STUDIES ON THE BIOLOGY OF PHLEBOTOMID SAND-FLIES.

by

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ABSTRACT.

Certain aspects of the biology of sand-flies were investigated. The findings contributed to the successful establishment of a colony of L. longipalpis.

Other species proved more difficult to rear because of larval diapause, unfavourable larval conditions or the unwillingness of laboratory-reared females to take blood-meals.

L. longipalpis females were found to be opportunist feeders: They took blood-meals from a wide range of mammals and fed equally well throughout the day, over a range of temperature (21·5° - 30°C) and humidity (70% - 100% RH).

Host breath and heat, alone, could induce L. longipalpis females to probe an artificial membrane, after which physiological saline stimulated gorging. With or without ATP, serum albumen was only as attractive as saline. The conclusion that blood salts and/or mechanical bending of the labium normally stimulates blood gorging was supported by finding that whether a fluid was dispatched to the crop or mid-gut depended less on its nature than on the manner of feeding.

Results for L. longipalpis supported the idea that the blood-meal is the source of nutrients for vitellogenesis. The maximum number of oocytes matured in the first gonotrophic cycle depended also on female body-weight, sugar-feeding and age, but not mating.

The blood-meal ingredients essential for egg maturation by L. longipalpis were amino acids. The number of eggs matured per female was proportional to the weight of protein in the meal, but varied between different vertebrate bloods.

Sugar-feeding and mating increased the proportion of mature eggs laid by L. longipalpis females. More eggs were laid in dark than in light periods, and results suggested that egg-laying was governed by a circadian rhythm.

Field studies concerning the resting sites, seasonal prevalence and biting habits of some sand-fly species in Italy and Brazil were also reported and discussed.

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Special thanks are due to my mother who painstakingly and cheerfully typed the thesis from an almost illegible manuscript, and to my wife for her ungrudging help during the preparation of the thesis.

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GENERAL INTRODUCTION.

Phlebotomid sand-flies are dipterous insects of the sub-order Nematocera. The imagos are small (of about 3mm. length), brown and slender-bodied. Their nearly-erect wings and the scales which cover their bodies give them the appearance of small, hairy moths. Indeed some taxonomists still prefer to treat them as a sub-family, Phlebotominae, of the Psychodidae or moth-flies (D.J.Lewis pers.comm.).Rodendorf (1964) raised them to family rank, distinguished in part by their blood-sucking adaptations and the relatively dry breeding places of many species.

The life of the imago is concerned almost entirely with mating and, for the female, egg laying. Only the female sucks blood, which she uses to develop a batch of eggs. Autogeny has been recorded but is thought to be the exception (Johnson 1961). The eggs are laid on the larval food - organic matter found in soil, leaf litter and about animal dwellings. The sculpturing on the outer chorion of the egg may be important for plastron respiration (Ward & Ready 1975).

Like most dipterous larvae those of the sand-fly require a high humidity. This they find within soil or rubbish heaps, and in rock or wall crevices where there is condensation. Consequently, in the tropics and other warm mainland areas where they occur, sand-flies are widely distributed, with some species inhabiting arid plains and equatorial rain-forests. There are four larval instars:

The first stage larva is characterized by a single pair of black, caudal setae and an egg-tooth on the

crown of its translucent head capsule, which it uses to break out of the egg. The other stages have two pairs of caudal setae and larger, darker head capsules. A black, pigmented 9th tergite distinguishes the last stage larva from the others. All larvae are legless, but numerous stiff, pinnate hairs facilitate locomotion.

Before pupating the 4th instar larva stops feeding and crawls upwards. It attaches itself to the substratum with a secretion from its enlarged salivary glands. The free, non-motile pupa is usually upright. Its cuticle, at first white, soon turns yellow and then brown. The eyes and wing-setae of the developing adult blacken shortly before emergence.

The blood-sucking habit of sand-flies has made them a pest to man, owing to the irritation of their bites and their transmission of several diseases, including bartonellosis and sand-fly fever (Lewis 1974). Sand-flies are the only known invertebrate vectors of Leishmaniasis, a group of diseases (often zoonoses) caused by species of the protozoan genus Leishmania.

The morphology and taxonomy of sand-flies have been well studied (Fairchild 1955; Lewis 1965, 1974; Theodor 1965; Perfiliev 1968; Abonnenc 1972; Forattini 1973), but despite the medical importance of sand-flies there are no compendia on their behaviour and physiology. Partly, this results from the historical priority given to the study of diseases transmitted by mosquitoes and tse-tse flies. The establishment in temperate countries of large closed colonies of tropical mosquitoes and tse-tse flies for parasitology

investigations was the stimulus for detailed research into many aspects of these insects' biology. In comparision, sand-flies have usually been reared only in small colonies in their country of origin. The ease with which ailing laboratory colonies could be supplemented by field collections made the refinement of rearing techniques unnecessary. Thus there has been a tendency to study sand-flies only as vectors of human diseases. Often, laboratory studies have referred only to rearing methods and life-histories. The work of Theodor (1934, 1936), Chaniotis (1967) and Gemetchu (1972), where the focus of the research was on the sand-fly and not its parasites, have been notable exceptions.

The establishment at Imperial College Field Station, Ascot, England in 1972 of a research unit to study Leishmania in the sand-fly gave me the opportunity to undertake research on closed colonies of sand-flies. Of necessity, much of my early work was concerned with improving rearing techniques. Later I was able to investigate in greater depth some aspects of the feeding behaviour and reproductive physiology of one species, Lutzomyia longipalpis (Lutz & Neiva, 1912).

For short periods, in 1973 and 1974, I was fortunate to visit two areas of endemic leishmaniasis, in northern Italy and in the lower Amazon Basin, Brazil, where I became acquainted with some aspects of the behaviour of sand-flies in the field.

SECTION ONE: THE ESTABLISHMENT OF A CLOSED LABORATORY COLONY OF SAND-FLIES.

INTRODUCTION.

From some larvae of Phlebotomus mascitti found in his Rome cellar, Grassi (1907) reared the first sand-flies in the laboratory. The rearing techniques employed to meet the needs of sand-flies in each of their developmental stages have altered little since then.

The general requirements of many species of sand-flies and the methods which have been used to provide them have been reviewed by Barretto (1942), Sherlock & Sherlock (1972) and Ward (1974). Sand-fly larvae require a humid atmosphere, a damp substrate, a relatively high temperature (22° - 30°C) and a supply of organic material for food. Drier sites away from the centre of larval activity must be provided for pupation; for this the walls of the larval container usually suffice. Adults thrive in a similar environment, and with a supply of sucrose can live for 1 - 2 months. Females usually require a vertebrate blood-meal before they can develop a batch of eggs. The eggs are laid on a damp substrate, and will only survive and hatch if they remain in contact with water.

Rearing methods fall into two categories: natural chambers, where all stages of a generation live in a single container, were favoured by Whittingham & Rook (1923), Unsworth & Gordon (1946), Najera (1951) and Sherlock & Sherlock (1959). Many workers have preferred to keep larvae and adults in separate containers (Bayma 1923; Adler &

Theodor 1927; Hertig & Johnson 1961). This entails the transfer of eggs to larval containers, a process that is laborious and often kills many eggs. However, it allows the productivity of a colony to be assessed, and generations to be synchronised in order to produce batches of flies for experimental work.

Attempts to colonize six species of sand-fly are described in this thesis. Only one, L. longipalpis was selected (by Dr. R. Killick-Kendrick) as a species suited to mass rearing in the laboratory. It had been bred through several generations in laboratories in Brazil (Sherlock & Sherlock 1959) and Panama (Christensen 1972). The population chosen was known not to diapause. The method used so successfully to rear L. longipalpis was employed, with mixed success, for two forest species in Pará, Brazil, and for three species collected from southern Europe.

MATERIALS AND METHODS.

Collection of sand-flies.

All colonies were started with eggs laid by wild, blood-fed females. These were usually collected on or near animal baits. Each female was placed in an oviposition tube (see below) and transported in a warm, moist environment within a box of expanded polystyrene.

Temporary mounts of dead females were made in gum chloral to display the cibarial armature, pharynx and spermathecae from which they were identified (Lewis 1974).

Rearing methods.

These were developed by Dr. R. Killick-Kendrick, Mr A.J. Leaney and myself for the routine maintenance of L. longipalpis, to which the following account refers (Killick-Kendrick et al. 1973, 1975). The methods employed were, for the most part, modifications of those used by Adler & Theodor (1935), Hertig & Johnson (1961), Harwood (1965) and Gemetchu (1971).

From its initiation the colony was divided. Adult females from the main line were fed on hamsters, while I maintained a line (to produce most of the material for my own work) for which the females were fed on myself.

Stock was reared at 25°C, with the larvae placed in continuous dark (except for observation) and the adults in a photoperiod of 12 hours (06·00 - 18·00 hrs GMT). The first four generations were kept in a constant-temperature (C-T) room. Later generations were maintained in

photothermo-static (or environmental) cabinets.

Immature stages. 5 days after they had been laid, eggs were counted and moved with a camel-hair brush on to small pieces of wet filter paper which were shaped to identify each batch. Approximately 100 eggs were placed to hatch in pots made from the plastic tubes of WHO insecticide testing kits (Plate 1). When cut in half, each tube made the sides of two open-ended pots with screw caps, 6 cm. in length and 4·5 cm. in diameter. The inside of the pots was roughened with acetone and lined with plaster of Paris. When the lining was set, the bottom of each pot was filled to a depth of 3.0 cm. with plaster. The lids were covered with fine gauze. The use of these pots was suggested by Dr.D.F.J. Hilton.

The plaster substrate on which the larvae moved was kept damp by standing the pots on filter paper wetted with distilled water. 1st stage larvae survived best when there was a film of moisture over the plaster surface. If this film was too deep (1mm) the larvae became immobile and starved. Older larvae preferred drier conditions. A high humidity (80-100%RH) and a control of moisture was achieved by placing the pots in trays of distilled water in a C-T room or on damp filter paper in snap-top, polyethylene food containers within environmental cabinets (Plate 1).

In the early generations, larvae were fed on boiled lettuce (Chaniotis pers.comm.) as well as desiccated liver powder (Gemetchu 1971). Lettuce was not fed to later generations when it was discovered not to be essential for growth and development. Small amounts of liver powder were

Plate 1:Rearing pots and container.

Plate 2:Oviposition tubes and container.
(Courtesy Dr.R.Killick-Kendrick.)

added daily to each pot. Care was taken not to starve the larvae, especially those of the 4th instar, because of cannibalism. Overfeeding encouraged the growth of fungi which were controlled by removing uneaten food and scraping faeces from the plaster. After tending a pot, instruments were flamed to avoid transferring harmful fungi. Some fungi were eaten by the larvae, but early stages became trapped, and died, when fungal growth was excessive.

Adult flies. On the day they emerged adult flies were counted and sexed before being released into a gauze-covered cage (18cm. cube). 60 - 100 flies were released into each cage over a 1 to 3 day period. The ratio of males to females was adjusted to 1·2 - 1·0 (the overall ratio per generation). Flies were handled by capturing them in clear plastic tubes. They were not aspirated since this damaged them.

A 30% (w/v) sucrose solution was freely provided on cotton wool in a dish and was changed thrice weekly. For those generations reared in a C-T room a high humidity was obtained by covering each cage with a damp cloth. Those cages kept in environmental cabinets were sealed in a clear plastic bag containing a swab of wet cotton wool.

The flies mated freely in the cages, and pairs could be seen in copula at any time from 24 hrs after emergence. Mating occurred before, during or after a female's blood-meal.

3 - 5 days after emergence female flies were offered a blood-meal. Hamsters were anaesthetised with sodium pentobarbitone given intraperitoneally (0·4 mg/10 g. body

wt, increased as tolerance developed), and pinned to a cork board through sticky tape attached to each leg. With the animal on its back, the abdominal hair was trimmed with scissors to present a large area of naked skin on which the female flies could feed.

The line fed on man was offered blood from a volunteer's hand and wrist inserted into the cage.

Oviposition. Each engorged female was individually collected in a small glass vial (4 x 2 cm) lined at one side with a triangle of filter paper, the tip of which covered the base of the tube (Plate 2). A 30% solution of sucrose was provided on swabs of cotton wool placed on the gauze covering the mouth of each tube. The humidity was controlled by standing the tubes over a tray of water (in the C-T room generations) or placing them in a "desiccator" over water or dilute sulphuric acid solutions (Solomon 1951). The filter paper in the tube was wetted with distilled water applied (through the gauze top) with a syringe and hypodermic needle. Eggs were laid on the damp filter paper, after which few females survived more than 24 hours.

Experiments to determine optimum rearing conditions.

Investigations into the feeding behaviour, egg production and oviposition of L. longipalpis produced findings that were used to modify early rearing methods. Most of these investigations are recorded in later sections of this thesis, but the improvements in rearing success caused by the modifications which they suggested are

detailed in this section. Other experiments are recorded here in full.

(i) Effect of density on the survival of larvae. In the 5th generation 25 larval pots were set up with a different number (26 - 110) of freshly hatched larvae in each. All the pots were kept in snap-top polyethylene food boxes placed in the dark at a constant temperature of 25°C. Liver powder, the only food, was placed daily in each pot so that it was always in excess.

The number of flies that emerged from each pot was counted. This figure is proportional to the number of 4th instar larvae that successfully pupated, since pupal mortality was low and more or less constant for each pot (see below).

(ii) Sucrose and adult longevity. Freshly emerged adult flies were released from larval pots into three gauze cages (18 cm cube) so that each contained 20 males and 20 females. On the floor of cage 1 was placed a petri dish (9 cm in diameter) containing cotton wool soaked in 30% (w/v) sucrose solution. Similarly, cage 2 was provided with distilled water. 30% sucrose solution was offered to cage 3 on a dental wick held in a tube containing the solution.

Solutions were renewed daily. Each cage was placed in a sealed, clear plastic bag containing a swab of wet cotton wool. The experiment was carried out in a C-T room at 25°C with a photoperiod of 12 hrs (06·00 - 18·00 GMT).

The number of each sex alive in the cages was

recorded daily until all were dead.

Monitoring the productivity of the colony of L.longipalpis.

Age specific life-tables were prepared for many of the first nine generations. Such tables, or budgets, should record absolute populations at each stage of a generation, and the action of known mortality factors (Southwood 1966). In this case, however, the different larval stages were not treated separately as the overlap between them was so great that it was impossible to estimate absolute populations.

After generation 9 the rearing techniques were modified little. Most changes were made between generations 4 and 5 when the colony was moved from a C-T room to the more tightly regulated conditions of environmental cabinets. The budgets were used to measure and analyse the changes in productivity that resulted from these modifications. For this, "Survivorship" curves were drawn and "Apparent" and "Indispensable" mortalities were calculated (Southwood 1966).

In addition to productivity, the qualitative success of the rearing methods was measured by determining the mean weights of male and female pupae of early and late generations. On the day of eye and wing-setae blackening (which is 3 days before adult emergence, at 25°C) pupae were taken at random from pots of equal density and weighed on a Beckman microbalance.

RESULTS.

Collecting and rearing sand-flies.

L. longipalpis. The colony was established with 1,726 eggs laid by 53 engorged females collected by Dr. R. Killick-Kendrick from one of the Lapinha caves, 60 Km. N.W. of Belo Horizonte, Minas Gerais, Brazil (Long. 43° 57', Lat. 19° 03' S). A caged cockerel was left in the cave for the two days preceeding the collection.

From October 1972 to December 1975 I reared 23 successive generations of this species at Imperial College, Ascot. Each generation after the eighth produced, on average, 600 adult flies (1·2 males to 1·0 female). Under the best conditions, at 25°C in environmental cabinets, the time of development from female engorgment to the first emergence of adults of the next generation was 40 days. The minimum developmental times of each stage are shown in Table 1 . For the larvae these are very approximate and were obtained by observing the first appearance of each stage.

P. perfiliewi. (For this species and those that follow, details of the number of flies reared through successive generations are given in Table 2 , and details of developmental times in Table 1).

During the summer of 1973, engorged females were captured from cattle or human baits in a cow-shed at Vedriano, a hamlet in the foothills of the Apeninnes near Bologna, Emilia-Romagna, Italy. The survival of the

SPECIES	CONDITIONS	DEVELOPMENT TIME (DAYS) PRE-OVIPOSITION PERIOD	EGG	LARVAE	PUPA	TOTAL DEVELOPMENT TIME EGG - ADULT (DAYS)
L.longipalpis	25°C.,95%RH.	5 - 9	5 - 9	12 - 24	7 - 10	24 - 43
L.longipalpis	28°C.,95%RH.	3 - 6	4 - 7	9 - 16	6 - 9	19 - 32
P.ariasi	25°C.,95%RH.	8 - 11	9 - 11	25 - 28	8 - 10	42 - 49
P.perniciosus	25°C.,95%RH.	5 - 7	6 - 11	17 - 18	9 - 10	32 - 39
P.perfiliewi	25°C.,95%RH.	2 - 7	8 - 10	16 - 27(168)*	9 - 27	33 - 64(205)*
L.flaviscutellata	25°C.,95%RH.	3 - 5	6 - 10	18 - 28	5 - 8	29 - 46
Ps.davisi	25°C.,95%RH.	3 - 7	7 - 12	19 - 30	7 - 9	33 - 51

( )* = Ca.16 weeks at 10°C. as diapausing 4th.instar larvae.

Table 1. Range of minimum development times for six laboratory-reared sand-fly species.

SPECIES	WILD-CAUGHT FLIES		LABORATORY GENERATION						
	No. OF FEMALES CAPTURED	No. OF FEMALES LAYING EGGS	TOTAL EGGS LAID	No. OF LARVAE	No. OF PUPAE	No. OF MALES	No. OF FEMALES	No. OF FEMALES FEEDING	No. OF FEMALES LAYING EGGS
P.ariasi	42	9	77	47	5	2	2	0	-
P.perniciosus	1	1	50	50	24	14	8	4	2
" second laboratory generation			61	46	38	20	18	16	2
" third laboratory generation			32	2	2	1	0	-	-
P.perfiliewi	77	25	650	(179)*	37	4	2	0	-
Ps.davisi	40	21	510	300	10	5	3	0	-
L.flaviscutellata	16	5	170	112	12	4	6	0	-

( )* = minimum number.

Table 2. Number of sand-flies of species other than L.longipalpis reared through successive generations.

engorged females was poor, and few laid any eggs. Most of the eggs were fertile and larvae were successfully brought to the 4th stage. By November 1973 there were only a few pupae; the majority of larvae had died or entered a state of dormancy.

The larvae had been reared at 25°C in constant dark. In November they were divided; some larval pots were placed at 25°C in a photoperiod of 16 hrs and others in a dark chamber at 10°C. After 6 - 14 weeks small numbers of chilled larvae were brought to 25°C in a photoperiod of 16 hrs. Mr. A.J. Leaney carried out much of this later work.

Most of the larvae from both treatments died. Only a few pupated. None of the females that emerged took a blood-meal or laid eggs.

P. ariasi and P. perniciosus. These species were captured in the southern Cévennes (near Roquedur-le-Bas, Languedoc), France, by Dr. R. Killick-Kendrick and Mr. A.J. Leaney in the summer of 1975.

42 engorged female P. ariasi were captured from the nose of a dog. Few eggs were laid; however, 61% of these hatched. The larvae did not fare well, and only few 4th instars pupated. The females that emerged refused on several occasions to take blood from human and hamster baits offered at different times of the day. No eggs were laid.

One engorged female P. perniciosus was captured after it had bitten a sleeping man in an isolated farm-house. 50 eggs were laid and all hatched. From these larvae 3

generations of flies were reared at Ascot. The colony faltered when many of the second generation females died during the digestion of their blood-meals.

Both of these species were reared at 26°C in a photo-period of 16 hrs light (05·30 - 21·30 hrs GMT) - the summer conditions of the region in which they were captured.

Psychodopygus davisi and L. flaviscutellata. These species were captured from human and rodent baits respectively, in Mocambo Forest, Belém, Pará, Brazil. They had been bred in small numbers in the Wellcome laboratories in Belém by Dr. R.D. Ward, but the colonies had never survived for more than 2 generations (Ward 1974).

During a visit to Belém in 1974 I attempted to rear these species using the methods that had proved so useful for L. longipalpis. Small numbers of both species were taken through one complete generation, but females reared in the laboratory refused to take a blood-meal.

Experiments to determine optimum rearing conditions for L. longipalpis.

(i) Effect of density on the survival of larvae. The percentage of 1st instar larvae successfully reared to pupation was found to be independent of density in the range of 26 to 110 larvae per pot (Fig 1).

(ii) Sucrose and adult longevity. Male and female flies lived longer when 30% (w/v) sucrose solution was offered in place of water (Fig ?). This difference was more

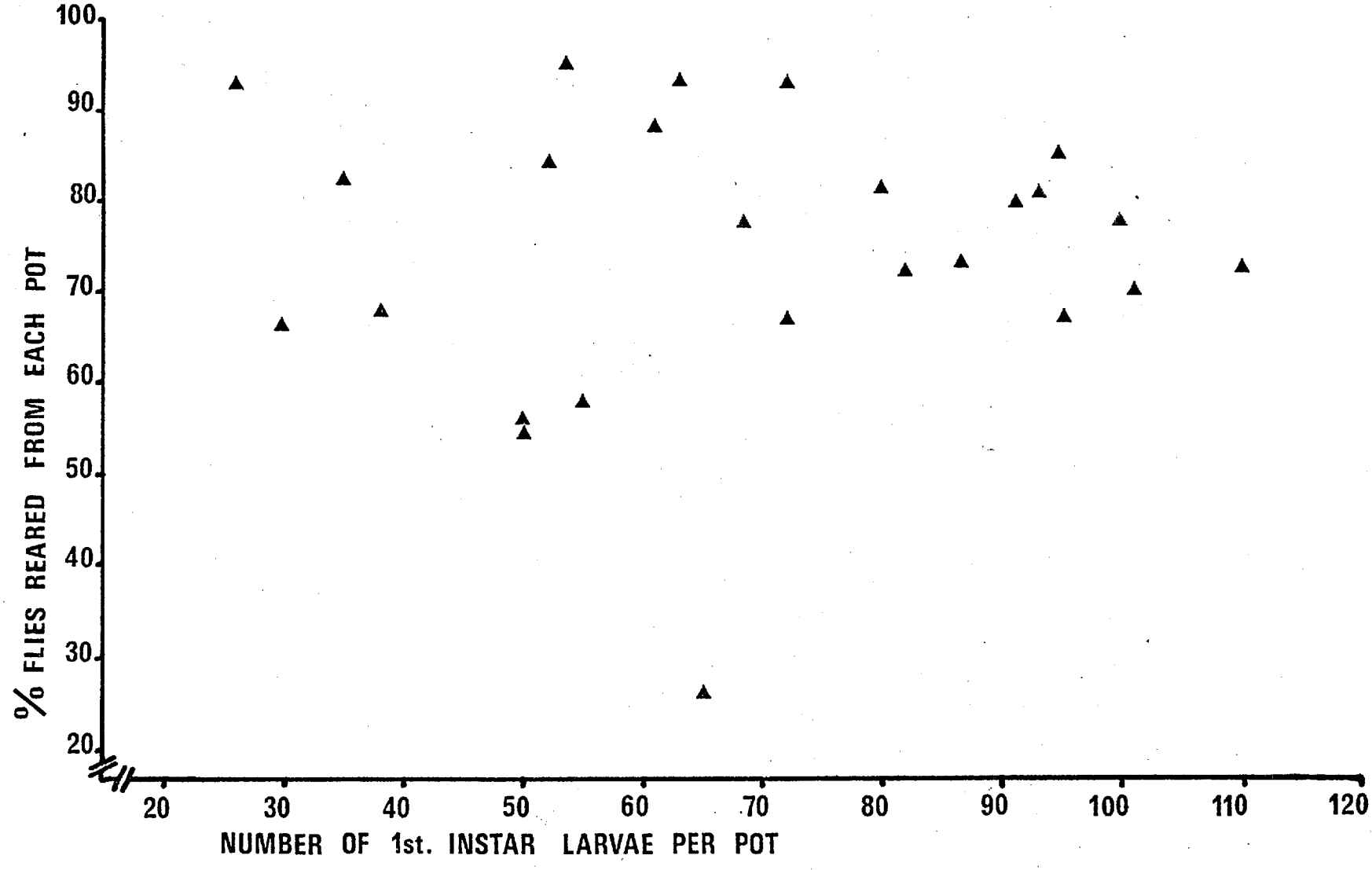


Fig. 1. Effect of density on the survival of the larvae of L.longipalpis.

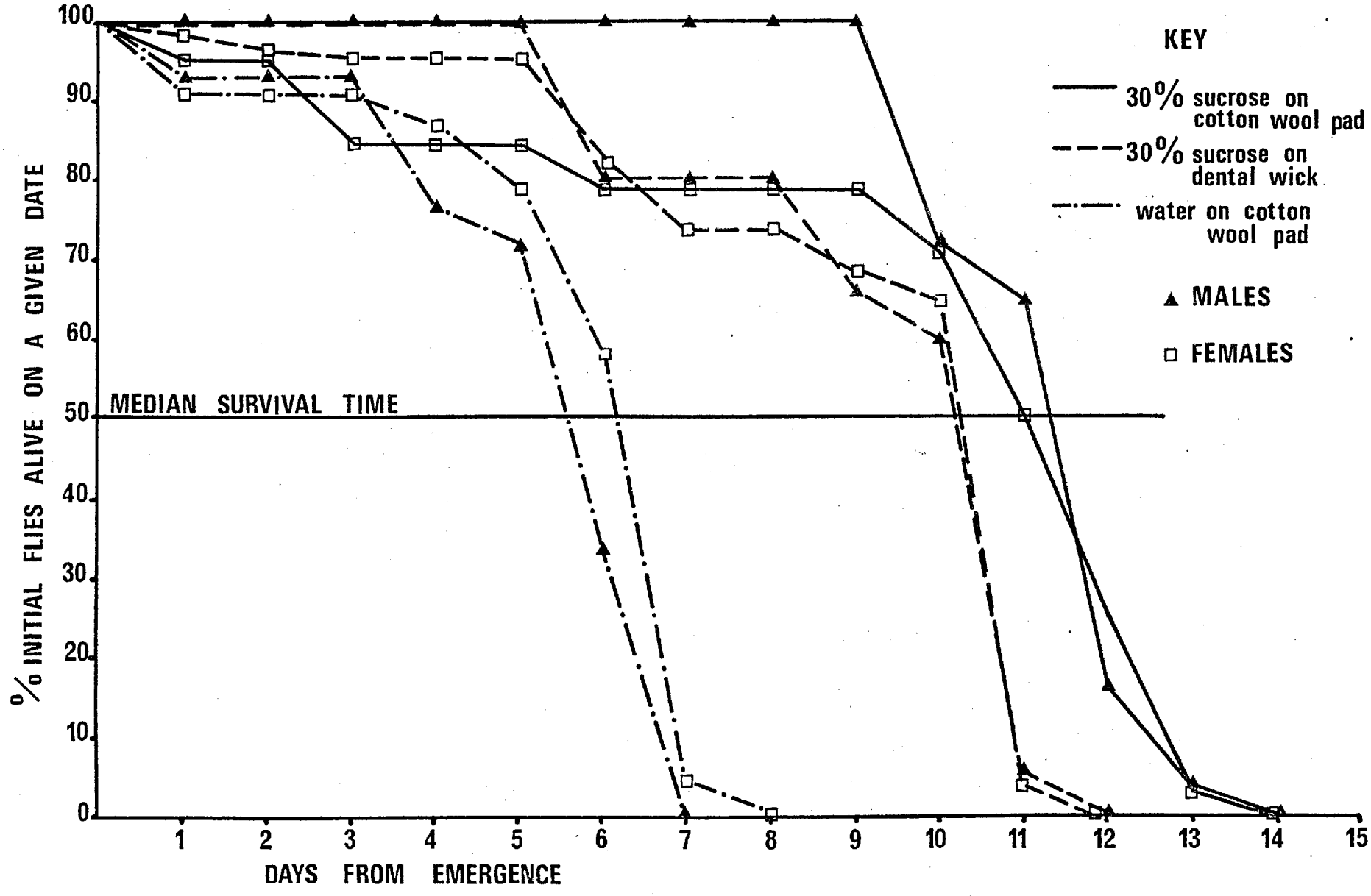


Fig. 2. Sucrose-feeding and the longevity of L. longipalpis adults.

marked when the sucrose solution was presented on a cotton wool pad rather than on a dental wick. The former method was therefore used for rearing.

The median survival time was 6, 10 and 11 days for flies offered water, 30% sucrose solution on a dental wick, or 30% sucrose solution on a cotton wool pad, respectively. (These times were considerably longer in environmental cabinets; sugar-fed flies lived as long as six weeks).

Monitoring the productivity of the colony of L. longipalpis.

Life-tables for generation 2/3 and generation 10/11 were prepared (Table 3). Using this data, curves were drawn in order to compare visually the productivity of each generation (Fig 3).

The "Apparent" mortality affecting each developmental stage is compared for the two generations in Fig. 4 .

The net reproductive rate of a population (R_o) is given by Nt/N_o where N_t is the number of individuals in a population at the end of a generation and N_o the number at the beginning of that generation (Southwood 1966).

For generation 2/3 $R_o = 206/220 = 0{\cdot}94$; and for generation 10/11 $R_o = 1{,}187/220 = 5{\cdot}39$.

The mean weights of male and female pupae in generations 1, 9 and 17 are shown, with confidence limits, in Fig 5 . The mean weights of both sexes in generations 9 and 17 are significantly higher than those for generation 1 ($p = 0.001$ for t test). There is no significant difference between the values for generations 9 and 17. The data from

Table 3.

Life tables for laboratory reared *L. longipalpis* fed hamster blood.

KEY:

a. Life table for generation 2/3, *L. longipalpis*.

Mean number of eggs matured per female = 93

Mean number of eggs laid = 38

b. Life table for generation 10/11, *L. longipalpis*.

Mean number of eggs matured per female = 93

Mean number of eggs laid = 68

Absolute data; Proportions are given because some flies were taken for experiments, and others not sampled at every stage.

Table3a.

GENERATION 2/3	ABSOLUTE DATA	DATA CORRECTED FOR 220 ADULTS OR 100 FEMALES	FROM CORRECTED DATA - No. OF POTENTIAL ADULTS	FROM CORRECTED DATA - INDISPENSABLE MORTALITY (%)	FROM CORRECTED DATA - APPARENT MORTALITY (%)
No. of females in feeding cages	336	100	9,300		
				3.6	62.0
No. of females taking blood	128/336	38	3,534		
				0.4	15.8
No. of females maturing eggs	93/110	32	2,976		
				7.4	77.0
No. of females laying eggs	58/106	18	684		
				2.1	48.1
No. of eggs hatching	1,150/2,204		355		
				1.4	39.2
No. of pupae	700/1,150		216		
				0.1	4.6
No. of adults	667/700		206		

Table 3b.

GENERATION 10/11	ABSOLUTE DATA	DATA CORRECTED FOR 220 ADULTS OR 100 FEMALES	FROM CORRECTED DATA - No. OF POTENTIAL ADULTS	FROM CORRECTED DATA - INDISPENSABLE MORTALITY (%)	FROM CORRECTED DATA - APPARENT MORTALITY (%)
No. of females in feeding cages	146	100	9,300	3.0	19.0
No. of females taking blood	118/146	81	7,533	0.3	2.5
No. of females maturing eggs	45/46	79	7,347	13.8	51.9
No. of females laying eggs	59/86	52	3,536	8.3	39.5
No. of eggs hatching	1,543/2,549		2,140	8.9	41.0
No. of pupae	590/1,000		1,263	0.8	6.0
No. of adults	555/590		1,187		

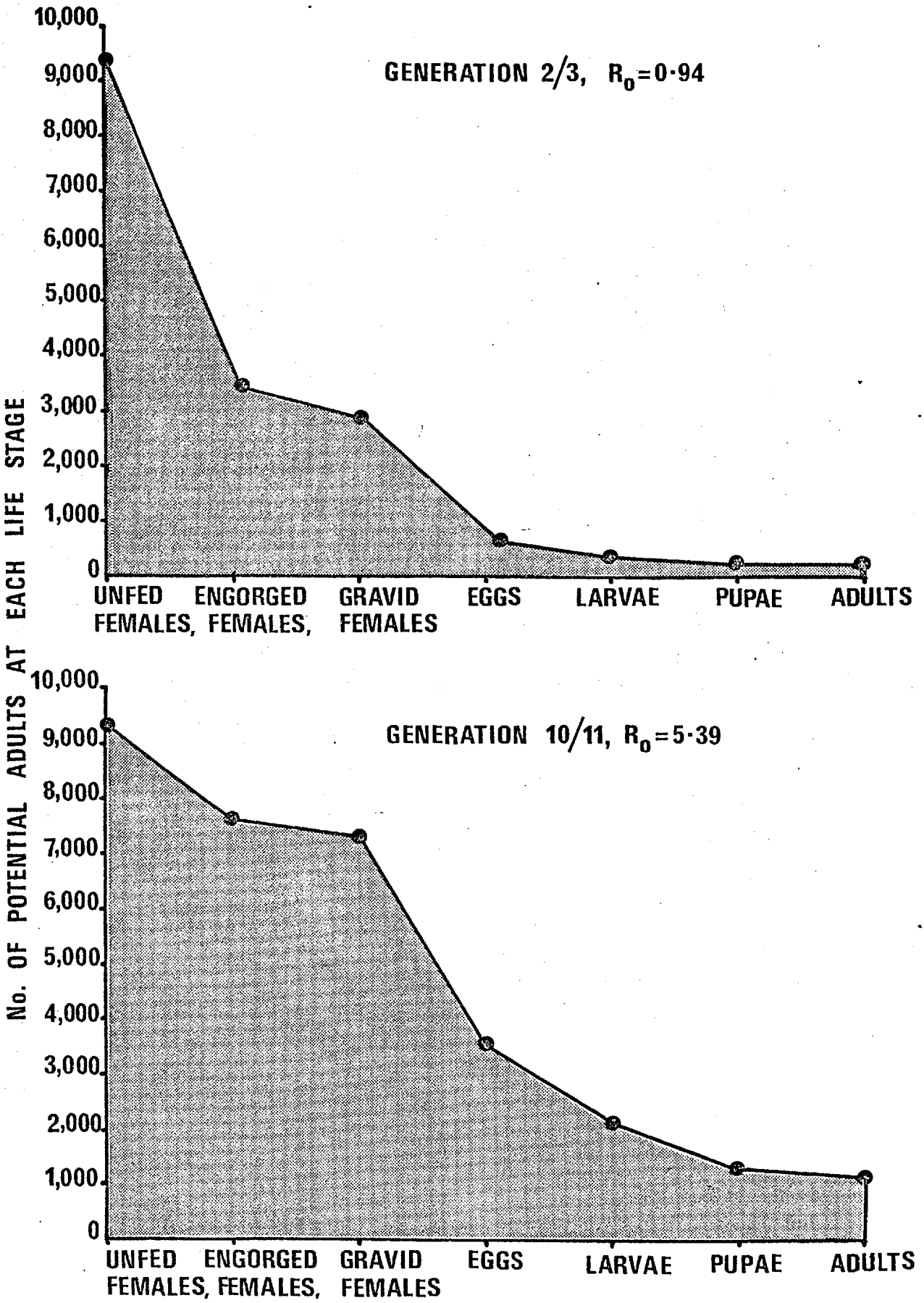


Fig. 3. Survivorship curves for two laboratory generations of L.longipalpis. Gen. 10/11 in improved conditions with environment cabinets.

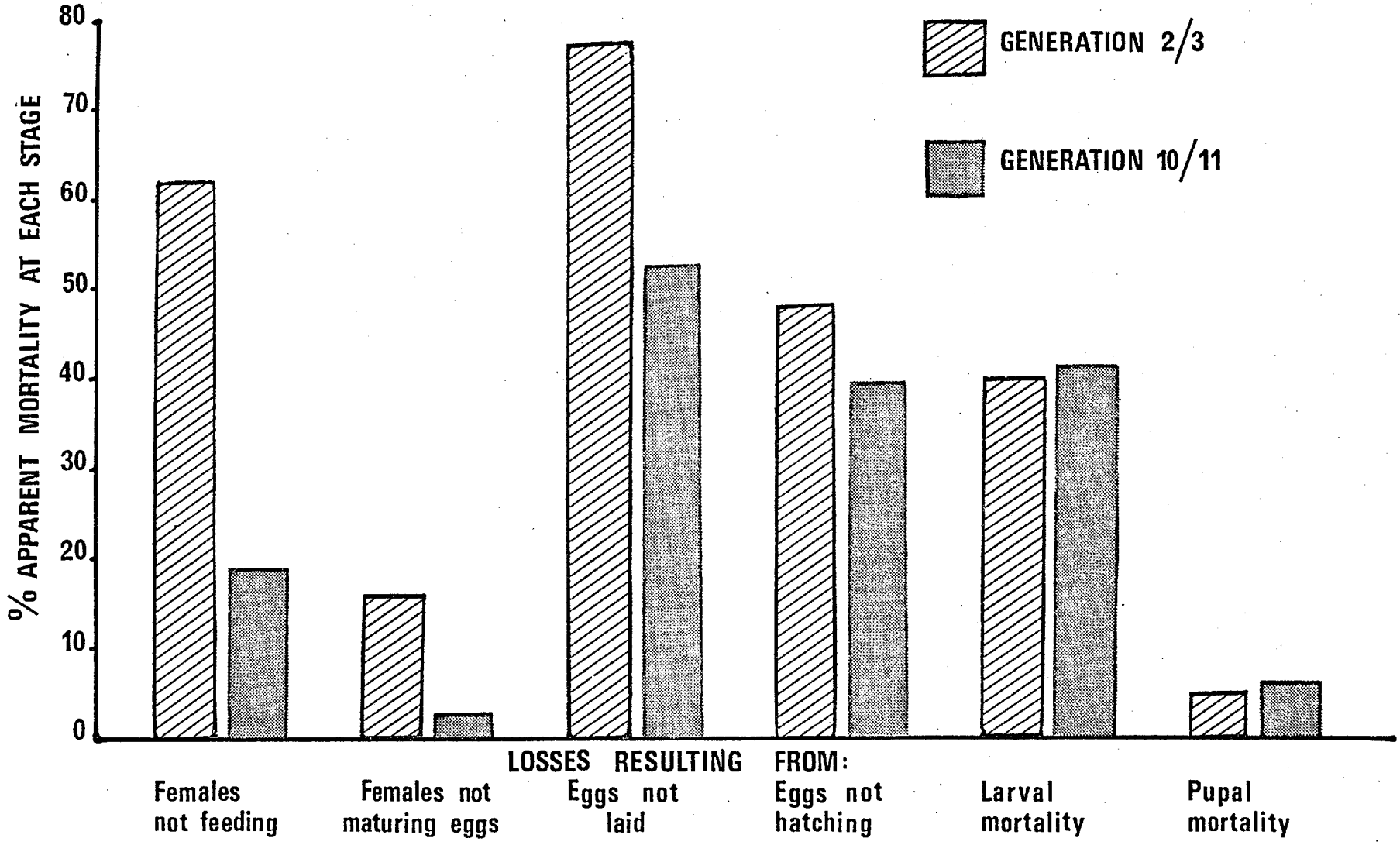


Fig. 4. The "Apparent" mortality affecting each development stage of two laboratory generations of L. longipalpis. Generation 10/11 in improved conditions within environment cabinets.

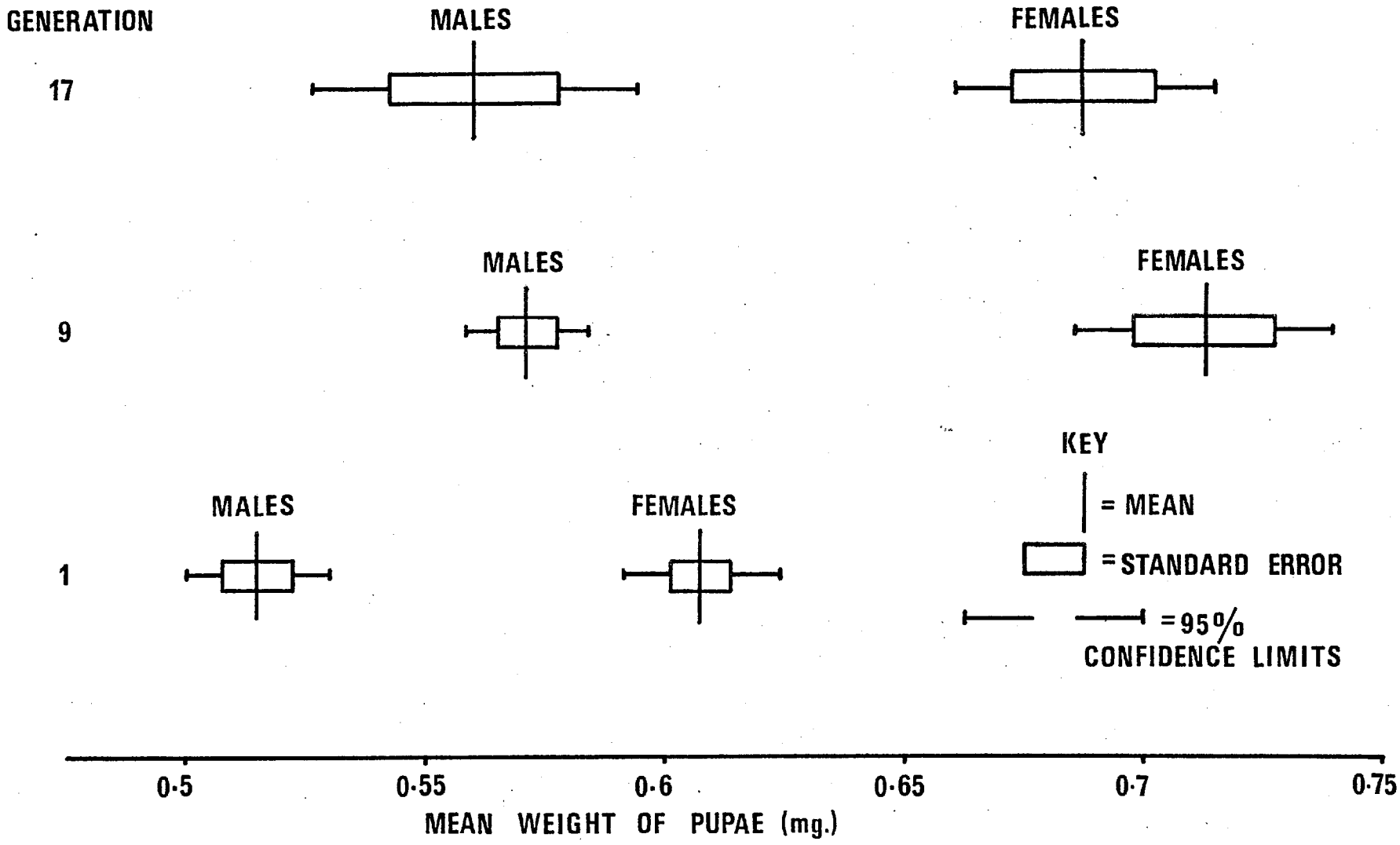


Fig. 5. Changes in mean weight of L.longipalpis pupae between different laboratory generations.

which these values were calculated are given in Appendix A.

DISCUSSION AND CONCLUSIONS.

The net reproductive rate (R_o) was only about 1·o for the generations (1 - 4) of L. longipalpis reared in the C-T room but rose to more than 5·o for the later generations kept in environmental cabinets. The increase in production resulted mainly from improved adult survival and egg production (see Section 4); the survival of pre-imagines was little changed (Fig 4).

The percentage of individuals lost to a generation through female flies refusing to feed or not digesting a blood-meal was significantly reduced in later generations (Table 3). Females reared in the C-T room were fed at widely varying times after their emergence, but in later generations each feeding cage contained only the flies emerging on 1 - 3 consecutive days so that all could be fed at the optimal age of 4 - 6 days (see Section 2). The higher and more constant humidity obtained by keeping the feeding cages in sealed polythene bags (rather than under damp cloths) undoubtedly increased the numbers of females surviving to the time when a blood-meal was offered. That a larger proportion of females took a blood-meal and succeeded in digesting it in later generations is, perhaps in part, due to the selection for females favouring the (human and hamster) hosts offered. In the Lapinha caves L. longipalpis does occasionally feed on man but its natural host is thought to be the rodent Cercomys cunicularis (A.R. Falcão pers. comm.).

The proportion of matured eggs that were successfully laid by a female was noticeably higher in later generations (Fig 4). The temperature of the C-T room fluctuated so much that the water applied daily to the filter papers lining the oviposition tubes formed droplets of condensation that trapped engorged/gravid flies. Such losses were reduced in later generations by : (1) placing the tubes over a dilute solution of sulphuric acid (S.G. 1·1) in a sealed desiccator to obtain a constant relative humidity of 95% (Solomon 1951); (2) maintaining a more constant temperature (within environmental cabinets); and (3) not watering the filter paper until the fourth day after the blood-meal. The latter step may have induced flies to take more sugar, which is beneficial to egg-laying (Section 4).

Up to 40% of the eggs laid by females kept in the improved conditions failed to hatch, compared with 48% in the C-T room (Fig 4). Infertility was not a major problem, however, for most of the eggs that failed to hatch were from otherwise fertile egg-lays. Infertile egg-lays were easily recognised, not only because all the eggs failed to hatch but also from the small number of eggs in each batch (Section 4). Much of the egg mortality was undoubtedly the result of damage in handling.

The survival of larvae and pupae from generation 5 onwards was not noticeably improved by the more careful control of temperature and humidity (Fig 4). However, the mean weight of male and female pupae did increase (Fig 5). The larvae were fed only liver powder (Gemetchu 1971)

after the first generation. This is probably a more concentrated and complete food than that available to larvae in natural populations. Thus the increase in weight of laboratory pupae is not surprising. Schmidt (1964) similarly found that laboratory-reared P. papatasii and P. orientalis were heavier than wild specimens.

The methods used at Ascot to rear L. longipalpis have been reasonably successful. This is more the result of paying close attention to detail than of innovation; the basic requirements of L. longipalpis and many other sand-flies are very similar and were known before (Sherlock & Sherlock 1959;1972; Safyanova 1964). It is, however, the first time that a New World sand-fly species has been reared for more than 10 generations in a closed colony outside its country of origin.

The aim was not just to breed L. longipalpis but to produce a flourishing colony that would provide a surplus of robust flies for experimental work. This was achieved. There was no fall in pupal weights in later generations, which is often the first sign that a colony is ailing through inbreeding. Further, the productivity of the colony compared favourably with that for a colony of P. argentipes bred by Shortt et al. (1926), and one of P. papatasii bred by Unsworth & Gordon (1946), but was only half that of a colony of P. papatasii reared by Schmidt (1964) who obtained a net reproductive rate (R_0) of 10.

In one respect, the rearing method used for L. longipalpis was unsatisfactory. About 50% of the eggs

matured by a female were not laid (Fig4). This is the most important mortality factor acting in each generation (Table 3). Moreover, nearly all the females died within a few hours of laying eggs. High losses at this stage of the life cycle have also been reported by Chaniotis (1967) and Ward (1974).

The author's attempts to rear other species of sand-flies have been less successful and illustrate some of the principal difficulties that have challenged other workers.

Many species of Holarctic sand-flies enter a period of dormancy in the winter when it is difficult to keep them alive in the laboratory (Theodor 1934; Harwood 1965; Chaniotis 1967). In the present study many larvae of P. perfiliewi were lost in this way. Surprisingly, the effect that photoperiod might have on sand-fly dormancy has not been investigated. The failure in the present study to rear the early larval instars of P. perfiliewi in a long "summer" photoperiod was probably a mistake.

Another difficulty in rearing sand-flies is to find the correct conditions for larval development. The moisture requirement depends on the species and can only be judged by experience (Theodor 1936; Unsworth & Gordon 1946). Failure to provide the correct larval environment (including moisture) probably accounted for the author's inability to rear P. ariasi, Ps. davisi and L. flaviscutellata. All are exophilic or "forest" species (Rioux et.al. 1967; Ward 1974), and their larvae live in leaf-litter or other damp organic matter. It would have been

better, perhaps, to rear these larvae in a humus/faeces medium (Hertig & Johnson 1961; Safyanova 1964) rather than the more artificial plaster pots favoured by the peri-domestic species, L. longipalpis and P. perniciosus, successfully reared in this study.

A problem that has been frequently encountered when trying to rear South American sand-flies is the reluctance of females reared in the laboratory to take a blood-meal (Hertig & Johnson 1961; Ward 1974). The small colonies of L. flaviscutellata and P. ariasi failed, in part, for this reason. The feeding behaviour of these species might be more complex than that of L. longipalpis and might require the participation of the males. For example, mating swarms of male sand-flies have been collected in animal-baited traps (Shaw & Lainson 1968; Ward 1974). In a small colony, the chances of bringing together sufficient individuals for such performances must be slight. Feeding through membranes is unlikely to provide the solution to this problem, and force feeding (Hertig & Mc.Connell 1963; Alekseev 1969) is probably too laborious for mass rearing. Progress is unlikely until more is known of the factors which stimulate sand-flies to seek a host and take blood.

A different problem was encountered when rearing P. perniciosus. A small colony was started with eggs from just one female. At first this flourished but was lost in the third generation. It is likely that this resulted from the expression of lethal homozygotes.

SECTION TWO:

ON THE FEEDING HABITS OF L. longipalpis AND OTHER PHLEBOTOMIDS.

INTRODUCTION.

The feeding habits of biting flies have been reviewed by Downes (1958), and in general his findings are applicable to Phlebotomids. In the laboratory, both sexes of sand-flies usually feed on sugar; this is imbibed as a free liquid and used to sustain life and activity (Barretto 1942; Chaniotis 1967; Gemetchu 1972). The importance of sugar-feeding to wild sand-flies is not known, although the crops of some species have been found to contain plant sugars (Lewis & Domoney 1966).

Only the female sand-fly sucks blood, which is usually taken to provide material for maturation of the oöcytes (Unsworth & Gordon 1946; Dolmatova & Demina 1971). Consequently, the piercing mouthparts (or fascicle) are much reduced in the male (Adler & Theodor 1926; Davis 1967). In both sexes the liquid, whether sugar or blood, is taken in through a tube formed primarily of the apposed, elongate (dorsal) labrum and (ventral) hypopharynx (Adler & Theodor 1926). In a recent review, Lewis (1975) has described the action of the fascicle: The mandibles cut the host's skin; and the maxillae, which are thin and distally barbed (Plate 3), engage the sides of the wound to steady the fly and to hold the wound open for the sucking tube to enter. Anti-

Plate 3: L.longipalpis. Tip of female maxilla. (x 5,000)

coagulant from the salivary glands (Adler & Theodor 1926) is pumped into the wound via a canal inside the hypopharynx, which from its structure in L. longipalpis (Plate 4) participates in the piercing of the skin. The morphology of the mouthparts have been well studied (Adler & Theodor 1926; Christophers et al.1926; Perfil'ev 1968; Lewis 1975).

Shortt & Swaminath (1928) observed the way P. argentipes females fed on a mouse. The fascicle probed deep enough to break the smaller blood vessels in the dermis and to form a blood pool from which the fly fed. Pool-feeding seems to be typical of sand-flies (Christensen & Herrer 1972), as the rigid labrum would suggest. After L. longipalpis has fed on man, haemorrhagic pools dot the skin. These are 2 - 4 mm in diameter and do not clot for some hours because of the injected anticoagulant.

Some sand-fly species have definite host preferences. Thus most species of Sergentomyia feed preferentially on birds or cold-blooded vertebrates (Lewis 1974). For other species, the preferences may change with geographical location, e.g. P. babu feeds on geckoes in India but is anthropophilic in Mauritius (Adler & Theodor 1957). Sometimes local host preferences refer to innate differences between races of a sand-fly species. For example, races of P. argentipes from India, where the species transmits Kala-Azar, bite many animals including man (lloyd et al. 1925; Napier & Smith 1926), but another race from Ceylon, where this species is not a Kala-Azar vector, refuses to

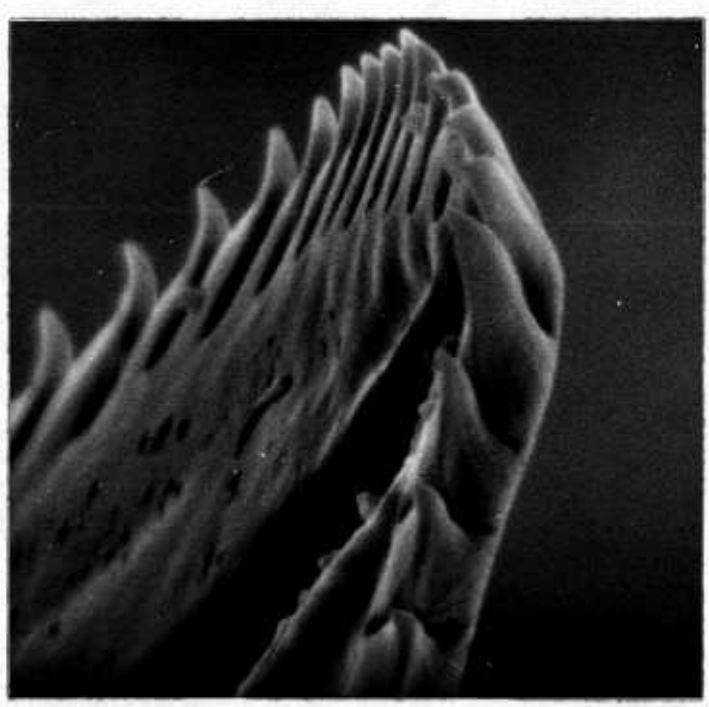


Plate 4: L.longipalpis. Tip of female hypopharynx.

Above: ventral view. (x2,500)

Below: dorsal view. (x7,500)

feed in the laboratory on man (R. Killick-Kendrick pers. comm.). Often, however, local host preferences are only a reflection of host availability (Ward 1974), and these may change from one time of the year to another (Tesh et al. 1971, 1972).

Habitat selection, too, affects host availability, and, therefore, can falsely suggest that some hosts are innately preferred. Thus L. gomezi rarely bites man at ground-level in the forests of Panama, but in the canopy it is the dominant anthropophilic sand-fly (Chaniotis et al. 1971a). In comparision, L. flaviscutellata rarely searches for hosts in the canopy and therefore its natural preference is for rodents and other ground-living mammals (Shaw et al. 1972); but when arboreal mammals were caged at ground-level they were bitten avidly by this species (Ward 1974).

Periodicity of activity also has a marked effect on sand-fly feeding habits; most species only bite at night, but many have a favoured time (Williams 1966; Chaniotis et al. 1971a, Ward 1974). Along with species - specific responses to small changes in the environment (Ward 1974), periodicity probably accounts for the difficulty in feeding some sand-flies in the laboratory.

In comparison L. longipalpis exhibits a great plasticity in its feeding habits which accounts, in part, for the ease with which it can be reared in the laboratory and for its wide geogrophical range - from Mexico to southern Brazil (Lewis 1971). Flies used in the present

investigation came from a forest cave near Belo Horizonte in southern Brazil, and (courtesy of Dr. R.D. Ward) from domestic habitats in the dry north-eastern state of Ceará, Brazil. In nature, the cricetid rodent, Cercomys cunicularis, is believed to be the principal host for the Belo Horizonte flies, but the latter will leave the cave to bite man even during the day (Killick-Kendrick et al. 1975). L. longipalpis from Ceará have been captured biting man, dogs, chickens, bulls, horses (Chagas et al. 1938) and the fox, Lycalopex vetulus (Deane 1956); in the laboratory they have been fed on man, opossum, sloth, hamster and spiny rat (Ward 1974).

In view of the fact that L. longipalpis is an opportunistic feeder and the large amount of knowledge that has accumulated on the general stimuli that attract biting insects to their hosts (Clements 1963; Hocking 1971; Gillies 1972) it was considered unprofitable to examine the host-seeking behaviour of this species in depth. However, some observations are offered, and these are compared with field and laboratory notes that were made on other species.

The principal aim of the present investigations was to establish methods by which L. longipalpis could be fed blood and blood-like solutions experimentally. A natural outcome of these studies was the identification of blood-feeding stimuli, which prompted a preliminary investigation of the sensilla on the fascicle.

At first, the pipette technique of Hertig & Hertig

(1927).was used to try to artificially feed L. longipalpis. This method has proved useful in infecting sand-flies with Leishmania cultures (Napier 1930; Alekseev 1969). Indeed, Hertig & Mc.Connell (1963), who redescribed the technique, found it the only practical way of artificially feeding Panamanian sand-flies: 70% of most species took larger than "half feeds" from the pipette-feeder, whereas only one out of 150 fed when offered blood/cultures through a variety of membranes. In comparison, the author has been able to feed L. longipalpis only on sucrose using the pipette-technique, but has successfully employed a membrane-feeding technique.

Membranes have been used to feed many haematophagous insects in the laboratory (Tarshis 1958), but only rarely for sand-flies. Adler and associates successfully fed P. papatasii on serum and blood fluids through animal membranes (Adler & Theodor 1927; Adler & Ber 1941). In an attempt to improve the feeding performance of wild-caught Amazonian sand-flies, Ward (1974) offered warmed blood across a number of animal membranes, but in four trials only 10 females fed. R. Killick-Kendrick (pers.comm.) has offered hamster plasma to L. longipalpis through a hamster-skin membrane and found that they accepted it. Recently, Ms.M. Jennings of the Animal Virus Research Institute has been using the technique routinely to infect L. longipalpis with viruses.

MATERIALS AND METHODS.

4 - 6 day old, laboratory-reared females of L. longipalpis were used for all experiments. These were from the colony originally collected in Belo Horizonte (Section 1), unless stated otherwise in the text.

The methods of providing cagedfemales of L.longipalpis with blood-meals have been described in Section 1.

Individual blood-feeds from animals.

Female sand-flies were weighed on a Beckman micro-balance before and shortly after a blood-meal in order to determine the weight of blood ingested. A procedure was developed empirically to ensure that the maximum number of females fed.

10 - 15 females were individually captured in small glass tubes (2 cm diameter) with gauze caps. Carbon dioxide gas was blown into each tube until the female collapsed, when she was tipped onto the scale-pan of the microbalance and thence into a clean tube. It took 30 minutes to narcotize and weigh 15 flies, during which time they were at 60% RH and 18° - 20°C. At the end of this period the tubes were placed in an incubator at 100% RH and 25°C. 10 minutes later individual females were taken from the incubator in the order they had been weighed and offered a blood-meal in shaded light. Few fed in bright light.

The blood-meal was offered by inverting the opened

tube onto the bare skin of the forearm or, for animals other than man, the shaved belly skin. The tube was lightly tapped to encourage a female to descend to the skin, but more vigorous knocking was avoided for it led to agitated flight and a complete disinterest in feeding. Those females that had not started to feed within 5 minutes were counted as non-feeders.

Individually-tubed females that had not been narcotised (and given a short recovery period) rarely took a blood-meal. For this reason, the above procedure was used as a basis for feeding females of L. longipalpis on hamsters infected with Leishmania spp (Killick-Kendrick et al. 1976).

Membrane feeds.

The above method was modified to feed L. longipalpis on solutions in a membrane-feeder. It was found empirically that more females fed if they were placed in small groups in gauze-topped pill boxes (6 cm diameter) rather than in individual tubes.

Preliminary observations with densities of 1 to 10 showed that as many as 7 females could engorge on human blood in the 15 minute feeding period allowed to each batch, but groups of only 2 - 5 were used to ensure that there was ample opportunity for all to feed. Each group contained females of similar body weight, i.e. ± 0·005 mg. A 15 minute feeding period was chosen as the maximum possible without significant weight changes occurring through diuresis (see Section 3).

The feeding unit used was designed by Mellor (1971) for infecting Culicoides with Onchocerca. It consisted of an outer glass cylinder, of 1·5 cm internal diameter, over the bottom of which a stretched membrane was attached. Using a pasteur pipette the test solution was introduced until it covered the membrane. A hollow glass "finger" was inserted into the outer collar so that its tip (7 mm diameter) rested on and slightly stretched the membrane. An immersion thermostat with pump[1] circulated water through the "finger" to warm the test solutions; the temperature could be controlled to ± 1°C.

The feeding unit was clamped so that the membrane slightly dented the taut nylon gauze covering the pill box. The flies were activated by gently breathing into the box; without this few of the females would move from the sides of the box where they rested. In an attempt to standardize this stimulus each box was given only 3 gentle breaths.

All membrane feeds were carried out in dim light with an ambient temperature and humidity of 23°C and 70% RH respectively. For all but one of the experiments reported in this section the temperature of the test solution was 37·5°C.

Preparation of membranes. Two types of membrane were used: Chick skin and parafilm 'M'[2] were chosen as the natural and artificial membranes that had given the best results

1. "Thermomix II" by B. Braun, Melsungen, W. Germany.
2. American Can Co., Menosha, Wisconsin, U.S.A.

with other blood-sucking Nematocera, e.g. Rutledge et al. (1964) and Mellor (1971).

Freshly killed or deep-frozen day-old chicks were plucked and their skins removed. A preliminary investigation showed that even when the sub-cutaneous fat had been peeled off, a large amount of material leached into the test solution during the feeding period. Therefore, each skin was soaked for a total of one hour in three changes of distilled water and one of 0·85% (physiological) saline. The leachings from these "leached chick membranes" were slight (Section 3). Chick skins were always attached to the feeding unit so that the epidermis was presented to the flies; if fitted the other way the skin soon dried.

Parafilm 'M' was stretched to 2½ x its normal size to form thin, uniformly translucent membranes. Care was taken not to touch the parts of these that were to be used for feeding experiments, so that no olfactory stimulants became associated with them. Parafilm membranes became dry and brittle if left on the feeding unit for more than 3 hours, whereas chick membranes retained their character even when left over-night.

Test solutions. 0·85% saline (NaCl) was used as a diluent. Human blood: Transfused whole human blood was obtained from a local hospital on the day of its expiry, and kept at 4°C for no more than a week. Citrate phosphate dextrose (CPD) was the anticoagulant; 100 ml of blood contained 2·63g of sodium citrate (BP), 327 mg of citric acid (BP) and 251 mg of sodium acid phosphate (BP).

Hamster blood: Heart blood was obtained by cardiac puncture and heparin used as anticoagulant (300 USP units per 5 ml). The whole blood and fractions from it were used to feed flies on the day the blood was drawn.

Red cell suspensions: Whole blood was centrifuged for 20 minutes at about 2,500 RPM in a graduated tube. The volumes of red cells and plasma were noted, and the plasma removed with a pasteur pipette. The cells were resuspended in about four times their volume of saline and centrifuged as before. This washing process was repeated twice more; between each washing the supernatant fluid and the buffy coat (of white cells) were discarded. The cells from several centrifuge tubes were pooled in a minimum of saline and centrifuged at 3500 RPM for 30 minutes in a graduated tube to determine their exact volume. They were then resuspended in saline to form a 45% (v/v) suspension. This was the mean red cell concentration in the human blood used.

Red cell extract: The method of Whitman (1948) was followed. A freshly prepared 45% (v/v) suspension of red cells was transferred to a stoppered, wide-mouthed flask so that the suspension was approximately 1 inch deep. This was placed in a deep-freeze until the suspension was frozen, and then thawed in warm water. The suspension was again frozen and thawed before being centrifuged for 30 minutes at 3500 RPM to remove the ~~nuclei and~~ stroma. In addition, the supernatant was passed through a 1·2 μ millipore filter to remove large cell

fragments.

Plasma: This was obtained while preparing red cell suspensions and diluted as required.

Denatured human plasma proteins: Plasma from CPD human blood was heated at 60°- 90°C for 30 minutes. The small volume of whey was decanted, and the coagulant freeze-dried for 24 hours before being ground to a fine powder with a pestle and mortar. 0·08 gm of dried and powdered protein was suspended in saline so that it was at half plasma concentration, but only one-tenth formed a stable suspension, i.e. a final concentration one-twentieth that in plasma. Solutions of amino acids: Lea et al.(1958) defined the diet of amino acids that are essential for a high level of egg production in Ae. aegypti. This diet, called here "Lea 1", was fed to L. longipalpis. Its exact amino acid composition per 100 ml of distilled water was: L arginine mono HCl 0·38g, L cystine 0·15g, L glutamic acid 1·00g, L histidine mono HCl 0·15g, DL isoleucine 0·50g, L leucine 0·75g, L lysine mono HCl 0·75g, DL methionine 0·15g, DL phenylalanine 1·20g, DL threonine 0·30g, L tryptophan 0·30g, DL valine 1·00g. All amino acids were sigma grade chemicals.[1] To this was added 5g of glucose, 5g of fructose and 0·1g of a salt mixture. (Mammalian ringer's was used:- 0·9% NaCl, 0·024% $CaCl_2$, 0·042% KCl). In practice, the chemicals were dissolved in 10 ml of the 1% mammalian ringer's and

1. Sigma (London) Chemical Co., Ltd., England.

70 ml of distilled water, and then dilute sodium hydroxide and distilled water were added so that the final solution had a pH of 7·2 - 7·5.

A similar solution was prepared which differed only in its concentration of sugar. "Lea 2", as it is called here, contained only 0·09g of glucose and 0·005g of fructose per 100 ml. This is their concentration in human blood (Spector 1956).

Other solutions: These were prepared from Sigma grade chemicals, refrigerated and desiccated to the manufacturers instructions.

Meals taken from cotton-wool pads.

Flies were deprived of sucrose and water for 2 days before an experiment. A petri dish (9cm diameter) containing a cotton-wool pad soaked in test solution was placed on the base of the nylon-gauze feeding cage (18 cm cube) into which the flies were introduced. The cage was left undisturbed for 2 hours in an illuminated incubator at 25°C and 70% RH. At the end of this period all the flies were dissected and the proportion feeding and the location of their meals noted.

Solutions not naturally coloured were mixed with cochineal red food-colouring[1]. This contained glycerin, isopropyl alchohol and potassium hydroxide as well as E120 cochineal in aqueous solution. (Manufacturers notes did not state concentrations).

1. Rayners Ltd., London.

Its concentration in the test solution was 1% (v/v).

This colouring was used to facilitate the identification of the meals in the flies. Its colour is not believed to have affected the results: Thus, "colourless" sucrose is often taken by L. longipalpis from white cotton-wool, the bulging crop being visible through the pleura of the abdomen. A.J.Leaney (pers.comm.) has shown that L. longipalpis does not discriminate between sucrose coloured red (cochineal) and blue (Trypan Blue). Similarly, L. trapidoi does not discriminate between red and green coloured sucrose (Chaniotis 1974). Finally, 1% cochineal red in 10% sucrose was neither an attractant nor a repellent when offered to L. longipalpis across chicken-skin (Table 5).

Dissections - to locate meals in sand-flies.

The fly was narcotised and tipped onto a drop of saline on a microscope slide. A small surgical scalpel and fine entomological pins (mounted on match sticks) were used for the dissection. For this the fly was pinned by the lower thorax and shallow cuts made in the abdomen (Fig 6). This helped to release from the cuticle tracheae and connective tissue which otherwise tended to retain or rupture the gut and ovaries when they were removed. Next, the head was removed by gently pulling it from the secured thorax. Any food in the pharynx or oesophagus could be seen before the gut broke just anterior to its junction with the crop. Finally, the crop, remaining gut and ovaries were drawn backwards by traction while a needle

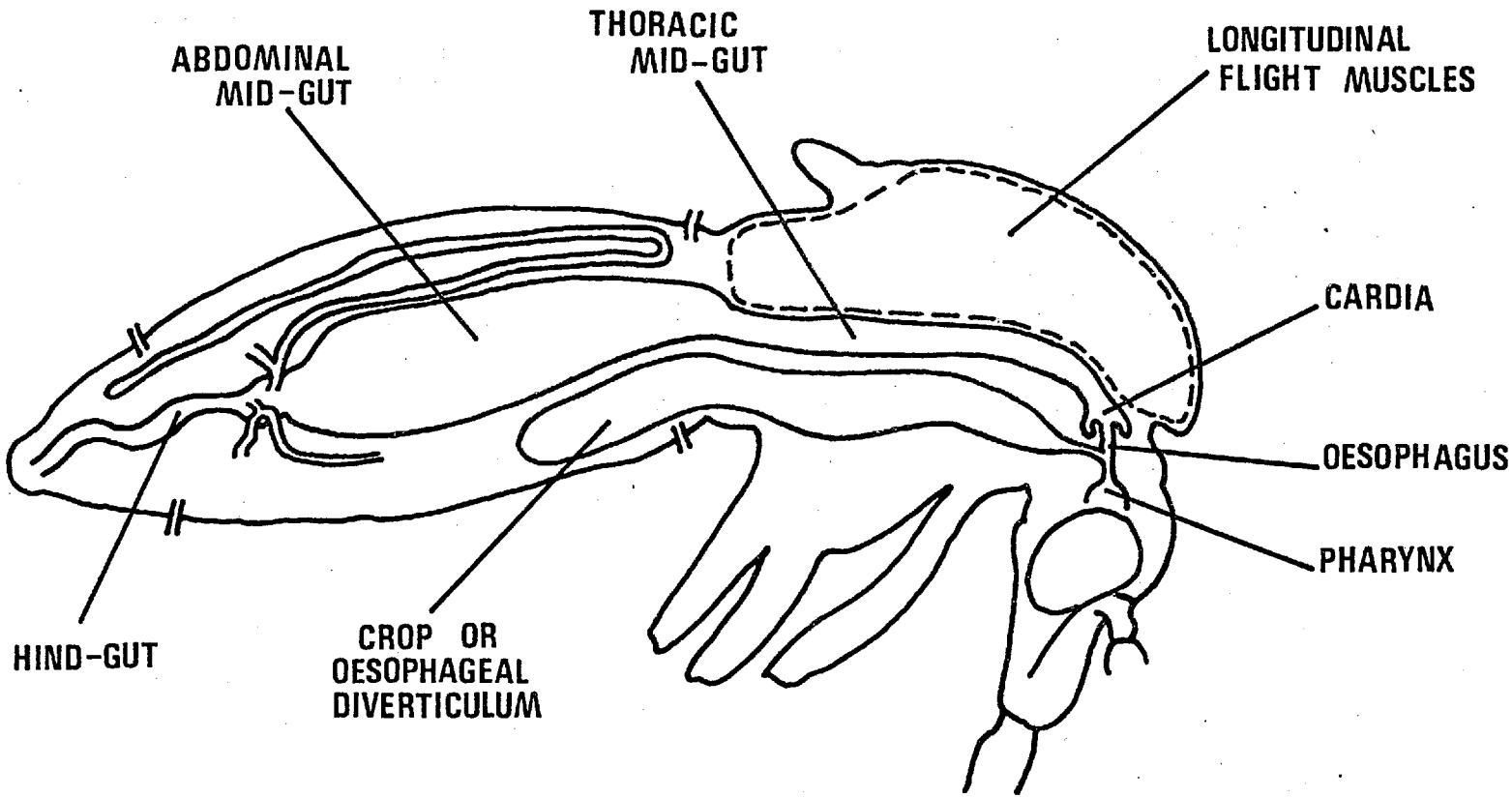


Fig. 6. Schematic sagittal section of L.longipalpis female to show location of alimentary tract.

(// Preliminary cuts for dissection to remove gut.)

on the thorax held the rest of the sand-fly in position.

Preparation of fascicle for stereoscan electron microscopy (SEM).

Newly-emerged, unfed flies were killed in the vapour of industrial ether so that the mouthparts gaped (C.T. Lewis pers.comm.). The heads were severed, fixed in alcoholic Bouin's (2 days) and dehydrated in absolute alcohol (3 - 7 days). Stylets were mounted individually or attached to the head, each being stuck to the viewing platform (or "stub") with a high-conductivity adhesive (Durofix). After mounting, the material was air-dried over silica gel for 24 hours and then "sputter-coated" with gold-palladium. A Cambridge Stereoscan 2A electron microscope was used to examine and photograph the material.

RESULTS.

Size of female and size of blood-meal.

Body size significantly affects the size of meal ingested by some mosquitoes (Barlow 1955; Bar-Zeev 1957). Therefore, it is a factor that must be considered when designing feeding experiments.

On four occasions the effect of female size (weight) on the weight of human blood ingested by L. longipalpis was investigated. Only once was there a significant positive association between the two (Fig 7 ; Appendix B).

From these results it was concluded that for practical purposes blood-meal size was independent of body weight within the range 0·28 - 0·40 mg. Only females whose weights were within this range were used for feeding experiments.

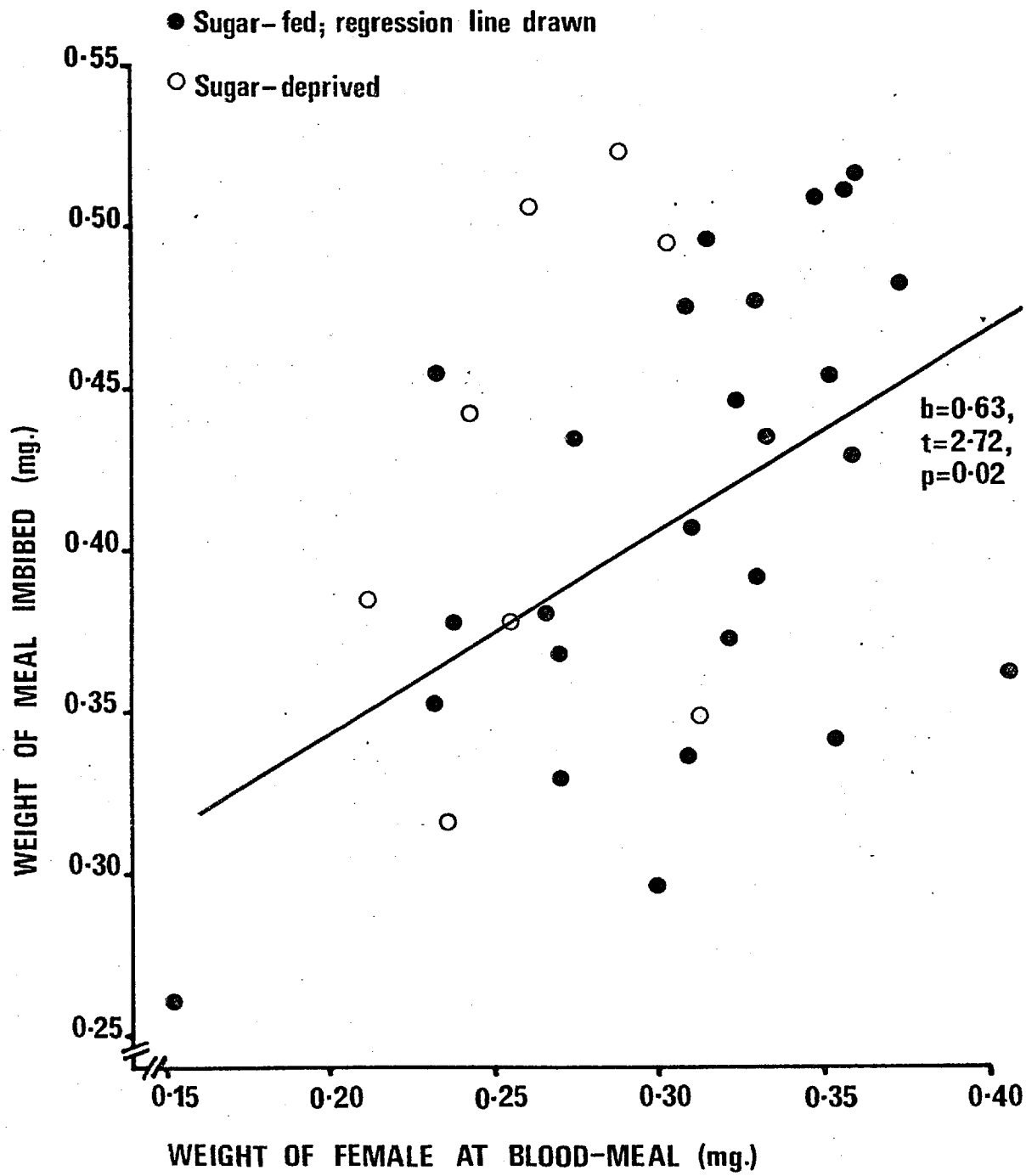


Fig. 7. The effect of female body-weight on the weight of blood imbibed from man by females of L.longipalpis.

Free feeding of females of L. longipalpis in cages.

During the course of normal rearing, the majority of 3 - 10 day old females accepted a blood-meal from an anaesthetized hamster or the arm of a man. The mean proportion feeding on either host during the 23 generations of the study was 80% (30% - 100%). Poor feeds were irregular and unexplained. Unfavourable physical conditions were not responsible - 100% feeds occurred just as frequently over a range of temperature(21·5° - 30°C), humidity (70% - 100% RH) and at different times of the day. Feeding was unaffected by bright light.

The mean weight of blood imbibed from hamster and man was 0·46± 0·04mg (90 observations) and 0·41± 0·05mg (90 observations) respectively.

Under normal rearing conditions only age had any constant effect on feeding, which was poor (< 50%) for groups of freshly-emerged and old (> 16 days) females.

Individual feeding of females of L. longipalpis after narcotisation.

After narcotisation rarely more than 30% of females took a blood-meal in bright light, but the figure was 90% (60% - 100%) for both human and hamster hosts placed in shaded light. Again, feeding was equally good, and occasionally inexplicably poor, over the range of stated physical conditions. Mating, sugar-feeding and nature of host had no noticeable effect on the meal weight, e.g. data for the fourth laboratory generation:-

Host	Man.	Man.	Man.	Hamster.
Condition of female.	Sugar-fed/ mated.	Sugar-fed/ unmated.	No sugar/ mated.	Sugar-fed/ mated.
Mean weight of meal(mg)	0·43	0·42	0·42	0·41
95% confidence limits.	±0·07	±0·05	±0·05	±0·04
Range of meal weight.	(0·26-0·55)	(0·32-0·51)	(0·32-0·52)	(0·34-0·51)
No. of observ-ations.	30	18	16	30

Second feeds. I have been unable to encourage engorged L. longipalpis (either individually in tubes or in groups in cages) to take a second blood-meal, although A.J. Leaney (pers.comm.) reports that this is sometimes possible.

With a few exceptions, females of L. longipalpis only survived after the first gonotrophic cycle if they were prevented from laying their eggs by denying them a damp oviposition surface. In one experiment, six days after the first human blood-meal 13 out of 18 females were still alive. 5 of these took a second human blood-meal the size of which (0·18 - 0·30mg) was unrelated to the size of the first (0·18 - 0·72 mg).

L. longipalpis females from Ceará (Courtesy of Dr. R.D. Ward). The percentage of females and the mean weight of the meals inbibed was recorded for different hosts (Table4). From each batch of flies, a small sample (5 - 8) was fed on man as a control.

HOST	No. OF FEMALES OFFERED MEAL	% FEEDING	MEAN WEIGHT OF MEAL (mg.)	95% CONFIDENCE LIMITS FOR MEAN WEIGHT	RANGE OF MEAL WEIGHTS (mg.)
Spiny Rat, *Proechimys* sp.	29	76%	0.45	±0.08	(0.36 - 0.58)
Man	33	95%	0.47	±0.06	(0.31 - 0.72)
Capuchin monkey, *Cebus apella*	25	92%	0.53	±0.10	(0.32 - 0.63)
Opossum, *Didelphis marsupialis*	28	93%	0.55	±0.05	(0.45 - 0.65)
Three-toed sloth, *Bradypus tridactylus*	16	62%	0.49	±0.20	(0.30 - 0.70)

Table 4. The feeding response, after narcotisation, of females of *L.longipalpis* (Ceará strain) to different hosts.

The spiny rat and the three-toed sloth both have coarse, stiff hair, which even after it had been trimmed was off-putting to some females. This, together with the tough skin of the sloth, explains the relatively low percentage of females feeding on these two hosts. For the females that fed there was no significant difference between the mean meal weight taken from each host.

Feeding behaviour and speed of blood-sucking.

During routine culture the following picture emerged. 60 or more females of L. longipalpis would take a blood-meal from an arm inserted in a cage for just 20 minutes. Both males and females were activated by the breath of the donor, but it was not until 2 - 5 minutes after the arm was first placed in the cage that the first female bit. Both females and males congregated on the side of the cage nearest the human host, from where they hopped onto the arm. Males would dash from one female to another, facing them and wing "fluttering" until accepted. On landing on the skin a female usually made an exploratory walk, describing ever-decreasing circles about the point where she finally probed. Most found a "suitable" spot within a minute and then engorged in 2 - 6 minutes, but a few probed several times and took up to 15 minutes to engorge.

When an anaesthetized hamster was the bait the individual feeding time was the same, but the females took longer to find the bait. Only about 30 fed in one hour.

There was often a close aggregation on the bare skin of the hamster nose.

Most females offered a meal individually in tubes usually settled to feed within 1 - 2 minutes. Although some descended immediately to the skin, most displayed an initial bout of activity which was followed by a calmer exploration of the tube.

Membrane feeds.

The percentage of females of L. longipalpis that accepted from the membrane feeder meals of various protein and sucrose solutions, and the mean weight of each solution ingested, are recorded in Table 5 .

The feeding behaviour was similar to that of females offered vertebrate hosts in cages. Activation by human breath brought them to the gauze top of the pill-box. Again some females went straight to the membrane, but most walked in ever-decreasing circles, often "shying" away two or three times, before finally approaching it. For most solutions, females were satiated within 5 - 10 minutes, but 30% sucrose was unusual in that all the females remained feeding for 20 - 30 minutes.

The destination in the fly of protein and sucrose meals ingested through membranes are set out in Tables 6 & 7 respectively.

Miscellaneous results: On four occasions the feeding unit was placed inside a feeding cage (18 cm cube) containing 30 - 50 L. longipalpis females. These were then

Table 5.

The feeding response of females of L.longipalpis to solutions offered in the membrane feeder.

* For all flies, including interrupted feeders.

γ Only those females not interrupted.

NR Not recorded.

Table 5.

TEST SOLUTION (VOL./VOL. 0.85% SALINE)	MEMBRANE	NUMBER OFFERED FEED *	% * PROBING	% * FEEDING	NUMBER γ SATIATED	MEAN WT. OF MEAL (mg.) γ	95% CONFIDENCE LIMITS FOR MEAN WEIGHT γ	RANGE OF MEAL WEIGHTS (mg.) γ
HUMAN								
Whole blood	Leached chick skin (LCS)	24	NR	58	14	0.38	±0.06	(0.18 - 0.55) .
Whole blood	Parafilm	24	54	21	5	0.42	±0.14	(0.31 - 0.59)
Plasma	LCS	16	NR	75	12	0.27	±0.04	(0.11 - 0.37)
50% plasma	LCS	19	NR	58	8	0.37	±0.07	(0.26 - 0.46)
45% red cell suspension	LCS	38	NR	58	20	0.40	±0.16	(0.09 - 0.56)
45% red cell extract	LCS	34	NR	53	18	0.23	±0.07	(0.09 - 0.36)
10% (w/v) haemoglobin	LCS	33	NR	39	13	0.19	±0.06	(0.02 - 0.32)
HAMSTER								
Whole blood	LCS	20	NR	65	10	0.36	±0.05	(0.13 - 0.46)
Plasma	LCS	57	NR	54	30	0.32	±0.04	(0.17 - 0.60)
45% red cell suspension	LCS	35	NR	51	18	0.41	±0.07	(0.16 - 0.62)
45% red cell extract	LCS	52	NR	40	21	0.27	±0.02	(0.18 - 0.36)

Table 5, continued.

TEST SOLUTION (WT./VOL. 0.85% SALINE)	MEMBRANE	NUMBER OFFERED FEED *	% * PROBING	% * FEEDING	NUMBER γ SATIATED	MEAN WT. OF MEAL (mg.) γ	95% CONFIDENCE LIMITS FOR MEAN WEIGHT γ	RANGE OF MEAL WEIGHTS (mg.) γ
5% denatured human plasma	LCS	11	NR	45	5	0.28	±0.08	(0.21 - 0.36)
" + 0.005M ATP.	LCS	23	NR	56	13	0.23	±0.06	(0.07 - 0.38)
10% bovine serum albumen (BSA)	LCS	40	33	33	13	0.38	±0.08	(0.12 - 0.56)
10% BSA	Parafilm	35	63	57	19	0.20	±0.07	(0.01 - 0.46)
10% BSA + 0.005M ATP.	Parafilm	37	32	16	6	0.24	±0.22	(0.05 - 0.45)
10% BSA, fatty-acid free	Parafilm	20	50	25	4	0.16	±0.23	(0.02 - 0.32)
10% BSA, fatty-acid free	LCS	20	65	60	12	0.36	±0.08	(0.04 - 0.50)
5% BSA	LCS	37	70	70	24	0.30	±0.05	(0.04 - 0.47)
20% BSA	LCS	42	69	64	22	0.25	±0.05	(0.03 - 0.45)

Table 5, continued.

TEST SOLUTION (WT./VOL. 0.85% SALINE	MEMBRANE	NUMBER OFFERED FEED *	% * PROBING	% * FEEDING	NUMBER γ SATIATED	MEAN WT. OF MEAL (mg.) γ	95% CONFIDENCE LIMITS FOR MEAN WEIGHT γ	RANGE OF MEAL WEIGHTS (mg.) γ
"Lea 1"	Parafilm	15	46	13	2	0.15 and 0.16 mg.		
"Lea 1"	LCS	23	48	43	10	0.07	±0.02	(0.04 - 0.15)
"Lea 2"	LCS	28	39	11	3	0.05	±0.03	(0.04 - 0.06)
0.85% saline	Chick skin - not leached	20	55	50	8	0.42	±0.06	(0.08 - 0.53)
0.85% saline	LCS	21	62	62	10	0.36	±0.04	(0.18 - 0.53)
0.85%saline	Parafilm	45	49	44	18	0.21	±0.06	(0.06 - 0.43)
10% sucrose	LCS	25	52	52	12	0.15	±0.03	(0.08 - 0.22)
10% sucrose + cochineal red	LCS	45	76	76	25	0.14	±0.02	(0.03 - 0.23)
10% sucrose	Parafilm	49	35	25	12	0.12	±0.03	(0.03 - 0.19)
30% sucrose	LCS	20	70	70	14	0.04	±0.01	(0.02 - 0.07)

Table 6.

Destination of protein meals imbibed through leached chick skins by females of L.longipalpis. Meals coloured with 1% (v/v) cochineal red.

MEAL W./VOL. 0.85% SALINE	No. OF FEMALES	DESTINATION OF MEAL: CROP	MID-GUT
20% Bovine serum albumen	5	-	++
	5	-	++
	5	-	++
10% Bovine serum albumen	5	-	++
	5	-	++
	5	-	++
5% Bovine serum albumen	5	-	++
	5	-	++
	2	-	++
0.85% Saline	10	-	++

Key for this and tables 7 and 8:

- \- empty of meal
- (+) trace of meal
- \+ <0.05mg. of meal) estimated when split between mid-gut and crop.
- ++ >0.05mg.of meal)

Table 7.

Destination of sucrose solutions imbibed through membranes by females of L.longipalpis. Meals coloured with 1% (v/v) cochineal red.

MEAL W./VOL. 0.85% SALINE	MEMBRANE	No. OF FEMALES	DESTINATION OF MEAL: CROP	MID-GUT
10% Sucrose	Leached chick-skin	11 All	-	++
10% Sucrose	Leached chick-skin	10	-	++
			(+)	++
			+	++
			(+)	++
			-	++
			-	++
			-	++
			-	++
			-	++
			-	++
10% Sucrose	Parafilm	8	+	+
			+	+
			+	+
			-	++
			-	++
			-	++
			-	++
			-	++

Table 7, continued.

MEAL W./VOL. 0.85% SALINE	MEMBRANE	No. OF FEMALES	DESTINATION OF MEAL: CROP	MID-GUT
30% Sucrose	Leached chick-skin	14	+	(+) Cardia only
			-	(+) Cardia only
			-	+
			+	-
			-	+
			-	+
			-	+
			+	(+) Cardia only
			+	+
			-	+
			-	+
			-	+
			-	+
			-	+

Table 8.

Destination of solutions imbibed from cotton wool by females of L.longipalpis. Solutions coloured with 1% (v/v) cochineal red. No meals were larger than 0.15mg.

MEAL W./V. 0.85% SALINE	TIME DISSECTED (HR.) AFTER FEEDING PERIOD	PROPORTION FEEDING	LOCATION OF MEAL: CROP	CARDIA	MID-GUT
30% Sucrose	0 - 0.2	10/18	+	-	-
			+	(+)	-
			++	(+)	-
			+	(+)	-
			++	(+)	-
			++	(+)	-
			+	(+)	-
			+	(+)	-
			++	(+)	-
			+	(+)	-
	0 - 0.2	11/20	+	(+)	-
			++	(+)	-
			++	(+)	-
			+	(+)	-
			+	(+)	-
			+	(+)	-
			+	(+)	-
			+	(+)	-
			++	(+)	-
			+	(+)	-
			+	(+)	-
	6.0 - 6.2	9/22	All +	+	+

Table 8, continued.

MEAL W./V. 0.85% SALINE	TIME DISSECTED (HR.) AFTER FEEDING PERIOD	PROPORTION FEEDING		LOCATION OF MEAL: CROP	CARDIA	MID-GUT
10% Sucrose	0 - 0.2	11/20		+	(+)	-
				+	(+)	-
				+	(+)	-
				+	(+)	-
				+	(+)	-
				+	+	+
				+	+	+
				+	(+)	-
				+	(+)	-
				+	+	+
				+	(+)	-
	0.5 - 0.7	10/18	All	+	+	+
Human plasma	0 - 0.2	0/20		-	-	-
		2/20	Both	-	-	+
		0/10		-	-	-

activated with human breath: none ever fed, but several probed the rubber tubing carrying warm water to the unit.

On one occasion a group of L. longipalpis females was offered lizard blood which could not be warmed above 30°C for fear of damaging the parasites it contained. With the blood warmed to 28° ± 1°C, 26 out of 34 took a blood-meal.

Meals taken by L. longipalpis from cotton-wool pads.

The percentage of females imbibing different solutions from cotton-wool pads and the destination in the fly of each solution, is set out in Table8 .

In addition, coloured sucrose was found in the crops, but not the mid-guts, of 6 out of 7 males dissected shortly after a two hour period in which a cotton-wool pad soaked in coloured, 30% (w/v) sucrose had been left in their cage.

Sensilla on the fascicle of L. longipalpis.

Sensilla were only found on the labrum. For each of 3 males and 3 females the length and width of the labrum (as seen under the light microscope) were measured *in situ* and after dissection. The length recorded was the distance from the tip of the labrum to its articulation with the clypeus; the mean length was 0·32mm for females and 0·28mm for males. The maximum width was 0·04mm for each.

From these measurements and the structures seen under the stereoscan electron microscope, a semi-diagrammatic illustration of the ventral side of the male and female labrum was drawn to show the number and location of all

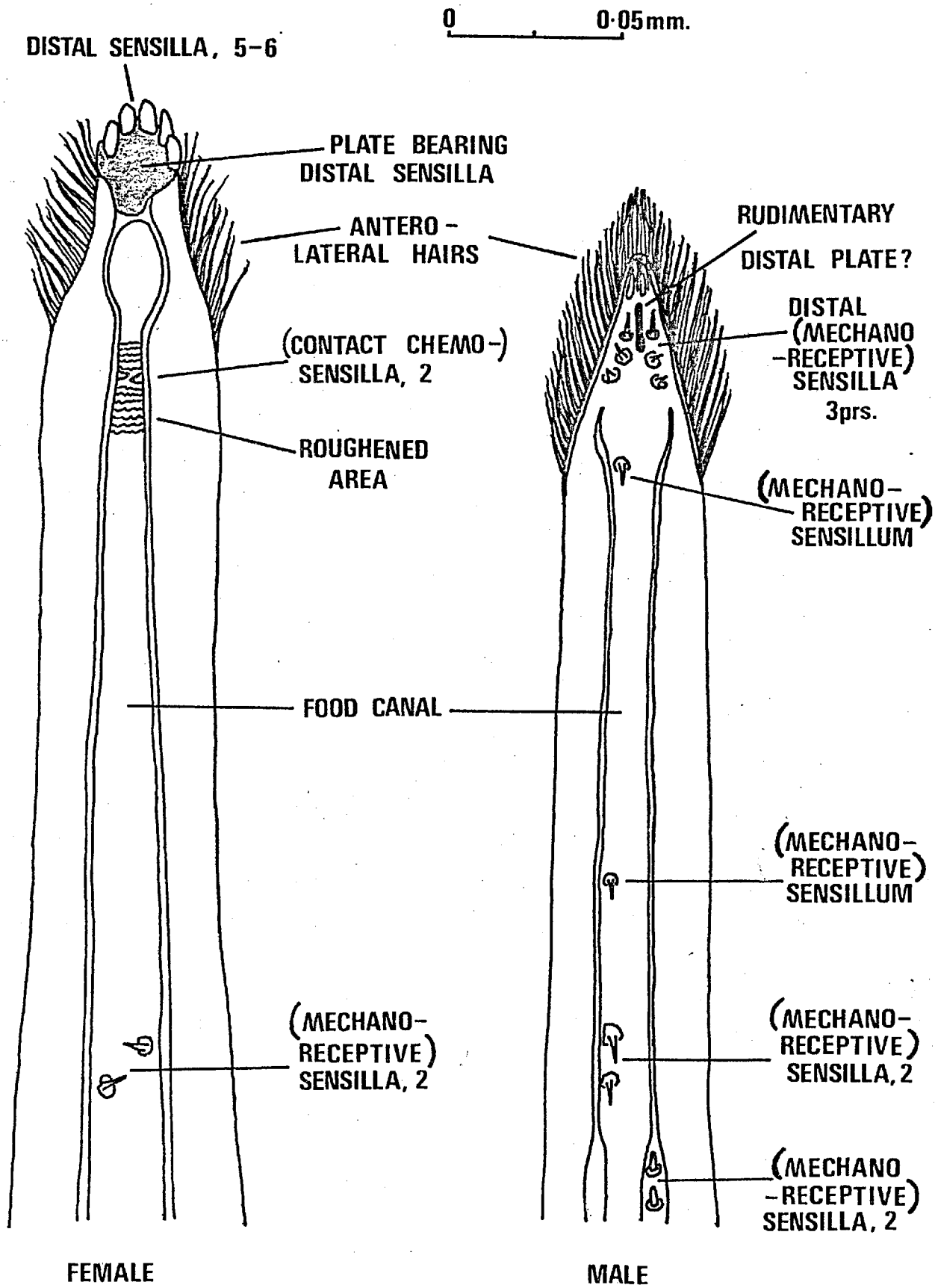


Fig. 8. Ventral view, labrum of L. longipalpis.

Plate 5: L.longipalpis. Ventral tip of labrum, of female.

Above: distal plate and "teeth". (x3,200)

Below: distal "teeth". (x7,500)

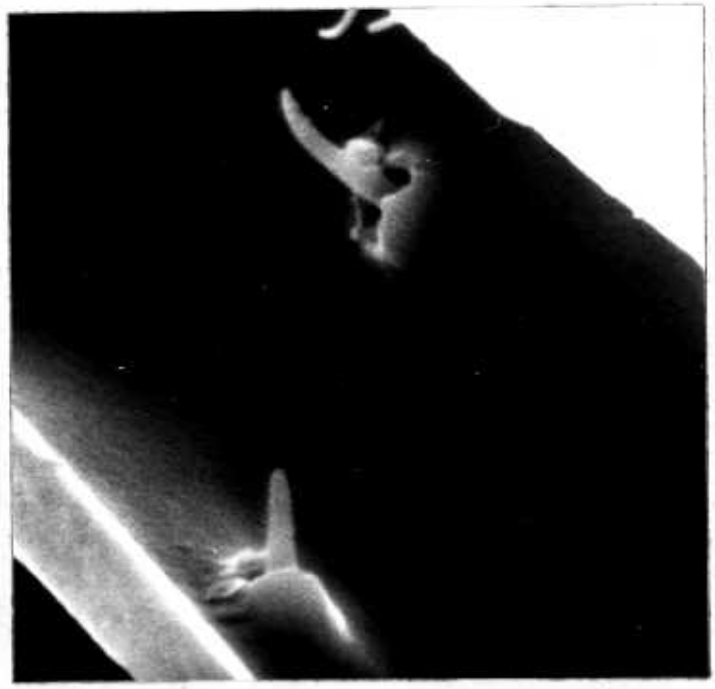


Plate 6: L.longipalpis. Labral food canal and sensilla, of female.

Above: proximal trichoid sensilla. (x8,500)

Below: distal trichoid sensillum. (x10,000)

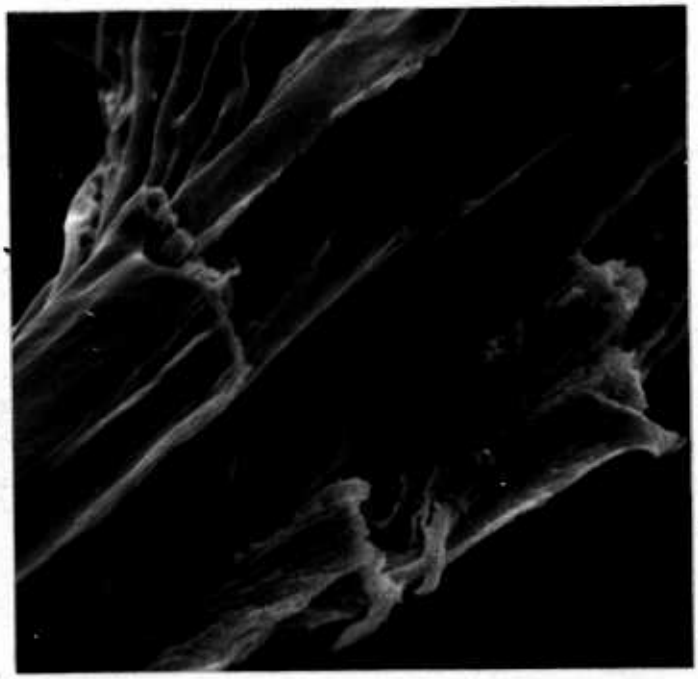


Plate 7: L.longipalpis. Labral food canal and sensilla, of male.

Above: proximal group of trichoid sensilla. (x2,600)

Below: enlarged view of a single trichoid sensillum. (x11,000)

the sensilla seen (Fig 8). The exact nature of these sensilla will not be known until their internal structure has been revealed by transmission electron microscope studies and (if this is possible) their electrophysiological response to different stimuli recorded. They have been provisionally identified by comparing their external structure with that of the sensilla of other Diptera.

PLATES 5 - 7 show the structures seen with the SEM.

Observations on the feeding habits of other sand-fly species.

4 - 6 days after their emergence only 4 out of 17 laboratory reared females of P. perniciosus took a blood-meal from an anaesthetised hamster when this was offered between 10.00 hr and 12.00 hr GMT (16 hour photoperiod, 04·30 - 20·30 GMT). The remainder refused to feed at the same time on the following day, but that evening all 12 remaining flies fed when the same hamster was offered in the dark between 21·45 hr and 23·30 hr GMT.

12 females of Sergentomyia bedfordi reared from eggs obtained from Ethiopia, were narcotised and individually offered a human blood-meal during the "day". All settled on the skin and probed within five minutes. However, although many probed several times and spent as long as twenty minutes "up-ended", with their probosces fully inserted into the flesh, none succeeded in obtaining a blood-meal.

Females of Ps. davisi were captured at night as they probed on human baits in Mocambo Forest near Belém, Brazil.

On two occasions groups of 30 were taken in a dark humid box to a nearby laboratory where within 1 - 2 hours of capture they were narcotised and offered a human blood-meal. Only 15% and 3% (respectively) fed, although the physical conditions in the laboratory were similar in every respect to those in the forest. On two other occasions, 62% and 64% of similarly captured and narcotised females of Ps. davisi accepted human blood-meals when these were offered in the forest one hour after capture.

In the summer of 1973 in the foot-hills of the Apennines in Emilia-Romagna, Italy, females of P.perfiliewi only started to bite after dark (Section 5). This species fed on a wide range of hosts, including man and rodents, but on windy or cold nights ($<14^{o}C$) few females were captured away from human dwellings. The same species was common in cow sheds: Those feeding on cows were often aggregated on fleshy parts of the body, and on the spine, out of range of the tail. Large numbers of males of P. perfiliewi were found alongside the females in the cowsheds and were also caught in oiled "sticky traps" (Killick-Kendrick et al. 1975 and Section 5); this suggests that mating swarms of males are common in this species and play an important part in feeding behaviour.

DISCUSSION.

On the feeding habits of L. longipalpis and other sand-flies: The catholic feeding habits of wild L. longipalpis have been described already. The results of the present investigations show that in the laboratory, too, females of this species will feed on a variety of hosts in a wide range of physical conditions. Ward (1974) reached a similar conclusion. Moreover, it was shown that once a female had probed, one blood was as attractive as another. The nature of the host's hair and skin affected the ease with which a female could probe; this and host availability are probably the principal factors controlling the host preferences shown by L. longipalpis.

L. longipalpis is disinclined to feed again until it has matured a batch of eggs, unlike P. papatasii (Adler & Theodor 1935; Schmidt & Schmidt 1965) and P. longipes (Gemetchu 1972) which can take more than one blood-meal during a gonotrophic cycle. The laboratory conditions did not meet its oviposition requirements, which are more exacting than those for feeding (Section 4), and therefore females could only be kept alive after the first gonotrophic cycle by preventing them from laying their eggs. Under these conditions about 40% accept a second blood-meal (Killick-Kendrick et al.1976). This finding is of epidemiological significance for it suggests a natural limitation to the transmission of Leishmania by L. longipalpis.

The observations on the feeding habits of other species

reflect some of the points made in the introduction and show that L. longipalpis is by no means typical. Thus, although P. perfiliewi, too, fed on a wide range of hosts, feeding was limited by photoperiod, climate and host behaviour. Moreover, there was a suggestion that the presence of mating swarms of males was an essential part of the feeding behaviour, as has been proposed for P. argentipes (Shortt et al.1926).

P. perfiliewi only bit at night, but it fed as readily in a lighted cow-shed as it did in the natural gloom. In comparision, P. perniciosus also usually bites at night, but is not "attracted" to light-traps or to lighted dwellings (Rioux et al.1969). This species was reluctant to bite during the day in the laboratory; it would be interesting to know if the success of the night feeds resulted from a true periodicity and/or the dark.

Like other species of the genus, Sergentomyia bedfordi usually bites cold-blooded vertebrates, and at least in Ethiopia does not attack man (Ashford 1974). When laboratory-reared females were artificially brought into contact with the skin of man, however, all probed; but they were unable to take blood, possibly because their probosces were too short. This illustrates that the immediate stimuli emanating from potential hosts are not necessarily responsible for host preferences. Indeed, for many haematophagous insects the habits and ecology of insect and host are equally important, or more so (Hocking 1971; Gillies 1972).

Ward (1974) described the difficulties of inducing some laboratory-reared Amazonian sand-flies to feed. This

probably relates to fine and specific differences in feeding behaviour which enable a diversity of sand-fly species to co-exist in the Amazon forests. The sensitive nature of the feeding behaviour of one of these species, Ps. davisi, was noted by the author.

Host seeking behaviour of L. longipalpis: Human breath was sufficient to activate females of L. longipalpis who then explored their containers. In a pill-box this brought them into contact with the membrane feeding unit, but in a cage the initial activation was followed by movement towards the source of host breath. For mosquitoes, carbon dioxide in the breath is probably the main activator, but heat, moisture, host odour and various visual factors all contribute to activation as well as to orientation and alighting (review Clements 1963; review Lewis 1972). It is probable that these stimuli are also involved in host-seeking by sand-flies, for sensilla have been found on their antennae and palps that are structurally analogous to those of mosquitoes which are involved in host-seeking (C.T. Lewis pers.comm.).

The strength as well as the nature of the 'odour' plume determine its power to attract (Gillies 1972). Thus groups of caged L. longipalpis took longer to feed on an anaesthetized hamster than they did on the arm of a man from whom a stronger plume emanated. The importance of the 'odour' plume in host location was shown again when females failed to locate the feeder unit placed in a cage.

It is speculated that narcotisation was a necessary preliminary to feeding females in tubes because it supressed

the normal demands for orientation and alighting stimuli. Thus females who were not narcotised usually flew frantically about the tube and rarely settled on the skin even if they touched it. Subjectively, it seemed that the tube was not large enough to enable them to perform their usual searching behaviour.

'Alighting' and probing: Some of the females fed in cages alighted on the host, but in all three containers most walked onto the target. Heat (and host breath) were sufficient to stimulate probing. Thus females were seen probing on warm rubber tubing, and many fed through parafilm membranes whose only attraction was their warmth. Females of L. longipalpis only probed on the skin or target membrane, but Foster et al. (1972) found that P. longipes probed after alighting on clothing. This suggests that probing may sometimes follow automatically after the host has been located.

Heat may not be the only stimulus for L. longipalpis to probe. Thus females successfully fed through a chick-skin when this was warmed to 28°C, only 5°C above the ambient temperature. An olfactory stimulus for probing is suggested. Unfortunately, the response of L. longipalpis to artificial membranes at ambient temperatures was not tested. Similarly, Schmidt (1964) found that P. orientalis successfully fed through animal membranes on blood at ambient temperatures.

Food recognition and feeding by L. longipalpis females: Preliminary observations showed that when groups of 4 - 6

day old females were exposed to human blood over a chick-skin, the proportion that fed was very nearly equal to the proportion that probed. However, this proportion varied from 30% to 82% depending on the batch of flies used. Likewise, when various solutions were offered through chick-skins the percentage of flies that fed was more-or-less equal to the percentage that probed, and varied from 32% to 76% (Table 5). Therefore, "% feeding" cannot be used to compare the 'acceptability' of different test solutions. For this, the mean weight of the freely-ingested meals will be used.

Dethier (1968) found that for the blowfly, 0·6% and 1·2% solutions of NaCl were recognised as 'salt and water' and 'salt' respectively, and that they were accepted. A 3% solution however was rejected as 'salt'. Hosoi (1959) concluded that 0·85% saline was a low-order stimulant for Culex pipiens. The same can be inferred from the results of Salama (1966) who found that 1·29M NaCl had to be added to 0·1M sucrose before 50% of Ae. aegypti females would reject the mixture.

By analogy, the ingestion of 0·85% saline through a parafilm membrane is taken by the author to represent the membrane-feeding response of L.longipalpis to a low-order phagostimulant. The mean weight of saline so ingested (Ca. 0·2 mg) was equivalent to a "half-feed" from a natural host, but saline taken through chick-skin was significantly more attractive. This suggests that there is something about chick-skin which stimulates gorging.

Certainly, L. longipalpis fed through chick-skin more

avidly than through parafilm: With one exception, the proportion of females feeding through chick-skin was roughly equal to that probing it, whereas the ratio of those feeding to those probing parafilm was extremely variable (13: 46 to 44: 49). It is possible that the physico-chemical nature of the chick-skin but not of the parafilm provided stimuli which encouraged the females to remain in the feeding position. Alternatively, the physical nature of the parafilm may have prevented many of the sand-flies from feeding. Thus, sand-flies have a preference for feeding from pools of blood in the skin rather than directly from capillaries (Lewis 1975). Pools did not form in the thin, one-layer parafilm, and feeding through this membrane must have been similar to piercing a capillary. From the observed behaviour of L. longipalpis feeding through parafilm both explanations are equally likely: Many females probed several times before settling to feed, and others that did not feed returned repeatedly to probe before they lost interest.

A second alternative to explain the more avid feeding, and the larger meals taken, through chick-skin, is that phago -stimulants leached from the skin into the test solution. That this happened is suggested by the fact that saline meals were larger still when a chick-skin was not leached prior to use.

With the difficulty of comparing feeds from the two membranes in mind, the relative attractiveness of the various solutions will be discussed. For feeds through parafilm, a 10% solution of bovine serum albumen (BSA) in saline was

only as attractive as the saline itself, whereas whole blood was significantly more attractive than either. These results indicate that there is a phagostimulant in the red cell fraction of blood. Support for this contention comes from the tendency for meals containing red cells to be slightly larger than those of plasma. A significant difference between the two is perhaps masked because plasma meals contained the contents of some lysed red cells and the leaching from chick-skin.

Bishop & Gilchrist (1946), Greenberg (1951) and Day (1954) all noted that for mosquitoes the phagostimulants in blood were contained in the red cells. The blood stimulant for C. pipiens was isolated from ox red cells by Hosoi (1959), who identified it as adenylic acid. He showed that the phosphate salts of adenylic acid were also phagostimulants. These included ATP, which is a phagostimulant for Ae. aegypti (Galun et al. 1963). There is further circumstantial evidence for the role of ATP. Thus, haemolysed blood or haemoglobin are ingested by mosquitoes to a lesser degree than intact erythrocytes (Bishop & Gilchrist 1946; Day 1954; Hosoi 1959). This could be the result of the rapid degradation of ATP that is known to occur in lysed red cells (Hosoi 1959).

Similarly for L. longipalpis, meals of erythrocyte extracts and haemoglobin suspensions were noticeably smaller than those containing whole red cells. However,

the addition of 0·005M[1] of the sodium salt of ATP to 10% BSA[2] did not make this solution more attractive when offered through parafilm. Similarly, 0·005M of the sodium salt of ATP added to a suspension of denatured human plasma had no effect. These results indicate that ATP is not a high-order phagostimulant for L. longipalpis, but there remains the possibility that it is a low-order one.

Of all the solutions offered to L. longipalpis through membranes only those of amino acids (at blood pH of 7·2 - 7·5) were repellent. When the amino acid solution contained only 0·1% of sugars, 28 of the 39 females probing rejected it. All probing flies fed when 5% glucose and 5% fructose were added, but the meal size was still small. It may have been the neutralizing NaOH and/or the amino acids themselves that repelled the flies.

As well as glucose and fructose, fatty acids may be low-order phagostimulants for L. longipalpis - for 10% BSA was less attractive when it did not contain them.

It is generally believed that mosquitoes determine whether to dispatch food to the crop or the mid-gut by recognising its chemical nature (Clements 1963; review, Lewis 1972); and by analogy this has been assumed for Simulids and Phlebotomids (Lewis & Domoney 1966). In mosquitoes, blood and like substances are sent to the mid-gut and sugary fluids are stored in the crop (Clements

1. Optimal concentration for Ae.aegypti (Rutledge etal.1964).
2. It is believed to be "essentially free" of ATP, but Sigma Chemicals (London) are confirming with their head office (U.S.A.).

1963). The discriminating sensilla are located in the cibarium (Galun et al.1963; Salama 1966), and there seems to be an integration of sensory input in the central nervous system, for when mixtures of blood and sugars are ingested they are sent to the mid-gut, crop or both depending on their relative concentrations (Day 1954; Hosoi 1959). Recently, sensilla superficially similar to those of mosquitoes have been identified in the cibgria of sand-flies (D.J.Lewis pers.comm.). It seems, therefore, that a consideration of food destination should be a means of identifying phagostimulants for L. longipalpis.

L. longipalpis females feeding from cotton-wool dispatched sucrose solutions to the crop and plasma to the mid-gut. 51/98 voluntarily accepted sucrose but only 2/50 took plasma. This is the result expected when mosquitoes feed from droplets: Only their tarsal and labellar chemosensilla usually come into contact with free liquids (Clements 1963), and as these respond to sugar, water and saline but not "blood" (Owen 1963; Salama 1966), the latter is rarely imbibed, e.g. Hosoi (1954).

During the course of many dissections of blood-fed L. longipalpis in the Ascot laboratory, blood has only rarely been found in the crop, and then only in small quantities (R. Killick-Kendrick & A.J. Leaney pers.comm.; the author, Section 3). The same was concluded for P. papatasii by Adler & Theodor (1926). An early report by Waterson (1922) that blood can be seen in the crop of P.

papatasii for about 48 hours after a meal has not been substantiated. Therefore it can be assumed that blood is normally dispatched to the mid-gut in sand-flies as it is in mosquitoes. This must be treated as a generalization to which there will be exceptions, e.g. Genetchu (1974) stated that ".... some blood has been observed to enter the crop in at least some P. longipes".

However, the results of feeding artificial diets to L. longipalpis through membranes indicate that there are differences as well as similarities between the feeding mechanisms of sand-flies and mosquitoes. Plasma and 10% BSA were as attractive as whole blood to L. longipalpis feeding through chick-skins. In contrast, Ae. aegypti has been found to refuse or only reluctantly take plasma and serum when these were offered through animal membranes (Bishop & Gilchrist 1946; Greenberg 1951; Day 1954) : The same species only fed voluntarily on 10% bovine serum offered through an animal membrane when ATP was added (Rutledge et al. 1964). Similarly, Hosoi (1959) could only feed C. pipiens on plasma by forcibly placing its fascicle in the medium; then the bulk of the plasma went to the crop. In comparision, 42/42 L. longipalpis ingested BSA (5% - 20%) into the mid-gut as if it were blood. Also, Adler & Theodor (1927) found no difficulty in infecting the mid-guts of P. papatasii with Leishmania by feeding females serous fluids through a rabbit skin.

Saline was ingested in substantial amounts (half-feeds) by 20/22 L. longipalpis probing parafilm membranes. Visual

inspection of these flies and the dissection of 10 others which had fed through chick-skin showed that saline was sent to the mid-gut alone. In comparision, only 11% of C. pipiens whose fascicles were forcibly held in 0·85% saline accepted a meal, and saline was found in the crop as well as the mid-gut (Hosoi 1959).

Likewise, sucrose solutions were ingested through membranes by L. longipalpis, and these went predominantly to the mid-gut. Only one of 156 Ae. aegypti tested by Bishop & Gilchrist (1946) gorged on glucose through membranes, and although almost 100% of C. pipiens imbibed 2·5% glucose when their fascicles were introduced to it the meals were mostly dispatched to the crop (Hosoi 1959).

These results can be explained on a chemical basis by assuming that 0·85% saline and blood salts are stronger "blood" stimulants for L. longipalpis than they are for mosquitoes. Plasma proteins and BSA would then be ingested because they do not inhibit the fly's response to the salts ingested with them. Similarly, the ingestion of 10% sucrose predominantly into the mid-gut can be explained in terms of competition between the "sugar" and "blood" responses. Dissection of L. longipalpis that had fed through membranes on 10% sucrose solutions showed that some of them dispatched small amounts of sucrose to the crop, and that this trend was more noticable when the meal was taken through parafilm rather than chick-skin. In the latter case, the dominance of the "blood" response (stimulated by the saline) over the "sugar" one would have been reinforced by the leachings

from the chick-skin which are "blood" stimulants (see above).

Only small meals of 30% sucrose were ingested through chick-skin, but as all the females that probed remained feeding for 20 - 30 minutes it seems unlikely that this solution was rejected. Unlike any other solution, 30% sucrose went entirely or predominantly to the crop in some flies (4 of the 14 dissected), which indicates that it was recognised as "sugar". At such a concentration it is difficult to believe that it was recognised as anything else. However, 9 out of 14 females dissected had dispatched it solely to the mid-gut.

This finding suggests an alternative explanation to the differences in feeding behaviour between mosquitoes and sand-flies, namely that in sand-flies "blood" feeding might be stimulated mechanically by probing. A possible mechanism is suggested by an observation of Hertig & McConnell (1963). They noted that a sand-fly whose fascicle was placed in a fine capillary tube and then covered with blood fluid would only feed if the labium was bent back; fluid was then ingested into the mid-gut. The auther has found that 10% sucrose fed in this manner is dispatched to the mid-gut. The inference is that the bending of the labium stimulates gorging, possibly via a chordotonal organ.

It is generally agreed that in mosquitoes it is the nature of the food and not the method of feeding which determines whether the meal goes into the mid-gut or crop (Bishop & Gilchrist 1946; Trembley 1952; Day 1954; Hosoi

1959). This may be true for L. longipalpis, with inorganic salts being the major chemical stimulants for "blood" ingestion. However, a mechanical stimulus for triggering blood-feeding should not be dismissed until the innervation and sensilla of the sand-fly labium have been investigated. Both mechanisms preclude the need for ATP as a high-order stimulant, which is unusual amongst blood-sucking insects. Besides the mosquitoes already quoted, ATP has been shown to induce gorging in many haematophagous species, including Rhodnius, Tabanidae and Glossina (cited in Lewis 1972).

Sensilla on the fascicle of female L. longipalpis: Sensilla were only seen on the labrum. The females examined had 5 or 6 large "teeth" or blunt spines set in a plate at the distal tip of the labrum. These were not present in the males which suggests that they are involved with blood-feeding. Adler & Theodor (1926) called them teeth and considered the labrum and hypopharynx of P. papatasii to be piercing stylets. After examining the labra of many New World sand-flies Lewis (1975) agreed with Christophers et al. (1926) that the apical spines were too soft and blunt to be used in penetration and were probably sensory; he postulated that they have a tactile and mechanical function, enabling the fascicle to locate a crevice in the host's skin, and quoted a personal communication from B. Jobling which said that the apical spines are innervated. However, the robust plate in which these "distal sensilla" are set in L.

longipalpis strongly suggests that they are used to exert a force on the host's skin, perhaps not to penetrate but merely to stretch the skin to facilitate the entry of the other stylets. The distal plate was not seen by Lewis (1975) on the labra of L. flaviscutellata that he examined by SEM.

From a drawing by Lewis (1975) the tip of the labrum of L. flaviscutellata differs from that of L. longipalpis in being considerably longer and bearing two pairs of subapical sensilla. These and other sensilla in the labral food-canal (number and location unspecified) are apparently similar to the proximal trichoid sensilla in L. longipalpis. All are very like some of the sensilla in the buccal cavity of Glossina which are believed to be flow monitors (Rice et al. 1973).

The uneven surface of the pair of sensilla at the distal end of the labral food canal of L. longipalpis females suggests that they may be contact chemoreceptors of the basiconic type described by Dethier (1963). It is in the region of these sensilla that the salivary duct enters the food canal from the hypopharynx (Plate8), and it may be that they monitor the fluid nature of the blood and thus ensure that the optimal amount of anticoagulant is released.

Most female mosquitoes have 2 pairs of apical, thick-walled chemoreceptors which are believed to taste blood (Salama 1966; Lee 1974). Further studies may reveal that the distal "teeth" of L. longipalpis are chemoreceptors

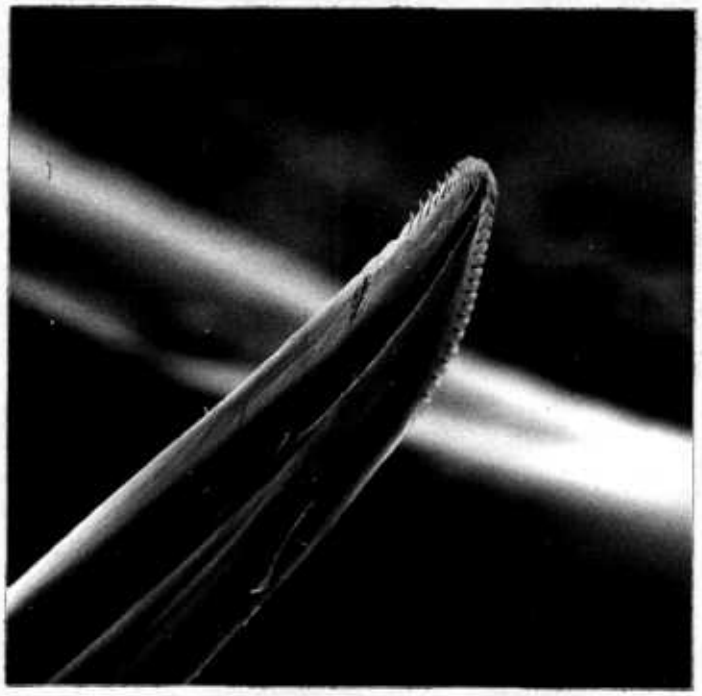


Plate 8: L.longipalpis, female.

Distal opening of salivary duct in hypopharynx. (x900)

but this does not seem likely in view of the less discriminating membrane-feeding behaviour of this species. A pair of campaniform sensilla are present on the labrum in most mosquitoes, and may monitor the bending of the labrum during feeding (Lee 1974). Unlike the mosquito, the sand-fly does not usually feed from capillaries; therefore it is not surprising that L. longipalpis does not have campaniform sensilla on the labrum.

The significance of sugar-feeding in sand-flies: The males and females of most sand-fly species probably feed on plant sugars (Lewis & Domoney 1966). Sugar meals are taken by most blood-sucking insects largely to sustain life and activity, not for growth (Hocking 1953; Downes 1958). That this is true for sand-flies has been shown by finding that more females of L. longipalpis successfully digest a blood-meal, mature eggs (Section 3) and lay eggs (Section 4) when they are fed 30% sucrose rather than water. Moreover, longevity has been improved by feeding sugar to L. longipalpis (Section 1), and other species (Barretto 1942; Gemetchu 1972; Chaniotis 1974).

It is generally assumed that haematophagous insects store sugars in the crop (Downes 1958; Clements 1963). To date, only the crop contents of wild-caught sand-flies have been tested, and sometimes found positive, for sugars (Lewis & Domoney 1966; Lewis 1971). In the laboratory, L. trapidoi accepted a wide range of naturally occurring sugars from droplets, and dispatched them all to the crop.

Similarly, sucrose was ingested from cotton-wool directly into the crop by L. longipalpis females, and only later did it appear in the mid-gut.

However, it has not been proved that sand-flies naturally obtain sugars as free solutions. Indeed, some species have been seen feeding on leaves (Minter & Wijers 1963; Ashford 1974). The results of feeding L. longipalpis females on sucrose through membranes indicates that if sugars are obtained by piercing plant vessels most of the meal will go to the mid-gut and not the crop. This finding is relevant not only to the physiology of the fly but also to the epidemiology of leishmaniasis. Thus, if sugar goes into the crop it is a possible energy store and perhaps an osmotic regulator (Lewis 1971), but ingested directly into the mid-gut it loses this function, and might impair the development of Leishmania by introducing bacteria (Adler & Theodor 1957; Lewis & Domoney 1966).

The males of P. papatasii (M.Mariani, cited in Downes 1958), P. longipes (Gemetchu 1972) and some Lutzomyia species (Chaniotis 1967, 1975) are known to take sugars in the laboratory. Sugar (30% sucrose) was also ingested (into the crop) by males of L. longipalpis, but there is no definite proof that sugar-feeding occurs in nature. However, the presence of a large number of probable flow monitors (trichoid sensilla) on the labrum of the male of L. longipalpis, suggests that some fluids are taken by wild flies.

Ashford (1974) reported that females and, more often, males of P. longipes and P. orientalis commonly probe, and apparently feed, on plants in Ethiopia. From the structure

of the male sand-fly's mouthparts it is difficult to believe that they can be used to pierce plant vessels in order to obtain sap. Firstly, the male fascicle lacks mandibles, which are the cutting stylets; this is definitely so for L. longipalpis, and for P. orientalis (Davis 1967). Second, the tip of the male labrum (at least in L. longipalpis) has not teeth or spines but a fringe of hairs (Plate 9). In fact, the description that Ashford (1974) gives of leaf-probing is reminiscent of the behaviour of male and female L. longipalpis when they feed from droplets of sugar or water.

L. longipalpis readily ingested 30% sucrose, and L. trapidoi did not discriminate between 10% and saturated fructose (Chaniotis 1974). As pointed out by Chaniotis (1974), the acceptance of highly concentrated sugar solutions by sand-flies suggests that they feed in nature on nectar and other sweet liquids. Many insects visit flowers, and Hocking (1953) has estimated that sugars constitute (by weight) up to 76% of insect crop contents and 77% of floral nectar.

Plate 9: L.longipalpis, male.

Tip of fascicle. (x2,250)

SECTION THREE:

ON THE REPRODUCTIVE BIOLOGY OF L. longipalpis.

INTRODUCTION.

The early Diptera are believed to have originated in the Permian and to have been biting insects feeding on the body fluids of insects and/or vertebrates.(Downes 1958). It is generally held that feeding by the adults of present-day nematocerans is supplementary to that of the larvae: The primitive blood-feeding habit of the female is only retained when the protein provided by the larva is insufficient for the maturation of eggs; but both males and females take water and carbohydrate (usually nectar) for flight and general metabolism (Downes 1958; Oldroyd 1964).

The Phlebotomidae have retained the blood-sucking habit. Dolmatova (1942) described the digestion of a blood-meal and the concurrent development of the ovaries and accessory glands in P. papatasii. She found that a small blood-meal led to the maturation of a few follicles only, and that unfertilized females produced normal eggs. The importance of the blood-meal for egg maturation by sand-flies has been widely accepted. Indeed, at one time it was thought that females could not be induced to produce eggs unless they had taken a blood-meal (Unsworth & Gordon 1946). More recently it has been found that (like their cousins the Culicidae) females of certain populations of sand-flies can lay eggs without taking a blood-meal

(Johnson 1961; Schmidt 1964; Dolmatova & Demina 1971).

An integral part of the digestion of a blood-meal by a female sand-fly is the formation of a peritrophic membrane (Adler & Theodor 1926). Recently, Gemetchu (1974) has made a comprehensive study of the morphology and fine structure of the mid-gut and peritrophic membrane of the adult female of P. longipes.

Many laboratory investigations have shown that the digestion of blood and the concurrent maturation of oocytes proceed most smoothly if female sand-flies are kept in a high humidity (70% - 100% RH) and at a temperature of 21° to 30° (Adler & Theodor 1935; Dolmatova & Demina 1971; Gemetchu 1972). These conditions are similar to those in the shady nooks where the engorged females of some species usually rest (Perfil'ev 1968).

The nature and function of the blood-meal constituents which stimulate egg maturation by sand-flies is not known. Adler & Theodor (1926) concluded that only the plasma was essential for egg maturation by P. papatasii; they based their conclusion on the observation that haemolysis rarely occurred before the third day after a blood-feed.

For sand-flies, blood-feeding is not always intimately associated with egg maturation. Thus Adler & Theodor (1935) found that wild-caught P. papatasii took several blood-meals between gonotrophic cycles, whereas other species, notably P. perniciosus, refused to feed until a batch of eggs was laid. They related this to the ability of P. papatasii to survive in lower humidities than the other

species and postulated that it maintains its water balance by taking frequent blood-meals. Similarly, Oldroyd (1964) has argued that sand-flies have retained the blood-sucking habit because it may have been an advantage at the end of the last Ice Age when conditions became drier in the sub-tropical latitudes where they evolved. The failure of some P. papatasii females to mature eggs following a blood-meal has also been recorded by Dolmatova; "gonotrophic dissociation", as the phenomenon has been called, is particularly common in autumn sand-fly populations in non-tropical climates (Dolmatova & Demina 1971).

This section reports on some aspects of the reproductive biology of colonized females of L. longipalpis. The course of blood digestion and vitellogenesis were investigated first, as a basis for relating egg maturation to the size and nature of the blood-meal, to sugar-feeding, mating and to other physical factors. In particular, investigations were made to determine the ingredients of a blood-meal that were essential for egg maturation.

The quantitative effect that different bloods have on the egg production of Culicidae is well known (Clements 1963), but the possibility that blood composition might influence the nature of the yolk protein synthesised does not appear to have been investigated. Such differences were sought in the eggs of L. longipalpis by the electrophoretic separation of water-soluble yolk proteins and by attempting to identify host antigens.

MATERIALS AND METHODS.

The rearing and feeding techniques used were those that have been described in Sections 1 & 2, unless otherwise stated in the text.

The digestion of the blood-meal.

Estimation of the red cell concentration in a blood-meal. A freshly narcotised fly was weighed before its unbroken, blood-filled gut was dissected out and placed in a well-slide containing 0·14 ml of physiological saline. The gut was broken and the blood teased out and thoroughly mixed with the saline using fine, mounted entomological pins. The blood suspension was immediately sucked into an erythrocyte pipette where it was shaken for a minute to ensure an even dilution of the cells.

The red cell concentration was estimated in an improved Neubauer counting chamber in the usual manner (Darmady & Davenport 1963). 80 small squares were counted.

The SGs of most vertebrate bloods are similar to that of human, i.e. 1·056; and the same holds for the SGs of red cells, for which the value for human cells is 1·098 (Spector 1956; Hill 1973).

Therefore, the dilution of the blood-meal in the counting chamber was taken to be

$$\frac{M}{1 \cdot 077} \text{ ul in } 0 \cdot 14 \times 10^3 \text{ ul},$$

where M = weight of meal at dissection, and 1·070 = a mean value for the SG of the meals.

For each blood-meal, the mean of 3 different red-cell estimations was calculated.

The identification of haemoglobin and haptoglobins using gradient polyacrylamide gel electrophoresis. A technique for identifying haemoglobin (Hb) and haemoglobin-bound haptoglobins (Hp) by gradient gel electrophoresis was perfected by Baxter & Rees (1974). The same system, using commercially prepared gradient gels , has been used by the Immunology Laboratory, Imperial College at Silwood Park, to detect and identify human blood-meals in anopheline and culicine mosquitoes (Boreham & Lenahan 1976). The separated Hb and Hp appear as blue-green bands after staining in orthotolidine (1% in 45ml ethanol/5ml acetic acid/50ml water) and then hydrogen peroxide (2% in water).

Mr. Lenahan of the Immunology Laboratory kindly allowed me to use his apparatus to trace the fate of Hb and Hp in the blood-meals of L. longipalpis.

Universal Scientific Ltd., London.

Egg production in relation to size and nature of feed. Feeding. Meals of different sizes were obtained by disturbing some flies before they had become fully engorged. The weight of the meal imbibed was measured by weighing (on a Beckman microbalance) the female shortly before and after a meal.

Meals taken through membranes. The methods of preparation

of the meals tested for their effect on egg production have been described in Section 2. Each type of meal was offered on at least two occasions through two (or more) different leached chick membranes to check the constancy of its effect on egg production. All solutions were warmed to $37^{\circ} \pm 1^{\circ}C$.

Egg counts. Only the eggs matured by flies that survived for at least 72 hours after a meal were counted. The fly's egg production was then taken to be the sum of the fully matured (stage 5) eggs that had been laid and those still remaining within the oviducts. Flies that did not lay any eggs were killed on the tenth day and their retained eggs counted.

Statistical analysis. Scatter diagrams were drawn for the number of eggs matured by a female on a measured weight of meal. Any linear association between these parameters was calculated using regression analysis (Bailey 1968). Transformations (i.e. $\sqrt{y}$, $1/y$, $\log y$) were attempted, but as they did not consistently improve the regressions were rejected.

Regression coefficients for different meals were compared using a modified 't' test (Bailey 1968). Alternatively, the mean number of eggs matured on different meals was compared within a small range of meal weights. This alternative was employed when the data covered too narrow a range of meal weights for regression analysis to be meaningful, or when it was thought that there might be a constant but not proportional difference between the effects of different meals.

The development phases of egg follicles and their identification.

For anophelines, Christophers (1911) separated the development of the ovaries into 5 phases. This scheme was used by Dolmatova (cited in Dolmatova & Demina 1971) as a basis for describing the maturation of Phlebotomid eggs, and was followed by the author in the present study.

Narcotised sand-flies were dissected and the follicles identified using a monocular microscope with top-lighting and a calibrated eye-piece graticule.

Stage 1. The follicle has the smallest diameter, about 40 μ, and contains no yolk; it appears as a translucent sphere.

Stage 2. The first yolk granules appear; the follicle has a diameter of 40 - 100 μ and an oval shape (in 2-D); one half is filled with yolk, but the other half (containing the food cells) appears translucent.

Stage 3. The follicle becomes slightly elongate; yolk (i.e. the egg) occupies 2/3 - 3/4 of the follicle; maximum diameter of 0·1 - 0·12 mm.

Stage 4. The follicle is distinctly elongate.

(a) the food cells are just visible and occupy only 1/10 of the follicle,

(b) yolk granules occupy all of the follicle; diameter 0·12 - 0·3mm.

Stage 5. The egg is mature, covered with a chorion, and slightly concave/convex along its long axis.

Separation of the water-soluble, yolk proteins of L. longipalpis by disc-gel electrophoresis.

Preparation of the protein homogenate. Approximately 200 one day-old eggs were washed, twice in distilled water and once in TRIS-buffer electrolyte (see below), and placed in a microcentrifuge tube. 40 µl of TRIS-buffer were added from an Oxford micropipette/dispenser, and the mixture left to cool at 4°C for 30 minutes. The yolk proteins were "liberated" by ultrasonication of the eggs (kept cool in a beaker of ice to prevent protein denaturation). Egg shell fragments were separated from the protein homogenate by centrifugation at 4,000 g for 10 minutes. Samples were stored at -20°C if not immediately used.

Other concentrations were tried, but this was the optimal.

The electrophoresis apparatus. The Shandon analytical polyacrylamide gel electrophoresis apparatus[1] was used. This consists of an anode lid and a cathode core placed, respectively, in upper and lower electrolyte reservoirs which are linked by 12 glass running tubes. Each precision-bore tube (5 mm diameter; 75 mm long) is filled with polyacrylamide gel, which separates the proteins that migrate down it according to molecular size and charge.

[1] Shandon Southern Instruments Ltd., Camberley, Surrey.

Preparation of the polyacrylamide gels. These were cast in the running tubes. I followed the simplified method proposed by Clarke (1964), for which only one, uniform, small-pore (7·5%) gel is used. The monomeric mix was prepared from the following stock solutions.

1 volume of A, containing 36·3 g TRIS, 0·46 ml TMMED (N_4- tetramethylethylene diamide) and 48 ml 1·0 N Hcl, made up to 100 ml with distilled water;

2 volumes of B, containing 30g acrylamide monomer, 0·8g N, N - methylene/bisacrylamide made up to 100 ml with distilled water;

4 volumes of C, containing 0·14g ammonium persulphate per 100 ml of distilled water; and

1 volume of distilled water.

The electrolyte was a 1: 10 dilution of stock prepared from 29g glycine, 6g TRIS, and 9·75 ml of distilled water. Final pH was 8·1.

The gel system of Ornstein & Davis (1962) was tried, but did not improve resolution. For this, a large-pore (5%) gel was cast on top of the small-pore in order (in theory) to help concentrate (or "stack") the proteins at the face of the small-pore gel.

The electrophoretic run. 25 µl of the protein homogenate were mixed with 25 µl of 40% (w/v) sucrose, and a drop of 0·05M bromophenol blue was added. With the running tubes in their place in the apparatus, the test mixture was carefully run into the space above a gel. This space was

then filled with electrolyte. The sucrose gave the test mixture sufficient density to ensure that it was not washed away.

The sample proteins were stacked for 15 minutes at 0·5 ma. per gel, and separated at 1·5 ma. per gel for 1·5 - 2 hours. The bromophenol blue acted as a marker for the stacking process and the ion-front. The current was provided by a regulated DC power supply. The apparatus was placed at 4°C during the run to prevent overheating and possible protein denaturation.

Staining. The gels were removed from their tubes by "rimming" and pulling with a blunt dissecting needle, and then placed in 0·1% naphthalene black 10B (in 7% acetic acid) or 0·25% coomassie brilliant blue (1·25g in a mixture of 454ml of 50% methanol and 46ml of glacial acetic acid). These solutions stained and fixed the protein bands.

Excess stain was removed (overnight) in one of the following destaining solutions: 7% acetic acid for naphthalene black; and 5% methanol in 8% acetic acid for coomassie blue.

Mobilities of the separated protein bands. The gels can swell up to 8% in the acidic solutions used for staining and destaining. Therefore, the calculation of a band's mobility has to include the length of the gel before and after staining as well as the measured mobility of the band and the marker dye, e.g. Weber & Osborn (1969).

Significant differences in band mobility result from

small changes in gel structure, current and temperature (Gordon 1971). Therefore, comparisions between the yolk proteins of different egg batches were only made when they had been separated on the same electrophoretic run and using gels made at the same time from the same monomeric mix. One assumption made in calculating band mobilities was that gels of the same batch swelled evenly between the end of a run and after staining. Band mobility = (Distance of protein migration)(length of gel before staining) / (length of gel after destaining)(distance of dye migration).

The immunodiffusion test.

A micro-immunodiffusion test (Weir 1967) was used in an attempt to demonstrate host antigens in the egg-yolk of L. longipalpis. Homogenates of egg-yolk were prepared as described above.

Thin layers of agar gel were cast on clean glass slides. In each gel there was cut a central well and four peripheral wells set in a circle round it. Host anti-serum was placed in the central well, and egg-yolk homogenate and host sera (as controls) were separately placed in the peripheral wells.

The theory behind the test is that precipitin bands will form in the gel in the positions where the antigen and antibody molecules reach optimal proportions after diffusion.

Two slides were run with hamster anti-serum against hamster serum, human serum, homogenate of egg-yolk from

hamster-fed flies and homogenate of egg-yolk from human-fed flies. Similarly, two slides were run with human anti-serum.

RESULTS.

All results, unless otherwise stated, refer to L. longipalpis maintained (before and after blood-meal/test feed) at 25°C, 95% RH and in a photoperiod of 12 hours (06·00 - 18·00 hrs GMT) with a constant supply of 30% (w/v) sucrose solution.

Even-size (0·280 - 0·400 mg) groups of 4 - 6 day-old females were selected for these experiments.

AUTOGENY.

Some females were not offered a blood-meal. For the first four days after emergence they were kept with males in rearing cages, and on the fifth day set up in oviposition tubes alongside a control group of blood-fed females. A few eggs were matured autogenously. (Table 9).

THE COURSE OF BLOOD DIGESTION AND VITELLOGENESIS.

A cage of stock flies was divided. Each group was offered blood, one from a human and the other from a hamster. Thereafter, no sucrose or water was offered. At various times after the meals small samples (2 - 5) of each group, which had taken approximately equal meal weights, were weighed and immediately dissected. The following details were recorded (Table 10):1)stage of oocyte growth; 2) colour and position in the gut of the blood-meal. 3) concentration of red cells in the meal, and 4) the colour and presence of faeces in the oviposition tubes. Control counts were made of the red cell

TABLE 9.

Autogeny in L. longipalpis.

GENERATION	NUMBER OF FEMALES TESTED	NUMBER OF FEMALES SHOWING AUTOGENY	NUMBER OF EGGS MATURED
1	10	3	5, 2, 3
6	10	0	0
9	24	1	2
18	10	0	0
TOTAL	54	4	12

TABLE 10.

The course of blood digestion and vitellogenesis, at 25°C., in L. longipalpis following a meal of (a) human blood and (b) hamster blood.

TABLE 10(a).

TIME AFTER MEAL (HRS.)	No. IN SAMPLE	OOCYTE STAGE	COLOUR OF MEAL	POSITION OF MEAL	CONCENTRATION OF RED CELLS x 10^6 (PER C. MM.)	PRESENCE AND COLOUR OF FAECES
-1.0	5	1	-	-	4.40	(Control Blood)
0.1	4	-	Cherry Red	Abdominal Midgut	4.22 (5.49-3.25)	Clear Fluid
8.5	3	1/2	"	"	3.62 (4.71-2.53)	-
20.0	4	2/3	Red inside Black	"	2.40 (3.10-1.92)	White/Yellow
30.0	2	3	"	"	1.17 (1.51&0.82)	White/Yellow
51.0	5	4(a)	"	"	0.59 (1.77-0.30)	White/Yellow
53.0	4	4(a)	Trace of Red inside Black	"	0.16 (0.29-0.14)	White/Yellow
67.0	2	4(b)/5	Brown/Black	Mid- and Hind-gut	0	Brown
70.0	3	5	Brown/Black	"	0	Brown

TABLE 10(b).

TIME AFTER MEAL (HRS.)	No. IN SAMPLE	OOCYTE STAGE	COLOUR OF MEAL	POSITION OF MEAL	CONCENTRATION OF RED CELLS x 10^6 (PER C.MM.)	PRESENCE AND COLOUR OF FAECES
-1.0	5	1	-	-	5.10	(Control Blood)
0.1	4	-	Cherry Red	Abdominal Midgut	4.83 (6.28-2.89)	Clear Fluid
9.0	2	1/2	"	"	3.60 (3.90&3.31)	-
20.5	4	3	Red inside Black	"	1.19 (1.34-0.14)	-
29.0	4	3/4(a)	Trace of Red inside Black	"	0.19 (1.42-0.00)	White/Yellow
50.0	8	4(b)	Brown/Black	"	0	White/Yellow
56.0	3	5	Brown/Black	Mid- and Hind-gut	0	White/Yellow
69.0	6	5	Brown/Black	"	0	Brown
74.0	2	5	Clean Gut	-	0	Brown

concentrations in blood taken directly from the human and hamster hosts.

The figures for the concentration of red cells in the meals up to 9 hours old are not very accurate because there was considerable agglutination (or "clumping") of red cells. Agglutination was not so marked in older meals.

The growth of the ovaries in the posterior part of the abdomen tended to push the decreasing remains of the blood-meal forward. This gave the illusion that digestion of the blood-meal was forward-moving, as quoted by Dolmatova & Demina (1971). In fact, digestion occurred from the outside of the meal inwards in a fairly uniform manner, as could be seen from the colour changes in the meal - the inside was still red 30+ hours after ingestion.

Vitellogenesis and the digestion of the meal were complete by the same time. Both were markedly faster when hamster blood rather than human blood was imbibed. This was confirmed by further observations (Table 11).

The fate of haemoglobin in the blood-meal.

Haemoglobin was easily detected in the abdominal contents of females of L. longipalpis up to 47 hours after they had taken a meal of human blood. Haptoglobin bands (of the 2 - 1 pattern of the author, who had fed the flies) were faint from 42 hours after the meal, but along with the haemoglobin bands could not be detected in the meals of 66hours or more (Table12).

Haemoglobin was detected in only 2 of 3, 47 hour

TIME AFTER MEAL (HRS.)	HAMSTER BLOOD No. IN SAMPLE	HAMSTER BLOOD OOCYTE STAGE	COLOUR OF BLOOD-MEAL	POSITION OF BLOOD-MEAL	FAECES	HUMAN BLOOD No. IN SAMPLE	HUMAN BLOOD OOCYTE STAGE	COLOUR OF BLOOD-MEAL	POSITION OF BLOOD-MEAL	FAECES
20 - 21	4	3	Red inside black	Mid-gut	White	4	2/3	Red inside black	Mid-gut	White
	6	3	"	"	"	4	2	"	"	"
	6	3	"	"	"	4	2/3	"	"	"
48 - 49	6	4(a)/4(b)	Brown	Mid-gut	White	5	4(a)	Trace of red inside black	Mid-gut	White
	4	4(b)	"	"	"	4	4(a)	"	"	"
	4	4(b)	"	"	"	3	4(a)	"	"	"
70 - 74	5	5	Brown/black	Mid-gut/hind-gut	Brown	4	4(b)	Brown	Mid-gut	White
	4	5	"	"	"	5	5/4(b)	Brown/black	Mid-gut/hind-gut	Brown

Table 11. The faster rate of blood digestion (and concurrent vitellogenesis) in L. longipalpis females that had ingested hamster rather than human blood-meals. (All at 25°C.)

Table 12.

The presence of "haemoglobins" in human and hamster blood-meals of L.longipalpis at various times after the meal.

HUMAN BLOOD TIME AFTER FEED (HRS.)	PROPORTION OF MEALS CONTAINING: HAEMOGLOBIN	HAPTOGLOBINS
0.5	4/4	4/4
6	4/4	4/4
18	4/4	4/4
24	4/4	4/4
31	4/4	4/4
42	4/4	(4)/4 FAINT
47	6/6	(6)/6 FAINT
66	0/6	0/6
72	0/6	0/6

HAMSTER BLOOD TIME AFTER FEED (HRS.)	PROPORTION OF MEALS CONTAINING HAEMOGLOBIN
0.5	4/4
47	2/3

hamster blood-meals tested. Hamster blood does not contain haptoglobins.

No haemoglobin was detected in the faeces of females that had digested human blood (0/4) or hamster blood (0/3). Following the separation of faeces or of old meals that did not contain haemoglobin there was a considerable "streak" of stain just behind the point on the gel where the haemoglobin band would have been. This "streak" indicated the presence of some of the products of haemoglobin breakdown; it was only very faint following the separation of meals containing haemoglobin.

Haemoglobin in sand-fly eggs. In 3/3 tests haemoglobin or haemoglobin-type compounds were detected in the water-soluble extract from the washed eggs of females of L. longipalpis which had been fed on human blood. In each case, a faint band with the same mobility as haemoglobin from whole human blood was demonstrated; this band was not detectable in controls.

Decrease in female weight following a blood-meal.

Females that had taken a human or hamster blood-meal were weighed at different times from 1 to 72 hours after the meal. Their loss in weight has been expressed as a % of the weight of the meal, referred to as "% loss in meal-weight" (Fig 9). Similarly, the % loss in meal-weight was calculated 20 and 60 minutes after a human blood-meal (Appendix 1), when the mean values were 25·5% and 38% respectively.

Females that had taken a human blood-meal were found to

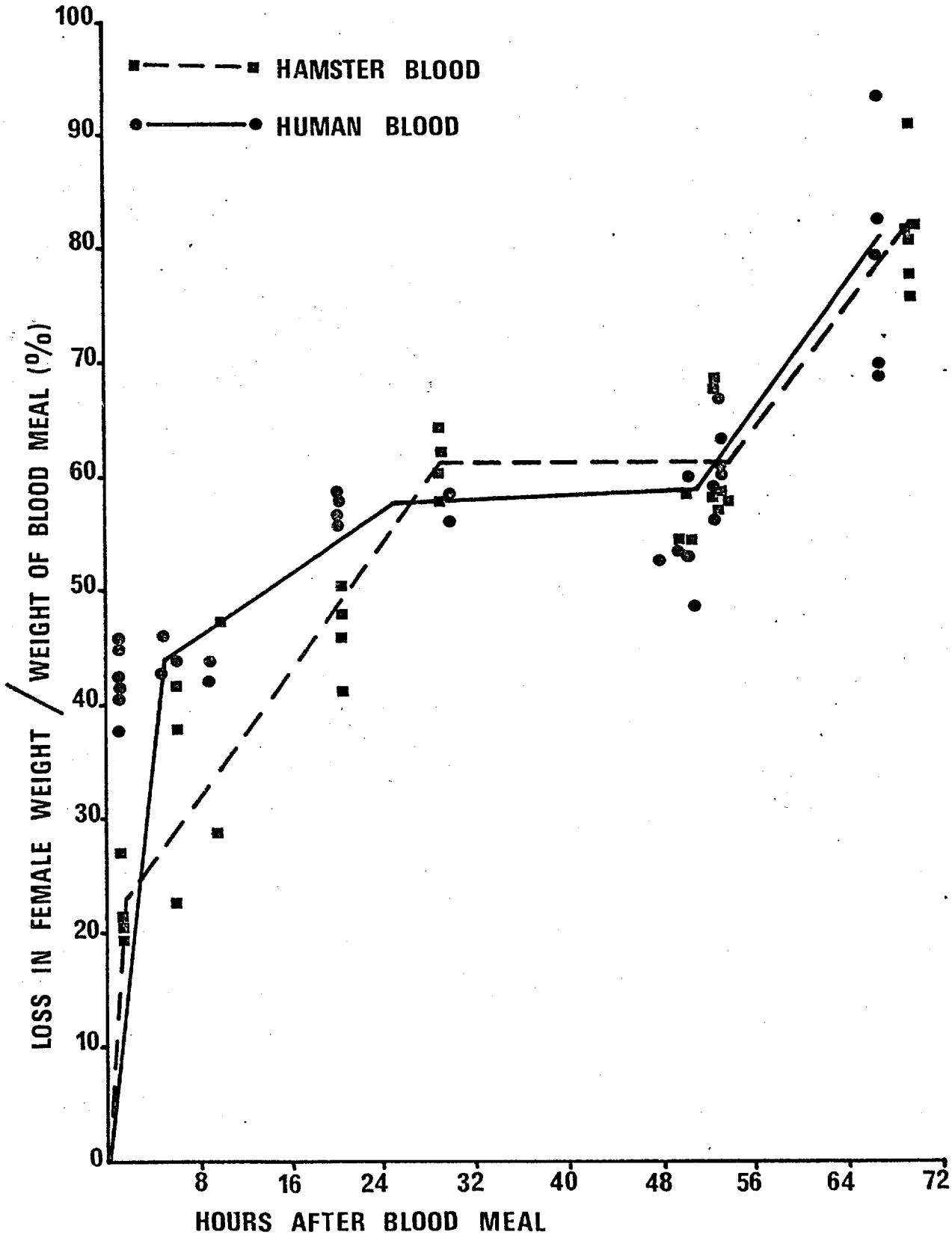


Fig. 9. Loss in weight of L.longipalpis females following a blood-meal. Loss is expressed as % loss in meal-weight.

lose weight faster in the first 20 hours than those with a hamster blood-meal. However, the mean weight of the hamster blood meals (0·344mg) was considerably less than the human (0·464mg). Therefore, the relationship between % loss in meal-weight after 1 hour and the weight of human blood-meal was determined (Fig 10). For this, there was only a 5% rise in the "% loss in meal-weight" for a 0·15mg rise in the weight of blood imbibed ($p = 0·02$; $t = 2·58$).

Oösorption.

It was not possible to count accurately the number of ovarioles or stage 1 oocytes in each ovary of a newly emerged female of L. longipalpis because of their small size and translucence. However, from the size of the larger egg batches (120 - 130) recorded from the colony there would appear to be a maximum of 65 oocytes that can be matured in each ovary in a gonotrophic cycle. Usually, fewer were matured, the number depending largely on the size of the blood-meal imbibed (see below).

Females that had taken known weights of human blood were dissected 14, 30, 72 and 96 hours after engorgement in order to investigate the time at which the number of oocytes to be matured was determined. This was found to be about 30 hours after engorgement, between stages 2 and 3 of vitellogensis (Fig 11). For a given weight of blood, more oocytes initiated vitellogenesis than eventually completed it.

Following a meal of hamster blood the number of oocytes to be matured was more-or-less set by 20 hours. Thus :-

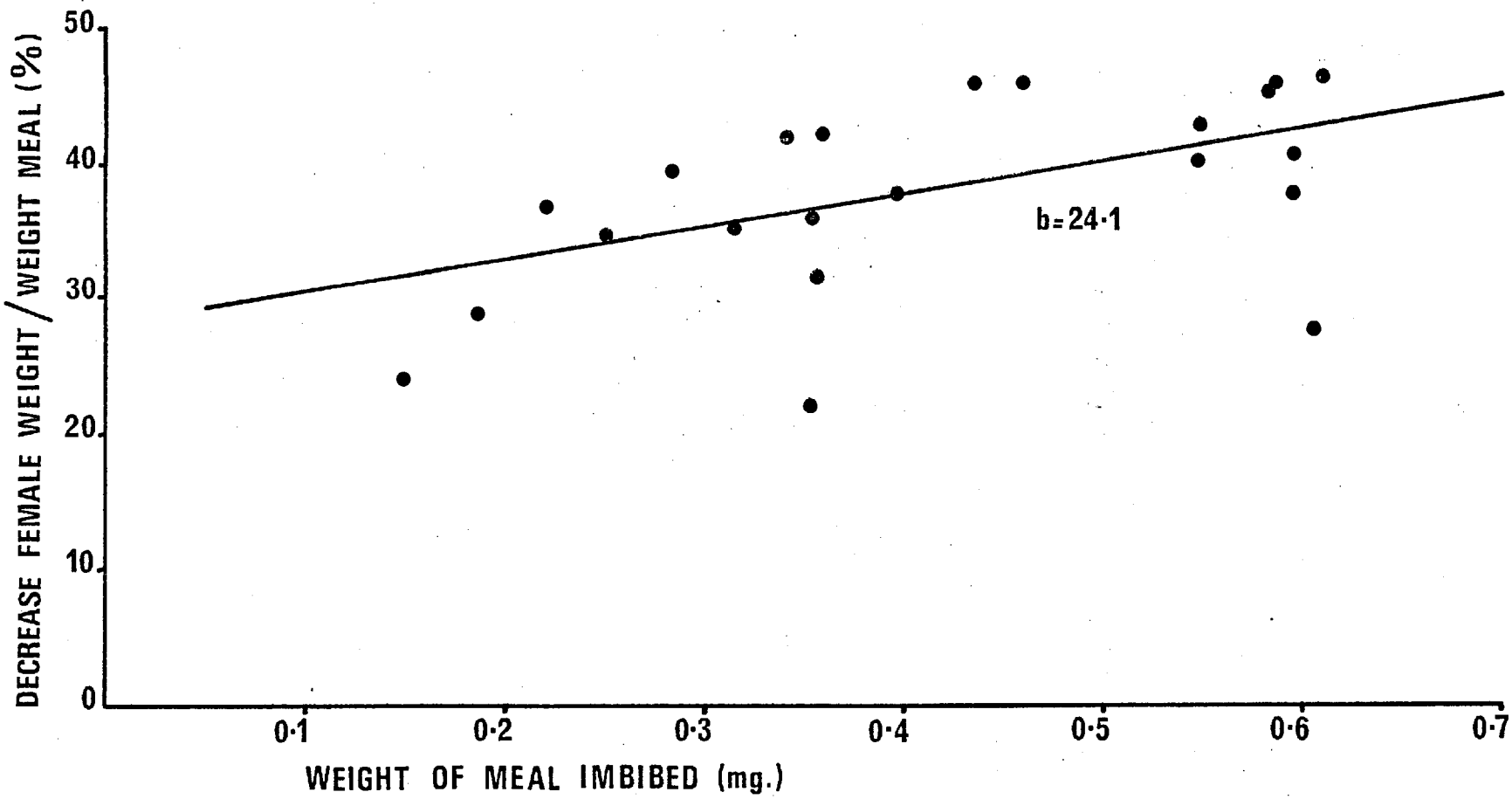


Fig. 10. The % loss in blood-meal weight after one hour in relation to the weight of blood imbibed.

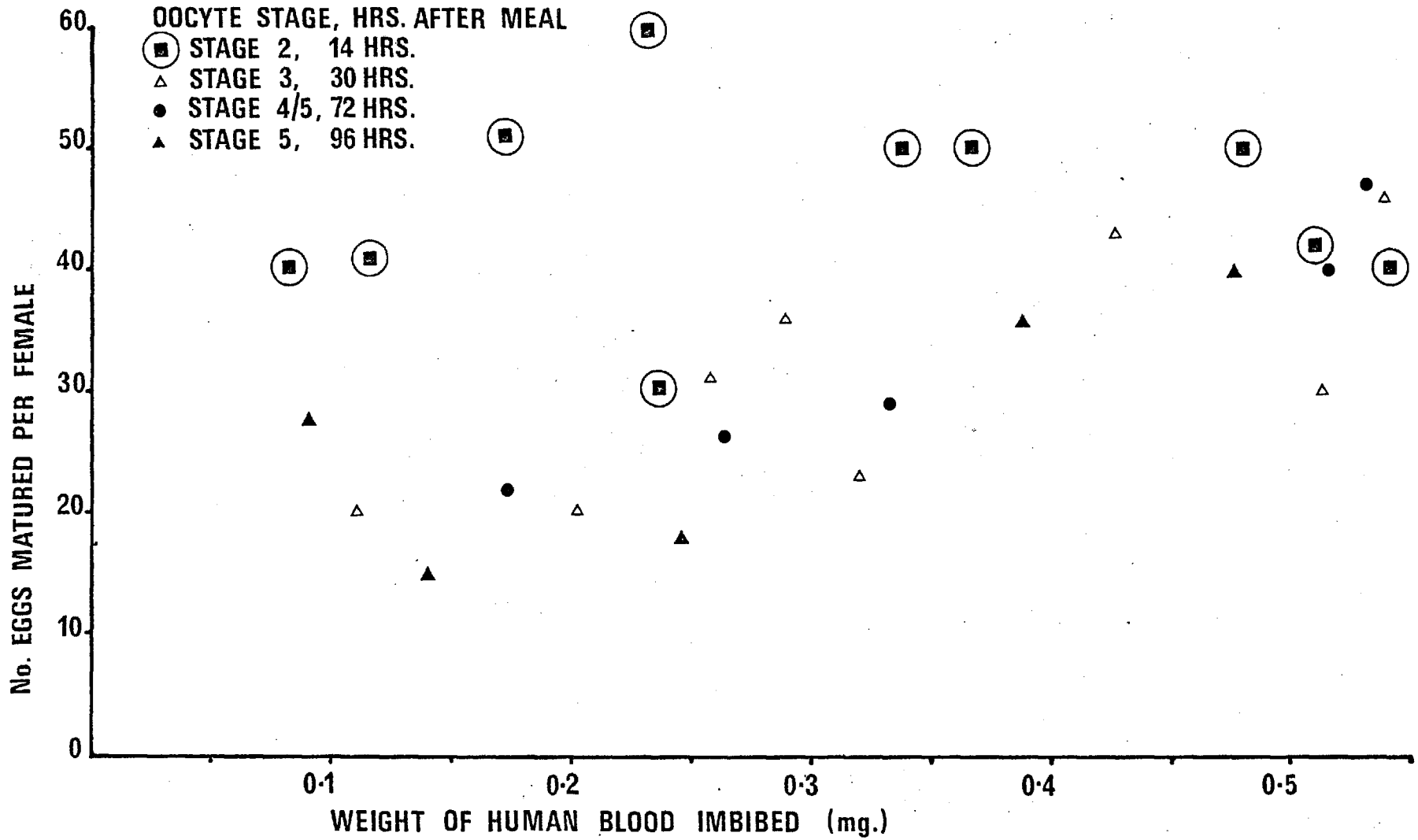


Fig. 11. For given weights of human blood, the number of oocytes containing yolk at different times after the blood-meal.

Weight of hamster blood imbibed (x)	No. of oocytes developing.	Oocyte stage.	Expected No. of eggs matured. (From Fig.13).
0·372	80	3	70
0·409	67	3	75
0·180	55	3	45
0·343	73	3	68

THE NUMBER OF EGGS MATURED BY A FEMALE IN RELATION TO THE SIZE AND NATURE OF THE MEAL IMBIBED.

"Natural" blood-meals from warm-blooded vertebrates.

Females of L. longipalpis of the Belo Horizonte (B.H.) strain were fed on three mammal species - laboratory hamsters and mice, and man. In each case the number of eggs matured by a female was proportional to the weight of blood imbibed (Fig 12); the linear regression coefficients were significant, usually at the 0·1% level (Appendices 2 - 8). The regression coefficients for eggs matured on the weight of blood imbibed by flies of different generations were not significantly different for any one host (Fig 13) but differed between hosts. This difference was significant for 2 out of 3 comparisions between hamster and human blood-meals (Table 13). For the third, the average number of eggs matured on 0·3 mg hamster blood, 70, was significantly greater than that on human blood, 45 ($p = 0·001$; $t = 4·67$). Mouse blood was intermediate in effect between that of hamster and human.

L. longipalpis of the Ceará (c) strain were fed on six animal species, viz: man; Proechimys sp, the spiny rat; Didelphis marsupialis, an opossum; Bradypus tridactylus, the three-toed sloth; Saimiri sciureus, the squirrel monkey; and Cebus apella, a capuchin monkey. Again, the number of eggs matured by a female L. longipalpis was proportional to the weight of blood imbibed (Fig 14 and Appendices 9-15). The significance of the differences between the regression

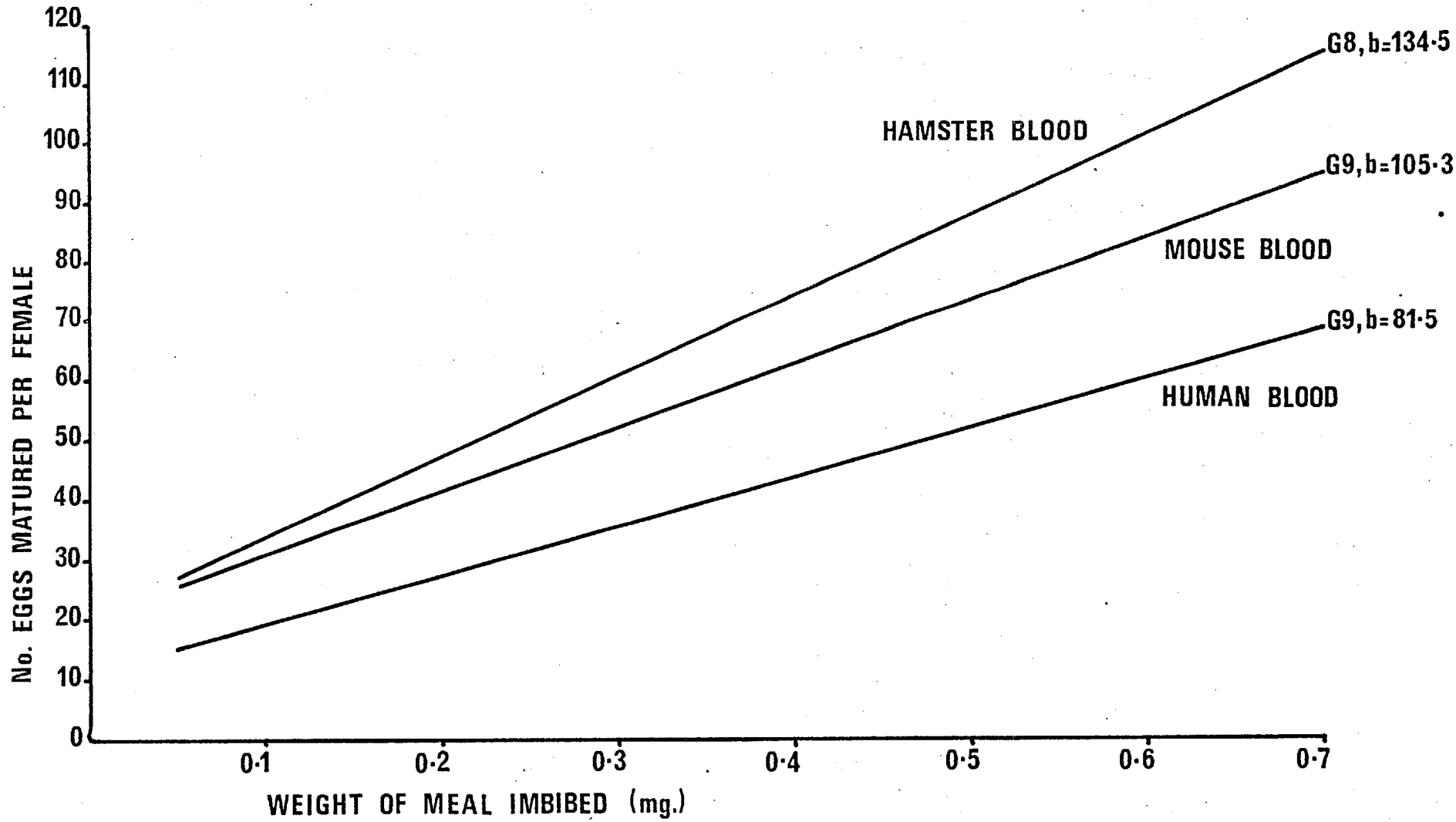


Fig. 12. The number of eggs matured by L. longipalpis females which had ingested measured weights of three different bloods.

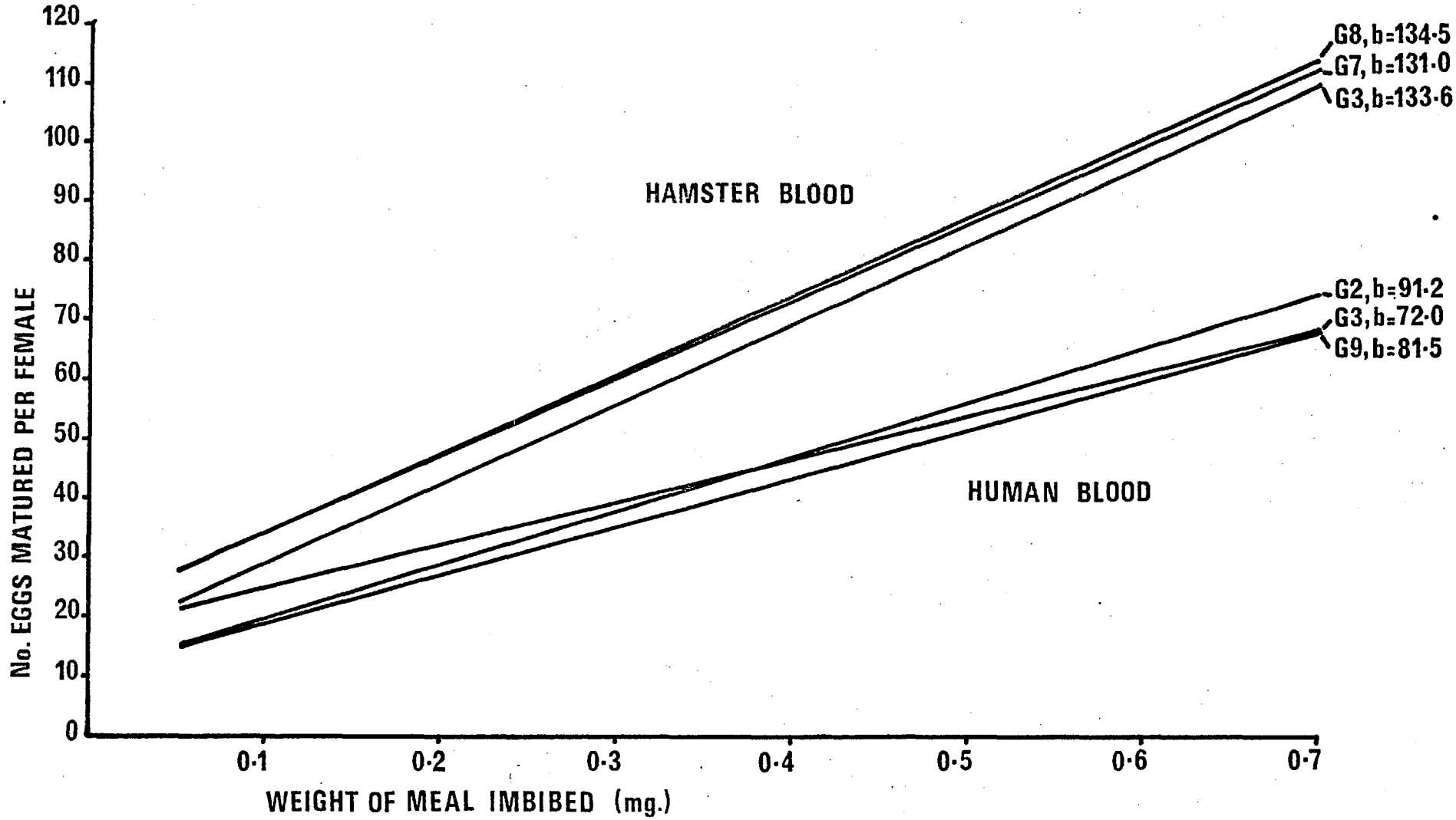


Fig. 13. For L. longipalpis, the variation between laboratory generations (G) for the number of eggs matured on given weights of blood.

COMPARISON OF						STUDENT'S 't'	PROBABILITY OF DIFFERENCE THROUGH 'CHANCE'	DEGREES OF FREEDOM
REGRESSION COEFFICIENT	BLOOD MEAL	STRAIN OF L.longipalpis	WITH REGRESSION COEFFICIENT	BLOOD MEAL	STRAIN OF L.longipalpis			
91.2	HUMAN (MAN)	B.H.	72.0	HUMAN (MAN)	B.H.	1.21	0.30	30
91.2	HUMAN (MAN)	B.H.	64.3	HUMAN (WOMAN)	C.	1.66	0.20	27
133.6	HAMSTER	B.H.	91.2	HUMAN (MAN)	B.H.	1.89	0.10	30
133.6	HAMSTER	B.H.	81.5	HUMAN (MAN)	B.H.	2.25 *	0.05	32
133.6	HAMSTER	B.H.	72.0	HUMAN (MAN)	B.H.	2.60 *	0.02	32

Table 13. Comparison of regression coefficients for number of eggs matured by L.longipalpis on weight of different bloods imbibed. *Comparisons which are significantly different.

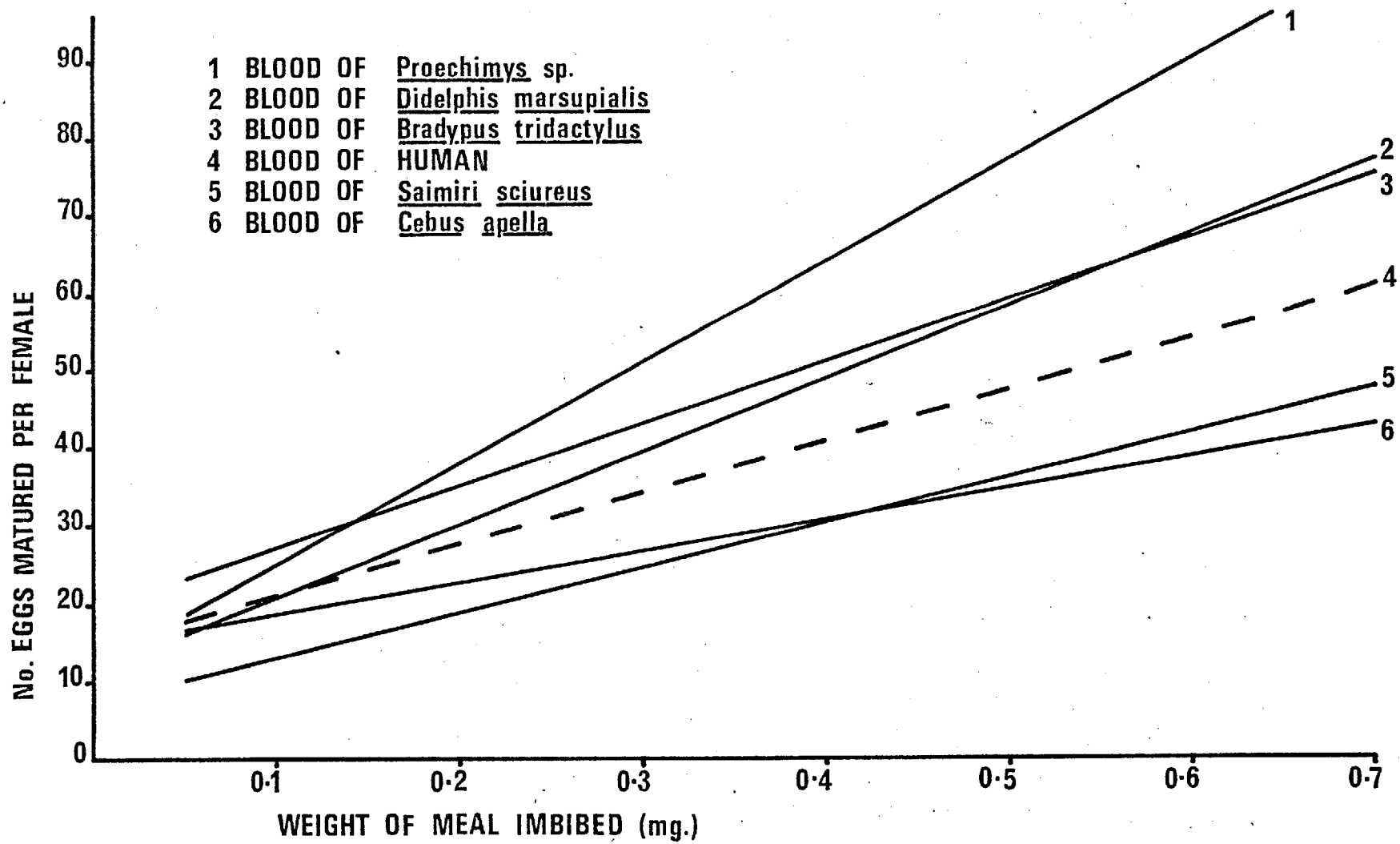


Fig. 14. Regression lines for number of eggs matured by L. longipalpis (Ceará strain) on weights of different bloods imbibed.

coefficients for the number of eggs matured on meal weights are recorded in Table 14. The average number of eggs matured on 0·45 - 0·65 mg of each blood was (with ranges): Spiny rat, 78 (80 - 76); three-toed sloth, 66 (69 - 64); opossum, 56 (76 - 40); man 47 (52 - 31); capuchin monkey, 38 (44 - 27); and squirrel monkey, 37 (42 - 32).

The number of eggs matured on the blood of man was not significantly different for the two strains (BH & C) of L. longipalpis (Fig 15; Table 13). Similarly, there was no significant difference between the number of eggs matured on the blood of man and woman (Fig 15; Appendices 9 & 10).

The number of eggs matured by wild-caught Psychodopygus davisi when fed on known weights of human blood are shown in Fig 16. This species is considerably smaller than L. longipalpis (Appendix 18) and took smaller meals. The regression coefficients for eggs matured on $<0·3$ mg of human blood imbibed were not significant for either species (Appendices 16 & 17), but the mean number of eggs matured by Ps. davisi (38·6) was significantly higher than that for L. longipalpis (28·1) (Appendix 18).

The number of eggs matured on two blood-meals.

15 female L. longipalpis engorged with different amounts of human blood were maintained in dry oviposition tubes so that they did not lay the eggs which they matured. (This would have led to their 'premature' deaths). Six days after the first meal, 5 of the 13 survivors took a second blood-meal from man (Table 15). At the end of a

COMPARISON OF				STUDENT'S 't'	PROBABILITY OF DIFFERENCE THROUGH 'CHANCE'	DEGREES OF FREEDOM
REGRESSION COEFFICIENT	BLOOD MEAL	WITH REGRESSION COEFFICIENT	BLOOD MEAL			
131.6	SPINY RAT	94.5	OPOSSUM	1.25	0.3	30
131.6	SPINY RAT	79.7	3-TOED SLOTH	2.00	0.10-0.05	20
131.6	SPINY RAT	67.0	MAN	2.66 *	0.02	18
94.5	OPOSSUM	41.4	CAPUCHIN MONKEY	3.12 *	0.01	26
94.5	OPOSSUM	57.2	SQUIRREL MONKEY	2.03	0.10-0.05	20
79.7	3-TOED SLOTH	57.2	SQUIRREL MONKEY	1.28	0.3	12
79.7	3-TOED SLOTH	41.4	CAPUCHIN MONKEY	2.38 *	0.05	12
70.0	MAN	41.4	CAPUCHIN MONKEY	1.99	0.10-0.05	36

Table 14. Comparison of regression coefficients for number of eggs matured by L.longipalpis (Ceará strain) on weight of different bloods imbibed. *Significant differences.

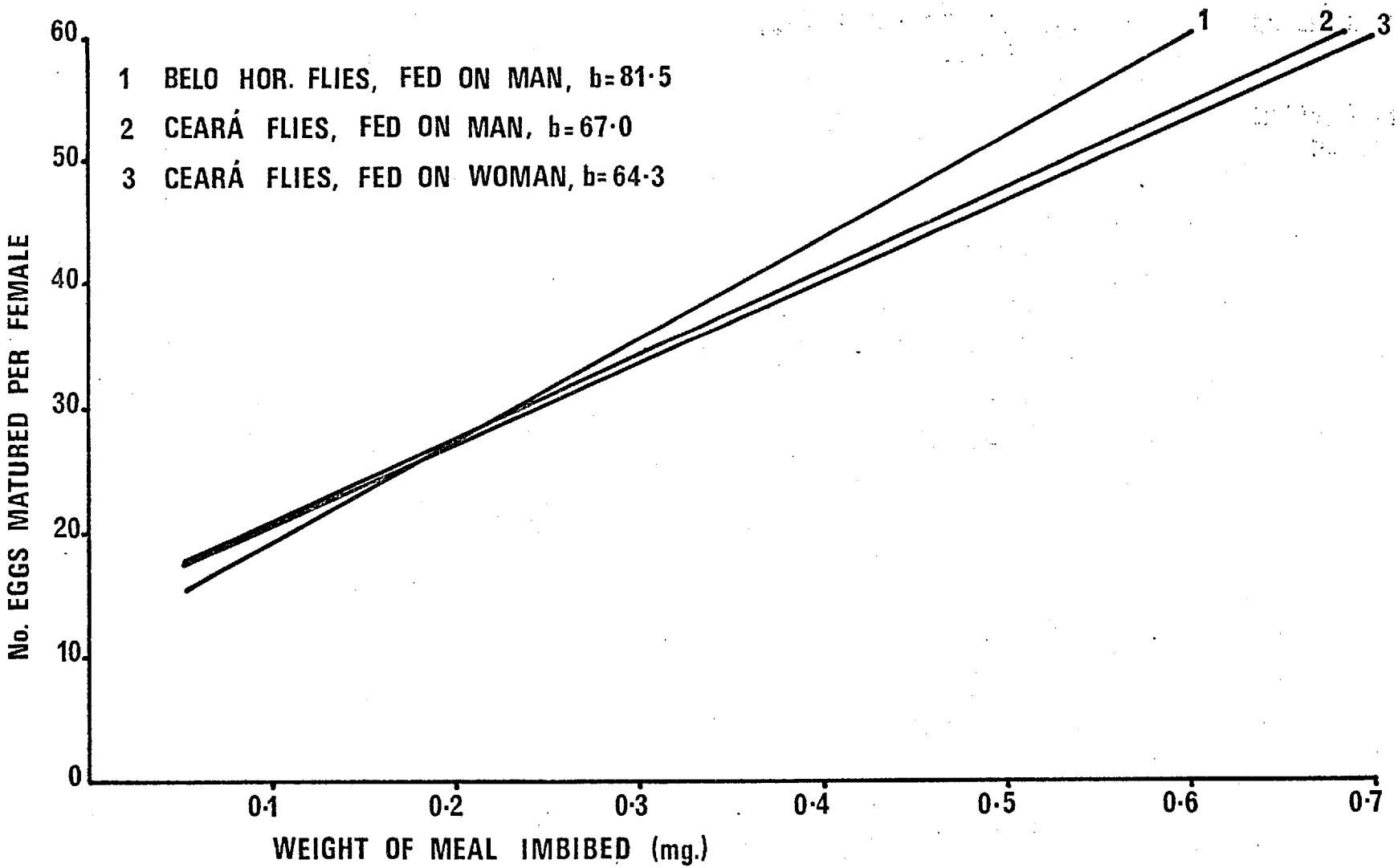


Fig. 15. The number of eggs matured by L. longipalpis females which had imbibed measured weights of human blood.

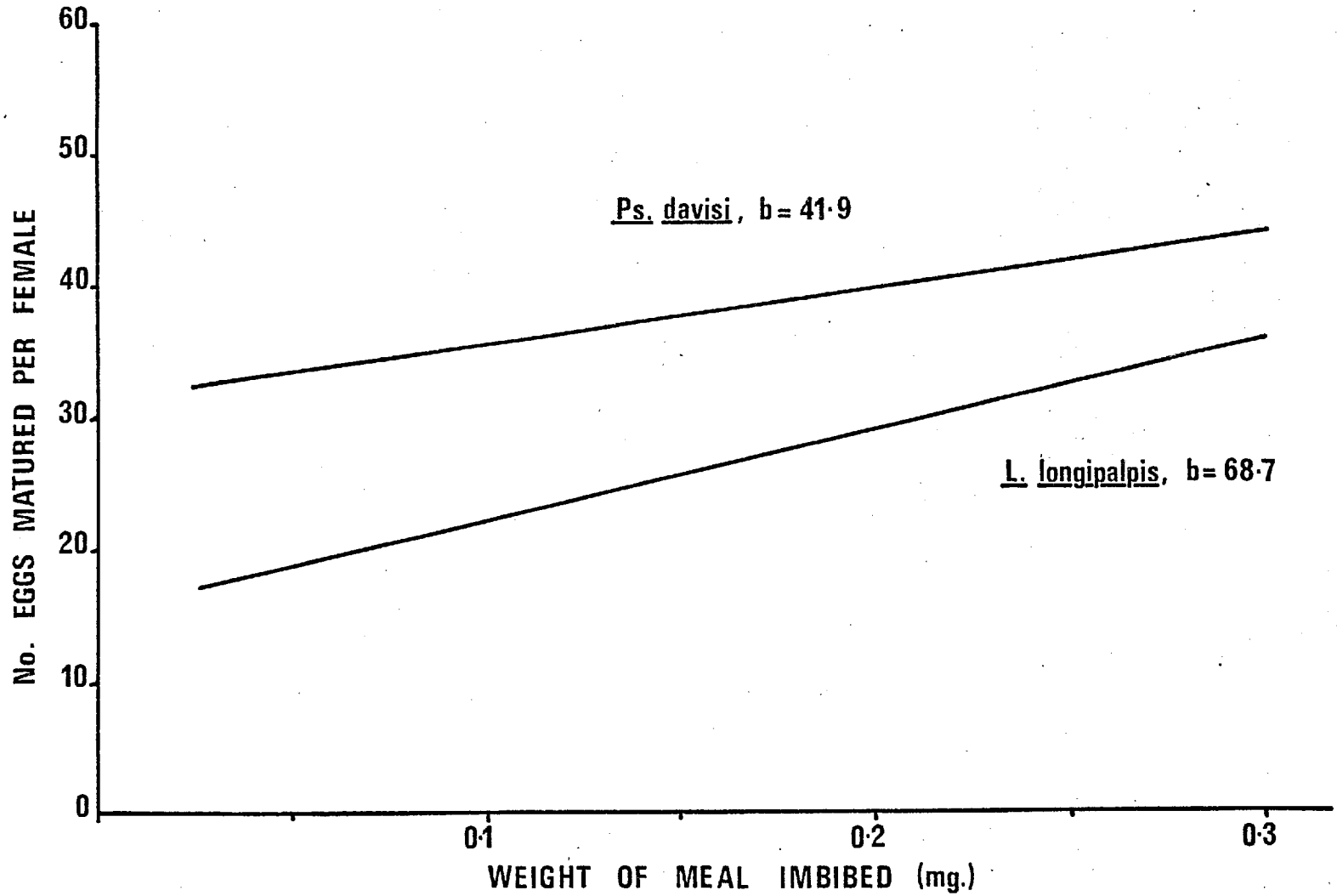


Fig. 16. The number of eggs matured by L.longipalpis and Ps.davisi on measured weights of human blood.

Table 15.

Eggs matured after one or two meals of blood from man.

5	11	11	15 DAYS AFTER EMERGENCE
Weight of 1st. meal (mg.)	Weight of 2nd. meal (mg.)	No. of eggs matured	No. of eggs matured
0.649	Refused	58	-
0.701	Refused	62	-
0.306	Refused	30	-
0.565	Refused	43	-
0.716	Refused	58	-
0.235	Refused	41	-
0.347	Refused	41	-
0.482	Refused	45	-
0.719	0.187	-	67
0.505	0.181	-	49
0.272	0.253	-	39
0.183	0.295	-	23
0.217	0.280	-	27

further four days all 5 had digested their second meal and defaecated. They were then dissected and the number of eggs matured by each counted. The latter was proportional to the weight of the first meal, the relationship being the same as that for the flies that had taken only one meal (Fig17).

Meals taken by L. longipalpis through membranes.

The number of eggs matured on each type of meal was tested at least twice, but no significant differences between results were detected. The results below refer to pooled data. Where even spreads of meal weights were not achieved, comparisions between treatments were made for means and not regression coefficients.

For practical reasons the flies were fed in small groups of 2 - 4. Therefore some flies were not weighed until as long as 15 minutes after they had fed. Fig. 18 shows the changing relationship between the number of eggs matured and the weight of human blood imbibed when the latter was calculated for flies weighed 1, 20 and 60 minutes after feed. The linear regression coefficient for each is highly significant (Appendix 19). The difference between the regression coefficients for the 1 and 20 minute relationships are not significant ($p = 0{\cdot}2$; $t = 1{\cdot}35$).

Table16 sets out the number of eggs matured when 0·85% saline was imbibed from three different membranes. 24 hours after the meal the abdomens of many of the flies that had fed through the defatted chick membrane were still noticeably swollen, whereas the abdomens of those that had fed through

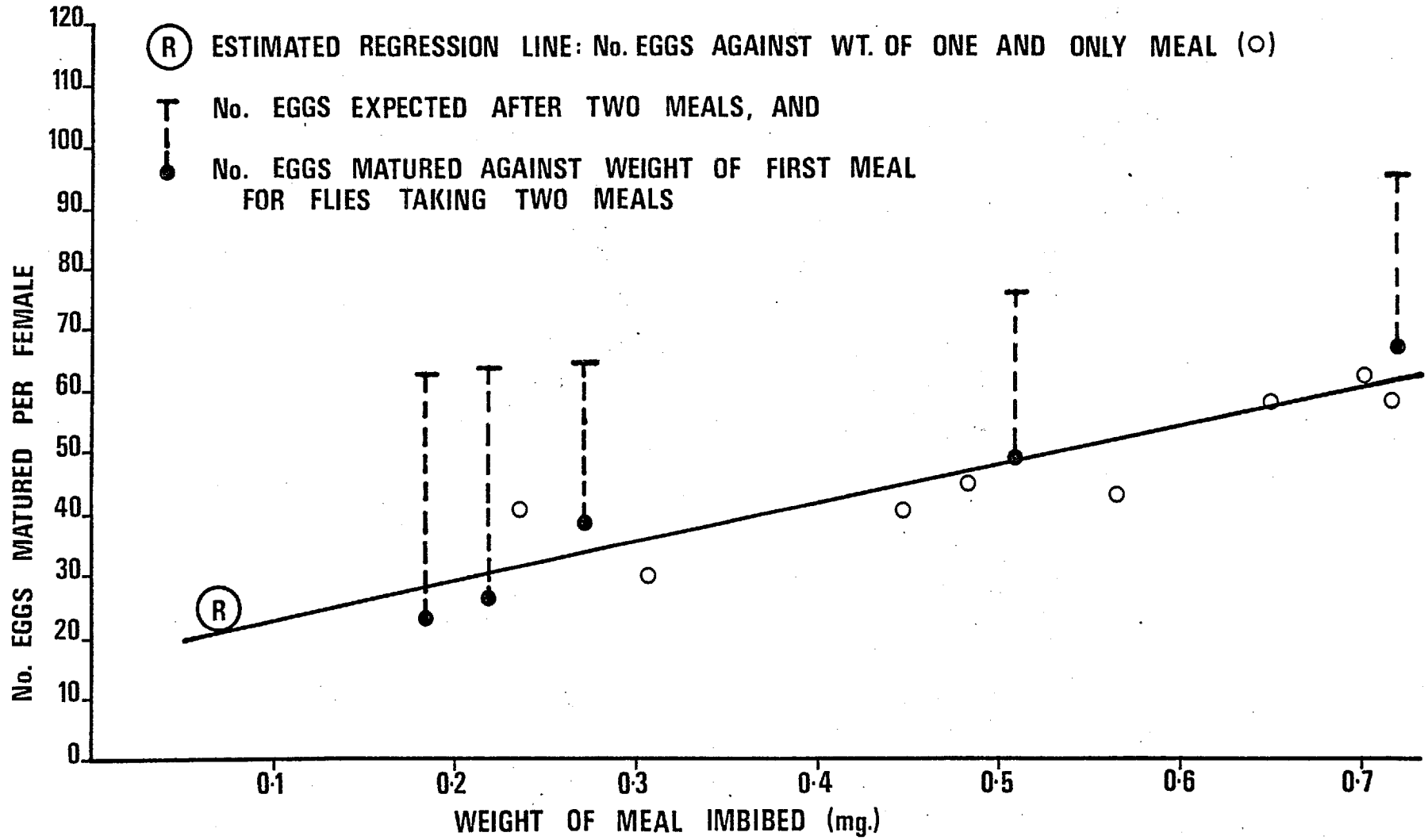


Fig.17. For L.longipalpis, the number of eggs matured by females that had imbibed one or two meals of human blood. (Cf. Table 15.)

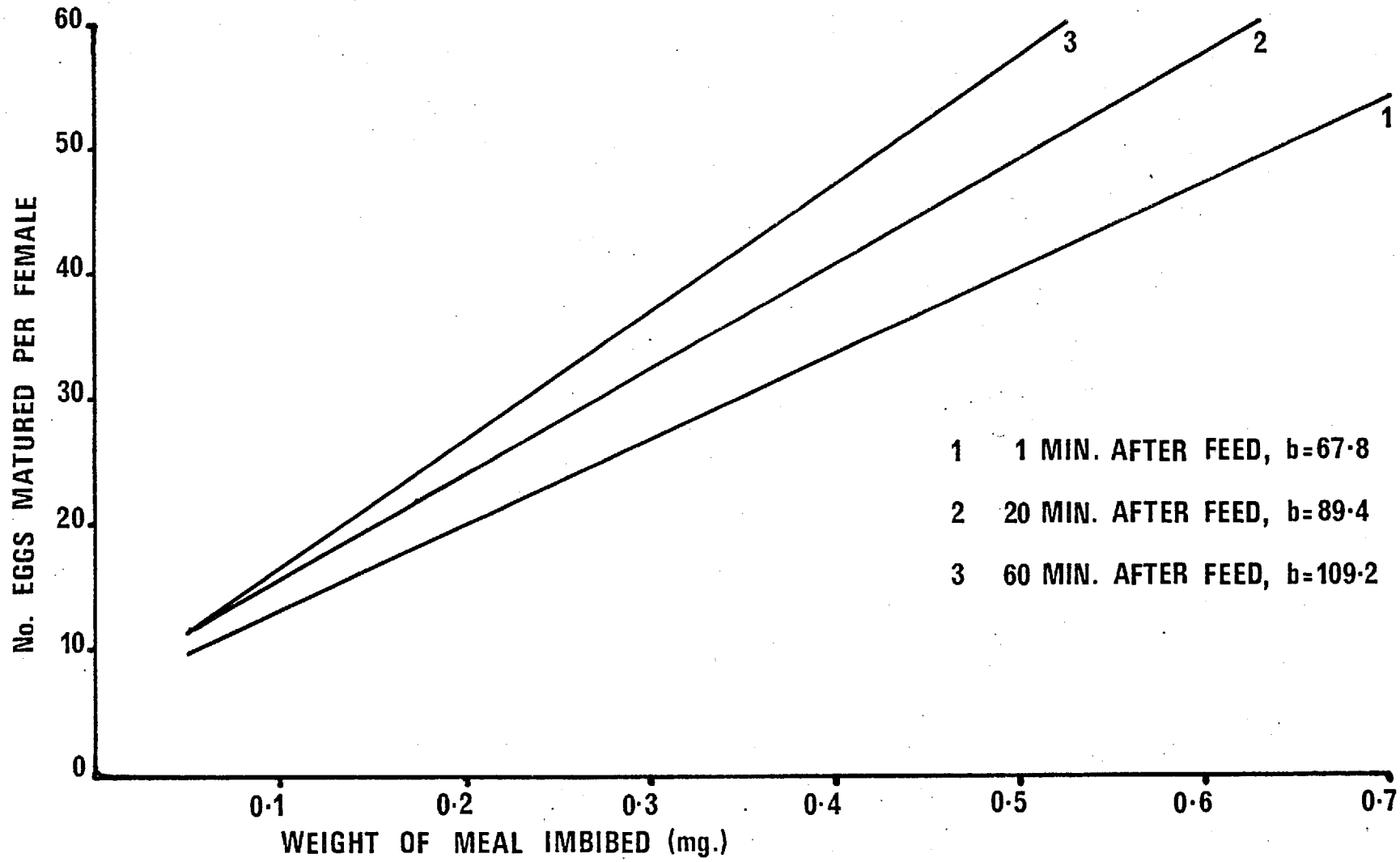


Fig. 18. The changing relationship between the number of eggs matured and the weight of human blood imbibed when the latter was measured at different times after the meal.

Table 16.

L. longipalpis; human line; generation 23.

Eggs matured when 0.85% saline imbibed through:

DEFATTED CHICK SKIN		DEFATTED AND LEACHED CHICK SKIN		PARAFILM 'M'	
Wt. meal	No. eggs	Wt. meal	No. eggs	Wt. meal	No. eggs
0.445	16	0.481	4	0.427	0
0.533	14	0.415	1	0.406	0
0.493	14	0.401	7	0.277	0
0.422	9	0.386	0	0.176	0
0.398	13	0.374	0	0.157	0
0.388	9	0.329	0	0.147	0
0.317	8	0.323	0	0.126	0
0.371	1	0.309	0	0.118	0
0.126	4	0.306	0	0.090	0
0.084	0	0.292	0		
		0.249	0		
		0.244	0		
		0.178	0		
n=10		n=13		n=9	

parafilm had returned to their pre-feed size. On the basis of these results all chick membranes were leached before being used for the following experiments.

The effect on egg production of blood taken through a membrane.

For a given weight of blood, there was no significant difference in egg production between flies fed through a membrane and those fed directly on the host (Fig 19); all regression coefficients were significant (Appendices 20 & 21). The slight difference in regression coefficients between membrane feeds and "natural" feeds was expected but not significant (see above).

The number of eggs matured when human blood was taken through parafilm was not significantly different from that obtained by feeding through a leached chick membrane (Fig 20 ; Appendix 22).

The effect on egg production of different blood fractions. The associations between the number of eggs matured on measured weights of different fractions of human blood are shown in Fig.21 and Appendices 23 &24 . Similarly for hamster blood, the associations are detailed in Fig. 22 and Appendices 25 -27 . The regression coefficient for eggs matured on the weight of 50% solution of hamster plasma imbibed has been calculated by dividing that for undiluted hamster plasma (Fig.23). The latter was obtained for eggs matured by females fed through hamster belly skin (Appendix 25), and although the regression

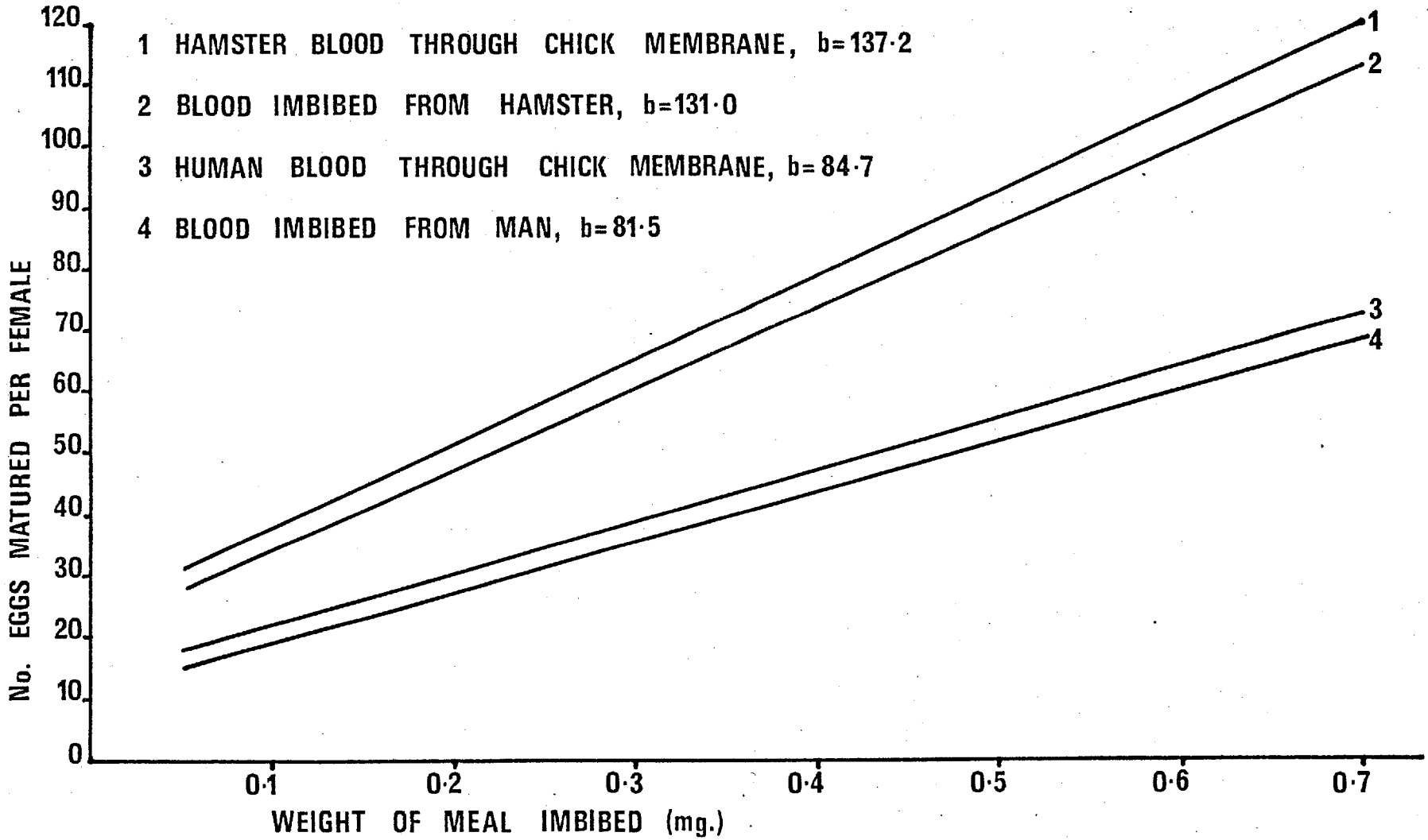


Fig. 19. For L. longipalpis, the number of eggs matured on given weights of bloods when these were imbibed in different manners.

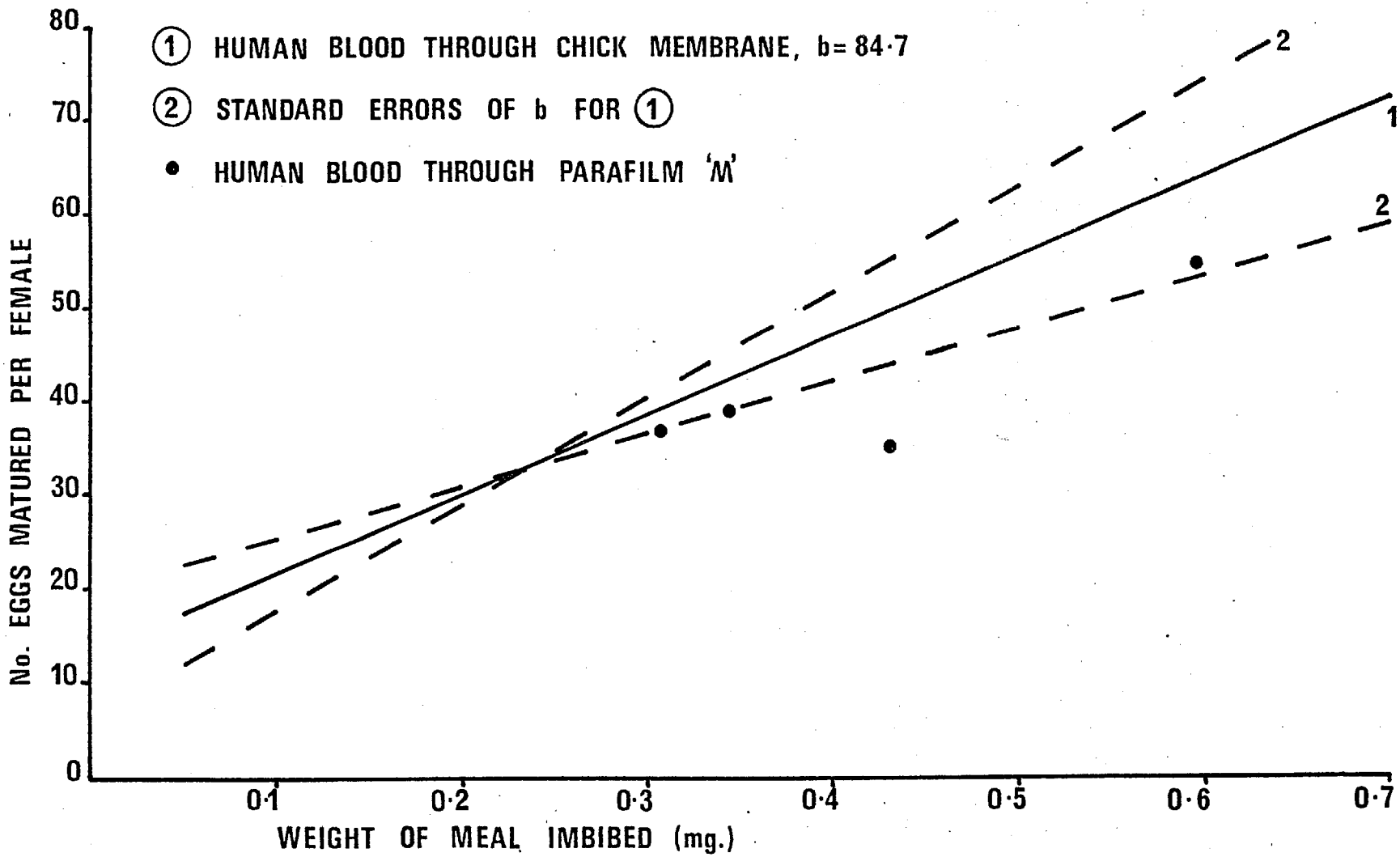


Fig. 20. Eggs matured by L. longipalpis females which imbibed human blood through two different membranes.

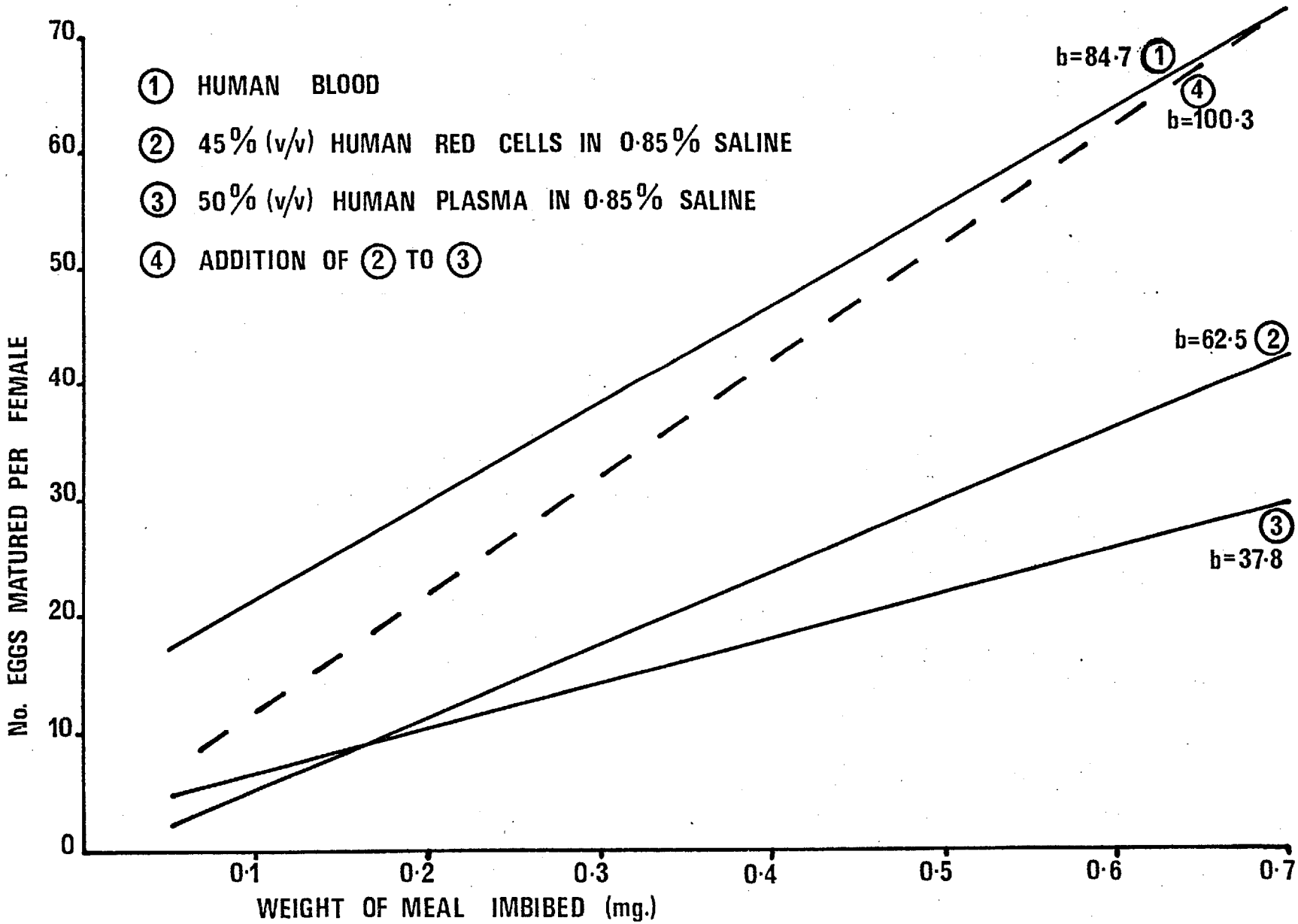


Fig. 21. The number of eggs matured by L. longipalpis females which had imbibed different fractions of human blood through leached chick skins.

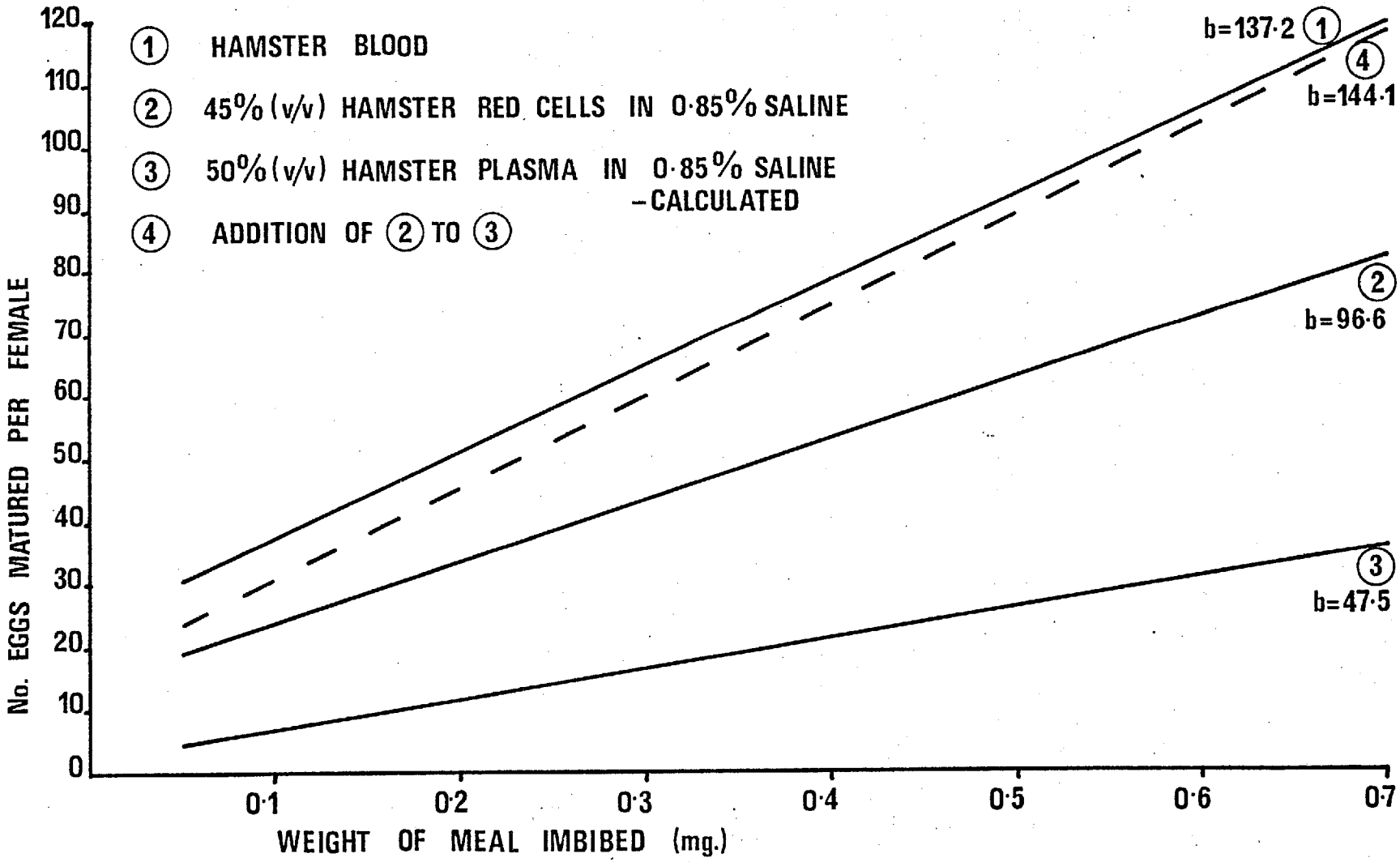


Fig. 22. The number of eggs matured by L. longipalpis females which had imbibed different fractions of hamster blood through leached chick skins.

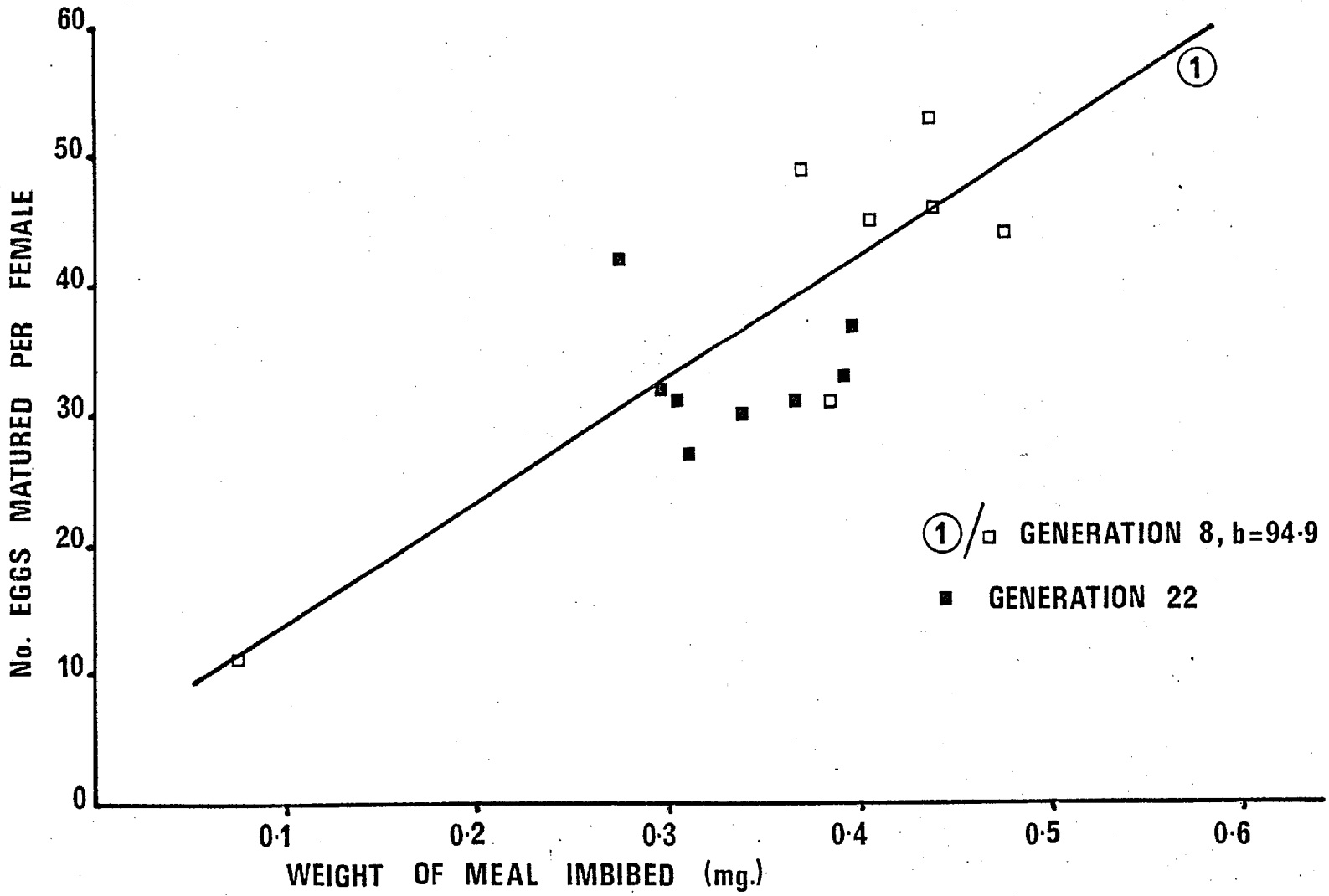


Fig. 23. The number of eggs matured by L.longipalpis females which had imbibed measured weights of hamster plasma.

coefficient is not significant (because of the small spread of blood-meal sizes) it is likely to be valid as it is supported by the results of feeding undiluted hamster plasma through a leached chick membrane (Appendix 26).

The results show that both the red cell and the plasma fractions of a blood-meal can initiate egg maturation and that the sum of the number of eggs matured on an equal weight of both (each diluted to blood concentration) more-or-less equals that obtained on the same weight of whole blood (Fig. 21& 22).

The effects on egg production of the different fractions in hamster and human blood are compared in Fig. 24. The regression coefficients are highly significant except for hamster plasma (Appendices 23 - 25, 27), but are not significantly different from each other. However, comparisons (by the Student's 't' test) of the mean number of eggs produced by the different fractions over smaller ranges of meal weight show that there are highly significant differences between :

human blood (mean of 33·6 eggs) and a 50% suspension of human plasma (mean of 13·5 eggs) over the range 0·25 - 0·35 mg (Appendix 28);

human blood (mean of 33·6 eggs) and a 45% suspension of human red cells (mean of 18·0 eggs) over the range 0·25 - 0·35 mg (Appendix 29); and a 45% suspension of hamster red cells (mean of 56·5 eggs) and a 45% suspension of human red cells (mean of 25·4 eggs) over the range 0·33 - 0·52 mg (Appendix 30).

Fig. 24. A comparison of the effects of different fractions in hamster and human bloods on the egg production of L.longipalpis.

LEGEND:-

① Hamster Blood

② 45%(v/v) Hamster Red Cells in 0.85% Saline

③ Human Blood

④ 45%(v/v) Human Red Cells in 0.85% Saline

⑤ 50%(v/v) Hamster Plasma in 0.85% Saline - Calculated

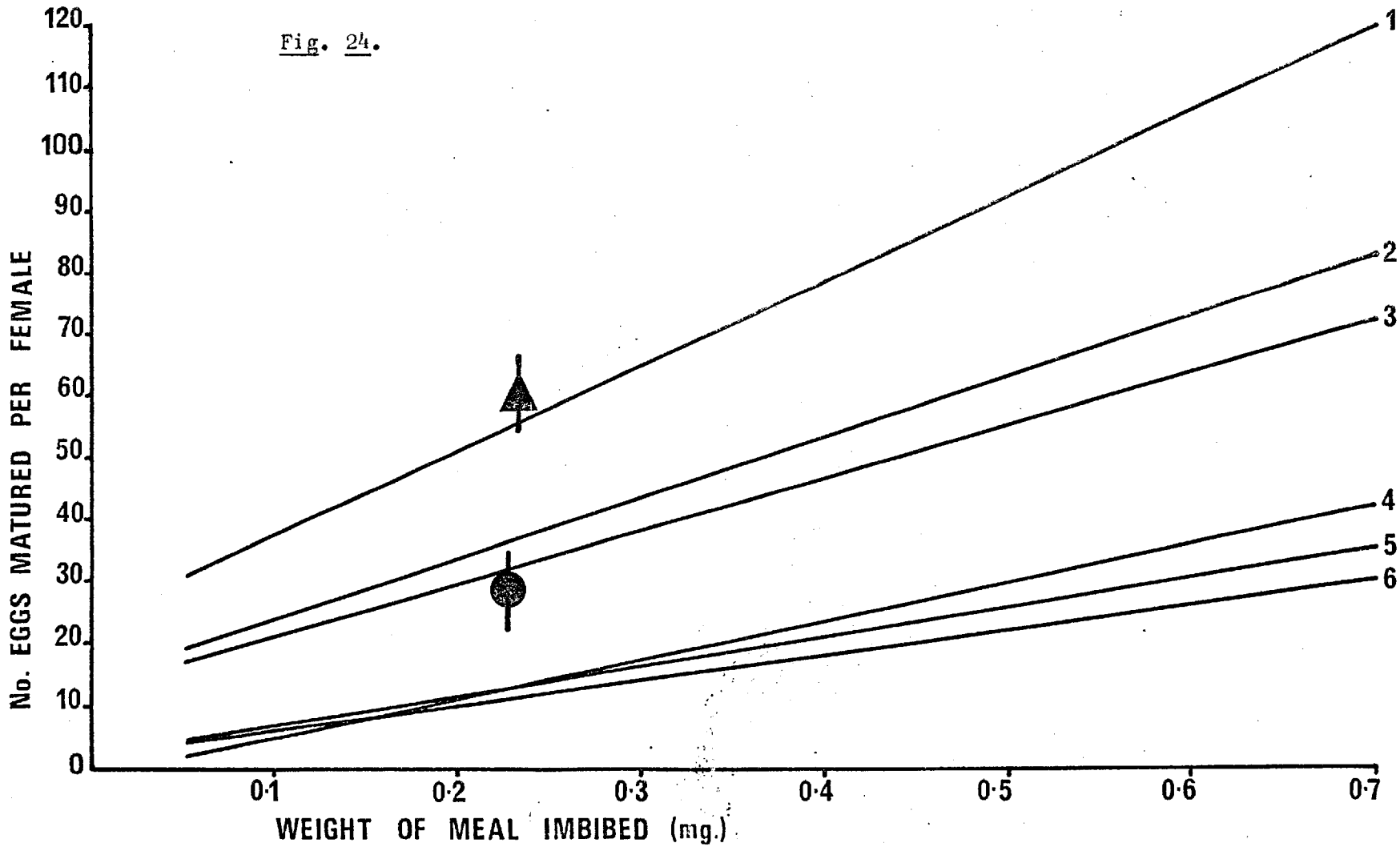
⑥ 50%(v/v) Human Plasma in 0.85% Saline

45%(v/v) Solution of Hamster Erythrocyte Extract

45%(v/v) Solution of Human Erythrocyte Extract

* Vertical lines refer to 95% confidence limits.

Fig. 24.


No. EGGS MATURED PER FEMALE
120
110
100
90
80
70
60
50
40
30
20
10
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
WEIGHT OF MEAL IMBIBED (mg.)
1
2
3
4
5
6

Similarly, there was no significant difference between: a 45% suspension of human red cells (mean of 18·0 eggs) and a 50% suspension of human plasma (mean of 13·5 eggs) over the range 0·25 – 0·35 mg (Appendices 28 & 29); or undiluted hamster plasma (mean of 32·4 eggs) and undiluted human plasma (mean of 33·4 eggs) over the range 0·25 – 0·35 mg (Appendix 31).

From these comparisions, it is clear that the marked difference in egg production following the ingestion of equal weights of hamster and human blood results from differences in the red cell fractions of these bloods.

The number of eggs matured on the water soluble extract of erythrocytes (EE). A given weight of a 45% solution of human EE produced significantly more eggs than a 45% suspension of whole red cells (Appendix 32).

Egg production following a meal of hamster or human EE (Appendices 33 & 34) equalled that on the respective whole blood (Fig. 24). A mean of 60·3 eggs per female was matured following meals of 0·18 – 0·27 mg of hamster EE, but a mean of only 28·4 eggs per female was matured on equal amounts of human EE; the difference was highly significant (Appendix 35).

Eggs matured on 10% (w/v) human haemoglobin in normal saline. Only 1 out of 13 females that had imbibed this solution matured more eggs than one would expect from an equal weight of saline (Table 17).

Addition of L – isoleucine to human blood. The addition of

Table 17.

Eggs matured by L. longipalpis at 25°C on weight of 10%(w/v) human haemoglobin in 0.85% saline imbibed through a leached chick membrane.

Weight of meal (mg.)	No. of eggs
0.321	1
0.317	16
0.312	0
0.261	0
0.241	0
0.195	0
0.169	1
0.142	0
0.142	0
0.122	1
0.114	0
0.095	0
0.020	0

n=13

L - isoleucine to some bloods has been shown to increase the fecundity of mosquitoes feeding on them (Lea et al 1958). Therefore, the effect of this amino acid on L. longipalpis was tested. 6 females that had taken 0·28 - 0·39 mg of 0·6% (w/v) L - isoleucine dissolved in a 75% solution of human blood in physiological saline matured a mean of 34·2 eggs (Appendix 36). Another 6 females that had taken an equal amount of similarly diluted blood that did not contain any added L. - isoleucine matured a mean of 32·8 eggs (Appendix 37). The difference in egg production between the two groups was highly unsignificant ($p = 0·9$; $t = 0·126$).

Eggs matured on solutions of bovine serum albumen (BSA). Eggs were matured on 10% solutions of BSA and essentially fatty-acid free BSA when these were imbibed through leached chick and parafilm membranes; in each case the linear regression coefficient for the number of eggs matured on the weight of solution imbibed was highly significant (Appendices 38 - 40).

There was no significant difference between regression coefficients for the solutions that did and did not contain fatty-acids ($p = 0·6$; $t = 0·57$), or for the meals taken through the different membranes ($p = 0·6$; $t = 0·57$). (Fig. 25).

Eggs matured on protein solutions of different concentrations. The number of eggs matured by flies that had imbibed known weights of an undiluted and a 50% solution of human plasma are shown in Fig. 26. The eggs-matured/meal-weight

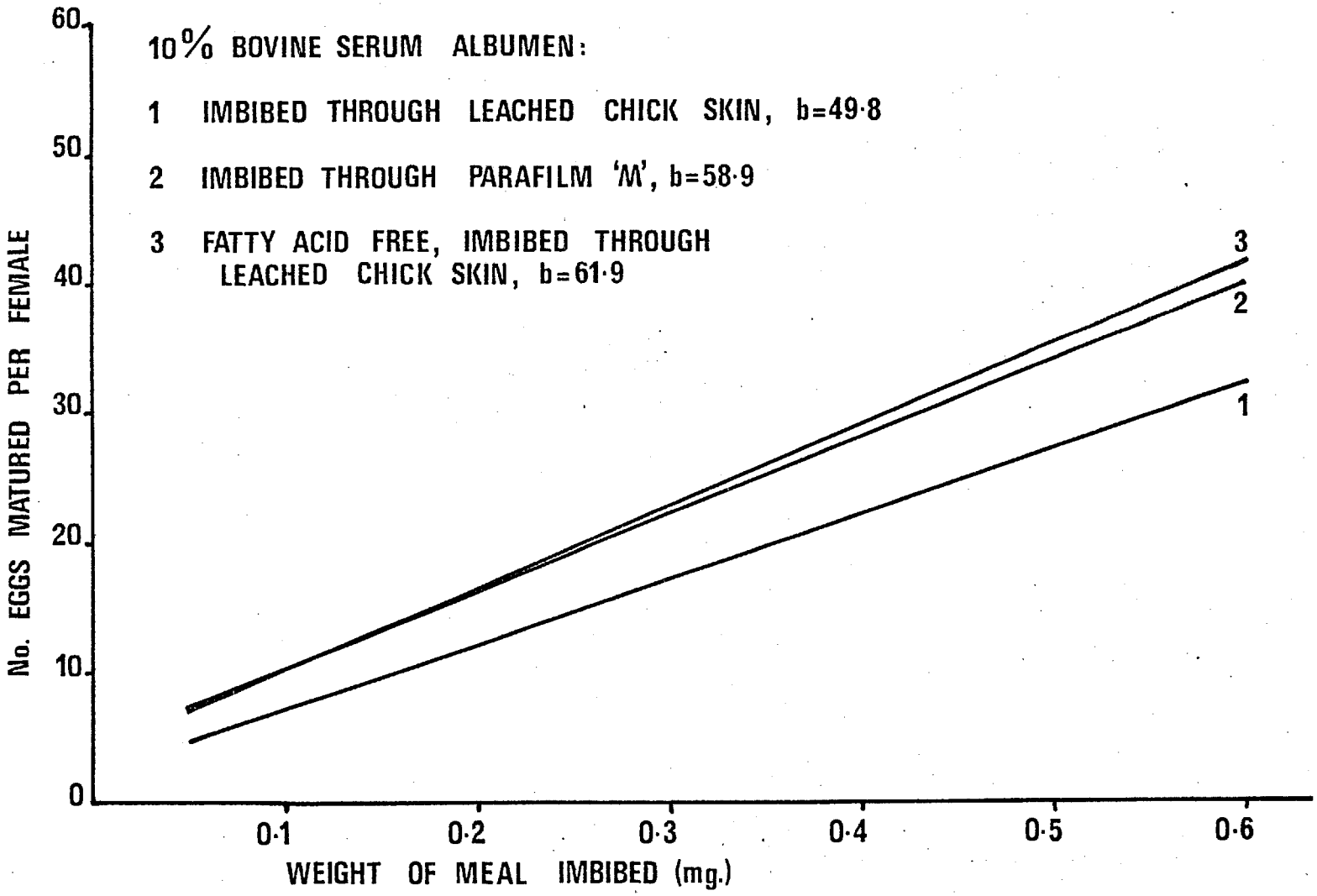


Fig. 25. Eggs matured by L. longipalpis females which had imbibed solutions of Bovine Serum Albumen.

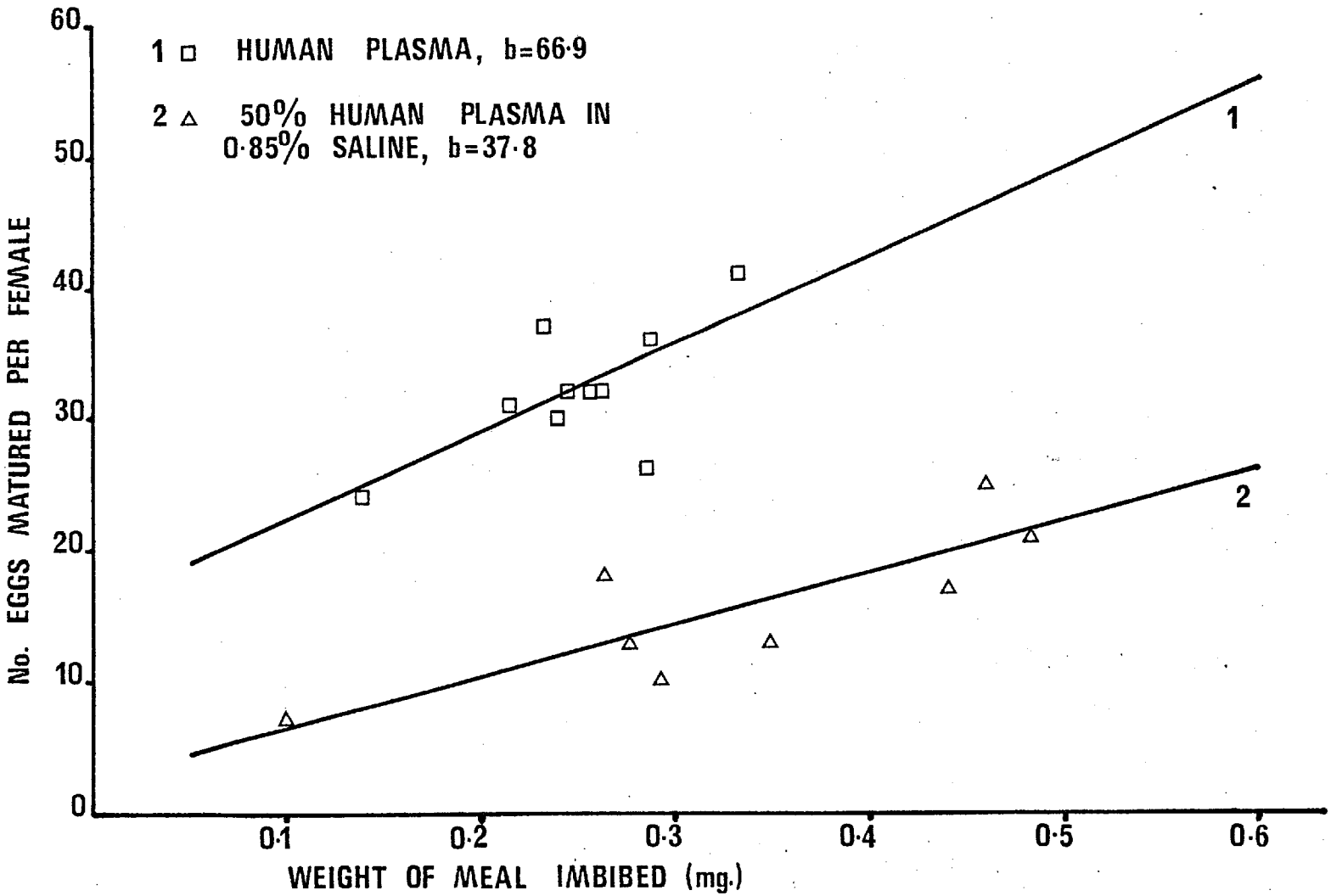


Fig. 26. The number of eggs matured by L. longipalpis females which had imbibed known weights of 50% and undiluted human plasma.

regression coefficients are significant for both meals (Appendices 23 & 41), but are not significantly different from each other ($p = 0{\cdot}2$; $t = 1{\cdot}35$). However, the mean number of eggs matured on 0·25 - 0·35 mg of undiluted plasma was 33·4, whereas it was only 13·5 for the 50% solution of plasma. The difference is significant at the 0·1% level (Appendix 42).

There is a similar difference between the number of eggs matured on 10% and 5% (w/v) solutions of BSA (Appendices 38 , 43 & 44). Again, the number of eggs matured was approximately halved for a 50% dilution (Fig 27).

The number of eggs matured on a 20% (w/v) solution of bovine serum albumen was not proportional to the weight of meal imbibed (Fig. 28 ; Appendix 45).

Eggs matured on denatured plasma protein. Eggs were matured on a suspension of denatured human plasma proteins (Table 18). It was not possible to draw any conclusions on the quantitative effect of this suspension on egg production because it was not homogeneous.

Eggs matured on solutions of amino acids. The number of eggs matured by flies that had imbibed these solutions across leached chick skins and parafilm "M" are shown in Table 19. The composition of these solutions is given in Section 2.

The fertility of eggs matured on artificial meals. Of the eggs matured on the various diets offered only those resulting from meals of human haemoglobin and amino acids

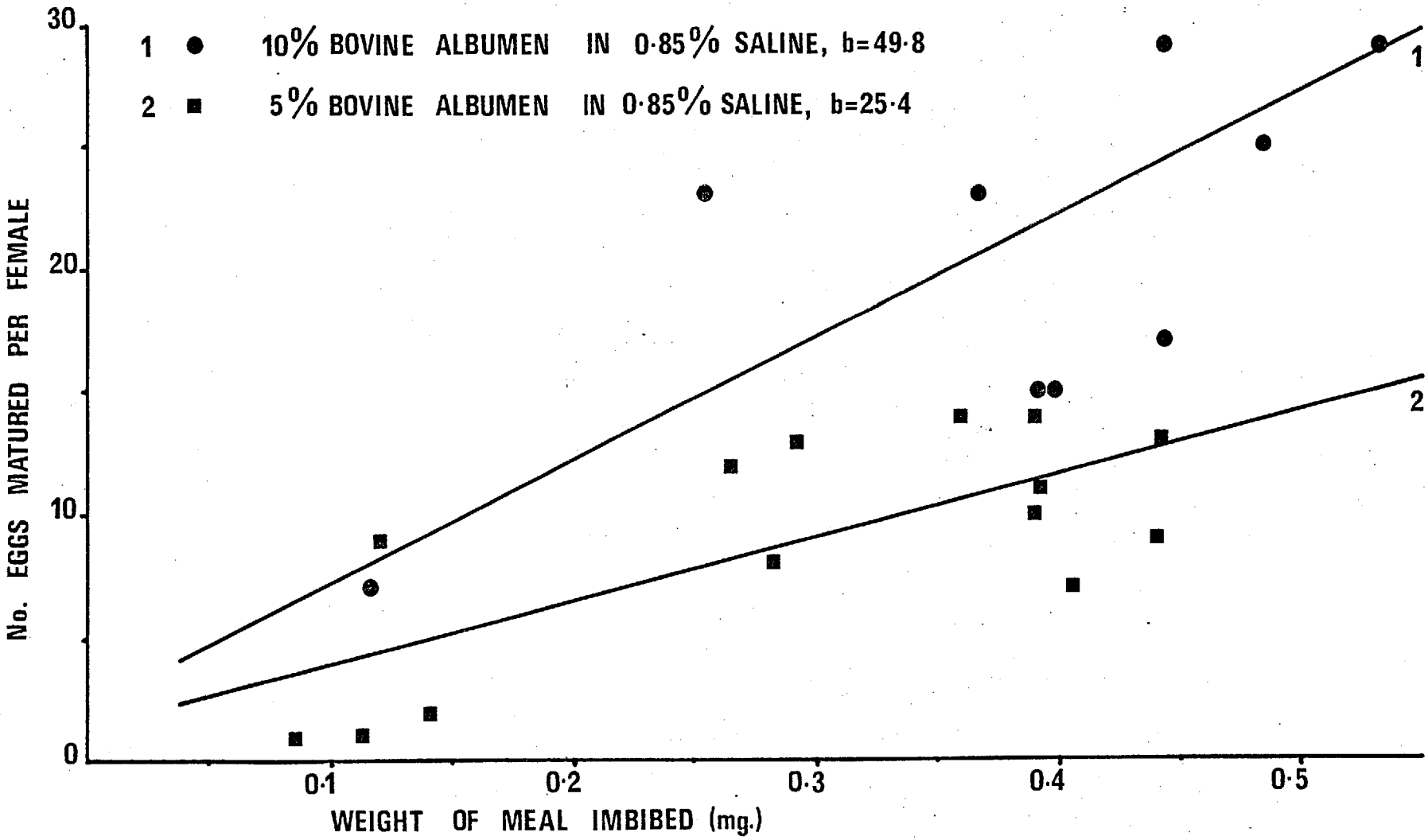


Fig. 27. The number of eggs matured by L.longipalpis females which had imbibed known weights of 5% and 10% Bovine Serum Albumen.

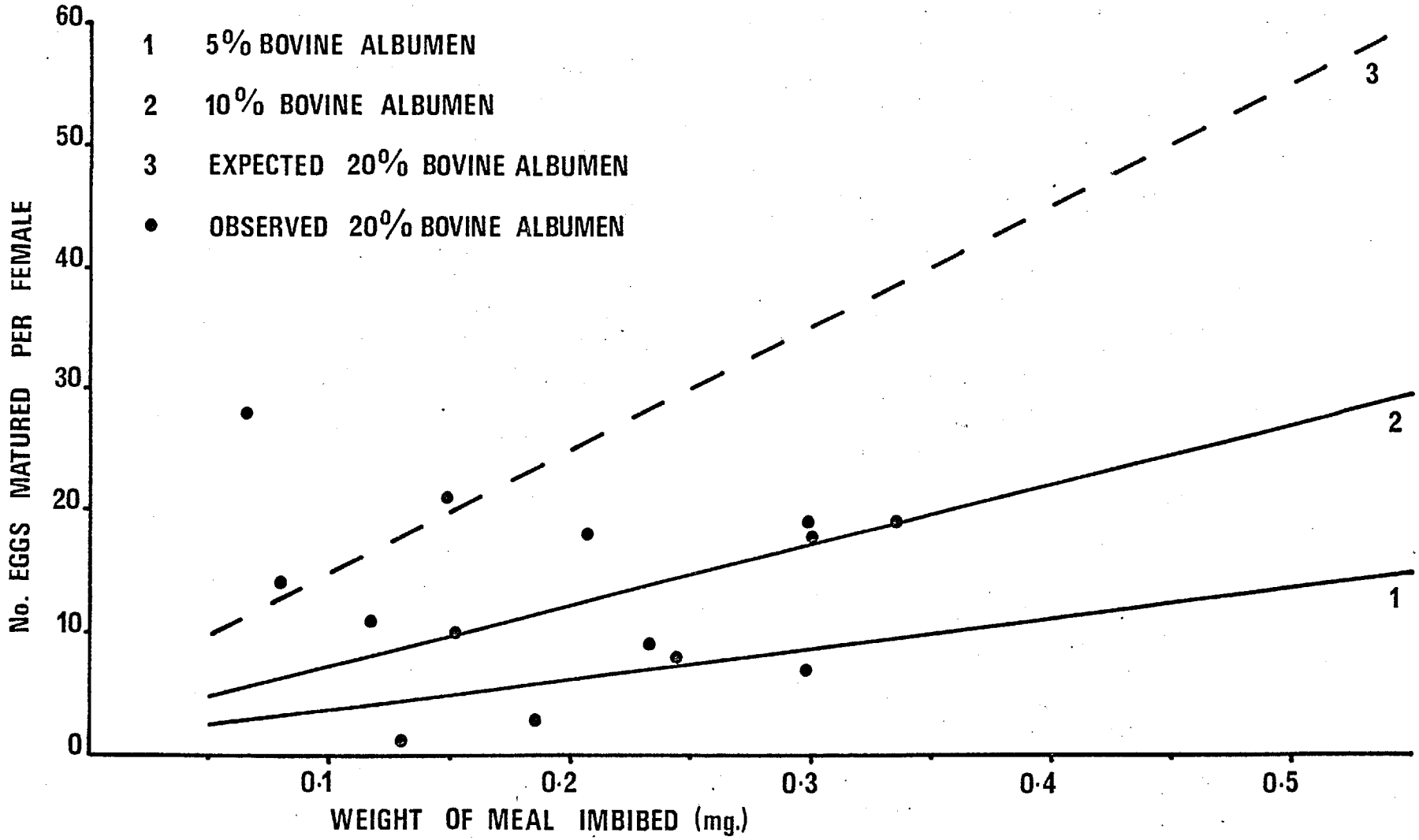


Fig. 28. The number of eggs matured by L.longipalpis females which had imbibed known weights of 5%, 10%, and 20% solutions (in 0.85% saline)of Bovine Serum Albumen.

Table 18.

Eggs matured by L.longipalpis at 25°C on weight of a suspension of denatured plasma proteins in 0.85% saline imbibed through a leached chick membrane.

Weight of meal (mg.)		No. of eggs
0.287		18
0.250		13
0.380		13
0.124	PLASMA	14
0.229	SUSPENSION	10
0.068	PLUS	8
0.165	0.005M	13
0.229	A.T.P.	10
0.330		7
0.295		8
0.246		12
0.123		6
0.212	PLASMA	24
0.317	SUSPENSION	23
0.305	ALONE	14

Table 19.

Eggs matured by L. longipalpis at 25°C on weight of amino acid solution imbibed.

SOLUTION	MEMBRANE	WEIGHT OF MEAL (mg.)	NUMBER OF EGGS
Lea 1	Parafilm 'M'	0.130	10
		0.165	0
Lea 1	Leached chick skin	0.100	0
		0.050	0
		0.075	0
		0.062	0
		0.065	0
		0.044	0
		0.100	11
Lea 2	Leached chick skin	0.046	11
		0.058	10
		0.037	9

were never laid. For egg batches that were laid, observations during routine larval culture indicated that there were no major differences in fertility between treatments.

THE NUMBER OF EGGS MATURED IN RELATION TO FACTORS OTHER THAN BLOOD-MEAL.

The weight of a female at blood-meal.

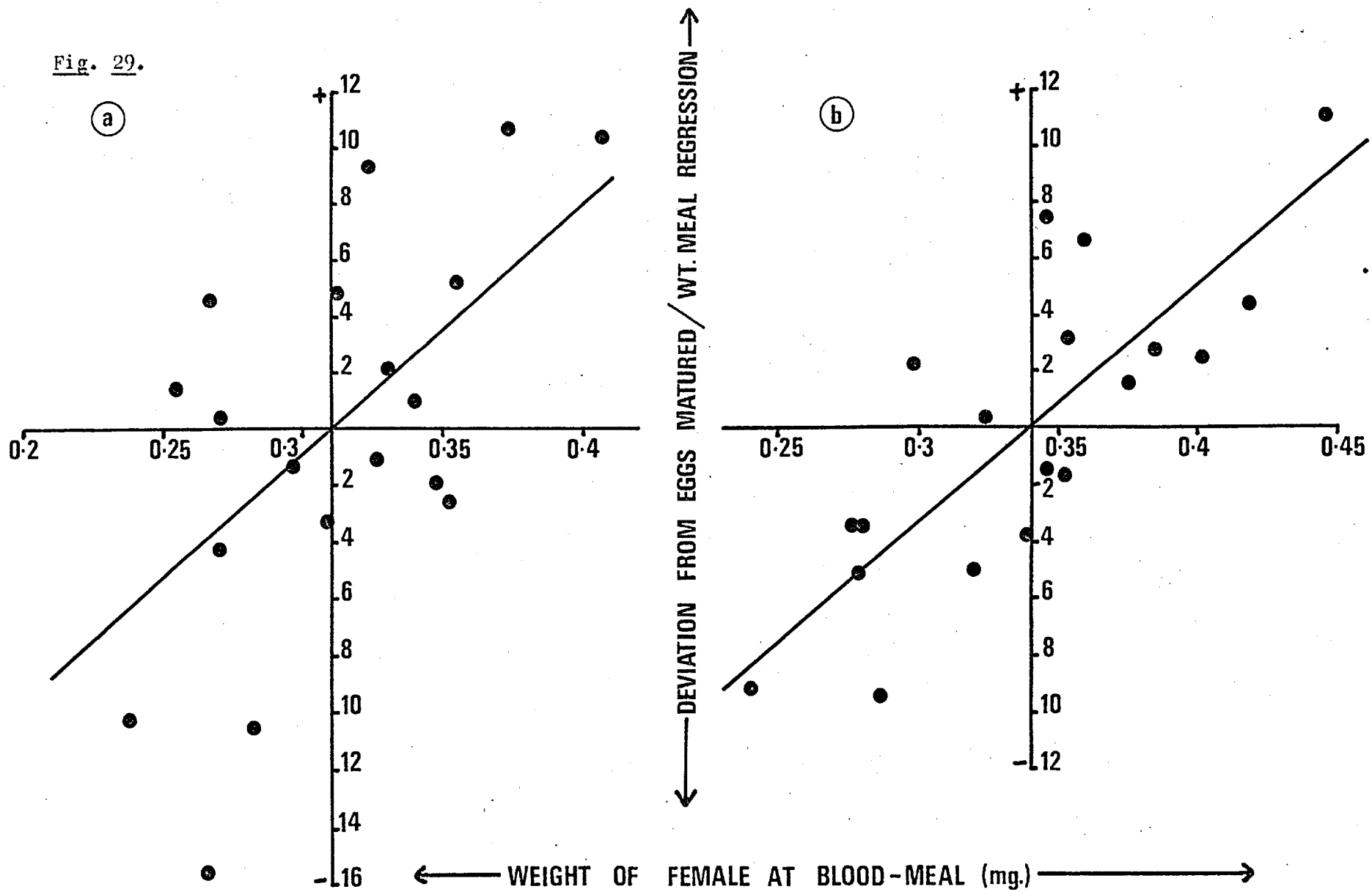
There was found to be a positive association between the weight of a female at the time when she took a blood-meal (4 - 6 days after emergence) and the number of eggs that she matured on human blood. In two experiments the positive or negative deviation of each point from the eggs-matured/meal-weight regression line was calculated (Appendices 46 & 47). When the deviations were plotted against the females' prefeed weights there was a highly significant positive association between the two (Fig. 29). The regression coefficients were similar in each case (b = 87·8 and b = 83·6). For these experiments groups of females with the widest possible weight range were chosen.

Factors affecting the weight of adult flies. Some flies were weighed as 4th instar larvae one day before they pupated, again on their last day as pupae and once more on the day they emerged as adults(Appendix 48). There was a significant, positive association between the weight of a fly as an adult and its weight at the end of the larval period (Fig 30).

Fig. 29.

For L. longipalpis, on two occasions (a and b), the effect of the female's body-weight at the time of the blood-meal on the number of eggs matured. (The effect of blood-meal size has been eliminated.)

Fig. 29.


a
b
+
12
10
8
6
4
2
0·2
0·25
0·3
0·35
0·4
0·45
16
14
DEVIATION FROM EGGS MATURED / WT. MEAL REGRESSION
WEIGHT OF FEMALE AT BLOOD-MEAL (mg.)

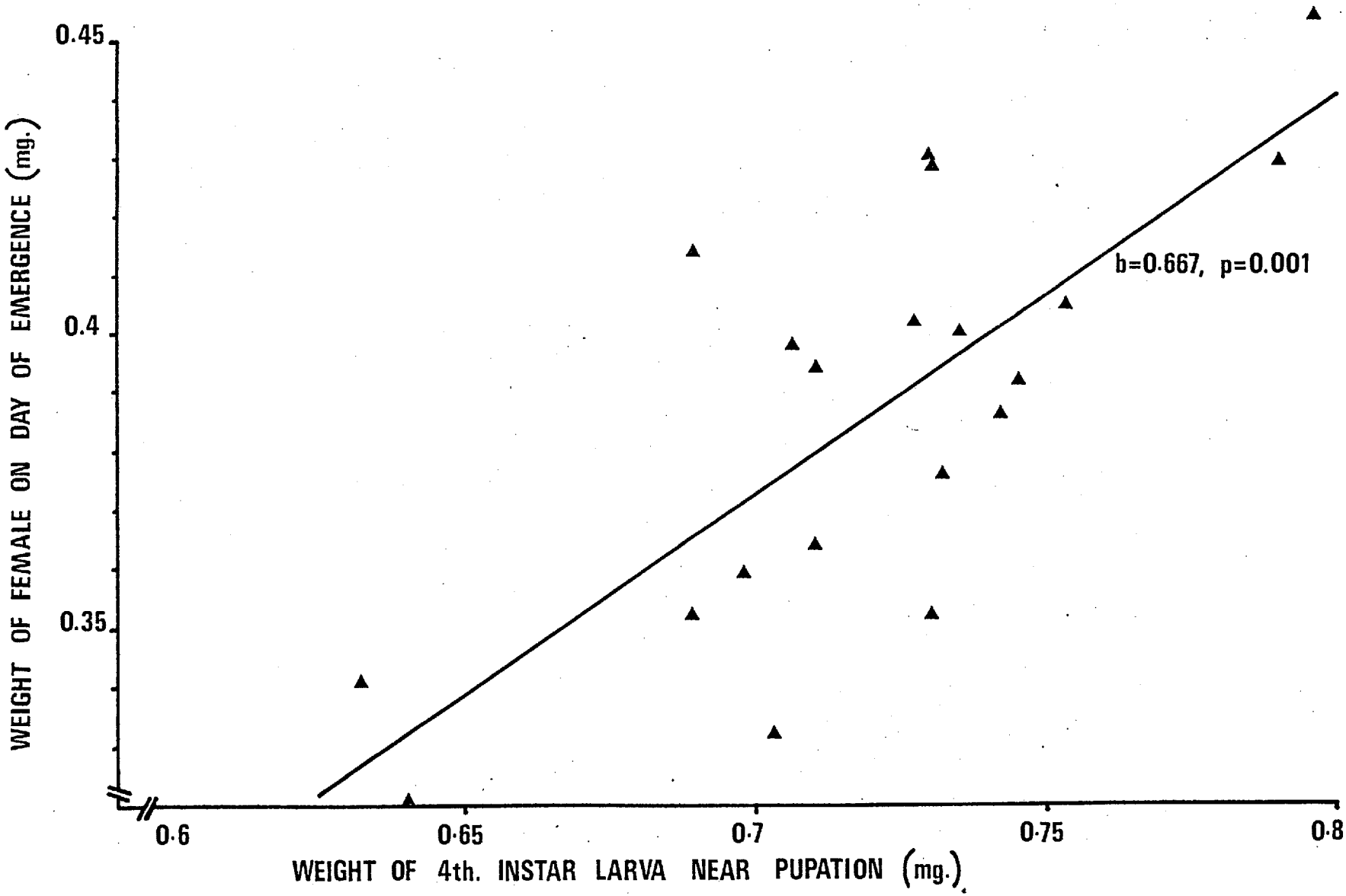


Fig. 30. Relationship between weight of female L. longipalpis as a late larva and as an adult.

60 freshly hatched larvae were taken at random from a number of rearing pots and 30 placed in each of two clean pots, one of which was placed at 25°C and the other at 28°C. Liver powder was provided in excess. Adults from these pots were weighed on the day they emerged (Appendix 49). The mean weight of those reared as larvae at 28°C (0·324 mg) was significantly less than that of those reared at 25°C (0·393 mg).

The effect of sucrose feeding on egg production.

From emergence to blood-meal the mean weights of females that were offered a 30% (w/v) sucrose solution on cotton-wool pads fell by 12·3%, whereas the similar figure for those offered only water was 24·1% (Table 20). The difference in loss of weight is significant at the 1% level.

When the weight of a female fly at blood-meal (4 - 6 days after emergence) was plotted against her weight at emergence there was found to be a significant, positive association between the two, whether sucrose or only water had been offered (Fig. 31 ; Table 20). A closer inspection of the plot for flies that had been offered sucrose (Fig. 31a) showed that the points fell along two distinct regression lines, one of which was very similar to that for sucrose-deprived flies (b = 0·70) while the other approached 45° (b = 0·95).

For both groups of flies - those offered and those deprived of sucrose - there was a highly significant

Table 20.

Changes in weight of female L.longipalpis between emergence and blood-meal.

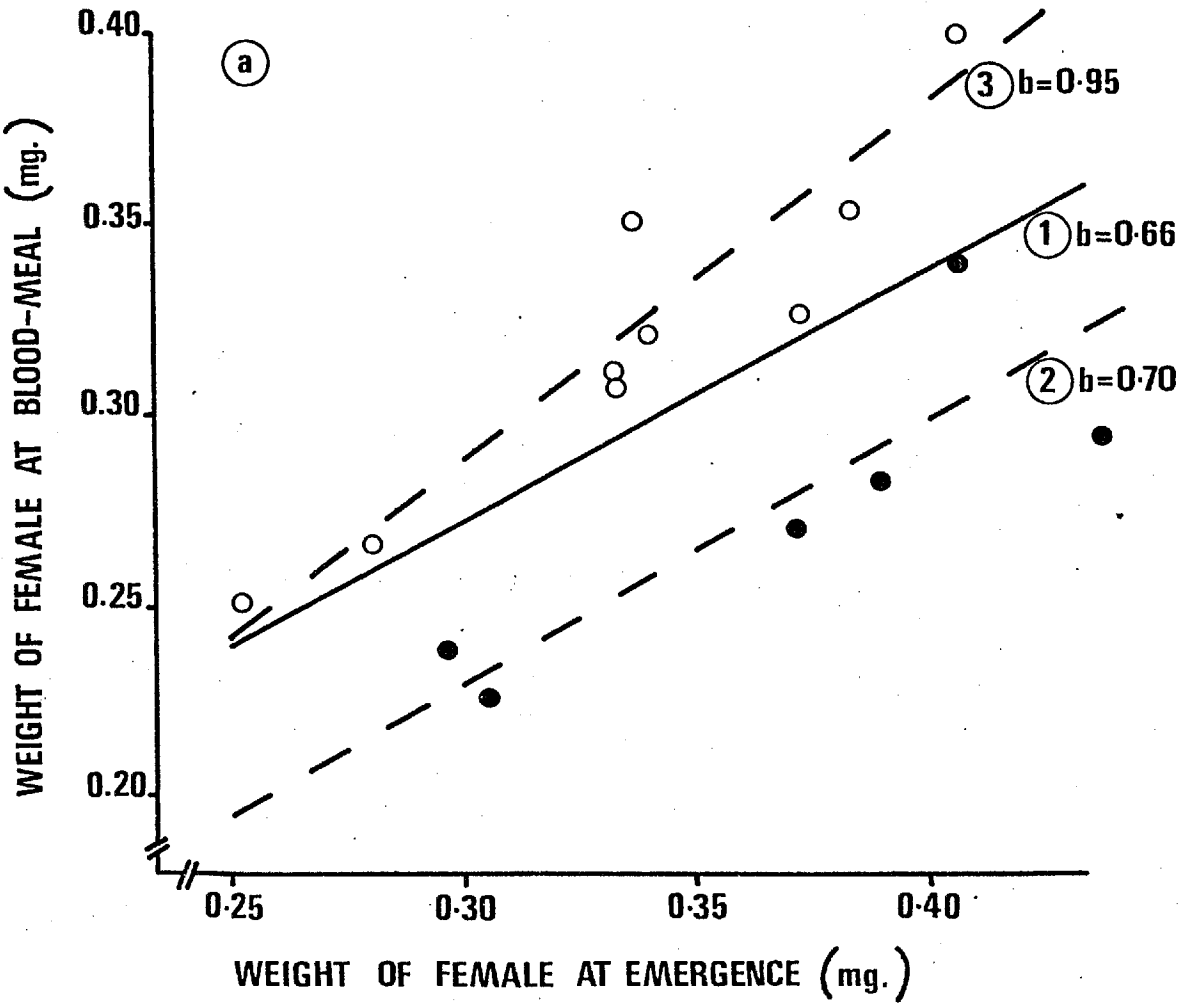
FEMALES OFFERED 30% SUCROSE			FEMALES OFFERED WATER ONLY		
a Weight at emergence	b Weight at b.-meal	c % change in weight	d Weight at emergence	e Weight at b.-meal	f % change in weight
0.382	0.354	-7.33	0.344	0.236	-31.40
0.297	0.238	-19.87	0.359	0.268	-25.35
0.406	0.406	0.00	0.319	0.250	-21.63
0.332	0.313	-5.72	0.396	0.294	-25.76
0.279	0.266	-4.66	0.382	0.290	-24.08
0.336	0.352	+4.76	0.294	0.241	-18.03
0.338	0.322	-4.73	0.297	0.212	-28.62
0.368	0.326	-11.40	0.381	0.301	-21.00
0.437	0.295	-32.49	0.329	0.261	-20.67
0.372	0.270	-27.42			
0.332	0.309	-6.93			
0.406	0.340	-16.26			
0.252	0.254	+0.79			
0.304	0.226	-25.66			
0.390	0.282	-27.69			
n=15			n=9		
$\sum a=5.220$	$\sum b=4.553$	$\sum c=-184.61$	$\sum d=3.105$	$\sum e=2.349$	$\sum f=-216.54$
$\bar{a}=0.348$	$\bar{b}=0.304$	$\bar{c}=-12.31$	$\bar{d}=0.345$	$\bar{e}=0.261$	$\bar{f}=-24.06$
$S_a=0.052$	$S_b=0.050$	$S_c=11.81$	$S_d=0.038$	$S_e=0.030$	$S_f=4.22$

Fig. 31.

Changes in the body-weights of L.longipalpis between emergence and blood-meal:

a) for females offered 30%(w/v) sucrose solution, where
(1) is the regression line for all points (t=3.5; p=0.01)
(2) is the regression line calculated for (b), and
(3) is the regression line for O points only (t=10.6; p=0.001)

b) for females offered only water, where
(2) is the regression line for all points (t=5.0; p=0.002)


a
3 b=0·95
1 b=0·66
2 b=0·70
0.40
0.35
0.30
0.25
0.20
WEIGHT OF FEMALE AT BLOOD-MEAL (mg.)
0·25
0·30
0·35
0·40
WEIGHT OF FEMALE AT EMERGENCE (mg.)

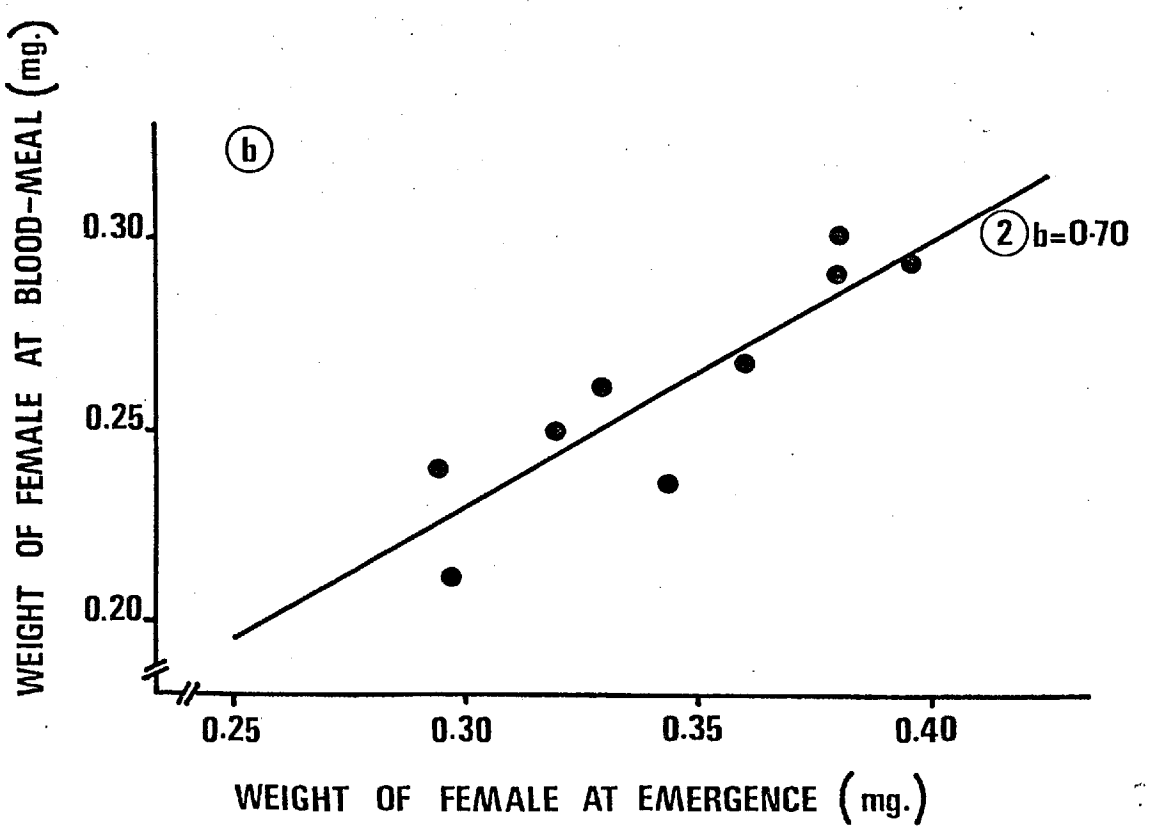

b
2 b=0·70
0.30
0.25
0.20
WEIGHT OF FEMALE AT BLOOD-MEAL (mg.)
0.25
0.30
0.35
0.40
WEIGHT OF FEMALE AT EMERGENCE (mg.)

Fig. 31.

positive relationship between the number of eggs matured and the weight of human blood imbibed (Appendix 50). The difference between the regression coefficients for each group was highly unsignificant ($p = 0{\cdot}9$; $t = 0{\cdot}17$), but for a given weight of blood significantly fewer eggs were matured by the flies that had been deprived of sucrose (Fig 32).

The effect of female age on egg production.

Groups of L. longipalpis from the same rearing cage, where 30% (w/v) sucrose was freely provided, were fed on a human volunteer 2/3, 5/6, 11/12 and 15/16 days after emergence. The weight of each female at the time of the blood-meal, the weight of blood she imbibed and the number of eggs she matured, were recorded (Appendix 51 -54). The mean weight of the flies fell as they got older (0·396, 0·387, 0·340 and 0·329 mg respectively). The number of eggs matured was proportional to the weight of blood imbibed, but for a given weight of blood younger flies tended to mature more eggs than older flies (Fig 33). This difference was only significant for the smaller blood-meals. Only 45% of the 15/16 day-old flies survived sufficiently long after the meal to mature any eggs: therefore, there was insufficient data to compare quantitatively their performance with that of the other groups.

The effect of temperature on egg production.

Stock flies, reared at 25°C from eggs, took measured

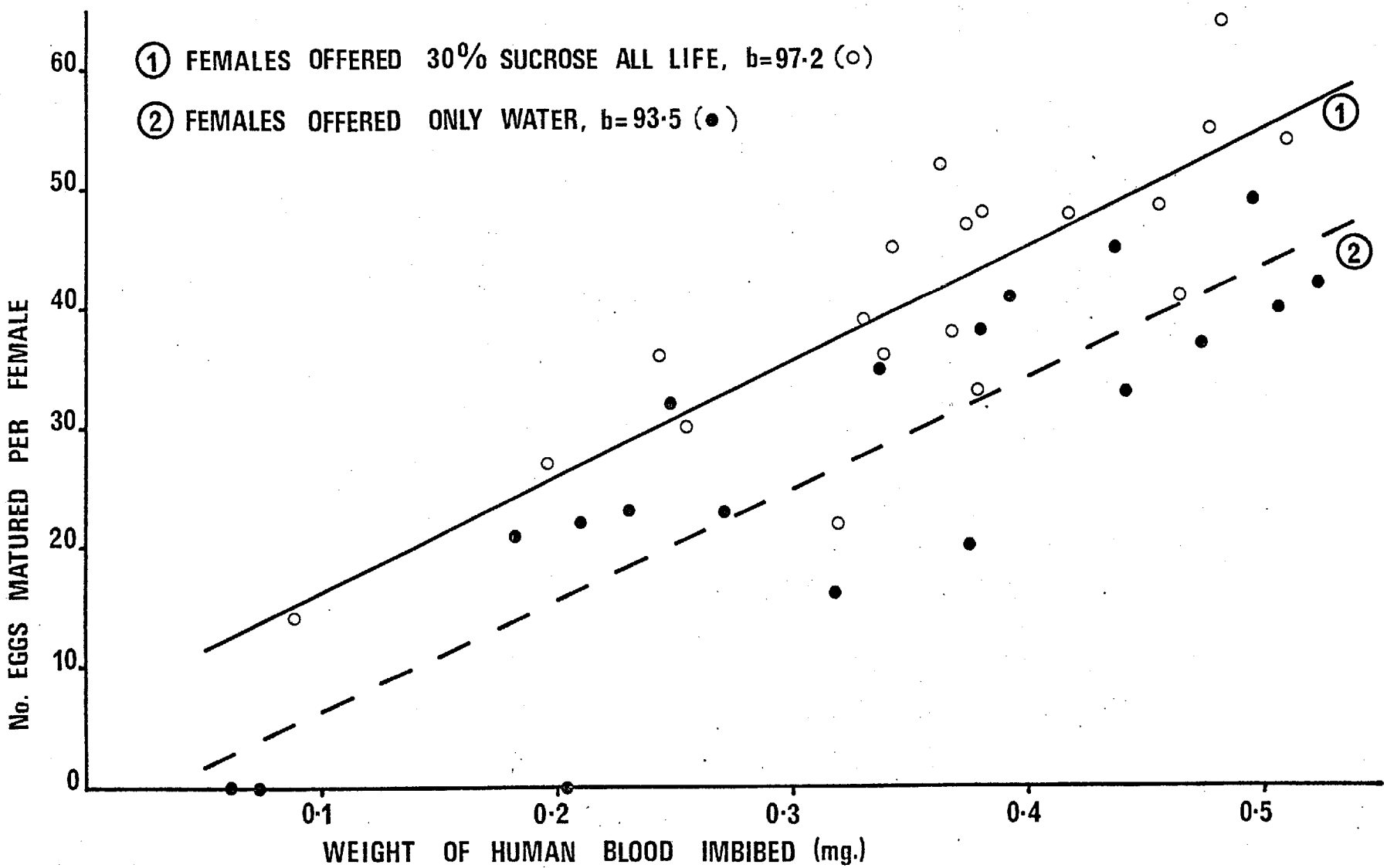
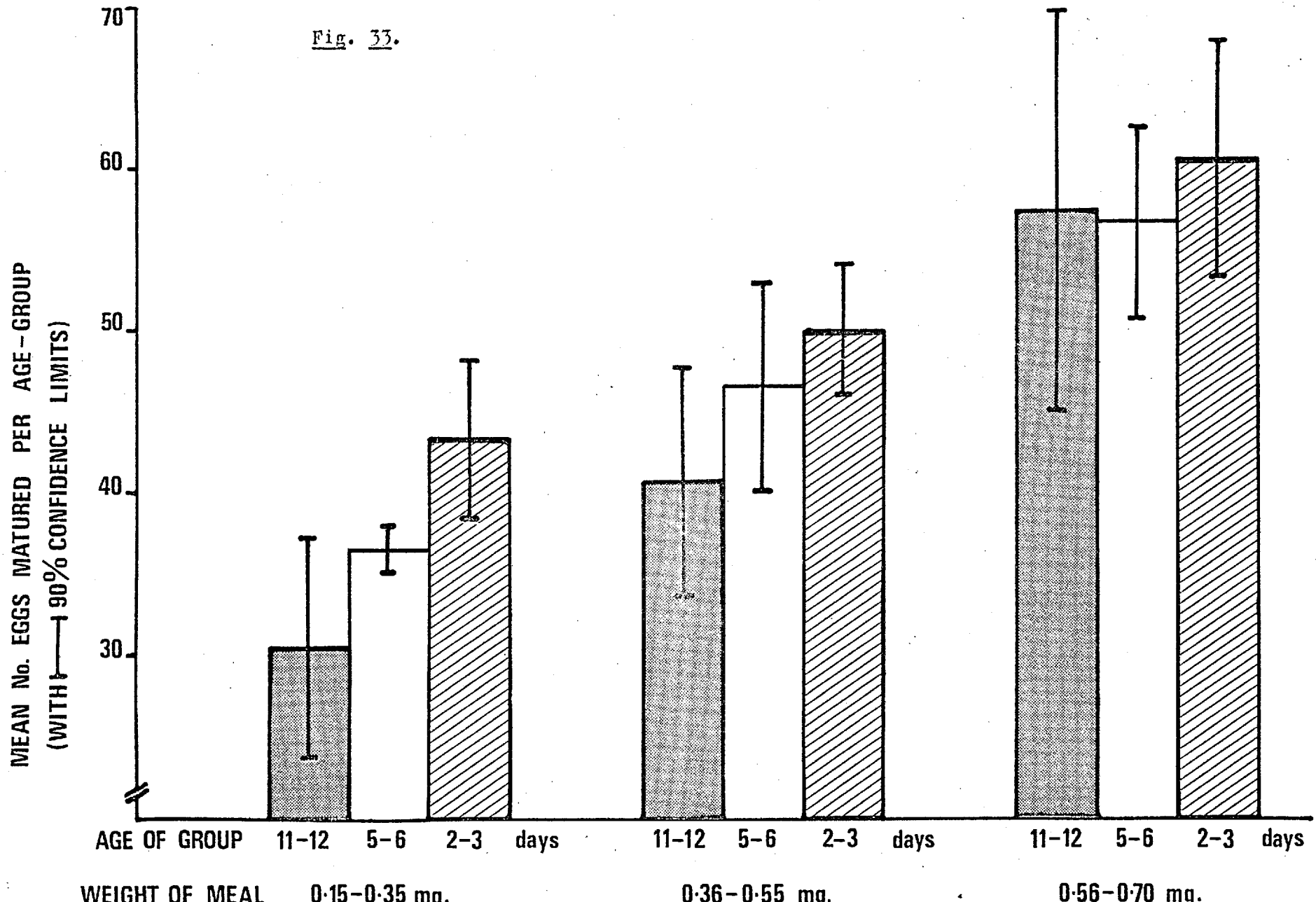


Fig.32. The number of eggs matured on known weights of human blood taken by L.longipalpis females offered or deprived of 30%(w/v) sucrose solution.

Fig. 33.

The number of eggs matured by L.longipalpis females of different ages which had taken known weights of human blood.

Fig. 33.


MEAN No. EGGS MATURED PER AGE-GROUP
(WITH ⊢ 90% CONFIDENCE LIMITS)
70
60
50
40
30
AGE OF GROUP 11-12 5-6 2-3 days 11-12 5-6 2-3 days 11-12 5-6 2-3 days
WEIGHT OF MEAL 0·15-0·35 mg. 0·36-0·55 mg. 0·56-0·70 mg.

weights of human blood and were maintained in three groups at 28°, 25° or 22·5°C. All were placed over dilute sulphuric acid in sealed desiccators so that the ambient relative humidity was 95% (Solomon 1951). Over the range tested, temperature had no effect on the eggs matured/meal-weight relationship (Fig 34).

In another experiment, flies which had fed on the arm of a volunteer were placed without regard to the size of meal taken at 20°, 25° or 30°C (All at 95% RH). The number of eggs matured by each female was counted on the day she died (Table 21). Even after five days some of the females kept at 20° contained large amounts of undigested blood. The average number of eggs matured by this group was appreciably less than the averages matured by those kept at 25°C and 30°C.

Temperature had a marked effect on the rate of blood digestion, as measured by noting (daily at 10·00 hrs) the first appearance of the dark faeces resulting from red cell digestion (Table 22). The rate of digestion of human blood at 30°C was approximately double that at 20°C, i.e. $Q_{10} = 2$ (Fig. 35).

The effect of humidity on egg production.

At 25°C there was no appreciable difference between the number of eggs matured on a given weight of human blood by females of L. longipalpis kept at 95% RH or 60% RH (Fig. 36).

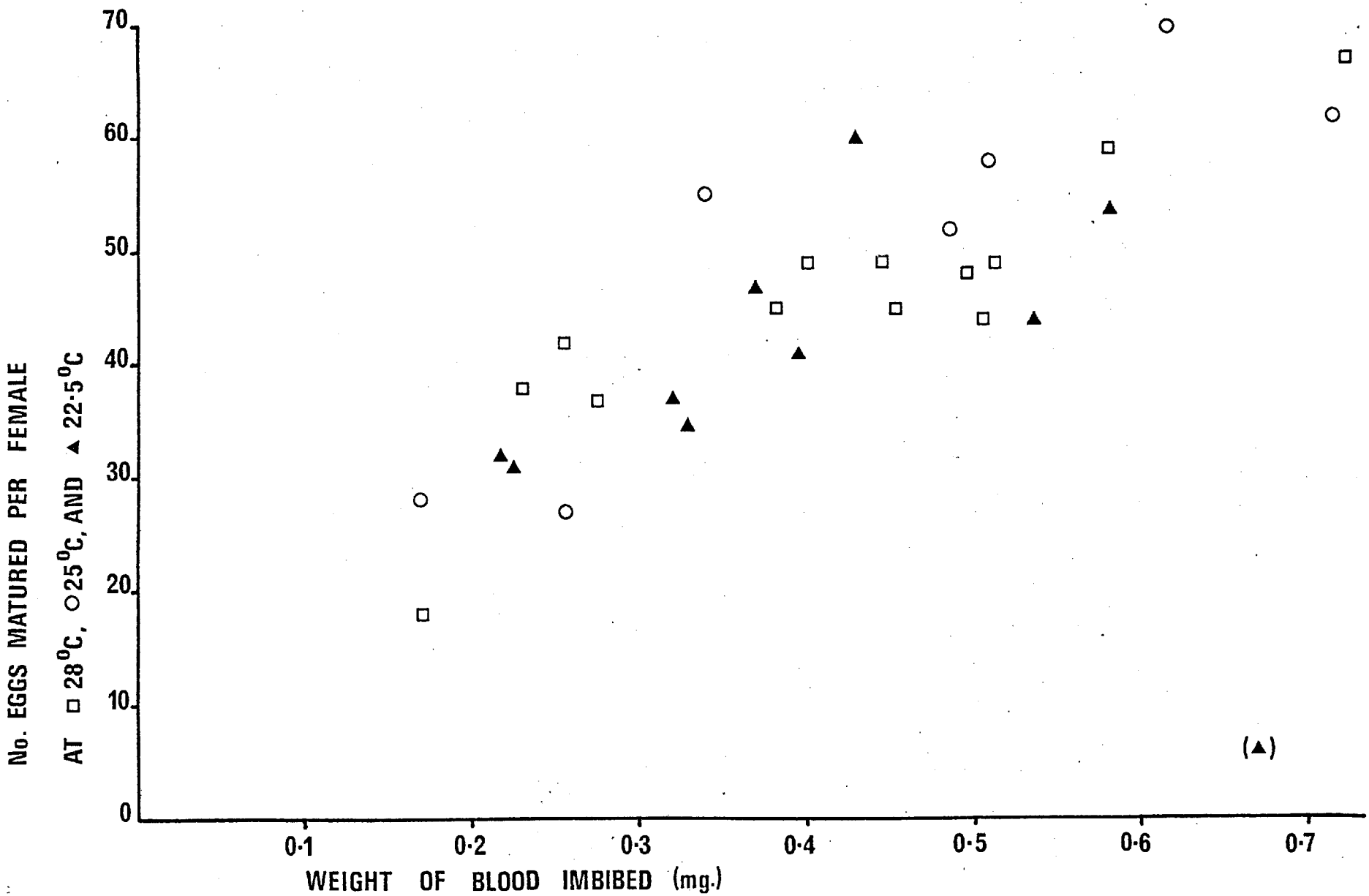


Fig. 34. The effect of temperature on the number of eggs matured by L. longipalpis females which had taken known weights of human blood.

Table 21.

The number of eggs matured by females of L.longipalpis at three different temperatures (95% RH.)

30°C.(x)	25°C.(y)	20°C.(z)
U.D. *	U.D. *	U.D. *
34	55	U.D. *
27	43	42
42	40	7+U.D. γ
40	43	50
42	48	17
40	46	46
14	35	3+U.D. γ
25	32	8+U.D. γ
39	35	65
39	45	48
39	49	42
55	48	50
73	10	24
43	49	
50	56	
$\bar{x}$=37.6	$\bar{y}$=39.6	$\bar{z}$=28.7
s=16.4	s=15.2	s=22.6

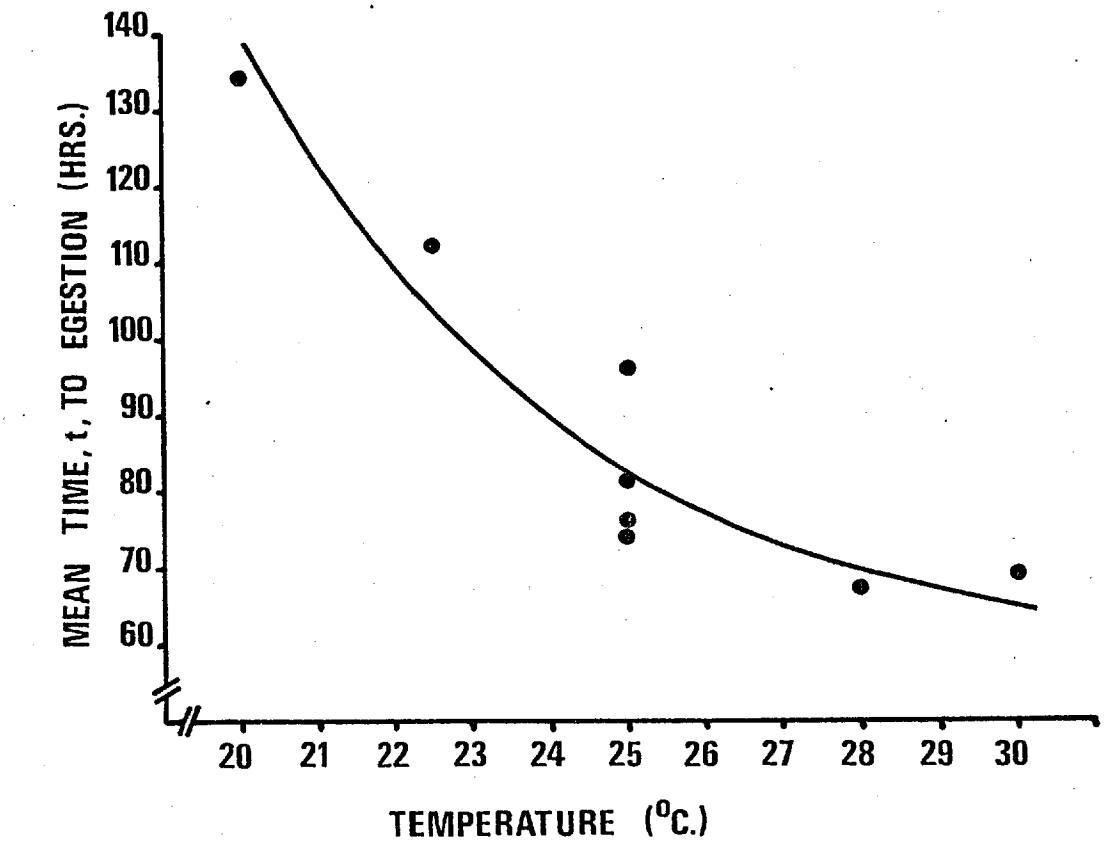
* Dead within 3 days of the meal.

γ Still alive 5 days after the meal.

TEMPERATURE	No. IN SAMPLE	MEAN TIME, t, TO EGESTION (HRS.)	RANGE (HRS.)	MEAN RATE OF DIGESTION (1/t)
30°C.	18	69.6	72 - 48	0.0144
28°C.	11	67.7	72 - 65	0.0148
25°C.	19	76.4	91 - 71	0.0131
25°C.	14	74.4	96 - 72	0.0134
25°C.	23	81.6	120 - 72	0.0123
25°C.	7	96.4	105 - 71	0.0104
22.5°C.	6	112.3	138 - 91	0.0089
20°C.	15	134.4	168 - 96	0.0074

Table 22.

The effect of temperature on the speed and rate of digestion of human blood by L.longipalpis



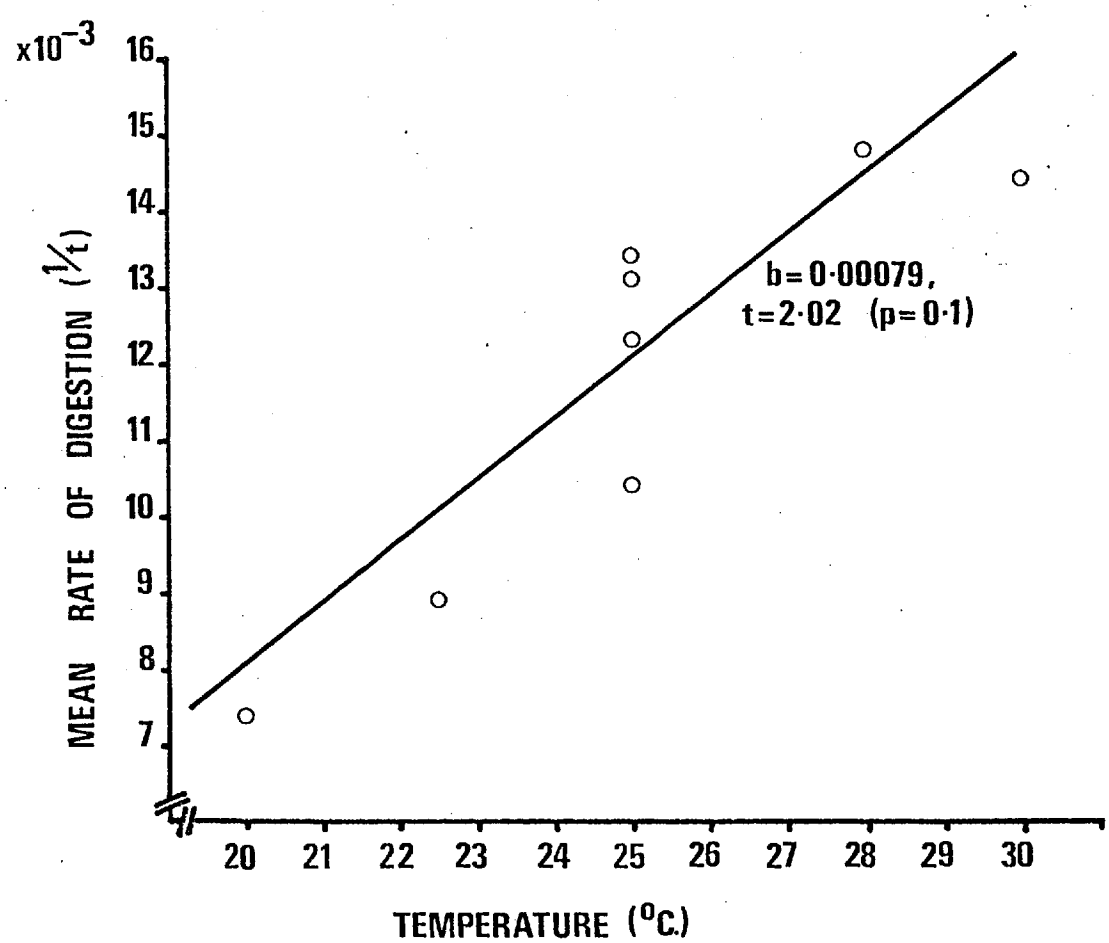


Fig. 55. The effect of temperature on the speed and rate of digestion of a human blood-meal by L.longipalpis.

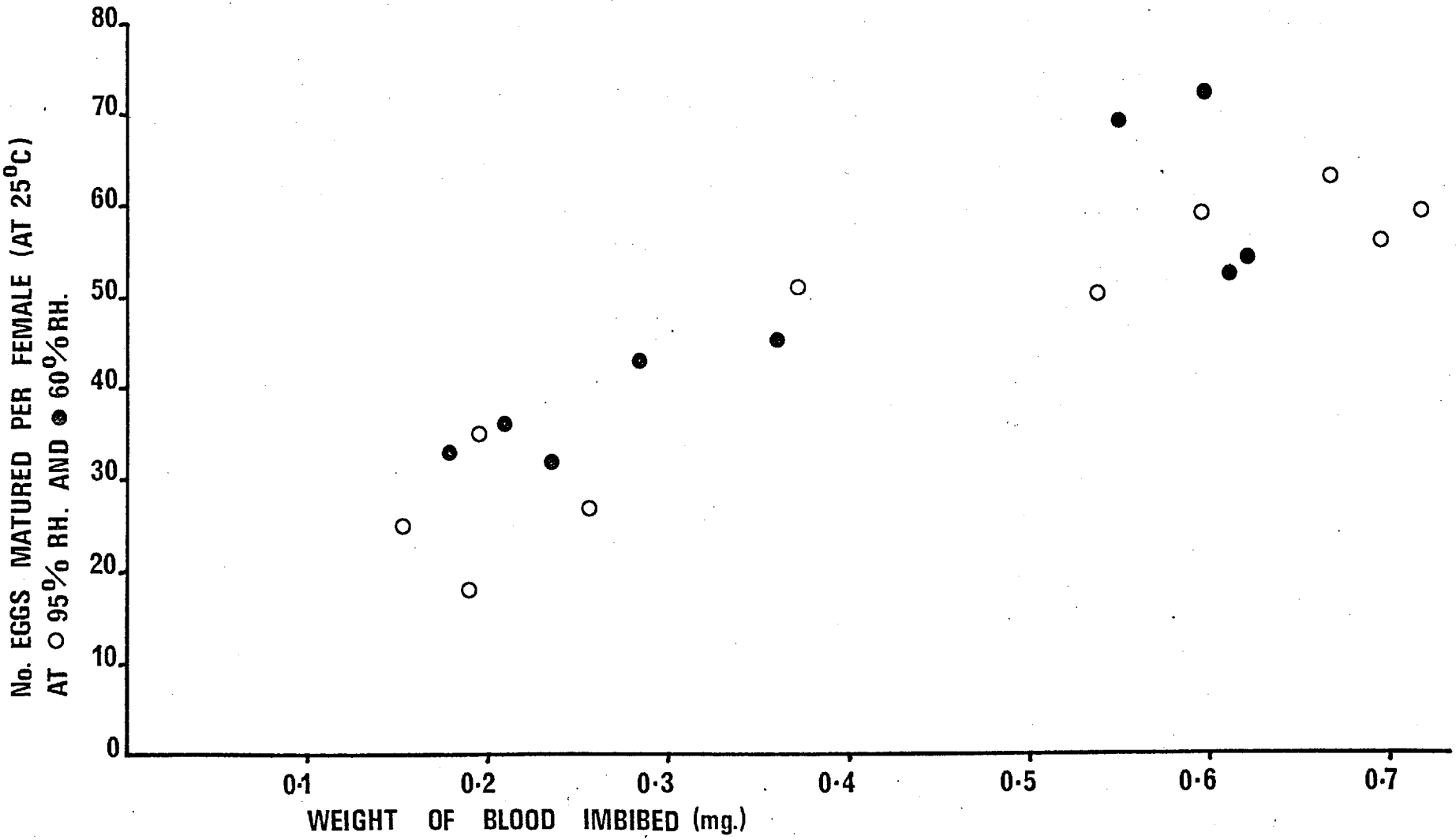


Fig. 36. The effect of humidity on the number of eggs matured by L. longipalpis females which had taken known weights of human blood.

On those females not digesting a blood-meal.

Under optimal conditions (25°C; 95% RH) 2 - 10% of female L. longipalpis failed to digest a blood-meal; all died within 3 days of taking the meal. In this respect, there was no marked difference between human and hamster blood-meals - both within and between the lines of flies normally reared on one or the other hosts - or for other blood types. Table 23 shows the percentages of females of L. longipalpis of different ages (and under different physical conditions) that failed to digest fully a blood-meal.

On unmated females and the number of eggs they matured.

Female pupae were separated from male on the basis of their larger size and greater weight (Section 1). The female flies that emerged were kept in separate rearing cages alongside those containing "mated" females and males. "Mated" and unmated females of the same age and from the same stock were fed on measured weights of human blood. On equal weights of blood there was no significant difference in the number of eggs matured by females of each group (Fig 36X). All the egg-batches laid by "mated" females were fertile.

Sand-fly species and egg productivity.

In Table 24 are recorded the size of the adults (weight) and eggs (volume) of 3 species of neotropical sand-fly, and the approximate number of eggs matured by each on 0·2 mg of human blood. The egg statistics are means (and ranges) for 50 eggs from 5 females of each

Table 23.

% females of L.longipalpis not fully digesting a blood-meal

CONDITIONS	BLOOD-MEAL	AGE AT MEAL (DAYS)	No. IN SAMPLE	% FAILING TO DIGEST BLOOD
Belo Horizonte strain - normal meal human blood				
95%RH.,30°C.	Human	4 - 6	16	6.3
95%RH.,25°C.	Human	4 - 6	49	7.0
95%RH.,22.5°C.	Human	4 - 6	25	18.1
95%RH.,20°C.	Human	4 - 6	14	35.7
95%RH.,25°C.	Human	2 - 3	21	4.8
95%RH.,25°C.	Human	11 - 12	18	16.7
95%RH.,25°C.	Human	15 - 16	19	52.6
60%RH.,25°C.	Human	4 - 6	11	9.1
95%RH.,25°C.) -NO SUCROSE)	Human	4 - 6	18	22.2
95%RH.,25°C.	Hamster	4 - 6	43	9.2
Ceará strain - normal meal human or hamster blood				
95%RH.,25°C.	Human	4 - 6	50	12.0
95%RH.,25°C.	Capuchin Monkey	4 - 6	23	8.7
95%RH.,25°C.	Opossum	4 - 6	18	11.1
95%RH.,25°C.	3-toed Sloth	4 - 6	10	0.0
95%RH.,25°C.	Spiny Rat	4 - 6	14	0.0

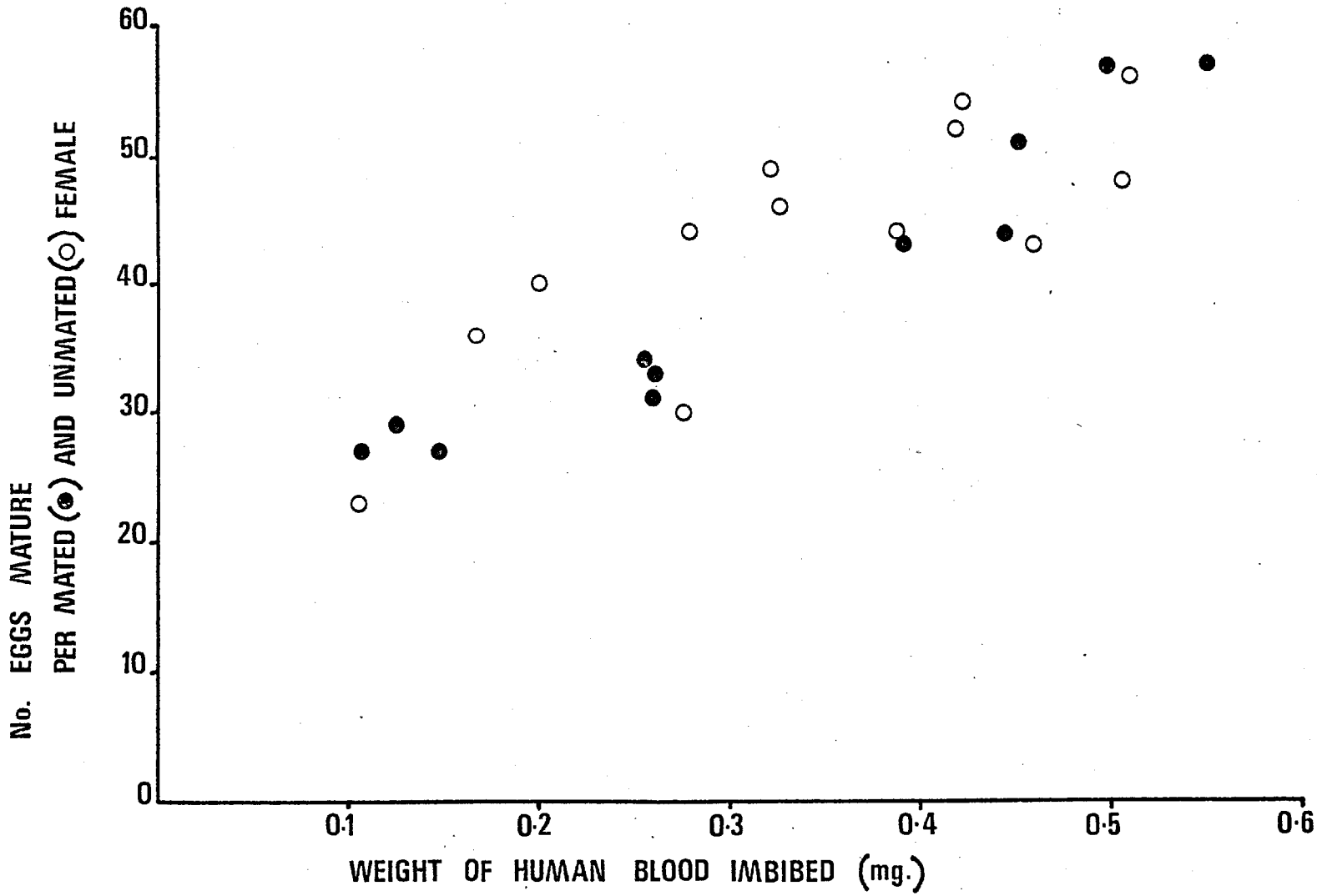


Fig. 36x. The effect of mating on the number of eggs matured by L. longipalpis females.

Table 24.

Egg statistics and productivity in relation to adult size for three neotropical species of sand-fly

SPECIES	MEAN WEIGHT OF FEMALE AT FEED (mg.)	MEAN NUMBER EGGS MATURED ON 0.2 mg. HUMAN BLOOD	AGE OF EGGS (DAYS POST OVIPOSITION) WHEN MEASURED	MEAN LENGTH OF EGGS (mm.)	MEAN WIDTH OF EGGS (mm.)	MEAN VOLUME OF EGGS (mm^3.)
Wild *L.yuilli*	0.140	32	1	0.341 (0.348-0.331)	0.068 (0.070-0.065)	0.007
Wild *Ps.davisi*	0.179	38.6	1	0.429 (0.443-0.414)	0.074 (0.086-0.071)	0.010
Laboratory *L.longipalpis* (from Ceará)	0.308	28.1	1	0.359 (0.371-0.342)	0.086 (0.092-0.084)	0.011
Laboratory *L.longipalpis* (from Ceará)	0.308	28.1	4	0.389 (0.399-0.377)	0.113 (0.116-0.108)	0.021

species; the eggs were measured the day after they had been laid. 30 eggs of L. longipalpis were measured again 3 days later to show the way they increased in volume after they had been laid.

The egg was treated as a prolate spheroid of long axis, a, and short axis, b; its volume = $4/3\ \pi\ a\ b^2$.

THE WATER-SOLUBLE YOLK PROTEINS OF L. longipalpis.

Origin of yolk-protein homogenates:-

Strain of L. longipalpis	Host
Belo Horizonte.	man, laboratory hamster,
Ceará.	man, laboratory hamster, Proechimys sp., Cebus apella, Bradypus tridactylus, Didelphis marsupialis.

Fig. 37 shows the bands of water-soluble proteins usually demonstrated after the electrophoretic separation of yolk-protein homogenates in 7·5% polyacrylamide gels and staining in coomassie blue or naphthalene black 10B. Bands c, f and g were not always present, and other faint bands occasionally appeared. However, from a number of paired separations (5 plus for each test homogenate) it was found that all the bands figured could be demonstrated in the extracts from the eggs of flies that had fed on any of the test animals. Other patterns were obtained by using different combinations of gel concentration, current and running time, but no consistent differences could be demonstrated between the yolks formed after different blood-meals. Further, there were no differences detected between the band-patterns obtained from the eggs of Ceará and Belo Horizonte strain flies. Plate (10) shows the results of a typical separation.

As has been pointed out in similar studies (Whitmore &

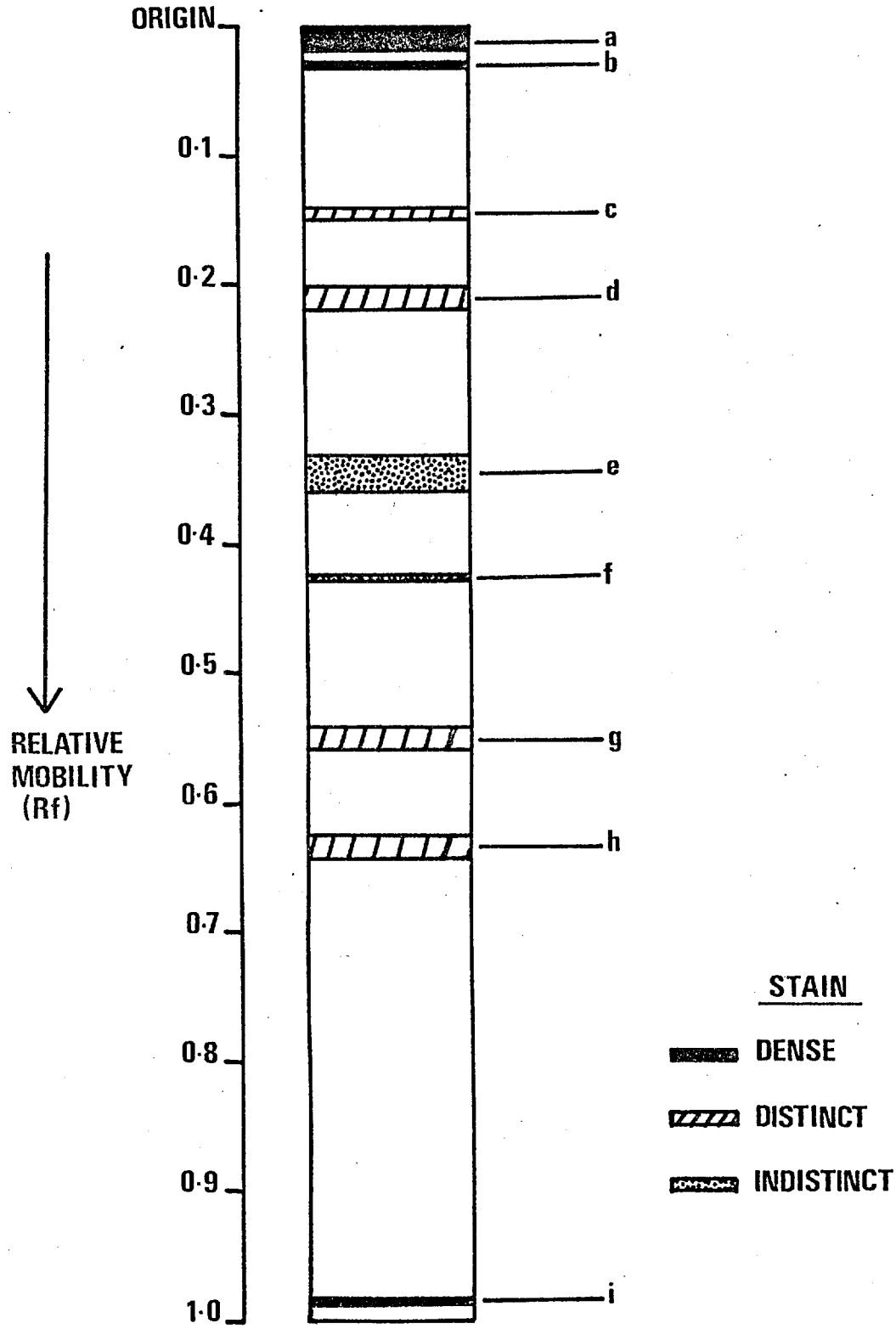


Fig. 37. The relative mobilities in 7.5% polyacrylamide gel of the water-soluble proteins from the egg-yolk of L.longipalpis.

Plate 10: Typical results for the electrophoretic separation of the water-soluble yolk proteins of L.longipalpis eggs.

Gilbert 1974) protein bands with different mobilities do not necessarily represent different proteins, and those with the same mobility (from different preparations) are not always the same. Moreover, as much as 10% by weight of the water-soluble proteins in preparations similar to that used often fail to enter the gel (Clarke 1964); this was obviously the case in the present investigation, where one of the most intense bands (a) was near the origin.

However, gel electrophoresis is a means of identifying possible similarities or differences in protein composition. Usually it is followed up by more sophisticated techniques which can identify the nature of individual proteins. In the present case it was decided not to do so, because the number of sand-fly eggs needed was prohibitive.

HOST PROTEIN IN THE EGG YOLK OF L. longipalpis.

No host antigens were demonstrated (by the micro-immunodiffusion test) in the water-soluble extracts from the eggs of L. longipalpis. All the controls were positive, showing that the antisera were active.

THE PROTEIN CONTENT OF SOME MAMMALIAN BLOODS.

The Instituto Evandro Chagas of Belém, Brazil, kindly carried out for me some tests to determine the red cell and protein concentrations in the blood of some of the mammals on which I fed sand-flies. These are set out in Table 25 .

Methods: haematocrit in Wintrobe tube.
protein by the Greenberg test.

Table 25.

Blood data for some hosts of Ceará strain of *L.longipalpis*

HOST BLOOD	HAEMATOCRIT (%)	SERUM ALBUMEN (g./100ml.)	SERUM GLOBULIN (g./100ml.)	TOTAL SERUM PROTEIN (g./100ml.)
Hamster	52.0 ± 3.6	4.9 ± 0.8	0.9 ± 0.4	5.8 ± 0.3
Cebus apella	47.2 ± 4.7	5.2 ± 1.2	2.0 ± 0.7	7.2 ± 0.5
* Human:				
Man	46.2 (52.0-36.0)	5.0 - 3.8	3.0 - 2.6	8.0 - 6.4
Woman	40.6 (46.0-35.0)			

* Normal Ranges

DISCUSSION AND CONCLUSIONS.

Autogeny: This term is used to describe the ability of insects to mature and lay eggs without protein ingestion, and was coined by Roubaud (1929). It is a phenomenon that has been particularly noticed in certain strains of many blood-sucking insects (Engelmann 1970), where it is of ecological significance in that eggs can be laid in the absence of a host for feeding.

Some populations of P. papatasii from Russia (Dolmatova 1946) and Egypt (Schmidt 1964), and 9 strains of L. gomezi from Panama (Johnson 1961) have been reared autogenously in the laboratory.

In the present work, a few eggs were matured by L. longipalpis fed only sucrose, but as no (fertile) eggs were laid, autogeny was not strictly demonstrated. Similar results have been obtained with many strains of P. papatasii (Dolmatova 1946), and therefore it appears that some populations of sand-flies have a latent capacity for autogeny. Control of autogeny in some insects is not solely genetic, e.g. larval and adult diets can have an important effect on its manifestation (Lea 1964). Thus it is perhaps significant that most of the present results refer to the first laboratory generation, i.e. the one closest to the wild stock.

Blood digestion and the gonotrophic cycle: The loss in female weight (at 25°C and 95% RH) following a blood-meal

closely reflected the observed stages in blood-digestion. Thus for a human blood-meal as much as 40% of the meal-weight was lost in the first hour. Drops of clear fluid were discharged from the anus during this period. These probably consisted almost entirely of water, for they did not gel or tan as later faeces did. L. longipalpis does not continue feeding when fully engorged and then pass out blood straight from the mid-gut as some mosquitoes and lizard-feeding sand-flies do (Parrot 1922). Following the initial diuresis, there was a steady decrease in weight so that some 60% of the meal-weight had been lost by 30 hours, when white/yellow faeces first appeared. Vertebrate blood is hypotonic to the haemolymph of insects and has a higher salt concentration (Edney 1957). The rapid diuresis that follows a blood-meal removes these imbalances and gives the insect greater mobility.

During the first 50 hours of digestion the red cell concentration in the meal fell steadily but no obvious remains of their digestion could be detected in the hind-gut or oviposition (OP) tubes. Serum proteins cannot be detected in the blood-meal of L. longipalpis 36 hours after a meal (A.J. Leaney pers. comm.) and therefore it seems likely that most of the weight loss between 10 and 24 hours results from malpighian secretions rather than the egestion of blood-meal remains.

There was little loss in the meal-weight during the middle third of digestion (30 - 50 hours) when the water requirements of the fly seemed to be nearly balanced by

that available in the blood-meal.

There was a dramatic decrease in weight between 52 and 72 hours when the undigested/unabsorbed remains of the meal were egested; dark brown (haem-containing) faeces first appeared in the hind-gut and OP tube at this time.

The initial diuresis following a hamster blood-meal was less rapid than that following a human one. Differences in blood-meal weight between the two groups can only account for 5% of this. The rate of diuresis might have an effect on egg production by concentrating the meal.

Theodor (1936) carried out similar studies with P. papatasii and found that on the first day a clear fluid was passed, and one-half (at 23°C) to two-thirds (at 30°C) of the meal-weight was lost. Unfortunately, he did not report the origin of the blood-meals. Similar figures have been obtained for the digestion of vertebrate bloods by Glossina (Lester & Lloyd 1928) and Rhodnius (Maddrell 1964) and Aedes (Boorman 1960).

Gemetchu (1974) reported that the peritrophic membrane in P. longipes only becomes a discrete envelope 24 hours after a meal, and then breaks down when the gut is evacuated on the sixth day. At the same temperature (25° ± 1°C) digestion in L. longipalpis takes only half this time, but it is interesting to note that the period of little weight loss occurs from about 24 hours to gut evacuation. Therefore, it would appear that protein digestion starts before the peritrophic membrane has been fully formed in L. longipalpis.

Adler & Theodor (1926) found that the digestion of a blood-meal by P. papatasii was a "relatively slow process", with erythrocytes remaining intact in the gut for four days after a feed; haemolysis did not start until the third day, after which unaltered haemoglobin was passed in the faeces. The red cell count fell steadily in the blood-meals of L. longipalpis, and although some erythrocytes were still intact in human blood-meals after 3 days it does seem that this species can lyse red cells more easily than P. papatasii. No haemoglobin was detected in faeces, and in some females of L. longipalpis this protein was shown to stimulate egg maturation.

The epithelium of the mid-gut of P. papatasii probably releases a powerful anticoagulant, and the salivary glands certainly do (Adler & Theodor 1926). When dissected out, the blood-meal of L. longipalpis was easily broken up to form a nearly homogeneous suspension in physiological saline. There was some agglutination but it was not pronounced. From these observations it would seem that L. longipalpis, too, has an anticoagulant.

Concordance. Egg maturation and blood digestion were concordant in L. longipalpis, with fully matured (stage 5) eggs first appearing when the last of the blood-meal remains were egested. Dolmatova & Demina (1971) reported that parallel digestion of blood and maturation of eggs is the rule for P. papatasii, P. sergenti, P. caucasicus and P. chinensis during the Russian summer. In the autumn, however, these species (which hibernate as larvae)

displayed "gonotrophic dissociation" i.e. eggs were not matured following a blood-meal. This phenomenon has been studied in mosquitoes and probably results from the inhibition of gonadotrophic hormone production by photoperiod and temperature (Clements 1963).

Other species, too, do not always exhibit gonotrophic concordance. Adler & Theodor (1935) found that P. papatasii will often take a number of blood-meals between gonotrophic cycles, and Hertig & Johnson (1961) reported the same was true for several Panamanian sand-fly species. This probably reflects nutritional deficiencies in some populations.

Females of P. ariasi, collected in July 1974 from southern France, were unusual in that eggs were matured following a blood-meal but only after a delay of 6 days. Chaniotis (1967) noticed a similar pattern of development in Californian sand-flies.

Oosorption: The degeneration of a certain number of egg follicles in each gonotrophic cycle seems to be a normal occurrence in mosquitoes (Detinova 1949; Hosoi 1954) and many other insects (Engelmann 1970). Degeneration can occur when there is a shortage of nutrients and/or gonadotrophic hormone (Highnam & Hill 1969), or much later if the eggs are not laid (Roth & Stay 1962). The author's unpublished observations show that L. longipalpis does not resorb fully matured eggs under laboratory conditions.

There are two phases of oocyte development in mosquitoes, the "initiation" phase when the oocytes in the

newly-emerged female are brought to the resting state, and the "promotion" phase when the "resting" oocytes expand by forming yolk (Gillett 1956). The number of oocytes that reach the resting stage is determined by larval nutrition and adult sugar/nectar feeding (Clements 1963). From a study of An. maculipennis, Detinova (1949) suggested that after a blood-meal only those "resting" follicles proceed to develop which have a significantly low threshold of sensitivity to the stimuli initiating oogenesis; if later on the material needed for "ripening" the eggs is not sufficient for all, then some of the maturing follicles degenerate.

Dolmatova & Demina (1971) state that sand-flies (presumably P. papatasii) and Aedes differ from anophelines in that only those "resting" oocytes that will complete maturation ever start it. This is not true for L. longipalpis: It was shown that 14 hours after a human blood-meal more oocytes had passed out of the resting stage (1) and into stage 2 than eventually reached maturity. 20 hours after a hamster blood-meal the number of oocytes to be matured had been set.

As in mosquitoes, the number of terminal oocytes entering the resting stage (or rather, not being resorbed before a blood-meal) depends on larval nutrition and adult sugar-feeding (see later).

The "blood"-meal and egg maturation: In most blood-sucking species of insects the number of eggs matured is determined

largely by the quantity of food imbibed, e.g. Stomoxys, Haematopota, Rhodnius, Cimex and most mosquitoes (cited in Wigglesworth 1965). Roy (1936) found that the number of eggs laid by Aedes aegypti was roughly proportional to the size of blood-meal. Others showed that the number of eggs matured by this species was proportional to the weight of human blood ingested over the range 0.1 - 2·9 mg (Woke et al.1956; Colless & Chellapah 1960). Larger meals were taken but did not result in the maturation of any more eggs.

Similarly, for Phlebotomus chinensis (Dolmatova & Demina 1971) and three North American species of sand-flies (Chaniotis 1967) it was noticed that the larger egg batches resulted from the larger blood-meals, although no specific relationship was determined. In the present study, it was established that the number of eggs matured by L. longipalpis was proportional to the weight of the meal imbibed. Even for the most efficacious meal (hamster blood) this relationship did not plateau, i.e. the flies never took more blood than was sufficient to mature a full egg batch (Ca. 130). The largest meals (Ca. 0·7 mg) seemed to fully stretch the abdomen. The relationship between the nutrient requirements of each egg, the (species-specific) number of ovarioles per ovary and the maximum possible extent of gut distension may make it physically impossible for L. longipalpis to "over-feed". Alternatively, the diet offered to the colonized L. longipalpis (as larvae and adults) might have been nutritionally deficient - obliging the adult females to use so much of their blood-meal for non

egg-specific metabolism that their guts were physically incapable of imbibing a surfeit of nutrients in one meal.

Goeldi (1905) found that mosquitoes have host preferences, and suggested that the blood of some species of animals may be superior to that of others for egg production. Later, Roy (1931) noted that the number of eggs laid by Anopheles stephensi fed human blood was only half that of those fed the blood of rat, guinea-pig or rabbit. For Ae aegypti Woke (1937) showed that significantly fewer eggs were matured on human blood than on rabbit, guinea-pig, frog, turtle or canary blood.

Chaniotis (1967) noted that the sand-fly L. vexator matured a mean of 71 eggs when fed on a lizard but only 63 when fed on a snake. This difference was not significant, and he attributed it to blood quantity not quality. In the present study, L. longipalpis was fed on 8 species of warm-blooded vertebrates. For each species, the number of eggs matured by a female was proportional to the amount of blood imbibed. When tested, the regression coefficient for a particular host was constant between generations of L. longipalpis, and for different individual hosts of either sex. Similarly for Ae. aegypti, Woke (1937) and Woke et al (1956) found that egg production was constant for different individuals and that fasting did not affect the blood's efficacy. In this connection, the author has found that the egg production of L. longipalpis was not reduced when the blood-meals were taken from a human volunteer 3 hours after

he had donated a pint of blood (unpublished data).

Broadly, the regression coefficients for the different hosts fell into three groups: the highest were for the rodents, Proechimys and laboratory hamster, which were significantly different from the lowest which were for the two monkey species. Intermediate were regression coefficients for mouse, opossum, three-toed sloth and man.

It is usually dangerous, but always tempting, to interpret such results in terms of host preferences and epidemiological significance. Mathis (1934) found that Ae. argentatus (= aegypti) laid 25% more eggs on human blood than on any other kind, and concluded that humans were sought preferentially by this species because their blood was more favourable for reproduction. Woke (1937) showed that to the contrary Ae. aegypti (the yellow-fever vector) found human blood far less useful for egg maturation than that of most other animals including turtle and frog!

L. longipalpis collected from Belo Horizonte (BH) came from a natural cave where the cricetid rodent, Cercomys cunicularis, and bats were common but human hosts were infrequent (R. Killick-Kendrick pers. comm.). The strain of L. longipalpis from Ceará, however, was collected from under roof tiles (Ward 1974); man and dogs are their natural hosts. However, egg production for both was poor on human blood compared with that of rodents. The results of feeding human-line and hamster-line females of the BH strain on non-complementary hosts do not suggest that the colonization of these strains had selected flies for hamster blood. Host

adaptationhas been reported for An. stephensi (Stahler & Seely 1971) and for Culex pipens (McCray & Schoof 1970).

The effect on egg production of the blood of monkeys, man and rodents was surprisingly similar for Ae. aegypti (Woke 1937) and L. longipalpis. Fewest eggs were produced (per mg) on monkey blood; human blood was possibly slightly better; rodent blood was significantly better than either of the other two. This suggests that there are intrinsic differences between blood types which are more important than any differences in the efficiency of the digestive processes of these two very different insect species.

During my stay in Belém, Brazil, I had hoped to compare the egg production of different sand-fly species when fed on preferred and other hosts. This was not achieved because of the disinclination of the local species to feed (experimentally) on any but their preferred hosts - see Section 1 and Ward (1974). Psychodopygus davisi and L. y uilli did take measured weights of blood from one of their preferred hosts (man). With regard to the weight of the meal, Ps. davisi produced significantly more eggs than L. longipalpis. The relationship between the size of the adults and eggs, and the egg productivity of these sand-fly species shows that absolute egg production depends as much on species-specific characters as on the blood type.

The results of the membrane feeds show that L. longipalpis is able to mature eggs on both the red cell and plasma fractions of vertebrate blood. The egg production on blood-concentrations of these two fractions was additive.

That is, it was the concentration and not the total volume of the meal that determined the number of eggs that were matured. This was also shown by feeding different concentrations of sera and artificially prepared bovine serum albumen. The "buffy" layer (white cells and platelets) was not fed to the flies, but the results suggest that it can only be of minor importance for egg maturation.

The difference for egg production between human and hamster bloods was shown to result from differences in the red cells of the two hosts. From Table 25 it can be seen that the haematocrit values for hamster blood were generally higher than those for human blood. Red cell concentrations for the membrane feeds were 45% for both species. Thus the observed difference in their effects on egg production will be effectively greater for a comparision of whole bloods. Human red cells have a far greater sedimentation rate than those of hamster and other small mammals (Spector 1956). This may have reduced their effective concentration in the membrane-feeds and/or the extent of their digestion in the mid-gut of the fly.

The results suggest that there is both a chemical difference between the contents of each type of cell and the degree to which the fly can lyse them. Thus egg production on the water-soluble contents of lysed red cells was significantly greater than that on red cell suspensions. This difference exists for hamster cells (as well as human) and is too great to be explained in terms of cell sedimentation, for there was no significant

difference between egg production on heparinized hamster blood from a membrane and blood from the hamster. Therefore, it is concluded that the difference between red cell contents and whole cells results from the flies inability to lyse all the cells within the period of oögenesis. This is supported by the observation that red cells were still present in the meal after the egg number had been set.

The increase in egg production following a meal of lysed red cells compared with a cell suspension was disproportionately more for human blood (111·1%) than for hamster blood (62·6%) which supports the observation that hamster red cells are lysed at a faster rate than human. However, even if all the human red cells in a blood-meal were lysed, the number of eggs produced (per mg of meal) would still be substantially less than that produced on hamster blood. This suggests a further chemical difference (for oögensis - inducing substances) in red cell contents.

L. longipalpis matured eggs when fed a suspension of denatured proteins or a solution of the "essential" amino acids. Therefore, protein per se. is not essential for egg maturation; polypetides, peptones and amino acids, i.e. the normal products of insect protein digestion (Wigglesworth 1965) are sufficient.

The significance of these results can best be discussed in relation to what is known for mosquitoes, because no similar results have been reported for other sand-flies, and other blood-sucking insects are too dissimilar. Thus the hemimetabolous families Cimicidae and Pediculidae have

gut symbionts that affect blood-digestion and nutrition. (Koch 1956), and Glossina ingests blood into the diverticulum not the mid-gut (Langley 1966).

A series of experiments carried out this century have shown that the products of protein digestion are alone sufficient to stimulate egg maturation in mosquitoes. Fielding (1919) established that Ae. aegypti will lay eggs when fed on peptone-sugar solution. For Ae. elutus, the egg maturing factor was found to be in the stroma of the erythrocytes and in the cell-free serum of donkey blood, and was not inactivated when the serum was heated at 100°C for 1 hour (Yoeli & Mer 1938). Ae. aegypti laid eggs when fed either the corpuscular or serous fractions of rabbit blood (Woke 1937), and when fed suspensions in saline of erythrocytes, sera, plasma albumen, horse haemoglobin and bacto-peptone (Greenberg 1951). Later, Dimond et al.(1956) and Singh & Brown (1957) found that only amino acids were essential to stimulate egg maturation in Ae. aegypti.- Bellamy & Bracken (1971) showed that the number of eggs matured by C. pipiens depended on the concentration of protein in the meal and not the volumetric size of the meal. L. longipalpis is similar in all these respects.

The origin of yolk proteins: For mosquitoes, as for many other insects with polytrophic ovarioles the most important source of yolk protein is the fat body, which synthesises and secretes female-specific protein (or vitellogenins) that are then absorbed by the oocytes in preference to other haemolymph proteins (Hagedorn & Judson 1972; Hagedorn &

Fallon 1973; Rockstein 1973). There is good but circumstantial evidence that the blood-meal is the source of nutrients for yolk-protein synthesis in mosquitoes: Briegel (1969) demonstrated that after a blood-meal the level of free amino acids in C. pipiens fatigans increases rapidly and reaches a maximum within 24 hours. The results of several other studies on mosquitoes similarly indicated a peak of metabolites about 24 hours after a blood-meal (Fisk & Shambaugh 1952; O'Gower 1956; Hudson 1971[1]; Thayer 1972).

For L. longipalpis, the results of the immuno-diffusion test and the fact that amino acids alone will initiate oögensis suggest that host proteins do not usually enter the oöcytes without undergoing some degradation. That certain large molecules can enter the haemolymph and then the oöcyte was shown by detecting haemoglobin (or like compounds) in matured eggs. This indicates that yolk proteins are taken up by pinocytosis. Similarly, Wigglesworth (1943) noted that some parahaematin (a breakdown product of haemoglobin) is taken up by the yolk of the eggs of Rhodnius, but not by An. maculipennis or Ae. aegypti. A possible route for the uptake of large molecules from the gut into the haemocoel of sand-flies has been described by Gemetchu (1974); he demonstrated a ".... deep, convoluted invagination of the basal cell membrane and basement membranes" which formed ".... a duct-like extension of the haemocoel into the epithelium" of the mid-gut of a blood-fed P. longipes.

1. Quoted in Bellamy & Bracken, (1971).

Further evidence that the process of yolk formation in L. longipalpis is similar to that in mosquitoes comes from the results of the separation of yolk proteins experiments. These indicated that the water soluble yolk-proteins present in largest quantities are similar when different vertebrate bloods are the source of nutrients, which suggests that only certain proteins are deposited in the yolk. It follows that the essential difference between vertebrate bloods for egg production could be the suitability of the amino acid balance.

As yet there is no explanation why the blood of some hosts is more favourable than that of others for oögenesis in mosquitoes. The difference does not lie between nucleate and anucleate red cells (Woke 1937), and the addition of nucleic acids to an artificial diet of amino acids fed to Ae. aegypti did not raise egg production (Dimond et al.1958). The most likely explanation is that the amino acid balance is more suitable in the blood of some hosts than in others (Clements 1963).

Both Greenberg (1951) and Lea et al. (1958) found that the number of eggs laid by females of Ae. aegypti could be increased if L-isoleucine was added to their meals prepared from red cells deficient in this. For L. longipalpis, however, the addition of L-isoleucine to diluted human blood did not increase egg production. This could reflect a specific difference between Ae. aegypti and L. longipalpis, but is more likely to result from the

inadequacy, or otherwise, of the larval diet.

From the results of the yolk-proteins investigation it was postulated that the efficiency of different vertebrate bloods for egg production could depend on the suitability of their amino acid balance. This is unlikely to depend solely on their gross protein content, which is comparable for most bloods (Spector 1956). Haemoglobin provides another example: it forms 90% by weight of the proteins in human red cells (Spector 1956) but as a suspension in saline it stimulates the maturation of few eggs in females of L. longipalpis or of Ae. aegypti (Greenberg 1951).

The suitability of the amino acid balance obtained from different meals for egg production could depend as much on the physico-chemical nature of the meal as its intrinsic composition. Thus it was shown that egg production became erratic when the concentration of bovine serum albumen solution was increased from 10% to 20%. There were also differences in the ability of L. longipalpis to obtain the nutrients contained in the fractions of different bloods. The proportion of females digesting a meal and maturing eggs was only less than 90% when the blood fraction was a suspension (in saline) of red cells (Table 26). This could have resulted from the inability of the sand-fly protease to lyse red cells when most of the complement had been washed off. Alternatively, protein/amino acids contained in the red cells may not have been recognised as food. Thus Shambaugh (1954) found that after females of Ae. aegypti had fed on a suspension of sheep erythrocytes in saline,

Table 26.

% females of human-line L.longipalpis not digesting a meal taken through a leached chick membrane. All those not digesting a meal also failed to mature any oocytes beyond stage 2 and died 1 - 3 days after the feed.

MEAL	No. IN SAMPLE	% FAILING TO DIGEST MEAL
Citrated human blood	19	5.3
45% suspension of human red cells in saline	22	31.8
45% extract of human red cells in saline	18	0.0
10%(w/v) human haemoglobin in saline	13	0.0
50% human plasma in saline	11	0.0
Human plasma	12	0.0
Heparinised hamster blood	13	8.0
45% suspension of hamster red cells in saline	18	66.7
45% extract of hamster red cells in saline	21	9.5
Hamster plasma	31	6.5
10% bovine serum albumen	26	3.8
20% bovine serum albumen	12	0.0
5% bovine serum albumen	14	0.0

protease activity was only a little higher than the residual level found in unfed mosquitoes; protease secretion was stimulated by the non-dialysable plasma proteins. Similarly for Glossina morsitans protease secretion was stimulated by the serum and not the red cells of guinea-pig blood (Langley 1966).

Some females of L. longipalpis were able to digest suspensions of red cells, the proportion being higher for human than for hamster red cells. This seems to contradict the conclusion that the digestion of a meal of hamster red cells was more complete than that of a meal of human red cells. This need not be so if initiation and degree of red-cell lysis in a meal are independent.

The initiation of oögenesis: So far it has been assumed that the stimulus for oögenesis in L. longipalpis is the arrival of nutrients in the haemolymph. This is not necessarily so, for in many insects there are "token" stimuli which do this (Rockstein 1973).

It is unlikely, on the basis of what is known for mosquitoes, that oögenesis in sand-flies is triggered by the sensory perception of nutrients as they are imbibed. Thus, females of Ae. aegypti (Larsen & Bodenstein 1959); and of C. pipiens (Bellamy & Bracken 1971) mature eggs if a protein meal is given as an enema. Differences in host stimuli may affect oögensis in L. longipalpis by determining the size of the meal ingested, but it is unlikely that they have any direct effect, for egg production was unchanged when a protein meal was taken across an artificial membrane rather

than from a living host.

Gillett (1957) and Larsen & Bodenstein (1959) proposed that the sustained stretching of a mosquito's gut by a (blood-) meal is the token stimulus for the release of gonadotrophic hormone without which oögenesis cannot proceed. This has been accepted as proven by many authors (Clements 1963; Jones 1968; Harris & Cooke 1969), but not by Bellamy & Bracken (1971). The evidence for the gut-distension theory is worth considering for it bears on several of the observations made for L. longipalpis.

Recently, Gillett et al. (1975) have restated the gut-distension theory: The stimulus for gonadotrophic hormone release is distension of the mid-gut beyond a threshold value; and it is the adequacy or otherwise of the blood-meal in stimulating hormone release that determines whether ovary development will follow, not the size of the blood-meal per se. This is an all-or-nothing theory; it does not propose to explain the quantitative nature of the ovarian response.

Gillett et al.(1975) give no figures for the weight of blood imbibed by the females of Ae. aegypti they used, but state that ovary development was halted prematurely in 83% of "half-feeders". This is not compatible with the findings of Woke et al. (1956) who showed that the % of females not maturing eggs was 26% for those that took 1·0 - 1·4 mg (half-feeds), 69% for those that took 0·5 - 0·9 mg and 90% for those that took 0·0 - 0·4 mg of human blood. Thus it would seem that the threshold for adequate gut-

distension must be rather low.

There is a critical period (up to 2 - 8 hours after the meal) during which the gonadotrophic hormone must be liberated if oögensis is to be initiated (Gillett 1957); Larsen & Bodenstein 1959). As Ae. aegypti shows rapid diuresis during the first few hours after a blood-meal (Boorman 1960), any process regulating oögensis according to the degree of gut-distension would have to be highly uncritical.

That gut-distension is not a "token" stimulus for oögensis in one strain of C. pipiens was shown by Bellamy & Bracken (1971): Eggs were not matured when the mid-gut was expanded by sealing the anus and feeding sugar, or giving an enema of agar. However, eggs were matured when the enema contained protein, and when a solution of amino acids were injected into the haemocoel. They interpreted these results as showing egg maturation as a direct response to nutrients, and speculated that "peaking" of nutrients in the haemolymph in the 24 hours following a meal set the quantitative ovarian response.

Eggs were matured by females of L. longipalpis which had imbibed only 0·006[1] mg of hamster blood. Quantities of other bloods and artificial diets of less than 0·01 mg usually led to the maturation of eggs. L. longipalpis and P. papatasii (Theodor 1936), like Ae. aegypti, exhibit rapid

1. By using standard weights it was shown that the microbalance used was accurate to ± 0·005 mg.

diuresis during the first few hours after a blood-meal. Therefore, if it occurs in sand-flies, the gut-distension stimulus for oögenesis is highly uncritical, and for L. longipalpis operates at a very low threshold. Moreover, gut distension could not have had a quantitative effect on the oögenesis of L. longipalpis because this depended on the concentration of protein in the meal not on the meal size.

What reason is there to suppose that a "token" stimulus for the control of oögenesis is necessary in blood-sucking mosquitoes and sand-flies? Usually a "token" stimulus mediated by the neuroendocrine system is the means by which a female insect ensures that oögenesis only proceeds when there is (or will be) a sufficiency of nutrients for her to complete a gonotrophic cycle and/or for larval nutrition. Examples are the arrest of oögenesis in Leptinotarsa fed on ageing potato leaves, and abnormal egg production by the sugar-beet moth, Scrobipalpa ocellatella, when it is not stimulated by the leaves of its host plant (cited in Rockstein 1973).

In comparision, the blood-sucking mosquito or sand-fly receives a single large meal of concentrated nutrients. As proposed, the "token" stimulus of gut-distension will not provide it with any useful information as regards the quantity or quality of the meal, except that it is adequate in size or otherwise. But simply because the meal is immediate and nutrient-rich, control could be achieved more

efficiently if the neuroendocrine system responded directly to the nutrients once they had been absorbed into the haemolymph.

All the elements of such a mechanism have been shown. Thus, protease release in the mid-gut of Ae. aegypti is not under humoral control but is a direct response to protein in the meal (Shambaugh 1954). Eggs were matured by C. pipiens when amino acids were injected into the haemolymph without distending the gut (Bellamy & Bracken 1971), and by females of Ae. aegypti when they ingested into the diverticulum amino acids from a cotton-wool pad (Dimond et al. 1956).

The fact that some populations of mosquitoes (Clements 1963) and sand flies (Lewis 1971) are autogenous is another piece of evidence to support the contention of Bellamy & Bracken (1971) that the initiation of oögenesis is a direct response to nutrients. The occasional autogeny recorded in this study suggests that the same is true for L. longipalpis.

Gillett et al.(1975) showed that for Ae. aegypti the gut must be distended beyond a threshold if the blood-meal is to be retained. From some of the present observations on L. longipalpis it is likely that gut or abdomen distension plays a part in ensuring that the blood-meal is retained. Thus, even though many females that had imbibed a suspension of red cells failed to recognise the meal as "food" and died without digesting it, all retained the meal in the mid-gut until the normal time of digestion or death.

However, the recognition of food (and the physiological

changes that follow) may also be sufficient to ensure the retention of the meal. Most females that had imbibed a meal of saline through parafilm did not retain the meal for more than 24 hours. However, when the saline was taken through an already leached chick skin, the very small amounts of material that leached into it during the experiment (the saline was not noticeably opaque by the end) was sufficient to stimulate egg production; after 24 hours these flies were still noticeably swollen. In this connection, Gemetchu (1974) observed that even a partial bloodmeal stimulated the production of a well-formed peritrophic membrane in P. longipes.

Critical assessment of the membrane feeding technique.

1. The period between feeding and reweighing was as long as 15 minutes for some flies. As the rate of diuresis is not necessarily the same for all meals, there may have been considerable differences between actual and observed meal weights.

On one occasion when this error was tested it was not found to be critical: The regression coefficients for eggs matured on meal-weight was not significantly different when engorged flies were weighed 1 minute and 20 minutes after they had fed on man. Moreover, ~~similar~~ regression coefficients for membrane feeds and host feeds were not significantly different for any blood type.

2. Some of the feeds were carried out over a 2 hour period. There must have been some settling of red cells in

the blood preparations despite frequent shaking. However, the close similarity between natural and membrane feeds suggests that this could have only resulted in minor inaccuracies. A magnetic stirrer would have improved the technique.

For these reasons red cell suspensions were used for much shorter periods - never more than one hour.

3. It has been assumed that the blood (or other meal) reaching the mid-gut of a fly was essentially similar to that in the feeder. There is evidence to support this assumption.

The estimates of erythrocyte concentration in normal blood and that in the mid-gut of L. longipalpis 1 minute after feed were similar. Further evidence that mammal-biting sand-flies do not filter red cells out of the meal was provided by Shortt & Swaminath (1928), who concluded that the fascicle canal of P. argentipes allowed the passage of at least three red cells abreast. From its known habits and the structure of its mouthparts, L. longipalpis is a typical mammal-biting sand-fly; these normally have a wide fascicle canal (Lewis 1975). Most fat cells (Lewis 1975) and the larger white cells (R.W. Ashford pers.comm.) are probably the only constituents filtered out of a meal.

4. The results of section 2 support the assumption that the proteinaceous solutions tested for their effect on egg maturation were ingested into the mid-gut, and were therefore available for digestion.

A large proportion of the flies fed on the solution of

amino acids "Lea 1" failed to nature eggs. This was not surprising because the meals were very small. Later, however, it was realised that a part of such solutions (containing 20% sugar) was often dispatched to the diverticulum. For the few feeds obtained, egg production on "Lea 2", which contained less than 10% of sugars, was more consistent. This suggests that not all of the measured meal of "Lea 1" was available for egg production.

5. The plasma for these experiments was prepared by centrifuging heparinised hamster or citrated human blood, and therefore undoubtedly contained some red cells and their contents. Therefore, the contribution of plasma to egg production could have been overestimated.

6. Sterile conditions are required in order to observe actual nutritional requirements in insects. Such conditions were not maintained during these experiments, but every effort was made to limit microbial growth.

The membranes were prepared from freshly killed or deep-frozen chicks and used immediately. They should have been no less sterile than the skin of a living host. The feeding apparatus was cleaned in a surfactant to remove proteins (Decon 90 from BDH), washed several times in boiling water and then methanol to remove fats and amino acids, and heat-dried at 200°C before use. All solutions were prepared from refrigerated and/or desiccated pure reagents, or blood freshly drawn from the host (hamster) or a sterile pack (human).

Singh & Brown (1957) were able to rear the larvae of

Ae. aegypti in aseptic conditions and thus determined the nutritional requirements (larval and adult) for oögenesis. The conditions necessary for rearing sand-fly larvae do not lend themselves so easily to sterile technique.

7. It has been assumed that the ovaries respond quantitatively to changes in adult nutrition solely by maturing different numbers of eggs and not eggs of different size. This was not proven, although the variation between the few egg batches measured was slight. According to Shannon & Hadjinicalao (1941) the size of the egg does not vary with the size of the female in Anopheles, but does vary considerably between species. Johansson (1964) in a review of reproductive processes and nutrition in insects stated ...."In contrast to the great flexibility in fecundity and fertility is the constancy of the egg dimensions under varying nutritional conditions".

The number of eggs matured on two blood-meals: L. longipalpis could only be kept alive after the first gonotrophic cycle by preventing oviposition. Therefore all the females taking a second feed (6 days after the first) contained fully matured eggs. No eggs were matured on the second meal, although this was fully digested.

Similarly, Detinova (1953) found that the follicle anterior to a retained egg in An. maculipennis degenerates during the following gonotrophic cycle. In this example, the retained eggs were laid with the next batch or resorbed. There is evidence that L. longipalpis does not resorb

matured eggs (Unpublished data), but as at least a few of the retained eggs are often passed into the oviducts (Section 4) it is surprising that there was not a small increase in the number of eggs matured.

Body weight of females of L. longipalpis and egg maturation: Christophers (1960) found, from his own results and those of others, no suggestion of any close relation between the number of eggs and the size of the female of Ae. aegypti. Other reports are contradictory: Barlow (1955) concluded that for Ae. hexodontus female body-weight was correlated more closely than meal-weight with the number of eggs matured. For Malay and Australian colonies of Ae. aegypti, Colless & Chellapah (1960) established that the number of eggs matured depended equally on female body-weight and meal-weight. However, working with another colony of the same species Woke et al. (1956) could not find a statistically significant association between body-weight and the number of eggs matured. Similarly, no significant relationship was found for C. salinarius (Shelton 1972).

Body-weight as a measurement of female or fat-body size, may exert its effect on egg production either at the "initiation" or "promotion" phase of oögenesis. Thus the number of oöcytes that reach the resting stage in the ovary of a mosquito can depend on the size of the ovary, which depends on the physical size of the female (Colless & Chellapah (1960), the nutrition of the larva and the nutrition of the adult (Clements 1963). The fat-body of

Ae. aegypti synthesises yolk-protein (Hagedorn & Fallon 1972) and therefore it is possible that stored reserves contribute directly to the maturation of resting stage oöcytes; they may do this indirectly by allowing the mosquito to use more of a blood-meal for egg maturation instead of for general metabolism. That a blood-meal can be used for general metabolism and not for egg maturation has been shown for mosquitoes (MacDonald 1956) and suggested for P. papatasii (Adler & Theodor 1935).

In the present study, it was shown that for sugar-fed females of L. longipalpis there is a small but significant, positive correlation between body-weight (at feed) and the number of eggs matured. This relationship is usually masked by that of meal-weight to egg production.

There is evidence that for this sand-fly species female body-weight is governed by larval nutrition: Thus, the mean weights of female pupae increased after colonization - almost certainly because the laboratory diet for larvae was better balanced than the natural one (Section 1). Further, the mean weight of females at emergence (which is proportional to the larval weight at the end of the feeding period) was higher when they were reared as larvae at 25°C rather than 28°C; at 28°C the metabolic rate was higher and the feeding period shorter.

However, as the resting stage oöcytes in each ovary could not be counted it can only be speculated whether larval nutrition affects egg production in L. longipalpis. In natural populations, variation in female body-weight as

a result of differences in larval nutrition are probably far greater. This variation could help, through its effect on egg production, to co-ordinate the size of larval populations with food supply.

The effect of sucrose feeding on egg production: The weight of a L. longipalpis female at the time of blood-meal was proportional to her weight at emergence (4 - 6 days before), but those feeding on a sucrose solution lost little or no weight in this period, whereas the weight of those offered only water fell by 24%. The most likely explanation is that those deprived of sucrose lost weight because food reserves in their fat bodies were utilized but not replenished. In this connection, Clements (1956) found that the fat body of mosquitoes shrinks to almost nothing in the first few days of adult life, but later becomes massive from nectar feeding. Dolmatova (1946) reported a similar phenomenon in P. papatasii.

Alternatively, it might be argued that as sucrose is a phagostimulant for sand-flies (Chaniotis 1975) the weight differences could simply reflect the amount of fluid imbibed by each group. However, for the weight differences involved (0·05 - 0·10 mg) the abdomens of sugar-fed flies would have been noticeably swollen; this was not so. It is also unlikely that those offered only water lost weight through failing to replenish their metabolic water - for females that have been kept without water will drink when damp filter paper is offered.

Sugar-feeding was not essential for oögenesis in females of L. longipalpis. Unsworth & Gordon (1946) found the same to be true for laboratory-reared P. papatasii.

The regression coefficients for eggs matured on weight of human blood-meal were the same for the groups offered and denied sucrose, However, fewer eggs were matured (per mg of blood) by those which had been denied sucrose, i.e. there was a more-or-less constant (not proportional) difference between groups. The constancy of this difference suggest that without a supply of carbohydrate before the blood-meal, females of L. longipalpis resorb some of the resting stage oöcytes in their ovaries. Presumably, in nature a female's need to find a source of carbohydrate will depend on larval nutrition and how soon after emergence she obtains a blood-meal.

The effect of female age on egg production: With increasing age the mean weight of groups of females of L.longipalpis fell, even though sucrose had been offered from emergence. Also, the number of eggs matured on a given weight of human blood was less for older flies. That these differences were only significant for the smallest blood-meals suggests that they resulted from oösorption in the "promotion" phase and not the "initiation" phase of oögenesis. This also indicates that the fat-body reserves of the females do, in part, influence egg production.

The effect of environmental factors on egg production:

Temperature affects a multiplicity of enzymatic reactions in insects and, as each has its own characteristics, the observed effect on egg production is the overall balance among them (Wigglesworth 1965).

It is true for many insects (Engelmann 1970), and L. longipalpis is no exception, that the temperature limits for reproduction are much narrower than those for other activities. In its cave of origin, the strain of L. longipalpis from Belo Horizonte rarely encounters a daily temperature outside the range of 30° to 18°C. In the laboratory, females survive for several days when kept at 18°C without sucrose, but oögenesis is impaired at temperatures of 20°C or less. Between 22·5° and 30°C the relationship between the number of eggs matured and the weight of blood imbibed was constant, but at 20° ± 1°C some of the females did not digest all the blood imbibed and therefore matured fewer eggs.

By recording the first appearance of the dark faeces that mark the end of digestion, the rate of digestion at these different temperatures was estimated. These figures are very approximate because observations were made only once a day (at 10.00 hrs). However, a trend appeared which indicates that the rate of digestion increases proportionally with temperature. Between 20° and 30°C there was a Q_{10} of about 2·0. This is of the same order found for Ae. aegypti (Williams 1956), An. maculipennis (Shlenova

1938) and P. papatasii (Dolmatova & Demina 1971) for the temperature range 15° to 35°C. For P. papatasii, however, the Q_{10} relationship did not hold below 20°C - digestion at 18° to 21·5C took twice as long as it did at 22° to 25°C. Interestingly, temperate species of sand-flies are only active as adults when the daily temperature rises above about 20°C e.g. Rioux et al. (1969) and Dolmatova & Demina (1971).

The effect of temperature on egg production through larval nutrition has already been mentioned but it also affects the egg production of a population by controlling the number of gonotrophic cycles that a female is able to complete in her life.

Humidity. In many insects a significant proportion of the total water loss occurs through the respiratory surfaces; during periods of active metabolism the spiracles are open and water loss increases (Edney 1957). Therefore, in dry environments one might expect the digestion of a blood-meal to take longer - the oxygen supply being restricted by the insect's reluctance to open its spiracles. Observations on several Indian species of Anopheles showed that during the hot dry months blood digestion took 2 - 3 days longer than it did in the more humid months (Mayne 1928).

A slower rate of digestion and/or a water imbalance resulting from a low humidity could reduce egg production. However, as pointed out by Engelmann (1970) unless the atmosphere has a very low humidity most blood-sucking insects obtain sufficient water with their food . For

females of L. longipalpis completing digestion of the blood-meal the number of eggs matured (per mg of human blood) was not affected at 25°C by humidity in the range 60% - 95% RH . The effect of the atmosphere of 60% RH on egg production was all-or-nothing - significantly more females failed to digest their blood-meals at 60% RH compared with 95% RH.

The egg production of a population of L. longipalpis: This depends, in part, on the proportion of females that digest their blood-meal, which is influenced by environmental factors, the physiological condition of females and perhaps the nature of the meal. In the present study no host adaptation was observed but the % of females failing to digest a blood-meal was higher for those groups that were deprived of sugar, kept at a low temperature or not offered a blood-meal within 6 days of emergence.

Mating and egg maturation: For the females of many hemimetabolous insects mating is a stimulus for egg maturation, but for most holometabolous species it affects oviposition rather than oögenesis (Engelmann 1970). After revieving this subject Clements (1963) concluded that the evidence for the belief that some species of Anopheles will not mature eggs if unmated was inadequate; it was only oviposition that was affected by insemination. ~~More recently, however, Heale & Lea (1972) have shown that the sperm of some mosquitoes contains a factor which stimulates egg maturation.~~

Females of P. papatasii (Dolmatova 1942) and of three North American species of sand-flies (Chaniotis 1967) have been shown to mature normal-size eggs when unmated, and there is circumstantial evidence that the same is true for L. gonezi and L. sanguinarius in Panama (Johnson & Hertig 1961). Unmated females of L. longipalpis, too, mature and lay normal-size eggs after a blood-meal. There may have been a tendency for the unmated females to mature more eggs than the mated for human blood-meals of less than 0·3 mg. Without any interference from males, unmated females may have been able to take sugar meals more regularly and not wasted energy in mating activities. Such group interactions have been reported for Drosophila melanogaster (Pearl 1932).

When seen, wild populations of L. longipalpis have contained a good proportion of males (Killick-Kendrick et al. 1975). Mating occurs before, during or after a blood-meal in the laboratory. Thus unless male-density is a means of regulating population size it cannot be to the advantage of the relatively short-lived sand-fly to delay egg maturation until mating has occurred.

In conclusion: In L. longipalpis, the digestion of the blood-meal and the maturation of the eggs are concurrent. The results of the present experiments support the idea that the blood-meal is the source of nutrients for vitellogenesis, and that the maximum number of oöcytes that can be matured depends on female body-weight, sugar-feeding and

age. Egg maturation is normal within a wide range of temperature (22·5° - 30°C) and humidity (60% - 100% RH), and does not depend on mating.

The ingredients in the blood-meal essential for egg maturation are amino acids . The number of eggs matured by a female is proportional to their concentration in the meal.

The red cell fraction of a blood-meal is digested less rapidly than the plasma which means that much of it is not made available for vitellogenesis. In this respect, there are differences between the red cells of hamster and human blood which may explain in part the greater efficacy of hamster blood for egg production. However, differences in the composition of the stroma of the two types of cell account for some of this superiority.

For L. longipalpis the quantitative effect of different vertebrate bloods on egg production is surprisingly similar to that reported for the mosquito Ae. aegypti. Supported by the findings from artificial feeding experiments, this suggests that these blood-sucking nematocerans do not have nutritional needs for egg production that can be met only by specific hosts.

It is usually argued that the blood-sucking habit has been retained to supplement a poor larval diet (Downes 1958), but its retention would seem to be a positive advantage even when larval nutrition is adequate. Blood-meals enable the female sand-fly to maintain its water-balance when the humidity is low (Theodor 1936), and being a concentrated

source of nutrients can provide for additonal egg batches without causing the fly to spend long periods away from the optimal environment - a humid nook where predators and violent fluctuations of temperature are rare.

SECTION FOUR :

THE EFFECT OF PHOTOPERIOD AND OTHER FACTORS ON EGG-LAYING BY L. Longipalpis.

INTRODUCTION.

The difficulty of inducing female sand-flies to lay most of their eggs and thereafter to survive sufficiently long to begin a second gonotrophic cycle has long been recognised as the most serious hindrance to establishing a sand-fly colony (review, Unsworth & Gordon 1946). None of the reports of attempts to rear sand-flies have stated that this phenomenon is unnatural, but from the efforts made to improve oviposition success one might assume that it was thought so.

Working with P. papatasii, Waterson (1922) developed the method of confining engorged females in damp, earthenware pots to provide both the humidity necessary for their survival, and a surface damp enough to stimulate egg-laying without trapping them on droplets of condensation. In a similar vein, Shortt et al.(1926) carefully regulated the physical conditions in which they maintained engorged P. argentipes and thereby induced many to pass through 2 or 3 gonotrophic cycles. Adler & Theodor (1935) were similarly successful with P. papatasii. However, later workers have shown that a fine regulation of temperature and humidity are not sufficient to induce many other species to survive more than one gonotrophic cycle (Chaniotis 1967; Foster

et al. 1970; Gemetchu 1972; Ward 1974).

One of the commonest explanations for the "premature" death of female sand-flies is that egg-laying is "exhausting" (Newstead 1911; Whittingham & Rook 1923; Chaniotis 1967; Dolmatova & Denina 1971). In this connection, Chaniotis (1975) has shown that for L. trapidoi sugar-fed females lay more eggs than those deprived of sugar.

Often, attempts have been made to improve the oviposition performance of sand-flies by maintaining them in "natural" chambers containing pebbles, soil and faeces (Smith 1925; Unsworth & Gordon 1946; Najera 1949; Safyanova 1964; Vattier-Bernard 1968; Foster et al.1970). From the knowledge available of habitats where sand-fly larvae live, such conditions should be optimal for egg-laying. For example, Whittingham & Rook (1923) reported that P. papatasii females usually lay their eggs underneath pieces of earth, stones or insect remains. Similarly, Hanson (1961) collected Panamanian sand-fly larvae from the soil at the base of trees, and Perfil'ev (1968) records that sand-fly larvae have been found in Russia in the soil and faeces on the floor of rodent burrows. However, despite all the work that has been carried out only twice has it been reported that natural chambers improved egg-laying success. Foster et al.(1970) found that engorged P. longipes laid more eggs and lived longer after oviposition when they were kept in cages (not vials or tubes) containing damp pots filled with organic materials. In "natural" chambers,

females of P. papatasii have survived for 4 gonotrophic cycles (Perfil'ev 1968).

In the present study, some external factors that affect egg-laying by laboratory-reared L. longipalpis are described. Particular attention was paid to the effects of photoperiod. The influence of photoperiod on egg-laying does not seem to have been investigated before for sand-flies, although its effect of entraining circadian egg-laying rhythms has been well studied for Ae. aegypti (Gillett et al. 1959; Haddow et al.1961) and other insects (review, Engelmann 1970).

METHODS.

The rearing methods and oviposition tubes have already been described in Section 1. Unless otherwise stated, females were fed on human blood and then maintained in carefully controlled conditions in environmental cabinets set at 25°± 1°C and 12 hours light (06·00 - 18·00 hrs GMT).

In some early experiments the filter papers provided for oviposition were dampened with water daily from the day of the blood-meal. Later, water was only applied from the 4th day after a blood-meal; in this way fewer females were lost through trapping themselves on damp surfaces.

For the photoperiod experiments, eggs were counted at 06·00, 12·00, 18·00 and 24·00 hours. A dark-red light was used when counting egg-lays during a dark period. A photoperiod of 13 hours (22·00 - 09·00 hrs GMT) was chosen as that most close to the natural one in Belo Horizonte at the time of year when the experiments were carried out. Later, 18 hour photoperiods were used, so that the dark period was not interrupted even by dark-red light.

RESULTS.

Time of ovulation.

4, 5 and 6 days after a human blood-meal 12, 8 and 8 (respectively) females that had not oviposited were killed and dissected to determine the location of the eggs they had matured.

With one exception, the eggs in females dissected 4 days after a feed lay closely stacked in two discrete groups. In contrast, many of the eggs in those females dissected 5 and 6 days after a feed were more loosely associated and often floated away singly once the abdomen had been opened.

It is concluded that at 25°C many eggs were passed from the ovaries into the oviducts during the 48 hours after they had reached maturity.

Fertility of retained eggs.

7 days after a human blood-meal 5 living females and 5 females that had died in the preceeding 24 hours were dissected and the eggs they contained set up in larval rearing pots. All of these females had laid some eggs, and each egg batch was la ter found to be fertile. In addition, 10 females which had not laid any eggs were killed 7 days after a blood-meal and their eggs similarly removed and set up to observe if they hatched.

For the retained egg-batches from living flies, 0/54, 2/33, 0/26, 0/15 and 0/40 eggs hatched. For the retained egg-batches from dead flies which had laid some eggs, 0/9, 0/27, 2/19, 0/42 and 0/21 eggs hatched. All of the retained eggs from females that had not oviposited failed to hatch.

Most of the eggs dissected from all of these flies had tanned (to a dark brown) within 2 hours of their removal from the fly. In other experiments, too, it was

noted that most (often all) of the mature eggs removed from flies which had been dead 1 - 10 days were tanned. A similar rate of tanning has been observed for normally laid eggs.

Notes on behaviour before and during oviposition.

During the first day in the oviposition tubes engorged L. longipalpis were noticeably active. It was at this time that many died after squeezing themselves between the dampened filter paper and the side of the tube. In the following 2 - 3 days most females remained inactive, either resting on the filter paper or inverted on the gauze top of the tube. From the 4th day after a blood-meal (at 25°C) the gravid females became more active, and the first eggs were laid.

For females that survived at least 12 hours after the first egg had been laid, the following behaviour was observed: Each egg was gently laid by the female squatting and drawing the tip of her abdomen forward over the damp surface as the egg was extruded; the female waited for at least several minutes before walking a short distance and laying another. The result was that eggs were widely scattered on any damp surface within the tube.

Often, however, a female died shortly after the first egg had been laid. Sometimes she was found trapped on a damp surface with a pile of eggs beside her, and more being rapidly extruded. At other times dead females were discovered with a small trail of attached eggs stretching

behind them like a string of sausages.

Indices of egg-laying success.

For many of the experiments to determine the effect of various factors on egg-laying there was no significant difference in the proportion of females that laid at least some eggs. Nevertheless the mean number of eggs laid in different conditions showed a wide variation. Therefore, the index of "% of females laying" was not considered helpful.

The use of "the number of eggs laid" as a way of recording the periodicity of egg-laying in different photoperiods was considered valid because the females concerned had all taken large meals. However, the groups of females used for the other experiments showed a wider range of meal-weights. Thus although these experiments were carried out by randomly splitting otherwise uniform stock, there remained the possibility that an index of egg-laying success based on the absolute number of eggs laid could depend on the chance selection of uneven blood-meal weights.

With these considerations in mind, the mean % of mature eggs retained by a group of flies was used as an index of the unsuitability of different egg-laying conditions. When tested in two different laboratory-reared generations (2 and 6) it was found that this index was independent of the number of eggs matured (Fig. 38), which confirmed its suitability.

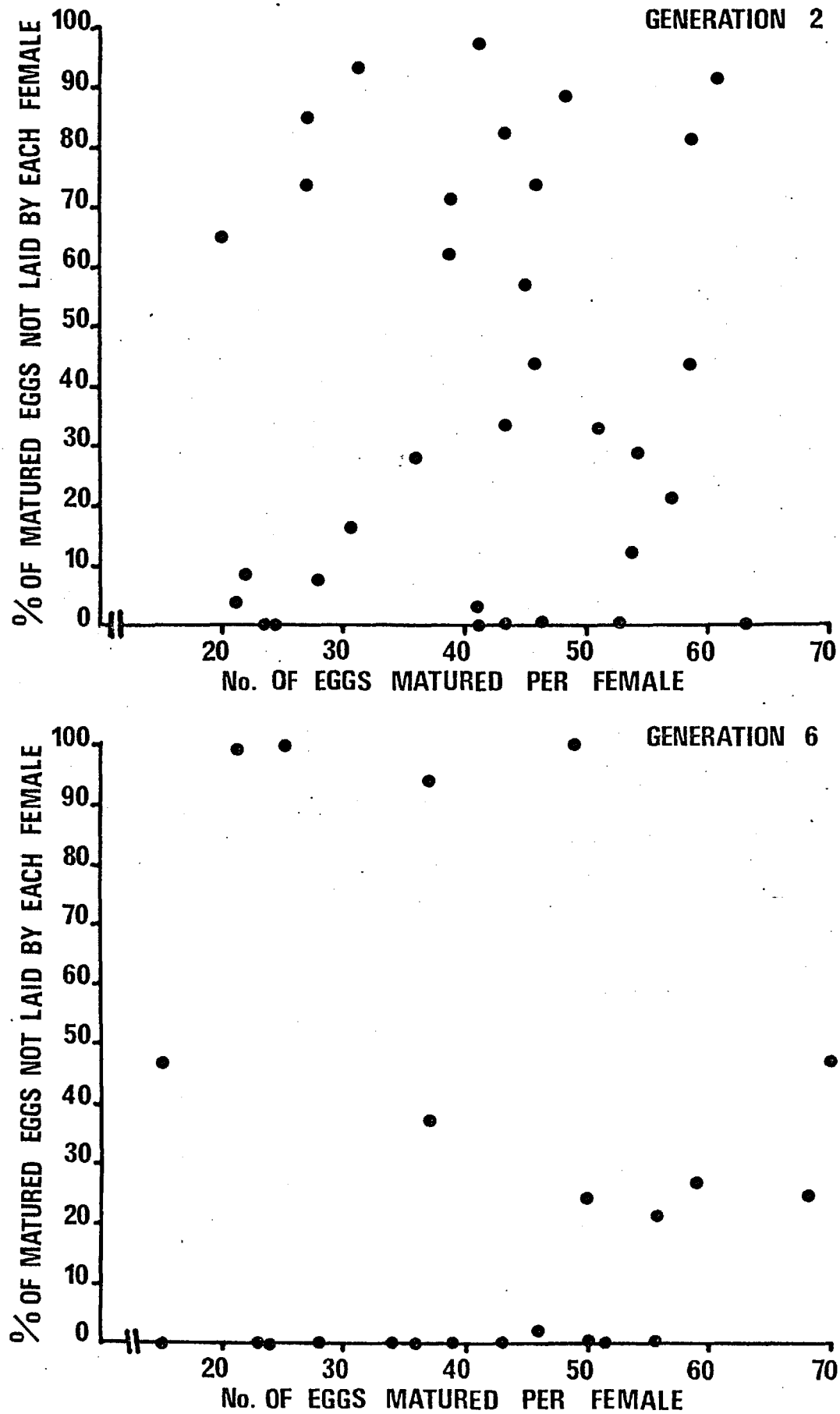


Fig. 38. The effect of egg-batch size on the % of matured eggs that are successfully laid.

Factors controlling the retention of matured eggs.

These results refer to paired (i.e. controlled) experiments; they are set out in Table 27. The "% of eggs not laid" is a mean for all females in a group which survived to digest a blood-meal; this includes those that failed to lay any eggs.

The post-oviposition longevities have not been set out because they were always low (1 - 5 days) and varied within groups as much as they did between treatments. 80 - 90% of all females died within 24 hours of oviposition.

The effect of photoperiod on egg-laying.

The effect of photoperiod on the periodicity of egg-laying is set out in Table 28. Each experiment has been given a number so that the effect of photoperiod on the duration of egg-laying (Table 29) and the distribution (in time) of egg-lays (Figs. 39 - 42) could be set out without repeated reference to the conditions experienced by the preimagines and prefeed adults. Therefore, Table 29 and Figs 39 - 42 should be used with reference to Table 28. All these results refer only to eggs laid by females in the first 6 hour period of egg-laying. This has been done deliberately, because relatively few eggs were laid after the first spell, and those few were likely to have been laid involuntarily by dying females.

For experiment 11, many of the eggs laid in the day quarter 18·00 - 24·00 must have been deposited before the start of the dark period (at 22·00 hrs) because they

were well-tanned at 24·00 hours.

With reference to Table 28 the relative effects of different photoperiods on the periodicity of egg-laying can be tested by comparing the total % of eggs laid in each 6 hour observation period with the even-distribution value of 25%. Assuming a binomial distribution, the standard deviation (S.D.) for each reading is given by $\sqrt{\frac{pq}{n}}$ where p = % value expected, q = (100 - p) and n = total number of observations (Bailey 1968). In the present situation S.D. = $\sqrt{\frac{25 \times 75}{100}}$ = 4·3. Therefore, the 95% confidence limits are ± 8·8, i.e. any percentage outside of the range 16·2% - 33·8% is significantly different from the expected mean of 25%.

The mean % of eggs not laid by the groups of flies experiencing different photoperiods varied from 30·1% to 44·9 %. There was no obvious pattern: Thus, a mean of 44·9± 15·6% of matured eggs were retained by 22 flies ovipositing in a regular 24 hour cycle (Experiment 11a), and a mean of 35·6± 14·2% of matured eggs were retained by 28 flies ovipositing arhythmically (Experiment 12(b)).

Table 27. Factors controlling the retention of matured eggs by L.longipalpis. (*Significant differences between pairs of readings.)

EXPERI-MENT	CONDITIONS	TREATMENTS	No. OF FEMALES TESTED	MEAN % OF EGGS NOT LAID (RANGE 0-100 FOR ALL)	±	95% CONFIDENCE LIMITS	POST-FEED LONGEVITY (DAYS)	+	RANGE (DAYS)
1.	25°C.,70%RH. 4 - 6 days old at blood-meal. Watered from blood-meal.	30% sucrose all life	19	47.5 *	±	19.4	9.5		(4 - 15)
		No sucrose all life	16	83.4 *	±	17.1	6.5		(4 - 11)
2.	25°C.,70%RH. 4 - 6 days old at blood-meal. Watered from blood-meal.	Sugar all life	15	38.6 *	±	19.5	8.5		(4 - 12)
		No sugar all life	13	76.2 *	±	12.1	6.0		(4 - 10)
3.	4 - 6 days old at blood-meal. Watered from blood-meal. Sugar all life.	30°C. (100%RH.	15	39.0	±	22.2	4.5		(3 - 6)
		(80%RH.	14	38.5	±	22.0	7.5		(3 - 11)
		25°C. (100%RH.	15	48.3	±	19.4	6.0		(4 - 8)
		(95%RH.	13	20.9)*	±	16.2	5.0		(4 - 8)
		(80%RH.	16	34.5)*	±	17.1	7.0		(4 - 15)
		(60%RH.	14	49.0	±	22.7	10.5		(5 - 16)
		20°C. (100%RH.	12	53.8	±	24.1	11.0		(8 - 15)
		(80%RH.	11	62.9)*	±	23.0	12.5		(7 - 16)
		(60%RH.	9	76.8)*	±	19.8	11.0		(9 - 15)

Table 27, continued.

EXPERI-MENT	CONDITIONS	TREATMENTS	No. OF FEMALES TESTED	MEAN % OF EGGS NOT LAID (RANGE 0-100 FOR ALL)	±	95% CONFIDENCE LIMITS	POST-FEED LONGEVITY (DAYS)	+	RANGE (DAYS)
4.	25°C.,95%RH. Sugar all life. Watered from 4th. day after blood-meal.	Age at blood-meal:							
		2 - 3 days	20	26.4	±	15.7	5.0		(4 - 11)
		4 - 6 days	12	27.0	±	16.7	7.0		(4 - 11)
		11 - 12 days	15	36.2	±	19.5	4.5		(4 - 6)
		15 - 16 days	9	64.4	±	31.2	4.5		(4 - 5)
5.	25°C.,95%RH. Sugar all life. All females 4 - 6 days old at blood-meal. Watered from 4th.day after blood-meal.	Oviposition surface:							
		White filter paper.	14	28.6	±	18.0	6.0		(4 - 8)
		White filter paper impregnated with rabbit faeces.	13	33.9	±	20.2	5.5		(4 - 8)
		Rabbit faeces.	15	37.0	±	24.8	6.0		(4 - 9)
6.	25°C.,100%RH. Sugar all life. All females 4 - 6 days old at blood-meal. Watered from 4th. day after blood-meal.	Hamster blood-meal	18	38.4	±	16.8	5.0		(4 - 7)
		Human blood-meal	13	34.0	±	18.5	7.0		(5 - 11)

Table 27, continued.

EXPERI-MENT	CONDITIONS	TREATMENTS	No. OF FEMALES TESTED	MEAN % OF EGGS NOT LAID (RANGE 0-100 FOR ALL BUT γ)	±	95% CONFIDENCE LIMITS	POST-FEED LONGEVITY (DAYS)	+ RANGE (DAYS)
7.	25°C.,100%RH. Sugar all life. All females 4-6 days old at blood-meal. Watered from 4th. day after blood-meal.	Mated - fertile eggs laid	11	37.1 *	±	23.6	8.0	(7 - 11)
		Unmated	14	80.0 *	±	18.8	10.5	(7 - 14)
8.	25°C.,100%RH. Sugar all life. All females 4-6 days old at blood-meal. Watered from 4th. day after blood-meal.	Mated - fertile eggs laid	17	32.9 *	±	15.7	-	-
		Unmated	17	62.7 * γ (30.3 - 100.0)	±	12.2	-	-
9.	25°C.,100%RH. Sugar all life. All females 4-6 days old at blood-meal. Watered from 4th. day after blood-meal.	Mated - fertile eggs laid	12	34.5	±	24.7	-	-
		Unmated	8	65.7	±	24.5	-	-

EXPERIMENT	CONDITIONS FOR PREIMAGINES	CONDITIONS FOR PREFEED ADULTS	CONDITIONS FOR POSTFEED ADULTS	TOTAL EGGS LAID
	24 6 12 18 24HR.	24 6 12 18 24HR.	24 6 12 18 24HR.	
			% OF TOTAL EGGS LAID IN EACH QUARTER	
10 a	26.5°C	28°C ↓	28°C	909
			84·7 0 5·9 9·3	
10 b	26.5°C	28°C ↓	28°C	611
			69·1 8·2 0 22·7	
11 a	26.5°C	26.5°C ↓	26.5°C	453
			42·0 0·2 0 57·8	
11 b	26.5°C	26.5°C ↓	26.5°C	1,214
			63·7 7·8 10·1 18·6	
11 c	26.5°C	26.5°C ↓	26.5°C	963
			35·2 18·7 23·1 23·0	

Table 28. The effect of photoperiod on the periodicity of egg-laying by L.longipalpis.

↓ = time of feed.

EXPERIMENT	CONDITIONS FOR PREIMAGINES (24 6 12 18 24HR.)	CONDITIONS FOR PREFEED ADULTS (24 6 12 18 24HR.)	CONDITIONS FOR POSTFEED ADULTS (24 6 12 18 24HR.)	TOTAL EGGS LAID
			% OF TOTAL EGGS LAID IN EACH QUARTER	
12 a	26.5°C	26.5°C	26.5°C ONCE — 22·6 11·1 13·1 53·3	752
12 b	26.5°C	26.5°C	26.5°C — 14·9 12·6 36·6 36·1	1,003
13 a	26.5°C	26.5°C	26.5°C — 17·0 16·2 56·1 11·0	926
13 b	26.5°C	26.5°C	26.5°C — 10·3 24·2 9·4 46·6	820

Table 28, continued.

Table 29.

The effect of various photoperiods on the duration of egg-laying

EXPERIMENT	No. OF FEMALES TESTED	NUMBER OF 6-HOUR PERIODS FROM FIRST TO LAST EGG-LAY								
		1	2	3	4	5	6	7	8	9
10 a	27	12	3	1	3	3	1	2	1	1
b	18	10	1	3	1	2	0	1	0	0
11 a	18	18	0	0	0	0	0	0	0	0
b	36	22	6	7	0	1	0	0	0	0
c	32	25	4	1	2	0	0	0	0	0
12 a	21	19	0	0	0	0	2	0	0	0
b	24	23	1	0	0	0	0	0	0	0
13 a	32	21	5	0	1	3	0	0	0	0
b	28	13	9	4	2	0	0	0	0	0

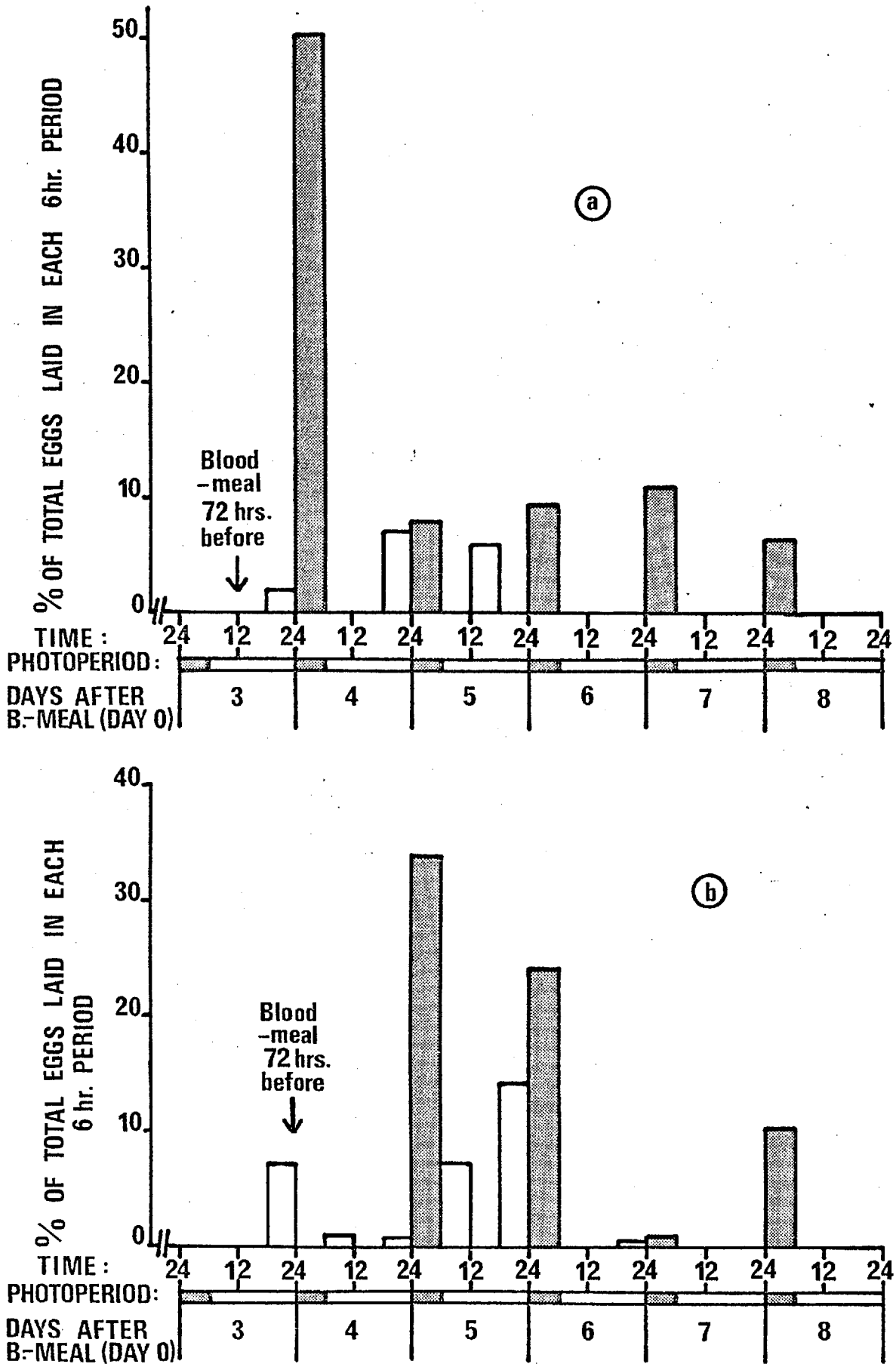


Fig. 39. Distribution of egg-lays for Experiment 10.
(See Table 28 for details.)

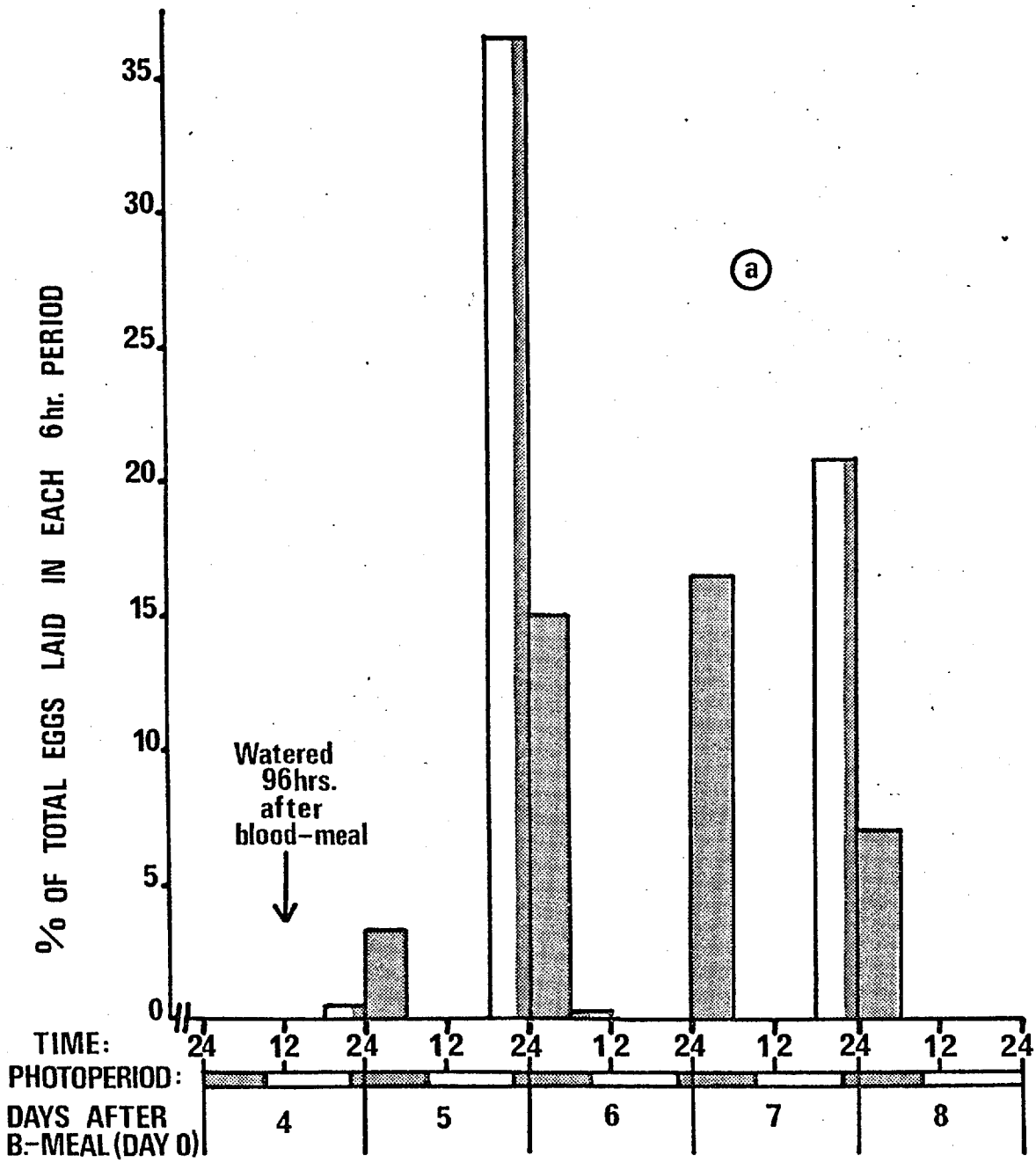


Fig. 40. Distribution of egg-lays for Experiment 11.
(See Table 28 for details.)

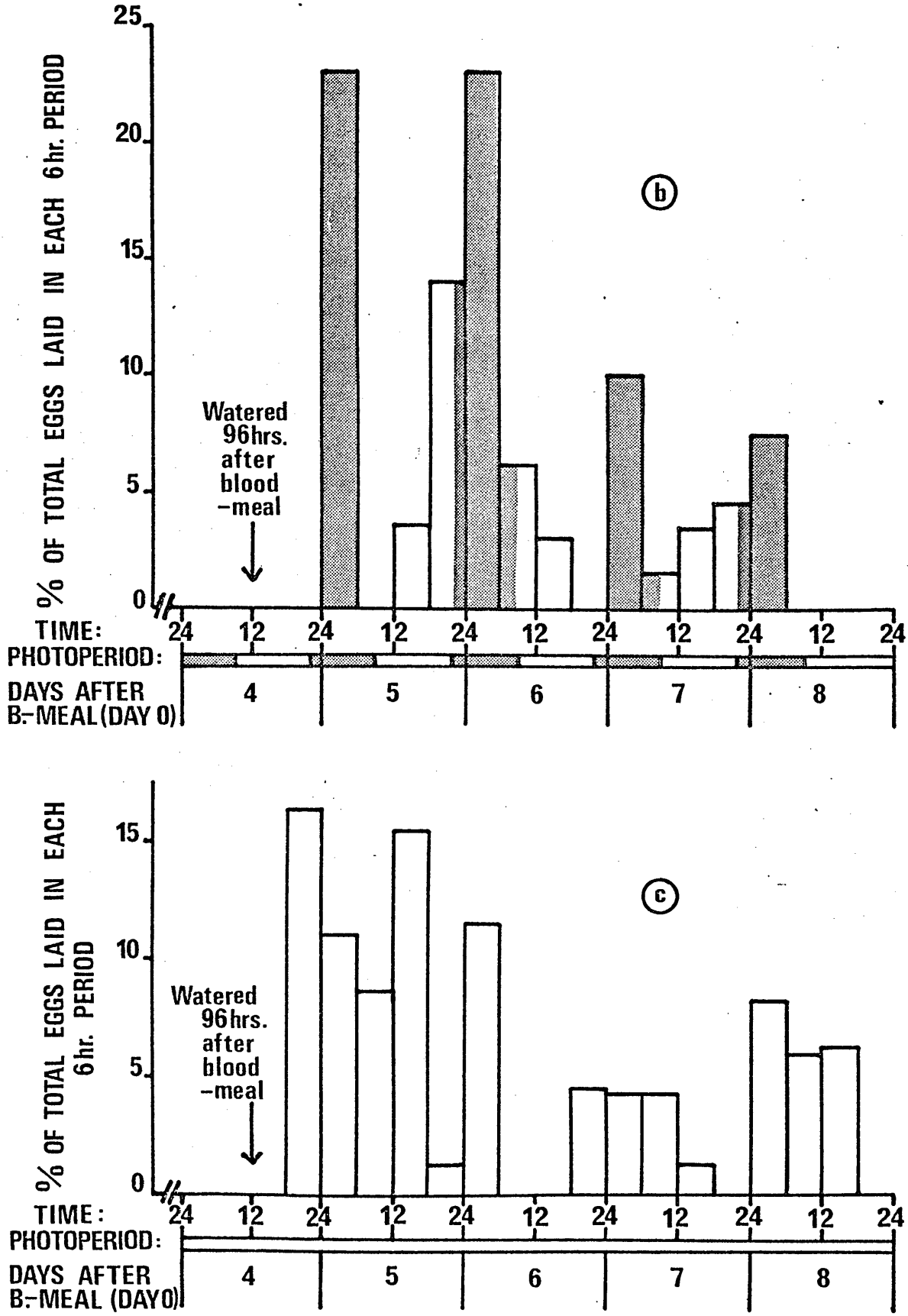


Fig.40, continued. Distribution of egg-lays for Experiment 11.

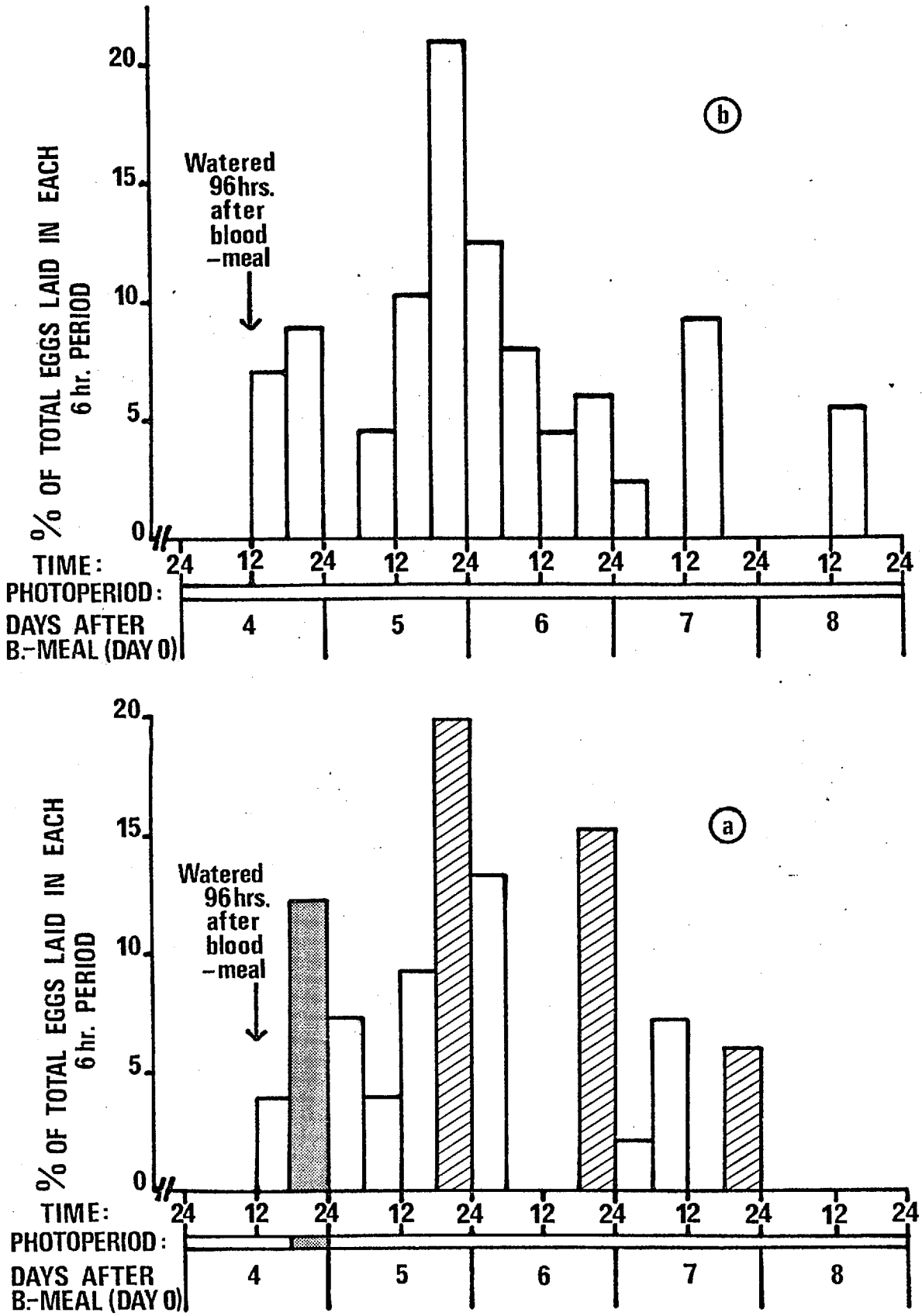
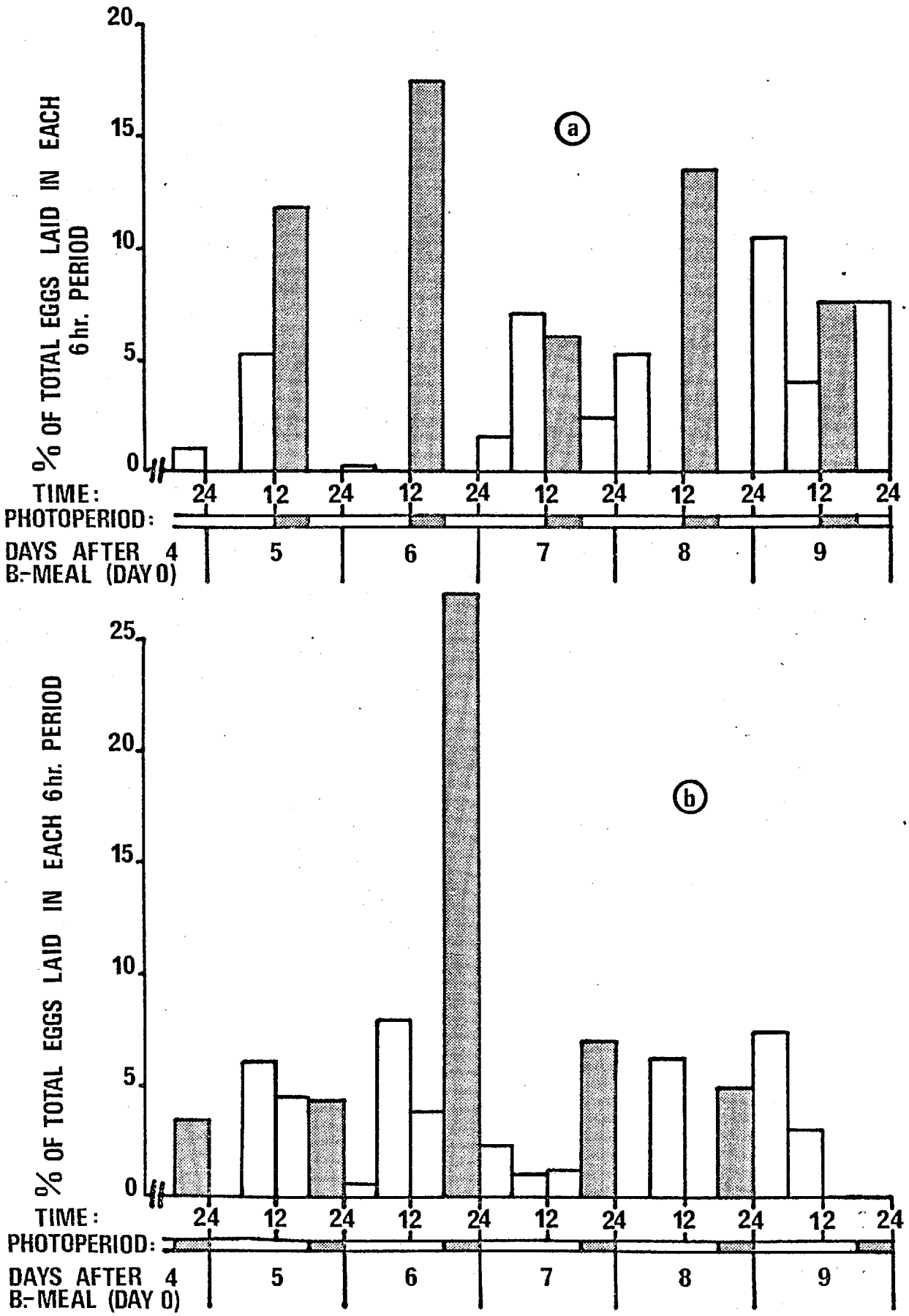


Fig. 41. Distribution of egg-lays for Experiment 12.
(See Table 28 for details.)

Fig. 42. Distribution of egg-lays for Experiment 13.
(All oviposition filter-papers watered 96 hours after blood-meal. (12.00,Day 0) See Table 28 for details.)



DISCUSSION.

Ovulation, the process by which a matured egg is passed out of the ovary into the oviduct, can be dependent on an external stimulus, like the need for inpregnation in Glossina; in many insects, however, the eggs are set free in rapid succession as they reach maturity (Wigglesworth 1965). Ovulation in L. longipalpis follows shortly after the eggs are matured, and is largely independent of mating; the eggs are not normally fertilized until shortly before they are laid.

Most reports of egg-laying by sand-flies have failed to distinguish between ovulation and oviposition, e.g. Whittingham & Rook (1923), Unsworth & Gordon (1946), Chaniotis (1967), Foster et al. (1970), Ward (1974). They have tacitly assumed that the difficulty with which laboratory-reared sand-flies lay their eggs results from an environment unfavourable for oviposition. The effect on egg-laying success of ovulation and other internal processes could be a profitable study. For example, some L. longipalpis females laid strings of eggs laid end-to-end; Foster et al. (1970) observed the same for P. longipes. The accessory gland secretions may have been unusually glutinous in these instances and thereby prevented normal oviposition.

Some external factors were shown to have an effect on the egg-laying success of L. longipalpis. In 2 out of 3 experiments, unmated females retained a significantly

higher percentage of eggs than mated females. The virgin females of Diptera often retain most of the eggs they mature, (review, Engelmann 1970), although one notable exception is the Lagos strain of Aedes aegypti (Gillett 1956). Infertility is a factor that has not been considered before when the egg-laying performance of sand-flies has been examined. For the strain of L. longipalpis from Belo Horizonte, however, it can only have been of minor importance (see Section 1).

On several occasions it has been suggested that many sand-fly females fail to survive after the first gonotrophic cycle because egg-laying is an exhausting process (Whittingham & Rook 1923; Chaniotis 1967; Dolmatova & Demina 1971). There is some evidence to support this view. Chaniotis (1975) has shown that females of L. trapidoi laid significantly fewer eggs when they were deprived of sugar. Similarly, the percentage of eggs retained by L. longipalpis was significantly higher for groups of flies deprived of 30% sucrose. Further, there may have been a tendency (not significant) for old (15 - 16 days) L. longipalpis females to retain a higher percentage of eggs, than younger flies, and for the younger, sugar-fed flies to have a higher post-feed longevity. The finding that the % of mature eggs laid by a female is independent of the number of eggs she matures, seems to contradict the exhaustion hypothesis. This could be a misleading result, however, for it is possible that many of the larger egg-lays come from dying flies; the last-minute extrusion of

eggs by dying sand-flies is reminiscent of the rapid oviposition of decapitated or "shocked" mosquitoes (Clements 1963).

The optimum physical conditions for egg-laying by L. longipalpis females were a temperature of 25°C and a high humidity. Similar results have been recorded for P. papatasii (Adler & Theodor 1935) and P. longipes (Gemetchu 1972). As discussed earlier these conditions probably relate to those occurring in the humid nooks where engorged sand-flies are believed to rest (Whittingham & Rook 1923; Unsworth & Gordon 1946). Under experimental conditions, there was a suggestion that an atmosphere of 95% RH might have been better than one of 100% RH for egg-laying by L. longipalpis. Empirically, it has been found that 95% is the optimum RH for the routine, mass culture of this species; the slight saturation deficiency compensates for any excess dampening of the oviposition filter-papers, which commonly occurs when large numbers of flies are being handled.

Even under optimum physical conditions, sugar-fed L. longipalpis females often failed to lay many of their eggs, as did many females of P. papatasii (Dolmatova & Demina 1971), L. vexator (Chaniotis 1967), P. longipes (Foster et al.1970; Gemetchu 1972) and L. tranidoi (Chaniotis 1975). For sand-flies, "premature" death and poor oviposition success may not be an unnatural occurrence; it is not incompatible with the fact that a species is a known vector of leishmaniasis because the females of several vector species have been shown to suck blood more than once in a

gonotrophic cycle (Adler & Theodor 1935; Johnson & Hertig 1961; Gemetchu 1972). Moreover, since Foster et al. (1970) and Ward (1974) have shown that the presence of granules in the accessory glands is not a certain indication of a parous condition doubt has been thrown on the belief that many species pass through more than one gonotrophic cycle in nature.

Some sand-fly populations have been induced to pass through 2 or more gonotrophic cycles in the laboratory: Up to 50% of wild-caught P. argentipes did so (Shortt et al. 1926); and P. papatasii females have been reported as not infrequently laying 4 egg batches (Perfil'ev 1968). The reasons for these successes are unclear.

Recognising that the female sand-fly probably requires a number of oviposition stimuli, many workers have provided mixtures of soil and faeces, often in large breeding chambers, to simulate natural breeding sites (review, Unsworth & Gordon 1946; Najera 1949; Safyanova 1964; Vattier-Bernard 1968; Foster et al. 1970). In such conditions it is always difficult to count the number of eggs that have been laid. However, only Foster et al. (1970) has claimed that these more natural oviposition environments benefit egg-laying and post-oviposition survival. In the present study, the provision of rabbit faeces or filter-papers impregnated with rabbit faeces did not improve the egg-laying success of L. longipalpis held in glass vials.

Ward (1974) showed that females of the Ceará strain of

L. longipalpis laid significantly more eggs when the white filter-paper on which they oviposited was folded to simulate the cracks and crevices in which they naturally lay their eggs. Similar experiments have been carried out with the Belo Horizonte strain of L. longipalpis, but the worth of corrugated papers has not been proven for this strain (A.J. Leaney pers.comm).

For the first day after a blood-meal, L. longipalpis females held in glass vials were active. In nature they are probably seeking a resting site during this period (cf. the relocation of tagged flies described in Section 5). The need for an engorged female to spend some time after a blood-meal searching for resting sites might be a "drive" that is not being satisfied in laboratory conditions. However, when L. longipalpis females have been kept in rearing cages for 1-4 days after a blood-meal many have died and the others have not laid any more eggs than normally reared flies (Unpublished data; and A.J. Leaney pers.comm.).

The effect of photoperiod on egg-laying by L. longipalpis:

There were no significant differences between the overall percentages of matured eggs that were successfully laid by groups of females held in different photoperiods. Further, none of the photoperiods significantly improved the post-oviposition longevity over that obtained using routine rearing methods.

Although the oviposition success of L. longipalpis was not altered by photoperiod, the periodicity of egg-laying was markedly affected. Significantly more eggs were laid in

day-quarters containing dark spells compared with those of continuous light. Also, when one half of a batch of flies was fed 12 hours after the other half, the last group to be fed showed a peak of egg-laying 24 hours and not 12 hours after the first; in both cases the peak coincided with the period of darkness between 24·00 hr and 06·00 hr.

Egg-laying was acyclical when females were kept from emergence in constant light. In contrast, for those maintained after a blood-meal in regularly alternating periods of 13 hours light and 11 hours dark, egg-laying was cyclical, with peaks in the dark periods. That most peaks in the cycle started just before a dark period strongly suggests that these were not solely a direct response to darkness; an endogenous, circadian rhythm is indicated, although more results will have to be obtained to confirm this.

The results for females kept in 18 hour photoperiods are equivocal, but suggest several points which should be clarified by further experiments using larger samples. Thus, when females were kept all their lives in an 18 hour photoperiod there was a tendency for egg-laying to be cyclical. This tendency was less noticeable when flies were reared as preimagines and pre-feed adults in continuous light. These results suggest that the cyclical egg-laying rhythm can be strengthened if it is entrained over a longer period.

There was a normal distribution of egg-lays when females were kept in constant light all their lives. In

comparision, females kept in constant light except for one 6 - hour period of dark (4 days after the blood-meal) laid their eggs in a more cyclical fashion. 53·3% of their eggs were laid between 18·00 hr and 24·00 hr on successive days, during the day-quarter when the solitary dark period had been given. Unfortunately, no firm conclusions can be drawn from this result because the peak of the normal distribution of egg-laying by the females kept in continuous light happened to coincide with the day-quarter 18·00 - 24·00 hr (see Fig. 41).

Undoubtedly, the results of the photoperiod experiments described here are less conclusive than they might have been because many of the eggs were laid involuntarily. Until a method is devised for keeping L. longipalpis females alive beyond the first gonotrophic cycle the full significance of photoperiod for egg-laying is unlikely to be discovered.

SECTION FIVE:

FIELD WORK.

During the period when I was working for this thesis, I was also able to investigate the behaviour of sand-flies in field conditions. The aspects of sand-fly behaviour studied were those that have a particular relevance to the epidemiology of leishmaniasis.

In 1971 - 72 there was an outbreak of Kala-Azar (K-A), or visceral leishmaniasis, in the foothills of the Apennines near Bologna, in the province of Emilia-Romagna, Italy (Pampiglione et al.1974a). Not only was the epidemic unprecedented (60 cases were reported, of which 13 were fatal) but the form of the disease was unusual (Pampiglione et al.1974b).

Foreign scientists of various disciplines were invited by Professor Pampiglione of Bologna University to visit the region to study the surviving cases and the probable vectors. A team from Imperial College Field Station investigated the biology of the region's sand-flies in an attempt to assess their potential as vectors. A wide range of trapping techniques were used to gather data on sand-fly distribution, prevalence and behaviour in July and September 1973 (Killick-Kendrick et al.1975).

It was discovered that P. perniciosus, the suspected vector of K - A in Italy (Corradetti 1962) was virtually absent from the region. Only P. perfiliewi was a potential vector in 1973. Two reports out-lining the results and recommendations arising from the visits were submitted to

the administration of Emilia-Romagna (Appendix 56).

An entirely different investigation of sand-fly behaviour was carried out in Pará State, Brazil, in 1974. The specific aim was to identify the places in the forest where anthropophilic sand-flies rested during the day so that engorged females could be collected. These were to be precipitin-tested to identify their hosts - the potential forest reservoirs of human leishmaniasis.

ON THE RESTING SITES OF ANTHROPOPHILIC SAND-FLIES IN PARÁ STATE, BRAZIL.

INTRODUCTION.

Leishmaniasis is a major health problem for the poor of Amazonian Brazil (Lainson & Shaw 1973). Extensive epidemiological investigations have been made to identify the Phlebotomid vectors and vertebrate reservoirs of the several forms of this disease found in Pará State (Lainson & Shaw 1968; Shaw & Lainson 1968; Ward et al 1973; Ward 1974).

Human and animal baits have been used to study the host preferences of the common sand-flies (including known vectors of leishmaniasis). However, a more direct way of associating sand-flies with their hosts is to precipitin-test the blood-meals of engorged sand-flies collected from their natural resting places. In the New World this has been successfully achieved in Panama by Tesh et al (1971; 1972). Such studies are particularly important when they identify the natural hosts preferred by anthropophilic sand-flies, and thereby indicate possible reservoir hosts of human leishmaniasis.

Using catching techniques similar to those employed in Panama, Ward (1974) collected large numbers of sand-flies from their diurnal resting sites in the forests of Pará. Unfortunately, only a handful of these were engorged females of species known to bite man.

During 1974 I attempted to find the resting sites of

anthropophilic sand-flies in Pará not only by continuing Ward's collections but also by following and searching for marked flies. An ultra-violet spot-light was used to detect flies dusted with fluorescent powders - a technique that has been used in similar ecological studies on mosquitoes (Zukel 1945; Pal 1947).

STUDY AREAS.

Fig 43 shows the locations of the study areas.

Forests near Belém.

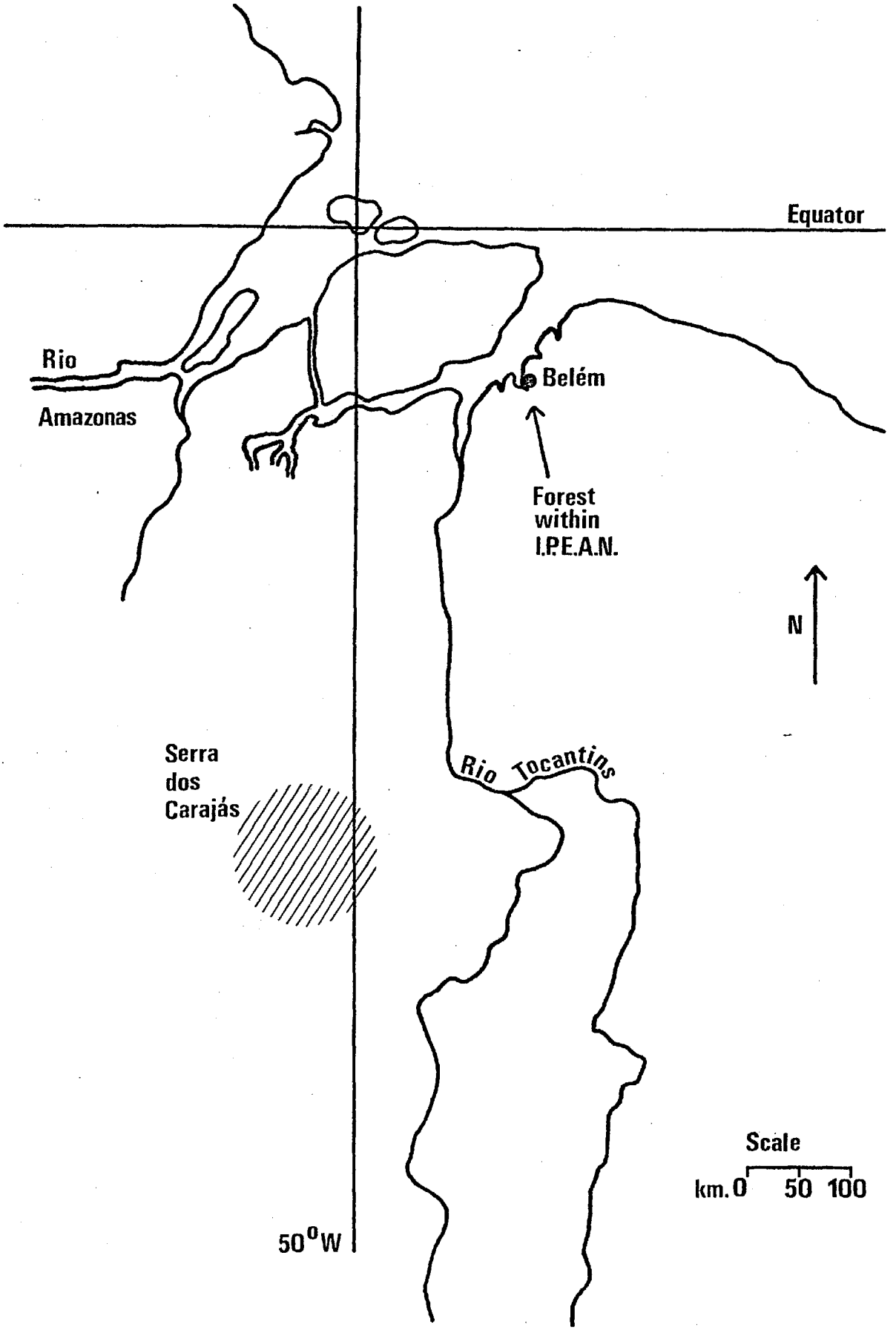
Sand-flies were collected in the Utinga Forest and adjoining forests in the grounds of the Instituto de Pesquisas e Experimentação Agropecuarias do Norte (I.P.E.A.N.) situated 4 Km to the east of Belém.

The area is part of the Amazonian river flood plain and has an elevation of only 12 - 20m above sea level. 4/5th of the annual rain falls between January and June, but the second half of the year is not a true dry season for there are 500mm of rain.

Elevation and drainage largely determine the flora of these forests. A web of rivers and streams frequently flood large areas of low-lying terrain, which is covered with Varzea forest. Here, many of the trees have "stilt" roots or large buttresses. Igapo (or moor swamp) forest covers the poorly drained inland areas, which are water-logged by rain for all but the driest months. Mature tropical rain-forest, the "Terra-firme", is found only on the better drained river terraces. Much of this has been felled and has been replaced by secondary growth or capoeira (Richards 1966).

Much of the present work was carried out in the "terra-firme" forests, where anthropophilic sand-flies are most numerous (Ward 1974). The main features of this type of forest are the three vegetation storeys (at about 1·5, 20·0

Fig.43. Location of study areas in Pará state, Brazil.



and 35·0 m), a closed canopy and a very high density of tree species. Many of the trees have trunks of moderate or small diameter, with long, unbranched boles and relatively small crowns. Ground cover is sparser and epiphytes are fewer than they are in the wetter forests. A layer of leaf litter (rarely deeper than 3 - 4 cm) is present all the year (Richards 1966).

In and around Belém there is little seasonal variation in temperature (a daily range of 18°C to 27°C), humidity (daily range of 70% to 98% RH) or the length of daylight (05·30 hr - 18·30 hr local time),

Serra Norte (Serra dos Carajás).

Further studies were made in the northern hills of the Serra dos Carajás, a part of the Brazilian Shield. This area is endemic for Leishmania braziliensis (Ward et al.1973; Lainson et al.1973). Since 1970 about 20 human cases of this disease have been reported annually from the area (Dr. R. Lainson, pers.comm.).

At altitudes of 300 - 500 m, the iron-rich laterite of the range supports a tall (30 - 45 m), montane rain-forest similar to the "terra-firme" forests of the lowlands. However, it is considerably drier and less humid than the "Terra-firme" of the Belém area, especially between June and September when it has a distinct dry season. There is little seasonal fluctuation in temperature.

METHODS.

For these studies it was necessary to find areas in the forests where anthropophilic sand-flies were abundant. Human baits were used to identify foci of abundance within sections of the forests found suitable by Ward (pers.comm.).

The species composition of each focus was determined. Female sand-flies, mounted in Berlese fluid, were identified from the morphology of their spermathecae and foreguts (Lewis 1974).

The capture of sand-flies from human bait.

Men worked in pairs. One man stripped down to the waist and exposed his legs from below the knees. The other remained fully-clothed and caught (in plastic vials) all female sand-flies that fed on his partner. Following Shaw et al. (1972), I have treated the catch made by each pair in one hour as representing "2 man-hours".

Usually, both men were seated 20 - 30 cm above the ground, and, at night, each kept his electric torch switched on for the whole catching period.

Daytime collections of resting sand-flies.

An open-bottomed cage (Chaniotis et al. 1972; Ward 1974) was used to trap sand-flies resting on leaf-litter or herbaceous plants on the forest floor. The cage was constructed of fine, nylon gauze covering a wooden frame (1·0m x 0·5 x 0·5m). Plants and leaf-litter covered by the trap were agitated with a stick, and any insects disturbed were aspirated from the gauze of the cage where they came

to rest.

The surfaces of shrubs and trees were gently tapped to disturb resting insects which were collected with a pooter. Usually, tree trunks were searched only to a height of 2·0m. Above this level few anthropophilic sand-flies have been captured in Pará (Shaw et al. 1972; Ward 1974).

A wide-nozzled, battery operated aspirator of the type described by Freitas et al. 1966 was used to "vacuum" over tree trunks and above leaf-litter as it was disturbed with a stick.

Investigations with fluorescent markers.

Engorged sand-flies were collected at night from human and animal baits, and were dusted with a fine, coloured powder[1] that fluoresced in ultra-violet (u-v) light. After their release they were detected with a powerful u-v spot-light[2] and followed, or later sought, in attempts to find their resting places.

Freshly engorged females were captured in 6·0 x 2·0 cm snap cap vials. One in four was retained for identification. The others were sprayed with fluorescent powder from a fine Pasteur pipette introduced through the gauze top of each tube. Marked flies were released on the ground near the bait within an hour of their capture. Flies damaged during the

1. "Flare" fluorescent pigments by Industrial Colours Ltd., London. S.W.6.

2. By P.W. Allen & Co., London NI INA; a 125 watt mercury discharge lamp, peaking at 365nm.

marking were not used further.

The u-v spot-light was powered by a portable petrol generator, supplied with 30m of flex. At night, marked flies could be detected up to 10m from the spotlight. By day, the range of detection was only 2m.

The fluorescent powders used were ZnS based, and had an average particle size of 5μ. They were only slightly hygroscopic and adhered well to sand-flies. Thus after 7 days dusted sand-flies kept in dry or damp tubes in the laboratory could be detected in the field as easily as freshly dusted individuals.

The suppliers advertized these powders as being non-toxic to mammals and "other animals". In the laboratory, dusted blood-fed sand-flies (of the species studied in the field) survived and produced fertile eggs as successfully as undusted controls.

RESULTS.

I have followed the classification of Theodor (1965) but have adopted the genus Psychodopygus (Mangabeira 1941), as advocated by Forattini (1971), for those sand-flies previously placed in the subgenus of that name by Theodor.

The capture of sand-flies from human bait.

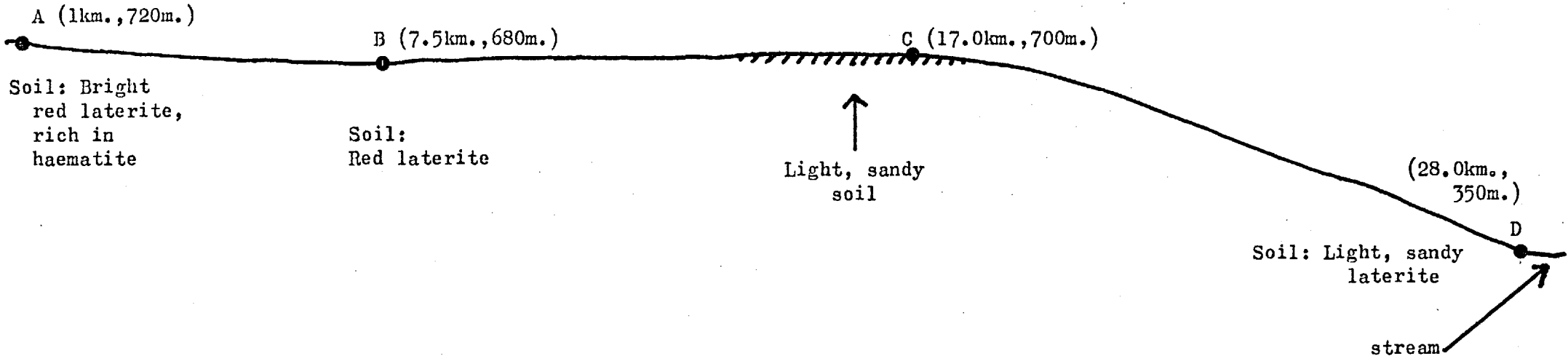
"Terra firme" forest (Mocambo), near Belém. From March to June 1974, 476 sand-flies were captured in 72 man-hours. 360 of these were identified, viz: Ps. davisi (86%), Lutzomyia yuilli (9%), Ps. paraensis (3%) and other species (2%).

"Serra dos Carajás (17th - 26th September 1974.) Captures from human baits were made at four altitudes along a 30 Km stretch of track cut (by the Meridional Mining Co.,) through the montane rain-forest clothing one of the hills of the Serra Norte (Fig 44).

In both weeks of this investigation catches were made at each of the four locations for one 2 hour period in the morning (approximately 10·00 hr - 12·00hr local time) and in the evening (approximately 20·30hr - 22·30 hr local time).

At each location a path was cut for 100m through the secondary growth bordering each side of the road until the more open floor of the forest was reached. During a 2 hour catching period a pair of men stationed themselves for ½ hour spells in two spots, 20m apart, near the end of each path.

Fig. 44. Profile of Serra Norte, to show catch-sites.



A (km., m.) = Catch-site designation,
with road distance from mining camp and altitude.

Only once was a sand-fly captured in the daytime, when one female Ps. wellcomei was caught at catch site B on 25th September. On this occasion the pair of men had spent 30 minutes clearing the site of its ground-cover before the catch.

The results of the night-catches are recorded in Table 30.

Daytime collections of resting sand-flies.

"Terra firme" forests, near Belém. 2 male Ps. davisi and 1 unengorged female L. flaviscutellata were collected from leaf-litter during 8 man-hours of searching.

No sand-flies were found resting on the leaves or stems of herbs, shrubs or saplings during 4 man-hours of searching.

98 L. antunesi, 48 L. dendrophila, 39 L. gomezi and 14 L. shannoni were collected from the larger, scaly-barked or buttressed trees in Utinga Forest during 4 man-hours. Of these, however, most were males or unengorged females. 2 female L. gomezi were gravid, while 2 female L. dendrophila and 6 female L. gomezi contained partly-digested blood-meals.

Serra dos Carajás. No sand-flies were found in leaf-litter or on the trees.

Investigations with fluorescent markers.

"Terra firme" forest (Mocambo) near Belém.

$\frac{121}{135}$ (95%) of sand-flies caught and marked in this forest during the period of these experiments were Ps. davisi.

Table 30.

Phlebotomid sand-flies captured during September 1974 on human bait in the Serra dos Carajás

CATCH-SITE:	A		B		C		D			TOTALS OBTAINED
DATE OF CATCH:	19/9	20/9	17/9	23/9	16/9	22/9	18/9	24/9	TOTAL	BY WARD (1973)
SAND-FLY SPECIES										
L.sp.260.43	2	7	-	87	-	34	-	-	130	125
L.gomezi	1	-	-	18	-	-	-	2	21	20
L.dendrophila	-	-	-	6	-	-	-	-	6	-
L.shannoni	1	-	-	2	-	-	-	-	3	5
L.brachyphalla	-	-	-	-	-	1	-	-	1	6
Ps.wellcomei	1	-	-	1	-	4	-	-	6	2,230
Ps.amazonensis	-	-	-	-	-	4	-	2	6	187
Ps.carrerai	-	-	-	1	-	6	-	2	9	284
Ps.davisi	-	-	-	1	-	7	-	1	9	314
Unidentified	-	-	-	-	-	1	-	-	1	260
TOTAL	5	7	0	116	0	57	0	7	192	3,431

Only this species was recaptured. Other species identified in the samples were Ps. paraensis (10), L. dendrophila (3) and L. brachyphalla (1). Specimens of L. yuilli - distinguished from other species by their white thorax and smaller size - were also taken from human baits at this time (see above). They were not marked in order to ensure that most observations referred to Ps. davisi.

Following marked sand-flies. Sand-flies that had fed on human bait in the evening (19·30 hr - 22·30 hr) or early morning (03·30hr - 05·30 hr) were marked and followed on release. All immediately flew a short distance (0·5 - 2·0m) and settled either on leaf-litter or the base of a small sapling or shrub. This first flight was always low (not more than 0·5m above ground-level) and accomplished in a series of hops.

After 5 - 10 minutes the blood-fed female would do one of two things. In the evening she would fly off at a height of 1 - 3m. This flight lasted for about 10 minutes during which she would halt for short intervals, either on the ground or on vegetation. Most females flew 6 - 10 m from the release point, but one female was observed to travel 20m. In the morning, especially near dawn, the marked sand-fly would fly at a height of 0·5m or less, and rarely travel more than 5m.

The site at which the female finally came to rest was always within 1m of the ground, and was usually at ground-level on leaf-litter. Each site was marked and the fly observed at 15 minute intervals. (Constant surveillance

with the u-v beam was found to disturb the females at this stage). Within 3 hours the majority of females had been lost. A few were seen to crawl under dead leaves on the ground. 4 of these were recaptured. All were Ps. davisi. Detailed results are given in Table 31.

Searching for marked sand-flies. Each batch of sand-flies released in the above experiments had been marked with a powder of a different colour. For the three nights following their release, the marked flies were sought in an area of 20m radius centred on the release point. Each night's search lasted for 3 hours. Tree trunks, saplings and the lower canopy were scanned with the u-v spotlight. Next, ground vegetation and leaf-litter were carefully examined. Finally, saplings, shrubs and leaf-litter were agitated and cigarette smoke was blown into animal burrows to disturb resting sand-flies. However, none of the marked flies was found again.

On two occasions batches of marked flies were released but not followed. Then 6, 21 and 45 hours after release they were sought using the u-v spotlight. Only 2 of the 162 marked sand-flies were recaptured. Both were Ps. davisi. They were found, 21 hrs after release, 18 cm above the ground on the leaves of one of the common herbs, Piper sp, and only 3m from the release point.

Capoeira forest, near Belém.

In September 1974, L. flaviscutellata was captured with a box trap designed by Ward (1974). The hamster-baited trap

DATE	TIME OF RELEASE	NO.FLIES MARKED AND RELEASED	NO. FOLLOWED TO 1st RESTING SITE	NO.FOLLOWED TO 2nd RESTING SITE	NO. WITH KNOWN LOCATION 3HR AFTER RELEASE
26.3.74	22.00hr	10	10	4	2 Ps.davisi
23.4.74	22.30hr	20	20	9	0
27.4.74	22.00hr	25	25	11	0
11.5.74	04.30hr	25	25	19	0
18.5.74	05.30hr	20	20	20	2 Ps.davisi
TOTAL NO.		100	100	63	4

Table 31. "Recapture" of tagged, engorged sand-flies in Mocambo Forest, near Belém.

was placed on the forest floor in the early evening and the captured, blood-fed females were collected, marked and released on the following morning. Marked flies were sought 12 and 36 hours after their release. 3 of the 12 females marked were recaptured 12 hours after their release. All were found on leaf-litter within 2m of the release point, and were later identified as L. flaviscutellata.

Montane rain-forest (altitude 700m), Serra dos Carajás.

Between 21·00 hr and 23·00 hr 25th September, 1974, 100 engorged sand-flies were taken from human bait at site B (Fig. 44). All were marked and then released.

An extensive search for the marked flies was made from 05·00 hr to 07·00 hr on 26th September. 20 were found resting under leaf-litter near the release point. 9 of these were recaptured and identified as L. sp 260·43 (near anduzei), the species biting man in the largest numbers in this locality in September 1974.

No marked flies were found in a search from 22·00 hr to 24·00 hr on 26th September.

DISCUSSION AND CONCLUSIONS.

The capture of sand-flies from human baits.

"Terra firme" forests, near Belém. The species captured were those most commonly caught by Ward (1974), and occurred in approximately the same proportions as they did in his catches.

Montane rain-forest, Serra dos Carajás. During a visit to this region in December 1970/January 1971, Ward et al. (1973) captured 3,431 female sand-flies from human bait in 50·75 man-hours. In September 1974, however, only 192 female sand-flies were similarly taken in 32·0 man-hours (Table 30 . This excludes the 100 flies used for the marking experiment).

The enormous difference in results reflects the paucity of sand-flies of the genus Psychodopygus during the second survey. Ward et al.(1973) found that Ps. wellcomei formed 65% of the total catch off man, and that other species of Psychodopygus accounted for another 29%, i.e. a total of 3,266 flies. In September 1974 only 7 specimens of Ps. wellcomei and 25 other Psychodopygus were taken. In contrast, similar numbers of sand-flies of the genus Lutzomyia were taken off man in both surveys, and L. sp 260·43 (near anduzei) predominated on each occasion (Table 30).

From the exochorionic sculpturing of their eggs Ward & Ready (1975) suggested that the preimaginesof Psychodopygus spp are adapted to wet conditions. Ward et al. (1973) expressed the belief that the population density of L. flaviscutellata

(and perhaps other species of sand-flies) was not so much governed by the amount of rainfall but more by the degree of saturation of the top-soil in which the preimagines are assumed to live. Thus it is likely that the dry soils of the Serra dos Carajás in September 1974 did not favour the breeding of Psychodopygus spp. The large catches in 1970/1971, however, were made at the begininning of the wet season when the soils were damp.

The Serra dos Carajás has a distinct dry season from mid-May to mid-September. During this time it is likely that adult Psychodopygus are inactive or uncommon, and that the populations survive mostly as aestivating preimagines. New World sand-flies have been recorded entering dormancy (during dry seasons) as eggs or 4th instar larvae (Lindquist 1936; Johnson & Hertig 1961; Ward & Killick-Kendrick 1974).

The seasonal occurrence of Psychodopygus spp.in the Serra dos Carajás would limit the period when man is open to fresh inoculation with leishmaniasis. Unfortunately, no monthly analysis of the cases recorded from this area has yet been made.

Perhaps Lutzomyia spp, too, aestivate or remain quiescent during dry spells in the Serra dos Carajás - for it is interesting that only 5 of a total of 292 sand-flies caught in the present survey were taken in the dry, first week (Table 30). Only 9·6 mm of rain had fallen from 4th September to the end of that first week; but then 18·4 mm fell on the night 19th/20th September (Appendix 55). Perhaps

the increased moisture in the top-soil stimulated adult flies to emerge from aestivating pupae. Alternatively, the rain itself may have activated quiescent adults.

On the resting sites of anthropophilic sand-flies in Pará.

Approximately 124,000 sand-flies have been collected in Pará from their daytime resting places on tree trunks and in animal burrows (Damasceno et al.1949; Nery-Guimaraes et al 1966; Ward 1974). With few exceptions, the species found have been those that bite man infrequently, e.g. L. dendrophila, L. antunesi and L. gomezi, or (more usually) not at all, e.g. L. rorotaensis and L. tuberculata. Only rarely have blood-fed females been found.

Reports from Pará of anthropophilic sand-flies captured away from human or animal baits are few. From August 1942 to April 1945 Damasceno et al. (1949) collected 119,107 sand-flies of 54 species from tree trunks and animal burrows. Only 9 of these were of species known to bite man ardently, and all were males (8 Ps. davisi and 1 Ps paraensis). Similarly, of approximately 2,000 sand-flies collected from these micro-habitats by Ward (1974), 8 female Ps. paraensis (including 4 with blood-meals) and 3 female Ps. sp. near squamiventris (including 1 with a blood-meal) were the only representatives of strongly anthropophilic species.

Only Ward (1974) has reported collections of sand-flies from leaf-litter in Pará. He made intensive collections but found only 25 males and 5 females. Ps. davisi and Ps.

paraensis were the species most commonly found in the "terra firme" forests near Belém.

At first sight, it is puzzling that so few anthropophilic sand-flies have been found at rest in Pará. Large numbers have been collected in the forests of Belize (Williams 1965, 1970; Disney 1968), and in Panama Chaniotis et al. (1971b) have captured as many as 5,000 a year. Moreover, engorged females have been regularly collected in Panama, where Tesh et al. (1972) identified the blood-meals of 1,750 anthropophilic sand-flies from three localities.

I believe that the significant difference between Pará and the other two regions is the species composition of the common man-biters. Of the sand-flies that attack man in Pará, those of the genus Psychodopygus predominate both in numbers and species (Shaw et al. 1972; Ward et al. 1973; Ward 1974). Thus, in the "sand-fly season" in the Serra dos Carajás Ward et al. (1973) recorded that 92% of the sand-flies biting man were species of Psychodopygus. Similarly, the figure is 80 - 90% in the forests near Belém (Ward 1974). In comparision, the sand-flies caught biting man in Belize and Panama are fairly equally divided between the genus Lutzomyia and Psychodopygus (Williams 1965; Johnson et al. 1963; Chaniotis et al. 1971a).

From the experience of the investigators in Panama and Belize one would not expect to find engorged Psychodopygus easily or in great numbers. Thus, of the 1,750 blood-meals of anthropophilic sand-flies that have been precipitin-

tested in Panama, only 102 have come from Psychodopygus spp (Tesh et al.1972). In Belize, Disney (1968) collected 147 female Ps. panamensis from leaf-litter. However, only 5 contained blood-meals (pers. comm.).

Therefore, when one considers where anthropophilic sand-flies may be resting in the forests of Pará, one is really considering the whereabouts of species of Psychodopygus. The evidence available from Pará and elsewhere in the New World might suggest that they spend most of their life cycle at or near ground level.

In Belize, Ps. panamensis (the most abundant species of this genus there) is the principal man-biting sand-fly at ground level (Williams 1965). Williams (1970) found only one at rest above ground level, and all 147 females collected by Disney (1968) came from leaf-litter on the forest floor.

Ps. pessoana and Ps. panamensis are the principal man-biters at ground level in Panama (Johnson et al. 1963; Chaniotis et al. 1971a). The extensive investigations of Chaniotis et al.(1972) showed that these species rest almost exclusively on low green plants and leaf-litter: they collected only 1/2, 557 Ps. pessoana and 1/170 Ps. panamensis in other micro-habitats. Also in Panama, Hertig et al. (1960) flushed Ps. panamensis and Ps. bispinosus from green leaves within a few feet of the ground, and Johnson & Hertig (1961) reported that Ps. panamensis was often collected under leaf-litter.

Evidence that the ground layer is the breeding site of Psychodopygus spp. in Panamanian forests comes from the work of Hanson (1961), who found their larvae only under leaf-litter, and from Tesh et al. (1972), who collected engorged females only on low vegetation and leaf-litter.

In Belize and Panama, many Psychodopygus have been taken in castor oil traps baited with a wide range of animals, but only rarely when the traps have been set 10m or more above the ground (Disney 1966; 1968; Thatcher 1968; Williams 1970).

The information from Pará also associates Psychodopygus spp. with the forest ground layer. They come to human and animal baits only rarely when these are more than 5m above the ground and have been collected during the day only from leaf-litter and animal burrows (Ward et al. 1973; Ward 1974).

The present attempts to collect engorged, anthropophilic sand-flies from their diurnal resting places in Pará have been no more successful than previous ones, and only bear out the findings of Ward (1974). The experiments with fluorescent markers suggest that engorged females of Ps. davisi rest on or near the ground, at least in the first 24 hours following a blood-meal. If this is so, then the inability of Ward (1974) and myself to find large numbers of engorged Ps. davisi (in an area where this species bites man in large numbers) may result from the flies hiding in ground fissures and/or being widely dispersed. Alternatively, they may be resting in the canopy, where searches have not been made.

Williams (1970) provided evidence that in Belize Psychodopygus spp. do rest in the canopy, and that this may be their preferred resting place between blood-meals. Many female Psychodopygus were caught at night in light traps set at 25ft and 40ft above the ground while few were taken at ground level. Williams (1970) therefore considered that the females found under leaf-litter during the day were recently emerged adults or females near oviposition. Similarly, Christensen et al. (1972) found that the numbers of female Ps. panamensis caught in light traps in eastern Panama were 18 times greater at 35m than 3m. Working elsewhere in Panama, Chaniotis et al. (1971b) also reported large numbers of Ps. panamensis attracted to light traps set in the canopy.

The results of the investigations of Ward (1974) and the author in Pará seem to support Williams' hypothesis. Thus, in a "terra firme" forest (Mocambo) near Belém where Ps davisi bites man in large numbers, only 2 males and 2 (unengorged) females have been captured at ground level during the day. Further, fluorescent marking experiments showed that engorged females of this species could be found on low vegetation only up to 21 hours after a blood-meal.

It is suggested that many of the females that feed in the early or middle parts of the night (and have been recorded making flights at 3m or more above the ground) remain on or near the ground while they reduce (by diuresis) the volume of their blood-meal and then seek shelter in the

canopy later in the night. In contrast, those feeding late in the night (and recorded as never flying very far above the ground) might delay their ascent till the following night.

FINAL OBSERVATIONS.

Attempts were made to rear several sand-fly species in the laboratory but only L. longipalpis was established. This species presented only one major rearing problem, which was concerned with egg-laying. Few females survived beyond the first gonotrophic cycle, and during routine culture 50%, on average, of the eggs they matured were not laid.

The effects of photoperiod on egg-laying by sand-flies was investigated for the first time; in part, this was done in the hope of improving the egg-laying success of L. longipalpis. Photoperiod was shown to influence the periodicity of egg-laying but not the percentage of mature eggs that were laid.

Beginning with Newstead (1911) many workers have commented on the poor egg-laying performance of laboratory-reared sand-flies. This has not always been improved by providing near-natural conditions, e.g. Unsworth & Gordon (1946). In view of the many unsuccessful attempts to find an oviposition chamber suitable for sand-flies and the uncertainty as to whether many species commonly survive more than one gonotrophic cycle in nature, it was argued that the "premature" death of laboratory flies may be a natural occurrence.

Another major problem encountered when trying to rear sand-flies was diapause. The effects of temperature on the diapause of P. papatasii have already been investigated (Theodor 1934; Safyanova 1964), but surprisingly, photoperiod

has not been considered. This lack of knowledge has prompted the author to continue his studies on sand-flies by investigating the diapause of Mediterranean species.

Several species could not be reared because the females refused to take blood-meals in the laboratory. Field studies in Italy and Brazil indicated some of the complexities of sand-fly blood-feeding behaviour. Careful field observations will have to be made if such species are to be reared successfully. In comparison, L. longipalpis presented no feeding problems, and human breath was sufficient to stimulate females to probe warm, artificial membranes.

An interesting finding was that the stimulus for gorging in L. longipalpis was significantly different from that in many other haematophagous insects: ATP was shown not to have any effect on gorging, which was probably stimulated by blood salts and/or the mechanical bending of the labium as the sand-fly probed. Further, in contrast to the findings for mosquitoes (review Clements 1963), it was the manner of feeding rather than the nature of the food which determined whether a given meal was dispatched to the mid-gut or the crop.

A preliminary investigation indicated that compared with some mosquitoes (Lee 1974) the fascicle of L. longipalpis females is not well-endowed with chemoreceptors. In view of the differences between the gorging behaviour of this sand-fly and mosquitoes a detailed study of the innervation, morphology and fine structure of the fascicular

sensilla is called for.

The investigation of egg maturation showed that in this aspect of its biology L. longipalpis is remarkably similar to many mosquitoes. For example, for both it is the weight of protein in the "blood"-meal that determines the number of eggs that are matured, and not the volume of the meal; a meal of amino acids alone is sufficient to initiate vitellogenesis; and the number of eggs matured depends not only on the size of the meal ingested but also on the nature of the host blood, and to a lesser degree on sugar-feeding and the weight of the fly. Such points have often been overlooked by those experimenting with sand-flies. Thus, the number of eggs laid by a female has been used as an index of egg-laying success (Ward 1974; Chaniotis 1975), but this is meaningless unless the host and meal-size (among other factors) are standardized.

The mechanism by which oogenesis is initiated in blood-sucking Nematocera is not well understood. The willingness with which L. longipalpis ingests unnatural solutions through membranes makes it a more suitable subject than Ae. aegypti for an investigation of this problem.

The identification of the host range of man-biting sand-flies is of paramount importance when investigating the epidemiology of leishmaniasis. In the forests of Pará, (Brazil), however, it has proved to be virtually impossible to find engorged, man-biting sand-flies (Ward 1974). The results of the author's marking and release experiments suggested that they may be resting in the forest canopy. This possibility is now being investigated by Dr. R.D.Ward of the Wellcome Parasitology Unit, Belém, Pará.

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APPENDIX A. Fresh weights (mg.) of pupae.

These were weighed on the day of eye- and wing-setae blackening, 3 days before adult emergence at 25°C.

FEMALE PUPAE					MALE PUPAE			
1		9	17	GENERATION	1		9	17
0.610	0.583	0.784	0.710		0.572	0.528	0.545	0.567
0.700	0.603	0.738	0.756		0.431	0.470	0.600	0.636
0.615	0.489	0.755	0.764		0.476	0.483	0.584	0.575
0.682	0.531	0.650	0.605		0.438	0.529	0.538	0.526
0.670	0.592	0.626	0.720		0.537	0.486	0.572	0.604
0.627	0.341	0.679	0.637		0.501	0.541	0.620	0.567
0.655	0.605	0.747	0.697		0.544	0.525	0.592	0.540
0.585	0.677	0.710	0.642		0.476	0.523	0.543	0.506
0.622	0.686	0.665	0.656		0.506	0.517	0.577	0.517
0.622	0.668	0.696	0.773		0.510	0.515	0.643	
0.474	0.661	0.780	0.683		0.533		0.535	
0.601	0.715	0.696	0.650		0.463		0.543	
0.607	0.576	0.779	0.672		0.500		0.610	
0.631	0.642	0.711	0.657		0.494		0.620	
0.586	o.623	0.631			0.508		0.565	
0.606	0.600	0.754			0.532		0.605	
0.629	0.580				0.535		0.553	
0.641	0.596				0.543		0.523	
0.566	0.642				0.572		0.540	
0.561	0.650				0.539		0.533	
0.608	0.633				0.526		0.520	
0.553	0.597				0.654		0.575	
0.565					0.501		0.587	
							0.578	
n=	45	16	14		33		24	9

APPENDIX B. The effect of female body-weight on the weight of blood imbibed from man by females of L. longipalpis.

INDEPENDENT (x) Weight of female at time of blood-meal (mg.)	DEPENDENT (y) Weight of blood imbibed (mg.)
0.275	0.436
0.330	0.477
0.310	0.407
0.354	0.342
0.309	0.475
0.360	0.516
0.238	0.378
0.406	0.362
0.300	0.296
0.152	0.261
0.333	0.435
0.315	0.496
0.266	0.380
0.352	0.454
0.324	0.446
0.322	0.373
0.348	0.509
0.373	0.482
0.231	0.353
0.270	0.330
0.270	0.368
0.309	0.337
0.233	0.455
0.357	0.511
0.359	0.429
0.330	0.392

$n = 26$

$\sum x = 8.010$ $\quad$ $\sum y = 10.700$

$\bar{x} = 0.308$ $\quad$ $\bar{y} = 0.411$

$(\sum x)^2 = 64.160$ $\quad$ $(\sum y)^2 = 114.490$

$\sum x = 2.550$ $\quad$ $\sum y = 4.530$

$\sum xy = 3.350$ $\quad$ $b = 0.625$

$\sum x \sum y = 85.700$ $\quad$ $S_b = 0.065$

$t = 2.72$

$p = 0.020$ $\quad$ $df = 24$

APPENDIX 1.

Decrease in the weight of female L. longipalpis at 25°C after engorgement on human blood as a % of the weight of meal.

Generation 19/22; human-line.

Time after meal imbibed (mins).	Weight of meal(mg) 1.	Decrease in wt of female/ Wt of meal (%). 20.	60.
	0·605	18·8	27·8
	0·548	31·6	43·1
	0·221	19·0	37·1
	0·342	-	42·1
	0·283	32·9	39·6
	0·250	24·8	34·8
	0·582	33·2	45·4
	0·187	17·1	28·9
	0·357	25·8	31·7
	0·595	26·1	37·8
	0·353	19·0	22·1
	0·610	36·7	46·4
	0·547	15·9	40·2
	0·457	41·6	46·2
	0·316	20·6	35·5
	0·149	18·8	24·2
	0·596	-	40·8
	0·353	-	41·1
	0·434	-	45·9

	0.359	–	42.3
	0·397	–	37.8
	0·586	–	45·7
TOTAL =	9·127	381.9	836·500
MEAN =	0·415	25.5	38·023
N =	22	15	22

REGRESSION ANALYSIS.

INDEPENDENT (x) Weight of meal (mg)		DEPENDENT (y) Decrease wt female/wt of meal (%) after 60 mins.	
n =	22		
Σx =	9·127	Σy =	836·5
$\bar{x}$ =	0·415	$\bar{y}$ =	38·023
$(\Sigma x)^2$ =	83·300	$(\Sigma y)^2$ =	699,732·25
Σx^2 =	4·255	Σy^2 =	32,893·59
Σxy =	358·327	b =	24·070
$\Sigma x \Sigma y$ =	7,634·736	S_b =	6·387
p =	0·020	t =	2·581
		df =	20

APPENDIX 2.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from male hamster. Generation 3; Hamster line; L. longipalpis from BH.

INDEPENDENT (x)	DEPENDENT. (y)
Weight of meal (mg)	No. of eggs.
0·392	76
0·510	92
0·440	80
0·363	66
0·058	29
0·361	78
0·045	18
0·033	20
0·056	16
0·382	68
0·463	97
0·470	96
0·244	65
0·453	85
0·288	70
0·201	49
0·444	59
0·122	36
0·382	51
0·234	25
0·384	45

0.438	42
0.444	59

n	= 23		
Σx	= 7.207	Σy	= 1,322.00
$\bar{x}$	= 0.313	$\bar{y}$	= 57.478
$(\Sigma x)^2$	= 51.941	$(\Sigma y)^2$	= 1,747,684.000
Σx^2	= 2.797	Σy^2	= 90,174.000
Σxy	= 486.249	b	= 133.586
$\Sigma x \Sigma y$	= 9,527.654	S_b	= 14.751
		t	= 6.649
p	= 0.001	df	= 21

APPENDIX 3.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from male hamster. Generation 7; hamster line; L. longipalpis from B.H.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg).	No.of eggs.
0.373	96
0.605	101
0.030	17
0.278	64
0·184	55
0·224	82
0·551	90
0·310	61
0·325	90
0·202	60
0·565	114
0·422	50
0·311	57
0·600	70
0·096	22
0·085	25
0·138	27
0·212	25

n = 18

Σx	= 5·508	Σy	= 1105·992
$\bar{x}$	= 0·306	$\bar{y}$	= 61·444
$(\Sigma x)^2$	= 30·338	$(\Sigma y)^2$	= 1,223,218·304
Σx^2	= 2·248	Σy^2	= 375,357·981
Σxy	= 412·191	b	= 131·008
$\Sigma x \Sigma y$	= 6,091·804	S_b	= 18·746
		t	= 5·244
p	= 0·001	df	= 16

APPENDIX 4.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from male hamster. Generation 8; human line; L. longipalpis from B.H.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No. of eggs.
0.040	19
0.530	82
0.627	118
0.076	26
0.363	95
0.006	19
0.623	90
0.013	9
0.324	50
0.307	97
0.104	43
0.500	97
0.446	69
0.521	81
0.032	22
0.497	76
0.128	38
0.131	45
0.111	35

n = 19

Σx	= 5·377	Σy	= 1,111·006
$\bar{x}$	= 0·283	$\bar{y}$	= 58·474
$(\Sigma x)^2$	= 28·912	$(\Sigma y)^2$	= 1,234,334·332
Σx^2	= 2·426	Σy^2	= 80,845·913
Σxy	= 433·324	b	= 134·537
$\Sigma x \Sigma y$	= 5,973·879	S_b	= 14·122
		t	= 9·058
p	= 0·001	df	= 17

APPENDIX 5.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from man. Generation 2; human-line; L. longipalpis from BH.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·063	24
0·088	15
0·103	23
0·152	23
0·226	28
0·263	39
0·303	40
0·321	31
0·327	37
0·328	43
0·347	41
0·353	38
0·392	47
0·396	42
0·402	50
0·440	62
0·458	57
0·467	44
0·510	63

n = 19

Σx	= 5·938	Σy	= 747·000
$\bar{x}$	= 0·313	$\bar{y}$	= 39·316
$(\Sigma x)^2$	= 35·260	$(\Sigma y)^2$	= 558,009·000
Σx^2	= 2·173	Σy^2	= 32,539·000
Σxy	= 262·367	b	= 91·199
$\Sigma x \Sigma y$	= 4,435·686	S_b	= 5·602
		t	= 9·165
p	= 0·001	df	= 17

APPENDIX 6.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood from man. Generation 3; hamster-line; L. longipalpis from B.H.

INDEPENDENT. (x) Weight of meal (mg)	DEPENDENT (y) NO: of eggs.
0·353	37
0·337	43
0·349	45
0·370	41
0·425	48
0·230	30
0·249	36
0·361	45
0·531	50
0.458	52
0·145	33
0·300	52
0·326	52
0·131	25
0·077	17

$n = 15$

Σx	= 4·642	Σy	= 606·000
$\bar{x}$	= 0·309	$\bar{y}$	= 40·400
$(\Sigma x)^2$	= 21·548	$(\Sigma y)^2$	= 367,236·000
Σx^2	= 1·655	Σy^2	= 26,064·000

$\Sigma xy = 203 \cdot 223$

$\Sigma x \Sigma y = 2813 \cdot 052$

$p = 0 \cdot 001$

$b = 71 \cdot 959$

$S_b = 5 \cdot 902$

$t = 5 \cdot 693$

$df = 13$

APPENDIX 7.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from man. Generation 9; human-line; L. longipalpis from BH.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No. of eggs.
0.666	65
0.542	46
0.633	65
0.404	47
0.447	64
0.665	60
0.505	62
0.650	65
0.437	40
0.413	34
0.324	34
0.472	54
0.675	65
0.095	20
0.262	22
0.344	49
0.270	34

$n = 17$

$\Sigma x = 7{\cdot}803$ $\Sigma y = 826{\cdot}000$

$\bar{x}$	= 0·459	$\bar{y}$	= 48·588
$(\Sigma x)^2$	= 60·887	$(\Sigma y)^2$	= 682,276·000
Σx^2	= 4·035	Σy^2	= 3,503·189
Σxy	= 416·047	b	= 81·486
$\Sigma x \Sigma y$	= 6,445·278	S_b	= 7·745
		t	= 7·081
p	= 0·001	df	= 15

APPENDIX 8.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from male mouse. Generation 9; human-line; L. longipalpis from BH.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No. of eggs.
0·454	57
0·420	83
0·652	81
0·282	39
0·537	93
0·482	80
0·393	59
0·138	36
0·568	70

n	= 9		
Σx	= 3·926	Σy	= 598·000
$\bar{x}$	= 0·436	$\bar{y}$	= 66·444
$(\Sigma x)^2$	= 15·413	$(\Sigma y)^2$	= 357,604·000
Σx^2	= 1·904	Σy^2	= 42,946·000
Σxy	= 280.964	b	= 105.251
$\Sigma x \Sigma y$	= 2,347.748	S_b	= 12.130
		t	= 3.792
p	= 0.010	df	= 7

APPENDIX 9.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from man. Generation 7; L. longipalpis from Ceará.

INDEPENDENT (x) Weight of meal (mg).	DEPENDENT (y) No. of eggs.
0·156	22
0·383	32
0·481	52
0·543	53
0·422	34
0·441	43
0·389	33
0·177	40
0·193	31
0·414	47
0·494	51
0·507	52
0·406	44
0·277	24
0·324	29
0·209	30
0·400	43
0·220	39
0·076	15
0·351	34
0·185	24

n = 21

Σx	= 7·048	Σy	= 7·720
$\bar{x}$	= 0·336	$\bar{y}$	= 36·760
$(\Sigma x)^2$	= 49·674	$(\Sigma y)^2$	= 595,984·000
Σx^2	= 2·724	Σy^2	= 30,770·000
Σxy	= 283·148	b	= 66·990
$\Sigma x \Sigma y$	= 5,441·056	S_b	= 6·410
		t	= 6·260
p	= 0·001	df	= 19

APPENDIX 10.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a woman. Generation 7; L. longipalpis from Ceará.

INDEPENDENT.(x) Weight of meal (mg)	DEPENDENT (y) NO. of eggs.
0·489	31
0·319	57
0·559	50
0·559	47
0·210	29
0·735	71
0·302	41
0·120	20
0·174	21
0·554	52
0·297	34
0·200	24
0·425	40
0·496	37
0·283	25
0·224	35

$n = 16$

$\Sigma x = 5.946$ $\quad \Sigma y = 614.000$

$\bar{x} = 0.372$ $\quad \bar{y} = 38.375$

$(\Sigma x)^2$ =	35·355	$(\Sigma y)^2$ =	376,996·000
Σx^2 =	2·678	Σy^2 =	26,578·000
Σxy =	258·249	b =	64·254
$\Sigma x \Sigma y$ =	3,650·844	S_b =	8·798
		t =	4·996
p =	0·001	df =	14

APPENDIX 11.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a male squirrel monkey, Saimiri sciureus.

Generation 9; L. longipalpis from Ceará.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·480	32
0·384	31
0·645	40
0·545	42
0·537	42
0·137	14

$n = 6$

Σx	2·728	Σy	201·000
$\bar{x}$	0·455	$\bar{y}$	33·500
$(\Sigma x)^2$	7·442	$(\Sigma y)^2$	40,401·000
Σx^2	1·398	Σy^2	7,309·000
Σxy	100·426	b	57·200
$\Sigma x \Sigma y$	548·328	S_b	4·050
		t	5·650
p	0·010	df	4

APPENDIX 12.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a male capuchin monkey , Cebus apella.

Generation 8; L. longipalpis from Ceará.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·525	41
0·567	30
0·580	37
0·601	36
0·613	40
0·353	33
0·174	21
0·579	35
0·320	26
0·389	27
0·210	29
0·557	44
0·470	41
0·485	36
0·631	43
0·157	24
0·135	22
0·281	22
0·278	12

n = 19

Σx	= 7·905	Σy	= 599·000
$\bar{x}$	= 0·416	$\bar{y}$	= 31·526
$(\Sigma x)^2$	= 62·490	$(\Sigma y)^2$	= 358,801·000
Σx^2	= 3·821	Σy^2	= 20,297·000
Σxy	= 271·259	b	= 41·430
$\Sigma x \Sigma y$	= 4,735·095	S_b	= 5·400
		t	= 5·600
p	= 0·001	df	= 17

APPENDIX 13.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a female opossum, Didelphis marsupialis.

Generations 8/9; L. longipalpis from Ceará.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·210	50
0·454	54
0·632	76
0·050	2
0·436	60
0·244	35
0·016	0
0·239	69
0·223	35
0.152	22
0·012	0
0·067	13
0·085	14
0·554	40
0·444	59
0·213	37
0.195	35
0·378	62
0·687	61
0·474	49

n = 20

Σx =	5.765	Σy =	773.000
$\bar{x}$ =	0.288	$\bar{y}$ =	38.650
$(\Sigma x)^2$ =	33.240	$(\Sigma y)^2$ =	597,529.000
Σx^2 =	2.470	Σy^2 =	40,497.000
Σxy =	299.16	b =	94.48
$\Sigma x \Sigma y$ =	4,456.35	S_b =	13.760
		t =	6.17
p =	0.001	df =	18

APPENDIX 14.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a female three-toed sloth, *Bradypus tridactylus*. Generation 7; L. *longipalpis* from Ceará.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No.of eggs.
0·186	47
0·557	69
0·301	37
0·700	72
0·247	51
0·371	50
0·040	8
0·553	64
0·056	30
0·337	38

n =	10		
Σx =	3·348	Σy =	466·000
$\bar{x}$ =	0·335	$\bar{y}$ =	46·600
$(\Sigma x)^2$ =	11·209	$(\Sigma y)^2$ =	251,128·000
Σx^2 =	1·548	Σy^2 =	217,156·000
Σxy =	1,560·168	b =	79·720
$\Sigma x \Sigma y$ =	190·057	S_b =	9·347
		t =	5·580
p =	0·001	df =	8

APPENDIX 15.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from a female and male Proechimys sp.

Generation 7/9; L. longipalpis from Ceara.

INDEPENDENT(x) Weight of meal (mg)	DEPENDENT (y) No.of eggs.
0·361	50
0·411	64
0·191	27
0·504	80
0·578	76
0·267	66
0·405	82
0·548	79
0·130	28
0·373	68
0·422	73
0·366	63
0·251	32
0·247	39

$n = 14$

$\Sigma x = 5{\cdot}504$ $\qquad \Sigma y = 827{\cdot}000$

$\bar{x} = 0{\cdot}361$ $\qquad \bar{y} = 59{\cdot}070$

$(\Sigma x)^2 = 25{\cdot}540$ $\qquad (\Sigma y)^2 = 683{,}929{\cdot}000$

$\Sigma x^2 = 2{\cdot}051$ $\qquad \Sigma y^2 = 54{,}073{\cdot}000$

Σxy	= 328·416	b	= 131·580
$\Sigma x \Sigma y$	= 4,179·66	S_b	= 10·371
		t	= 6·045
p	= 0.001	df	= 12

APPENDIX 16.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of blood imbibed from man.

Wild-caught Ps. davisi.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No of eggs.
0.236	48
0.243	44
0.166	58
0.148	55
0.263	26
0·169	38
0.199	55
0.208	18
0.119	15
0·113	27
0·141	34
0·183	24
0·193	42
0·139	29
0·164	26
0·192	45
0·191	45
0·124	43
0·173	48
0·174	37

	0·136		33
	0·160		60
n	= 22		
Σx	= 3.834	Σy	= 850.000
$\bar{x}$	= 0.174	$\bar{y}$	= 38.636
$(\Sigma x)^2$	= 14.700	$(\Sigma y)^2$	= 722,500.000
Σx^2	= 0.702	Σy^2	= 36,326.000
Σxy	= 149.557	b	= 41.9
$\Sigma x \Sigma y$	= 3,258.900	S_b	= 13.1
		t	= 0.57
p	= not significant	df	= 20

APPENDIX 17

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight (<0·300 mg) of blood imbibed from man.

Generation 7; L. longipalpis from Ceará.

INDEPENDENT.(x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·156	22
0·177	40
0·193	31
0·277	24
0·209	30
0·220	39
0·076	15
0·185	24

n	= 8		
Σx	= 1·493	Σy	= 225·000
$\bar{x}$	= 0·187	$\bar{y}$	= 28·125
$(\Sigma x)^2$	= 2·229	$(\Sigma y)^2$	= 50,625,000
Σx^2	= 0·302	Σy^2	= 6,843·000
Σxy	= 43·573	b	= 68·696
$\Sigma x \Sigma y$	= 335·925	S_b	= 7·939
		t	= 1 312
p	= not significant.	df	= 6

APPENDIX 18

Eggs matured on $<$0·300 mg of blood imbibed from man.

	x	y
	L. *longipalpis*, Ceara strain.	Ps. *davisi*.
Σx =	225·000	Σy = 850·000
$\bar{x}$ =	28·125	$\bar{y}$ = 38·640
Sx =	8·576	Sy = 12·882
n =	8	n = 22

Comparision of means:

t = 2.053, p = 0.05 for df = 26.

Size (mg) of females at time of meal.

	x	y
	L. *longipalpis*, Ceara strain.	Ps. *davisi*.
Σx =	2·464	Σy = 3·939
$\bar{x}$ =	0·308	$\bar{y}$ = 0·179
S_x =	0·041	S_y = 0·032
n =	8	n = 22
Range =	0·361 – 0·242	0·232 – 0·133

APPENDIX 19

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of human blood imbibed from arm of volunteer.

Generation 19; human-line; L. longipalpis from BH.

INDEPENDENT (x) Weight of meal (mg) after			DEPENDENT (y) No. of eggs.
1 min (x_1)	20 mins (x_2)	60 mins (x_3)	
0.605	0.491	0.437	47
0.548	0.375	0.312	50
0.221	0.179	0.139	24
0.283	0.190	0.171	33
0.250	0.188	0.163	18
0.582	0.389	0.318	49
0.187	0.155	0.133	10
0.357	0.265	0.244	35
0.595	0.440	0.370	41
0.353	0.286	0.275	28
0.610	0.386	0.327	46
0.547	0.460	0.327	44
0.457	0.267	0.246	33
0.316	0.251	0.202	25
0.149	0.121	0.113	22
n = 15	15	15	15

$\Sigma x =$	6.060	4.443	3.777	$\Sigma y =$	505.000
$\bar{x} =$	0.404	0.296	0.252	$\bar{y} =$	33.667
$(\Sigma x)^2 =$	36.724	19.740	14.266	$(\Sigma y)^2 =$	255,025.000
$\Sigma x^2 =$	2.839	1.516	1.082	$\Sigma y^2 =$	19,139.000
$\Sigma xy =$	230.527	167.455	141.458		
$\Sigma x \Sigma y =$	3,060.3	2,243.715	1,907.385		
$b =$	67.793	89.370	109.160		
$S_b =$	5.124	6.451	6.664		
$t =$	8.273	6.196	5.929		
$p =$	0.001	0.001	0.001		
$df =$	13	13	13		

APPENDIX 20.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of human blood (CPD) imbibed, through leached chick membrane.

Generation 20; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg).	DEPENDENT (y) No. of eggs.
0·354	53
0·340	45
0·277	35
0·277	35
0·204	35
0·214	39
0·141	32
0·269	30
0·159	29
0·229	31
0·239	28
0·214	27
0·168	24
0·265	23

$n = 14$

$\Sigma x =$	3·350	$\Sigma y =$	466·000
$\bar{x} =$	0·239	$\bar{y} =$	33·286
$(\Sigma x)^2 =$	11·223	$(\Sigma y)^2 =$	217,156·000
$\Sigma x^2 =$	0·853	$\Sigma y^2 =$	16,374·000

Σyx =	115.827	b =	84.706
$\Sigma x \Sigma y$ =	1,561.100	S_b =	6.435
p =	0.020	t =	2.973
		df =	12

APPENDIX 21.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of heparin-treated hamster blood imbibed through leached chick membrane.

Generation 22, human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) NO. of eggs.
0·140	15
0·340	64
0·358	53
0·424	77
0·372	86
0·134	42
0·222	73
0·381	73
0·463	92
0·250	77
0·397	86
0·252	62

$n = 12$

Σx	3·733	Σy	800.000
$\bar{x}$	0·311	$\bar{y}$	66.667
$(\Sigma x)^2$	13·935	$(\Sigma y)^2$	640,000.000
Σx^2	1·292	Σy^2	58,510.000
Σxy	266·843	b	137.221
$\Sigma x \Sigma y$	2,986·400	S_b	16.463
		t	3·017
p	0·020	df	10

APPENDIX 22.

Eggs matured at 25°C on weight of human blood (CPD) imbibed through parafilm "M" membrane.

Generation 20; human-line; L. longipalpis from B.H.

Weight of meal (mg)	No. of eggs.
0•594	54
0•430	35
0•343	39
0•307	37

APPENDIX 23.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 50% (v/v) human plasma in 0·85% saline imbibed through leached chick membrane. Generation 23; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs
0·098	7
0·274	13
0·482	21
0·292	10
0·262	18
0·348	13
0·440	17
0·460	25

n	= 8		
Σx	= 2·656	Σy	= 124·000
$\bar{x}$	= 0·332	$\bar{y}$	= 15·500
$(\Sigma x)^2$	= 7·054	$(\Sigma y)^2$	= 15,376·000
Σx^2	= 0·997	Σy^2	= 2,166·000
Σxy	= 45·510	b	= 37·757
$\Sigma x \Sigma y$	= 329·344	S_b	= 3·280
		t	= 3·904
p	= 0·020	df	= 6

APPENDIX 24.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 45% (v/v) suspension of human red cells in 0·85% saline imbibed through leached chick membrane.

Generation 20/23; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No.of eggs.
0·333	24
0·214	16
0·261	12
0·345	20
0·304	16
0·426	18
0·496	30
0·490	35

n	= 8		
Σx	= 2·869	Σy	= 171·000
$\bar{x}$	= 0·359	$\bar{y}$	= 21·375
$(\Sigma x)^2$	= 8·231	$(\Sigma y)^2$	= 29,241·000
Σx^2	= 1·104	Σy^2	= 4,081·000
Σxy	= 66·010	b	= 62·470
$\Sigma x \Sigma y$	= 490·599	S_b	= 4·712
		t	= 3·631
p	= 0·020	df	= 6

APPENDIX 25.

Eggs matured at 25°C on weight of hamster plasma imbibed through belly skin of hamster.

Generation 8; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No.of eggs.
0.383	31
0.074	11
0·474	44
0·404	45
0·437	46
0·435	53
0·376	49

n	=	7			
Σx	=	2·583	Σy	=	279.000
$\bar{x}$	=	0·369	$\bar{y}$	=	39.857
$(\Sigma x)^2$	=	6·672	$(\Sigma y)^2$	=	77,841.000
Σx^2	=	1·062	Σy^2	=	12,369.000
Σxy	=	113·304	b	=	94.982
$\Sigma x \Sigma y$	=	720·657	S_b	=	18.196
			t	=	1.723
p	=	not significant	df	=	5

APPENDIX 26.

Eggs matured at 25°C on weight of hamster plasma imbibed through leached chick membrane.

Generation 22, human-line; L. longipalpis from B.H.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No.of eggs.
0·295	32
0·273	42
0·338	30
0·391	33
0·364	31
0·395	37
0·310	27
0·303	31

$n = 8$

$\Sigma x = 2{\cdot}669$ $\quad\quad$ $\Sigma y = 263{\cdot}000$

$\bar{x} = 0{\cdot}334$ $\quad\quad$ $\bar{y} = 32{\cdot}875$

APPENDIX 27.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 45% (v/v) suspension of hamster red cells in 0·85% saline imbibed through leached chick membrane.

Generation 23; human-line; L. longipalpis from B.H.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No.of eggs.
0·158	28
0·338	50
0·411	59
0·439	54
0·520	63

n	=	5			
Σx	=	1·866	Σy	=	254·000
$\bar{x}$	=	0·373	$\bar{y}$	=	50·800
$(\Sigma x)^2$	=	3·482	$(\Sigma y)^2$	=	64,516·000
Σx^2	=	0·771	Σy^2	=	13,650·000
Σxy	=	102·039	b	=	96·613
$\Sigma x \Sigma y$	=	473·965	S_b	=	3·95o
			t	=	6·69o
p	=	0·010	df	=	3

APPENDIX 28.

Eggs matured on weight of meal imbibed for range 0·250 – 0·350 mg.

HUMAN BLOOD.

(a) Weight of meal (mg)	(b) No. of eggs.
0·265	23
0·269	30
0·277	35
0·277	35
0·340	45

$n = 5$

$\Sigma a = 1{\cdot}430$ $\quad \Sigma b = 168{\cdot}000$

$\bar{a} = 0{\cdot}286$ $\quad \bar{b} = 33{\cdot}600$

$(\Sigma b)^2 = 28{,}224{\cdot}000$

$\Sigma b^2 = 5{,}904{\cdot}000$

$S_a = 0{\cdot}031$ $\quad S_b = 8{\cdot}050$

50% HUMAN PLASMA IN 0·85% SALINE

(c) Weight of meal (mg)	(d) No. of eggs.
0·262	18
0·274	13
0·292	10
0·348	13

$n = 4$

Σc	= 1·176	Σd	= 54·000
$\bar{c}$	= 0·294	$\bar{d}$	= 13·500
		$(\Sigma d)^2$	= 2,916·000
		Σd^2	= 762·000
S_c	= 0·038	S_d	= 3·317

Comparision of $\bar{b}$ and $\bar{d}$:

p = 0.01; t = 5.07; df = 7

APPENDIX 29.

Eggs matured on weight of meal imbibed for range 0·250 – 0·350 mg.

HUMAN BLOOD

(a) Weight of blood (mg)	(b) No. of eggs.
0·265	23
0·269	30
0·277	35
0·277	35
0·340	45

$n = 5$

$\Sigma a = 1{\cdot}430$ $\qquad$ $\Sigma b = 168{\cdot}000$

$\bar{a} = 0{\cdot}286$ $\qquad$ $\bar{b} = 33{\cdot}600$

$(\Sigma b)^2 = 28224{\cdot}000$

$\Sigma b^2 = 5904{\cdot}000$

$S_a = 0{\cdot}031$ $\qquad$ $S_b = 8{\cdot}050$

45% HUMAN RED CELLS IN 0·85% SALINE

(c) Weight of blood (mg)	(c) No. of eggs.
0·333	24
0·261	12
0·345	20
0·304	16

$n = 4$

Σc	= 1·244	Σd	= 72·000
$\bar{c}$	= 0·311	$\bar{d}$	= 18·000
		$(\Sigma d)^2$	= 5,184·000
		Σd^2	= 1,376·000
S_c	= 0·037	S_d	= 5·164

Comparison of $\bar{b}$ and $\bar{d}$:

p = 0·01; t = 4.67; df = 7

APPENDIX 30

Eggs matured on weight of meal imbibed for range 0·330 – 0·520 mg.

45% (w/v) HAMSTER RED CELLS IN 0·85% SALINE

(a) Weight of meal (mg)	(b) No.of eggs
0·338	50
0·411	59
0·439	54
0·520	63

$n = 4$

$\Sigma a = 1{\cdot}708$ $\quad \Sigma b = 226.000$

$\bar{a} = 0{\cdot}427$ $\quad \bar{b} = 56.5$

$(\Sigma b)^2 = 51{,}076.000$

$\Sigma b^2 = 12{,}866.000$

$S_a = 0{\cdot}075$ $\quad S_b = 5.686$

45% (w/v) HUMAN RED CELLS IN 0·85% SALINE

(c) Weight of meal (mg)	(d) No.of eggs.
0·333	24
0·345	20
0·426	18
0·496	30
0·490	35

$n = 5$

Σc	= 2·090	Σd	= 127·000
$\bar{c}$	= 0·418	$\bar{d}$	= 25·400
		$(\Sigma d)^2$	= 16,129·000
		Σd^2	= 3,425·000
S_c	= 0·077	S_d	= 7·057

Comparison of $\bar{b}$ and $\bar{d}$:

$p = 0·001$; $t = 7·12$; $df = 7$

APPENDIX 31

Eggs matured on weight of meal imbibed for range 0·250 – 0·350 mg.

HAMSTER PLASMA

(a) Weight of meal (mg)	(b) No. of eggs.
0·295	32
0·273	42
0·338	30
0·310	27
0·303	31

$n = 5$

$\Sigma a = 1.520$ $\quad \Sigma b = 162$

$\bar{a} = 0.304$ $\quad \bar{b} = 32.4$

$(\Sigma b)^2 = 26,244.000$

$\Sigma b^2 = 5,378.000$

$S_a = 0.024$ $\quad S_b = 5.683$

HUMAN PLASMA

(c) Weight of meal (mg)	(d) No. of eggs
0·257	32
0·255	32
0·282	36
0·284	26
0·332	41

Σc	= 1·410	Σd	= 167·000
$\bar{c}$	= 0·282	$\bar{d}$	= 33·400
		$(\Sigma d)^2$	= 27,889·000
		Σd^2	= 5,701·000
S_c	= 0·031	S_d	= 5·550

APPENDIX 32

Eggs matured on weight of meal imbibed for range 0·200 - 0·350 mg.

45% (w/v) HUMAN E.E.

(a) Weight of meal (mg)	(b) No. of eggs
0·240	17
0·230	36
0·235	41
0·250	37
0·302	35
0·241	25
0·298	24
0·203	30
0·264	32

$n = 9$

$\Sigma a = 2·259$ $\quad \Sigma b = 277·000$

$\bar{a} = 0·251$ $\quad \bar{b} = 30·778$

$(\Sigma b)^2 = 76,729·000$

$\Sigma b^2 = 8,985·000$

$S_a = 0·32$ $\quad S_b = 7·579$

45% (w/v) HUMAN RED CELLS IN 0·85% SALINE

(c) Weight of meal (mg)	(d) No. of eggs
0·333	24
0.214	16
0·261	12
0·345	20
0·304	16

$n = 5$

Σc	= 1·457	Σd	= 88,·000
$\bar{c}$	= 0·291	$\bar{d}$	= 17·600
		$(\Sigma d)^2$	= 7,744·000
		Σd^2	= 1,632·000
S_c	= 0·054	S_d	= 4·561

APPENDIX 33

Eggs matured at 25°C on weight of 45% solution of the water soluble extract of hamster red cells imbibed through leached chick membrane.

Generation 21; human-line; L. longipalpis from B.H.

Weight of meal (mg)	No. of eggs.
0·242	65
0·258	73
0·283	51
0·182	56
0·343	78
0·322	84
0·215	45
0·257	74
0·215	52
0·306	74
0·285	75
0·198	59
0·222	58
0·255	67
0·262	54
0·270	61
0·325	58
0·318	61

n = 18

APPENDIX 34.

Eggs matured at 25°C on weight of 45% solution of the water soluble extract of human red cells imbibed through leached chick membrane.

Generation 21; human-line; L. longipalpis from B.H.

Weight of meal (mg)	No: of eggs.
0·240	17
0·356	28
0·195	19
0·180	19
0·150	29
0·230	36
0.130	34
0·235	41
0·250	37
0·152	27
0·302	35
0·241	25
0·298	24
0·172	36
0·203	30
0·264	32

n = 16

APPENDIX 35.

Eggs matured on weight of erythrocyte extract imbibed for range 0·180 – 0·270 mg.

HUMAN E.E.

(a) Weight of meal (mg)	(b) No. of eggs
0·180	19
0·195	19
0·203	30
0·230	36
0·250	37
0·241	25
0·235	41
0·264	32
0·240	17

$n = 9$

$\Sigma a = 2{\cdot}034$ $\qquad$ $\Sigma b = 256{\cdot}000$

$\bar{a} = 0{\cdot}226$ $\qquad$ $\bar{b} = 28{\cdot}444$

$(\Sigma b)^2 = 65{,}536{\cdot}000$

$\Sigma b^2 = 7{,}906{\cdot}000$

$S_a = 0{\cdot}028$ $\qquad$ $S_b = 8{\cdot}833$

$SE = 2{\cdot}940$

(c) Weight of meal (mg)	(d) No. of eggs.
0·198	59
0·215	45
0·215	52
0·182	56
0·222	58
0·242	65
0·255	67
0·257	74
0·258	73
0·262	54

$n = 10$

$\Sigma x = 2{\cdot}310$

$\bar{c} = 0{\cdot}231$

$S_c = 0{\cdot}028$

$\Sigma d = 603{\cdot}000$

$\bar{d} = 60{\cdot}300$

$(\Sigma d)^2 = 363{,}609{\cdot}000$

$\Sigma d^2 = 37{,}145{\cdot}000$

$S_d = 9{\cdot}334$

$SE = 2{\cdot}950$

Comparison of $\bar{b}$ and $\bar{d}$:

$p = 0{\cdot}001$; $t = 7{\cdot}43$; $df = 17$

APPENDIX 36.

Eggs matured at 25°C on weight of 75% human blood imbibed through leached chick membrane.

Generation 20; human-line; L. longipalpis from B.H.

Weight of meal (mg)	No. of eggs.
0·295*	22*
0·366	32
0·344*	30*
0·335*	33*
0·312*	31*
0·380*	42*
0·438	45
0·252	23
0·398	26
0·278	34
0·355*	39*
0·395	35

$n = 12$

* for weight of meal 0·280 - 0·390 mg

$\Sigma x = 2·021$ $\Sigma y = 197·000$

$\bar{x} = 0·337$ $\bar{y} = 32·833$

$S_x = 0·030$ $(\Sigma y)^2 = 38,809·000$

$\Sigma y^2 = 6,719·000$

$S_y = 7·083$

APPENDIX 37.

Eggs matured at 25°C on weight of 0·6% (w/v) L-isoleucine in 75% human blood imbibed through leached chick membrane.

Generation 20; human-line; L. longipalpis from B.H.

x Weight of meal (mg)	y No. of eggs.
0·444	26
0·282*	23*
0·470	63
0·465	43
0·428	48
0·344*	41*
0·388*	55*
0·365*	38*
0·344*	29*
0·136	5
0·300*	19*

$n = 11$

* For weight of meal 0·280 – 0·390 mg

$\Sigma x = 2·023$ $\qquad$ $\Sigma y = 205·000$

$\bar{x} = 0·337$ $\qquad$ $\bar{y} = 34·167$

$S_x = 0·039$ $\qquad$ $(\Sigma y)^2 = 42,025·800$

$\Sigma y^2 = 7,881·000$

$S_y = 13·242$

APPENDIX 38.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 10% (w/v) bovine serum albumen in 0·85% saline imbibed through leached chick membrane.

Generation 23; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) NO.of eggs.
0.399	15
0·537	29
0·443	17
0·253	23
0·367	23
0·443	29
0·391	15
0·117	7
0·562	37
0·484	25

n	= 10		
Σx	= 3·996	Σy	= 220·000
$\bar{x}$	= 0·399	$\bar{y}$	= 22·000
$(\Sigma x)^2$	= 15·968	$(\Sigma y)^2$	= 48,400·000
Σx^2	= 1·755	Σy^2	= 5,522·000
Σxy	= 95·774	b	= 49·759
$\Sigma x \Sigma y$	= 879·120	S_b	= 6·029
		t	= 3·280
p	= 0·020	df	= 8

APPENDIX 39

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 10%(w/v) bovine serum albumen in 0·85% saline imbibed through parafilm 'M' membrane.

Generation-23; human-line; L. longipalpis from BH.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No. of eggs
0·012	1
0·077	10
0·105	9
0·131	14
0·078	11
0·142	9
0·455	31
0·357	30
0·432	29
0·365	23
0·296	22
0·103	13
0·062	11

n	=	13			
Σx	=	2·615	Σy	=	213.000
$\bar{x}$	=	0·201	$\bar{y}$	=	16.385
$(\Sigma x)^2$	=	6·838	$(\Sigma y)^2$	=	45,369.000
Σx^2	=	0·817	Σy^2	=	4,585.000

$\sum xy$	= 59.968	b	= 58.952
$\sum x \sum y$	= 556.569	S_b	= 2.760
		t	= 11.520
p	= 0.001	df	= 11

APPENDIX 40.

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 10% (w/v) fatty-acid free bovine serum albumen in 0·85% saline imbibed through parafilm 'M' and leached chick membrane.

Generation 23; human-line; L. longipalpis from B.H.

	INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
Parafilm 'M'	0·315	27
	0·235	23
	0·046	8
Leached chick membrane.	0·258	13
	0·448	35
	0·387	23
	0·376	30
	0·301	16
	0·459	40
	0·503	23
	0·475	42
	0·271	23
	0·309	24

n = 13

Σx	= 4·381	Σy	= 327·000
$\bar{x}$	= 0·337	$\bar{y}$	= 25·154
$(\Sigma x)^2$	= 19·193	$(\Sigma y)^2$	= 106,929·000
Σx^2	= 1·664	Σy^2	= 9,399·000
Σxy	= 121·837	b	= 61.904
$\Sigma x \Sigma y$	= 1,432.587	S_b	= 6.420
		t	= 4·181
p	= 0·002	df	= 11

APPENDIX 41

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of human plasma imbibed through leached chick membrane.

Generation 20; human line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No of eggs
0·332	41
0·232	37
0·282	36
0·242	32
0·255	32
0·257	32
0·213	31
0·239	30
0·284	26
0·137	24

n	= 10		
Σx	= 2·473	Σy	= 321.000
$\bar{x}$	= 0·247	$\bar{y}$	= 32.100
$(\Sigma x)^2$	= 6.116	$(\Sigma y)^2$	= 103,041.000
Σx^2	= 0.635	Σy^2	= 10,531.000
Σxy	= 80·921	b	= 66.870
$\Sigma x \Sigma y$	= 793·833	S_b	= 3·938
		t	= 2·575
p	= 0·050	df	= 8

APPENDIX 42

Eggs matured on weight of human plasma imbibed for range 0·250 – 0·350 mg.

HUMAN PLASMA

(a) Weight of meal (mg)	(b) No. of eggs
0·257	32
0·255	32
0·282	36
0·284	26
0·332	41

$n = 5$

$\Sigma a = 1{\cdot}410$ $\quad \Sigma b = 167.000$

$\bar{a} = 0{\cdot}282$ $\quad \bar{b} = 33.400$

$(\Sigma b)^2 = 27{\cdot}889$

$\Sigma b^2 = 5{,}701{\cdot}000$

$S_a = 0{\cdot}031$ $\quad S_b = 5{\cdot}550$

$SE = 2{\cdot}480$

50% HUMAN PLASMA IN 0·85% SALINE.

(c) Weight of meal(mg)	(b) No. of eggs
0·262	18
0·274	13
0·292	10
0·348	13

$n = 4$

$\Sigma c = 1.176$ $\quad\quad$ $\Sigma d = 54.000$

$\bar{c} = 0.294$ $\quad\quad$ $\bar{d} = 13.500$

$(\Sigma d)^2 = 2,916.000$

$\Sigma d^2 = 762.00$

$S_c = 0.038$ $\quad\quad$ $S_d = 3.317$

$SE = 1.660$

Comparison of $\bar{b}$ and $\bar{d}$:

$p = 0.001$; $\quad t = 6.28$; $\quad df = 7$

APPENDIX 43

REGRESSION ANALYSIS.

Eggs matured at 25°C on weight of 5% (w/v) bovine serum albumen in 0·85% saline imbibed through leached chick membrane.

Generation 23; human-line; L. longipalpis from B.H.

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No. of eggs.
0·282	8
0·113	1
0·359	14
0·392	11
0·389	14
0·120	9
0·085	1
0·266	12
0·141	2
0·442	13
0·440	9
0·406	7
0·292	13
0·389	10

$n = 14$

$\Sigma x = 4{\cdot}116$ $\quad \Sigma y = 124{\cdot}000$

$\bar{x} = 0{\cdot}294$ $\quad \bar{y} = 8{\cdot}857$

$(\Sigma x)^2 = 16{\cdot}941$ $\quad (\Sigma y)^2 = 15{,}376{\cdot}000$

Σx^2	= 1.429	Σy^2	= 7,856.000
Σxy	= 42·026	b	= 25.434
$\Sigma x \Sigma y$	= 510·384	S_b	= 3.367
		t	= 3.535
p	= 0·010	df	= 12

APPENDIX 44.

Eggs matured on 0·350 – 0·450 mg of bovine serum albumen solution (in 0·85% saline) imbibed.

10% (w/v) SOLUTION

(a) Weight of meal (mg)	(b) No. of eggs
0.399	15
0.443	17
0.367	23
0.443	29
0.391	15

$n = 5$

$\Sigma a = 2.043$ $\quad \Sigma b = 99.000$

$\bar{a} = 0.409$ $\quad \bar{b} = 19.800$

$(\Sigma b)^2 = 9{,}801.000$

$\Sigma b^2 = 2{,}109.000$

$S_a = 0.034$ $\quad S_b = 2.469$

5% (w/v) SOLUTION

(c) Weight of meal (mg)	(d) No. of eggs
0.359	14
0.392	11
0.389	14
0.442	13
0.440	9
0.406	7
0.389	10

$n = 7$

Σc	= 2·814	Σd	= 78·000
$\bar{c}$	= 0·402	$\bar{d}$	= 11·140
		$-(\Sigma d)^2$	= 6,084·000
		Σd^2	= 912·000
S_c	= 0·030	S_d	= 1·635

Comparison of $\bar{b}$ and $\bar{d}$:

p = 0·010; t = 3·67; df. = 10

APPENDIX 45

Eggs matured at 25°C on weight of 20% (w/v) bovine serum albumen in 0·85% saline imbibed through leached chick membrane.

Generation 23; human-line; L. longipalpis from B.H.

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No. of eggs
0·233	9
0·186	3
0·243	8
0·117	11
0·079	14
0·065	28
0·131	1
0·148	21
0·207	18
0·298	7
0·152	10
0·299	19
0·300	18
0·335	19

n = 14

APPENDIX 46

The association of female weight at blood-meal and the number of eggs matured for L. longipalpis fed on human blood.

Weight of female at blood-meal (mg)	Deviation from eggs matured/blood weight regression (CALCULATED)
0.330	+ 2.19
0.354	+ 5.30
0.238	-10.20
0.406	+10.36
0.313	+ 5.92
0.266	+ 4.61
0.352	- 2.58
0.322	+ 9.29
0.348	- 1.92
0.373	+10.70
0.270	+ 0.47
0.326	- 1.02
0.295	- 1.25
0.270	- 4.22
0.309	- 3.21
0.340	+ 1.01
0.254	+ 1.49

	0·266		− 15·56
	0·282		− 10·55
n	= 19		
Σx	= 5·91	Σy	= 0·83
$\bar{x}$	= 0·31	$\bar{y}$	= 0·04
$(\Sigma x)^2$	= 34·98	$(\Sigma y)^2$	= 0·69
Σx^2	= 1·88	Σy^2	= 899·28
Σxy	= 3·77	b	= 87·75
$\Sigma x \Sigma y$	= 0·26	S_b	= 5·90
		t	= 2·97
p	= 0·010	df	= 17

APPENDIX 47

The association of female weight at blood-meal and the number of eggs matured for L. longipalpis fed on human blood.

Weight of female at blood-meal (mg)	Deviation from eggs matured/blood weight regression
0·345	+ 7·5
0·339	- 3·8
0·384	+ 2·8
0·352	- 1·7
0·277	- 3·4
0·353	+ 3·2
0·375	+ 1·6
0·240	- 9·1
0·280	- 3·4
0·298	+ 2·3
0·346	+ 1·4
0·319	- 5·0
0·323	+ 0·4
0·278	- 5·1
0·401	+ 2·5
0·446	+ 11·1
0·418	+ 4·4
0·286	- 9·4
0·359	+ 6·7

n = 19

Σx	= 6·42	Σy	= 0·20
$\bar{x}$	= 0·34	$\bar{y}$	= 0·01
$(\Sigma x)^2$	= 41·22	$(\Sigma y)^2$	= 0·04
Σx^2	= 2·22	Σy^2	= 540·64
Σxy	= 4·25	b	= 83·60
$\Sigma x \Sigma y$	= 1·28	S_b	= 3·35
		t	= 5·58
p	= 0·001	df	= 17

APPENDIX 48

The weights of individuals of L. longipalpis at different stages of development.

x		y
Weight of 4th instar larva a day before pupation	Weight of pupa a day before emergence	Weight of female on day of emergence
0·730	0·653	0·428
0·698	0·650	0·359
0·753	0·700	0·405
0·632	0·507	0·341
0·735	0·634	0·400
0·640	0·567	0·320
0·689	0·582	0·352
0·742	0·632	0·386
0·730	0·632	0·352
0·710	0·607	0·394
0·703	0·605	0·332
0·706	0·592	0·398
0·754	0·643	0·392
0·732	0·690	0·376
0·689	0·664	0·414
0·790	0·663	0·429
0·729	0·630	0·430
0·727	0·620	0·402
0·710	0·597	0·364
0·796	0·688	0·454

n = 20

Σx	= 14·395	Σy	= 7·728
$\bar{x}$	= 0·720	$\bar{y}$	= 0·386
$(\Sigma x)^2$	= 207·216	$(\Sigma y)^2$	= 59·722
Σx^2	= 10·392	Σy^2	= 3·011
Σxy	= 5·583	b	= 0·677
$\Sigma x \Sigma y$	= 111·245	S_b	= 0·024
		t	= 4·967
p	= 0·001	df	= 18

APPENDIX 49

Wet weights of adult females of L. longipalpis on day of emergence when the larvae were reared at 28°C and 25°C.

x	y
At 28°C	At 25°C
0·296	0·347
0·367	0·436
0·283	0·385
0·350	0·364
0·350	0·419
0·335	0·423
0·320	0·383
0·290	0·405
0·330	0·342
	0·393
	0·400
	0·402
	0·416
	0·453
	0·368
	0·415
	0·346
	0·375
n = 9	n = 18

Σx	= 2·921	Σy	= 7·074
$\bar{x}$	= 0·324	$\bar{y}$	= 0·393
$(\Sigma x)^2$	= 8·532	$(\Sigma y)^2$	= 50·041
Σx^2	= 0·955	Σy^2	= 2·796
S_x	= 0·029	S_y	= 0·032

Comparison of $\bar{x}$ and $\bar{y}$:

$p = 0{\cdot}001$; $t = 5{\cdot}62$; $df = 25$.

APPENDIX 50

FEMALES OFFERED

30% (w/v) sucrose all life

INDEPENDENT (x) Weight of meal (mg)	DEPENDENT (y) No.of eggs.
0·477	55
0·342	45
0·378	33
0·362	52
0·243	36
0·380	48
0·454	48
0·373	52
0·509	54
0·482	64
0·330	39
0·088	14
0·255	30
0·368	38
0·337	36
0·417	48
0·196	27
0·320	22
0·464	41

$n = 19$

$\Sigma x = 6.775$ $\quad \Sigma y = 782$

$\Sigma x^2 = 2.620$ $\quad \Sigma y^2 = 35,018$

$\Sigma xy = 298.670$

b = 97·16 t = 6·01

p = 0·001 df = 17

FEMALES OFFERED ONLY WATER

INDEPENDENT (x)	DEPENDENT (y)
Weight of meal (mg)	No.of eggs
0·349	35
0·317	16
0·523	42
0·182	21
0·442	33
0·378	38
0·495	49
0·061	0
0·202	0
0·074	0
0·436	45
0·392	41
0·271	23
0·375	20
0·209	22
0·506	40
0·473	37
0·248	32
0·230	23
Σx = 6·163	Σy = 517
Σx^2 = 2·358	Σy^2 = 18,261
Σxy = 201·270	
b = 93·5	t = 7·12
p = 0·001	df = 17

APPENDIX 51

Number of eggs matured on weight of human blood imbibed by 2 - 3 day old L. longipalpis. 30% (w/v) sucrose offered from emergence.

z Weight of female at blood-meal (mg)	x Weight of blood imbibed (mg)	y No. eggs matured
0.367	0.361	BLOOD NOT DIGESTED
0.414	0.444	46
0.380	0.530	48
0.332	0.536	51
0.413	0.472	53
0.440	0.235	43
0.360	0.680	58
0.411	0.265	35
0.482	0.286	60
0.402	0.466	37
0.352	0.418	52
0.390	0.278	39
0.380	0.432	62
0.477	0.271	47
0.316	0.602	63
0.468	0.152	35
0.353	0.302	43
0.377	0.358	51
0.315	0.351	48

0·313	0·264	38
0·385	0·308	49

$n = 20$

$\bar{z} = 0{\cdot}396$

$S = 0{\cdot}054$

APPENDIX 52

Number of eggs matured on weight of human blood imbibed by 5 - 6 day old L. longipalpis. 30% (w/v) sucrose offered from emergence.

z Weight of female at blood-meal (mg)	x Weight of blood imbibed (mg)	y No.eggs matured
0·442	0·270	BLOOD NOT DIGESTED
0·395	0·563	60
0·394	0·224	37
0·373	0·702	53
0·342	0·221	34
0·354	0·186	37
0·345	0·460	53
0·347	0·558	57
0·319	0·453	49
0·455	0·408	42
0·475	0·313	37
0·415	0·342	38
0·427	0·418	42

$n = 12$

$\bar{z} = 0·387$

$S = 0·049$

APPENDIX 53

Number of eggs matured on weight of human blood imbibed by 11 - 12 day old L. longipalpis. 30% (w/v) sucrose offered from emergence.

z	x	y
Weight of female at blood-meal(mg)	Weight of blood imbibed (mg)	No.of eggs matured
0.322	0.370	BLOOD NOT DIGESTED
0.365	0.460	DITTO
0.371	0.302	DITTO
0.380	0.537	51
0.332	0.448	43
0.315	0.710	49
0.342	0.348	40
0.328	0.452	30
0.371	0.610	60
0.385	0.475	48
0.304	0.198	17
0.326	0.269	33
0.332	0.333	37
0.375	0.287	30
0.300	0.510	41
0.395	0.790	63
0.340	0.178	26
0.280	0.495	31

$n = 15$

$\bar{z} = 0.340$

$S = 0.034$

APPENDIX 54

Number of eggs matured on weight of human blood imbibed by 15 - 16 day old L. longipalpis . 30% (w/v) sucrose offered from emergence.

z	x	y
Weight of female at blood-meal(mg)	Weight of blood imbibed (mg)	No.eggs matured
0·341	0·459	BLOOD NOT DIGESTED
0·283	0·359	DITTO
0·378	0·470	DITTO
0·337	0·683	DITTO
0·379	0·318	DITTO
0·290	0·100	DITTO
0·335	0·226	DITTO
0·427	0·378	DITTO
0·313	0·587	DITTO
0·326	0·477	DITTO
n = 10		
$\bar{z}$ = 0·341	$\bar{x}$ = 0·406	
S = 0·044	S = 0·169	
0·374	0·466	65
0·316	0·275	33
0·277	0·560	50
0·338	0·475	45
0·347	0·305	55

0.295	0·168	16
0.261	0·252	25
0.300	0·493	48
0.350	0·184	19

$n = 9$

$\bar{z} = 0·318$

$S = 0·037$

$\bar{x} = 0·353$

$S = 0·146$

Appendix 55.

A record of temperature and humidity in the Serra dos Carajás (Brazil) during September 1974. (Courtesy Dr. J. Texeira)

DATE (Sept. 1974)		TEMPERATURE, °C. MAX.	MIN.	RAINFALL (mm.)
01	day	27.5	21.5	0.0
01	night	26.0	21.0	0.0
02	day	28.0	21.0	0.0
02	night	26.0	22.0	0.0
03	day	27.0	22.0	0.4
03	night	24.0	20.0	15.0
04	day	25.0	20.0	0.0
04	night	26.0	20.5	0.0
05	day	27.0	21.0	0.0
05	night	26.5	21.5	0.0
06	day	27.0	23.0	4.2
06	night	24.5	21.5	0.0
07	day	27.0	21.0	0.0
07	night	26.0	21.0	0.0
08	day	27.0	21.0	0.0
08	night	25.0	20.0	0.0
09	day	28.5	21.0	0.0
09	night	27.0	21.5	0.0
10	day	27.5	21.5	0.0
10	night	26.5	21.0	0.0
11	day	30.0	21.0	0.0
11	night	28.5	21.5	0.0
12	day	29.0	21.5	0.0
12	night	28.0	22.0	0.0
13	day	27.0	20.5	0.0
13	night	26.0	22.0	0.0
14	day	28.0	22.0	0.0
14	night	26.0	18.5	2.0
15	day	26.5	20.5	0.0
15	night	26.0	21.5	0.0
16	day	28.0	21.5	0.0
16	night	25.5	22.5	0.0
17	day	27.0	22.0	0.0
17	night	26.0	22.0	0.0
18	day	28.0	22.0	3.4
18	night	27.0	21.5	0.0
19	day	27.5	22.0	0.0
19	night	26.0	22.0	15.4
20	day	23.0	21.0	3.0
20	night	24.0	20.5	0.0
21	day	27.0	21.5	0.0
21	night	25.5	21.0	0.0
22	day	27.0	21.5	0.0
22	night	25.0	22.0	0.0
23	day	26.5	22.0	0.0
23	night	25.0	21.5	0.0
24	day	27.5	22.0	0.0
24	night	26.0	22.0	0.0
25	day	27.0	23.0	0.0
25	night	26.5	22.0	0.0

Appendix 56.

Unpublished reports of fieldwork carried out in Emilia-Romagna, Italy, during the summer of 1973.

The author played a major role in the work described in the second report and collaborated fully in that set out in the first report.

First report on an investigation of phlebotomid sandflies of Emilia-Romagna, Italy, in July 1973

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Contents

Summary

In July 1973, an investigation was made of the sandflies in the south of the province of Emilia-Romagna, Italy, where there had been an outbreak of kala-azar in 1971-72. Only one species, P.perfiliewi, was found. It was widespread and abundant in domestic situations and in fields and woods. In natural conditions it was shown to feed on a wide range of wild and domestic animals. It came readily to man in varying temperatures and humidities and was shown to sustain the attack from shortly before sunset, through the night, until a few hours after dawn. Engorged females were collected and a laboratory culture initiated.

From its distribution and habits it could be suspected of being a vector in Emilia-Romagna. Confirmation of its susceptibility to Leishmania is necessary before a conclusion can be reached. Similar studies are planned on P.perniciosus, the assumed vector of kala-azar in other parts of Italy.

It is recommended that spraying should be continued, and current work aimed at revealing the vector be encouraged. It is unlikely that transmission to man can be prevented in conditions such as in 1971 without knowing the vector and reservoir hosts.

Background

In 1971-72 there was an outbreak of visceral leishmaniasis in the region of Emilia-Romagna, Italy. 60 cases were diagnosed and 13 patients died. During the previous 50 years, only 4 autochthonous cases of the disease had been reported in the region. The outbreak, which has been described by Pampiglione and colleagues (Ref. 1), differed from typical Mediterranean kala-azar in that: (i) a high proportion (60%) of the patients were adult men: (ii) in some patients the disease was rapidly fatal: (iii) from serological studies there is evidence of asymptomatic cases: and (iv) there is a strong suggestion that the usual reservoir host, the domestic dog, was not the source of infection.

In 1971 there was the most severe drought in Emilia-Romagna within living memory. One explanation of the outbreak is that this abnormal weather led to changes in the numbers and behaviour of the sandflies which altered the man-fly contact. Another is that the drought affected the behaviour of an as yet unknown reservoir host and the parasite was thus brought closer to man. Both changes may have occurred (Ref. 1).

In 1972, a team led by Professor A. Coluzzi collected 4,777 sandflies in the area and identified 4 species, viz.: Phlebotomus perfiliewi (54% of the catch), P. perniciosus (4.8%), P.papatasi (0.1%) and Sergentomyia minuta (41%) (Ref. 2). From indirect evidence of the distribution of leishmaniasis and sandflies, it is generally assumed that in Italy the vector of kala-azar is P.perniciosus, and that of cutaneous leishmaniasis (seldom seen in Emilia-Romagna), P.perfiliewei (e.g. Ref. 3). P.papatasi, though a vector of visceral and cutaneous leishmaniasis in other parts of its range, has not been implicated in Italy. S.minuta feeds predominantly on reptiles and is nowhere suspected of being a vector of leishmaniasis of mammals, including man. However, a closely related species, S.arpaklensis, from the Middle East and western USSR is thought to be important in the maintenance of epizootics of cutaneous leishmaniasis of rodents; it occasionally bites man.

Control measures taken in Emilia-Romagna include: (i) diagnosis and prompt treatment of cases; (ii) destruction of foxes and, in selected instances, of dogs; and (iii) spraying houses in the affected area with insecticides.

Objectives

At the invitation of Professor S. Pampiglione of the Cattedra di Parasitologia, Universita di Bologna, we spent the month of

July, 1973, in Emilia-Romagna studying the biology of the sandflies. Our aim was to gather data on their distribution and behaviour (especially biting habits), and to establish laboratory colonies of any sandflies suspected of being involved in transmission. We hope to be able to compare the susceptibilities of different flies to Leishmania in the laboratory, and evaluate their potential as vectors of kala-azar.

Observations

During the period July 9th-31st we collected 3,680 sandflies from 8 sites in the northern foothills of the Apennine range (see map, appendix 1). We have so far identified 2,500 specimens (500 ♀♀, 2,000 ♂♂). All were P.perfiliewi. Methods of collection are listed in appendix 3.

Domestic sites

Numerous P.perfiliewi were collected in a stable of a house (site 1, appendix 2) which had been sprayed in April, 1973. Only few sandflies were taken from inside the house. Female P.perfiliewi came readily to man outside a house in the Zena Valley (site 7), and in the garden of a villa belonging to a man who was the last case diagnosed (February, 1973) (site 6). In the latter locality, one of the highest man-biting rates of any sandfly ever recorded was observed. Both houses had been sprayed in the spring, and there were only few sandflies within the houses. Several female P.perfiliewi were taken in the garden of a house (site 3) at an altitude of 500 m., 200 m. higher than in previous collections in Emilia-Romagna (Ref. 2).

Silvatic sites

The preponderance of adult male patients in the 1971-72 outbreak suggests that at least some of the infections may have been acquired away from houses. Hunting and farming expose man to the bites of silvatic sandflies which may have become infected by feeding on wild mammals. We therefore collected from three silvatic sites, each more than 1 km. from the nearest house (sites 2, 4 and 5). In all three P.perfiliewi was abundant. There appear to have been no collections of P.perfiliewi from such sites in previous work.

Biting preferences of P.perfiliewi

Tent traps and oiled trays (see appendix 3) were baited with a variety of mammals and set at several places to determine which

animals P.perfiliewi will feed on in natural conditions, and to discover which were preferred hosts. The flies fed on the field mouse (Sylvaemus sylvaticus), the hare (Lepus capensis), the rabbit (Oryctolagus cuniculus), the beech marten (Martes foina), the fox (Vulpes vulpes) and the dog (Canis familiaris) (see appendix 4). In addition, flies were seen to feed readily on man and cattle. No fed females were taken from traps baited with voles (Pitymys savii) or the domestic cat (Felis catus). No observations were made on sheep, goats or pigs. An unusually high proportion of female flies took blood from the hare (20%) and the rabbit (15%). From the few experiments we had time to undertake, we conclude that the populations of P.perfiliewi studied may have a preference for leporids - the hare in the wild, and the rabbit in domestic situations. (Wild rabbits are not present).

Conditions in which P. perfiliewi attacks man

In all sites, at altitudes ranging from 90 to 500 m., P.perfiliewi attacked man in a remarkable variety of weather conditions. Light winds did not prevent attack, although there was a suggestion that in such conditions the movement of the flies was restricted to a level close to the ground. Females fed readily on man at temperatures ranging from 14°C to 25°C. Humidity seemed not to affect biting activity and we were attacked at R.H. from 50% to 100%. Light rain did not deter biting.

Biting activity of P.perfiliewi

P.perfiliewi never came to feed in bright light, but began before sunset and fed until a few hours after sunrise (at a time when farmers or hunters are often active). In an all-night man-biting catch at site 6, all flies which began to feed on a man stripped to the waist were collected during the first 15 minutes of each hour from 21.00 hrs until 07.15 hrs the following morning. Collectors used insect repellent, and 2 torches were kept on throughout each collecting period. The results are shown graphically in appendix 5. In suitable weather conditions, such as on that occasion, P.perfiliewi was found to attack man throughout the night. Peak biting activity was at 03.00-03.15 hrs when 118 flies probed on the bait - an unusually high number. During the eleven 15-minute collecting periods (total time $2\frac{3}{4}$hrs) 462 probing flies were taken.

In other localities when man-biting catches of engorged females were made for 1 hour from about 22.00-23.00 hrs 40-60 flies were commonly collected. These fed females provided eggs with which the laboratory culture was initiated using the methods described by Killick-Kendrick and colleagues (Ref. 4).

Conclusions

Since we encountered only P.perfiliewi in the places studied,

our conclusions must be limited to a provisional assessment of this species alone. It is likely that other man-biting sandflies will be found at other times of the year and that we were collecting when the population of P.perfiliewi was approaching a peak.

The picture of P.perfiliewi is of an abundant, widely distributed species with opportunistic feeding habits. It will take blood from a variety of wild animals - some of which could be reservoir hosts - and is a persistent and determined man-biter.

From its distribution and habits P.perfiliewi could be a vector of kala-azar in Emilia-Romagna. The Italian subspecies (P.p.perfiliewi) is a known or suspected vector of visceral leishmaniasis in Greece and Macedonia, and another subspecies (P.p.trancaucasicus) is a vector in Azerbaidzan, USSR (Ref. 5). However, it should not be assumed that this fly was responsible for the Italian outbreak unless the susceptibility of P.perfiliewi from Emilia-Romagna to Leishmania is demonstrated experimentally or Leishmania infantum is isolated from a naturally infected fly.

Recommendations

1. Control measures

Spraying houses with DDT seems to have greatly reduced the numbers of sandflies within the houses. If spraying is repeated in future years, it may be even more effective if it is undertaken in June, near the seasonal appearance of sandflies, rather than in March or April. In addition to the danger of leishmaniasis, sandflies are a serious pest in the area studied; spraying may be considered desirable on these grounds alone.

Some stables, often close to or integral parts of houses, appear not to have been sprayed. Large numbers of sandflies feeding on cattle were found in one such stable. This maintenance of a large population of flies close to houses is obviously undesirable, and the numbers could be reduced, at least in some places, by spraying all stables, pigsties, henhouses and rabbit hutches whenever the construction lends itself to such a measure.

The presence of small numbers of sandflies in houses sprayed only 3 months before our visit is difficult to explain. Possibly they were an 'overflow' from nearby stables. Since there was no spraying while we were in Emilia-Romagna, we did not see how it was done.

Russian workers, who have much experience of controlling sandflies with insecticides, suggest that:

(i) the behaviour and favoured resting sites of the flies should be determined;

(ii) rooms should be prepared for spraying by moving furniture away from walls and removing dust from surfaces to be sprayed;

(iii) insecticide should be sprayed on walls from the floor to the ceiling, on frames and sills of doors and windows, on a 1 metre strip of the ceiling along the walls and above lights, on the back of furniture and pictures which are against walls, and on the lower parts of chairs and tables;

(iv) occupants should be encouraged to keep sprayed surfaces free from dust with soft brushes or rags, and should understand that rough cleaning or re-painting will remove the insecticide;

(v) spraying in the open air should be done in dry, windless weather towards the end of the day as the heat subsides.

A safe dose-rate of DDT for mammals is 1-2 gms active compound per metre^2 which, according to the 1963 W.H.O. Report of The Expert Committee on Insecticides, should remain active against sandflies for up to 2 years. Russian workers suggest respraying every 30-40 days throughout the season, but we believe this to be uneconomic in Emilia-Romagna. The large populations of flies away from houses will not be controlled by house-spraying.

It is generally thought that sandflies do not become resistant to insecticides. Different species do, however, exhibit varying degrees of susceptibility (Ref. 6). Moreover, isolated populations of flies are said to be resistant to chlorinated insecticides in the USSR. In Emilia-Romagna, therefore, it would be prudent annually to test the susceptibility of the sandflies to DDT.

It is therefore RECOMMENDED that:

(i) the interiors of houses, stables and other structures housing domestic animals should be sprayed with DDT in June of each year;

(ii) the occupants should be advised how to prepare their houses for spraying and how best to maintain the activity afterwards;

(iii) the susceptibility of the sandflies to insecticides should be monitored.

2. Future studies

Although spraying can be expected to control domestic transmission, the danger of infections being acquired away from houses remains - and may, indeed, be the most likely place of transmission to man in Emilia-Romagna. Until the vector is discovered and its habits known, and the source of infection is discovered (probably a wild animal or - and this seems less likely - the domestic dog), the only effective control measure is to continue with the swift recognition of cases and prompt treatment. If the causes of the outbreak are to be understood and steps taken to ensure there are no more outbreaks, an intensive study of the sandflies, of reservoirs of infection and of strains of the parasite is desirable.

From the point of view of pinpointing the vector, it is RECOMMENDED that:

(i) cultures of P.perfiliewi and P.perniciosus be established in the laboratory, and a comparison be made of their susceptibilities to strains of Leishmania from patients.

This work has been started and will continue in collaboration with the workers in Bologna. For this and other work it is essential that new strains of the parasite be isolated from patients. In our view, this last step must be given high priority.

(ii) studies on the distribution and habits of P.perniciosus be made.

In September, 1973 one of us (F.D.R.) will attempt to find this fly and undertake a study similar to the one on P.perfiliewi. It is regrettable that there appear to be no records of the precise places from which P.perniciosus was collected in 1972, nor of the time of year that it was found. This information would greatly help future work.

(iii) flies be dissected and examined for the presence of Leishmania.

This should perhaps await an assessment of the susceptibilities of P.perfiliewi and P.perniciosus to Leishmania in the laboratory. Parasites from wild-caught flies should be established in culture and laboratory animals, so that they may be compared with strains isolated from man or reservoir hosts.

(iv) bloodmeals of engorged wild-caught flies be collected from fields and woods, and the source of blood identified.

Dr. P.F.L.Boreham of Imperial College, Ascot, England has kindly agreed to identify the bloodmeals. New techniques for collecting fed flies in other than domestic sites may have to be devised, e.g. the provision of artificial resting places. Such methods would be an important new contribution to the field study of sandflies.

Acknowledgments

We are most grateful to the administration of the Emilia-Romagna Region and the Wellcome Trust, London, for financial support.

Special thanks for many kindnesses and for arranging and supporting the field work are due to a number of people in Bologna and Castel San Pietro. We specially wish to thank Professor A.Mantovani, Professor S.Pampiglione, Professor F.Giungi, Dr. A.Parenti, Mr.M.Parenti and Mr. C.Varignana.

The Council of Castel San Pietro helped us greatly by providing transport, guides and animal accommodation.

Finally, it is a pleasure to thank Professor P.C.C. Garnham for help in planning the work.

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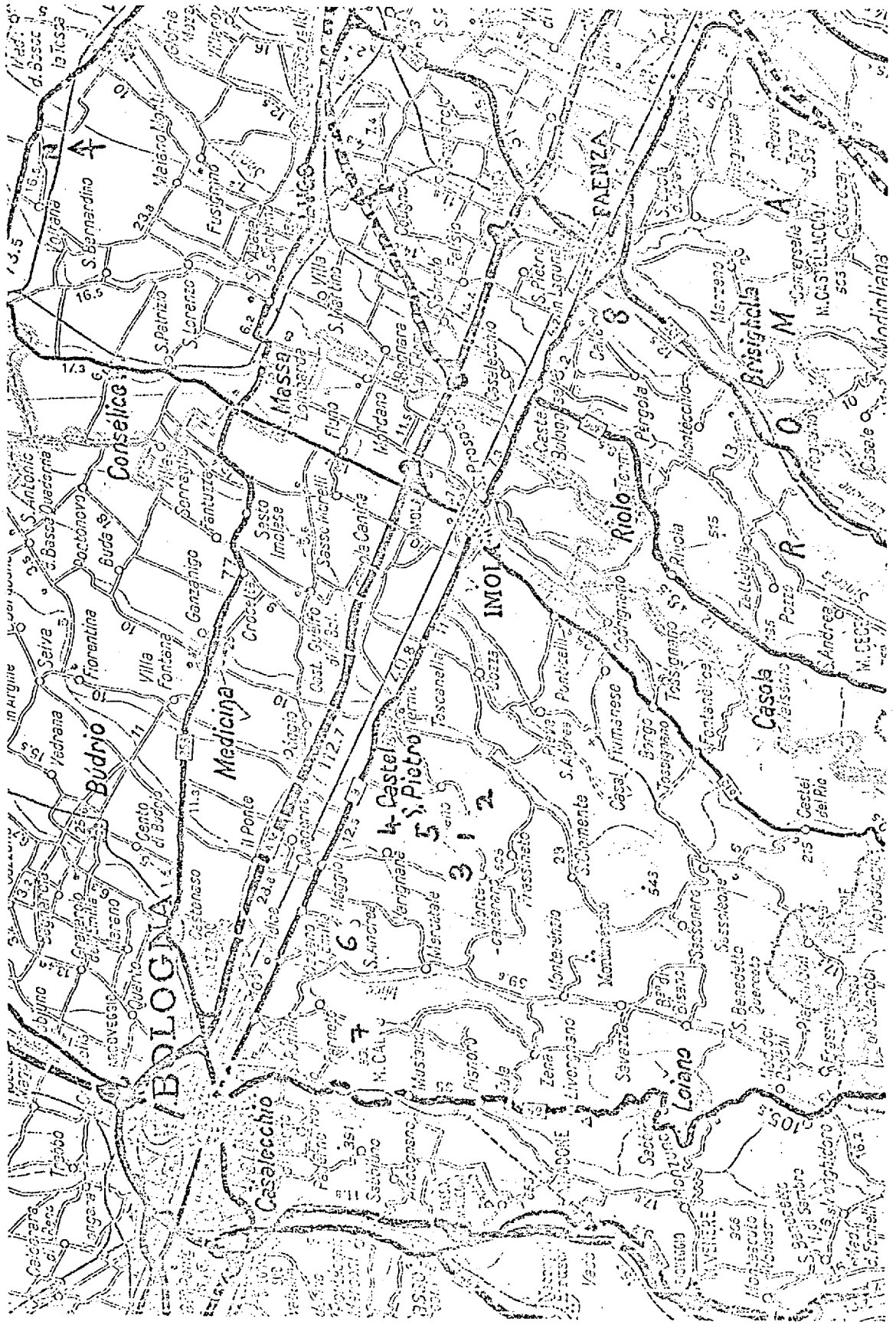
Map of Emilia-Romagna Province.



The outbreak was south of the via Emilia, in the foothills of the Apennine zone.

Appendix 2

List of collecting sites of P.perfiliewi

Site Number	Locality	Nature of collecting site	Trapping methods
1	Casa Beatuzzi and adjacent house, Vedriano. Via Gitanari	Cattle-sheds, farmyard, rabbit-hutches, chicken-houses, inside houses.	Sticky traps, tent traps, catches from man and catt
2	Near Ca Paderna, off via Gitanari	The wooded bottom of a dry valley (of Rio di Paderna)	Light/CO_2 sticky traps, Disney traps, catches fro man.
3	Montecalderaro. Villa of Dr. A.Parenti	Garden, and inside house.	Catches from man
4	Duzzola, Casalecchio Dei conti, Via Malvezza	Wooded hillside and stubble field	Light/CO_2 sticky traps, Disney traps, catches from man
5	Casalecchio Dei Conti, off Via Luogo	Dry, rocky river-bed and surrounding woods of Rio Della Varone	Light/CO_2 sticky traps, Disney traps, catches from man.
6	Casa Ortolani, Via Del Poggio	Hillside orchard and garden of a Villa	Catches from man, collecti of fed females from resting sites
7	Casa Rastignano, Zena Valley	Farmyard, rabbit-hutches, chicken-houses	Catches from man, collecti of fed females from resting sites.
8	Faenza Villa of Dr. M.	Garden	A catch from man

Appendix 3.

Collecting methods

1. Tent Trap (after Rioux)

Two, 2 metre poles support a pegged tent of mosquito-netting (2 metres long, and 1 metre wide at its base, whence it narrows to a point). Sandflies gain entry to the bait-animal within through a framed opening (60 cm x 30 cm) at ground level; trapped flies are collected with an aspirator from the top of the tent.

2. Modified Disney Trap

A caged animal is placed in the centre of a tray of castor oil (on the ground). Sandflies attracted to the bait are trapped when they land on the sticky surface.

3. Light and Carbon Dioxide Sticky Trap (after Rioux)

A paper lantern containing a dim white light and solid carbon dioxide attracts sandflies, which become stuck to the trap's castor-oil-impregnated walls.

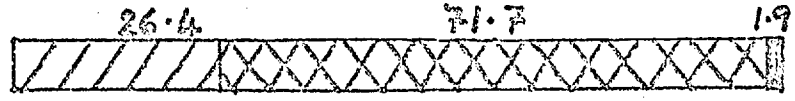
4. The collection with tube or aspirator of female sandflies attracted to bait animals.

5. Searching for engorged females in their resting sites.

Appendix 4.

Host preferences of P.perfiliewi.

'RIOUX' TENT TRAPS

		TOTAL FLIES CAUGHT	PERCENT FEMALES ENGORGED
BEECH MARTEN		126	8.9
FOX		53	2.6
DOG		60	14.3

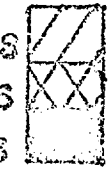
(SITE 1.) VERRIANO. 12.7.'73. 21.00 – 23.30 hrs.

FARMYARD, DIRECTLY OUTSIDE LIGHTED CATTLE SHED.

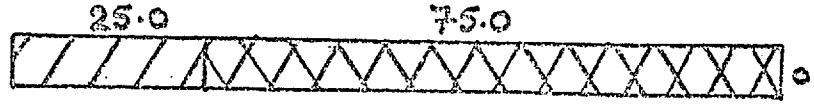
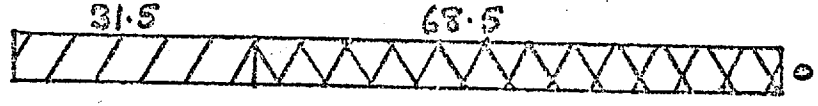
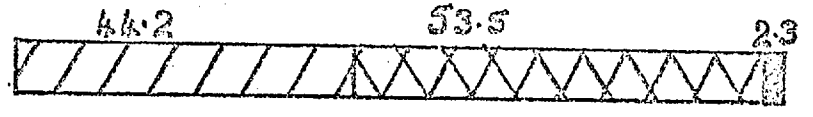
R.H. 60–65 %

TEMP. 22°C FALLING TO 20°C.

KEY



'RIOUX' TENT TRAPS

		TOTAL FLIES CAUGHT	PERCENT FEMALES ENGORGED
CONTROL		8	0
CAT		19	0
DOG		43	4·2

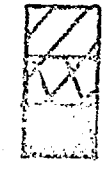
(SITE 1.) VEDRIANO. 16.7.'73. 21.10 — 23.10 hrs.

FARMYARD, AWAY FROM CATTLE SHED.

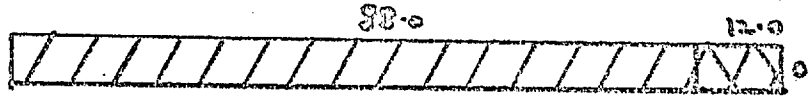
R.H. 60%

TEMP. 27·5°C. FALLING TO 22·0°C.

KEY



DISNEY TRAPS

		TOTAL FLIES CAUGHT	PERCENT FEMALES ENGORGED
SAVI'S VOLES (2)		270	0
RABBIT		360	13·2
HARE		398	19·3

(SITE. 4). CASALECCHIO DEI CONTI. 25-26.7.'73. 21.00hrs.— 08·30hrs.

STUBBLE FIELD.

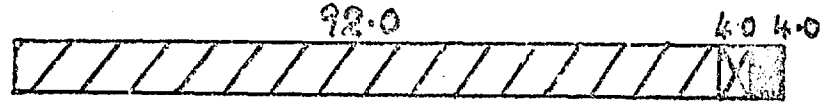
R.H. 100%.

TEMP. MAX. 22°C, MIN. 12°C.

KEY.

% MALES
% UNFED FEMALES
% FED FEMALES

DISNEY TRAPS

		TOTAL FLIES CAUGHT	PERCENT FEMALES ENGORGED.
CONTROL		25	[50]
BROWN RAT		67	0

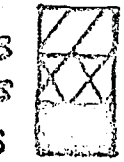
(SITE 5.) CASALECCHIO DEI CONTI. 15–16. 7. '73. 21·30 — 07·30 hrs.

DRY RIVER-BED.

R.H. 60 %

TEMP. MAX. 25°C, MIN. 17°C.

KEY

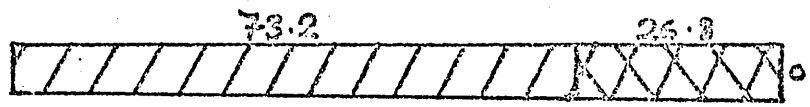
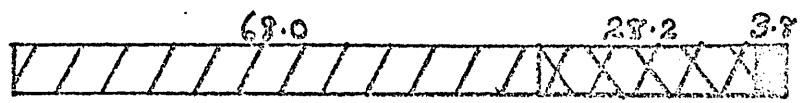


% MALES

% UNFED FEMALES

% FED FEMALES

DISNEY TRAPS

		TOTAL FLIES CAUGHT	PERCENT FEMALES ENGORGED
FIELD MOUSE		33	[50]
BROWN RAT		23	0
RABBIT		78	12

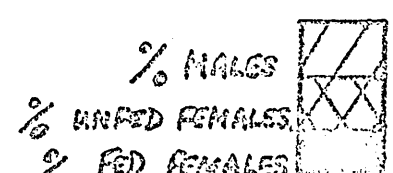
(SITE 2) CA PODERNA 20-21.7.'73. 21.15 hrs. – 09:00 hrs.

DRY RIVER-BED.

R.H. 50 %.

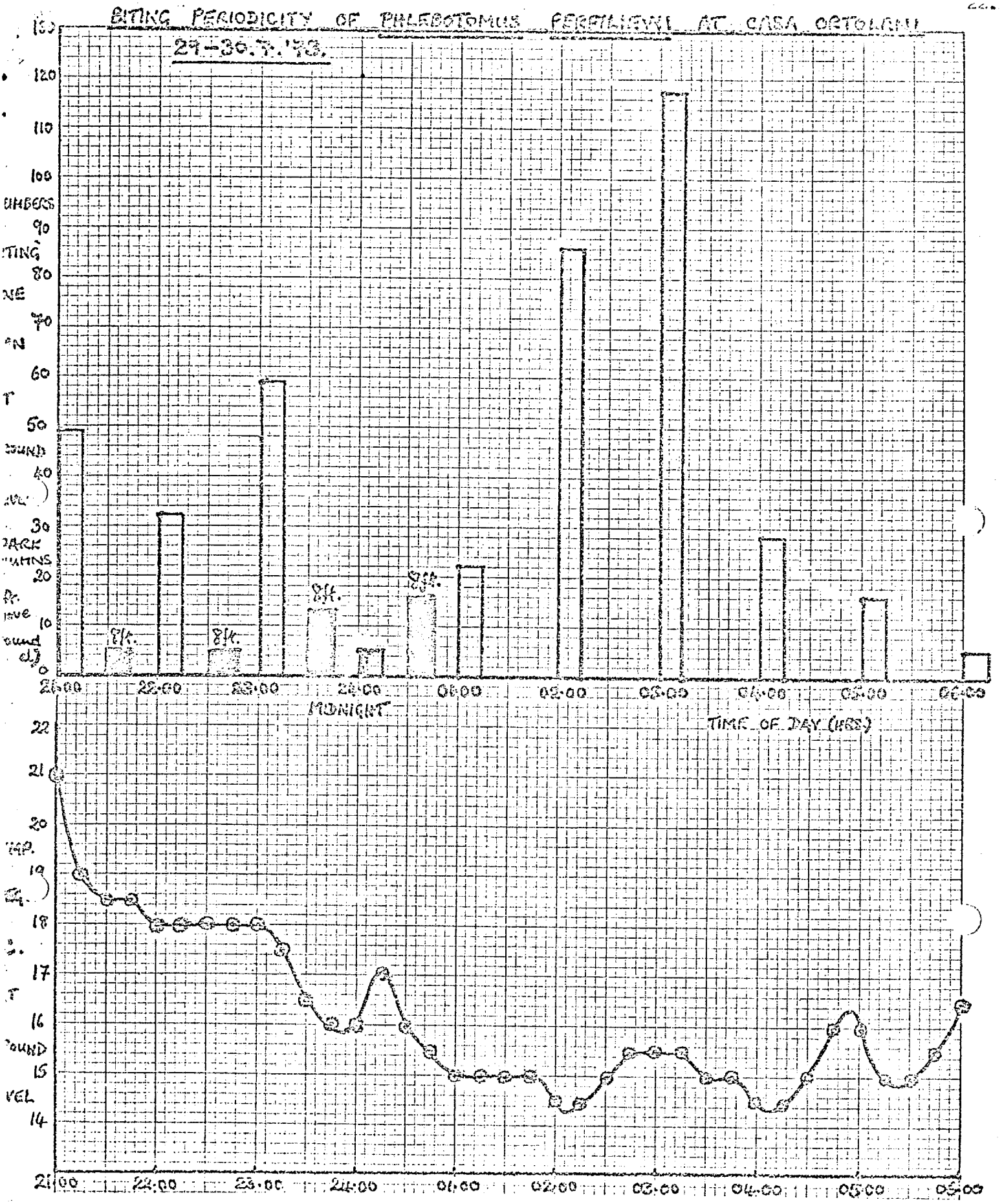
TEMP. MAX. 25·5°C, MIN. 12°C.

KEY.



Appendix 5.

Biting activity of *P.perfiliewi*.


BITING PERIODICITY OF PHLEBOTOMUS PERFILIEWI AT CASA ORTOLANI
29-30.7.'23.
NUMBERS BITING
8/4.
8/4.
8/4.
5/4.
MIDNIGHT
TIME OF DAY (HRS)
22-00
23-00
24-00
01-00
02-00
03-00
04-00
05-00
06-00

Second report on an investigation of phlebotomid sandflies of Emilia-Romagna, Italy, in September 1973.

P.D.READY

A.J.LEANEY

Imperial College Field Station,

Ashurst Lodge,

Ascot, Berks.,

England.

December, 1973.

Contents

Summary

In September 1973 the investigation of sandflies in the south of Emilia-Romagna started by R. Killick-Kendrick et al (Ref.1) in July was continued.

Once again Phlebotomus perfiliewi was found to be widespread, but in September it was nowhere abundant and was restricted largely to domestic situations. It is thought that the populations of this species in 1973 were not large enough to transmit Leishmania to man on a large scale. Engorged females were collected to supplement a laboratory colony initiated in July. The progress of this colony is reported.

Only one specimen, a male, of P.perniciosus was found out of a total of 3,845 sandflies captured from 15 localities during July and September. The importance of this species as a potential vector of Kala-Azar in 1973 must have been negligable.

It is recommended that DDT should be sprayed in domestic situations in September as well as in June. Field studies on sandfly biology should continue.

Introduction

An investigation of sandflies of Emilia-Romagna in July 1973 by R.Killick-Kendrick et al (Ref.1) resulted in a provisional assessment of Phlebotomus perfiliewi as a potential vector of Kala-Azar in the region. This report summarizes a second visit to the same area (the northern foothills of the Apennines around Bologna) made in September 1973.

Our purpose was to make a similar assessment of P.perniciosus - a species that is generally believed to be the vector of Kala-Azar (K-A) in Italy (Ref.2), but which we did not find in July. The potentials of P.perfiliewi and P.perniciosus, the two most numerous man-biting phlebotomids in the region in past years (Ref.3), as vectors of K-A could then be compared.

The late date of our visit was chosen to establish any seasonal differences in the abundance of P.perfiliewi and P.perniciosus.

Observations

13 sites were visited from Sept. 8th to Sept. 22nd (Map 1, Appendix 1). 7 of these had not been visited in July; they were chosen as sites known to contain P.perniciosus in 1972 (Ref.3).

Only 166 sandflies were collected (P.perfiliewi 51♂♂, 114♀♀; P.perniciosus 1♂). The trapping techniques used are described in the first report (Ref.1).

1. P.perniciosus

Only one specimen, a male, was collected. This was taken from a light/carbon dioxide trap placed at ground-level outside a cattle-shed at site 12 (Appendix 2). This site was in no way remarkable when compared with the other sites that were visited.

Intensive trapping with light/carbon dioxide traps, animal-baited sticky-trays and man-baits on following evenings produced no more P.perniciosus, although P.perfiliewi was found.

2. Seasonal changes in the abundance of P.perfiliewi

The numbers of this species collected in September were significantly less than those in July (165 and 3,680 respectively). This comparison is a valid one even if differences in the use of the various trapping techniques are considered (Appendix 3). The use of a larger number of sites in September obviated the danger of invalidating comparisons by sampling populations that had been reduced by intensive removal trapping in July.

Reliable sources report that sandfly numbers (as measured by the degree of annoyance to stock and people) fell drastically in the second week of August. Numbers remained low throughout the rest of August and the early part of September when the weather was colder and wetter than it had been for many years.

During July and September we recorded temperatures and humidities at collecting sites (Appendix 4). The mean evening and night temperatures for September were 3°C to 5°C lower than those for July. The relative humidity was very variable (50-100%) in both months. The amounts of cloud cover, wind and rainfall were comparable for both months.

3. Seasonal changes in the local abundance of P.perfiliewi

Some of the largest populations of P.perfiliewi found in July were recorded from silvatic habitats. In September sandflies were found in large numbers only in domestic habitats (Appendix 2). The temperatures of cattle-sheds and farm-buildings remained several degrees higher than the ambient in September, although they had fallen some 4°C to 5°C below July temperatures (Appendix 4).

4. Biting activity of P.perfiliewi

Dusk heralds the first biting P.perfiliewi, which arrive at a human bait from 21.00hrs in the middle of July but as early as 19.30hrs in the middle of September. This synchronisation of activity with dusk is neither by chance nor is it related to temperature, which varies greatly from one day to the next.

Conclusions

1. P.perniciosus

P.perniciosus appears to be thinly distributed in the study area. Collecting in 15 localities, at different times of the year and using a wide range of trapping techniques only one specimen was caught. Moreover, no females came to human baits in silvatic and domestic habitats.

Previous studies in Emilia-Romagna in 1948-1960 (Ref.2) and 1972 (Ref.3) indicated that P.perniciosus was not at all abundant. All studies suggest that it can only be locally important to man as a vector of K-A in Emilia-Romagna, although its possible role in maintaining an epizootic has not been investigated.

Comparing our findings with those of Prof.A.Coluzzi (Ref.3) P.perniciosus was not as common in 1973 as it was in 1972. Such a reduction (if real) was to be expected because P.perniciosus is an endophilic species and would have suffered greatly from the widespread spraying of DDT in domestic situations in 1972 and early 1973.

2. P.perfiliewi

Usually, a female sandfly becomes a potential transmitter of Leishmaniasis to man only after she has fed on an infected host and survived oviposition. Only a small percentage of such females will actually transmit Leishmania. Thus, in part, large-scale transmission will depend on the presence of large numbers of parous female sandflies.

In 1973 there were large populations of P.perfiliewi for a short period in the second half of July only. Therefore, it is extremely unlikely that any large-scale transmission of Leishmania to man by P.perfiliewi occurred in the study area in 1973.

In September sizeable populations of sandflies were found only in domestic habitats. Such sites might enable these populations to extend their breeding season. This would be detrimental to the effective control of these pests.

Recommendations

These are in addition to those recommendations made in our first report (Ref.1).

1. Control Measures.

Domestic habitats remain warm enough for the survival of adult sandflies long into the Autumn. In these sites sandflies might be able to extend their breeding season and add to their numbers. These sites do not lend themselves to thorough spraying; the DDT applied in the Spring does not seem to be effective in September.

It is therefore RECOMMENDED that the interiors of houses, stables and other structures housing domestic animals should be sprayed with DDT in September as well as June of each year.

2. Future studies.

The widespread spraying of DDT is wasteful financially and is potentially dangerous to humans and animals. In certain years when the survival of sandflies is not favoured applications of DDT could be totally unnecessary.

It is therefore RECOMMENDED that:

a long-term field-study should be started (conducted over several years) to determine the major factors that control the survival in any year of large numbers of parous female sandflies (i.e. potential vectors of K-A).

Acknowledgements

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We thank most sincerely all those who helped us with our work.

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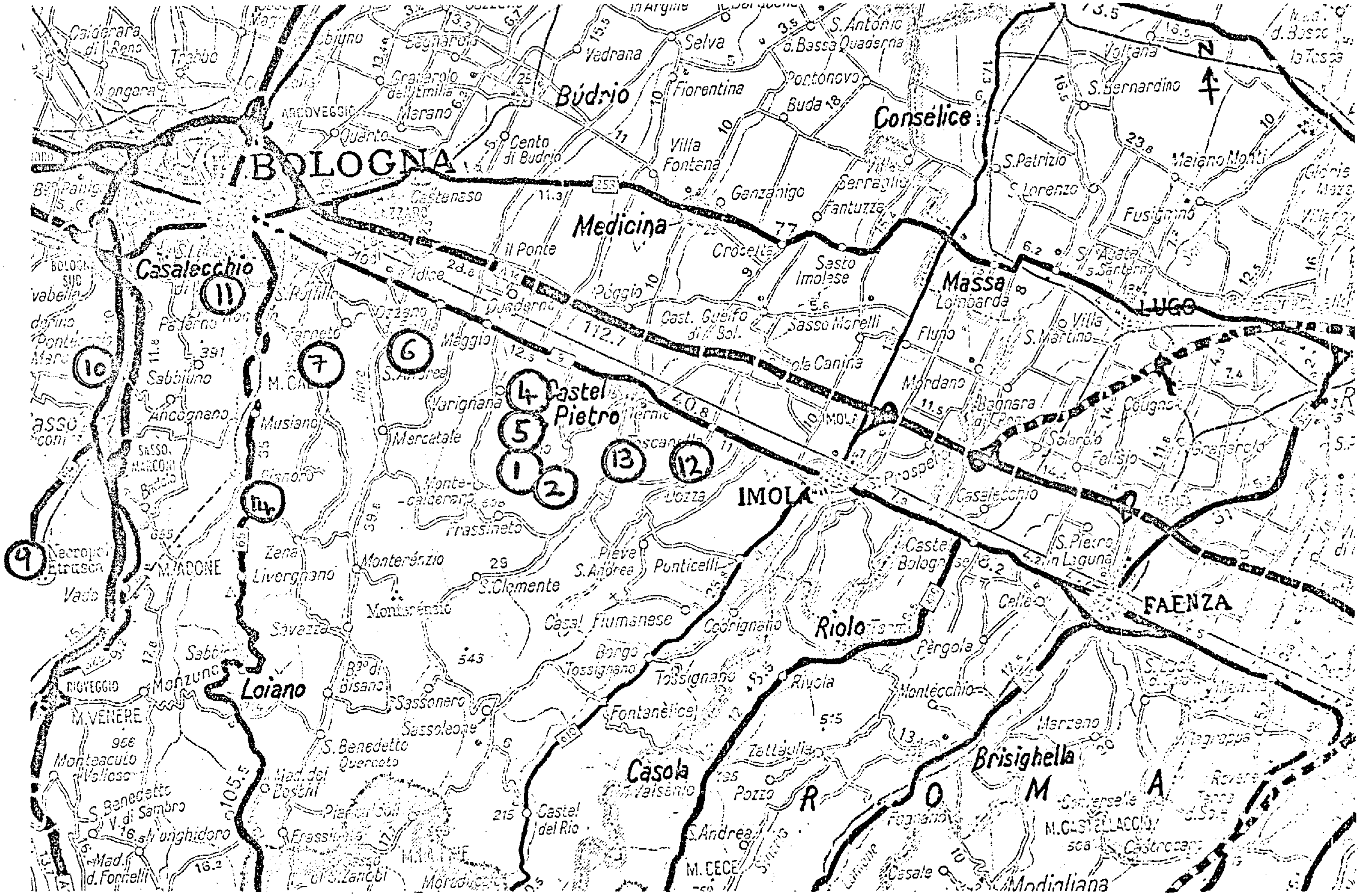
Appendix I

The study area, Emilia-Romagna Province. Numerals refer to the sites visited in September 1973. Site 15, in the Province of Modena, is not shown.


BOLOGNA
Casalecchio
Búdrio
Medicina
Consélice
Massa Lombarda
LUGO
Castel Pietro
IMOLA
FAENZA
Riolo
Casola Valsenio
Brisighella
Loiano
Castel del Rio
Monterènzio
Pianoro
Sabbiuno
Ancognano
Varignana
Ozzano
Maggio
Mercatale
S. Clemente
Casal Fiumanese
Tossignano
Fontanélice
Dozza
Toscanella
Poggio
Sesto Imolese
Mordano
Bagnara
Cotignola
Solarolo
S. Prospero
Castel Bolognese
Pergola
Rivola
Zattaglia
Modigliana
Monzuno
Vado
Necropoli Etrusca
M. VENERE
Montecchio
Marzeno
Fusignano
Maiano Monti
S. Patrizio
S. Lorenzo
S. Bernardino
Voltana
Selva
Vedrana
Fiorentina
Villa Fontana
Ganzanigo
Fantuzza
Crocetta
Sasso Morelli
Quaderna
Castenaso
Granarolo dell'Emilia
Quarto
Cento di Budrio
Buda
Fluno
Villa S. Martino
Casalecchio
S. Pietro in Laguna
Celle
Sassoleone
Sassonero
Savazza
Zena
Livergnano
Frassineto
Ponticelli
Pieve S. Andrea
Codrignano
Musiano
S. Benedetto Querceto
Monteacuto Vallese
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Appendix 2

List of Collecting Sites.

Site Number	Locality (altitude)	Nature of collecting site	Trapping Methods	*Abundance of P.perfiliewi
1	Casa Bertuzzi, Vedriano. via Gitanari (330 metres)	Cattle-shed	Catches from man and cattle	Numerous
2	Ca Paderna, off via Gitanari (90 metres)	The wooded bottom of a dry valley (of Rio di Paderna)	Catches from man	Absent
4	Duzzola, Casalecchio Dei Conti, via Malvezza (90 metres)	Wooded hillside and stubble field	Light/CO_2 sticky traps, catches from man	Few
5	Casalecchio Dei Conti, off via Luogo (140 metres)	Dry, rocky riverbed, and surrounding woods of Rio della Varone	Light/CO_2 sticky traps	Absent
6	Casa Ortolani, via Del Poggio (240 metres)	Hillside orchard and garden of a Villa	Catches from man	Absent
7	Casa Rastignano, Zena Valley	Farmyard, rabbit-hutches, chicken-houses	Catches from man, search of resting sites	Absent

*Numerous 20+ in 2 hrs
Common 5-20 in 2 hrs
Few < 5 in 2 hrs
Absent 0 in 2 hrs

The sites on this page were visited in July as well.

Site Number	Locality (altitude)	Nature of Collecting site	Trapping Methods	Abundance of P.perfiliewi
9	Panico (75 metres)	Cattle-shed, farmyard, house	Catches from man and cattle, search of resting sites	Absent
10	Casa Tonioli Pidne, Nr. Pontecchio (350 metres)	Cattle-shed, chicken-houses out-buildings and house	Light/CO_2 traps, catches from man, search of resting sites	Numerous
11	Casa Lambertini via Iola, Monte Donato (200 metres)	Cattle-shed, chicken-houses rabbit hutches, and away from farm	Light/CO_2 traps, catches from man, search of resting sites	Common
12	Cà Nova, Monte del Re, Nr Dozza (237 metres)	Animal houses and farm house	Light/CO_2 traps, catches from man, search of resting sites, Disney traps	Numerous
13	Casa Ferrari San Martino (75 metres)	Chicken-houses, farmyard	Light/CO_2 traps	Few
14	Casa Prati, via Liberta Pianoro (200 metres)	Animal houses, garden	Light/CO_2 traps, search of resting sites	Few
15	Casa Cornia, Fésta, Nr.Marano Vignola, Province of Modena, (350 metres)	Animal houses, farmyard, farmhouse	Light/CO_2 traps, search of resting sites	Few

Appendix 3

The abundance of *P.perfiliewi* in July and September using different trapping techniques.

Trapping Technique	July	September
Light/CO_2 sticky-trap	4.1	0.1
Man-bait	10.3	0.3
Disney trap	4.4	0.0
Tent trap	9.4	-
Search of resting places	1.9	0.4

Abundance is expressed as number of sandflies caught per location per trap-hour.

Appendix 4

Mean evening and night temperatures during the collecting periods.

(a) Mean temperatures (and ranges) in silvatic situations.

Collecting period \ Time	20.00hr	22.00hr	Minimum at night
July	26.0°C (28.0-25.0)	21.0°C (26.0-15.0)	14.5°C (17.0-12.0)
September	20.5°C (23.0-16.0)	17.5°C (21.0-16.0)	11.5°C (12.0-11.0)

(b) Mean temperatures (and ranges) in domestic situations.

Collecting period \ Time	20.00hr	22.00hr	Minimum at night
July	27.0°C (28.0-25.0)	25.0°C (25.5-24.0)	23.0°C (25.0-21.0)
September	22.2°C (23.0-22.0)	21.5°C (23.0-20.0)	19.0°C (22.0-18.0)

Appendix 5

The laboratory colony of P.perfiliewi started in July.

Of 162 engorged females taken from humans and cattle only 25 (or 15.4%) survived to lay eggs. A total of 955 eggs were obtained (Mean egg-lay 38; range 8-60). All egg-lays were fertile.

The larvae were reared under constant conditions (25°C; 95% R.H. 24 hr dark) using the methods described by Killick-Kendrick et al[1]. Larval mortality was high (60-80%). Larvae reached fourth instar stage 25-30 days after egg-lay. Only a few of these pupated; most became sluggish and entered diapause.

We are hoping to break the diapause of some of these larvae by maintaining groups of them at a low temperature (10°C) for different periods (from 1 to 6 months).

It is important to obtain female flies so that the susceptibility of this species to strains of Leishmania can be tested.

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