A STUDY OF SOME FACTORS AFFECTING BLOOD-FEEDING, AUTOGENY AND FECUNDITY OF SIMULIUM VITTATUM ZETTERSTEDT AND PROSIMULIUM MIXTUN SYME AND DAVIES

JOSEPH EDWARD MOKRY

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A STUDY OF SOME FACTORS AFFECTING BLOOD-FEEDING, AUTOGENY AND FECUNDITY OF *SIMULIUM VITTATUM* ZETTERSTEDT AND *PROSIMULIUM MIXTUM* SYME AND DAVIES.

by

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Abstract

The survival of female Simulium vittatum in vials was greater (85%) when males were present than when females were held alone (67%). A preponderance of males, however, resulted in decreased female survival (46%). Sucrose was found to greatly increase the fecundity of autogenous female S. vittatum (265 oocytes/female) compared to females given only distilled water (198/female). Prosimulium mixtum females were more fecund after feeding on human blood than on either duck or goose bloods, 276 oocytes/female and 253/female, respectively. Human blood was also digested more rapidly by these flies than was avian blood. A blood-meal significantly increased the number of mature oocytes that S. vittatum females developed during the autogenous gonotrophic cycle. It was shown that this blood-meal compensated females for poor larval nutrition. The effects of larval nutrition were correlated with adult female fecundity. Poorly-fed larvae produced small, less fecund females while larvae fed on richer diets yielded females that were larger and produced a greater number of mature oocytes/female. Larger and younger females of S. vittatum fed more avidly than did other females. The relationship of these results to autogeny and black fly behaviour in the field was discussed. Female S. vittatum and P. mixtum fed well on human blood (73% and 77%, respectively), pig blood (82% and 63%, respectively) and bovine blood (40% and 53%). In addition, P. mixtum fed readily on duck blood (59%). A

discussion of host preference based on field observations and the present results examined some aspects of host-seeking as related to feeding preferences. Preliminary experiments were carried out on the effects of light and possible circadian rhythms on the feeding rates of *P. mixtum* and *S. vittatum*.

Acknowledgements

I wish to express my appreciation to my supervisor, Dr Marshall Laird and committee, Drs Roger Gordon and Albert Undeen, for their advice and critical reading of this thesis. Dr Murray Colbo and Mr Gerald N. Porter kindly made unpublished data available for which I am grateful. Dr Marek Szczepanski, Director of Animal Care, Memorial University, very helpfully provided the animal bloods used in these experiments while the Canadian Red Cross Society provided outdated human blood.

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Introduction

In spite of the importance of black flies as pests and/or vectors of disease in many parts of the world, relatively little laboratory work has been done on adult biology. Since the most immediate impact of adult black flies comes from their blood-feeding activity, this aspect has perhaps been best studied. Blood-feeding studies have in general tended to concentrate on the active or potential role of black flies as vectors of both human and animal maladies as well as the sheer nuisance level of their persistent attacks. The relative paucity of information dealing with the factors both physical and physiological which elicit biting behaviour in simuliids has largely inspired this present research.

In the field, work on biting behaviour using bait or sentinel animals has led to a fairly sharp delineation between mammalophilic and ornithophilic species of black flies. Anderson and De Foliart (1961) used bait animals including potential avian and large mammal hosts to determine the host preferences of Wisconsin black flies. Host preferences for ornithophilic species were found to be reflected in their habitat preferences (Bennett, 1960; Pascuzzo, 1976). These authors showed the vertical stratification of bird biters which, although not a new concept (Haddow et al, 1961), was successfully applied to the monitoring of vector species at the forest canopy

level (Herman and Bennett, 1976). The relative specificity of some ornithophilic black flies such as Simulium rugglesi N & M and S. euryadminiculum Davies has allowed a more extensive examination to be made of their blood-feeding activities (Bennett, 1960, 1963; Lowther and Wood, 1964; Bennett and Fallis, 1971; Bennett et al, 1972 and Tarshis, 1972).

In contrast to the avian feeders, mammalophilic simuliids are relatively catholic in their choice of hosts. Generally, however, it is the larger mammals which are attacked (Davies and Peterson, 1956; Anderson and Dicke, 1961; Wenk and Schlörer, 1963 and Golini et al, 1976). Although host availability and relative abundance no doubt strongly influence ultimate host selection, the selection of preferred hosts may take place even when other choices are available (Downe and Morrison, 1957).

The results of field studies have been used to develop a model of host orientation (Smith, 1966 and Golini, 1970) which described the behavioural steps up to and including blood-sucking. As well, observations have been made on the environmental factors which affect biting behaviour (Wolfe and Peterson, 1960; Smith, 1966; Alverson and Noblet, 1976 and Pascuzzo, 1976). Yet field observations do not answer the questions regarding the nature of stimulants that elicit blood-feeding responses nor the physiological state of

hunger as perceived by the fly. It has been implicit in many laboratory trials that such factors were subject to definition (Friend, 1965; Wenk, 1965; McMahon, 1968; Dethier, 1976).

In Africa Wanson et al (1945), Muirhead-Thomson (1957), McMahon and Nelson (1967), McMahon (1968) and Raybould and Yagunga (1969) all experienced varying degrees of success attempting to feed Simulium damnosum Theobald s.l. on live hosts in the laboratory. Fallis et al (1973a, 1973b) also were partly successful in feeding ornithophilic simuliids on birds in Africa. The European species Boophthora erythrocephala De Geer and Wilhelmia lineata Meigen fed readily on the ear of a rabbit (Wenk, 1965; Wirtz, 1976). North American species have infrequently been induced to feed on live hosts in the laboratory. Tarshis (1972) fed five species of laboratory-reared ornithophilic simuliids on ducks with, in some cases, a high degree of success. Simulium vittatum Zetterstedt and S. venustum Say, reared from field-collected larvae were fed with some success (19-23%) on the ear of a rabbit (Wenk and Mokry, unpublished). In addition laboratory-reared S. vittatum have been successfully persuaded to feed on a human arm (Porter, pers. comm.).

Devices for in vitro feeding of hematophagous insects have been widely employed (Friend, 1965; Galun, 1967; Gatehouse, 1967; Mellor, 1971; Moloo, 1971) but have not been

used extensively to feed simuliids (McMahon, 1968; Sutcliffe and McIver, 1975; Mokry, 1976a). McMahon (1968) used the skin of two day old chicks as a membrane through which S. ornatum Meig. pierced and took blood. Sutcliffe and McIver (1975) examined the factors which induced probing and gorging in field-collected S. venustum using a latex membrane system. Mokry (1976a) also using a latex membrane, showed that lab-reared S. vittatum could be blood-fed soon after emergence.

Black fly physiology has not been extensively studied. Yang and Davies (1968a, 1968b, 1968c, 1974) carried out a series of experiments on the gut and salivary enzymes of several species of simuliids. Condon et al (1976) studied the neuroendocrine system of Prosimulium mixtum Syme and Davies and S. venustum larvae but comparable work has not been done on the adults.

Several investigations have been made on the fecundity of black flies in relation to the blood-meal and ovarian cycle (Davies and Peterson, 1956; Peterson, 1959; Abdelnur, 1968). Others have used the ovarian cycle as a guide to the age structure and bionomics of field populations (Davies, 1961; Fredeen, 1964; Chutter, 1970; Duke, 1975; Magnarelli and Cupp, 1978). Ovarian changes associated with vitellogenesis were studied by Liu and Davies (1975). Also, the effects of larval nutrition on adult female fecundity were observed under field conditions by Chutter (1970) and

Heckler and Rühm (1976).

Black fly biting cycles have not been studied in great detail but such reports as are available indicate that under field conditions there are morning and late afternoon peaks with a midday depression in feeding rates (Wolfe and Peterson, 1960; Lewis, 1960; Alverson and Noblet, 1976; Wenk and Mokry, unpublished). Whether the midday relaxation in biting activity is related to higher temperatures, lower humidity, increased solar radiation or is a reflection of endogenous rhythms is unknown.

The object of this present study was to investigate the ovarian development of simuliids in relation to autogeny and blood-feeding. A study was made of the factors that influence the fecundity of female S. vittatum and P. mixtum including the effects of blood-feeding on autogenous females. Some of the factors, physiological and environmental, that influenced the rate of blood-feeding were also examined. An attempt was made to determine whether black flies have an intrinsic biting cycle or whether the pattern of biting activity observed in the field is regulated by meteorological events.

Black Fly Collection Areas

The black flies used in this study were collected from several localities around St John's, Newfoundland. Referring to figure 1, site 1 is Shoe Cove Brook (47° 44.5'N, 52° 44.4'W) from which were collected large numbers of Simulium vittatum larvae. Site 2 is near the Flat Rock Road (47° 41'N, 52° 43'W) where several collections of attacking female Prosimulium mixtum were made. Site 3 is the Hughes Pond outlet (47° 35.6'N, 52° 50.9'W) which was a source for larvae and eggs of S. vittatum as well as larval P. mixtum. Site 4 was the major collecting site for attacking P. mixtum and S. vittatum. This is located at the Little Power's Pond outlet (47° 33.9'N, 52° 51.9'W). Site 5, on Mt Scio (47° 34.8'N, 52° 44.5'W), yielded numerous attacking females of both species besides P. mixtum larvae. The Bay Bulls-Big Pond road-side ditch (47° 23'N, 52° 47'W), which was the major source of larval and pupal P. mixtum, was site 6.

Larval and pupal collections of P. mixtum were done in April and May of 1976 and 1977. Adults of this species could be collected from late May until the end of June at most sites. Although S. vittatum larvae could be and often were collected in the fall or spring at Shoe Cove and Hughes Pond, the majority of flies of this species were obtained by the laboratory rearing of eggs collected in the fall from the sites listed.

Figure 1

Collection sites on the Avalon Peninsula for *Simulium vittatum* and *Prosimulium mixtum* eggs, larvae, pupae or adults

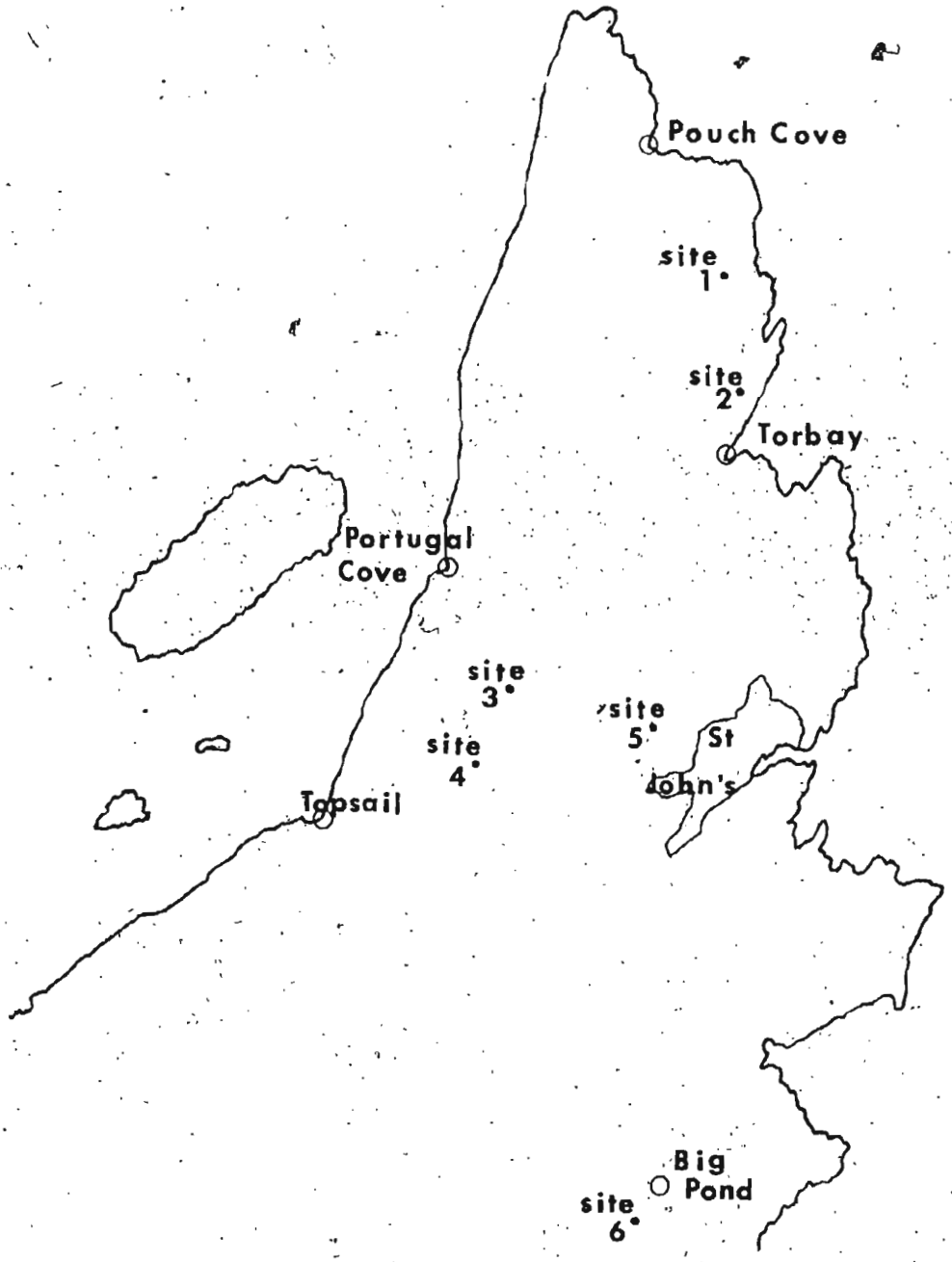
Pouch Cove
site 1
site 2
Torbay
Portugal Cove
site 3
site 4
site 5
St John's
Topsail
Big Pond
site 6

FIG 1

Materials and Methods

Adult female Prosimulium mixtum and Simulium vittatum which made up the bulk of the flies used in these experiments were obtained in either of two ways. Females were netted as they attacked in the field, put in vials or waxed ice-cream cartons and transported back to the laboratory in styrofoam boxes. These flies hereafter will be referred to as 'field-collected'. Field-collected eggs, larvae or pupae reared in bubblers (Hocking and Pickering, 1954; Fredeen, 1959; Mokry, 1976b) or a stir-bar apparatus (Colbo and Thompson, 1978) provided the second source of flies. These flies, referred to as 'lab-reared', emerged in the laboratory and were collected several times daily from the windows where they congregated or in net traps placed over the rearing cylinders. Flies were held in 3 cm x 5 cm vials with sponge bottoms beneath which was injected 1 ml of distilled water to ensure high humidity. A cotton-wool wick entering through a hole cut in the gauze tops of the vials was wetted daily with a 10% sucrose solution. All flies were treated in the same manner unless otherwise stated. In the case of S. vittatum, a daily record was kept of the number of surviving females.

In Newfoundland lab-reared females of both species begin the first gonotrophic cycle autogenously (Lewis and Bennett, 1973; Bradbury, 1972) and field-collected females, caught while seeking a blood-meal, are about to enter at

least the second gonotrophic cycle. The vast majority of field-collected females used in these experiments were P. mixtum. Since there was reason to suspect a priori that these females would show decreasing levels of fecundity with advancing physiological age, fecundity trials using this species were conducted only on females collected during the first week of the 'biting season'. It was thereby concluded that all females of both P. mixtum and S. vittatum netted at this time were uniformly entering the second gonotrophic cycle and that variations in potential fecundity were due to factors other than physiological age. When an individual experiment was terminated, the ovaries were examined and the age confirmed by the method of Detinova (1962).

Using lab-reared S.vittatum and P. mixtum, an attempt was made to describe the autogenous ovarian development during the first gonotrophic cycle. As the females were held in vials at a constant temperature (21° C) an attempt was also made to correlate the physiological age (Detinova, 1962) with the actual age in days.

The effect on ovarian development of a carbohydrate source was determined by allowing two groups of S. vittatum females daily access to a 10% sucrose solution while allowing only distilled water to two other groups. Four days after emergence the ovaries of the four groups were dissected out and the number of maturing oocytes counted.

More than 2100 field-collected and lab-reared females of both species were given the opportunity to take a blood-meal by means of an artificial membrane apparatus (Mokry, 1976a). The membrane system consisted of four 40 ml shell vials filled with pre-heated citrated blood and closed with squares of latex rubber (Sheik® regular prophylactics) which were held onto the vials with plastic rings. The exposed feeding area of each membrane was 4.5 cm^2. The vials were fixed into plastic bottles through which 40°C water was constantly circulated. Flies were introduced to the membrane by inverting the vial with blood and resting it on top of a 3 cm x 5 cm vial which contained the flies. During the feeding trials, the vial with the flies was kept covered except for a 1 cm space around the top which was in contact with the blood and membrane. Flies, attracted to the light, flew or climbed to the top of the vial where they came in contact with the warm membrane and began to feed. The system allowed four replicates to be run simultaneously on four different membranes. Flies were given access to the membranes for 20 minutes. Outdated human whole blood was provided by the Canadian Red Cross Society. Horse, cow, duck and goose bloods were supplied by Murdock Enterprises, Cambridge, Ontario while pig, sheep, dog and rabbit bloods were kindly provided by the Memorial University Medical School.

After having access to the feeding membranes for 20

minutes, flies were removed and dissected to determine the proportion which had fed. In one set of trials, P. mixtum females which had fed on human blood and avian (duck or goose) blood were kept in vials for five days. On the fifth day after feeding, the ovaries of females that had fed on the different host bloods were dissected out and the rate of development and number of maturing oocytes was compared between groups.

A trial was run to determine whether the average fecundity of P. mixtum and S. vittatum females changed with the number of gonotrophic cycles completed. Lab-reared examples of each species were held in vials until the fifth day when their ovaries were examined and the number of maturing oocytes counted for the first gonotrophic cycle. Field-collected females, i.e. those entering the second gonotrophic cycle, were fed on human blood in the laboratory and then likewise examined for comparison of their potential fecundity.

The effect of the calendar age on the blood-feeding rates of lab-reared S. vittatum females was investigated. Emerging females were held for periods ranging from 1-2 hours up to 8 days before being given the opportunity to feed. The flies in each case were dissected immediately afterward to determine the rate of feeding.

Previous experience had indicated that the size of flies used might influence the feeding rates and fecundity levels. A series of trials was designed, therefore, to determine the relationship between the size of the female and both its fecundity and feeding rates. A stir-bar apparatus devised by Colbo and Thompson (1978) was used to rear S. vittatum larvae under varying diet and population density regimes which were previously known to produce a range of adult sizes (Colbo and Porter, pers. comm.). Fifty 1st instar control larvae were put in the stir-bar system and fed 5 ml of a prepared (5gm/1 of water) Tetramin diet/day from the date of hatching. All other larvae used were transferred from a bubbled aquarium to the stir-bar system when they reached the third instar. These larvae were reared under the following diet and density conditions: Group 1, 50 larvae given 5 ml diet/day; Group 2, 100 larvae given 5 ml diet/day; Group 3, 50 larvae given 2 ml diet/day; Group 4, 100 larvae given 2 ml diet/day; Group 5, 200 larvae given 5 ml diet/day; Group 6, 200 larvae given 2 ml diet/day. Females from five replicates of each regime were used in the trials.

Emergent females were kept in vials and given free access to sucrose for five days. The ovaries were then dissected out, the number of maturing oocytes counted, and a mean established for flies reared in each regime. A measurement from wing tip to the proximal edge of the first

basal cell was used as an index of size for flies from each group. The adults obtained from these rearing regimes were used as a basis for determining the variation of potential fecundity in relation to adult size through the construction of regression lines.

Twenty-four of the females obtained from the above rearing regimes were given the opportunity to take a blood-meal a few hours to 24 hours after emergence. These females were likewise measured and the maturing oocytes counted on the fifth day and the results compared to the means for the unfed females.

To test the possibility that adult size was affecting the feeding rate as well as fecundity, a sample of 275 preserved S. vittatum females used in previous blood-feeding experiments (not recorded here) was examined. All females so measured had been lab-reared and used in human blood-feeding trials on the membranes. Wing lengths were measured as above and the condition of the fly, fed or unfed, was noted. Size distribution curves for fed and unfed flies were then constructed and the means for the two groups compared.

An attempt was also made to investigate the possibility that black fly biting activity was controlled by circadian rhythms. S. vittatum larvae were reared from eggs in

bubblers in a 12 hr light/12 hr dark regime. Emerged females were kept in vials in the same light regime at 21°C (the rearing temperature) in an environmentally controlled chamber. Flies, 24-48 hrs old, were given access to a blood-meal at two hour intervals starting at 0800 hr (artificial dawn).

An experiment was designed to determine the effects of light regime storage on the feeding rates of P. mixtum. Field-collected females were brought into the lab and divided into four groups. One group was blood-fed immediately and served as the control. The other three groups were stored overnight; one each in either 24 hrs dark, 12 hr light/12 hr dark, or 24 hrs light. They were then allowed the opportunity to feed on human blood. The trials continued for three consecutive days with a fresh collection of flies daily. All collections were made between 1130-1230 hr.

Results

I Survival of females in captivity

The results for daily mortality of lab-reared S. vittatum females held in vials for eight days are summarized on Table 1. Vials containing females alone had an average survival rate of 62%. Survivorship was slightly higher in vials with ≤5 females (65%) than in vials with >5 females (59%). When both males and females were stored in vials, density showed no obvious effect on the survival rate. The last three couplets shown on Table 1 were all derived from the (females + males) ≤5 and (females + males) >5 couplet (2) which represented the total vials with mixed sexes. When vials were divided into two groups based solely on sex ratios (couplet 3), it was clear that vials in which males outnumbered females showed poor survival rates for females. This held true regardless of the density of the flies (couplets 4 and 5). The greatest survival rate for females (85%) came from vials in which females equalled or exceeded males and where the total was ≤5.

II Ovarian development

There were morphological and rate of development differences between the ovaries of P. mixtum and S. vittatum especially during the early part of the autogenous ovarian

Table 1

Summary of daily mortalities of female S. vittatum in vials

	Number of females	Age in days 1	2	3	4	5	6	7	8	Total mortality	% Survival
Females only ≤5	60	1	1	6	5	4	2	0	2	21	65%
Females only >5	51	1	3	2	4	4	2	2	3	21	59%
(Females + males) ≤5	97	2	5	4	7	7	1	3	3	32	67%
(Females + males) >5	144	4	6	9	10	10	3	4	5	51	65%
Females ≥ males	130	3	4	5	6	5	0	0	0	23	82%
Females < males	111	3	7	8	11	12	4	7	8	60	46%
(Females ≥ males) ≤5	72	0	2	2	2	5	0	0	0	11	85%
(Females < males) ≤5	25	2	3	2	5	2	1	3	3	21	16%
(Females ≥ males) >5	58	3	2	3	4	0	0	0	0	12	79%
(Females < males) >5	86	1	4	5	7	6	6	3	6	38	56%

cycle. P. mixtum females were invariably observed with the ovaries in at least late Stage II though many females had Stage III ovaries. S. vittatum females were more variable than this but most females had early and middle Stage II ovaries. On occasion S. vittatum females were observed with ovaries that appeared to be Stage I (fig 2A). The difference between the ovaries of these species, however, was much less apparent after 72 hours at 21°C. Figure 2 illustrates the stages of development observed and attempts to apply the terminology of Christóphers' (1911) stages for mosquitoes.

A - Stage I — Egg follicle rounded with a faint granular appearance. Numerous (up to 15) nurse cells visible, however, nucleus of oocyte not distinguishable. (S. vittatum only)

B - Stage II (Newly-emerged) — 1) Early - enlargement of nurse cells to fill follicle, follicular epithelium formed, oocyte evident.

C - Stage II (Day 2) — 2) Middle - continued yolk deposition in oocyte, most yolk granules small.

D - Stage II (Day 3) — 3) Late - yolk deposition continues with larger rounded granules. Nurse cells beginning to contract.

Figure 2

Stages of autogenous development of ovaries of lab-reared *S. vittatum*

A - Stage I (?)

B - Early stage II

C - Mid-stage II

D - Late stage II

E - Stage III

F - Stage IV

G - Stage V

H - Stage I oocyte from attacking female

1 - nurse cells

2 - tunica

3 - germarium

4 - follicular epithelium

5 - nucleus of oocyte

6 - yolk granules

7 - follicular relic

E - Stage III (Day 4) Nurse cells contract further as yolk fills more than half of follicle. Nucleus of oocyte just visible.

F - Stage IV (Day 5) Yolk entirely fills follicle, nucleus obscured.

G - Stage V (Day 6) Shape changes to sub-triangular as chorionic layer is deposited.

The last follicle illustrated (fig 2H) is that of a field-collected P. mixtum. It indicates that the oocyte resting stage is Stage II. Also very clear was the follicular relic which showed that this female had completed one, presumably autogenous, gonotrophic cycle. The corpora lutea in the relic were distinctly yellow in colour.

III Factors affecting fecundity

A) Larval nutrition

Larval nutrition proved to be a factor strongly affecting adult size in S. vittatum. This in turn was correlated with the number of mature oocytes that a female could be expected to develop (Table 2 and figure 3). Control and Group 1 flies, which were the best fed, achieved wing lengths of 3.04 mm and 2.89 mm, respectively. Groups 5 and 6 (which were reared on near starvation diets) produced the smallest flies, 2.31 and

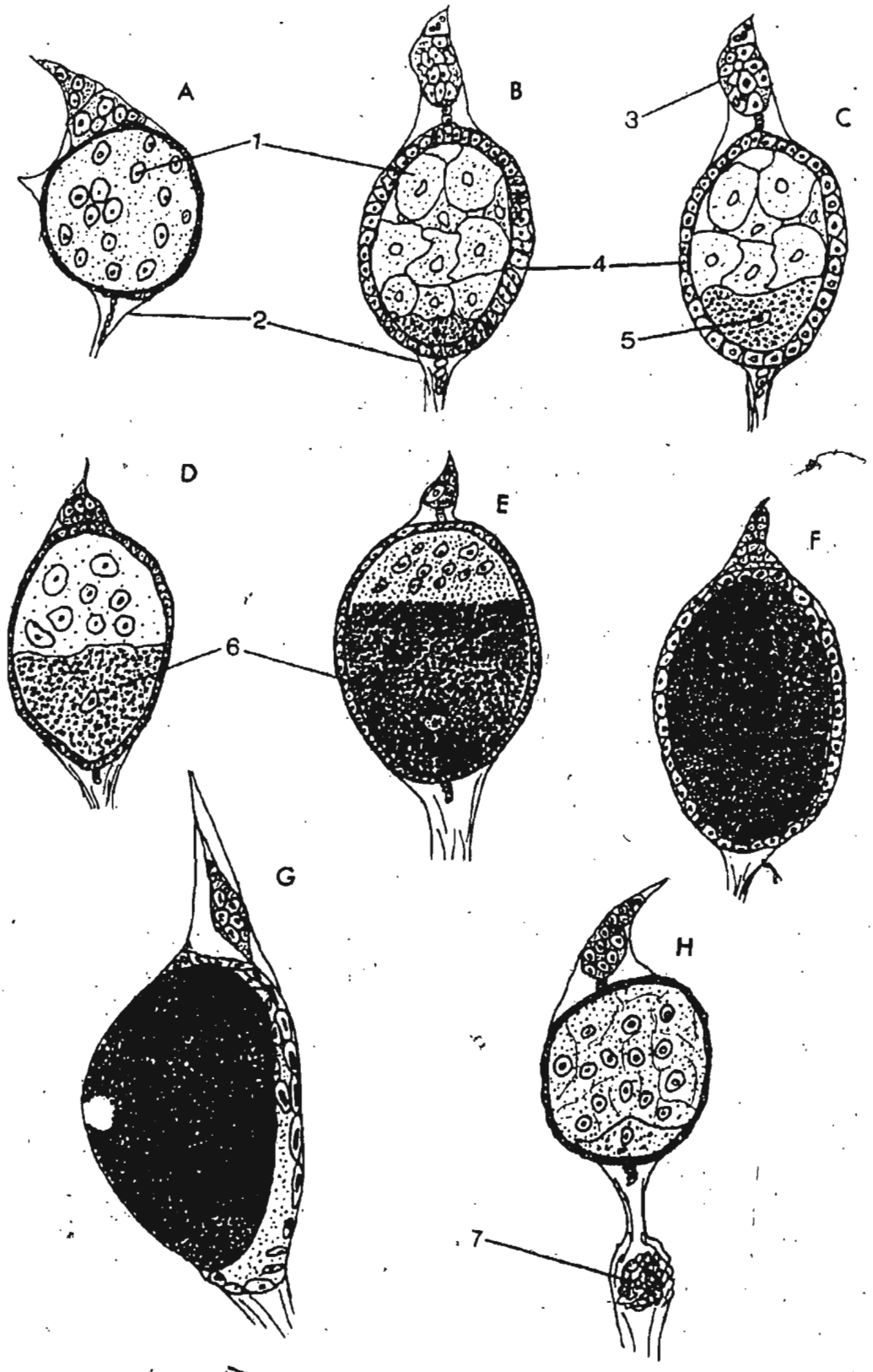

Fig 2

Table 2

Effect of larval diet on adult wing length and fecundity

*Group	Number measured	Wing length in mm	Oocytes
Control	8	3.04 ± 0.09	265 ± 24
1	6	2.89 ± 0.05	251 ± 9
2	9	2.69 ± 0.04	228 ± 36
3	9	2.51 ± 0.09	168 ± 31
4	11	2.43 ± 0.06	112 ± 34
5	11	2.31 ± 0.04	87 ± 25
6	13	2.21 ± 0.04	**77 ± 38

*Refer to Materials and Methods for details of diets supplied to each group

**Represents counts from 6 females only as 7 females did not develop any mature oocytes

Figure 3

Relationship between the number of mature oocytes/female and size of female as measured by wing length in laboratory-reared S. vittatum

C = Control females

1 = Group 1 females

2 = Group 2 females

3 = Group 3 females

4 = Group 4 females

5 = Group 5 females

6 = Group 6 females

see Materials and Methods for diets fed each group

2.21 mm, respectively. Groups 2,3 and 4 produced females with intermediate values in descending order.

These groups retained the same rank when the number of oocytes was counted and compared on the fifth day. The highest number of mature oocytes/female was found in the Control flies (265/female) and Group 1 flies (251/female). The small, poorly fed flies had the lowest numbers with Group 5 showing 87/female and Group 6, 77/female. Again, Groups 2, 3 and 4 demonstrated intermediate values in descending order.

The Group 6 flies, in addition to producing the lowest number of oocytes/female of any group, included seven females which did not produce any oocytes over the five day period. Only the six remaining females which did develop their ovarioles autogenously were included in the calculations of fecundity.

The correlation between wing length and fecundity was clear. A scatter-diagram and the calculated regression line (Y on X) are shown on figure 4. The calculated coefficient of correlation was $r = 0.81$, where the correlation constant = 248.27 and degrees of freedom = 45. The correlation equation was $Y = 247.7X - 472.8$.

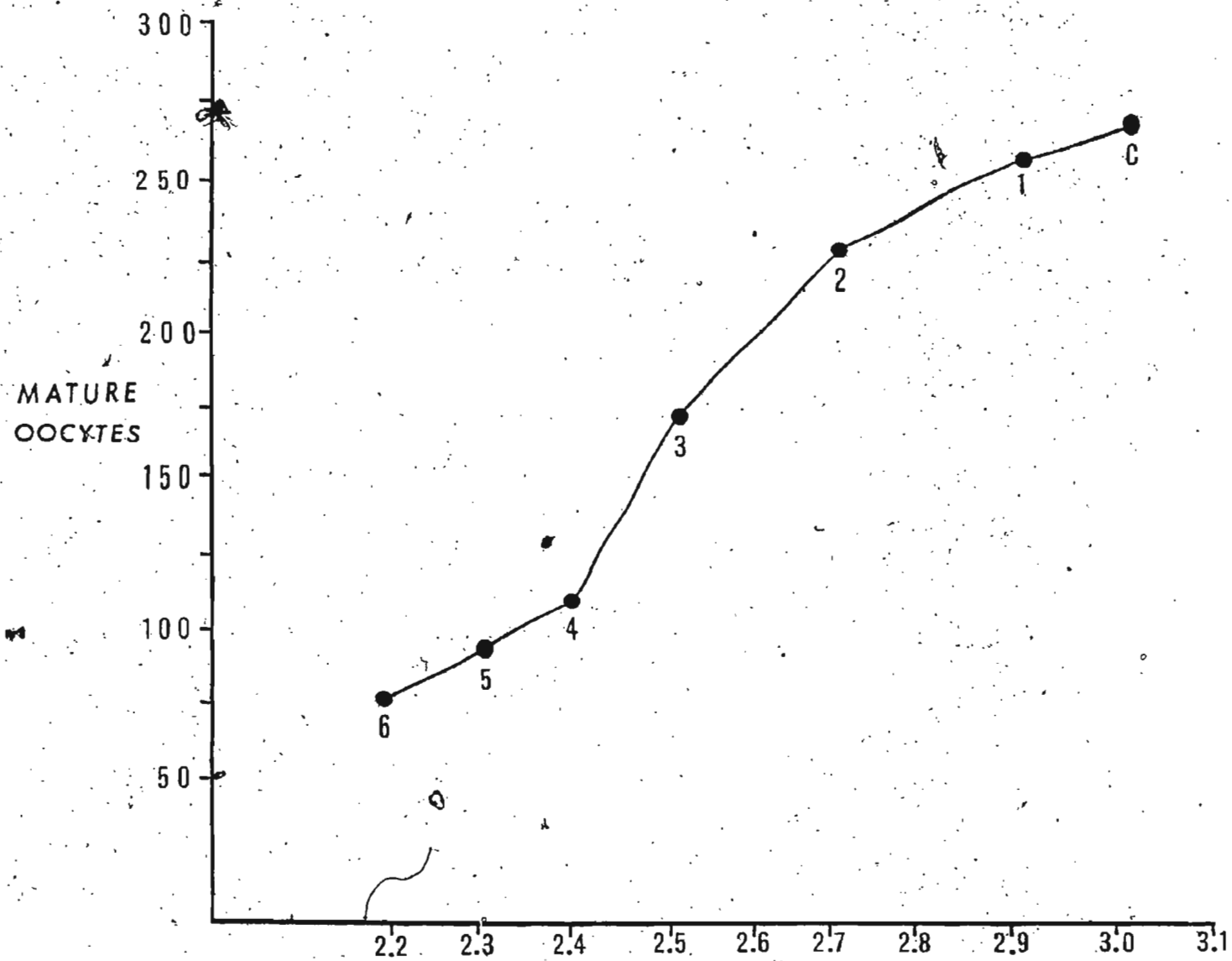
300
250
200
150
100
50
MATURE
OOCYTES
C
1
2
3
4
5
6
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
WING LENGTH IN mm

FIG 3

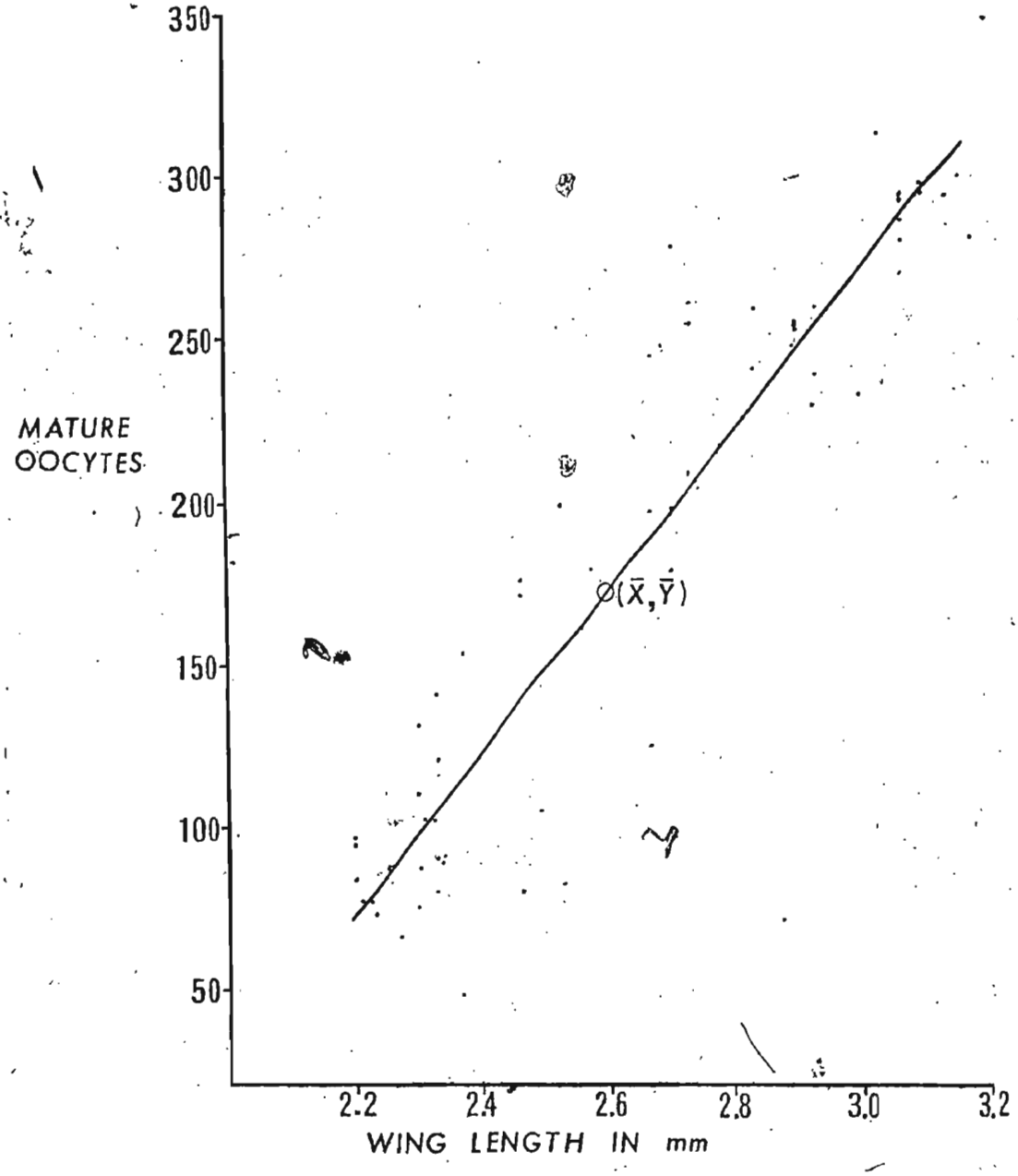
350
300
250
200
150
100
50
MATURE
OOCYTES
$\emptyset(\bar{X},\bar{Y})$
2.2
2.4
2.6
2.8
3.0
3.2
WING LENGTH IN mm

Fig 4

Figure 4

Scatter-diagram with regression line showing the relationship between wing length and number of oocytes.

Correlation equation, $Y = 247.7X - 472.8$; $r = 0.81$; $df = 45$

B) Blood-feeding autogenous females

An additional number of female S. vittatum from the above groups was given the opportunity to feed on human blood one or two days after emergence. The effect of this blood-meal on the fecundity of autogenous females is shown on Table 3. Data in the Oocytes Expected column were taken from Table 2 which gave the means of autogenous unfed fecundity for the appropriate size group. All the increases in mature oocytes were significant at $p = 0.01$. None of the Group 6 females (those that were physically the smallest) took a blood-meal. Consequently, data under the Oocytes Observed column are lacking for this group.

Figure 5 compares the level of fecundity of unfed and fed female S. vittatum from the same size groups. The percent increase in oocytes was inversely related to the size of the fly. Control females increased at the rate of 18% while the remaining groups increased at the following rates: Group 1, 29%; Group 2, 32%; Group 3, 57%; Group 4, 102% and Group 5, 70%.

C) Source of blood

Table 4 indicates that field-collected P. mixtum females which were fed on avian blood matured, on the average, fewer oocytes than did human-fed females. Three each of duck and goose blood-fed flies survived

Table 3

Effect of blood-meal on fecundity of lab-reared S. vittatum

Group	*Oocytes expected	Oocytes observed	
Control	265 ± 24	314 ± 10	t = 5, n = 12
1	251 ± 9	323 ± 9	t = 5.86, n = 9
2	228 ± 36	301 ± 26	t = 4.56, n = 16
3	168 ± 31	263 ± 23	t = 4.65, n = 7
4	112 ± 34	226 ± 32	t = 5.19, n = 11
5	87 ± 25	148	t = 7.12, n = 10
6	77 ± 38	**-	

*Values for Oocytes expected derived from Group means as shown on Table 5

**No Group 6 females could be persuaded to take a blood-meal

Figure 5

Relationship between number of oocytes/female and size of fly in autogenous S. vittatum. Upper curve represents blood-fed females; lower curve represents unfed females

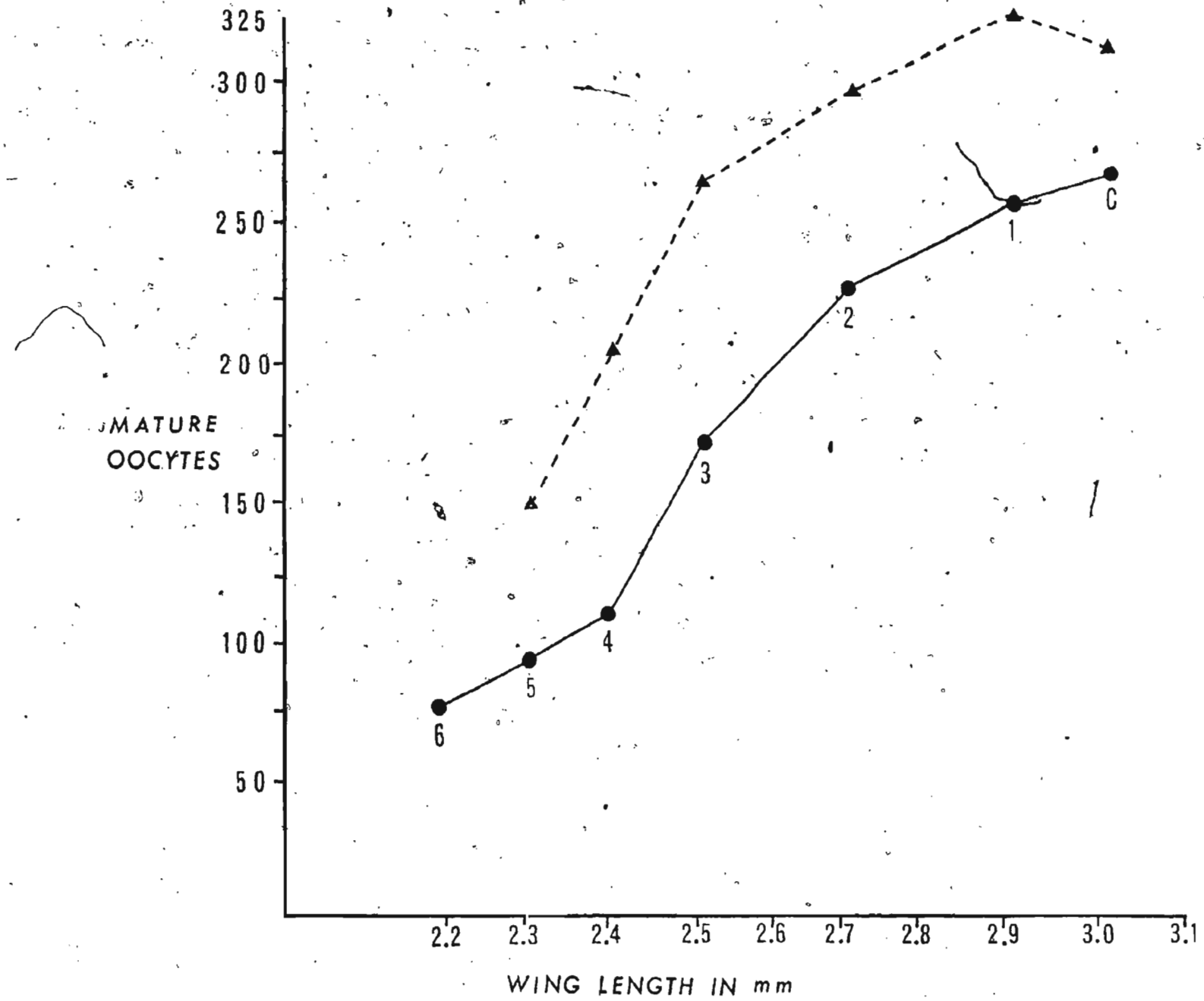
325
300
250
200
150
100
50
MATURE OOCYTES
C
1
2
3
4
5
6
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
WING LENGTH IN mm

FIG 5

Table 4

Fecundity* of individual P. mixtum females** with avian or human blood-meals

Avian (goose or duck)	Human
240 maturing oocytes	268 maturing oocytes
268	277
269	282
221	281
253	279
267	269
$\bar{x}$ = 253 ± 19	275
	277
	$\bar{x}$ = 276 ± 5

t = 2.84, significant at p = 0.05

*Fecundity was estimated by counting the number of maturing oocytes a female developed five days after taking the blood-meal.

**Females used were field-collected while attacking, hence, all were parous but of unknown physiological age.

the five days until the ovaries were dissected. There was no significant difference between the egg production for the two avian bloods at $p = 0.05$ so the two avian groups were combined and compared to human blood yields. An average of 275 ± 5 mature oocytes/female was produced by flies fed on human blood compared to 253 ± 19 for flies fed on avian blood. This proved to be significant at $p = 0.05$, $t = 2.84$, $n = 14$.

The blood-meal source also affected the rate of development of the ovaries in P. mixtum. All human-fed flies had reached Stage IV° while all six avian-fed females were found to be at Late Stage II on the fifth day when the experiment was terminated to conduct the examinations. Blood remains were found in the mid-gut of avian-fed flies while no traces of human blood were observed during the examinations.

Survivorship of field-collected blood-fed P. mixtum females was poor under lab conditions. Of the 29 duck blood-fed females and 21 goose blood-fed females, only three females from each group survived the five day period after feeding. Of the human blood-fed flies only 8 of 50 survived. This heavy mortality accounted for the small sample sizes in this experiment ($n = 14$).

D) Sucrose

Sucrose greatly influenced the number of mature oocytes a female S. vittatum could be expected to develop autogenously (Table 5). The flies used in this experiment were those which emerged from larvae fed either the Control diet or the Group 2 diet. Thus the flies shown in Table 5 as those having had free access to sucrose are the same flies listed under Control and Group 2 on Table 2. Those flies shown as having had only distilled water, however, are additional females of the same diet groups. Statistically, the sucrose-fed females served as the Expected data while the flies given only water provided the Experimental data.

Control-diet flies, which had an average wing length of 3.03 mm, developed an average of 198 ± 23 mature oocytes when given only distilled water to drink for four days. Sucrose-fed flies from the same diet group developed an average of 265 ± 24. The average decrease in fecundity of non-sucrose-fed females was 25% and was significant at $p = 0.01$, $t = 5.70$.

The results for the physically smaller Group 2 females were even more pronounced. The average wing length in this group was 2.70 mm. Flies given access to water only matured an average of 83 ± 34 oocytes compared to 228 ± 36 for sucrose-fed females. The decrease in the number of mature oocytes in females

Table 5

Effect of sucrose on the number of maturing oocytes in lab-reared S. vittatum

	Control Diet		Group 2 diet	
	Wing length	Oocytes	Wing length	Oocytes
Distilled water	3.00	201	2.67	91
	3.10	207	2.73	92
	3.07	213	2.70	97
	2.93	206	2.76	31
	2.97	151	2.73	31
	3.00	224	2.64	106
	2.97	179	2.64	91
	3.04	199	2.67	127
	3.01 ± 0.06	198 ± 23	2.69 ± 0.04	83 ± 34
Sucrose	3.00	233	2.70	198
	2.90	248	2.73	261
	2.93	230	2.67	197
	3.10	288	2.73	255
	3.17	281	2.70	279
	3.07	270	2.73	209
	3.07	287	2.67	245
	3.07	280	3.70	179
	3.04 ± 0.09	265 ± 24	2.70 ± 0.03	228 ± 36

not fed sucrose was 64% which was significant at $p = 0.01$, $t = 8.28$.

E) Physiological age

Female P. mixtum and S. vittatum developed significantly fewer mature oocytes during the second gonotrophic cycle as compared to the autogenous first cycle. The mean oocyte number for lab-reared autogenous P. mixtum and S. vittatum was 302 and 265, respectively. This compares to 276 and 218 for each, respectively, for the second gonotrophic cycle for which field-collected females were fed on human blood. These differences were significant at $p = 0.01$, $t = 5.81$ (P. mixtum) and $t = 4.81$ (S. vittatum). These data are presented on Table 6.

In the case of the field-collected S. vittatum females which were fed in the second gonotrophic cycle, the size of the flies used was equivalent to that of the females in the autogenous first cycle. That is, females between 2.95 - 3.14 mm in wing length provided the data for the second gonotrophic cycle. Their age was later confirmed by the ovarian relic technique of Detinova (1962).

P. mixtum females were also confirmed as being in the second gonotrophic cycle by the ovarian relic technique. At the time that these trials were done,

Table 6

Fecundity of P. mixtum and S. vittatum over two gonotrophic cycles

	*Autogenous cycle	*1st blood-fed cycle
P. mixtum	297 maturing oocytes	268 maturing oocytes
	301	277
	291	282
	289	281
	300	279
	321	269
	323	275
	295	277
	299	
	$\bar{x}$ = 301.8 ± 12	$\bar{x}$ = 276 ± 5.15
S. vittatum	233	201
	248	230
	230	227
	288	224
	281	189
	270	197
	287	210
	280	251
		232
	$\bar{x}$ = 264.6 ± 24	221
		210
		219
		$\bar{x}$ = 217.5 ± 17

*Autogenous females of both species were lab-reared, while females in the 1st blood-fed cycle (actually the second gonotrophic cycle) were field-collected when attacking

there was no standard in wing lengths for fecundity correlations for P. mixtum. However, a measured sample of 77 female P. mixtum captured and preserved at the same time and place of collection* for the females fed in this experiment gave a very consistent wing length ($\bar{x}$ = 2.89 ± 0.01 mm).

IV Factors affecting the blood-feeding rate

A) Size of the fly

The size of the fly was not only positively correlated with fecundity but with the rate of feeding as well. Figure 6 shows the results obtained when a sample of female S. vittatum used in some previous feeding trials was divided into two groups depending upon whether or not they had fed. All the females used in figure 6 were lab-reared from eggs and allowed the opportunity to feed on human blood. The unfed females (n = 144) had a mean wing length of 2.49 ± 0.18 mm while the females which blood-fed (n = 131) had a mean wing length of 2.64 ± 0.16. This difference was significant (t = 7.47) at p = 0.001, where t = 3.29 at df = ∞ (n = 275). The ranges of the two groups, however, greatly overlap so that the biological significance of these differences may be questionable.

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*June 10, 1977, Broad Cove River, Site 4, cf fig 1

Figure 6

Size distribution of lab-reared S. vittatum. Curve at left represents flies which refused to feed; curve at right shows flies which blood-fed.

Wing-length means: $\bar{x}_1$ unfed = 2.49 ± 0.18 mm, n = 144

$\bar{x}_2$ fed = 2.64 ± 0.16 mm, n = 131

B) Calendar age of fly

The age of fly in days also had an effect on the blood-feeding rate of lab-reared autogenous S. vittatum (Table 7). Newly-emerged females proved eager to feed while only 1-2 hours old (41.8%, 23/55), but the highest rate of feeding was observed in day-old flies, 84% (126/150). After the third day the feeding rate dropped abruptly and decreased daily to 9.1% (3/33) on the eighth day.

Although there was no quantitative record of the amount of blood taken by each age group of flies provided with a feeding opportunity, observations indicated that newly-emerged and day-old females became more bloated than older flies. In particular, seven- and eight-day-old females appeared to take considerably less blood than these younger females.

C) Lab-reared vs field-collected females

As stated in the previous section, lab-reared S. vittatum (24 hrs old) fed well on human blood (84%). Females field-collected while attacking or hovering near the collector, however, did not feed as successfully on the membrane (31/105, 29.5%). It is unclear if these females were simply less avid to take blood because of their physiological age or whether this was a result of the trauma of collection, transport and handling.

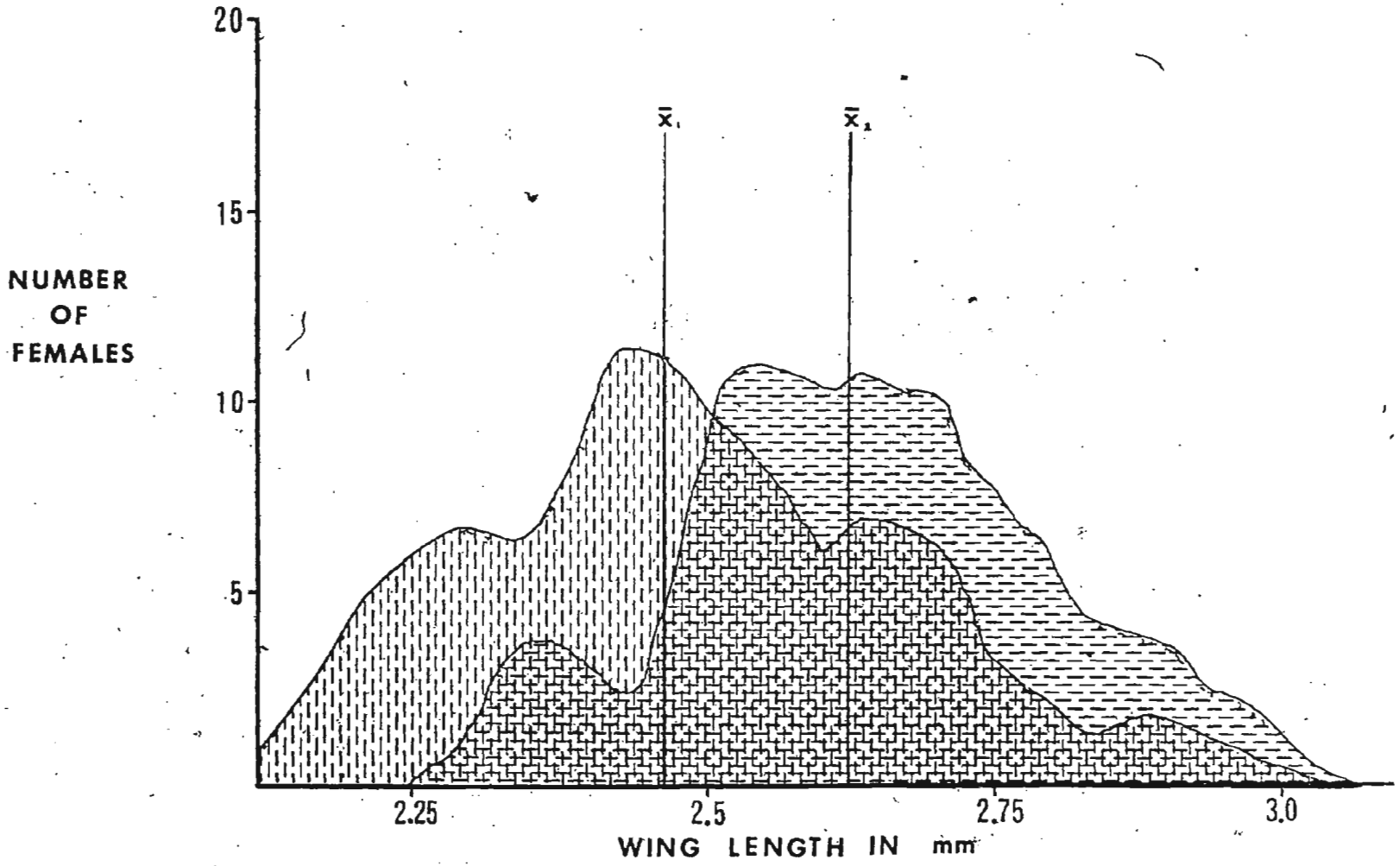
20
15
10
5
NUMBER OF FEMALES
$\bar{x}_1$
$\bar{x}_2$
2.25
2.5
2.75
3.0
WING LENGTH IN mm

FIG 6

Table 7

Effect of calendar age on the feeding rate of lab-reared S. vittatum

	Age in days								
	1-2 hours	1	2	3	4	5	6	7	8
Feeding rate	23/55♀♀	126/150	59/81	65/104	57/127	59/150	20/115	11/100	3/33
% blood-fed	42	84	73	62	45	39	18	11	9

However, attacking P. mixtum, collected at the same time and place, blood-fed eagerly in the laboratory (710/971♀♀, 73%). This situation is further complicated by the fact that lab-reared P. mixtum (2-24 hrs old) uniformly refused to blood-feed (0/182♀♀). Since these two species were handled in an identical manner throughout the course of these experiments, fault in technique would not appear to account for these decidedly opposite results.

D) Source of blood-meal

Table 8 summarizes the results of exposure of P. mixtum and S. vittatum to blood from various sources. Field-collected P. mixtum readily accepted blood from man (73%, 710/971), horse (72%, 63/88), pig (63%, 15/24), cow (53%, 18/34) and duck (59%, 29/49), but not from dog (10%, 11/110) or goose (14%, 3/21). Laboratory-reared females of P. mixtum uniformly refused to feed (0/182).

Simulium vittatum fed well on most bloods (human 77%, 264/345; pig 82%, 37/45; cow 40%, 14/35; dog 38%, 6/16 and sheep 36%, 8/22) but less well on others (duck 18%, 2/11; rabbit 20%, 12/60; horse 29%, 8/28). In the case of S. vittatum lab-reared flies fed more than twice as readily as field-collected ones: 65.5% (351/562) and 29.5% (31/105), respectively.

Table 8

The effect of different host bloods on the feeding rates of some simuliids

		Human	%	Rabbit	%	*Avian	%	Sheep	%	Total	%
P. mixtum	(A)	0/182	0	-	-	-	-	-	-	0/182	-
	(B)	710/971	73	-	-	32/70	46	-	-	742/1041	71
S. vittatum	(A)	264/345	77	12/60	20	2/11	18	8/22	36	286/438	65
	(B)	23/67	34	-	-	-	-	-	-	23/67	34

		Cow	%	Horse	%	Pig	%	Dog	%	Totals	%
P. mixtum	(A)	-	-	-	-	-	-	-	-	-	-
	(B)	18/34	53	63/88	72	15/24	63	11/110	10	107/256	42
S. vittatum	(A)	14/35	40	8/28	29	37/45	82	6/16	38	65/124	52
	(B)	3/9	33	-	-	3/27	11	2/2	-	8/38	21

(A) = females lab-reared from field-collected eggs, larvae or pupae
(B) = females caught in field while attacking

*Avian - includes goose or duck blood

E) Light regime storage

The effects on blood-feeding rates of storage for 24 hours of field-collected female P. mixtum are shown on Table 9. Freshly-caught control flies fed at consistently high rates (89%) compared to all stored flies (47%-22%). Among stored flies, those kept in 24 hrs darkness before feeding gave a 47% (31/66) blood-feeding rate which is somewhat higher than that shown by flies kept in 12/12 light/dark (41%, 19/46). Females kept in continuous light showed markedly reduced feeding rates with only 22% (12/54) taking blood.

F) Time of day

S. vittatum females which had been reared and stored for 24 hrs in a 12 hr light/ 12 hr dark photophase produced the results shown on Table 10 when given the opportunity to feed. Flies fed best during the two-hour period just after 'lights on' (89.6%). The three following two-hour periods showed slightly diminished feeding rates as compared to the first 'lights on' period although the three periods themselves produced similar results (82.3, 82.5, 80.6%). Females fed during the fifth two-hour period showed increasing reluctance to feed (63.6%), while those in the last period fed even less willingly (47.6%).

Table 9

Effect of light on stored blood-feeding *P. mixtum*

Control (fresh-caught)		24 hrs dark stored		12/12 light/ dark		24 hrs light stored	
17/19	89%	6/12	50%	11/25	44%	3/14	21%
7/8	87%	14/29	48%	3/9	33%	5/21	24%
23/26	88%	11/25	44%	5/12	42%	4/19	21%
47/53	88.7%	31/66	46.9%	19/46	41.3%	12/54	22.2%

Table 10

Effect of time of day on blood-feeding lab-reared S. vittatum

*Time	**Numbers used	% fed
0800 - 1000 hr	26/29 ♀♀ fed	89.6
1000 - 1200	14/17	82.3
1200 - 1400	25/30	82.5
1400 - 1600	25/31	80.6
1600 - 1800	21/33	63.6
1800 - 2000	10/21	47.6

*Artificial dawn at 0800 hr during 12 hr light/ 12 hr dark light regime

**Total of three replicates

Discussion

I Survival of females in captivity

The longevity of female black flies under laboratory conditions has obvious importance to any study of adult biology, especially when possible disease vectors are involved. For this reason recent studies on adult longevity have tended to concentrate on known vectors of human onchocerciasis (Raybould and Mhiddin, 1974 and Figueroa et al, 1977). The latter authors kept 45.9% of S. ochraceum females alive to the eighth day. Davies (1953) was able to keep 70-76% of field-collected S. venustum alive for five days in 20 cm x 3 cm tubes. Dry sucrose crystals and a distilled water supply were found to give the best results. Fly density apparently was not a factor affecting survival in these large tubes.

In the present work density again did not significantly influence the survival rates. What did affect female survival in this study was the relative proportion of females: males. Female survival was greatest (85%) in vials where a lesser number of males was present than in vials with females alone (65%). The poorest female survival (46%) was in vials where males outnumbered females.

Observations indicated that males were far more active than females and that their presence had an unsettling effect on the females in vials. The 'domino effect' was often

observed in that the activity of a single fly would displace or disturb a nearby fly which further disturbed yet another fly. In vials containing males, therefore, the level of activity was observed to be higher than in vials with females alone. It is likely that in vials in which males predominated the level of activity was such that stress-related mortality may have accounted for a large proportion of the dead flies.

II Ovarian development

The process of maturation of the ovarioles in both P. mixtum and S. vittatum followed well-defined stages which closely approximated similar stages of development in mosquito ovaries as defined by Christophers (1911) and Mer (1936). In the case of S. vittatum the developmental stages were in broad agreement with that of Pascuzzo (1976). Development was more uniform, however, in the black flies studied here than is generally true of mosquitoes (Clements, 1963). In autogenous Toxorhynchites spp. in particular, oocyte development is distinctly asynchronous as an ecological adaptation to the uncertain and changing tree-hole habitat (Thompson, 1976a; Watts and Smith, 1978). In one species of black fly, S. (S.) japonicum, oocyte development was observed to be irregular and asynchronous as a result of impartial or interrupted blood-meals (Takaoka, 1973).

The rather prolonged development period of eight days

at 20°C for both the autogenous and blood-fed cycles has implications for the potential vectorial capability of these flies. Since biological transmission of parasites relies upon repeated blood-feedings with the interspersed ovarian development and oviposition cycles, female S. vittatum and P. mixtum would have to survive at least 3½-4 weeks at 20° C to obtain and transmit a parasite. Anautogenous females would require one less ovarian cycle to be potential vectors. Magnarelli and Cupp (1977) found that many S. venustum and S. tuberosum (both anautogenous) completed two gonotrophic cycles with the rare female completing three cycles. If the same sort of physiological age structure exists for autogenous black fly populations, then these would be effectively eliminated from a disease transmitting role. It is, however, worth noting that Stone and Jamnback (1955) found that the second generation of S. vittatum produced by some streams in New York State was anautogenous for the first gonotrophic cycle. Both S. vittatum and S. decorum were shown to be anautogenous in the second generation in Alberta (Abdelnur, 1968).

III Factors affecting fecundity

A) Sucrose

Sucrose was previously reported to influence the expression of autogeny and the fecundity of some insects (Downes, 1958 and 1971; Nayar and Sauerman, 1975). Arctic Aedes communis de Geer was shown by Corbet (1964)

to require a carbohydrate source to develop its ovaries autogenously. The autogenous Australian black fly, S. ornatipes, has recently been shown to require a carbohydrate, preferably sucrose, in order to express autogeny (Hunter, 1977a). The present study with S. vittatum confirms the results of these authors. The lack of carbohydrates resulted in a significant reduction in the number of mature oocytes produced and greatly reduced female survival over the eight days that they were held. Chutter (1970) found that denial of sucrose for 48 hrs resulted in a decrease in the number of mature oocytes a female developed autogenously. Nayar and Sauerman (1975) showed that sucrose definitely increased fecundity when provided to mosquitoes both before and after blood-feeding. Also, more sugar-fed Culex nigripalpus developed eggs from partial blood-meals than did females which initiated oogenesis with previously depleted energy reserves (Edman and Lynn, 1975).

It is evident that although S. vittatum was in an autogenous gonotrophic cycle when tested in this study the same carbohydrate needs exist. It is suggested that in carbohydrate-deprived females, some nutrients that would have been utilized for ovarian development may be shunted to general maintenance and metabolism which ultimately prevents some ovarioles from becoming activated.

B) Source of blood

The source of blood had an effect on the fecundity of P. mixtum. Human blood-fed females produced a greater number of active ovarioles than did avian-fed flies. This difference in fecundity is probably a reflection of the species' known mammalophilic preference (Davies et al, 1962). The rates of blood digestion and ovarian development were retarded as well in avian-fed flies. The effects of different host bloods on fecundity in simuliids has, however, received very little attention considering the importance of the reproductive rate to control measures.

The effect of different host bloods on fecundity has been well-studied in mosquitoes. Bennett (1970) found that A. aegypti L. produced more eggs/female when fed on bird bloods than on guinea pig or human bloods. Woke (1937a), however, found that A. aegypti developed more eggs/mg of guinea pig or rabbit blood than on canary blood. Possibly this species routinely takes larger blood-meals from avian hosts than from mammals which would result in larger egg batches (Edman and Lynn, 1975; Takaoka, 1973). Culex tarsalis which has a strong avian preference (Reeves, 1971) clearly showed greater egg production when fed on chicken blood than when fed on guinea pig blood. Woke (1937b) demonstrated the same effect with Culex pipiens,

another avian feeder. In terms of rate of blood digestion, A. aegypti fared poorly compared to other species when fed human blood (O'Gower, 1955).

Bennett (1970) postulated that bird blood cells, being nucleated, are more nutritious than mammalian blood cells. However, Dimond et al (1958) did not find an increase in egg production when nucleic acids were added to an artificial diet fed to adult A. aegypti. Chang and Judson (1977) have shown the amino acid isoleucine to have a quantitative effect on egg production in A. aegypti. Isoleucine when added to human blood was found to increase the number of eggs produced to a number comparable to that produced by flies fed on guinea pig blood. Therefore the difference in egg production may be due to different amino acid concentrations in various bloods.

An alternative explanation is that the ability to utilize the nutrients present in blood may vary with the particular fly in question and that host preferences are a mutable physiological as well as behavioural adaptation for the blood-meal that is most nutritious for a given species at a given time. The very rapid adaptation of a blood-sucking insect to a specific host's blood would then show itself as a feeding preference. This was clearly demonstrated in mosquito

colonies that had been accustomed to feeding on rabbits and were subsequently switched to guinea pigs (Stahler and Seely, 1971). High mortality persisted for several months until the mosquito, Anopheles stephensi Liston, gradually became adapted to guinea pig blood. After four months the colony had returned to its former reproductive rate. McCray and Schoof (1970) found that Culex fatigans Weidemann, an avian feeder, was reluctant to feed on rabbits and those that eventually did take rabbit blood laid far fewer eggs than those fed on birds. After five generations of rabbit feeding, however, the egg production/female had quadrupled to very nearly the bird-fed level. The significance of this is that host preferences and fecundity due to a given host's blood represent physiological as well as behavioural and morphological adaptations to whatever is the preferred host.

C) Physiological age of female

A reduction in the number of eggs produced/female with increased age has already been noted in black flies (Abdelnur, 1968). In the present study both P. mixtum and S. vittatum were found to be less fecund in the second gonotrophic cycle compared to the first. This does not appear to be a difference in the quality of nutrients supplied to the adult, i.e. fat-body reserves from larval feeding in the autogenous cycle

vs blood-feeding in the second cycle. Rather, the phenomenon is wide-spread in biting insects including mosquitoes (Putnam and Shannon, 1934; Detinova, 1949) and is probably related to the aging process (Lambremont, 1960 and Lavoipierre, 1961). This same age-related decrease in fecundity has been utilized to determine the physiological age of S. damnosum in Africa (author's unpublished data). Up to six gonotrophic cycles could be distinguished based on the decreasing number of active ovarioles in successive cycles. It is believed (Calvin Lang, pers. comm.) that the decreasing facility for protein synthesis is one of the best biochemical monitors of the aging process. This would likely manifest itself in a reduced ability to synthesize the appropriate proteins for ovarian development.

D) Larval diet and autogeny

The size of adult S. vittatum was found to be directly related to the richness of the larval diet. This in turn greatly influenced the number of ovarioles a female activated on emergence. Chutter (1970) reported similar findings in field-collected specimens which he related to poor nutrition in certain streams. Contrary to the report of Anderson and Dicke (1960), Chutter (1970) also claimed that larval density in natural populations was not affected by poor nutrition but that the adults produced are small and less fecund.

In recent laboratory experiments, however, Colbo and Porter (pers. comm.) demonstrated that sustained high larval density was critically dependent on an adequate food supply. Poor larval nutrition not only affected the levels of fecundity reached by autogenous females in the present study, but also resulted in higher larval mortality (Colbo and Porter, pers. comm.).

The question of whether autogeny in black flies itself is a fixed character of a species, i.e. genetically determined, or whether it is an opportunistic response to a favourable larval environment has been debated (Davies, 1961; Rubtzov, 1955, 1956, 1958). A basic point in Rubtzov's argument was that the same species of black fly will produce both autogenous and anautogenous females depending on the nutrients available to the larvae. Autogeny was, therefore, environmentally induced and not inheritable. Davies (1961), however, showed that P. fuscum (autogenous in Ontario) and P. mixtum (anautogenous in Ontario) were produced from the same streams at the same time. Even when larvae developed on the same rock, P. fuscum was always autogenous and P. mixtum always anautogenous.

The present study generally supports the field results reported by Davies (1961), Chutter (1970) and

Heckler and Ruhm (1976) in that larval nutrition affects the size of the adult produced which in turn influences its fecundity. In the experiments conducted here, autogenous S. vittatum were produced regardless of the dietary conditions although the number of mature oocytes produced/female varied with the richness of the diet. Furthermore, previous experience (author's data) has shown that autogenous S. vittatum and anautogenous S. verecundum and S. venustum retain their autogeny or anautogeny even when reared together under the same conditions in the laboratory. Autogeny, then, seems to be a fixed character in S. vittatum.

Although autogeny is a genetically determined trait in S. vittatum, the present data indicated that there are in fact environmental limits to its expression. Group 6 larvae (Table 5) produced 7/13 females which failed to develop eggs autogenously. In other words more than half the females produced under these near starvation conditions were anautogenous. This does not, however, suggest that autogeny (or lack of it) is an environmentally induced response but rather that anautogeny under these conditions is an extreme example of a genotype masked by its environment.

Autogeny has been well-studied in mosquitoes. Spielman (1957) showed that autogeny was an inheritable

trait through crosses with anautogenous members of the Culex pipiens complex. Twohy and Rozeboom (1957) reported similar findings with the same complex. O'Meara and Krasnick (1970) studied the effect of larval diet on autogeny in Aedes atropalpus. These authors concluded that dietary deficiencies can mask autogeny in this species. Corbet (1967) reported a facultative autogeny in northern Aedes spp. Females that could not find a host reabsorbed a number of oocytes and used the total derived nutrients to develop a few eggs. In a field study on Aedes taeniorhynchus, O'Meara and Edman (1975) showed that this species existed in autogenous and anautogenous populations that were genetically isolated. Crossings experiments demonstrated that the polygenic controlling system was inheritable. In the laboratory Van den Heuvel (1963) and Sanburg and Larsen (1973) showed the effect on adult size and autogeny of mosquitoes reared under various photoperiod and temperature regimes. Linley et al (1970) showed by sampling field populations that autogeny in Culicoides furens (Poey) was inversely proportional to larval rearing temperature. Apparently, lengthening the larval life span by lowering the temperature allowed the larvae to feed longer and thus build up greater fat-body reserves. Fat-body nutrients were then used to develop autogenous eggs.

E) Blood-feeding autogenous females

Blood-feeding apparently augmented fat-body reserves in the present study. All S. vittatum females that took blood developed far more mature oocytes than did non-fed flies of the same size. The percentage increase in ovariole number was greater in smaller females. Equally, the actual amount of increase in number of oocytes was greatest in the small females. The single Group 5 female that blood-fed was exceptional in that its fecundity did not follow this trend. For the rest, however, it is apparent that the smaller flies probably took larger blood-meals since they demonstrated a greater increase in the number of oocytes after feeding than did the larger flies. The marked increase in fecundity of blood-fed females is evidenced by the following: Group 3 females, blood-fed, developed as many oocytes as did unfed Control group females. In a very real sense, then, blood-feeding during the autogenous cycle fully compensated these adults for relatively deprived larval conditions. (See table 3).

IV Autogeny and female behaviour

These results lead to several interesting issues by first of all begging the question of why nutritionally deprived autogenous black flies do not take blood under field conditions. That poor conditions exist such as to produce malnourished autogenous females has already been noted (Chutter,

1970; Heckler and Ruhm, 1976). Furthermore, the author is unaware of any reports to the effect that autogenous simuliid females in the first gonotrophic cycle have ever been caught attempting to feed. The other half of the question is why should nulliparous autogenous females feed at all under laboratory conditions as has been demonstrated in the present work.

The answer to these questions is that the physiological and hormonal changes that an autogenous female goes through during the gonotrophic cycle affect her behaviour. Failure of these females to blood-feed in the field is likely a result of an inhibition against host-seeking. The same suggestion has been made to explain the unresponsiveness to a host by blood-fed mosquitoes (Clements, 1963). The fact that in the laboratory S. vittatum females will feed readily (at least in the case of the younger females) is probably due to the more immediate stimulus of heat. Temperature is known to be the most important factor initiating probing (Dethier, 1954; Friend, 1965; Mellor, 1971; Sutcliffe and McIver, 1975). In effect, the females had found the host without the behavioural sequence of host-seeking. To put the above statements into context, however, it will first be necessary to review what is known about the physiological and behavioural changes a female endures during the gonotrophic cycle.

During ovarian development, a number of physiological activities take place under the influence of neurosecretory and humoral agents. In mosquitoes the ingestion of a blood-meal releases stored egg development neurosecretory hormone (EDNH) (Lea, 1967). EDNH apparently initiates the synthesis of a vitellogenin stimulating hormone (VSH) in the ovaries (Hagedorn, 1974; Hagedorn and Fallon, 1973). VSH, secreted by the ovaries, stimulates the fat-body to begin synthesizing the major yolk protein, vitellogenin, (Hagedorn et al, 1975) which is released into the hemolymph. The ovarioles absorb the yolk protein by pinocytosis and begin development of the oocytes.

One factor that affects feeding behaviour is the rate of digestion of a prior blood-meal. Edman and Lynn (1975) showed that six hours after ingestion of a partial blood-meal, Culex nigripalpus Theo. could not be induced to re-feed although before this time limit it would. Apparently blood-feeding activity had been interrupted as blood digestion and ovarian development began (Edman et al, 1975). Most likely, then, blood-feeding is also controlled by hormonal sequences.

The release of hormones that initiate the sequence of events leading to ovarian maturation is, as stated earlier, the ingestion of blood. In autogenous mosquitoes, however, females release EDNH shortly after emergence without the

addition of a blood-meal (Larsen, 1958). Presuming that the ovarian development does indeed interrupt or inhibit blood-feeding as suggested by Edman and Lynn (1975), then autogenous females of species which emerge with the oocytes at the resting stage might be induced to feed for a short period. This period would correspond to the time lapse after emergence before ovarian development had 'shut off' blood-feeding responses.

This was clearly proved by O'Meara and Evans (1973 and 1976) who showed that A. taeniorhynchus, an autogenous mosquito that blood-feeds readily on emergence, became increasingly reluctant to take blood as ovarian development proceeded. This is precisely the effect observed in this present study with S. vittatum. Females 1-2 hrs to 8 days old, given the opportunity to feed, showed increasing reluctance to blood-feed. The time required for the hormonal 'shut off' is probably a character specific to each species depending on the stage of the oocytes on emergence and is moderated by such factors as temperature and humidity. In the case of S. vittatum, at least, there is no definite 'on' or 'off' but a gradual decrease in the feeding rate from a high of 82% (24 hrs old) to a low of 9% (8 days) with a fairly abrupt decrease after the third day. This contrasts with the results of McMahon (1968) with the anautogenous S. ornatum. Under lab conditions this species did not reach its peak of biting activity until females were at least five

days old. This may also help to explain the consistently negative results obtained when trying to blood-feed lab-reared P. mixtum in the present study. As stated in the Results, P. mixtum females normally emerged with the ovarioles at Stage III, while females caught attacking in the field showed that the resting stage was Stage II. Clearly, ovarian development was begun in the pupa by the pharate adult in the case of P. mixtum. Equally clear is the fact that in this study S. vittatum females, emerging with Stage II ovaries, 'lagged' two to three days behind P. mixtum in oocyte development. In S. vittatum Stage III was usually reached on Day 4 which corresponds to the rather abrupt drop in feeding rates for this species (Table 7).

Host-seeking, on the other hand, is probably not allowed to be initiated until the first egg batch is laid. There are sound ecological reasons why this should be so. An autogenous female attracted to a host is likely to be drawn away from its site of emergence and, therefore, its likely oviposition site. As well, flight exposes it to predators, wastes energy that could be used for egg maturation and exposes it to environmental hazards such as hot, direct sunlight, low humidity and high winds. Besides these dangers, there are the host's defensive reactions to being bitten. Davies (1961), in fact, found that autogenous P. fuscum did not disperse more than a few metres from its stream of emergence until after the first gonotrophic cycle.

With these considerations in mind, it is easy to see that it is to the fly's 'advantage' to remain near the emergence site during oogenesis whether autogenous or as a result of blood-feeding. Since some flight must take place for activities such as nectar feeding and searching for a resting site, the lack of host-seeking is probably a lack of response to the host itself. Rather than a general 'flight arrest', the mechanism may work through inactivation of CO_2 and odour receptors. Since young female S. vittatum will blood-feed readily but do not seek a host, it is apparent that the two activities are under separate controls which come into effect at different times. Mattingly (1969) has already suggested, in an entirely different context, that blood-feeding and host-seeking are not necessarily related. If black flies do indeed possess a neuroendocrine system similar to that of mosquitoes, then these activities may be separable as follows.

Since the hormones that initiate ovarian maturation in autogenous mosquitoes are secreted shortly after emergence (Larsen, 1958), it is suggested that these hormones or the effects they produce, also inhibit host-seeking. Blood-feeding, on the other hand, is dampened gradually. This may be due to the delayed and gradual build-up of yolk proteins in the hemolymph destined for the ovaries which tells the fly whether she is sated or hungry. It may also be due to a build-up of the ENDH titre which inhibits feeding.

The mechanism in this case might be to inactivate, at least partially, the thermoreceptors which would stimulate the fly to probe. Autogenous S. vittatum will experience such a protein and hormone build-up in the hemolymph and might find its response to temperature gradually decreasing. McMahon's (1968) anautogenous females would, conversely, find the blood proteins diminishing as fat-body reserves were depleted and, as well, experience a build-up in the juvenile hormone titre which prepares the ovaries for development following the blood-meal (Gwadz and Spielman, 1973). These females would become 'hungrier' with time. The increase in feeding rates over the first nine days found by McMahon (1968) may be a reflection of this fact.

Currently, there are two attractive hypotheses to explain protein hunger in flies viz Phormia and calliphorids which feed on high protein content diets. First is that the neural mechanisms which mediate feeding behaviour are under hormonal control of a periodic nature (Strangways-Dixon, 1961). Second is that this behaviour is influenced by protein deficits in the blood or elsewhere (Dethier, 1961 and Belzer, 1970). While a version of the first hypothesis is favoured in blood-sucking flies (Hagedorn, pers. comm.), it seems that the stimulatory effects of low blood proteins has not received an equally intensive investigation.

Davies' (1961) observation of the fact that autogenous

females were not found to disperse until after the first gonotrophic cycle raises an interesting point. Streams that are, from a black fly's point of view, nutritionally poor will tend to produce smaller, less fecund females than richer streams. These same females are far more likely to oviposit in the stream of their emergence if they do not disperse. This means that fewer eggs will be laid in poor streams than in richer streams. The system is in fact self-regulating and does not permit an over-exploitation of limited resources or a wasteful overproduction of larvae. In richer streams, on the other hand, the larger females would leave a greater number of progeny which would allow the species the opportunity to expand with the food supply.

V Factors affecting feeding rate

A) Size of fly

As figure 5 illustrates, size had a great deal to do with the observed feeding rate. The reasons for this differential in feeding rates are not immediately obvious. In the case of the smaller flies, larval diet may have again had an effect. Groups 5 and 6 females, for example, were observed to be unsteady when moving around in the vials, constantly losing their grip on the sides. Their level of activity also seemed much reduced compared to other better-fed flies. They may in fact have been physically incapable of piercing the membrane in terms of energy requirements, musculature and armaments.

Females reared under conditions identical to Group 6 females (which were the most deprived as larvae) had significantly fewer maxillary teeth, than better-fed females (Porter, pers. comm.). This same argument may hold true for middle- vs large-size flies except that females in the middle range appeared to be in every respect 'normal'. At the present time there is no ready explanation as to why the larger flies fed more avidly than the average-size females.

B) Source of blood and host preferences

Table 8 shows that, although lab-reared S. vittatum and field-collected P. mixtum generally fed well (63% and 65% overall, respectively), both species showed preferences for certain host bloods. As might be expected, human, horse and cow bloods were all well-accepted by P. mixtum. These three are all naturally attacked by this species in the field. It was surprising, then, to see that duck blood proved to be so attractive to P. mixtum (59%, 29/49). This species is thought to be strictly mammalophilic (Davies et al, 1962). Goose blood was not very attractive to either P. mixtum (14%, 3/21) or S. vittatum (18%, 2/11). Another surprising result was the attractiveness of pig blood. P. mixtum fed well on pig blood (63%, 15/24) while S. vittatum fed in even higher rates (82%, 37/45). Pigs were used a number of times as bait animals in the

field (author's data) and were always found free from black flies. Meanwhile, other nearby farm animals were being attacked by these same species. Simulium arcticum, a fiercely biting fly in Western Canada, was rarely found to bite pigs even when fly populations were extremely high (Rempel and Arnason, 1947). Downe and Morrison (1957), using a serological test, analysed the blood found in the guts of black flies resting in a barn. They found that the vast majority of blood-meals were from horse and cow, with less than 1% from pig.

Another result inconsistent with field observations is the strong preference shown for human blood by S. vittatum. This species is not usually considered a biting pest of man but is often attracted to him in large numbers (Stone and Jamnback, 1955; Davies et al, 1962; author's data). Bites, however, are rare. A similar situation was observed by Peterson (1959) with several other species.

The above discrepancies suggest, as already put forward by Mattingly (1969), that host-seeking and blood-feeding are distinct and separate activities. The same author cites the example of zoophilic members of the Anopheles gambiae complex being attracted to, but not biting, man. As well, Culex pipiens fatigans, in spite of close associations with man, feeds extensively on birds, including fowl, even in urban areas. Yet,

even C. pipiens has been found to feed more by host abundance than by any real specificity (Edman and Downe, 1964).

The real difference, then, between these membrane feeding results and the feeding preferences established in the field is that here the acceptability of the host's blood is being measured, while in the field the attractiveness of the host itself is also considered. This is the danger of starting in the middle of a long sequence of reflexes (Hocking, 1971).

Carbon dioxide and host odour were shown to be flight stimulants for black flies (Golini and Davies, 1970; Bradbury and Bennett, 1974). Females are thought to be activated by CO_2 to fly upwind and trace the CO_2 and odour plume to its source. Once within visual range of the host, other close-range visual and chemical cues probably come into effect. These cues will either discourage further investigation or lead to landing. On the host's skin the fly, equipped with tarsal chemo- and olfactory receptors (Sutcliffe and McIver, 1975), may begin probing and subsequently take a blood-meal. Dethier (1957) has suggested that such a step-wise sequence of stimulus-response events was a requisite for feeding in some flies.

In the lab the long-range chemical cues such as odour and CO_2 do not elicit host-seeking behaviour. Perhaps because of the enclosed space or the fact that the females are unmated, the female's activity is to try to escape to the light. When placed in close proximity to the warm membranes, however, it is unnecessary for the females to go through the entire sequence of events that culminate in a blood-meal. The host, in effect, is found and more immediate close-range cues assume greater importance. Under laboratory conditions temperature has repeatedly been shown to be perhaps the single most important stimulus to elicit probing activity in hematophagous insects (Dethier, 1954; Christophers, 1960; Langley, 1972; Davis and Sokolove, 1975; Sutcliffe and McIver, 1975; Friend and Smith, 1977). Once probing has been initiated, other factors that stimulate gorging, such as ATP and ADP, come into effect (Hosoi, 1958, 1959; Lall, 1969; Smith and Friend, 1976).

The present results indicated that factors specific to the host's blood also have an effect. For example, only 10% (11/110) of P. mixtum fed on dog blood whereas 71% (838/1187) of P. mixtum fed on all other bloods. Dog blood was obviously rejected by P. mixtum but less strongly by S. vittatum, 44% (8/18, field-collected and lab-reared) vs 63% (345/546,

overall feeding, S. vittatum). The high feeding rate for both S. vittatum and P. mixtum on pig blood then, is a response to favourable blood factors while their non-biting of pigs is a (perhaps understandably!) negative response to the pig's odour plume.

There exists a sharp difference in preferences between ornithophilic and mammalophilic black flies (Fallis, 1965; Bennett, 1960). Results of the present study regarding responses to avian blood are not sufficient to lead to definite conclusions. In one series of trials, P. mixtum fed well on duck blood (59%, 29/49) but less well on goose (14%, 3/21). Other flies exposed to avian bloods were S. vittatum (chicken, 18%, 2/11), S. venustum (chicken,0/16) and the bird-feeder, Cnephia ornithophilia (chicken, 1/22). In the example of P. mixtum feeding well on duck blood, this seems to be another example of flies choosing a blood-meal in the absence of external factors which would ordinarily inhibit blood-feeding on a particular host in the field. However, Yang and Davies (1968b) found S. venustum, another mammalophilic species, far more receptive to human blood than to duck or chicken bloods under lab conditions. S. venustum, fed on avian blood, also produced a thinner peritrophic membrane than did ornithophilic simuliids (Yang and Davies, 1977).

Although heat and CO_2 obviously play an important part in eliciting biting behaviour, host odour is also important (Thompson, 1976b). Ornithophilic black flies are particularly specific (Bennett and Coombs, 1975; Bennett et al, 1972; Davies et al, 1962). Black flies have shown some refinements in terms of avian adaptations, not only in the classic 1:1 specificity of S. euryadminiculum to the common loon (Lowther and Wood, 1964), but also in adapting their biting activity to coincide with the resting periods of their avian hosts (Bennett, 1960). It was well-argued by Bennett and Coombs (1975) that, since in Newfoundland avian feeders are relatively scarce but that black fly-transmitted avian malarias are very common in local birds, ornithophilic black flies must be highly specific. This specificity would ensure that the host/vector contact remained high enough for simuliids to transmit a parasite efficiently.

C) Light

Black fly biting cycles have not been extensively studied but such reports as are available suggest that under field conditions there are morning and afternoon peaks with a midday depression (Davies, 1952; Lewis, 1960; Wolfe and Peterson, 1960; Alverson and Noblet, 1976; Wenk and Mokry, unpublished). Whether the midday depression is caused by elevated temperatures, reduced humidity, increased solar radiation or reflects an

endogenous rhythm of activity that is independent of meteorological factors, is presently unknown.

The series of trials designed to clarify some of these points was, unfortunately, limited. Table 9 clearly shows the depressant effect on blood-feeding of prolonged light storage of field-collected P. mixtum. A similar effect was noted by McMahon (1968) who found that exposure to continuous light decreased the feeding rate of S. ornatum in the laboratory. This was confirmed by Wirtz (1976) who fed Boophthora erythrocephala on the ear of a rabbit. Wolfe and Peterson (1960) and Wenk (1965) suggested that changes in light intensity rather than the intensity itself stimulated feeding activity. Tarshis (1972) found that blood-feeding by ornithophilic simuliids was increased under laboratory conditions by covering the flies to keep them in darkness. As with P. mixtum in the present study, freshly-caught S. damnosum females were shown to feed at higher rates than did stored females (Raybould and Yagunga, 1969). Certainly light or, more specifically, changes in light intensity are known to affect oviposition behaviour in simuliids (Colbo, 1974; Hunter, 1977b; Simmons and Edman, 1978).

There may also have been a disorientation effect involved in these present trials, as the flies used

were captured at midday and at that time put in the experimental 24 hour light regimes. Experiments with mosquitoes have shown that 48 hours are required to readjust to radically different light regimes (Jones et al, 1972) while at least 72 hours are required for tsetse flies (Brady and Crump, 1978).

In order to avoid the problem of re-orientation to a new light regime, trials were conducted with S. vittatum which were reared from eggs in 12 hr light/12 hr dark (Table 10). It was hoped that by keeping the emerged females in constant temperature, humidity and light intensity during the 12 hr day, that the only factor that would affect the feeding rate would be an intrinsic rhythm. From the limited data the apparent trend was that the feeding rate was highest (89.6%) just after 'lights on'. The rate declined to about 82% throughout the next three 2-hour trials. The drop continued to 47.6% just before 'lights out'. This may be the same effect that was seen with the P. mixtum stored in continuous light as shown on Table 9. On the other hand, the data are similar in their trend toward decreasing levels of biting in early afternoon as has often been observed in the field. Missing, significantly, from these laboratory data is the second arm which forms the 'V'-shaped trough of biting activity as observed in the field. Again, perhaps the change in

light intensity with approaching dusk stimulates the second peak in biting which was not duplicated in these experiments.

Ultimately, the data do not prove one way or the other whether an intrinsic biting cycle exists in black flies similar to that known to occur in mosquitoes and tsetse flies. The results were, at least, encouraging in that they show black flies to have a definite feeding response to both light and exposure time. The fact of the biting peak just after 'lights on' suggests dawn as a natural trigger. On the other hand, light may only act as a 'conditioner' to allow simuliids to react positively to other stimuli such as CO_2 and odour and thus begin appetitive flight and host-seeking. Clearly this is an area requiring further work.

Summary

1) Survival of female S. vittatum in the laboratory was enhanced (85%) when a lesser number of males was present than in vials with females alone (67%). When males outnumbered females, however, female survival was greatly reduced (46%). It is thought that when males were too numerous they contributed to stress-related mortality because of their greater activity. No explanation was found for the result that the presence of a lesser number of males increased female survival rates over the eight day period of storage.

2) P. mixtum females emerged with the ovaries at late Stage II or Stage III. S. vittatum females, although more variable than P. mixtum, usually had early or middle Stage II ovaries on emergence. Both P. mixtum and S. vittatum were found to require seven to eight days after emergence at 20°C to develop their ovaries autogenously. The developing oocytes passed through well-defined stages which are described according to the system and terminology established for mosquitoes.

3) Sucrose was shown to affect the number of oocytes an autogenous female could develop autogenously. Females of S. vittatum matured significantly fewer oocytes when given only distilled water compared to sucrose-fed females. The energy requirements of females, even under laboratory conditions, are thought to be met by the female's fat-body reserves and thus diminished

the amount of nutrients available for ovarian maturation in water-fed flies.

4) The source of blood taken by P. mixtum was found to affect the fecundity of the females. Significantly more mature oocytes were developed by flies fed on human blood than on either duck or goose bloods. It was argued that host preferences are physiological as well as behavioural adaptations to a host. Field studies observe the behavioural aspect which manifests itself as host-seeking for a limited selection of hosts. The blood-feeding experiments in the present work and those reviewed from elsewhere suggested that differential egg production based on different host bloods was the result of physiological adaptations. The feeding rate (% ♀♀ fed) on different host bloods was also affected which supports the idea of physiological adaptations. In some cases the responses of females to a particular blood were at variance with what is known about feeding preferences in the field. A discussion of host-seeking as the factor left out in laboratory feeding trials was thought to explain much of this inconsistency.

5) The physiological age of female P. mixtum and S. vittatum affected the number of mature oocytes produced. Females in the second gonotrophic cycle matured fewer oocytes than did females in the first cycle. It was concluded that the aging process made females less able to synthesize the appropriate yolk proteins for oogenesis.

6) Larval nutrition was shown to affect directly the size and fecundity of adult S. vittatum. Poorly-fed larvae produced smaller, less fecund females than did larvae fed on richer diets. It was also shown that females that blood-fed in the first gonotrophic (autogenous) cycle greatly increased the number of mature oocytes that they developed. The blood-meal apparently compensates females for nutrients lacking in their larval diet.

7) Larger females fed in higher rates than did smaller females. Some factors which might explain this difference in feeding rates are discussed but no conclusive explanation was found.

8) The calendar age of S. vittatum females during the first gonotrophic cycle was found to influence the feeding rate. Females were less likely to feed as the autogenous maturation of the ovaries neared completion. It is proposed that either the build-up in the hemolymph of yolk proteins bound for the ovaries or elevating titres of certain hormones signalled a 'satiated' condition in the female. This physiological condition made females less responsive to the opportunity to take a blood-meal.

9) Prolonged storage in the light greatly contributed to a decrease in the feeding rates of field-collected P. mixtum. Flies stored in 12 hr/12 hr light/dark or 24 hrs dark showed improved rates of blood-feeding.

A similar effect of decreasing willingness to feed was noted in lab-reared S. vittatum. These females, given the opportunity to feed at various times during a 12 hr. photophase, fed readily at 'lights on' but were increasingly reluctant after eight hours of exposure to light. The effects of light on biting rhythms of black flies and other blood-sucking flies are discussed.

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