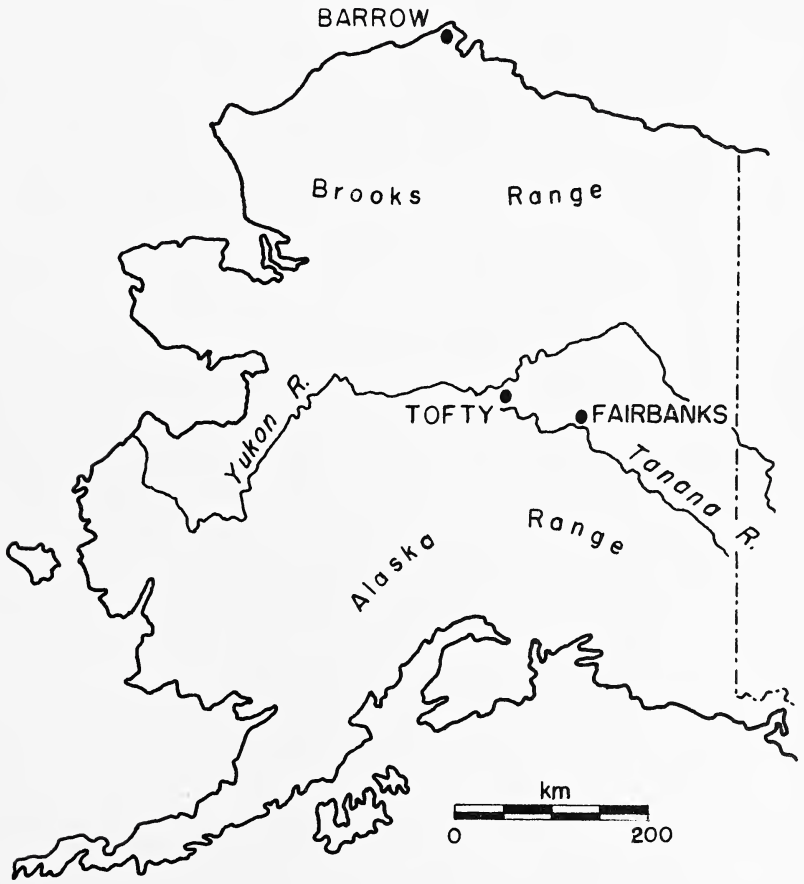

Fig. 1. Alaska. Localities mentioned in text.

The Eva Creek cut is of particular significance because it exposes all of the silt units which Péwé has described for the Fairbanks area (Péwé et al. 1965 and unpublished MS); however, since this report involves only samples from Wisconsin age sediments, details of the other stratigraphic units will not be discussed.

The thickness of the entire silt exposure at Eva Creek is approximately 35 m of which 11 m are of Wisconsin age. The Wisconsin silt is overlain by a Holocene silt unit containing, at some localities, buried forest beds (fig. 2). It rests unconformably on silt thought to be of Illinoian age (Péwé 1965, personal communication) (fig. 2). This unconformity is related to a long period of thaw and erosion (Péwé 1966a) probably representing the Sangamon interglacial.

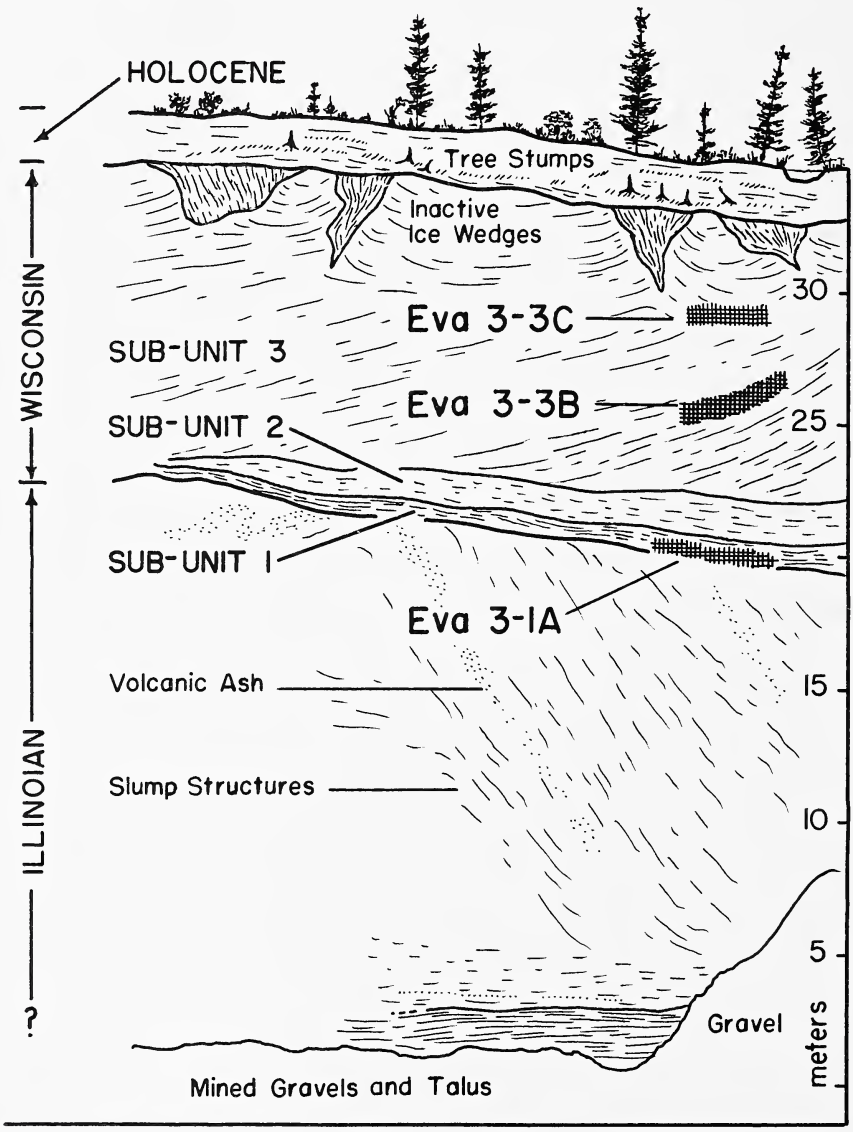

Fig. 2. Eva Creek Exposure, 1965.

Sample Localities within the Wisconsin Unit (Eva Creek)

The Wisconsin portion of the Eva Creek exposure may be divided into three sub-units (fig. 2) which are differentiated by color, ice content, and organic content. Some of these sub-units are probably only of local extent.

Sample Eva 3-1A comes from the basal two feet of Wisconsin silts. It is part of sub-unit 1 which is characterized by an extremely high content of wood (including a few stumps of small trees) and other plant fragments, most of which seem to be from mosses. This unit thickens toward the axis of the north-south trending valley and dips in a down valley direction. M.A. Geyh of Niedersächsisches Landesamt Für Bodenforschung, Hanover, has attempted C-14 analysis of a spruce stump (Hv. 1328) from several feet above the sample interval, but still within sub-unit 1. A final date is not yet available, but Geyh states (1967, written communication) that the first tests indicate an age greater than 56,900 years B.P. This date may not, however, indicate the time of deposition of the sediments in sub-unit 1 since the tree stumps in that unit could have been derived from older sedimentary units (Sangamon forest bed?).

A second sub-unit -- not sampled -- lies immediately above sub-unit 1. It consists of greenish, inorganic silt with a high content of seam ice (Taber ice, sirloin ice).

Sub-unit 3, from which samples 3-3B and 3-3C were taken is the thickest of the three sub-units and most like the dark brown Wisconsin "muck" at other frozen silt exposures in the Fairbanks area. Sample 3-3B was taken from a level 5.5 m above the base of the Wisconsin unit; sample 3-3C was collected 3.7 m above 3-3B. The only criteria used for selection of the two samples from sub-unit 3 was that they come from different levels in the sub-unit. A radiocarbon date of 24,400 ± 650 years B.P. (I-2116) was obtained on wood from the site of sample 3-3C. No radiocarbon date is available for 3-3B, but its position with respect to 3-3C and 3-1A indicates an age greater than 24,400 years B.P. and less than the age of the early Wisconsin sediments of sub-unit 1.

Sampling Procedure and Processing Notes

The insect fossils reported on in this paper were collected in conjunction with a search for small mammal fossils. The procedure followed for each sample was to take several thousand pounds of silt from a two foot interval at the exposure. This was then screened through 40 mesh per inch screens. A portion of the organic extract that remained on the screens was then processed for fossil insects. Since insect fossils are very abundant often only a small portion of the total residue from a sample was required in order to obtain a large number of specimens. This portion of the residue was washed through 80 mesh per inch screens in order to remove any remaining silt.

A process developed by G.R. Coope (1961) was used to concentrate the insect fossils in order that they might be more readily extracted from the organic residue. In this process, the residue remaining in the 80 mesh screens is immersed in light weight oil. When the oil soaked mass of residue is placed in hot water many insect fragments rise to the surface. The concentration of insect fossils obtained in this manner is

stored in alcohol and later examined with a binocular microscope. Insect fossils are mounted on slides similar to those used in the study of Foraminifera. This facilitates storage of the fossils, prevents their warping when they have dried, and allows detailed examination under high magnifications.

Identification Notes

The most striking feature of the faunal list (table 1) is the imbalance in the level of identifications. With few exceptions specific determinations were possible only among the carabid fossils. Generic determinations must suffice for the other families because of my unfamiliarity with many of these families and the real scarcity of knowledge concerning the coleopterous fauna of Alaska. Generic determinations of beetles often have little paleoenvironmental value inasmuch as few genera are restricted to specific habitats. Tabulation of the identified genera within each family and especially a recognition of the number of specimen types (species?) within each genus nevertheless permits an estimate of the total number of taxa present in each fauna. For the same reason, fossils which could not be identified to the familial or generic level are listed in table 1.

Were it not for the fact that the ecology and taxonomy of the carabid beetles of Alaska are now reasonably well documented, a paleoenvironmental analysis such as the one presented here would be impossible. Except for fossils of curculionid beetles (weevils) the carabids are the most abundant group within each of the fossil assemblages. Fortunately, the anatomical parts which are of particular value in carabid identification -- the pronotum, head, and elytra -- are often well preserved in organic silts and peats.

Most of the carabid fossils can be identified using C. H. Lindroth's detailed descriptions (Lindroth 1961, 1963b, 1966) and comparative museum specimens. Determinations within one group, however, the subgenus *Cryobius* of the genus *Pterostichus*, are very difficult. Specific identification of members of *Cryobius* is critical in this study because of the importance of *Cryobius* species in present arctic and subarctic habitats. A recent revision of *Cryobius* by G. E. Ball (1966) has placed the classification of the group on a firm basis, but the similarity among members of certain species pairs causes specific determinations of some fossils to be very uncertain. Indeed, to identify many living specimens to the specific level requires an examination of the median lobe of the male genitalia which is rarely encountered as a fossil in Pleistocene silts.

I have hesitated to assign specific names to many of the *Cryobius* fossils, this being especially the case where the fossils (pronota) were broken or otherwise damaged. Ball (1966) has divided the species of the subgenus *Cryobius* into species groups; therefore, in those cases in which I have not been able to make a specific determination, I have, nevertheless, carried the identification as far as the species group level. Certain of the species groups are restricted to specific habitats, and thus yield definite paleoenvironmental information. For example, the members of the *similis* subgroup are largely tundra inhabitants. A determination to the *ochoticus* subgroup level has certain special implications. One of the species of this group, *P. gerstlensis* Ball, may be eliminated from

consideration because it is quite distinct and readily identified. Thus, an *ochoticus* subgroup determination implies that *P. gerstlensis* Ball, a woodland inhabitant, has been ruled out as a possibility. All the remaining members of the group are tundra residents (Ball 1966). This is not the place to consider in detail the pitfalls of identification of fossil *Cryobius* specimens, but because the *Cryobius* identifications are so important for the conclusions reached in this paper and because my confidence in the specific identifications varies, I have indicated in table 1 with a question mark (?) those determinations which are suspect.

TABLE 1. Coleopterous fauna of Eva Creek samples 3-1A, 3-3B, 3-3C.

Taxa	Number of individuals			Ecology*
	3-1A	3-3B	3-3C	
Carabidae				
Carabus truncaticollis Eschz.	1		1	A
Carabus chamissonis Fisch.	2		1	Be
Notiophilus sp.	3			
Notiophilus semistriatus Say	2		3	BeF
Notiophilus borealis Harr.	2			BeF
Diacheila polita Fald.	6			BeF
Elaphrus riparius L. or *pallipes* Horn	1			
Dyschirius sp.	1			
Dyschirius nigricornis Mtsch.	1			BeF
Bembidion sp.			4	
Bembidion (Plataphodes) sp.		1		
B. (Plataphodes) arcticum Lth.	1			A
B. (Peryphus) sp.	2	2	5	
B. (Peryphus) grapei Gyll.			1	BeF
B. (Peryphus) dauricum Mtsch.	1	9	9	A
Pterostichus (Cryobius) sp.	18	2	19	
P. (Cryobius) pinguedineus grp.	5	3	2	
P. (Cryobius) ochoticus subgrp.		6	9	
P. (Cryobius) soperi Ball	5	4	4	Bx
P. (Cryobius) kotzebuei Ball	2	6	27	Bx
P. (Cryobius) tareumiut Ball	9	2		Bx
P. (Cryobius) gerstlensis Ball		2		CF
P. (Cryobius) chipewyan Ball	1?			
P. (Cryobius) similis subgrp.	3		3	
P. (Cryobius) similis Mann.	4			Ba
P. (Cryobius) parasimilis Ball	3	1		A
P. (Cryobius) pinguedineus Eschz.	2	1	2	BaF
P. (Cryobius) auriga Ball	1?		1?	

TABLE 1 (cont.).

Taxa	Number of individuals			Ecology*
	3-1A	3-3B	3-3C	
P. (Cryobius) ventricosus grp.	2			
P. (Cryobius) ventricosus Eschz.	8	1	4	Be
P. (Cryobius) caribou Ball		1		A
P. (Cryobius) brevicornis grp.	15	7	12	
P. (Cryobius) brevicornis Kby.	11	6	11	BaF
P. (Cryobius) mandibularoides Ball	2?	2?	2?	
P. (Cryobius) nivalis Sahlb.	34	6	5	A
P. (Stereocerus) haematopus Dej.	2	4	1	Ba
Agonum quinquepunctatum Mtsch.	1			CF
Amara alpina Payk.	3	5	5	A
Cymindis sp.			1	
Trichocellus porsildi Brown	2		6	Bx
Genus sp.	1	1		
Dytiscidae				
Colymbetes sp.			1	
Staphylinidae				
Staphylininae				
Acylophorus sp.	1		1	
Paederinae				
Lathrobium sp.	9			
Omaliinae				
Olophrum sp.	30		4	
Micralymma sp.	4	36		
Tachyporinae				
Tachinus sp. A	7	2	1	
Tachinus sp. B	2	5		
Genus sp.			1	
Steninae				
Stenus sp. (A and B)			6	
Stenus sp. A	2			
Stenus sp. B	2			
Dianous sp.		7		
Aleocharinae				
Genus sp.			8	

TABLE 1 (cont.).

Taxa	Number of individuals			Ecology*
	3-1A	3-3B	3-3C	
Silphidae				
Silpha trituberculata Kby. or *sagax* Mann.			1	
Scydmaenidae				
Veraphis sp.			2	
Scarabaeidae				
Aphodius sp. A	1			
Aphodius sp. B	1	1	35	
Byrrhidae				
Curimopsis sp.	1	17	2	
Caenocara sp.			1	
Simplocaria sp.	6			
Morychus sp. A	1	1	2	
Morychus sp. B		1		
Elateridae				
Genus sp. A	1		2	
Genus sp. B	1		2	
Genus sp. C		1		
Cryptophagidae				
Cryptophagus sp. A			2	
Cryptophagus sp. B			1	
Chrysomelidae				
Chrysolina sp. A	1	1	2	
Chrysolina sp. B		1		
Curculionidae				
Lepyrus gemellus Kby.	1	4	1	
Genus spp.	68	165	240	
	2 spp.	5 spp.	5 spp.	
Family and Genus unknown				
Genus sp. A	1			
Genus sp. B	2	13	1	
Genus sp. C	3			
Genus sp. D	2		1	
Genus sp. E	1			

TABLE 1 (cont.).

Taxa	Number of individuals			Ecology*
	3-1A	3-3B	3-3C	
Genus sp. F	1			
Genus sp. G	1			
Genus sp. H	1			
Genus sp. I	1			
Genus sp. J	2	2	5	
Genus sp. K	1	1	3	
Genus sp. L			16	
Genus sp. M			2	
Genus sp. N			4	
Genus sp. O			1	
Genus sp. P			1	
Genus sp. Q			1	
Total individuals	312	330	488	

** Ecologic class symbols are explained in the text.*

Notwithstanding the proven longevity of many coleopteran species, the similarity of some of the species of the subgenus *Cryobius* seems to indicate relatively late divergence; in fact, Ball (1963a) in his first zoogeographical paper on the group suggested that speciation may have occurred as late as Wisconsin time. Now (Ball 1966) he feels that the last episode of taxonomic splitting occurred earlier than 90,000 years ago, but he emphasizes that this must be supported by fossil evidence of which there was little available when he published the revision. Fossil evidence substantiating Ball's statements on speciation within *Cryobius* would add strength to the assumption that all of the *Cryobius* fossils in the three Eva Creek samples represent extant species. Such fossil evidence does exist, but not from Eva Creek.

A peat sample from the McGee cut (fig. 1) 100 miles west of Fairbanks in the Tofty mining district has yielded partially articulated specimens of carabid beetles, particularly *Cryobius*. In many specimens the male or female genitalia are preserved allowing positive specific identifications. The following species of the subgenus *Cryobius* were identified: *P. (Cryobius) similis* Mann., *P. (Cryobius) parasimilis* Ball, *P. (Cryobius) pinguedineus* Eschz., *P. (Cryobius) brevicornis* Kby., *P. (Cryobius) nivalis* Sahlb., *P. (Cryobius) mandibularoides* Ball, and *P. (Cryobius) tareumiut* Ball. Of these *parasimilis* and *similis* are thought by Ball (1966) to have been among the most recent to evolve. The sample comes from the base of the Wisconsin portion of the exposure (D. M. Hopkins 1966, personal communication). A radiocarbon analysis indicated an age greater than 39,900 years B. P. (I-2248) for the fossiliferous horizon.

The evidence from the McGee cut thus indicates that *P. parasimilis*

Ball and *P. similis* Mann. along with a few other species of *Cryobius* were in existence during early Wisconsin time. This evidence certainly does not confirm all of Ball's (1966) phylogenetic speculations concerning the time of evolutionary divergence within the subgenus *Cryobius*, but it does show that he was correct in those instances for which fossil evidence is now available. This enhances the probability that fossils of *Cryobius* in the oldest Wisconsin sample at Eva Creek do represent extant taxa. Unpublished work by the author dealing with Alaskan insect faunas much older than those reported on in this paper indicates that the other carabid taxa in the faunal list (table 1) have geologic longevities which greatly exceed the age of the oldest sample considered here (Eva 3-1A).

Derivation of Quantitative Data

In order that each of the three faunas might be compared quantitatively, the number of individuals represented by each taxon was tabulated. This number indicates the maximum number of individuals which could be represented by summing one diagnostic fragment (right elytron, pronotum, etc.). For example in Eva 3-3B the staphylinid genus, *Micralymma*, is represented by 36 heads. A tabulation of the elytra (seven right, six left) can account for only seven individuals. Thus, the greatest number of individuals (36) is represented by heads. In Eva 3-3C the staphylinid genus, *Stenus* is represented by six pronota, but an examination of *Stenus* elytra, which represent fewer than six individual beetles, indicates that two species are probably present. Therefore, the faunal list indicates six individuals of *Stenus* including at least two species.

The particular anatomical part that was used for tabulation varied from one group to another. The pronotum was used for all members of the genus *Pterostichus*, the elytra for members of the genus *Bembidion*, and in one exceptional case, *Silpha* sp. from Eva 3-3C, the scutellum was diagnostic. Only elytra were used for the tabulation of miscellaneous species. In many cases an elytron is represented by only a basal or apical fragment; therefore, tabulations were weighted so that two such fragments from the same beetle would not be counted as two individuals.

Coope (1961) has questioned the validity of using quantitative information for making faunal comparisons. His argument, based on tests, is that human bias is introduced during the extraction of fossils with the microscope. The biggest and most brightly colored species are often represented by the most fossils, while the small species are reciprocally under-represented. At Eva Creek this type of bias does not seem to be important. The largest and most spectacular species are often represented by very small fragments. Fortunately, such species (*Carabus trucaticollis* Eschz. is a good example) may be identified by examination of the small fragments, but very little may be said about individual abundance. Ten fragments could as easily have come from one insect as ten, but the faunal list must record the minimum number -- 1. The same situation applies to other large beetle fossils from the Eva Creek samples. Small fossils often are well preserved, and easily located at magnifications of 20X. While I do not claim that human bias of the type described by Coope (1961) is absent in this study, I do not believe that it is sufficiently significant to prohibit the use of quantitative data.

Ecologic Classification of Fossils

In order that the environment represented by each fossil assemblage can be discussed those fossils which have been positively identified to species (Carabidae) are assigned to ecologic classes A, B or C according to the habitat preference of their living counterparts (see table 1). The letter A indicates that a species is an obligate tundra inhabitant. Some of those species listed in table 1 do not now occur on the alpine tundra of interior Alaska, but this is probably an artifact of limited collecting.

The letter B indicates that a particular species occurs both in tundra and woodland areas. The suffix "e" is attached when it is known that the species prefers "open" environments when it occurs below coniferous treeline. For example, I have collected *Diacheila polita* Fald. in the Fairbanks area. It is most often found on the tundra, but at Fairbanks it occurs in a *Carax* bog -- an open (treeless) habitat. In addition to those species such as *Pterostichus (Cryobius) trevicornis* Kby. which occur both in closed boreal forest and on tundra (Ba), class B includes many species which are known to occur on tundra but about which there is some doubt concerning their occurrence at lower elevations. These cases of uncertainty are designated by the suffix "x".

The species placed in class "C" are those which have never been collected above the limit of coniferous treeline. One of these species, *Agonum quinquepunctatum* Mtsch., occurs in wet, open habitats (bogs). Species which have been collected at Fairbanks or nearby at the same elevation are indicated in table 1 by the letter "F".

Fossil Evidence, excluding Insects, from Eva Creek

Other fossil evidence is available with which information yielded by fossil insects may be compared. Large mammal fossils from many late Pleistocene localities near Fairbanks include extinct taxa such as *Mammuthus* sp., *Equus* sp., *Camelops* sp., *Bison priscuc* (*sensu lato*) and others which presumably required an open grassy habitat (Guthrie 1968, Péwé 1966a) as well as extant taxa -- *Rangifertarandus, Ovis nivicola* , and *Ovibos moschatus* -- that are now restricted to alpine or coastal tundra (Péwé 1966a). Small mammal fossils have been collected from the three sample intervals which yielded the coleopterous faunas reported in this paper. They, like fossils of large mammals indicate not only that the Wisconsin "mucks" of the Fairbanks area were deposited in an open, largely treeless environment, but specifically that the three samples (3-1A, 3-3B, 3-3C) at Eva Creek represent such an environment. Fossils of *Dicrostonyx* sp. and *Microtus gregalis* have been found in the three sampled intervals (Guthrie in press). Both of these microtines are now found on the tundra; however, neither occurs on the alpine tundra of the Tanana hills near Fairbanks.

Fossil pollen was extracted by the author from the silts of each of the three sampled levels. The pollen spectra from the three localities (table 6) are similar and indicate a treeless environment -- an environment too cold to support abundant dwarf birchs, alders, and ericaceous shrubs (Matthews MS).

Thus, fossils of vertebrates and pollen indicate that at the time dur-

which the three insect assemblages accumulated, treeline was significantly lower in interior Alaska. This conclusion will be compared with the environmental inferences derived from an analysis of the three fossil Coleoptera assemblages.

Discussion

The results presented in table 1 may be analyzed in two ways. One may compare the taxonomic content of each of the assemblages with the taxonomic composition of various contemporary Alaskan environments. The contemporary environment which most closely matches that of the fossil assemblage is judged to be the one that represents the paleoenvironment in which the fossils lived. This is the method that Coope has used in the analysis of fossil assemblages in England. Table 2 represents such an analysis using only specifically identified beetles from each assemblage. It is immediately apparent that the number of taxa available for a comparison of this type is small (maximum of 21 species). This results from the fact that specific determinations were possible only within the family Carabidae. In order to counter the effect of this small sample size I have chosen to compare the three assemblages quantitatively. The validity of the quantitative data presented in table 1 has been discussed in a previous section.

A quantitative approach similar to that used with the qualitative data (table 2) would be desirable. Unfortunately this is impossible since it requires that carabid faunas of the major environments in Alaska have been analyzed quantitatively. No such studies have been attempted. In lieu of such information I have proceeded on the basis of several assumptions relating to the way in which a fossil assemblage might theoretically reflect the environment in which it was deposited. First, in the three assemblages of fossils, the numerically dominant species will be those which resided at the site of deposition. A minority of the fossils will represent beetles which lived in habitats other than those existing near to or at the site of deposition. Whether this latter group of fossils is in fact a minority depends on the former diversity of the beetle fauna at the site of deposition, the powers of dispersal of species in former neighboring habitats, and the proximity of those habitats to the site of deposition. For example, a community proximal to the site of deposition of a fossil assemblage and possessing a great number of actively flying beetles might have contributed more fossils to the assemblage than a community with a beetle fauna composed of non-flyers. The beetles from the neighboring community might well form the majority of the members of a fossil assemblage if the fauna of that community was taxonomically diverse and numerically abundant relative to the fauna at the site of deposition of the sample. It is unlikely that a large fraction of the carabid portion of each Eva Creek assemblage consists of fossils of such allochthonous beetles, since the dominant element of each assemblage is of the subgenus *Cryobius* (genus *Pterostichus*), all species of which are constantly flightless.

Interpretation of the fossil assemblages from Eva Creek is made more complex by the retransported or colluvial character of the fossil bearing sediments. Theoretically, insects which lived in habitats at

higher elevations on the slopes near Eva Creek and which were penecontemporaneous with insects at low elevations could have been retransported with the silt into the valley bottom. Also, since deposition of sediments in one area, usually implies erosion in another, some of the fossils in each assemblage may have been derived from older sediments. Many insect fossils would probably not survive this type of redeposition especially when the sediments from which the fossils were derived were much older geologically than the sediments in which they were finally deposited. Contamination of the fossil insect assemblages by penecontemporaneous mixing during accumulation of the assemblage can be evaluated only indirectly -- by examining the consistency of the environmental implications of each assemblage.

TABLE 2. Abundance of species of Carabid beetles in each ecologic class.

Assemblage	Ecologic Class*						Total of A, B, and C
	A	Σ B	Be	Ba	Bx	C	
Eva 3-3C	4**	10	4	3	3	0	14
Eva 3-3B	5	7	1	3	3	1	13
Eva 3-1A	6	14	6	4	4	1	21

* *See text for explanation of class symbols.*
** *Number of species.*

Table 3 shows the relative abundance of beetles in each ecologic class. The dominance of class B is partly related to its containing species about which extralimital woodland occurrences are in doubt (class Bx). If more detailed ecologic information were available, class A would no doubt be larger and class Bx smaller. Nevertheless, the remarkable feature of table 3 is the preponderance of fossils in classes A and B and the paucity of fossils in class C.

To duplicate any of the fossil assemblages with a collection of modern carabid beetles, one would have to go to a locality near to or above the altitudinal or geographic limit of coniferous forest. A similar conclusion may be derived from the taxonomic analysis (table 2). But a statement that each of the assemblages represents a tundra environment might be challenged on the premise that all of the obligate tundra fossils (group A) are allochthonous -- having been included in the fossil assemblages by retransportation with the silt from higher elevations. If this were the case, the fossil assemblages might well have formed below treeline in the Hudsonian Zone. This is clearly not the case since the carabid fossils which would be expected to represent that zone are not present in any of the fossil assemblages. For example, *Pterostichus adstrictus*

Eschz., *Calathus ingratius* Dej., *Asaphidion yukonense* Wick., *Harpalus fulvilabris* Manh. *Bembidion mutatum* G. and H., *Carabus vietinghoffi* Adams, and *Trichocellus cognatus* Gyll., are common elements of the woodland carabid fauna of Alaska. Each of these beetles is morphologically distinct, they occur in a variety of habitats although most prefer open situations, and most of them range on to the tundra in parts of Alaska (Lindroth 1953, 1961, 1963a, 1963c, 1966). If any of the assemblages accumulated in a habitat which was below or near treeline, fossils of some of these species would be expected. They do not occur in any of the Eva Creek assemblages; therefore, all three of the assemblages no doubt represent a tundra environment. The position of coniferous treeline must have been below the present elevation of Eva Creek (230 m - 750 feet) during that part of Wisconsin time when each of the assemblages formed, and the Fairbanks area was largely, if not completely, treeless.

This conclusion agrees with the alternate fossil evidence presented earlier. The colder climate which must have been responsible for the lowered treeline is also indicated by inactive or remnant ice wedges in the sediments at Eva Creek. Currently such features are forming only in those areas where the mean annual temperature is at least 2 C colder than the present mean annual temperature of Fairbanks (Péwé 1966b).

TABLE 3. Abundance of individuals of carabid beetles in each ecologic class.

Assemblage	Ecologic Class*						Total of A, B, & C
	A (%)	Σ B (%)	Be	Ba	Bx	C (%)	
Eva 3-3C	20**(25.0)	60(63.7)	9	14	37	0 (00)	80
Eva 3-3B	22 (45.8)	24(49.9)	1	11	12	2(4.1)	48
Eva 3-1A	43 (42.1)	58(56.7)	21	19	18	1(0.9)	102

* *See text for explanation of class symbols.*
** *Number of individuals.*

Though all three of the fossil assemblages were evidently deposited in a tundra environment, each of the assemblages possesses certain distinctive characteristics. Statistical tests (table 4) are used to establish the mutual relationship, if any, of the assemblages, and an attempt is made to provide environmental interpretations for the observed differences among the three fossil assemblages.

Several of the statistical comparisons in table 4 indicate that samples Eva 3-3B and 3-3C are more closely related to each other than either is to Eva 3-1A. The basis for this conclusion is the lack of statistically significant differences between 3-3B and 3-3C. The failure of a com-

parison of two samples to be statistically significant implies that the two samples could have been drawn from the same parent population. In this paper such a conclusion will be taken to imply that the two compared assemblages represent the same type of paleoenvironment.

TABLE 4. Statistical tests*.

Type of comparison	N	Comparison	Computed χ^2	χ^2 .05
Relative preservation of Carabidae skeletal elements in each assemblage				
	180	3-3B, 3-3C	8.772	3.841
Pronota -	262	3-1A, 3-3C	*0.046* **	3.841
	196	3-1A, 3-3B	10.315	3.841
	198	3-3B, 3-3C	17.370	3.841
Right elytra -	129	3-1A, 3-3C	*3.637*	3.841
	179	3-1A, 3-3B	5.628	3.841
	189	3-3B, 3-3C	13.459	3.841
Left elytra -	237	3-1A, 3-3C	7.755	3.841
	190	3-1A, 3-3B	*1.427*	3.841
Number of individuals in Coleoptera families common to all assemblages	759	3-3B, 3-3C	66.640	12.592
	730	3-1A, 3-3C	120.250	12.592
	609	3-1A, 3-3B	79.962	12.592
Number of taxa in families of Coleoptera common to all assemblages	112	All three	*3.931*	21.026
Number of individuals of Curculionidae in each assemblage	819	3-3B, 3-3C	*0.108*	3.841
	805	3-1A, 3-3C	65.093	3.841
	652	3-1A, 3-3B	61.025	3.841
Number of individuals in the genera *Pterostichus, Amara* and *Bembidion*	197	3-3B, 3-3C	*0.788*	5.991
	260	3-1A, 3-3C	12.480	5.991
	205	3-1A, 3-3B	15.990	5.991
Number of individuals in the species groups of *Pterostichus (Cryobius)*	120	3-3B, 3-3C	*0.480*	9.488
	173	3-1A, 3-3C	25.431	9.488
	139	3-1A, 3-3B	17.375	9.488

* χ^2 *test of independence for binomial and multinomial populations.*

** *Nonsignificant* χ^2 *values are in italics.*

Differential preservation of certain fossils could bias conclusions concerning the taxonomic and numerical relationships of 3-3B and 3-3C. To test this contingency I have made a statistical comparison of the preservation of carabid fossils in the three assemblages (table 4). The results of these comparisons indicate that differential preservation does exist; however, the relationships of the three assemblages based on preservation are different from those based on qualitative and quantitative taxonomic composition. Thus, the qualitative-quantitative comparisons showing 3-3B and 3-3C to be related are probably not initially biased by differences in preservation of the fossils. It is interesting that the two samples which are shown to be related by the statistical analyses represent the same sub-unit in the Wisconsin portion of the Eva Creek exposure.

Samples Eva 3-3C and 3-1A display the greatest degree of taxonomic and quantitative difference. Some of the major differences cannot be explained at this time, yet their existence, established in part by the statistical tests, is assumed to be evidence of paleoenvironmental differences. For example, the obvious difference between the two assemblages is indicated in the miscellaneous species section of table 1. There are very few species common to both assemblages 3-1A and 3-3C. The relative abundance of individuals of curculionid beetles (weevils) is also markedly different in the two assemblages, but only one curculionid, *Lepyrus gemellus* Kby. was identified specifically, and this species is rare in all three assemblages.

Finally, Eva 3-3C differs from 3-1A (and 3-3B) by its relative abundance of fossils of the scarabaeid genus *Aphodius*. In England, Coope has observed a correlation of concentrations of large mammal fossils with an abundance of *Aphodius* fossils, the explanation being that *Aphodius* has copraphogous habits (Coope & Sands 1966). But Landin (1961) pointed out that some species of *Aphodius* are not coprophagous, and of the few species that are known from Alaska, some are associated with small mammals such as *Marmota* rather than ungulates (W. J. Brown 1967, written comm.). Thus, at the present time and until specific identifications are forthcoming, I am hesitant to invoke Coope's explanation for the abundance of *Aphodius* fossils.

Those differences between 3-1A and 3-3C which are subject to interpretation occur within the family Carabidae. The greatest contrast is within the subgenus *Cryobius*, genus *Pterostichus* . In 3-3C (and 3-3B) the species *P. (Cryobius) kotzebuei* Ball is abundant. In Eva 3-1A this species is rare, and *P. (Cryobius) nivalis* Sahlb. is abundant (see table 1). *P. (Cryobius) kotzebuei* Ball has been collected by Ball (1967, personal communication) on rather dry tundra, and since it is a dominant element in the carabid fauna of Eva 3-3C, I conclude that dry tundra was probably present at the site of deposition of that assemblage approximately 24,400 years ago. Supporting this conclusion is the presence in 3-3C (and 3-3B) of individuals of the species *Bembidion dauricum* Mtsch. which inhabits xeric tundra sites that are almost devoid of vegetation (Lindroth 1963b). *Notiophilous semistriatus* Say, occurring in both 3-1A and 3-3C, occupies similar habitats (Lindroth 1961) and *Cymindis* sp. represented by one fossil in 3-3C, often occurs in xeric habitats (Ball 1963b).

P. (Cryobius) nivalis Sahlb. has been collected on rather dry tundra, but

Ball (1966) states that it is also associated with "deep moss". The organic residue from which the fossils of 3-1A were extracted possessed an abundance of moss fragments. *P. (Cryobius) nivalis* Sahlb. evidently lived in the environment at the site of deposition of 3-1A -- hence its abundance in the fossil assemblage. *P. (Cryobius) tareumiut* Ball and *P. (Cryobius) similis* Mann., both of which occur in 3-1A and probably not in 3-3C are found presently on rather moist tundra (Ball 1963c, 1966). Several of the carabid fossils from assemblage 3-1A indicate that an aquatic environment may have existed near the site of deposition. *Bembidion arcticum* Lth. occurs now along the barren and gravelly margins of small streams (Lindroth 1963b). Both species of *Elaphrus* listed in table 1 for the one *Elaphrus* fossil in 3-1A are found in a similar habitat -- areas, usually near streams or ponds, which are devoid of vegetation (Lindroth 1961). *Agonum quinquepunctatum* Mtsch. is a hygrophilous species. It may not have lived at the site of deposition of 3-1A, but its presence in the assemblage is further evidence for the existence of an environment near by which was favorable for its survival. It is of interest that Lindroth (1966) lists *Dischirius nigricornis* Mtsch., another beetle restricted to assemblage 3-1A, as a contemporary associate with *Agonum quinquepunctatum* Mtsch. in a bog near Edmonton, Alberta. Eva 3-3C contains a fossil of an aquatic beetle, *Colymbetes*, but the absence of any other identified beetles which indicate an aquatic or near aquatic environment suggests that *Colymbetes* does not represent a community near the site of deposition of 3-3C.

With the exception of the fossils of *P. (Cryobius) kotzebuei* Ball and *P. (Cryobius) nivalis* Sahlb. the differences between the carabid portions of the two assemblages, 3-1A and 3-3C, are subtle; consequently, the environmental interpretations are somewhat speculative. A more definite concept of the meaning of the variation of the two assemblages might be obtained if they could be compared quantitatively and qualitatively with data from contemporary environments. As I have said earlier no such information is available, but the fossil assemblage from McGee Cut near Tofty, Alaska (fig. 1) is suitable for such a comparison (table 5).

Many of the beetle fossils extracted from the peaty silt at the McGee cut were partially articulated, and almost only those that were so preserved are included in table 5. Thus the assemblage is made up of those beetles which lived at or very near the site of deposition of the peaty silt. The fact that many of the fossils are better preserved than dead specimens collected in the contemporary environment indicates that some of the fossil beetles probably died during hibernation.

Pollen analysis of the sediments from which McGee fossils were extracted reveals an unusual pollen spectrum consisting of more than 70% sedge pollen and 20% willow pollen (Matthews MS). This is a clear case of over-representation in the pollen spectrum by elements of the local fossil plant community; that is, the plants growing at the site of deposition -- the habitat in which the fossil beetles originally lived.

Assemblage 3-1A is more similar to the McGee assemblage than is 3-3C. I consider this to be further evidence that the environment represented by assemblage 3-1A was more moist than that represented by assemblage 3-3C. The differences between 3-1A and the McGee assemblage are best explained by the depositional histories of the two samples.

The McGee assemblage, consisting of well preserved fossils derived from peaty silt, represents a local, monotypic habitat. The Eva 3-1A assemblage derived as it is from colluvial silt no doubt represents a polytypic habitat. This explains the association in 3-1A of fossils of *Pterostichus haematopus* Dej., *Carabus chamissonis* Fisch., *Notiophilus semistriatus* Say, and *Notiophilus borealis* Harr. (beetles normally found in areas of scant vegetation) with fossils of beetles indicative of a moist tundra habitat. The implication is that the taxonomic diversity of the carabid fauna of 3-1A is a reflection of the diversity of the paleoenvironment in which the fossil beetles lived. Compared to assemblage 3-1A the carabid fauna of 3-3C is much less taxonomically diverse, even though the sample size of 3-3C is larger and the diversity at the familial level is greater. Perhaps this implies that the paleoenvironment represented by 3-3C was both drier and more uniform than that represented by 3-1A.

TABLE 5. Fossil Coleoptera from the McGee cut.

Taxa	Number of individuals
Carabus truncaticollis Eschz.	3
Diacheila polita Fald.	4
Pterostichus (Cryobius) pinguedineus grp.	6
Pterostichus (Cryobius) ochoticus subgrp.	1
Pterostichus (Cryobius) tareumiut Ball	2
Pterostichus (Cryobius) similis subgrp.	10
Pterostichus (Cryobius) similis Mann.	22
Pterostichus (Cryobius) parasimilis Ball	11
Pterostichus (Cryobius) pinguedineus Eschz.	4
Pterostichus (Cryobius) ventricosus grp.	1
Pterostichus (Cryobius) ventricosus Eschz.	1
Pterostichus (Cryobius) brevicornis Kby.	13
Pterostichus (Cryobius) mandibularoides Ball	3
Pterostichus (Cryobius) nivalis Sahlb.	1
Agonum quinquepunctatum Mtsch.	1
Amara alpina Payk.	8
Total	91

Fossil assemblage 3-3B from Eva Creek was shown by the statistical tests to be allied with 3-3C; however, in some respects it differs from both 3-3C and 3-1A. Of these differences, one, fewer taxa in 3-3B, is probably related to the smaller sample size of 3-3B. Other characteristics of 3-3B may not be explained so easily. For example, it contains an abundance of fossils of the staphylinid genus *Micralymma*, the species of which are tundra inhabitants (M. Sanderson 1967, written communi-

cation) and the byrrhid genus, *Curimopsis*, presently a very rare insect. Though these differences are not subject to detailed environmental interpretation, I do believe they indicate that the paleoenvironment of Eva Creek was not identical when assemblages 3-3C and 3-3B accumulated. Minor differences in the content of plant macrofossils and the pollen spectra of 3-3C and 3-3B tend to support this conclusion (Matthews MS).

To what extent are the different tundra environments represented by the fossil insect assemblages indicative of climatic change? Palynologists working in arctic Alaska have found that late Pleistocene climatic changes produced only subtle modifications of the composition of former tundra plant communities (Colinvaux 1964, Livingston 1955). Also, there is abundant evidence that world wide climatic oscillations occurred in the time interval during which the three Eva Creek insect assemblages were deposited. These facts seem to imply that the observed variability of the insect assemblages is a reflection of such climatic change.

It is necessary to refer to evidence of fossil pollen in order to test this implication. Because pollen is more easily and more widely dispersed than insects, fossil pollen provides a more generalized picture of the environment of a region such as interior Alaska, and it is changes in the general environment which are coupled with macroclimatic change. Though the three pollen spectra associated with the insect fossils are not identical (table 6), they are similar enough to be placed in the same pollen zone according to the system of pollen zonation now in use in northern Alaska (Livingston 1955). This means that similar climates existed in the Fairbanks area when each insect assemblage formed. Accordingly, the differences among the insect assemblages cannot have major climatic significance. More than likely those differences reflect features of a local environment such as the Eva Creek watershed. The position of permafrost, proximity of water, exposure to sunlight, amount of vegetation cover, and rate of deposition of loess are just a few of the variables in such a local environment which may have influenced the composition of the insect assemblages.

TABLE 6. Fossil pollen spectra associated with Eva Creek fossil insect assemblages.

	Percentage of pollen types		
	Eva 3-3C	Eva 3-3B	Eva 3-1A
Spruce	-	+	-
Birch	+	9	12
Alder	+	4	9
Willow	10	26	24
Sedge	36	22	19
Grass	11	11	12
Artemisia	16	7	
Misc. pollen	10	9	7
Misc. spores	13	7	15

- *Indicates only a trace of pollen seen.* + *Indicates less than 3%.*

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EFFECTS OF THE SIZE AND FREQUENCY OF BLOOD MEALS ON *CIMEX LECTULARIUS* L.

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Successive small blood meals can induce moulting in C. lectularius. *Interval between meals is important in the effect on moulting. The food conversion efficiency in the different instars varies between 25.6 and 37%. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%. Unfed females did not lay eggs and the number of eggs laid per female as well as longevity showed good correlation with the amount of blood ingested.*

Accounts of the biology of *Cimex lectularius*, reared under various conditions, have been published by Bacot (1914), Hase (1917, 1919, 1930), Cragg (1923), Jones (1930), Kemper (1930), Janisch (1933, 1935), Kassianoff (1937), Johnson (1939, 1940a, 1940b, 1942), de Meillon and Golberg (1946, 1947), and Bell and Schaefer (1966). More recently Usinger (1966) published a monograph on Cimicidae reviewing most of the work done on this group of insects. Similarly studies on fertilization and physiology of reproduction of the adults have been published by Cragg (1915, 1920), Mellanby (1935, 1939a) and Davis (1964, 1965a, 1965b). Although a relationship between food supply and the growth of *C. lectularius* has been demonstrated (Titschack 1930 and de Meillon and Golberg 1946 and 1947), the effect of the size and frequency of blood meals has not been studied. This investigation was undertaken with a view to filling some gaps in the knowledge of bedbug biology.

METHODS

The threshold of hatching, nymphal development, and adult activity is between 13 and 15 C (Hase 1930, Mellanby 1935, Kemper 1936, Johnson 1942). The thermal death point is 44 C. Omori (1941) found that development ceased at 36 or 37 C. The temperature of 80 F (26.7 C) and 75% R.H. used for the stock culture proved to be in the range of optimum conditions for development, and was used for the experiments. The insects were kept in 4 x 4 x 1.5 cm plastic boxes with folded pieces of filter paper and were allowed to feed twice every week on human blood. The insects fed through the organdie covering a 3 cm diameter hole in the lid of the plastic box. The eggs were laid on the folded filter papers which were collected in new boxes where the eggs were allowed to hatch at the same temperature and relative humidity.

Two series of experiments were conducted for studying the effects of different blood meal sizes on *C. lectularius* .

In the first series newly moulted insects, taken from the standard culture, were kept singly in 2 x 7 cm specimen tubes with a 2 cm^2 piece of folded filter paper. The insects were fed only once on the second day after moulting. Feeding periods of five or six different lengths were used for each instar to provide the different meal sizes. For each feeding period 20 insects were used from every instar. Observations on subsequent moulting and longevity were recorded daily.

The efficiency of the various instars in converting human blood into body tissue and extra-cellular fluid was determined by the method given by Friend et al. (1965). The amount of blood required to produce 1 mg gain in body weight was calculated. The average difference in body weight between instars divided by the weight of the blood meal and multiplied by 100 is the food conversion efficiency % (Friend et al. 1965). Twenty first instar nymphs were taken from the standard culture immediately after hatching for determining the weight changes during development and the food conversion efficiency of the different instars. These insects were fed to capacity on the second day after hatching and once every ten days at least thereafter. The changes in body weight were recorded during development till the insects reached maturity. This way of estimating food conversion efficiency is subject to certain errors. Calculation of food conversion efficiency is always done on a dry weight basis, but is still subject to some errors. The most important of these arise from changes and differences in water content of both blood and insects, and from the presence of blood residues in the gut.

Newly moulted adults were used for studying the effects of different blood meal sizes on fecundity, longevity, and the duration of the preoviposition period. The following combinations of males and females and blood meal size were studied:

unfed female x engorged male
female fed for 60 seconds x engorged male
female fed for 120 seconds x engorged male
female fed for 240 seconds x engorged male
engorged female x unfed male
engorged female x engorged male
engorged female x male fed for 60 seconds
engorged female x male fed for 120 seconds
engorged female x male fed for 240 seconds.

For each combination 20 pairs were used and each pair was put in a single 2 x 7 cm specimen tube together with a 2 cm^2 piece of filter paper.

In the second series of experiments eggs were taken from the standard culture and put separately in 2 x 7 cm specimen tubes each with a piece of filter paper. The insects were fed on the second day after hatching. Eighty insects in four groups of 20 were used for the study of the effects of each feeding period. The four groups were given the blood meal of known duration at frequencies of 2, 4, 8, and 16 days respectively. Observations were carried out daily and the effects of the different feeding periods and their frequencies on the duration of the nymphal stadia, and on the preoviposition period; fecundity, longevity, and weight changes during development were recorded.

As most of the insects did not reach the adult stage when the feeding periods were 15, 60, or 120 seconds, at the different frequencies, an additional experiment was conducted. One hundred and sixty pairs in four groups of forty were taken from the standard culture as soon as they moulted from the fifth instar and used to complete the study of the effects of the size and frequency of blood meals on fecundity, longevity and the duration of the preoviposition period of the female and male. The feeding periods used were 15, 30, 60, and 120 seconds at the same

frequencies as before. Ten pairs were used for each frequency.

Effects of the Size of One Blood Meal

On the Nymphal Instars

The effects of the size of one blood meal per instar on per cent moulting, duration of the nymphal stadia, longevity and mortality rates were studied. Statistical analysis was undertaken to determine the level of correlation between the amount of blood ingested and each of duration of the nymphal stadia and longevity. A theoretical straight line relationship was assumed for the effect on the duration of the nymphal stadia and the longevity. The average decrement in the duration of the nymphal stadium and the average increment in the longevity per unit increase in the weight of the blood meal were estimated by calculating the coefficient of linear regression. The discrepancies between the observed values and the theoretical ones were shown by calculating the chi square to test the goodness of fit to the straight lines.

The effect of the size of one blood meal per instar on the percentage of nymphs which moulted in the different instars is shown in figs. 1a - e. No insect in any instar moulted if fed for 30 seconds or less. In all instars 100% moulting occurred if the insects were fed till engorgement. In all five nymphal instars there were positive correlations between the size of the blood meal and the percentage of nymphs which moulted; the correlation coefficients ranged between 0.916 and 0.999.

The minimum feeding period for a single blood meal to induce moulting was 60 seconds in the first and second instars, 40 seconds in the third instar, and 120 seconds in both the fourth and fifth instars. These periods are all less than half of the maximum feeding periods. Friend et al. (1965) stated that *R. prolixus* nymphs of all instars will moult normally when fed less than 50% of the maximum meal and for the third instar only 24.7% of the maximum was required. Locke (1958) caused fourth-instar nymphs of *R. prolixus* to moult by feeding them 35% of the maximum meal. The blood meal supplies the nutrients and stretches the abdomen. The latter initiates the hormone cycle that results in moulting (Friend et al. 1965). Wigglesworth (1963) demonstrated that nutrients alone do not stimulate moulting and this was supported by the work of Beckel and Friend (1964). The latter workers explained that release of the moulting hormone, and activation and division of the epidermis is caused by stretching and they claimed that in their experiments, because of either inadequate nutrient or some undiscovered factor, the moulting cycle was halted and the animal died prematurely. Locke (1958) studied the effect of the blood intake on the diameter of the tracheae produced. He found that the increase in the diameter at moulting in many of the tracheae in *Rhodnius* is proportional to the size of blood meal. From these studies on *C. lectularius* it was found that, in all the nymphal instars, moulting can be induced by blood meals much smaller than the full blood meal and that there is always a positive correlation between the size of the blood meal and the percentage of moulting insects.

Figs. 1a - e also show the effect of the size of the blood meal on the longevity of the different instars. The longevity of all five instars increased gradually with the increase in the size of the blood meal. The correlation coefficients ranged from 0.773 to 0.993. In the first four

instars chi square tests showed departure from a straight line relationship. In the fifth instar, on the other hand, the relationship proved to be a straight line.

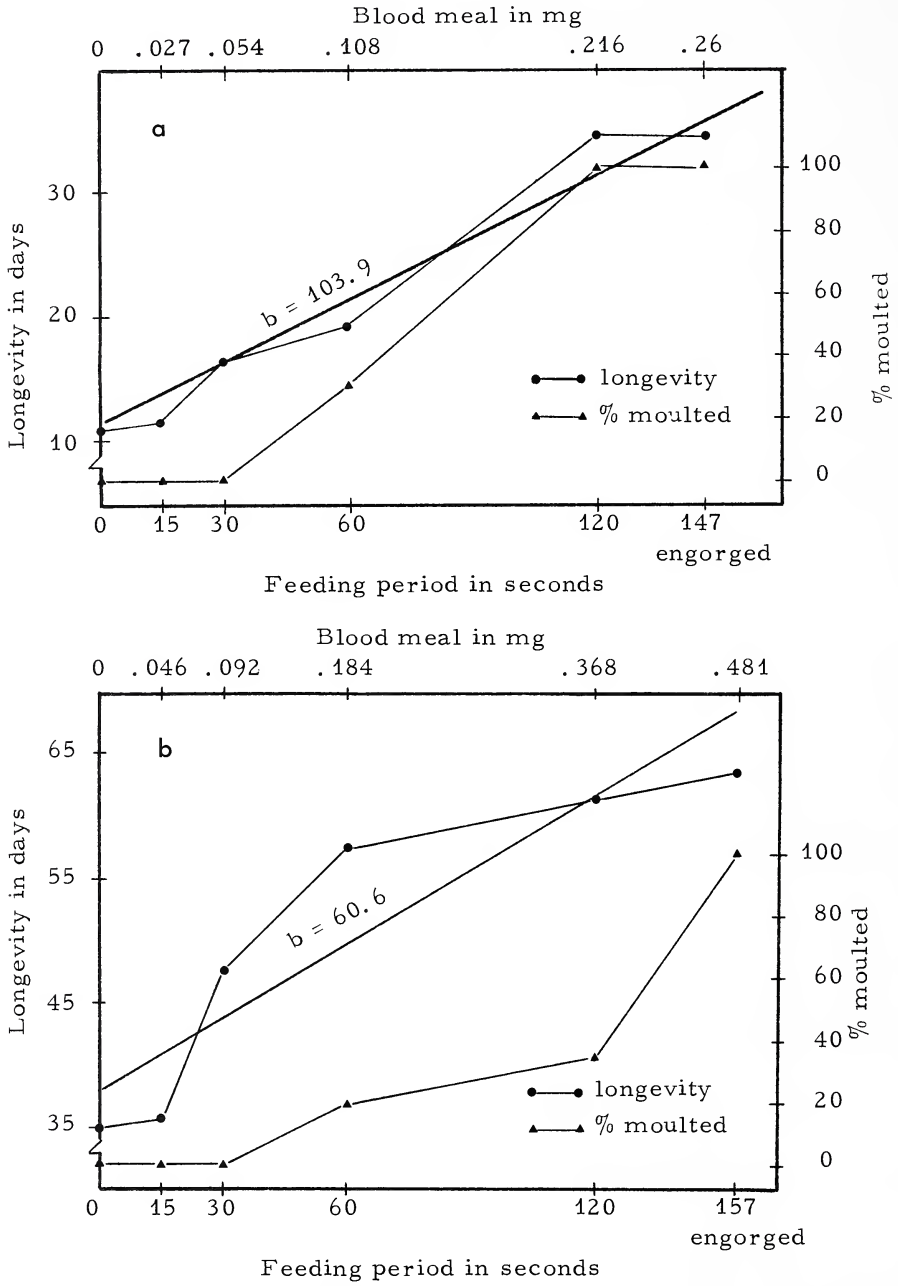
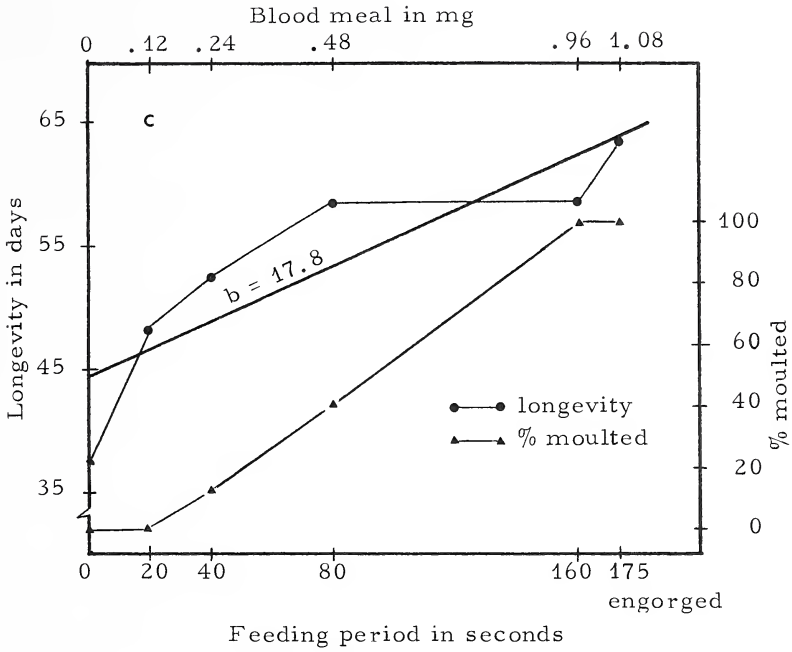


Fig. 1. Effect of the size of a single blood meal on the longevity and moulting of nymphs of *C. lectularius*. a. first instar; b. second instar.



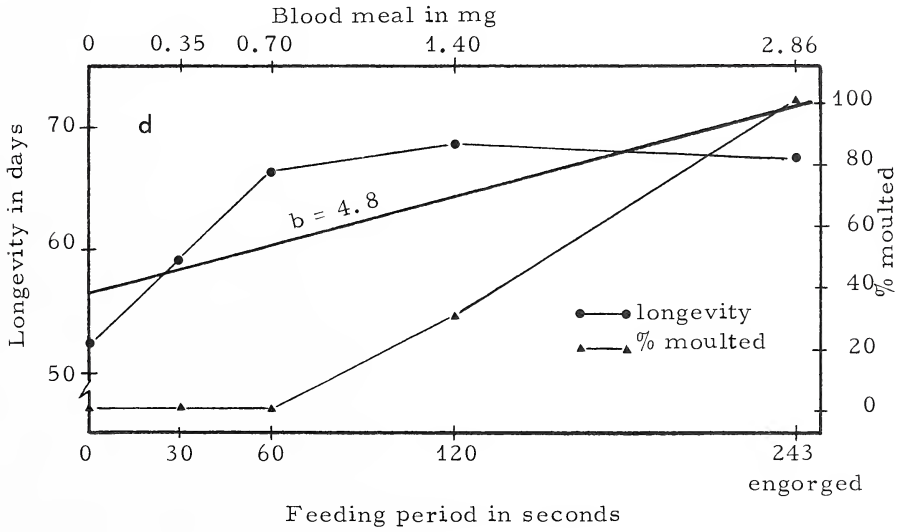


Fig. 1. c. third instar; d. fourth instar.

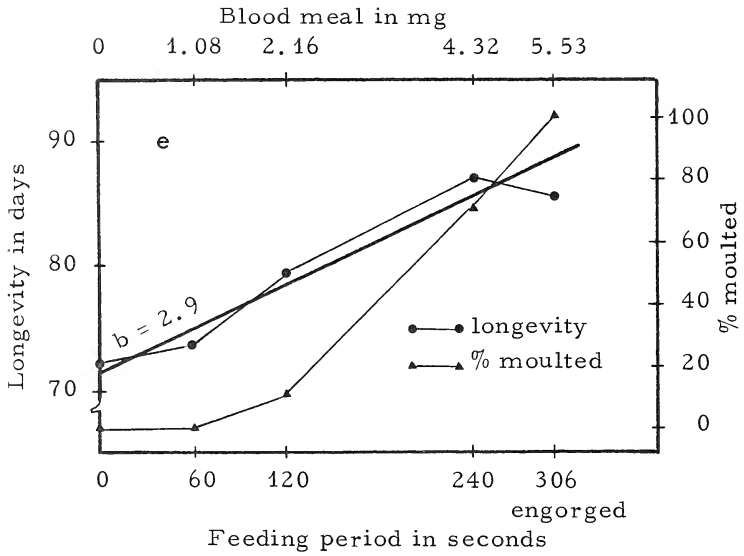


Fig. 1. e. fifth instar.

The effect of the size of the blood meal on the duration of the nymphal stadia was determined from those insects which moulted in each group. The duration of the nymphal stadium was taken as the period elapsing between the moult before feeding and that after feeding. The results are shown in table 1. In all the five nymphal instars there was a slight decrease in the duration of the nymphal stadium with the increase in the size of the blood meal. The correlation coefficient ranged between -0.79 and -0.99.

To estimate the effect of the size of a single blood meal on the mortality rate of the nymphal instars, the data were analyzed by probit method (Finney 1947). The LT50 was found for each blood meal size from the provisional probit line (figs. 2a to 2e). The reliability of the estimate is such that if the experiment is repeated there is 5% chance of getting an LT50 value that is not within the fiducial limits.

The LT50 values and their fiducial limits are shown in table 2. In all the five nymphal instars it was found that the LT50 increases with the increase in the size of the blood meal.

TABLE 1. Effect of the size of a single blood meal on the duration of the different stadia of *C. lectularius*.

Stadia:	Feeding period in seconds										
	0	15	20	30	40	60	80	120	160	240	engorged
1st											
x*	0	0.021		0.041		0.083		0.165			0.260
y**						5		4.7±0.15			4.5±0.13
	-	-		-		(6)		(20)			(20)
						5		4 - 5			4 - 5
2nd											
x	0	0.046		0.092		0.184		0.368			0.481
y						7		7			6.3±0.01
	-	-		-		(7)		(7)			(20)
						7		7			6 - 7
3rd											
x	0		0.124		0.248		0.496		0.992		0.087
y					7		7		6.4±0.01		5.6±0.01
	-		-		(2)		(8)		(20)		(20)
					7		7		6 - 7		5 - 6
4th											
x	0			0.035		0.704		1.408			2.856
y								7			6.6±0.01
	-			-		-		(6)			(20)
								7			6 - 7
5th											
x	0					1.086		2.172		4.345	5.533
y								8		6.2±0.08	6
	-					-		(2)		(14)	(20)
								8		6 - 7	6

* x = average weight of the blood meal in mg.

** y = mean duration of the stadium ± S.E.
(number of insects)
range

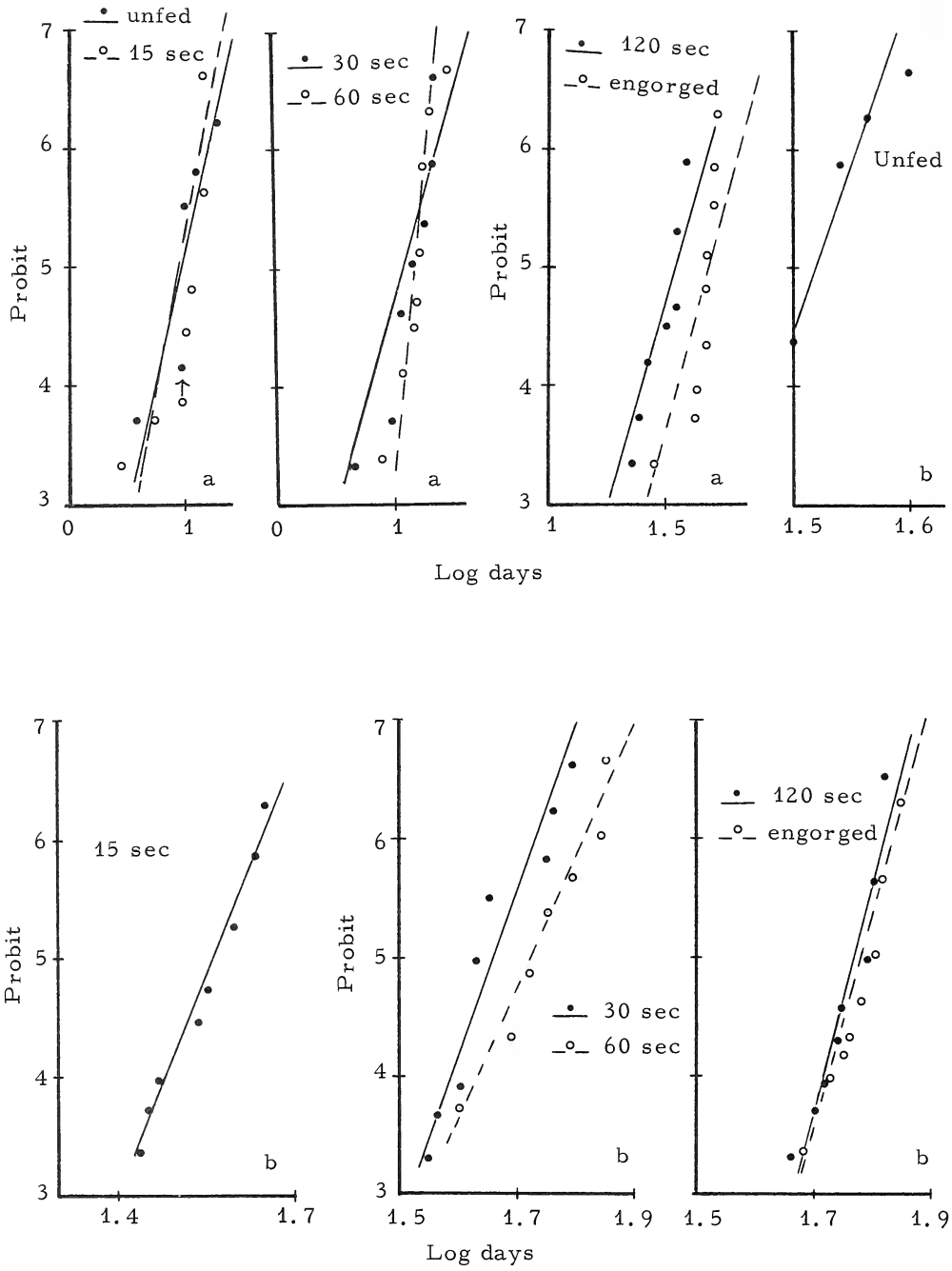


Fig. 2. Probit lines for longevity at various meal sizes in *C. lectularius*. a. 1st instar; b. 2nd instar.

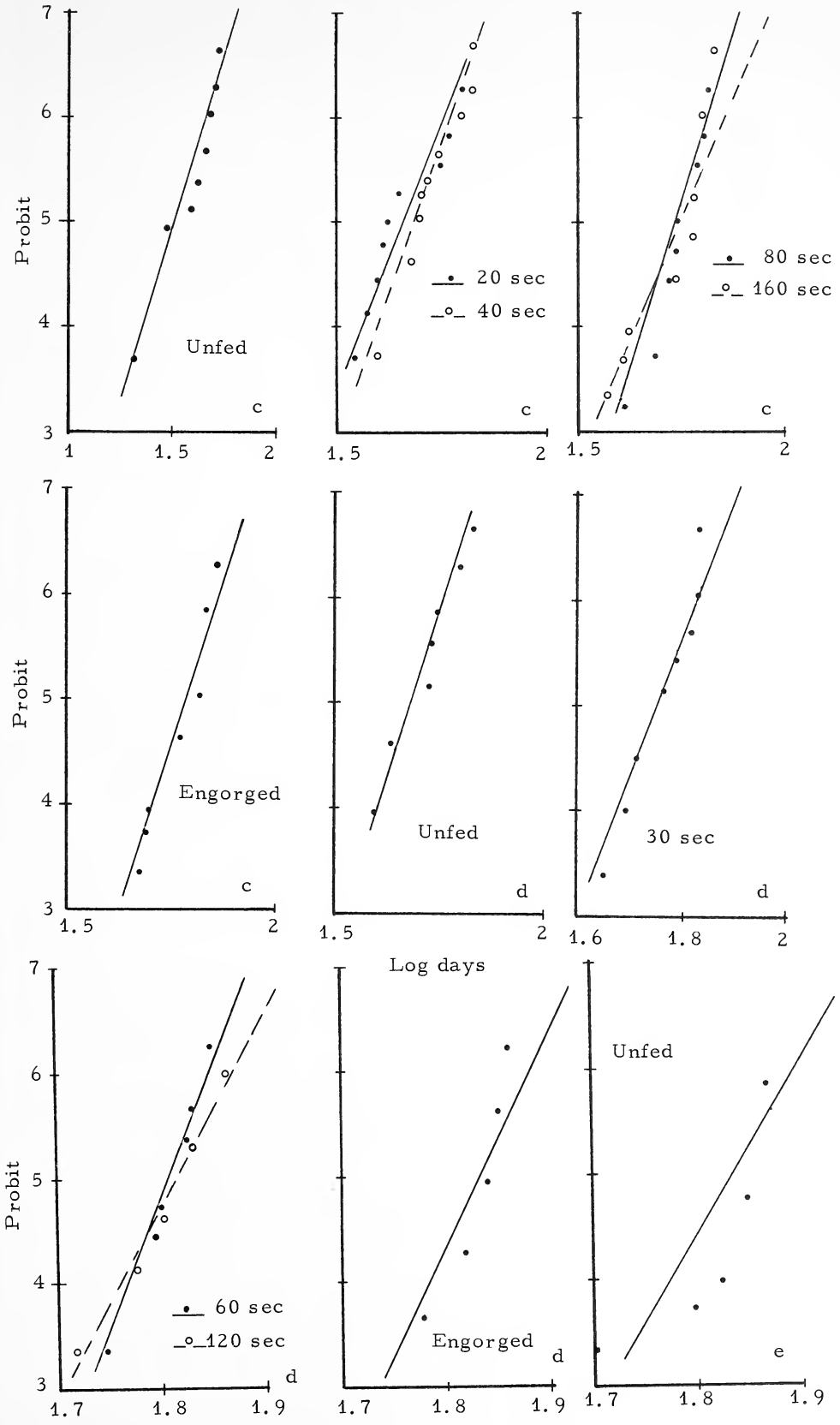


Fig. 2. c. 3rd instar; d. 4th instar; e. 5th instar.

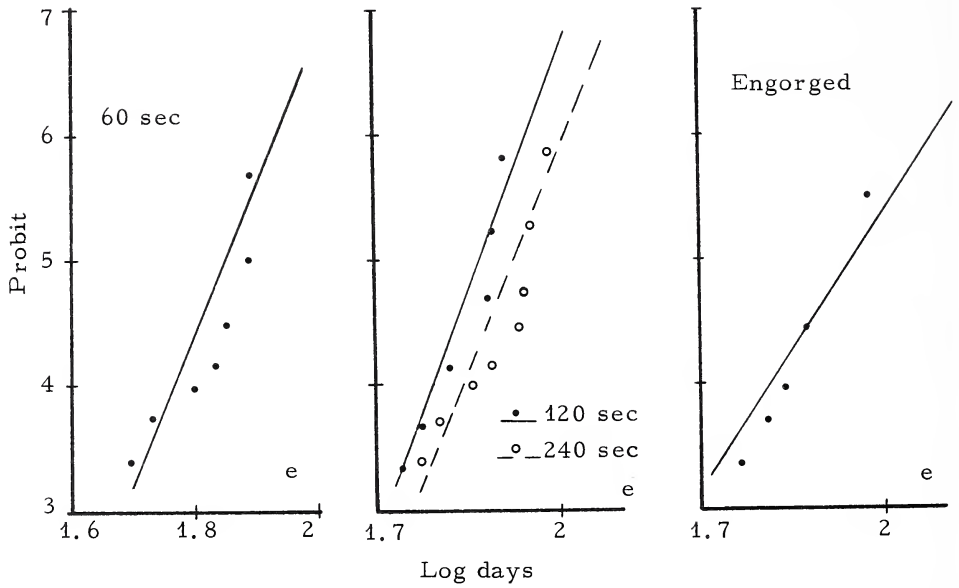


Fig. 2. e. 5th instar.

Fig. 3 shows the average weight changes during development of the nymphal instars when they take a full blood meal in each instar. The sudden rise in weight after taking the blood meal is followed by a rapid fall which is mostly due to the passage of the water and some other constituents of the blood during the first day after the meal. Over the period preceding the next meal the weight keeps dropping at a progressively slower rate. Titschack (1930) published a similar curve with small differences in weights. Przibram has proposed that the weight of an insect is doubled at each instar and the linear dimensions increased by 1.26 at each moult (Wigglesworth 1965). Although this rule is more applicable to Hemimetabola than to Holometabola, it is of doubtful value for *C. lectularius* because its growth curve appears discontinuous due to the ingestion of blood meals 3 to 5 times the body weight.

TABLE 2. Effect of the size of a single blood meal on the LT50 in the different instars of *C. lectularius*.

Instars	Feeding period in seconds										
	0	15	20	30	40	60	80	120	160	240	engorged
1st											
x*	0	1.021		0.042		0.083		0.165			0.260
y**	9.0	9.3		12.6		15.5		36.3			47.9
	(7.5-10.8)	(8.3-10.5)		(10.7-14.7)		(14.6-16.4)		(33.8-39.0)			(46.7-49.1)
2nd											
x	0	0.046		0.092		0.184		0.368			0.481
y	33.3	37.2		45.9		53.1		58.9			66.1
	(32.2-34.3)	(35.4-39.1)		(44.2-47.7)		(50.3-56.0)		(57.2-60.6)			(63.8-68.4)
3rd											
x	0		0.124		0.248		0.496		0.992		1.087
y	31.6		44.7		47.9		53.7		56.2		61.0
	(28.7-35.1)		(42.6-46.8)		(45.8-50.0)		(51.5-56.0)		(53.3-59.3)		(58.4-63.6)
4th											
x	0			0.352		0.704		1.408			2.856
y	48.4			58.9		64.4		65.3			27.6
	(46.2-50.8)			(57.1-60.7)		(63.0-66.0)		(63.1-67.7)			(65.9-69.4)
5th											
x	0					1.086		2.172		4.345	5.533
y	68.7					70.8		74.5		83.6	89.1
	(66.4-71.1)					(68.0-73.7)		(71.2-77.9)		(80.3-87.0)	(80.9-98.2)

* x = average weight of the blood meal in mg.
** y = LT50
(95% confidence limit)

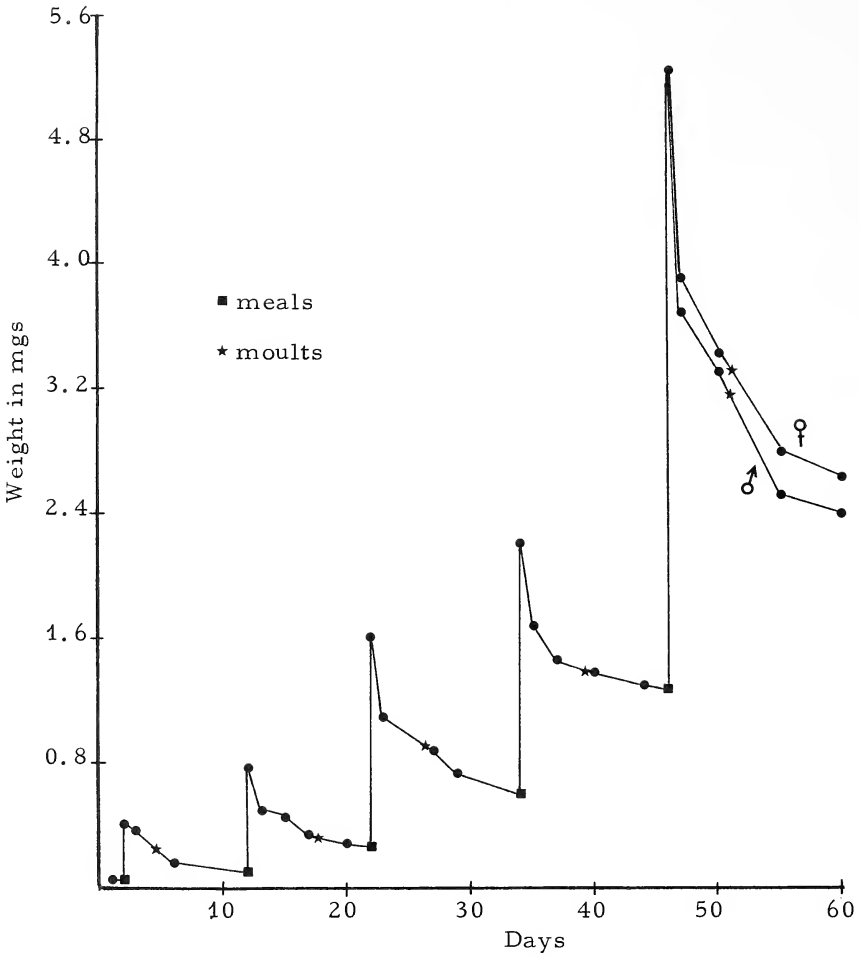


Fig. 3. Weight changes during development of *C. lectularius*.

Table 3 shows that the food conversion efficiency in the different nymphal instars of *C. lectularius* varies between 25.6 and 37.0%. It takes 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. The third instar is less efficient than are the others. Comparison of table 7 with the results of Jones (1930) shows a similarity in the trend of the food conversion efficiency in the different instars with small differences in the values. The values shown in Johnson's Table III (1960) are all higher than mine because he weighed the nymphs a short period after feeding. There is a difference in food conversion efficiency between the fifth instar and the adult male and female. This was overlooked by Jones (1930) and Johnson (1960).

TABLE 3. Food conversion efficiency in the nymphal instar of *C. lectularius*.

Moult*	Average difference in body wt. between instars (mg)	Average wt. of blood meal (mg)	Mg of human blood required to increase body wt. 1 mg	Food** conversion efficiency %
I to II	0.07	0.25	3.57	28.0
II to III	0.20	0.63	3.15	31.8
III to IV	0.27	1.02	3.90	25.6
IV to V	0.67	1.81	2.70	37.0
V to ♀	1.37	4.18	3.05	32.8
V to ♂	1.12	4.18	3.73	26.8
		Overall means:	3.70	30.3

* No. of insects was 20 in each test.

** Food conversion efficiency% = $\frac{\text{Average difference in body wt.}}{\text{Average wt. of blood meal}} \times 100$

On the Duration of the Preoviposition Period

The effect of the size of the blood meal on the duration of the preoviposition period is shown in figs. 4a and 4b. In fig. 4a the males were fed till engorgement and the females for different periods. No oviposition took place when the females were unfed. An increase in size of the female's blood meal caused a gradual decrease in the preoviposition period ($r = -0.908$). The highly significant value for chi square (49.8) shows departure from the theoretical straight line relationship.

In fig. 4b the females were fed till engorgement and the males for different periods. There was no significant difference in the duration of the preoviposition period.

On Fecundity

From fig. 5 it is clear that the size of the female's blood meal has a profound effect on the percentage of females which lay eggs when the mating males were fed till engorgement. No eggs were produced by unfed females. The more the females were fed the larger the number of females which laid eggs.

Fig. 6 shows no effect of the size of the mating males' blood meal on the percentage of females laying eggs when the latter were fed to capacity.

Figs. 5 and 6 also show the relationship between the size of the females' and mating males' blood meals and the number of eggs laid per female. Fig. 5 shows the effect of the size of the females' blood meal on the number of eggs laid per female when the mating males were fed to capacity. As shown in fig. 5 the more the females were fed the more eggs they laid.

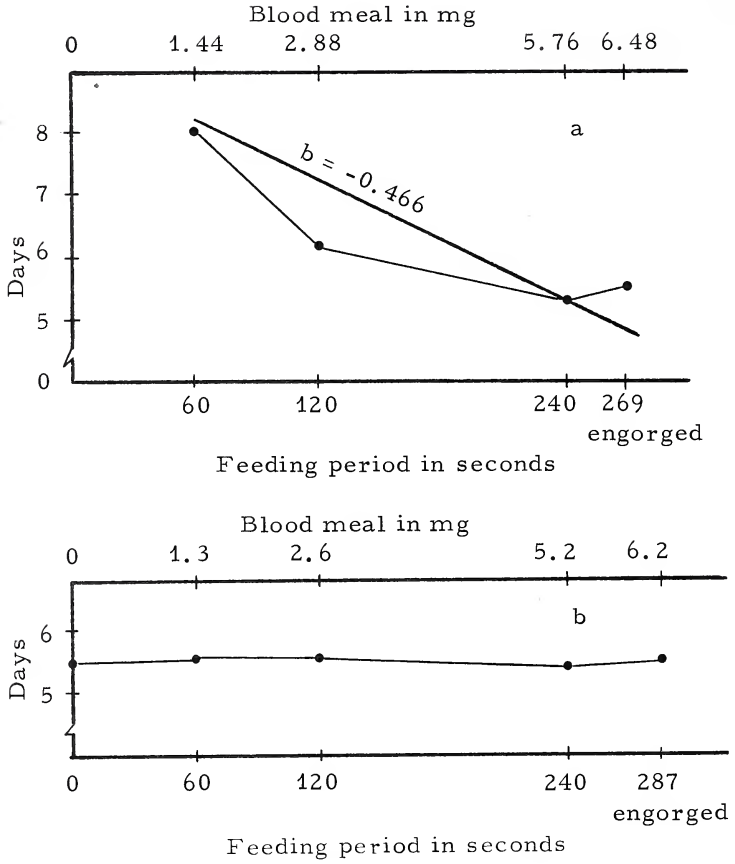


Fig. 4. a. Effect of the size of the female's blood meal on preoviposition period of *C. lectularius*; males engorged. b. Effect of the size of the mating male's blood meal on the preoviposition period of engorged female *C. lectularius*.

Fig. 5 also shows the relationship between the size of the females' blood meal and the number of eggs per milligram of blood. There is a slight increase in the number of eggs laid per milligram of blood with the increase in the size of the female's blood meal within the range of feeding periods from 60 and 240 seconds. On the other hand, there is a high increase when the feeding period increased from 240 to engorgement.

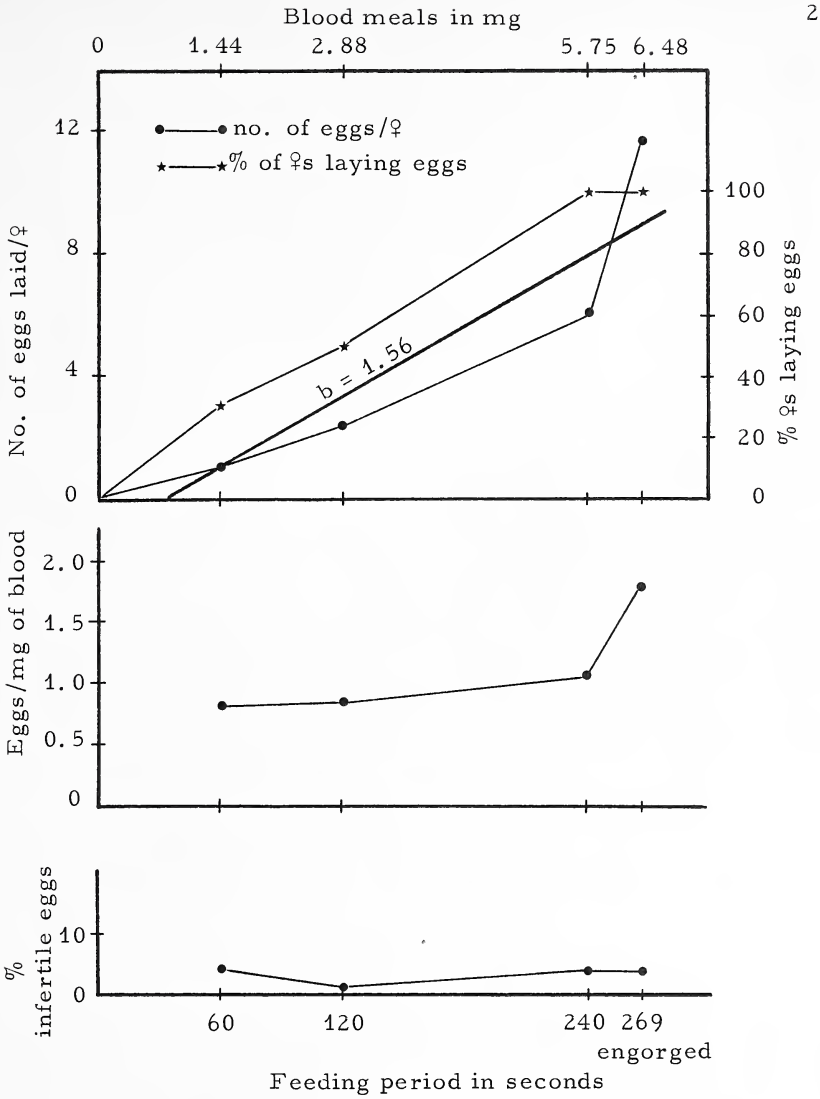


Fig. 5. Effect of the size of the blood meal of female *C. lectularius* on the percentage laying eggs, number of eggs laid per female, number of eggs laid per mg of blood, and percentage of infertile eggs.

Egg laying in *C. lectularius* has been studied under various conditions (Hase 1930, Titschack 1930, Omori 1941, Johnson 1942). In most blood-sucking insects, the number of eggs produced by a female is correlated, within limits, with the quantity of food taken (Wigglesworth 1960). The results shown in fig. 5 indicate that *C. lectularius* is no exception to this rule. Cragg (1923) wrote that the number of eggs is dependent on the amount of food obtained by the males as well as the females, and that females impregnated by unfed males do not produce as many eggs as females impregnated by fully nourished males. My studies confirm the

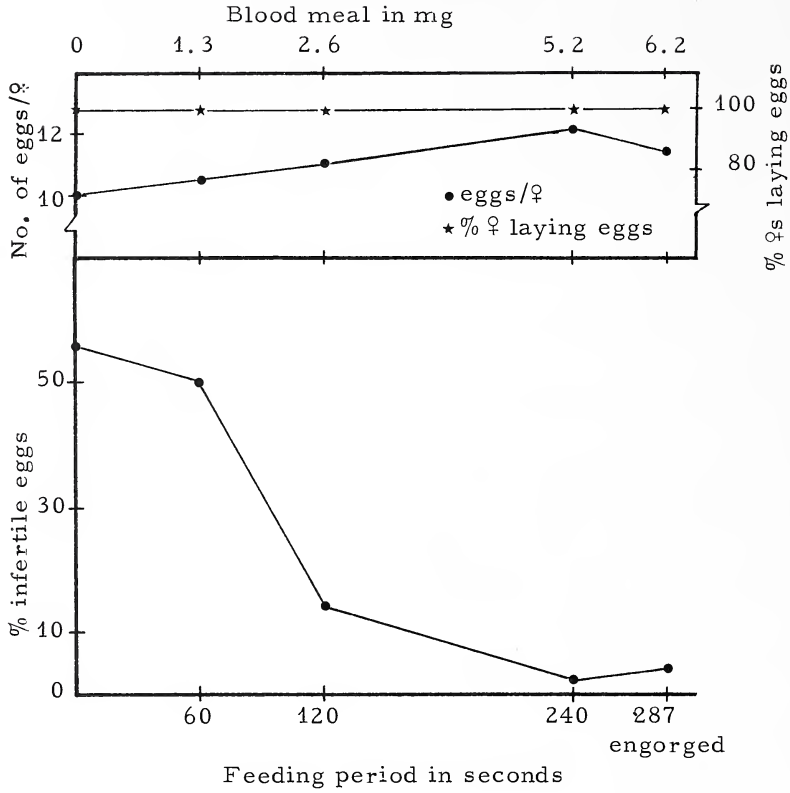


Fig. 6. Effect of the size of the mating males' blood meal on the percentage of females laying eggs, number of eggs, and the percentage of infertile eggs laid by engorged female *C. lectularius*.

former statement but indicate that the latter should be modified to read "fertile eggs". My unfed females did not lay eggs, although it was reported by Titschack (1930) and Johnson (1942) that unfed females can lay eggs. Jones (1930) claimed that the number of eggs produced by unfed females is probably related to the food reserves acquired in the previous instar. Davis (1964) studied the vitellogenesis of *C. lectularius* female that had mated but were starved and he found that no maturation occurs. Gooding (1963) postulated that although the most obvious function of the meal in human lice is that it provides the material and energy for growth of the nymphs and the maturation of eggs in the adult stage, it may also function as a stimulant resulting in the formation and release of hormones. Johansson (1964) said "after an adequate meal the 'nervous?' stimuli from the gut activate the corpus allatum by way of the brain.

Hormones from the corpus allatum and the neurosecretory cells in the brain stimulate the ovaries and accessory glands. In the absence of adequate food the brain inhibits the corpus allatum and the hormone titre remains too low for the ovaries and accessory glands to function normally." In *C. lectularius* it seems that there are more subtle factors involved in the production of eggs than mere quantities of blood taken. Bell and Schaefer (1966) found that the highest average egg production in *C. lectularius* (5.4) was by those fed on 9:1 mixture of whole rabbit blood and insect Ringer's, and the minimum was with females fed on Ringer's alone.

Figs. 5 and 6 show that although the size of the female's blood meal has no significant effect on the percentage of infertile eggs laid, the size of the blood meal of the mating male has a significant effect on the percentage of infertile eggs laid by the female.

Titschack (1930) found that the percentage of sterile eggs increased from 0 to 1% during the fertile period of *C. lectularius* . Mellanby (1939a) found that mating is a necessary process for egg production. Davis (1965b) studied the effect of insemination in activating the corpus allatum of female *C. lectularius* and he found that the mechanical aspects of insemination play no role in this activation and concluded that the presence of spermatozoa in the conceptacula or lateral oviducts stimulates the corpus allatum. He found that 3 to 4 hours are required for seminal stimulus to become effective and this period corresponds with the time required for the spermatozoa to reach the genital tract. He also suggested that the seminal stimulus results from summation of stimuli of many receptors in the genital tract.

On Longevity of the Adult

The effect of the size of the blood meal on the longevity of the adult female is shown in fig. 7. Analysis of the data was undertaken on the basis of dividing. The results were divided into females which did not lay eggs, females which laid eggs, and both together; the correlation coefficients were 0.99, 0.91 and 0.97 respectively. The straight line relationship for females which did not lay eggs and for all females did not hold for females which laid eggs. This indicates that when the females take smaller blood meals, egg production may have an influence on their longevity.

Fig. 7 also shows that the males lived longer when the blood meal was increased in size and that the relationship was a straight line.

Effects of the Size and Frequency of Blood Meals

On the Nymphal Instars

The effects of the size and frequency of the blood meals on moulting, duration of the nymphal stadia, and mortality were studied.

The size and frequency of blood meals have a profound effect on moulting of the insects in all instars (fig. 8). When the feeding period was 15 seconds on every 4th, 8th, or 16th day the first instar nymphs did not moult. At a frequency of every 2nd day only about 30% of the insects reached the second instar.

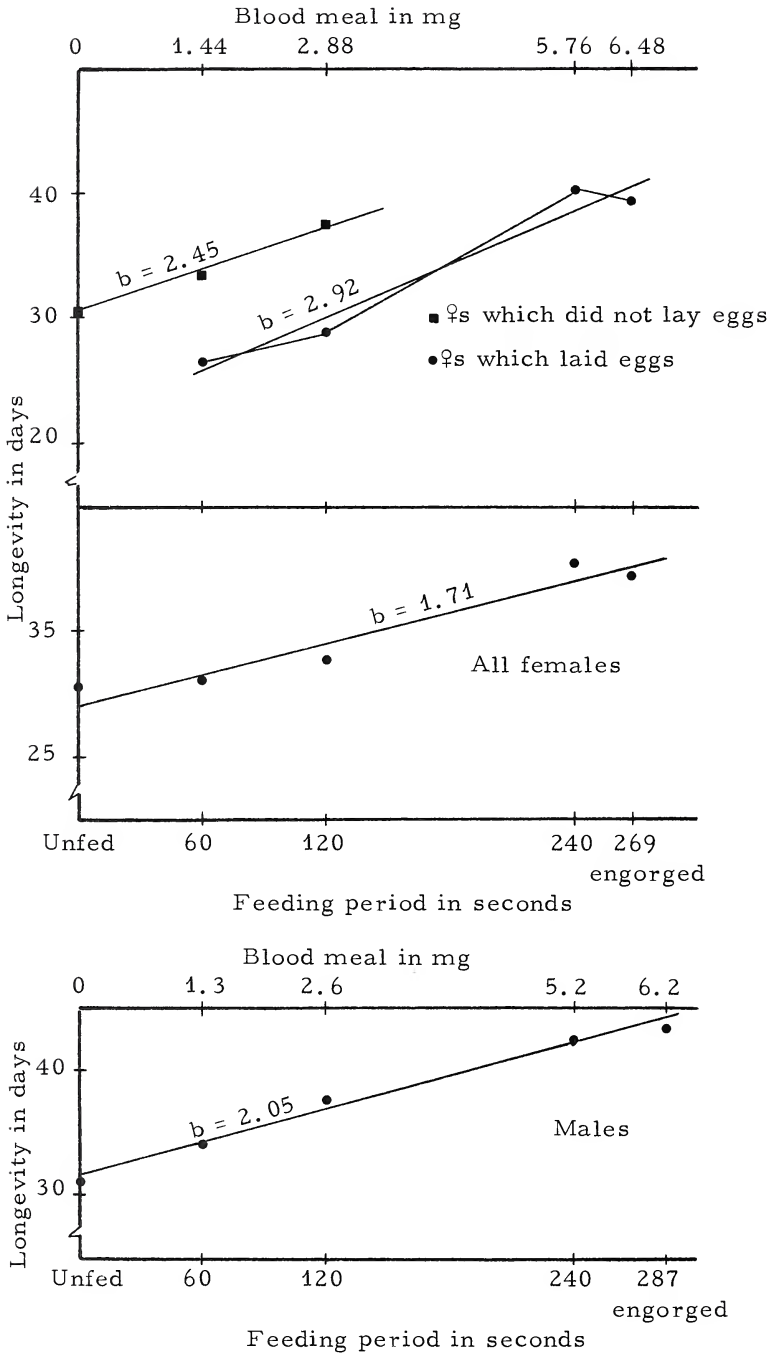


Fig. 7. Effect of the size of a single blood meal on the longevity of the female and male *C. lectularius*.

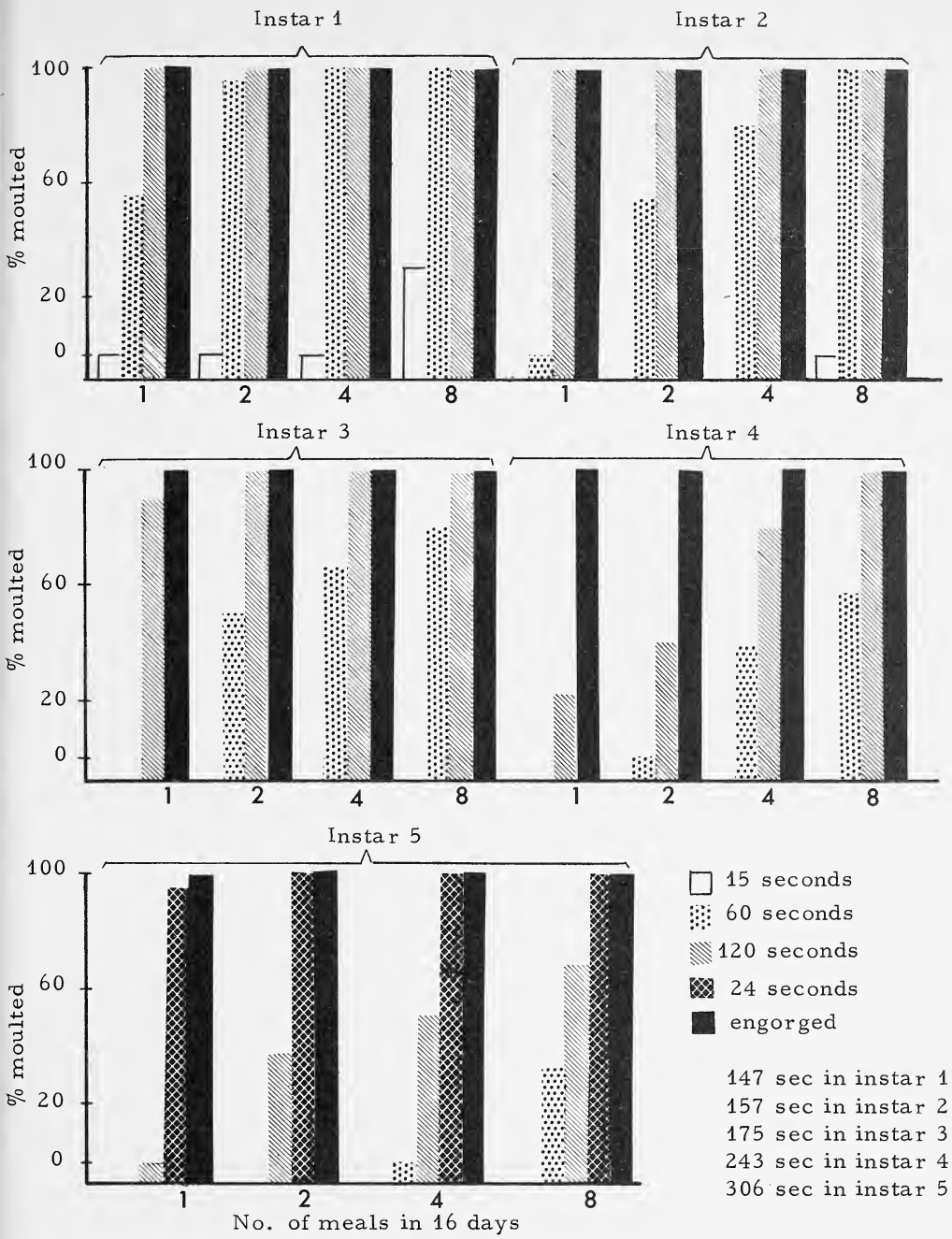


Fig. 8. Effect of the size and frequency of blood meals on moulting in the different instars of *C. lectularius*.

All insects fed for 60 seconds every 2 days reached the third instar but thereafter the number of nymphs that moulted decreased and only 15% of the insects reached the adult stage. When the interval between the blood meals was prolonged to 4 days all the insects reached the second instar and only 25% reached the fifth instar. At a frequency of every 8 days the number of insects that did not moult increased from the first to the fourth instar and 2.5% reached the fourth instar. When the insects were fed for 60 seconds every 16 days 55% reached the second instar.

All insects reached the third instar when fed for 120 seconds over the range of intervals between meals of 2 to 16 days. At a frequency of every 2nd day all the insects reached the adult stage. The number of insects that reached the adult stage decreased with the increase of the period between meals.

As the insects in the first four nymphal instar can engorge in less than 240 seconds, the effect of this feeding period and its frequency is shown in the fifth nymphal instar. For this experiment the insects were taken from the group which were fed till engorgement to the fourth instar at the different frequencies. When the fifth instar nymphs were fed for 240 seconds, in the range of frequencies studied, only one did not moult to the adult stage.

All insects which were fed till engorgement at any frequency reached the adult stage.

In general the number of insects which moult after the first meal at any instar increases with the increase in the size of the blood meal. Maximum moulting took place after taking the second meal, if the first meal did not bring about more than 50% moulting.

When the nymphs were given a series of blood meals, the percentage of moulting insects depended upon the size of blood meals as well as the intervals between them. Wigglesworth (1934) was unable to get the fifth instar nymphs of *R. prolixus* to moult by feeding them a series of small blood meals at intervals. Friend et al. (1965), on the other hand, found that third and fifth instar nymphs of *R. prolixus* can be induced to moult on a succession of small meals at 30 day intervals. Unfortunately they did not study the effect of the interval between feeding on moulting. My studies also show that successive small meals can induce moulting and that, in *C. lectularius*, the interval between feeding is very important. It seems that when the nymphs are fed a series of small meals within certain interval limits they achieve a physiological stage after which moulting can be induced by blood meals of small size. Friend et al. (1965) claimed that this might be due to some moulting hormone produced under the influence of small blood meals and stored until it reaches an effective titre when moulting results. They added that the cells may become more responsive to the moulting hormone after they have assimilated the nutrients supplied by small meals. This might be so in *C. lectularius* because the size and frequency of blood meals has no significant effect on the period between the last meal and the day of moulting.

The frequency of feeding has a profound effect on moulting and this effect is dependent on the size of the blood meal (fig. 8). The smaller the blood meal and the longer the interval between feeding the lower the percentage of the nymphs moulting. This may be due to the effect of the duration of the interval between feeding on the stored moulting hormone suggested by Friend et al. (1965).

The duration of any nymphal stadium increases with the increase in the number of meals required to induce moulting, and with the decrease of frequency of feeding. On the other hand, neither the size and interval between blood meals nor the number of meals have a significant effect on the period between the last meal and moulting. Jones (1930) reported that bedbugs that took two meals during the third stadium moulted

at a different time from others, but did not say whether this was earlier or later.

It was found that the mortality increased with the decrease in the size and the frequency of blood meals at any instar. Also the longevity of the insects that did not moult at any instar increased with the increase in the size and frequency of feeding.

On Weight Changes during Development

Figs. 9a to 9f show the relationship between the size and the frequency of blood meals and the difference in body weight of the different instars. The first instar was weighed one day after hatching and the other instars on the day of moulting.

The difference in body weight between any two successive instars increases with the increase in the size of the blood meals and with the decrease in the interval between feeding. Similarly, the ratio of the difference in body weight to the total weight of blood meals usually increases with the increase in the size of the blood meal and its frequency. This increase may be due to an increase in the food conversion efficiency with or without a difference in the weight of the unassimilated blood in the gut. The increase in this ratio with the increase in the size of the blood meals when the nymphs were fed every 16 days is mostly due to an increase in the food conversion efficiency.

On the Preoviposition Period

Fig. 10 shows the effect on the preoviposition period. The effect of frequency of feeding depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. In general, the more the insects feed and the shorter the interval between feeding the shorter the preoviposition period.

On Fecundity

The effect of the size and frequency of blood meals on fecundity is shown in fig. 11. Owing to the small number of insects which reached the adult stage as well as their small range in the size and frequency of blood meals, the experiment was completed using adults taken from the standard culture. The size and frequency of blood meals which the adults were fed had a marked effect on the number of females which laid eggs and the number of eggs laid per female. None of the females laid eggs when the feeding period was 15 seconds, at any frequency, nor when fed for 30 seconds every 8th or 16th day. The number of females which laid eggs and the number of eggs laid per female increase with the decrease in the interval between feeding and increases in size of the blood meals. There is a high positive correlation coefficient (0.99) between the weight of blood meals taken by the female and the number of eggs laid per female.

Fig. 12 shows the relationship between the number of eggs laid per milligram of blood and the size of the blood meal and its frequency. The data of the insects reached the adult stage alone did not show clearly the relationship and the correlation coefficient was small (0.45). When analysing these data together with those of the insects taken from the stan-

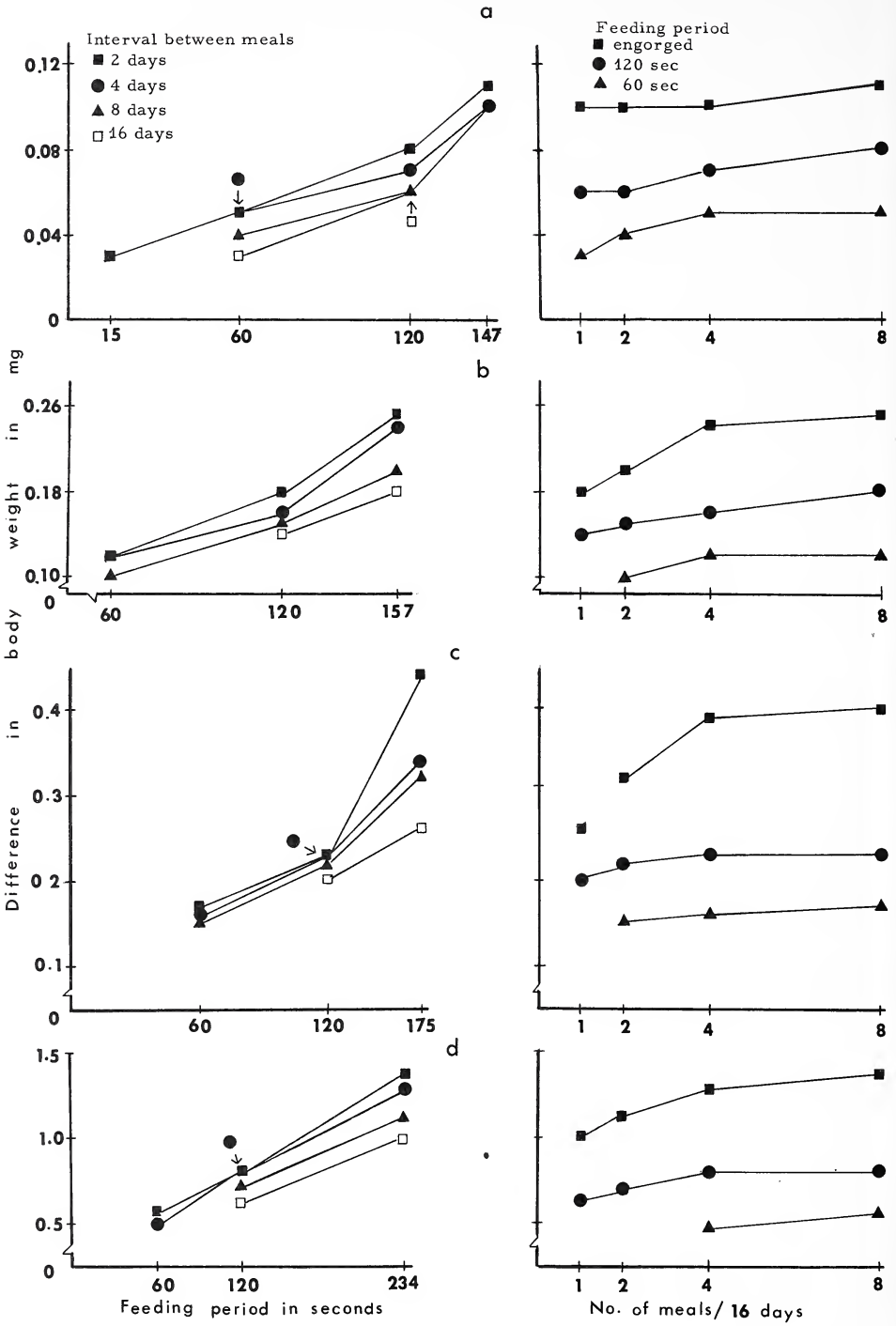


Fig. 9. Effect of the size and frequency of blood meals on the difference in body weight between successive instars of *C. lectularius*. a. 1st to 2nd instar; b. 2nd to 3rd instar; c. 3rd to 4th instar; d. 4th to 5th instar.

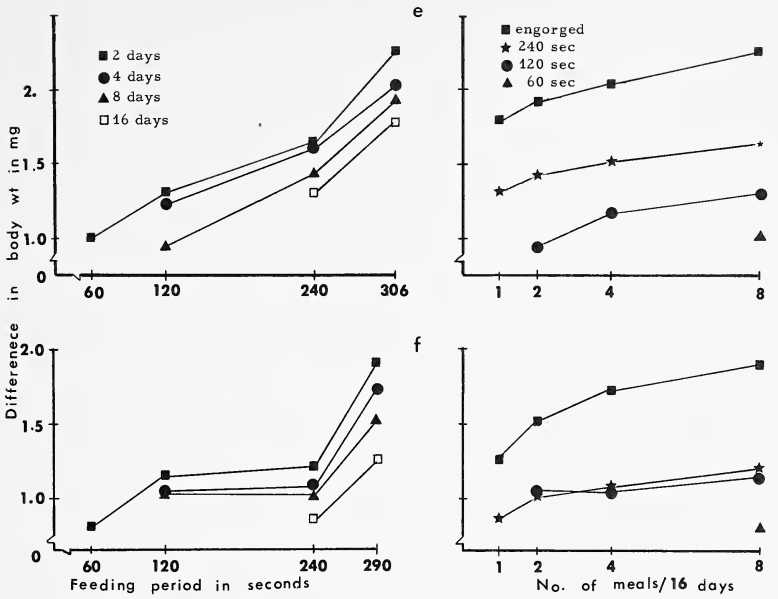


Fig. 9. e. 5th instar and adult female; f. 5th instar and adult male.

dard culture, to complete the experiment, the correlation coefficient was 0.70. The effect of frequency of feeding on the number of eggs laid per milligram of blood (fig. 12a) depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. When the insects were fed till engorgement the maximum number of eggs/mg of blood was when the insects fed every 4 days. Increasing the frequency of feeding causes a decrease in the number of eggs/mg of blood; the amount of blood taken is then more than enough for optimum egg production. Decreasing the frequency of feeding also decreases the number of eggs/mg of blood, because some of the blood is needed for maintenance metabolism. When the feeding periods were 240, 120, 60, and 30 seconds the number of eggs/mg of blood increased with the increase in frequency of feeding.

Also the effect of the size of the blood meals depends upon the frequency of feeding (fig. 12b). When the interval between meals was 2 days the number of eggs/mg of blood increases with the increase in the size of the blood meal and the optimum egg production was when the feeding period is in the range of 120 to 240 seconds. When the interval between feeding was four days the shape of the curve is changed from convex to step-like with a maximum number of eggs/mg of blood when the insects were fed till engorgement. This indicates that optimum egg production takes place when the feeding period is in the range between 240 seconds and engorgement. The change in the shape of the curve to concave and sigmoid when the intervals between meals were 8 and 16 days respectively shows that taking these blood meals at these intervals was not optimum for egg production and the number of eggs/mg of blood increases with the increase in the size of the blood meal.

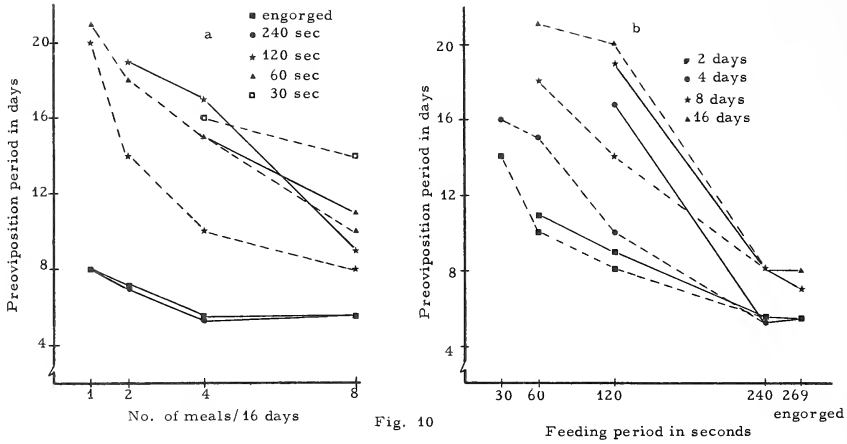


Fig. 10

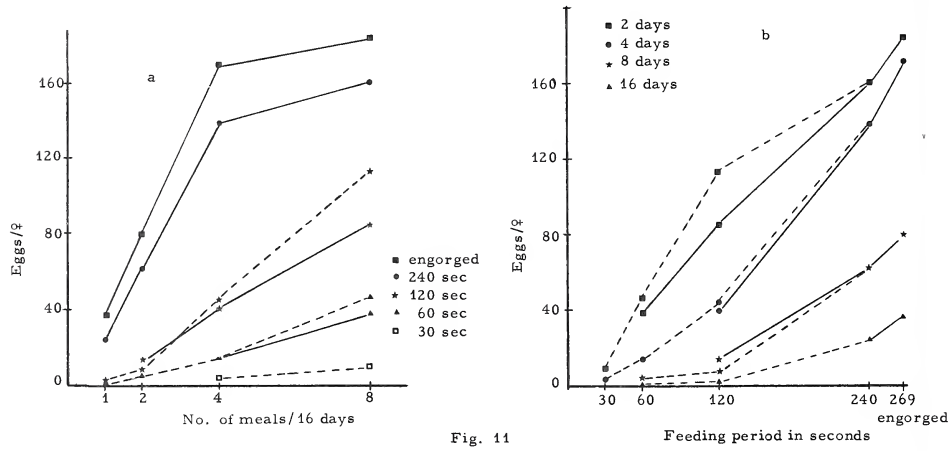


Fig. 11

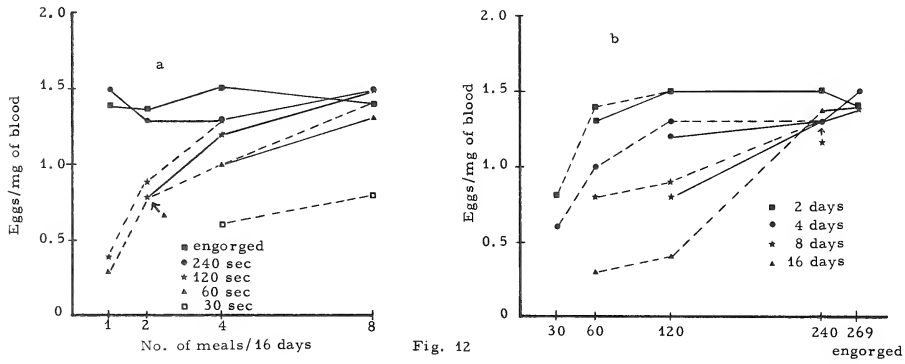


Fig. 12

Figs. 10, 11 & 12. Effects of the frequency (a) and the size (b) of (10) blood meals on the preoviposition period in *C. lectularius*; (11) blood meals on the number of eggs laid per female *C. lectularius*; (12) blood meals on the number of eggs laid per female *C. lectularius*. (--- insects taken from standard culture).

Comparing fig. 5, the relationship between the size of a single blood meal and the number of eggs laid per mg of blood, with the curve connecting size of the blood meal and the number of eggs laid per mg of blood when the interval between feeding was two days (fig. 12b), it is clear that the values when the insects were fed for 60, 120, and 240 seconds are lower than that in fig. 12b. This indicates that the amount of blood responsible for these differences in the values is not directed to egg production but might, for example, stimulate ovarian activity. On the other hand, the number of eggs laid per mg of blood when the insects were fed till engorgement is larger in fig. 5 than in fig. 12b. This indicates that the first feeding period required for optimum egg production is in the range between 240 seconds and engorgement.

There is little correlation (r = 0.32) between the weight of the female after the fifth moult and the number of eggs laid per female. On the contrary, there is a high positive correlation coefficient of 0.97 between longevity and the number of eggs laid per female.

Fig. 13 shows the effect of the mating male's blood meals and their frequencies on the percentage of infertile eggs laid by the females. The smaller the size of the blood meals the larger the effect of frequency of feeding. The correlation coefficient r between the size of the males' blood meals and the percentage of infertile eggs was -0.42 and -0.56 for the data of the insects which reached the adult stage alone, and these data together with those of the insects taken from the standard culture respectively.

The results of the experiments on blood intake and egg production in *C. lectularius* are similar to those reported by Roy (1936) and Friend et al. (1965). Roy (1936) studied *A. aegypti* and found that 0.82 mg of blood was required before the insect would lay eggs. In addition, the number of eggs laid per female was not dependent on the weight of the insect before feeding but there was a good correlation between the size of the blood meal and the number of eggs laid. Friend et al. (1965) found that female *R. prolixus* would not produce eggs until 56.6 mg of blood were fed and the correlation coefficient for the body weight before feeding and egg production was only 0.30.

In some other blood-sucking insects fecundity is influenced mainly by the weight of the female before feeding as long as a certain minimum meal is consumed. Barlow (1955) proposed that the reason for the correlation between the body weight of *Aedes hexodontus* and fecundity is because larger females tended to consume more blood than smaller ones.

On Longevity

Fig. 14 shows the effect of the size and frequency of blood meals on longevity of the female. Statistical analysis of these data showed that the correlation coefficient between the size of blood meals and longevity of the females was 0.97. The longevity of the males increases with the increase in the size of the blood meal and with the decrease in the interval between feeding (fig. 15). Analysis of the data showed a correlation coefficient (r) between the size of the blood meal and longevity of the males of 0.92.

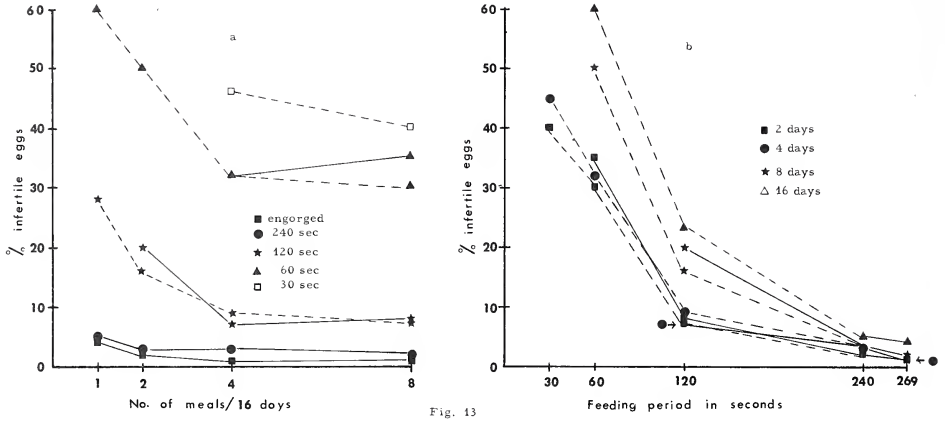


Fig. 13

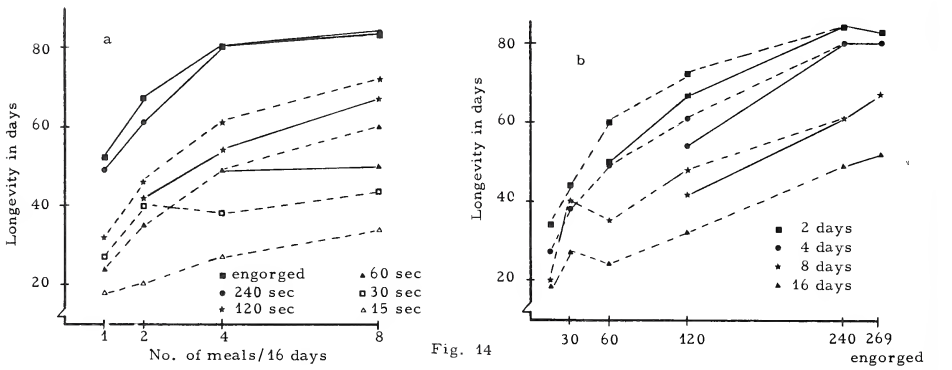


Fig. 14

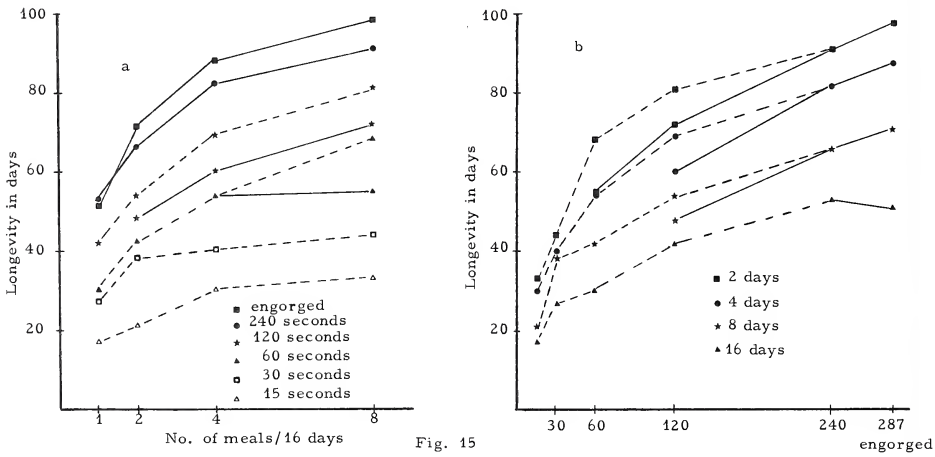


Fig. 15

Figs. 13, 14, & 15. Effects of the frequency (a) and the size (b) of (13) mating male's blood meals on the percentage of infertile eggs laid by female *C. lectularius* ; (14) blood meals on the longevity of female *C. lectularius*; (15) blood meals on the longevity of the male *C. lectularius*. (--- insects taken from standard culture).

PROTEIN CONTENT AND RESPIRATORY RATE IN THE DIFFERENT INSTARS OF *C. LECTULARIUS*

Methods

The protein content in the different instars of *C. lectularius* was determined using Folin phenol reagent (Lowry et al. 1951). Insects used in this experiment were taken from the standard culture. First instar nymphs were taken immediately after hatching and starved for five days. The other nymphal instars were taken on the day of moulting and starved for about ten days. For the adult stage two tests were undertaken. In the first test, fifth instar nymphs were taken from the standard culture and were given a full blood meal and put separately on 2 cm^2 piece of folded filter paper in 2 x 7 cm specimen tubes. After 12 days protein content of the females and males was estimated. In the second test, females and males were put together and given a full blood meal on the second day of moulting. Twelve days later the protein content of the females and males was estimated. The protein content of the eggs was also determined.

Oxygen consumption and carbon dioxide output in the different instars were measured in a Warburg constant volume respirometer using the direct method of Umbreit et al. (1964). Insects used were taken from the standard culture one day after moulting and were given a blood meal before starting the experiments. The respiratory quotient in the different instars was determined.

Results

Protein Content

The protein content in the different instars of *C. lectularius* is shown in fig. 16. The weight of protein per female was greater than that per male. Females which were kept separately had a higher protein content than those which were kept with males. Oviposition seems to be responsible for this difference because the females which were kept with males laid eggs while those which were kept alone did not. Similarly, the difference in protein content of the males may be due to sperm transfer as well as to the activity in courtship and mating. The percentage of protein ranged from 21.8 to 27% in the nymphal instars. In the females it was 26.1 in those which were kept alone and 18.2% in those which were kept with males. In the males it was 27.2 in those kept alone and 25.4% in those kept with females.

Spector (1956) gave the following chemical composition of the human blood:

water	83.000 g/100 ml
total protein	21.800 g/100 ml
lipids	0.560 g/100 ml
carbohydrates	0.439 g/100 ml

From this the amount of protein in the blood meal, in the different instars, was calculated. Lipids and carbohydrates contribute very little in the chemical composition of human blood as compared to protein.

Assuming that there is no conversion from the lipids and carbohydrates of the blood into protein, the efficiency by which the bedbugs, in the different instars, can convert the blood protein into body protein can be estimated (table 4). Protein conversion efficiency in the different instars of *C. lectularius* ranged between 28.0 and 65.3%. Similar results to those of the food conversion efficiency (table 3) would be expected only if the chemical composition of bedbug tissue were similar to that of human blood.

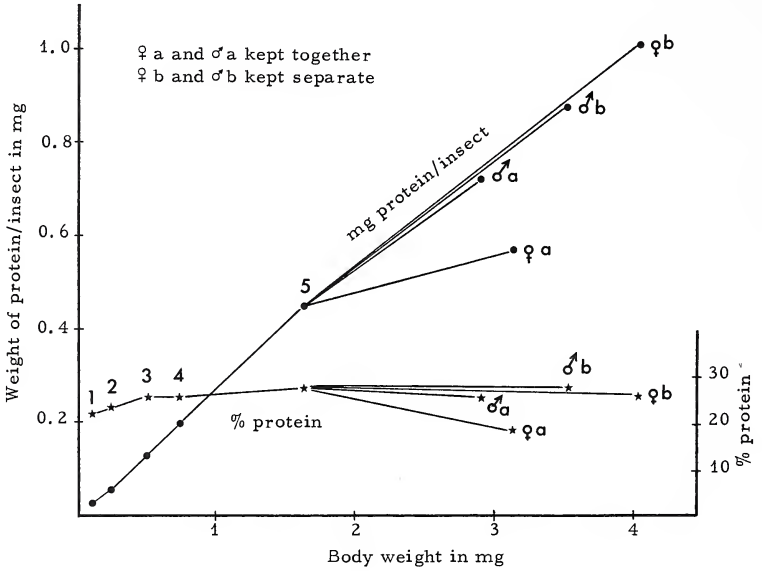


Fig. 16. Relationship between the body weight and protein content in the different instars of *C. lectularius*.

TABLE 4. Protein conversion efficiency in the different instars of *C. lectularius.*

Instar	Wt. of blood meal (mg)	Protein content of blood meal (mg)	Increase in body protein content (mg)	Protein conversion efficiency %
1st	0.26	0.054		
2nd	0.62	0.128	0.035	65.3
3rd	1.09	0.225	0.070	54.8
4th	2.86	0.589	0.068	30.3
5th♀	5.64	1.162	0.256	43.4
5th♂	5.42	1.117	0.603	51.9
			0.313	28.0

Rate of Respiration

The respiratory rates in the different instars of *C. lectularius* are shown in fig. 17. The oxygen uptake per insect per hour increases from the first nymphal instar to the adult stage. Oxygen uptake by the females was greater than by the males. The oxygen uptake, expressed as microliters/milligram of body weight/hour, was the same in the first two nymphal instars and then increased to a maximum in the adult stage. The respiratory quotient in the different instars ranges between 0.88 and 0.95 which indicates that either protein or fat or both are included in the metabolism.

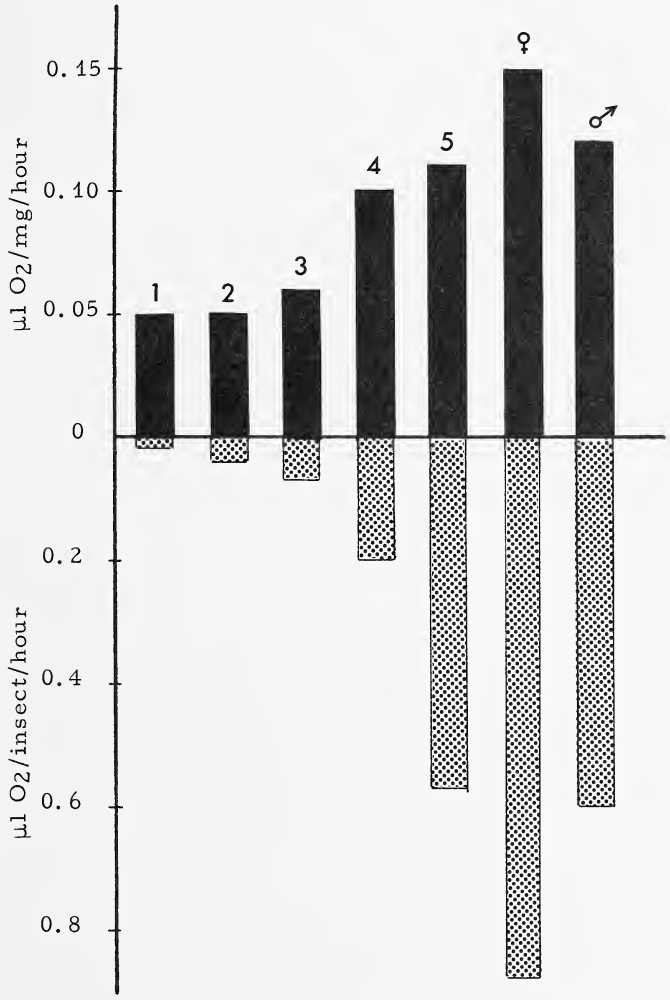


Fig. 17. Oxygen uptake in the different instars of *C. lectularius*.

From the weight of the total protein in the blood meal and the increase of protein content in the different instars, the weight of protein in the blood meal which is metabolized plus that which is not absorbed can be determined. Assuming that there is no interconversion between the chemical constituents of the blood meal during metabolism the weight of protein which is metabolized plus the protein which is not absorbed per day was estimated (table 5). It was found that the rate of metabolism, expressed as mg of protein/day, like that expressed as microliter oxygen/insect/day, increases from the first to the fifth nymphal instar.

TABLE 5. Rate of metabolism in the different nymphal instars of *C. lectularius.*

Instar	Wt. of protein metabolized + protein not absorbed (mg)	Mean longevity (days)	Wt. of protein metabolized + protein not absorbed per day (mg)	Rate of respiration μl O_2/insect/day
1st	0.019	34.9	5.4×10^{-4}	0.48 - 0.96
2nd	0.058	63.6	9.1×10^{-4}	0.96 - 1.68
3rd	0.157	63.9	2.5×10^{-3}	1.68 - 4.80
4th	0.333	68.9	4.9×10^{-3}	4.80 - 13.68
5th ♀	0.559	89.1	6.3×10^{-3}	13.68 - 21.12
5th ♂	0.804	89.1	9.0×10^{-3}	13.68 - 14.40

The method by which the weight of protein, which is metabolized plus that which is not absorbed, is shown in table 6. It is clear that the weight of protein which is metabolized plus that which is not absorbed, like the rate of respiration, is greater in the adult female than in any nymphal instar.

TABLE 6. A partial protein budget in the adult female *C. lectularius.*

Weight of blood meal	6.48 mg
Protein content of the blood meal	1.40 mg
Weight of protein/egg	0.034 mg
Number of eggs laid/female	11.55 eggs
Weight of protein in all the eggs	0.393 mg
Weight of protein metabolized + protein not absorbed	1.007 mg
Mean longevity	39.6 days
Weight of protein metabolized + protein not absorbed	2.5×10^{-2} mg/day
Respiratory rate	0.88 μl O_2/female/hour

GENERAL DISCUSSION AND SUMMARY

The size of the blood meal has its effect in two ways. The first one is the volume of the blood ingested that causes either stretching of the abdomen and moulting in the nymphal instars or stimulates oviposition in adult females. The second is the quantity of nutrients in the blood ingested and its effects on development or reproduction. It seems that taking a threshold quantity of blood as a single meal is more important to *C. lectularius* than taking the same quantity over a period of time in more than one blood meal. Also the blood of different hosts may have its effect on *C. lectularius* through the differences in the nutritive value of blood.

The results of the effects of the size and frequency of blood meals on *C. lectularius* can be summarized as follows:

1. Moulting can be induced in all the nymphal instars by blood meals smaller than the full blood meal and there is always positive correlation between the size of the blood meal and the percentage of insects moulting.
2. The duration of the nymphal stadia decreases with the increase in the size of the blood meals. It also decreases with the decreases in the number of successive meals required to induce moulting. Neither the size and interval between blood meals nor the number of meals have a significant effect on the period between the last meal and the day of moulting.
3. The food conversion efficiency in the different instars varies between 25.6 and 37.0% and the third instar is less efficient than the others. It takes about 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%.
4. The increase in the size of the females' blood meals causes a decrease in the preoviposition period. The size of the male's blood meal has no significant effect on the preoviposition period of a female mated with him.
5. No eggs were produced by unfed females. The more the females were fed the larger the number of eggs laid per female. There was no significant correlation between the body weight of the female and the number of eggs laid. There was no effect of the size of the mating male's blood meal on the percentage of females laying eggs. The percentage of sterile eggs increased with the decrease in the size of the mating males' blood meals.
6. There is always a significant correlation between the size of the blood meals and the longevity of any instar.
7. It seems important to study the role of crowding on frequency of mating and also the traumatic effects of repeated mating.

ACKNOWLEDGEMENTS

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