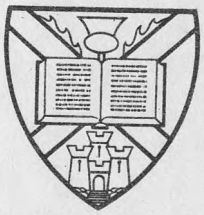
STUDY OF TSETSE FLIES (GLOSSINA spp.) AND THEIR MAINTENANCE IN LABORATORY COLONIES

A. R. MEWS



STUDY OF TSETSE FLIES (*GLOSSINA* spp.) AND THEIR MAINTENANCE IN LABORATORY COLONIES

ALASTAIR R. MEWS

Wellcome Research Fellow

Thesis submitted for the degree of M.Sc. in the University of Edinburgh, May 1969

CONTENTS

		PAGE
	Summary	
1.	Introduction	1.1
1.1	Synopsis	1.1
1.2	Reproductive physiology	1.2
1.4	Historical	1.5
1.4.1	Introduction	1.5
1.4.2	Feeding regimen and fly cages	1.5
1.4.3	Hosts	1.7
1.4.4	Temperature	1.7
1.4.5	Humidity	1.10
1.4.6	Light	1.11
1.4.7	Handling of pupae	1.12
1.4.8	Summary	1.13
2.	Materials and Methods	2.1
2.1	Environmental control	2.1
2.1.1	Ventilation	2.1
2.1.2	Temperature	2.2
2.1.3	Humidity	2.2
2.1.4	Lighting	2.3
2.2	Source of material	2.5
2.2.1	Pupa sources	2.5
2.2.2	Food sources	2.5
2.2.3	Feeding methods	2.5
2.3	Maintenance methods	2.7
2.3.1	Pupae	2.7

		PAGE
2.3.2	Flies: 1. Cages	2.7
	2. Eclosion	2.8
	3. Mating	2.9
2.4	Data recording and Analysis	2.10
2.4.1	Introduction	2.10
2.4.2	Identification of flies and pupae in the laboratory	2.10
3.	Results	
3.1	Introduction	3.1
	Factors affecting a single generation	3.1
3.2.1	Eclosion rate of 'wild' pupae and pupae introduced from other laboratories	3.1
3.2.2	Eclosion rate of Edinburgh bred laboratory pupae	3.2
3.3	Pupal period	3.3
3.4.1	Non-ecloded pupae	3.3
3.4.2	Non-expanded flies	3.4
3.5	Sex ratio	3.4
3.6	Mating	3.4
3.7	Longevity	3.5
3.7.1	Distribution of longevity	3.5
3.7.2	Survival curves	3.6
3.8	State of nutrition at death	3.6
3.9	Age specific fecundity	3.7
3.9.1	Interlarval period and number of days to first larviposition	3.7
3.9.1.1	Pupal production and mean interlarval period - Lisbon 1 colony	3.8
3.9.2	Pupal production	3.8

		PAGE
3.9.3	Abortions	3.10
3.9.4	Pupal weight	3.10
3.9.5	Correlation of pupal weight with pupal production and longevity	3.12
3.9.6	Correlation between pupal production and age of fly	3.12
	Factors affecting succeeding generations	3.13
3.10	Generation interval	3.13
3.11	Length of generations	3.14
3.12	Population increase	3.14
3.13	Number of flies culled	3.15
3.14	Number of flies alive at time of analysis	3.16
4.	Discussion	
4.1.1	Controlled environment of fly-room	4.1
4.1.2	Temperature	4.2
4.1.3	Water relations	4.3
4.2	Hosts	4.6
4.2.1	The blood type	4.7
4.2.2	Fly cage design and application of cage to host	4.7
4.2.3	Availability of blood source	4.11
4.3	Mating	4.12
4.3.1	Age of male and female at mating	4.12
4.3.2	Nutritional state of male and female	4.15
4.3.3	Duration of mating period	4.16
4.3.4	Mating procedure	4.17
4.3.5	Number of times a male may be used	4.18
4.4	Data recording and analysis	4.20

		PAGE
4.5	Eclosion rate/Pupal mortality	4.22
4.6	Longevity/Causes of death	4.22
4.6.1	Introduction	4.22
4.6.2	Male longevity	4.23
4.6.3	Female longevity	4.24
4.6.4	Early mortality	4.24
4.6.5	Late mortality	4.29
4.7	Age specific fecundity	4.31
4.7.1	Introduction	4.31
4.7.2	Time to first pupa	4.32
4.7.3	Interlarval period	4.34
4.7.4	Pupal production	4.36
4.7.5	Pupal weight	4.37
4.8	Population increase	4.39
4.9	Conclusion	4.41
5.	Appendix: Data recording and analysis	5.1
6.	Acknowledgements	6.1
7.	References	7.1

SUMMARY

This thesis describes the methods used in the establishment and maintenance of four closed colonies of the tsetse fly (Glossina Weidmann).

The pupae were sent by air from Africa or from the laboratory colonies in Lisbon and Bristol. The flies were kept in individual cages in a controlled environment fly-room at 25° ± 0.5°C and 65 ± 5.0% relative humidity.

When guinea-pigs were used as hosts for feeding the flies, the results were poor, but with the introduction of lop-eared rabbits the colonies began to expand rapidly, and large numbers of flies had to be culled to keep the size of the colonies within reasonable limits.

The records for each fly and pupa were stored individually on magnetic tape by computer, and daily registers recording the pupae produced, newly emerged flies and details of matings and deaths were used to update the fly or pupa records as necessary.

An analysis and comparison of the results between generations of flies of the same species and also between species is given. The results show that under the environmental conditions provided, the longevity of G. austeni and G. morsitans is comparable, but pupal mortality and "crippling" on emergence is higher in the former species. G. austeni had a shorter pre-reproductive period, shorter and more regular interlarval periods, and a higher mean pupal production than G. morsitans.

The performance of G. morsitans orientalis from the Zambesi valley in Rhodesia was better than that of G. morsitans morsitans from Singida in Tanzania.

1 Introduction

1 Introduction

1.1 Synopsis

The tsetse fly (Genus Glossina, Weidmann) is a blood sucking insect which because of its role in the transmission of trypanosomiasis to man ('sleeping sickness') and animals ('nagana') is one of the major remaining obstacles in the development of tropical Africa, of which over 4.5 million square miles are affected. Thus the study of the fly is of great importance in the overall attempt to eliminate trypasosomiasis.

Numerous attempts have been made over the last fifty years to establish a closed colony of Glossina; these met with little or no success until about ten years ago. The first completely successful attempt was described by Azevedo and Pinhao (1964) who established a colony of Glossina morsitans morsitans Westwood, in Lisbon in 1959. The idea in attempting this project sprang from their success.

The breeding of Glossina is required for two main purposes: first to provide uniform material for use in experimental work, and second, to provide flies in large numbers for biological control methods (Lumsden and Saunders, 1966)

Attempts to breed Glossina in the laboratory can conveniently be divided into those that were aimed at the establishment of a closed colony, and those that relied on the continuous reinforcement of the population with pupae or flies collected in the field. It is upon the/

the first of these groups that most emphasis will be placed in this thesis.

This thesis describes the successful establishment of closed laboratory colonies of Glossina austeni Newstead, two sub-species of Glossina morsitans, and a daughter colony of Azevedo and Pinhao's Lisbon population. An attempt has been made to examine the relationship of some of the different factors involved in the breeding of the fly in the laboratory, and to this end a detailed study has been made of the life history of individual females.

At the start of the project the only successful self-contained colony was that of Azevedo and Pinhao (1964): it was therefore decided to copy their methods as closely as possible. Accordingly the author spent three weeks working in Lisbon learning their techniques for the maintenance of Glossina. However, it soon became apparent that certain changes in techniques would have to be made. Later, because of poor results and the success of Nash et al. (1966c), and Itard and Maillot (1966), more drastic changes were made. These will be described later.

1.2 Reproductive physiology

The main reason for the difficulty of establishing a laboratory colony of Glossina lies in the method of reproduction - adenotrophic viviparity (Hagan, 1951).

An oviparous insect such as the house-fly Musca domestica L. lays an average of 120 eggs at a time and about 600 eggs in all (Howard, 1911). Under/

Under favourable conditions a generation may take as little as 8 - 10 days, and the capacity for increase is enormous. For instance, the progeny of one female has been estimated to be 1.875×10^{12} adults at the end of the eighth generation (Matheson, 1950).

In G. morsitans at 26°C the first larva is produced at 18 - 20 days after emergence, and subsequent interlarval periods are about 10 days (Saunders 1960). It has a mean length of generation of about 73 days (Glasgow, 1963).

The task of studying the overall rate of increase of a population of Glossina is complicated by the increasing overlap of succeeding generations. This was studied by Thomson (1931); Buxton (1955) used his method to calculate the potential rate of increase in a population. Buxton assumed an ambient temperature of 24°C, and an adult life of 70 days. His assumption that each female would therefore produce six offspring (assuming no pupal mortality) was used by Azevedo and Pinhao (1964) for comparison with the increase in size of their colony, and they found that the rate of increase was similar.

Apart from the results of Azevedo and Pinhao (1964) little was known of the reproductive potential of a successful laboratory colony of Glossina at the beginning of this project. For this reason it was decided to keep accurate records of the reproductive performance of each fly together with other details of its life history.

For the purpose of constructing a model with which to compare the reproductive/

reproductive performance of different groups of flies, it was assumed that at 25°C: (1) the first larva would be produced at 20 days, and (2) that subsequent larvae would be produced at 10 day intervals. (3) So that the effect of the size of generations could be examined the maximum longevity of a female was assumed to be 200 days and (4) that each female would produce a mean of 6 pupae per female, which assuming a 1:1 sex ratio, would give a threefold increase in the number of females from one generation to the next.

Starting with one female in the first generation, this model would produce three females in the second, 19,683 in the tenth, and $1 \times 3^{n-1}$ females in the nth generation. If 30 days is allowed for pupal development, and assuming the first larva deposited is a female, subsequent generations will start at 50 day intervals. The model is shown in diagramatic form in Fig. 1.2.1. Only the first five generations are shown, but by the time the fifth generation has ended, three years after the start of generation 1, the twenty-third generation will have begun. In reality, the picture is much more complex than this, and is affected by such factors as adult death rate, pupal mortality, reproductive losses and overlap of generations. For instance, an inter-larval period in excess of 10 days may be due to one of a number of causes including undetected abortion (Lumsden and Saunders, 1966) and the degeneration and resorption of egg follicles (Saunders, 1960b).

The figure of 200 days for the maximum longevity is an underestimate, but Nash et al. (1967b) showed that the reproductive performance is greatly reduced after this period. Similarly, the threefold increase in/

Fig. 1.2.1

Theoretical model of generation size and overlap (females only) based on the following assumptions:

1 One female fly in the 1st generation

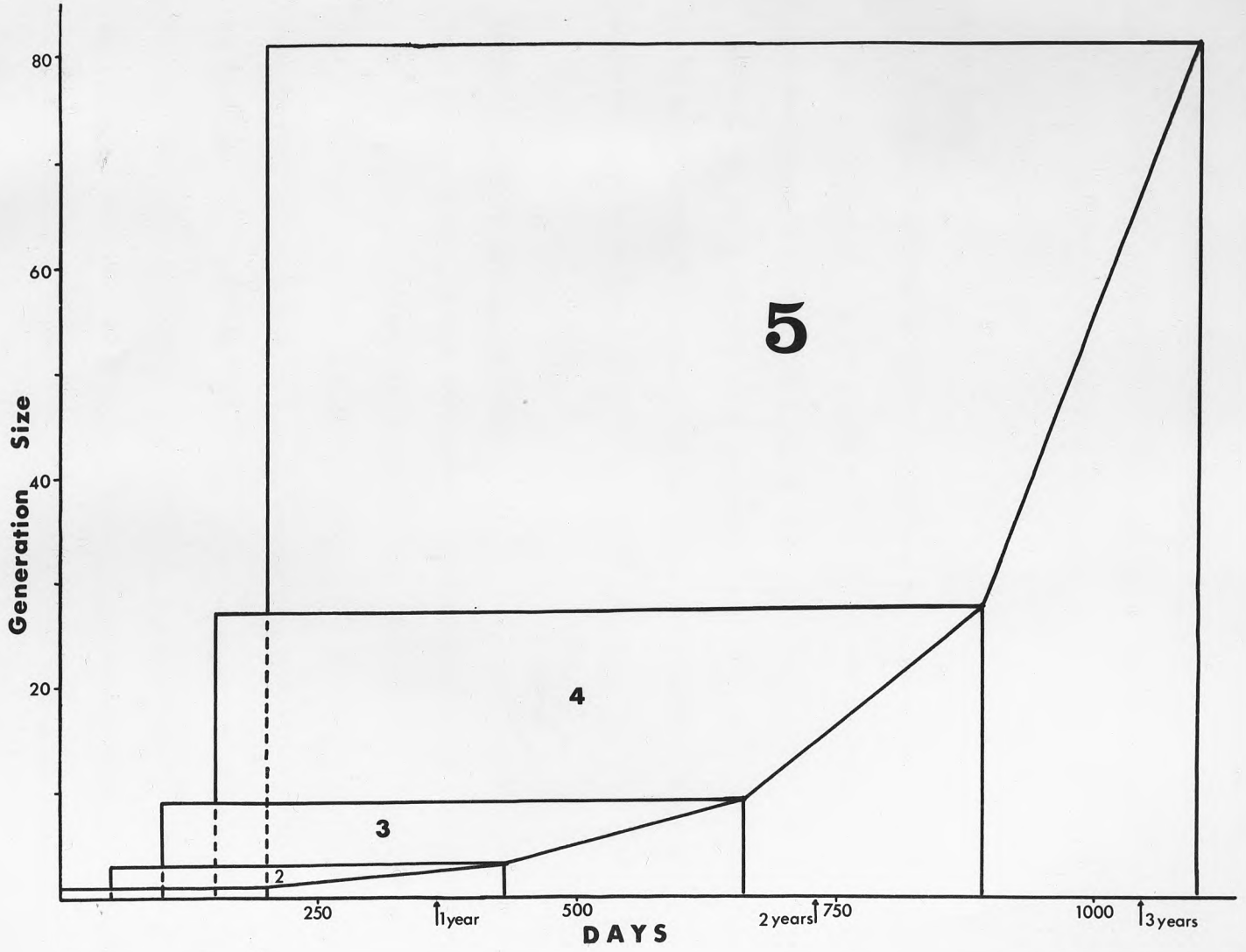
2 A tripling in size of each subsequent generation

3 A maximum longevity of any fly of 200 days

4 A generation interval of 50 days

Notes The first five generations only are shown

The figures inside each rectangle refer to the generation number (i.e. 1st, 2nd etc.)


80
60
40
20
Generation Size
5
4
3
2
250
1 year
500
2 years
750
1000
3 years
DAYS

in the number of females may be too low, for with G. austeni Jordan et al.(1967) achieved a 5.7-fold increase from one generation to the next but the recorded rate of increase for G. morsitans has so far been lower; Azevedo and Pinhao (1968) recorded a 2.6-fold increase in the number of females with this species, and the figure in the model is similar to the threefold increase suggested by Buxton (1955).

1.4 Historical

1.4.1 In reviewing the many previous attempts to establish a laboratory colony of Glossina, it is often difficult to be completely objective in assessing the reasons for their success or failure. The factors involved are both complex and interrelated, and many of the explanations given were necessarily subjective, and not clear cut. The more important factors involved are reviewed below:

1.4.2 Feeding regimen and fly cages

Feeding is of the utmost importance in determining the reproductive rate in Glossina, since the larva within the uterus is dependant upon the blood taken by the mother (Lumsden and Saunders, 1966). Because tsetse feed exclusively on blood, and in the laboratory may feed daily, it is important that the flies are offered food both regularly and often. Nash et al (1967b) found that G. austeni fed on average every second day, but that feeding was much more frequent after larviposition. Regularly breeding flies fed more often than irregular ones.

There are two methods of allowing the fly to feed on the host:

a/

a. Placing the host, either anaesthetised on unanaesthetised, in the same cage as the flies, and allowing them to feed at will. While this is successful for mosquitoes (Petersen, 1955), it has never proved so for Glossina. For instance, using this method Mellanby and Mellanby (1937) found that all the flies died after a few days and few ever fed. The use of large out-door cages placed in the natural habitat has recently been tried (A.R.C. Central Africa, Report 1964, E.A.T.R.O. Report, 1967) but reports so far are not very encouraging, and no such population appeared to be self-supporting.

b. Allowing the fly to feed through the mesh of its cage, which is applied directly to the skin of the animal. This is the method that has been used most commonly and a list of the types of cages used is given in Table 1.4.1.

The types of cages used can conveniently be divided into large and small:

Of the small cages, the most popular has been the 'Roubaud' or 'Geigy' cage, which has a wire frame over which a sleeve of mosquito netting is stretched. This type was used with success by Azevedo and Pinhao (1964), Itard and Maillot (1966) and Nash et al. (1966c). Azevedo and Pinhao (loc cit) considered that placing the females singly in such cages cut down the abortion rate due to disturbance of the flies, but the other workers kept more than one fly per cage. Nash et al (loc cit) considered that the minimum volume required per fly was 64 cc. and the performance of the flies fell if they were overcrowded.

Of/

Table 1.4.1

Common types of fly cage previously used

1 Small cages

2 Large cages

1 Small Cages

Description	No. of flies per cage	Volume (cc.)	Volume per fly (cc.)	Author
Glass tube 3" x 1" Covered with gauze netting Filter paper at bottom	1	37	37	Foster (1957) McDonald (1960)
Celluloid container 9 x 7.5 cm. in diameter Mosquito netting at one end, sleeve at other end	15 - 20	398	19.9	Buxton and Mellanby (1934) Mellanby K. (1936) Mellanby and Mellanby (1937)
"Roubaud" or "Geigy" cage. Wire frame 14 x 8 x 5 cm. Covered with mosquito netting with sleeve at one end	15 - 20 5 - 15 10 1 10 Up to 20	560	28 - 37 37 - 112 56 560 56 28	Roubaud (1915, 1917) Geigy (1948) Willett (1953) McDonald (1960) Azevedo and Pinhao (1964) Nash et al. (1966c) Itard and Maillot (1968)
"Minicage" Wire frame 3.8 x 3.8 x 7.6 cm.	1	110	110	Azevedo and Pinhao (1968)
"Mini-Geigy" Wire frame 3.8 x 3.8 x 5.1 cm.	1	74	74	Nash et al (1967b)
Bruce Box: Wooden frame 15.5 x 8 x 5 cm.	20	620	31	Nash et al (1958)

2 Large Cages/

2 Large Cages

Description	No. of flies per cage	Volume (cc)	Volume per fly (cc)	Author
Petena Box: Wooden frame 37.5 x 20 x 12.5 cm. with sloping floor	50	6,900	138	McDonald (1960)
'P.N.M 25' Wooden frame 28.6 x 13.3 x 10.2 cm. Wood sides with sloping floor for removal of pupae, dead flies etc.	25	3,880	155	Nash and Kernaghan (1964a)
'Geigy 25' Wire frame 25.4 x 12.7 x 5.1 cm. Nylon netting. End closed with hardboard and bung	25	1,650	66	Nash *et al.* (1966b)

Of the larger cages, most had wooden frames, and none proved very successful, and for instance, Nash et al. (1966b) found that their modified and enlarged 'Geigy' cage was far superior to their 'P.N.M 25' cage which was made of wood.

1.4.3 Hosts

Of the large animals, cattle (E.A.T.R.O. Reports, 1958 - 1963) and goats (Nash et al., 1958) have been most popular, but sheep (Willett, 1953) and calves (Jordan et al., 1966) have also been used. Of the small animals, guinea-pigs were used by Roubaud (1915, 1917) Geigy (1948), Willett (1953) and Foster (1957).

Azevedo and Pinhao (1964) and Itard and Maillot (1966) were the only two groups of workers to be successful in maintaining a colony on guinea-pigs. The flanks and abdomens of rabbits have long been used, (e.g. by Roubaud, 1917) but it was the use of the ears of lop-eared rabbits that proved to be the most successful method of feeding Glossina. This method originally used for feeding mosquitoes, was first used for feeding tsetse by Nash et al.(1966c) and has since been used by Itard (1968).

1.4.4 Temperature

An excellent review of the effects of temperature was given by Glasgow (1963). High temperatures appear to be more harmful than low ones. For instance, permanent sterility resulted in G. palpalis maintained at 29 - 30°C for 28 days (Mellanby H., 1937) and 32°C was found to be about the upper limit for the successful completion of development of G. morsitans/

G. morsitans pupae (Bursell, 1960c). Bursell considered that the most favourable temperature was about 24°C, but although in Africa the ambient temperature may rise as much as 12°C above this figure (Nash, 1963), these high temperatures are never constant, and the flies will be able to choose the most advantageous position to minimise their effects. Furthermore, at temperatures approaching the lethal limit of about 40°C, at which temperature death occurs in one hour (Jack, 1939), the opening of the spiracles causes a depression in body temperature of 1.6°C. By this mechanism the fly could survive for short periods in adverse conditions from which there is no escape (Edney and Barrass, 1962).

However, with flies confined to small cages, they will be unable to choose a suitable microclimate to avoid the effects of very high temperatures. For instance, Azevedo and Pinhao (1968) reported that, due to a malfunction of the temperature control system, the temperature on one occasion rose to 30°C - 32°C for two hours and this caused heavy mortality in the colony. Roubaud (1917) had a similar accident and when the temperature rose to 32°C, more than half the flies died.

At the lower end of the temperature range, spontaneous flights for food seeking and copulation in G.palpalis ceased at 20°C (Mellanby K., 1936) and McDonald (1960) found that at temperatures between 21 and 24°C, both feeding and pupal production were poor. At 8°C G. palpalis was immobilised but recovered rapidly on warming (Mellanby K., 1936).

It will be seen from these figures that the range of temperature tolerated/

tolerated by tsetse in the laboratory is quite narrow and appears to lie between about 24 and 28°C. However, it would appear that while a temporary rise in temperature above about 28°C may permanently affect the longevity and reproductive performance of a fly, a temporary fall in temperature has no lasting harmful effects.

Bursell (1960c) found that the optimum temperature for pupal development was 24°C at which temperature the emerging adults had maximum fat reserves. However, in the laboratory in order to obtain a high rate of reproduction, as high a maintenance temperature as is compatible with adequate longevity should be aimed at (Nash, 1963).

The temperature range most commonly used for the maintenance of Glossina in the laboratory has been between 24 and 26°C (e.g. Roubaud (1915): 24 - 25°C, Mellanby and Mellanby (1937): 24°C, McDonald (1960): 25 - 26°C, Itard and Maillot (1966): 25°C, Nash et al.(1966a): 25.5°C). Fluctuations in temperature within the tolerated range are obviously not harmful to the fly, and with adequate precautions it is possible to maintain Glossina in Africa without any artificial means of heating or cooling. For instance, Willett (1953) foun d that flies kept in a grass-roofed and open-sided hut did far better than those kept in the laboratory. Foster (1957) maintained G. morsitans at about 26.5°C for 3 - 4 hours during feeding in the morning, and then allowed the room to cool gradually to a night temperature of about 24°C. Azevedo and Pinhão maintained their colony at 26°C but opened the window for 3 - 4 hours daily during feeding and manipulation. A constant temperature is however essential for most experimental purposes.

1.4.5 Humidity

While the temperature requirements for adults and pupae are similar, their water relations are very different; pupae can only conserve water and never replenish it, but adults have a surplus of water to dispose of after feeding (Glasgow, 1963). Bursell (1958) showed that the resistance to desiccation of different species of tsetse pupae showed great variation. For instance G. longipennis was able to complete its development at 0% and G. morsitans at 10% relative humidity. On the other hand, G. brevipalpis pupae were non-viable at humidities below 60% R.H. and with G. austeni a high mortality occurred at humidities below 50% R.H. He showed that there was a close correlation between the resistance to desiccation of the pupae of different species of tsetse fly and the habitat in which they occurred and he suggested that the water balance of pupae may be a limiting factor in the invasion of semi-arid and arid habitats.

Azevedo and Pinhao (1964) considered that the humidity of the pupal environment was critical. When they placed pupae of G. morsitans in empty tubes, the eclosion rate was poor. From September 1963 they used damp sand as a pupal medium, and mortality fell from 31% in an earlier experiment (Azevedo et al., 1960) to almost zero. They therefore considered that this change in technique was the main reason for the increase in size of population that followed (Fig. 4.1). Later, however, when they again compared the eclosion rate of pupae kept in empty tubes with those kept in damp sand, the rates were identical. Furthermore, Nash and Kernaghan (1965a) found that using Azevedo and Pinhao's technique of burying the pupae in damp sand which was/

was then allowed to dry out, caused a pupal mortality of 7.8% which was double the figure obtained when the pupae were buried in dry sand suspended over wet sand.

The need to conserve water continues until the newly emerged fly has taken its first blood meal. The amount of water and fat present in the newly emerged fly depends to some extent on the humidity and the temperature respectively of the pupal environment (Bursell 1959, 1960c). The production of metabolic water from the combustion of fat will augment the existing water reserves at the rate of 1.08 mgs. of water for every 1.0 mgs. of fat until the fat reserves are exhausted. Teneral flies (i.e. imagines up to the time of their first blood meal) which were starved to death at 80% R.H. died from exhaustion of the fat reserves while those starved at 0% R H. died from desiccation, with substantial reserves of unused fat (Bursell, 1959). The critical water content at death is between 64.5 and 66% of the fatless wet weight, while the critical fat content is 4.2% of the total dry weight (Bursell, loc cit).

1.4.6 Light

The newly deposited larva is negatively phototactic (Glasgow, 1963) but the larvae of G. palpalis burrow just as well in darkness as they do in light (Parker, 1956). Glasgow (loc cit) suggested that the negative phototaxis guided the larva to the darkest available place on the surface, but that burrowing was in response to gravity.

The pupae normally develop in complete darkness, and in addition the puparial shell is almost black, and Glasgow therefore considered that the diurnal pattern of emergence was a temperature rather than a light effect/

effect. This view is supported by the finding that the normal rhythm of emergence was destroyed when the pupae were kept at a constant temperature (Bursell, 1959).

Adult flies are positively phototactic and need some light, for when Foster (1957) restricted G. austeni to 30 minutes of light per day, mortality increased and pupal production eventually ceased. For feeding they appear to prefer shady areas for Glasgow (1961) found that G. swynnertoni fed more readily in shade than in direct sunlight. Foster (loc cit) considered that in captivity a minimum of 10 - 20 lux were necessary at the feeding site. Itard, Azevedo and Nash considered that the flies fed better when the cages were covered while the flies were being offered food, and this must have severely reduced the amount of light at the feeding surface (personal observations).

1.4.7 Handling of pupae

Nash and Kernaghan (1965) considered that handling the pupae immediately after deposition might be harmful, and when the larvae were allowed to burrow into dry sand before pupating, and were then left for 9 - 16 days before weighing and reburying, they obtained a pupal mortality of only 1 (0.37%) out of 270 pupae.

Larvae that were allowed to pupate on the cage floor, weighed the next day and then buried in dry sand suspended over wet sand had a pupal mortality of 3.6%, midway between the previous figures.

1.4.8 Summary

From the foregoing, it will be seen that there is still much conflicting evidence as to the conditions which are most likely to favour the establishment of a laboratory colony of Glossina. The conditions that can be obtained in the laboratory are inevitably extremely artificial and furthermore, the requirements of different species vary greatly. However, apart from such considerations as temperature and humidity, the most difficult obstacle has been in overcoming the fact that the fly is unable to choose its own micro-climate and cannot seek out its food in the normal way. It must therefore be induced to take a blood meal under extremely artificial circumstances.

2 Materials and Methods

2.1 Environmental Control

The controlled environment fly-room was constructed in a room 4.3 m. long, 2.9 m. broad and 2.7 m. high. The adjacent room was employed as a service laboratory; the construction and layout of the fly-room is adequately described in Figs. 2.1.1 and 2.1.2.

The ceiling and external wall of the fly room were insulated with blocks of expanded polystyrene (7.6 cm. thick).

2.1.1 Ventilation

a Air Intake: Air from the outside was drawn into the room by a fan (Fig. 2.1.1., i) through coarse and fine filters. The coarse filter, Fig. 2.1.1, 1) consisted of a sheet of washable plastic foam, and the fine one (Fig. 2.1.2, m) a proprietary cotton-wool filter.

b Distribution and recirculation: The placing of the fan allowed air to be recirculated as well as drawing in fresh air from the outside.

The circulation of air in the room is shown in Fig. 2.1.3. The main current of air into the room was directed onto the corridor wall opposite, and therefore there was a relatively small draught of air along the other three walls, against which the flies and pupae were kept. Air escaped by positive pressure through the extract louvres at floor level on the outside wall (Fig. 2.1.1, k). The louvres were so constructed that they allowed air out of but not into, the room. The slight positive air pressure in the room prevented temperature fluctuations in the room when the communicating door was opened, (Fig. 2.1.2, a).

Fig. 2.1.1 - Constant temperature room: elevation

b Corridor

c Inside walls: 4" (10.2 cm.) concrete blocks finished in grey emulsion paint

d Stainless steel sink (See Fig 2.1.2 d)

e Fluorescent light (See Fig 2.1.2 e)

f Humidifier (See Fig 2.1.2 f)

g Ducting: galvanised steel

h Heater battery: 2 x 2 kw. elements
1 x 1 kw. element

i Fan: electric 12" (30.5 cm.) diameter blades. 1,350 revs. per minute

j Recirculation vent

k Extract louvre

l Coarse filter (See Fig 2.1.2 l)

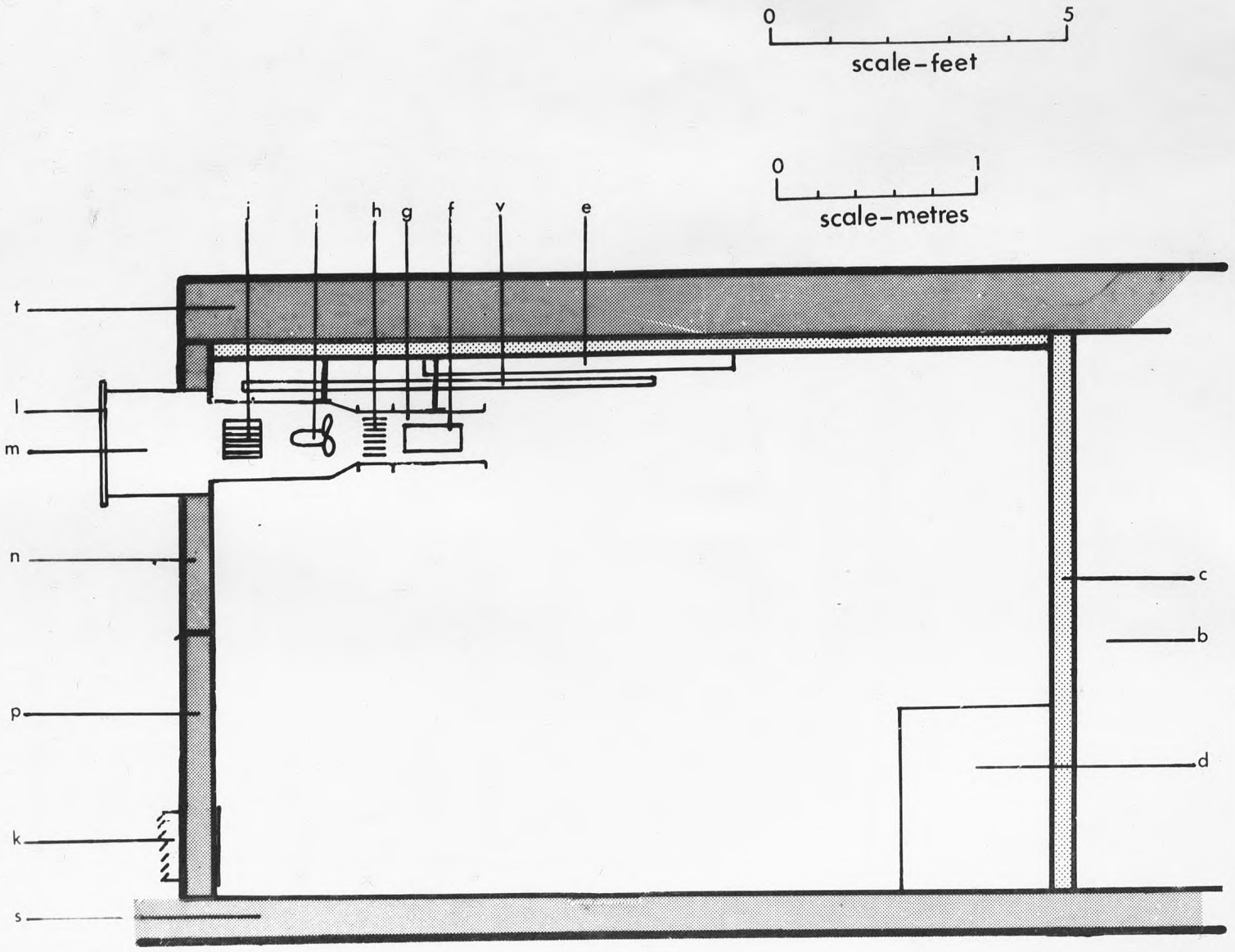
m Fine filter: pleated cottong wool (See Fig 2.1.2 m)

n Window replaced by $\frac{1}{4}$" (0.6 cm.) exterior grade plywood: lined with 3" thick (7.6 cm.) expanded polystyrene. Interior finish $\frac{1}{8}$" (0.32 cm.) plywood, finished in white gloss paint.

p Outside wall: stud wall construction 1" (2.54 cm.) lap jointed cedar boarding on building paper on 4" x 2" (10 x 5.1 cm.) stud. Lined with 3" thick (7.6 cm.) expanded polystyrene. Interior finish - 1 layer of foil backed $\frac{3}{8}$" (0.95 cm.) plasterboard.

s Ground floor construction: sheet vinyl on 2" (5.1 cm.) average screed on 6" (15.3 cm.) site concrete on 4" (10.2 cm.) hardcore

v Convector heater (See Fig 2 v)



ELEVATION

Fig. 2.1.2 - Constant temperature room: Plan

a_1 Door from service laboratory to corridor

a_2 Door between controlled environment room and service laboratory

b Corridor

c Inside walls (See Fig.2.1.1c)

d Stainless steel sink with draining board

e Fluorescent lights: 2 x 2 fluorescent tubes, 5' long with diffusers (each tube 80 watts)

f Humidifier: Defensor 2002 Rotary aerosol type

h Heater battery (See Fig.2.1.1 h)

i Fan (See Fig 1 i)

j Recirculation vent (See Fig.2.1.1 j)

k Extract vent (See Fig.2.1.1 k)

l Coarse filter (0.125" (0.32 cm.) plastic foam (washable)

m Fine filter: 'MV7' type. 7 longitudinal 'V' shaped pockets filled over a wire frame in duct

n Window replaced by insulated wall (See Fig.2.1.1 n)

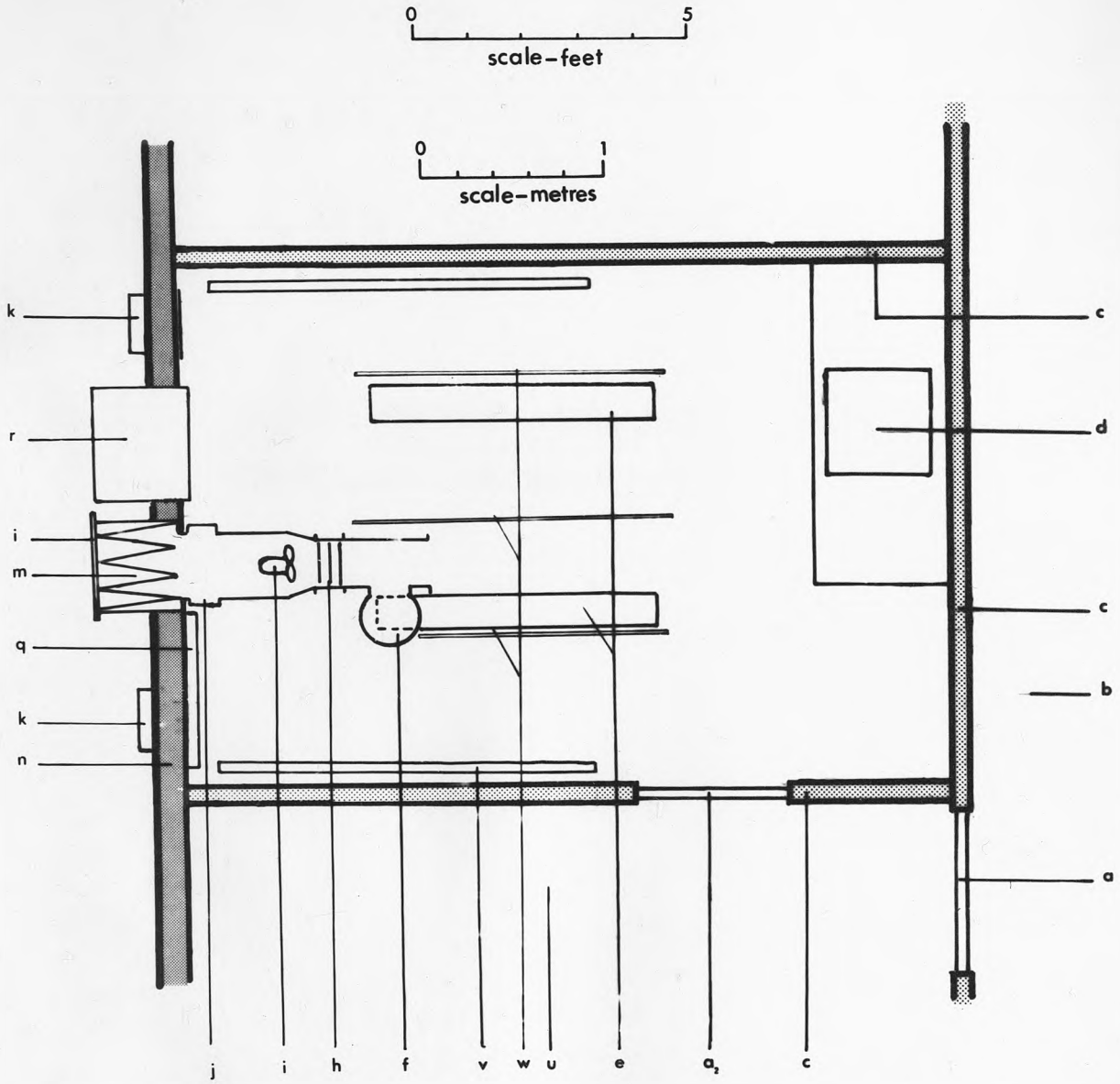
q Control panel containing thermostats, hygrostat, switch-gear and temperature/humidity recorder

r Air conditioner (Frigidaire 'ARH 11J5')

u Service laboratory (similar dimensions to constant temperature room)

v Convector heaters (2). Each of 1050 watts. Operating only during malfunction of temperature control system.

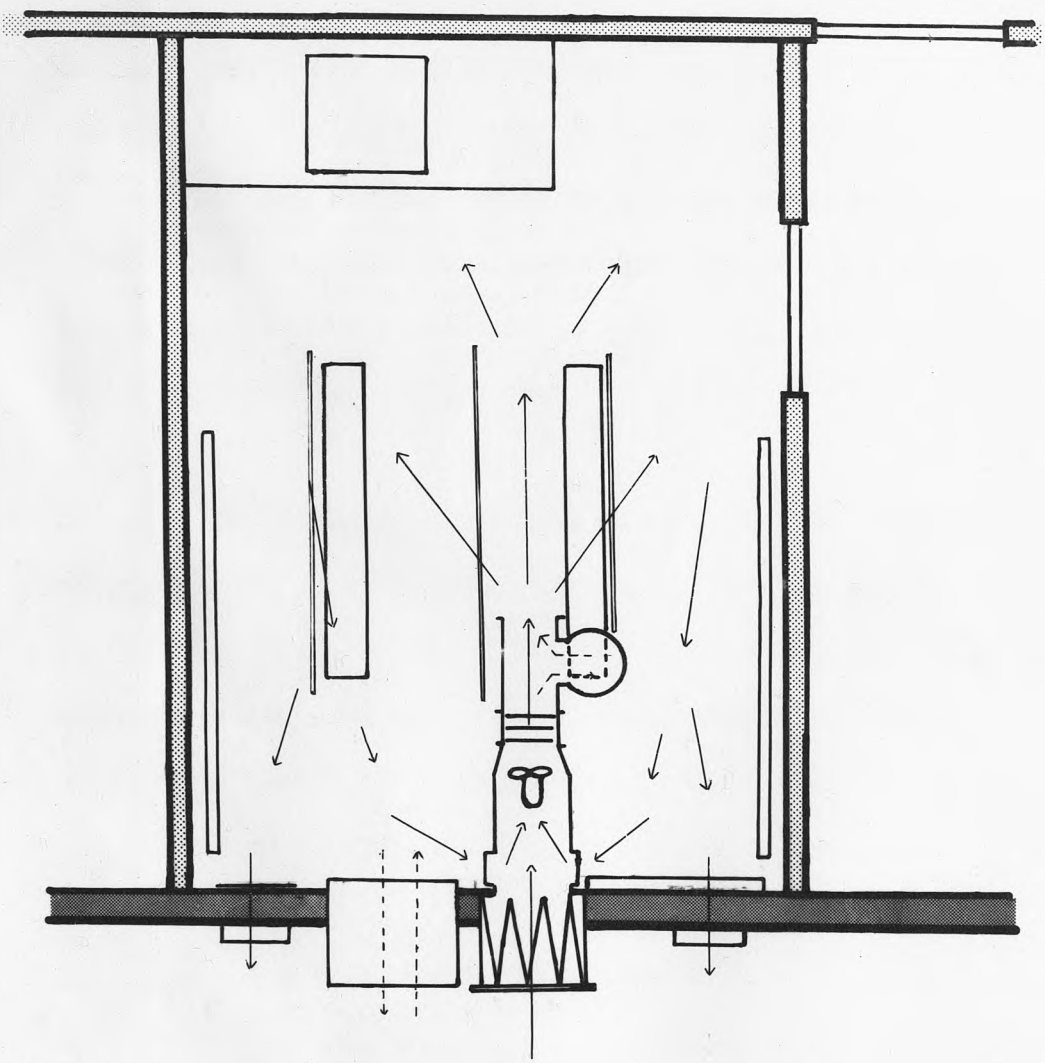
w Hardboard blinds (not shown in Fig.2.1.1) to shield direct rays of fluorescent light from shelves containing tsetse fly cages.



PLAN

Fig. 2.1.3 - Constant temperature room - plan

Movement of air currents in room



PLAN

2.1.2 Temperature

The room was maintained at 24.5 - 25.5°C. The fan drove the fresh and recirculated air across a three-step electric heater battery (Fig 2.1.1, h) which consisted of three elements of 2, 2, and 1 kilowatts respectively, making an available total of 5 kw.

When the temperature inside the room rose above the tolerated level, due to radiation of the sun on the roof or external walls, or to the heat generated by humans or animals inside the room, the air conditioning unit came into operation. This consisted of a 'Frigidaire' room air conditioner (Fig. 2.1.2 r) which was capable of removing 11,000 B.T.U.s per hour.

The temperature was controlled by a 4-way 12 volt thermostat mounted on the control panel (Fig. 2.1.2 q) allowing the recirculating air to pass over it. The thermostat operated the air conditioner and two of the three heater batteries. The third heater battery came on when the humidifier was operating. This counteracted the latent cooling effect of the water vapour from the humidifier.

The temperature differential between stages 1 and 4 of the thermostat was 0.5°C. The switch gear on the control panel stepped up the voltage from the thermostat (12 volts) to 240 volts at which point the heater battery and air conditioner worked.

Safeguards against failure of temperature control

a Should the temperature fall (due, for instance to a failure of the heater/

heater battery) a thermostat, mounted on the control panel and set at 22°C, cut out the fan when the ambient temperature fell below this.

b A thermostat positioned above the sink (Fig. 2.1.2 d) and also set at 22°C, brought the two 'Tuvec' convector heaters (Fig. 2.1.2 v) into operation. Each heater was of 1050 watts.

c Should, for any reason the heater batteries over-heat (for instance failure of the fan) a cut-out switch, mounted above the heater, cut off the current to the heater battery.

d In the event of an emergency, the air conditioner could be switched manually from its normal cooling function to heating, providing 11,000 B.T.U.s per hour. A thermostat in the air conditioner could be set to control the room temperature.

e Should, in the event of a power supply failure, the entire temperature control systems fail, the insulation of the room was such that over a period of a few hours, the temperature change within the room would not drastically affect the flies.

2.1.3 Humidity

Humidification was achieved by means of a rotary aerosol (Defensor 2002) type of humidifier which was capable of an output of approximately 1.8 kg. per hour. The quantity of water vapour produced varied with the external atmospheric conditions, but did not normally exceed 1 kg. per hour.

The water supply to the humidifier was filtered through a 10 micron pleater paper element to remove bacteria, fungi and algae, and then purified/

purified by means of an Elgostat B113/HU deioniser to remove dissolved solids.

The humidity in the room was controlled by a hair hygrostat (Honeywell 'H 64 A') mounted on the control panel (Fig. 2.1.2, q). Humidity was maintained at 65% relative humidity ± 5%.

2.1.4 Lighting

Natural light was excluded from the room. Lighting was by two fluorescent lights (Fig. 2.1.1, e). Each light was made up of two 80 watt fluorescent tubes with a diffuser. Direct light was prevented from falling on the shelves by means of pieces of hardboard hung from the ceiling (Fig. 2.1.2, w), The illumination of the working area was in the centre of the room and was approximately 800 lux and at the shelves and around the walls, 201ux. The illumination inside the cages on the shelves was not measured, but was considerably less than this.

2.2 Source of material

2.2.1 Pupae Sources

Details of the species of pupae received are given in Table 2.2.1.

Transport of pupae

Pupae sent from Tanzania and Rhodesia were at first sent in small tins or specimen tubes and packed with cotton wool. Since the eclosion rate of pupae despatched in this way tended to be very low, subsequent batches were sent in polystyrene boxes similar to those described by Kernaghan and Nash (1964) - Plate 2.2.2. Nylon shavings (Kernaghan and Nash, 1964) were used to pack the pupae in.

2.2.2 Food sources

Guinea pigs, and later, lop-eared rabbits were used for feeding the flies.

The guinea-pigs were initially kept in pens on the floor of the animal house, and later in four-tier racks of cages. The rabbits were kept in individual cages.

2.2.3 Feeding methods

1 Guinea-pigs

a Rack design: The design of the rack first used by Geigy (1948) was modified considerably as described below to allow the use of the 'tin' cage. The rack is fully described in Figs. 2.2.3 to 2.2.5.

The guinea-pigs were used for seven days and then rested for twenty-one before being used again. At the beginning of each week the guinea-pigs to be used for that week were shaved on both flanks with a pair of electric clippers. Each day, before use, the flanks of the guinea-pigs were cleaned with a small portable vacuum cleaner.

b Feeding routine/

Table 2.2.1 - Pupa Sources

	Species	Origin	Codename	No. of batches received	Dates of arrival: 1st batch	last batch
1	G. morsitans morsitans Newstead	E.A.T.R.O. (Singida, Tanzania)	SINGIDA	39	29 Dec 65	20 Oct 67
2	G. morsitans orientalis Vanderplank	Zambesi Valley Rhodesia	ZAMBESI	9	24 Feb 67	3 Aug 67
3	G. morsitans orientalis Vanderplank	Lisbon Colony (Prof. Azevedo)	LISBON	3	4 Aug 66	28 Jul 67
4	G. austeni Newstead	Bristol Colony (Dr. Nash)	AUSTENI	10	5 Jun 67	17 Aug 67
5	G. palpalis palpalis Robineau-Desvoidy	N.I.T.R. (Kaduna, Nigeria)	PALPALIS	1	24 Apr 67	

Plate 2.2.2.

Expanded polystyrene box with nylon shavings for the despatch of pupae.

Also shown are pieces of foam rubber which are damped and placed at top and bottom of the box. A roll of sticky tape for sealing the box is shown on the left, and a box ready for despatch shown on the right.

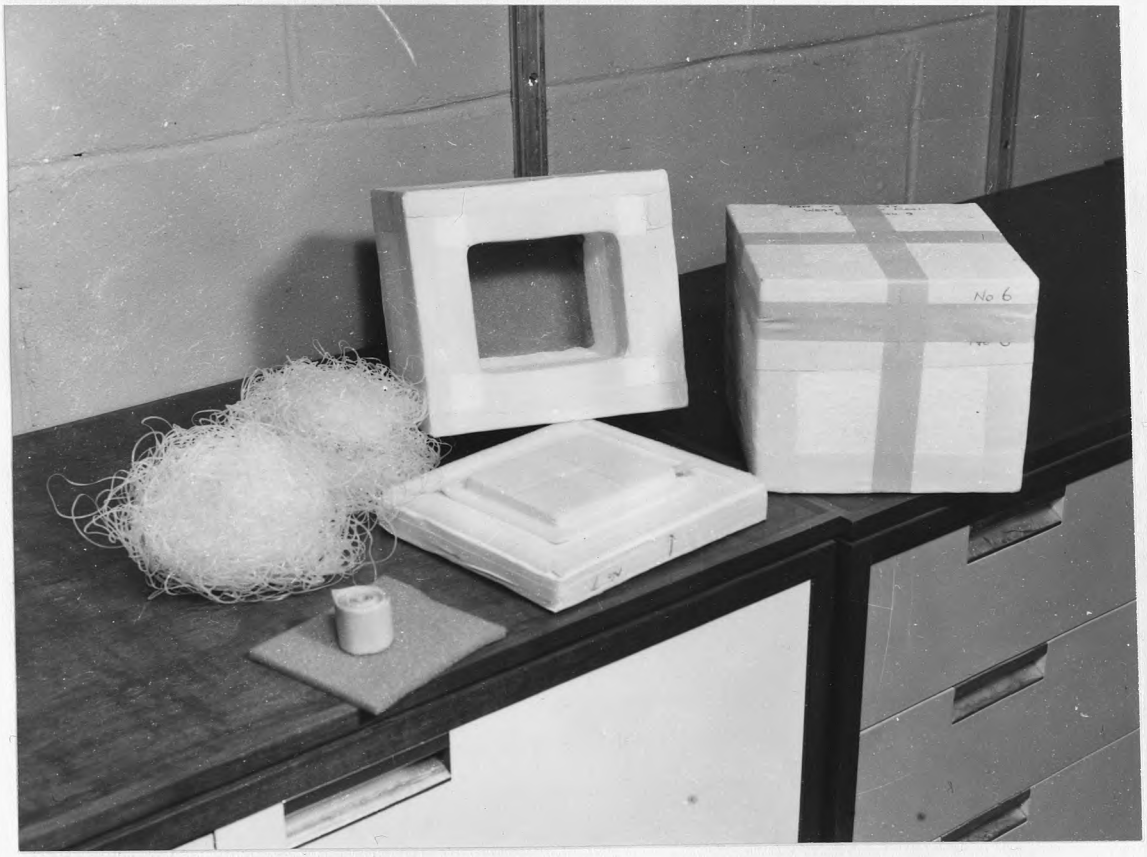


Fig. 2.2.3 - Front (elevation) view of modified 'Geigy' guinea-pig rack for feeding tsetse flies

a Aluminium gutter for guinea-pig to lie in

b Yoke for neck of guinea-pig

g Floor level of rack

h Neck halter (basin chain, 12" (30.5 cm.) long

i Chain fastener (modified 'Abro' fibre holder)

n Canvas strap for securing guinea-pig

m 'Velcro' fastener: attaches to 'Velcro' on back of rack (See Fig.2.2.4m)

q Loop in canvas strap (n) for chain (h) to go through

Fig 2.2.4 - Back (elevation) view of modified 'Geigy' guinea-pig rack for feeding tsetse flies

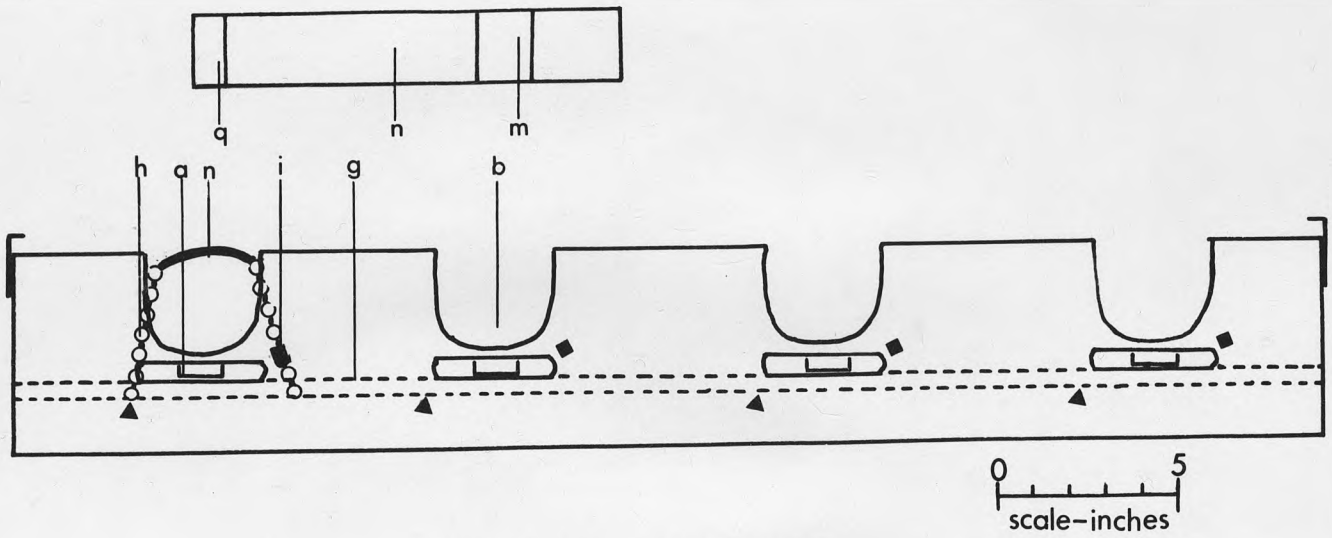
f Bracket for wooden strap (Fig. 8 p)

j Faeces tray

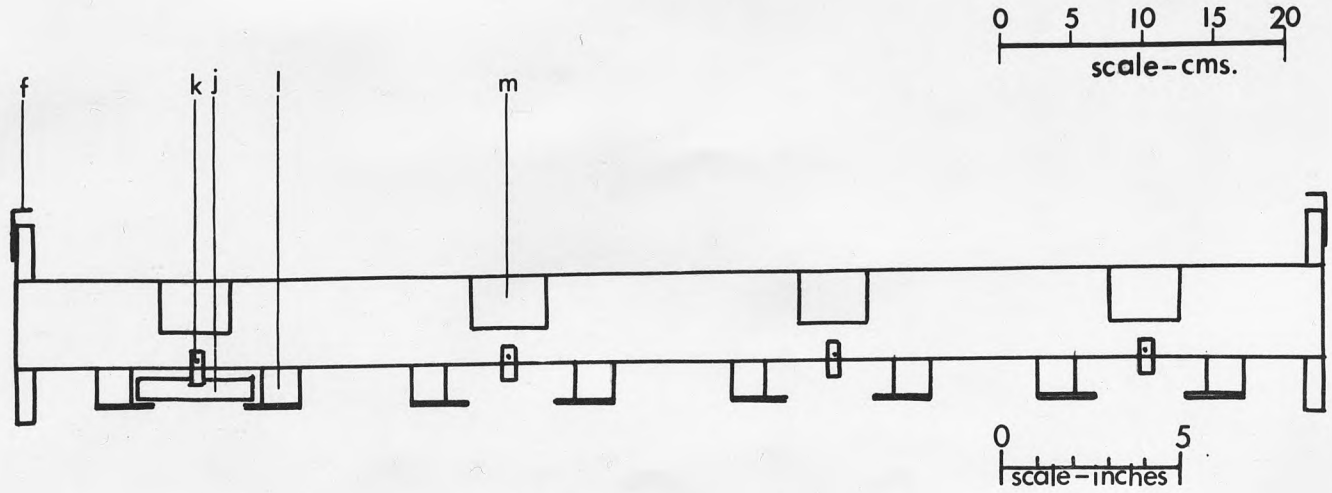
k Catch for faeces tray (j)

l Slide for faeces tray (j)

m 'Velcro' fastener: attaches to 'Velcro' on canvas strap (See Fig.2.2.3.m)



ELEVATION – FRONT VIEW



ELEVATION – BACK VIEW

Fig. 2.2.5 - Plan view of modified 'Geigy' rack guinea-pig rack for feeding tsetse flies

a Aluminium gutter for guinea-pig to lie in

b Yolk for neck of guinea-pig

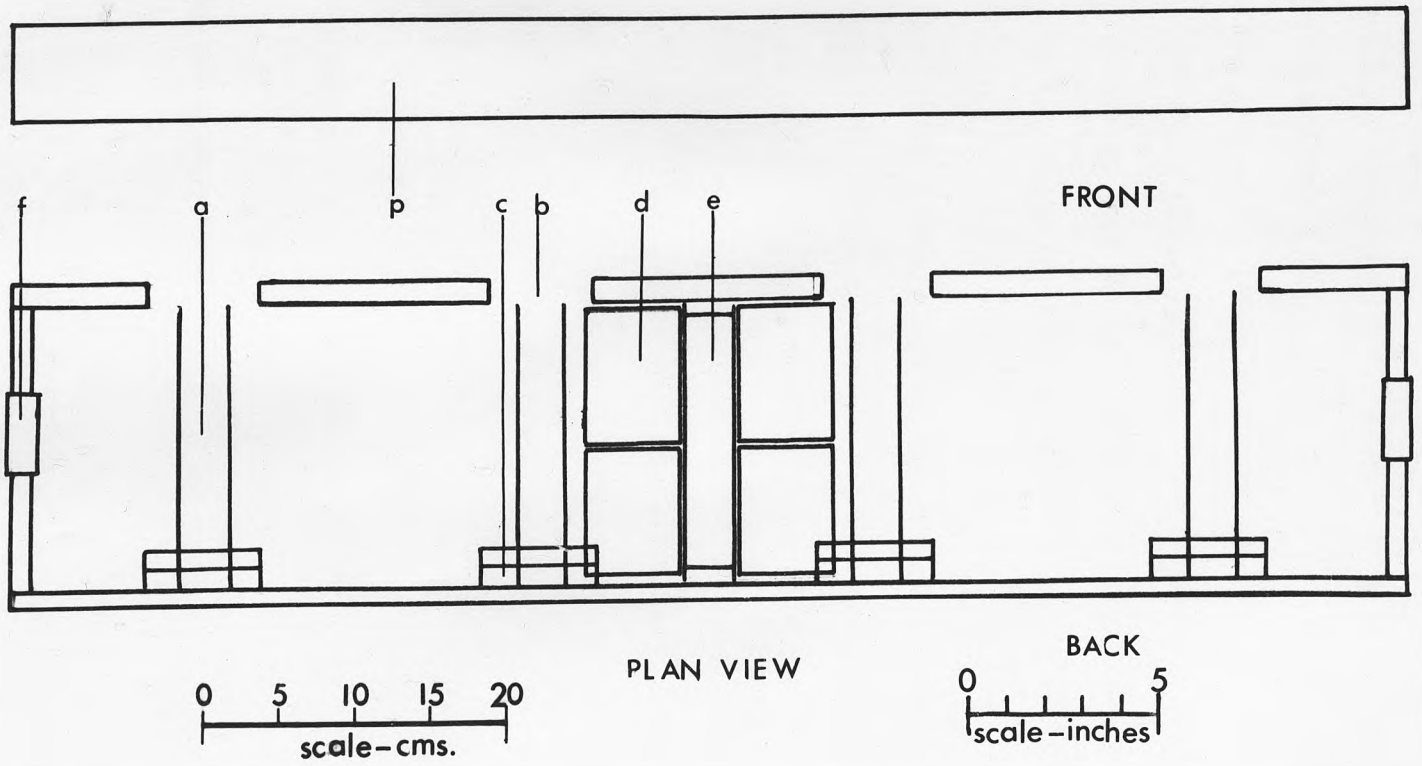
c Faeces channel

d Tsetse fly cage

e 'Spacer' to hold fly cages firmly against flank of guinea-pigs (plastic foam with thin plywood on either side)

f Bracket for wooden strap (p)

p Wooden strap to prevent cages 'riding up'


f
a
p
c
b
d
e
FRONT
BACK
PLAN VIEW
0 5 10 15 20
scale – cms.
0 5
scale – inches

b Feeding routine: Two tin fly-cages were placed at either side of the flank of each guinea-pig (i.e. four cages per guinea-pig) and were held firmly in position against the flank of the guinea-pig by means of a piece of plastic foam sponge to either side of which was glued a piece of thin plywood (Fig. 2.2.5 e). With all the cages in position, a further piece of plywood was fastened over the cages and held in position by clips (Fig. 2.2.5 p). The cages were applied for a period of about half an hour daily. The flies were then examined, and those that had not fed were given greater attention the next day.

2 Rabbits

The rabbits were held in a rack based on the design by Nash et al (1966) which allowed the flies to feed on the dorsal surface of the ears. A full description is given in Figs 2.2.6 to 2.2.8. Modifications to Nash's design were made in the yoke, and the removable ear boards. The ears of the rabbit were cushioned by 'pillows' made from plastic foam sponge. A template to take five cages and made from expanded polystyrene was placed on the dorsal surface of the ear and held in position with elastic bands.

a Feeding routine: The polystyrene fly cages were placed on the rabbits' ears and held in position by the template described above. The cages were applied for about 5 - 10 minutes daily. Female flies were offered food daily, and males every second day.

Fig. 2.2.6 - Rabbit rack: elevation - front

Key

a Adjustable yoke

b Removable ear board

c Ear board support

d Hole for rabbit's feet

e Pin for securing yoke

f Adjustable canvas strap

g Adjustable back

Elevation: Front

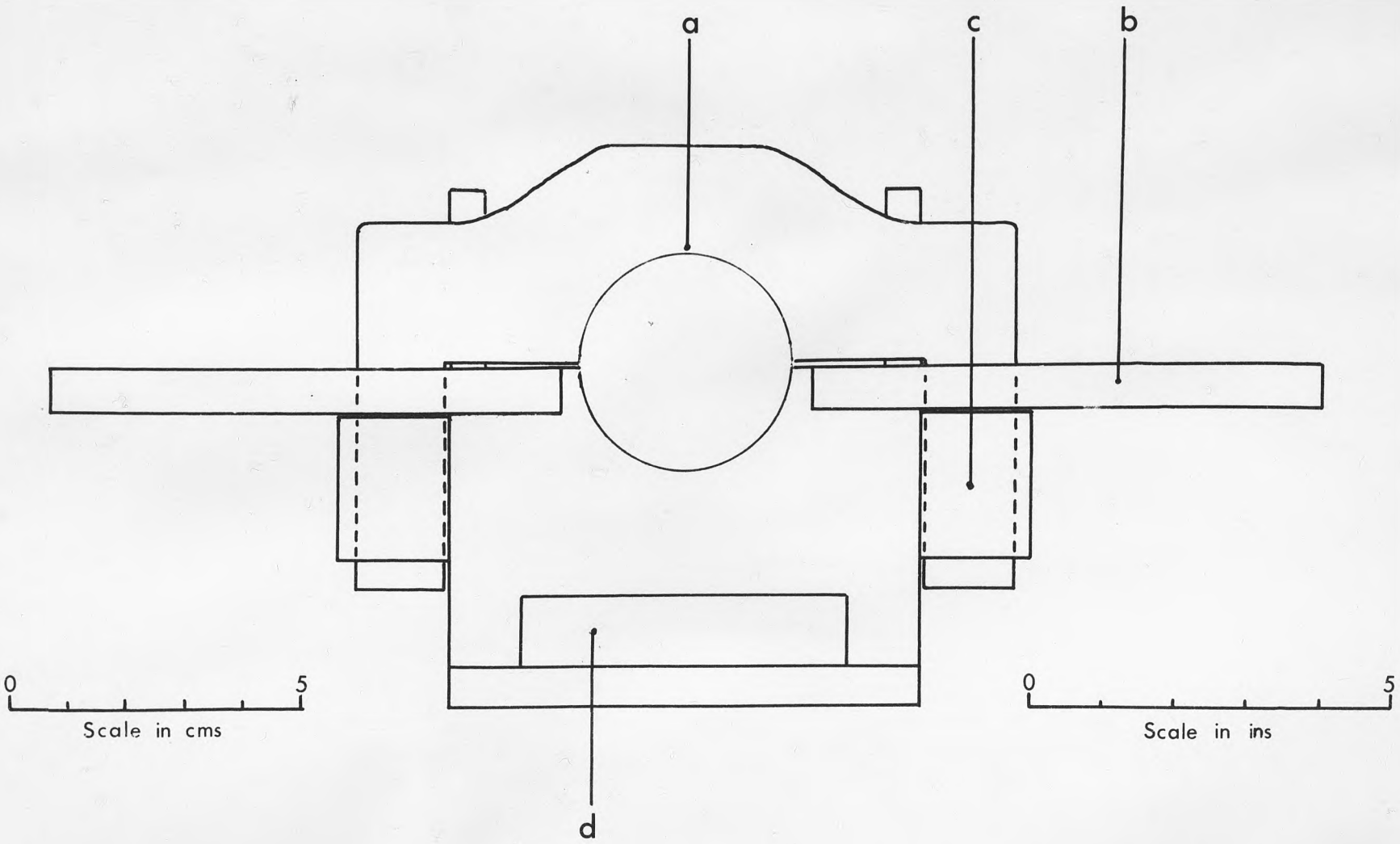
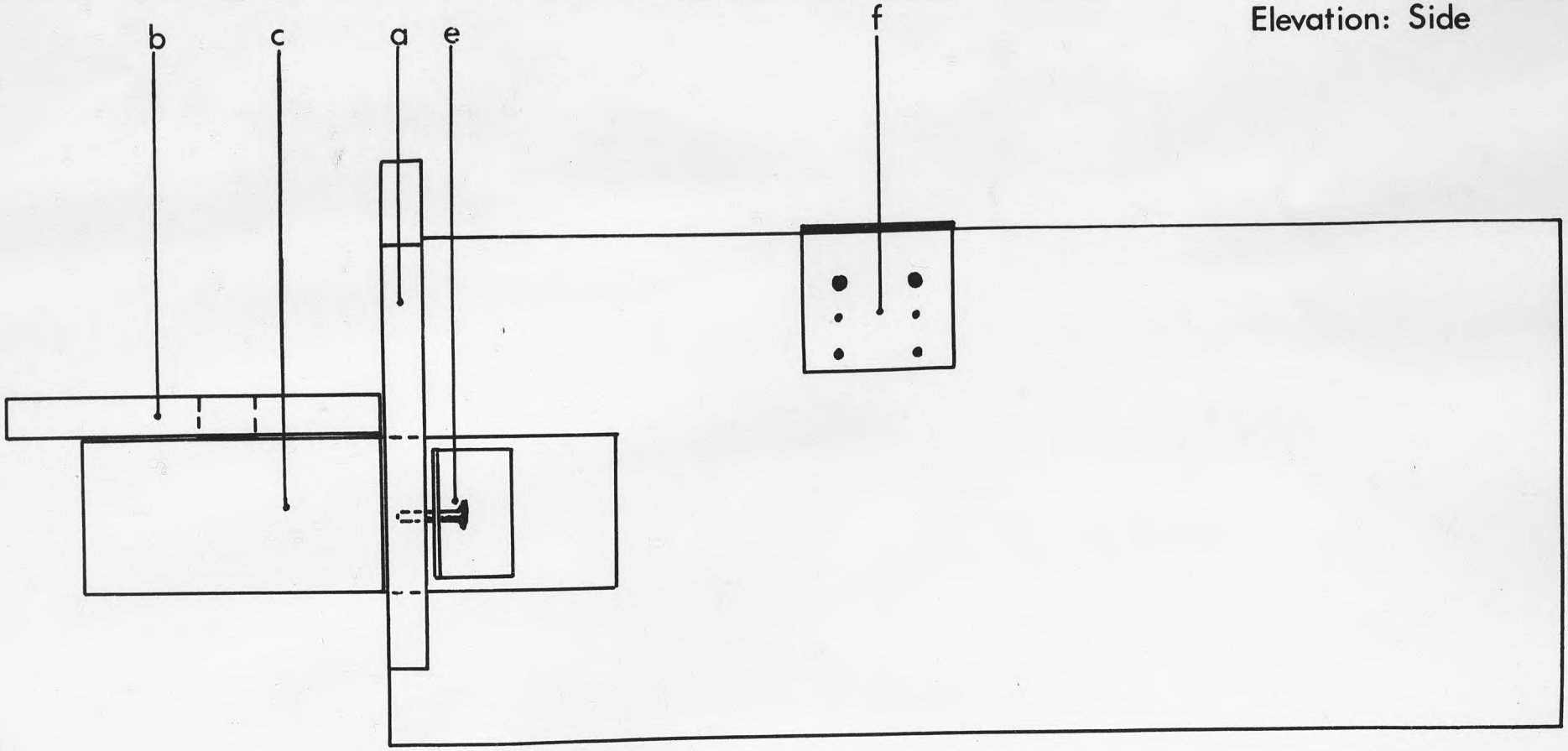


Fig. 2.2.7 - Rabbit rack: elevation - side

For key see Fig. 2.2.6

Elevation: Side



0 5

Scale in cms

0 5

Scale in ins

Fig. 2.2.8 - Rabbit rack: plan

For key see Fig. 2.2.6

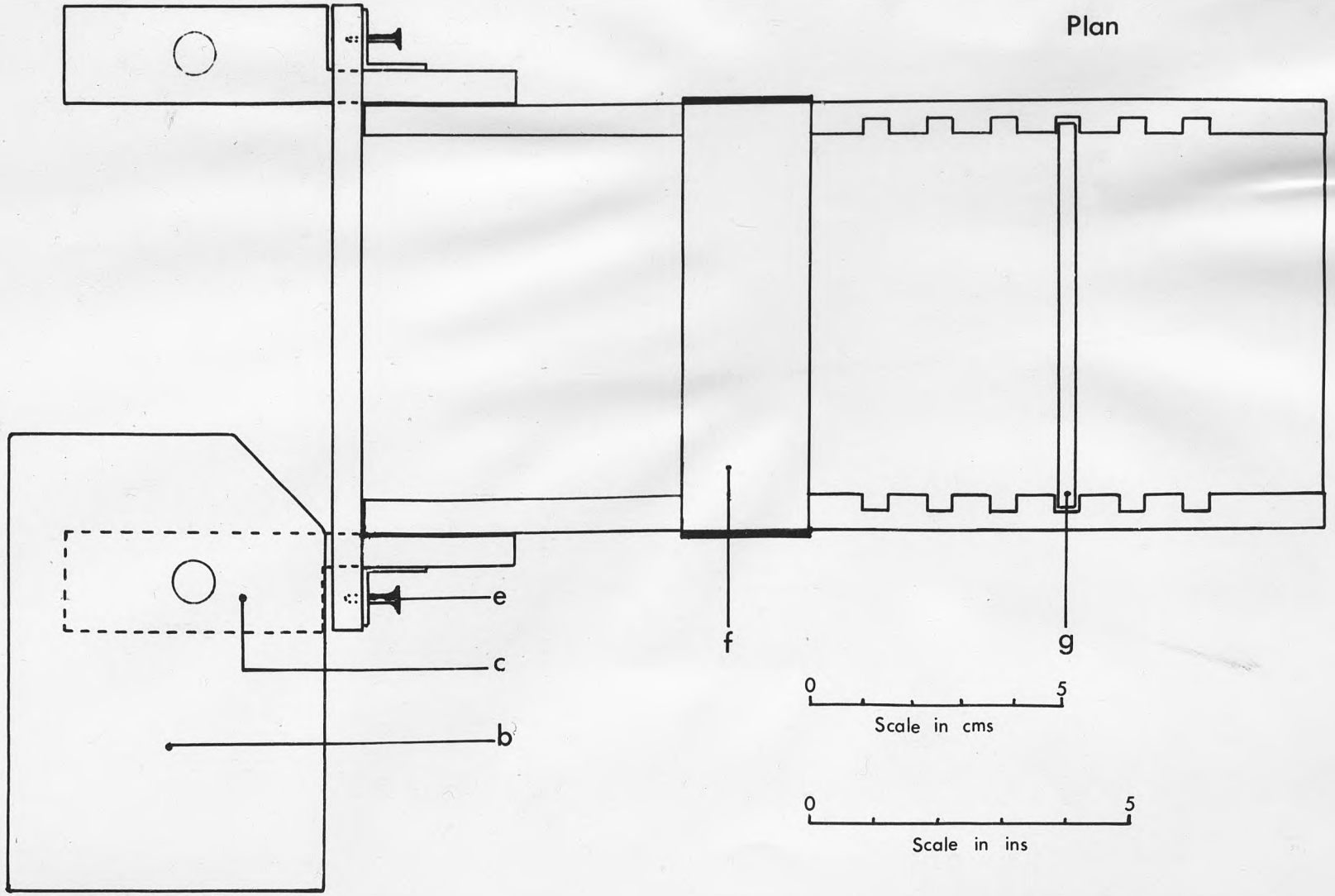

Plan
e
c
b
f
g
0
5
Scale in cms
0
5
Scale in ins

Fig. 2.3.1

Left - Perspex manipulation box for transferring flies.

Right - Torsion balance used for weighing pupae.

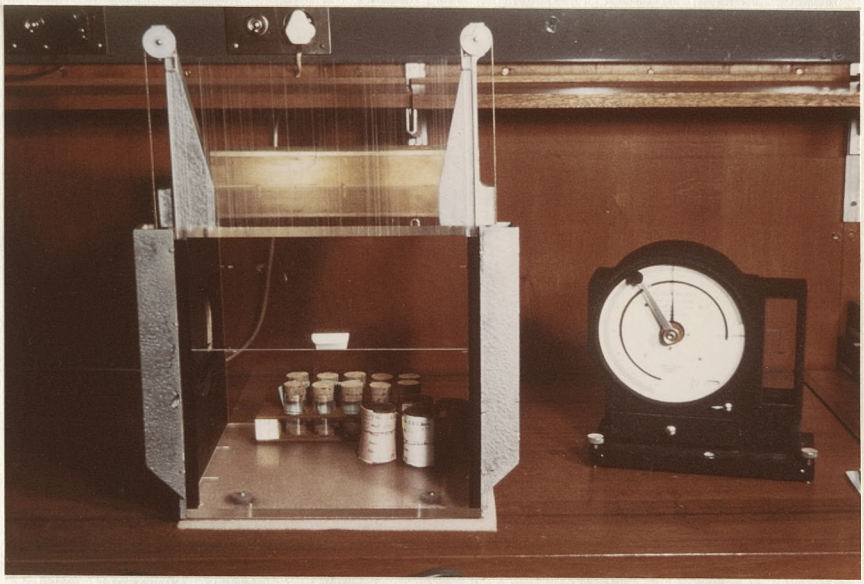


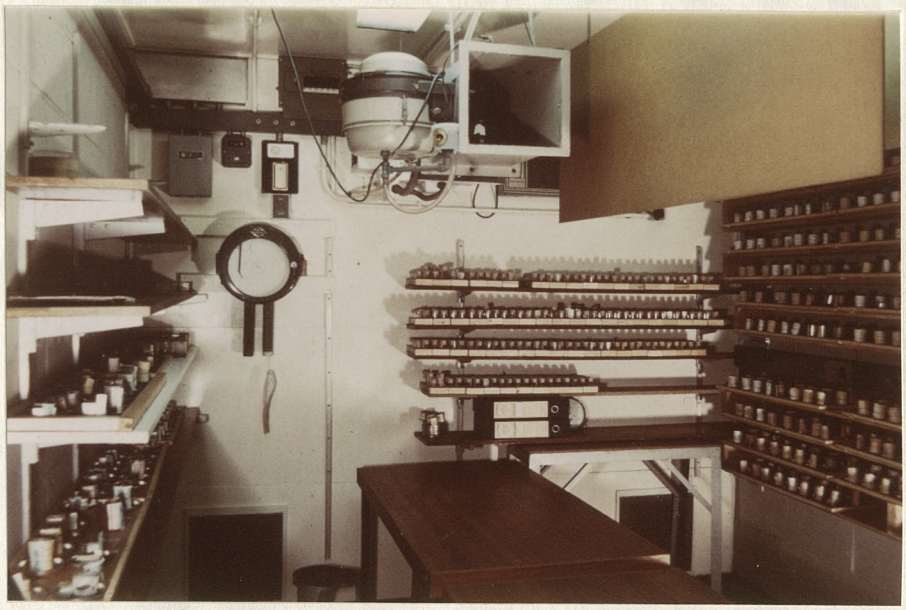
Fig. 2.3.2

General view of fly room.

Far wall, left hand side: temperature and humidity control panel.

Far wall, right hand side: pupa rack and tubes.

Right hand wall: fly cages.



2.3 Maintenance Methods

2.3.1 Pupae

Pupae, other than those bred in the constant temperature room, were unpacked on arrival and individually weighed on a 'White Electrical' Torsion Balance Model 0 (Fig. 2.3.1). Pupae under 25 mg. and which were found to be dead were discarded. Each pupa was then buried in dry sand in a 2" x 1" clear polystyrene tube. The tubes were then corked and placed in the fly room in racks designed to hold ten tubes (Fig. 2.3.2).

In a number of preliminary experiments this routine was varied, and some pupae were kept in damp sand, while in others they were kept in a number of different media, including damp sterilised compost and sawdust.

Laboratory-reared pupae were collected daily from the pupa trays beneath the fly cages, weighed, and placed in individual tubes as above. Abortions (including malformed pupae) were weighed when possible and then discarded.

2.3.2 Flies

1 Cages: At first it was proposed to use the 'Geigy' type of cage (Geigy, 1947) but for reasons discussed below (see Discussion) alternatives were sought. These included:

a A moulded plastic frame of the same overall dimensions to the Geigy cage. However, this proved too expensive to manufacture except in very large quantities.

b A cage made from a tinned steel container made by a local manufacturer

8.3/

8.3 cm. in diameter and 6.3 cm. deep (Plate 2.3.3). The bottom, and all except the rim of the lid were removed and replaced with black mosquito netting. A tinned steel lid was used as a pupa tray. The cage rested on a piece of clear plastic tubing, leaving a gap beneath the cage in which the deposited larva, after crawling through the mesh of the cage, could pupate.

c Polystyrene cage: Following the discovery by Azevedo and Pinhao (1967) and by Nash et al.(1966) that tsetse flies could be maintained in cages smaller than the standard Geigy type, the following design was adopted to allow the individual feeding of flies on rabbits' ears (Plate 2.3.4).

The bottom of a clear polystyrene tube (6 x 3.6 cm.) was removed using a band saw, and the end of the tube dipped in chloroform and then forced into one of the holes in a template over which a piece of terylene mosquito netting had been placed. The tube was removed from the hole 1 - 2 minutes later, and the netting, now stuck to the end of the tube, trimmed with a pair of scissors.

The top of the polythene stopper was also removed with a band saw. With the fly in the cage, the tube was closed with black terylene mosquito netting held in place by the polythene stopper. With the stopper end resting in a tray, the gap between the netting and the bottom of the cage allowed the deposited larae to crawl through the netting and pupate in the tray below.

2 Eclosion: The pupa racks were checked daily and the tubes with flies that had emerged, were removed. The flies were then transferred to cages/

Fig. 2.3.3 - Tin cage

Left - exploded view

Right - complete cage with record card and pupa tray and plastic tubing for resting the cage on

Fig. 2.3.4

Left Small Geigy cage (Nash et al, 1967 b)

Right Small Geigy cage (Azevedo and Pinhao, 1968)

Centre Two polystyrene cages, one with record card attached

cages using a perspex manipulation box (Plate 2.3.1).

3 Mating: Females of all species were mated when two to four days old, having taken at least one blood meal. Males were chosen that were at least seven days old, and were not offered food on the same day as they were used for mating.

The flies were separated 1 - 3 days later. When there was a shortage of suitable males, they were used more than once, but a gap of at least four days was left between each mating. Occasionally it was found necessary to use males less than seven days old.

A female was always mated with a male of the same origin. Males and females that emerged from pupae collected in Africa (hereinafter termed 'wild' or 'first generation' flies) were always mated with each other, and never with laboratory reared flies (i.e. second and subsequent generation flies). Laboratory reared flies were likewise mated only with each other.

2.4 Data Recording and Analysis

2.4.1 Introduction

One of the advantages of maintaining flies singly and not in large numbers per cage lay in the amount of information that could thus be obtained. However, the analysis of previous laboratory colony data had proved a formidable task, and much potentially valuable information could not be easily extracted (Pinhao - personal communication).

To overcome this, a series of computer programs were written to allow the storage and analysis of the data from the colony. The record for each fly and pupa were written onto magnetic tape and updated as each event in the fly or pupa's life history occurred.

On emergence each fly was allocated a number prefixed by the letter 'M' or 'F' according to its sex. Each pupa (or abortion) was likewise given a number prefixed by the letter 'P'. Events in the life history of a fly or pupa were recorded daily as they occurred on one of five different registers (Plate 2.4.2):

1 Eclosion register: Sex and number of each newly emerged fly and their respective pupa and female parent number.

2 Mating register: The fly-numbers of the flies mated.

3 Pupa register: Each pupa or abortion deposited together with its weight and female parent number.

4 Deaths register: The fly number and state of nutrition of each fly that had died.

2.4.2 Identification of flies and pupae in the laboratory

1 Flies: the sex and source of each fly was identified by the colour of the card attached to its cage. The layout and colour coding of these cards is shown in Fig. 2.4.1. The date of larviposition of the pupa deposited by the fly was recorded down the left hand side of the

fly/

Fig. 2.4.1 - Fly record cards (attached to cage with an elastic band)

Females	Males
Singida	Singida
Zambesi	Zambesi
Austeni	Austeni
Lisbon	Lisbon

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P M.

D.Ecl O/G

No.

Mated

Death

P.M.

D.Ecl O/G

No.

Mated

Death

P.M.

Plate 2.4.2

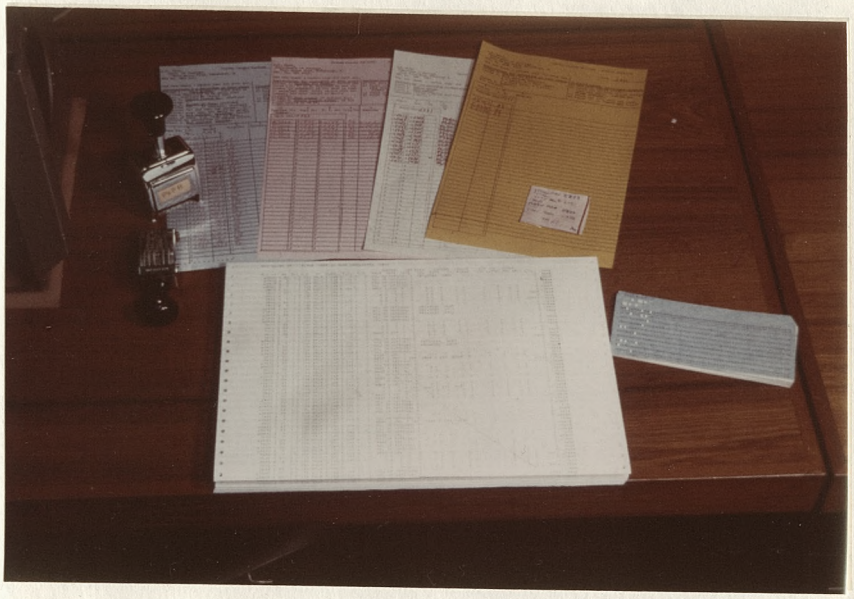
Data recording

Top — Daily registers. From left to right pupa (blue), eclosion (pink), mating (green) and death (yellow). A completed fly card is shown on top of the deaths register.

Left — Automatic numbering machine for recording pupae and newly emerged flies.

Below right — A daily register that has been transferred to punched cards.

Below centre — A print out of some of the fly records.



fly card.

2 Pupae: A description of the method of collection and weighing of the pupae is given in Section 2.3.1. The pupa was identified by a card with the relevant details attached to its tube.

The daily registers were transferred to punched cards and these were used to set up or update the records of the flies and pupae held on magnetic tape.

The records of any fly or pupa were printed out when desired, and detailed analysis of the records was achieved by modifying various standard computer statistical routines already available.

Full details of the methods used to record and analyse the data are given in Appendix 1.

3 Results

3.1 Introduction

Four principal colonies of flies were examined:

1. G. morsitans morsitans Westwood : SINGIDA (Batch 37)
2. G. morsitans orientalis Vanderplank : ZAMBESI (Batch 9)
3. G. austeni Newstead : AUSTENI (Batch 9)
4. G. morsitans morsitans Westwood : LISBON (Batch 1)

The first three colonies were maintained throughout on rabbits' ears, but the Lisbon colony was initially maintained on guinea-pigs, and then changed to rabbits' ears.

Some results from other colonies are shown were appropriate. For simplicity, the colonies will be referred to by their source name or species (i.e. Singida (S): Zambesi (Z): Austeni (A) and Lisbon (L)), followed by the batch number.

3.2 Factors affecting a single generation

3.2.1 Eclosion rate of 'wild' pupae and pupae introduced from other laboratories.

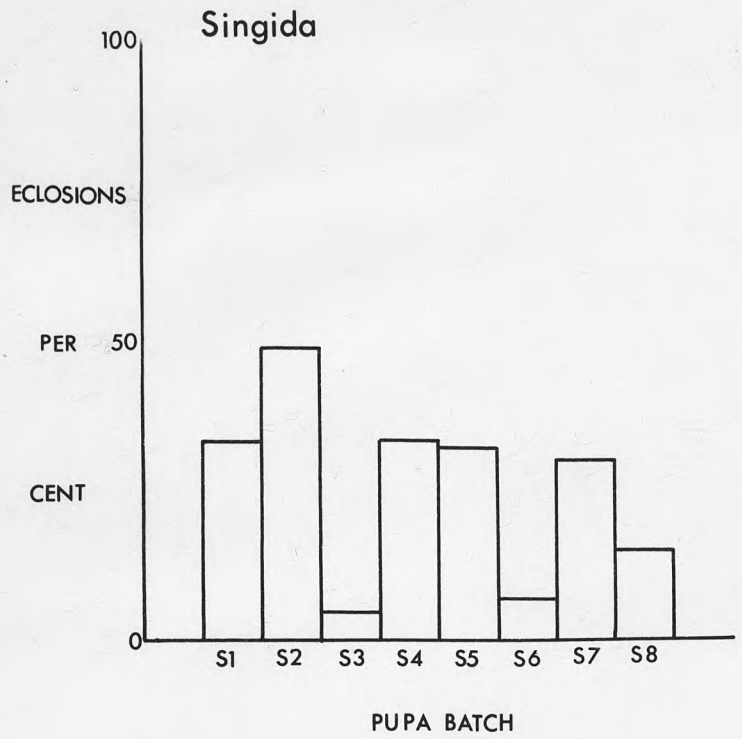
Figs. 3.2.1 and 3.2.2 show the eclosion rate of may of the batches of pupae received. In the case of the Singida batches (Fig. 3.2.1) the percentage eclosion before the introduction of the polystyrene boxes only reached 50% on one occasion, while after the introduction of the boxes, the percentage was only once below 50%.

In the case of the Zambesi pupae, the third and fourth batches were considerably better in this respect than the first and second, and the introduction of the polystyrene boxes (indicated by the arrow) did not have/

Fig. 3.2.1 - Percentage eclosion rate of batches of Singida pupae

Singida 1 - 8 Sent in tubes or tins

Singida 22 - 39 Sent in polystyrene boxes


Singida
100
ECLOSIONS
PER
50
CENT
0
S1
S2
S3
S4
S5
S6
S7
S8
PUPA BATCH

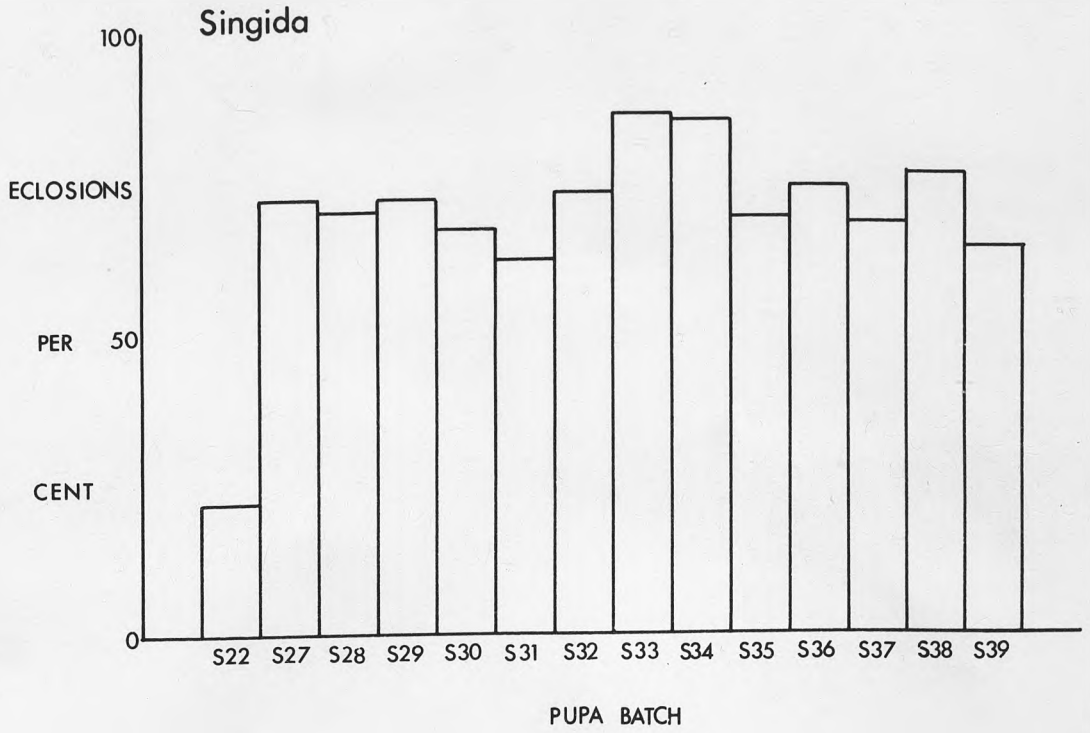
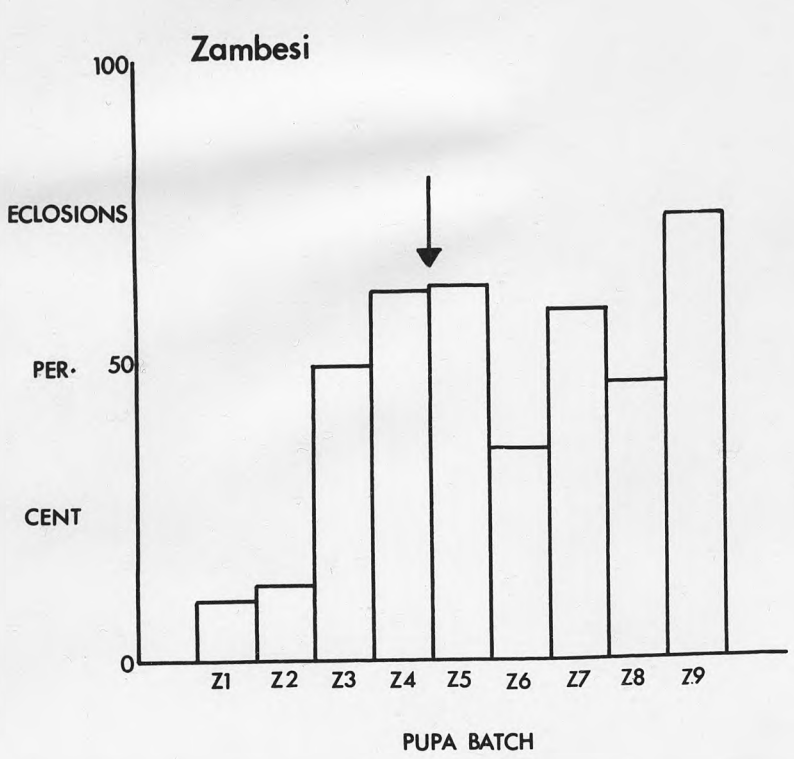
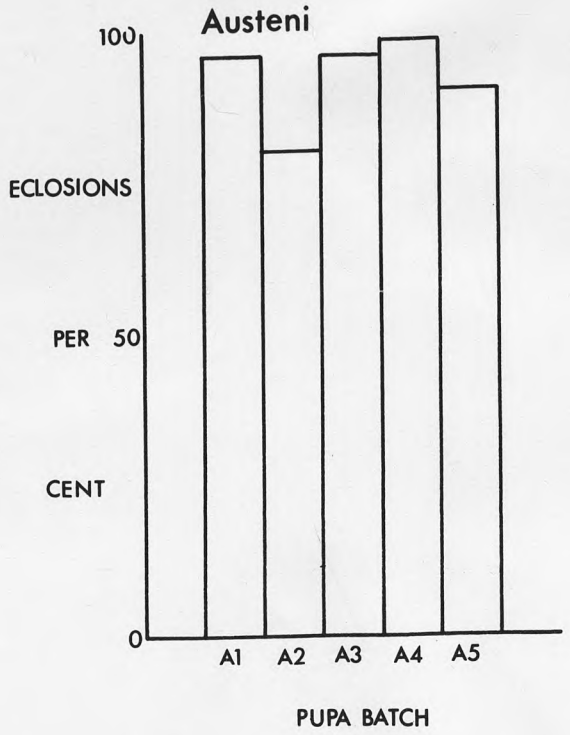
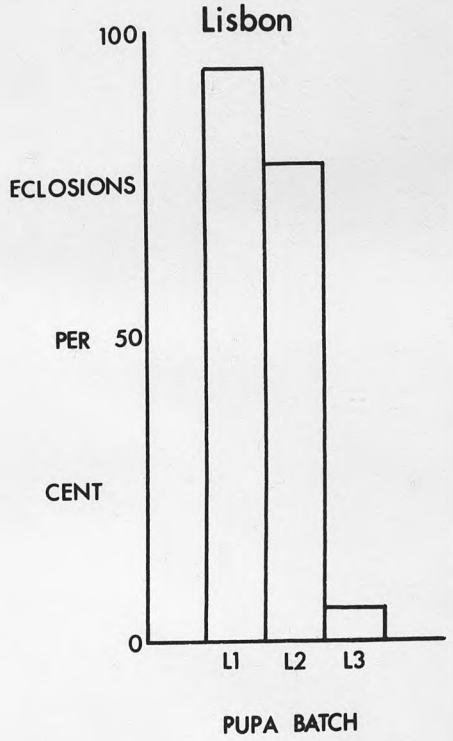

Singida
100
ECLOSIONS
PER
50
CENT
0
S22
S27
S28
S29
S30
S31
S32
S33
S34
S35
S36
S37
S38
S39
PUPA BATCH

Fig. 3.2.2 - Percentage eclosion rates of batches of pupae

Zambesi 1 - 4	Sent in tins
Zambesi 5 - 9	Sent in polystyrene boxes (the arrow indicates the time of introduction of polystyrene boxes)
Austeni 1 - 5 Lisbon 1 - 3	Sent in polystyrene boxes


Zambesi
100
ECLOSIONS
PER
50
CENT
0
Z1
Z2
Z3
Z4
Z5
Z6
Z7
Z8
Z9
PUPA BATCH


Austeni
100
ECLOSIONS
PER
50
CENT
0
A1
A2
A3
A4
A5
PUPA BATCH


Lisbon
100
ECLOSIONS
PER
50
CENT
0
L1
L2
L3
PUPA BATCH

have such a marked effect.

Polystyrene boxes were used throughout in the case of the Austeni and Lisbon batches and the eclosion rate was always extremely high, except in the case of Lisbon 3, which was unaccountably poor.

3.2.2 Eclosion rate of Edinburgh bred laboratory pupae

This is defined as the percentage of normal pupae from which flies ecloded.

The mean eclosion rate of pupae deposited by first and second generation flies is given in Table 3.2.3:

Table 3.2.3

	Generation 1		Generation 2	
	No. pupae	No. ecloded	No. pupae	No. ecloded
Singida 37	156	145 (92.8%)	377	335 (88.9%)
Zambesi 9	364	344 (94.6%)	783	737 (94.1%)
Austeni 9	168	132 (78.7%)	414	340 (82.1%)

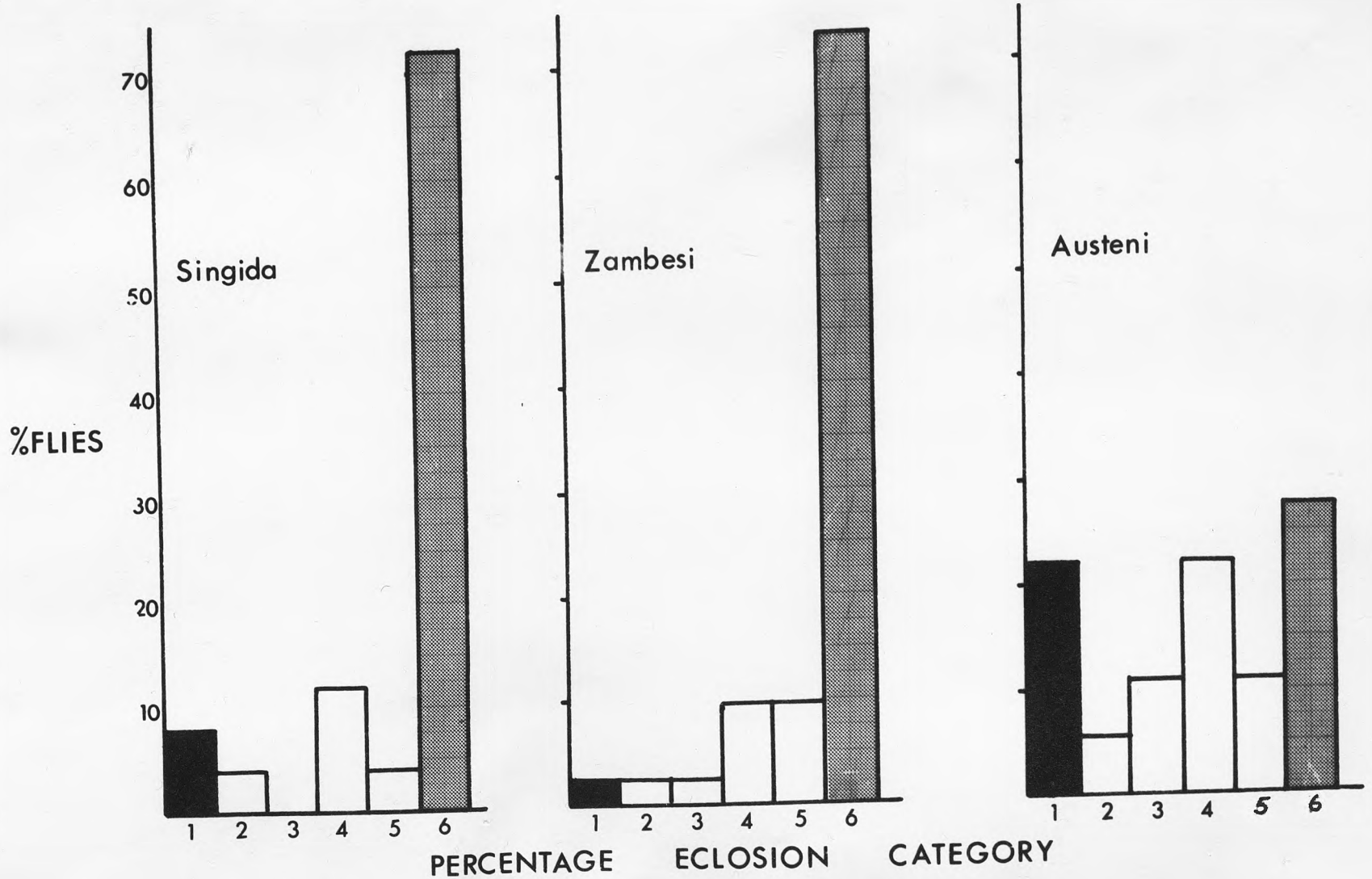
Table 3.2.4 shows that some females produced pupae, few of which emerged, whilst others produced pupae with a high rate of emergence. The results from Singida and Zambesi are similar, with the majority of the flies producing pupae, all of which ecloded. However, in the Austeni colony 22% of the flies deposited pupae of which less than 50% ecloded (Category 1) and only 28% deposited pupae all of which ecloded.

Fig. 3.2.4 - The percentage of the total number of flies contributing to each percentage eclosion category

Category	Percentage of pupae ecloded
1	0 - 49.9
2	50 - 59.9
3	60 - 69.9
4	70 - 79.9
5	80 - 89.9
6	90 - 99.9
7	100

Notes 1 The black columns represent an accumulation of the 0 - 50 percentage eclosion rate.

2 The stippled columns represent the 100 percentage eclosion rate.


Singida
Zambesi
Austeni
%FLIES
70
60
50
40
30
20
10
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
PERCENTAGE ECLOSION CATEGORY

3.3 Pupal period

The mean pupal period for Generations 2 to 5 are given in Table 3.3.1, and the distribution of the pupal period for generation 3 pupae (i.e. those deposited by generation 3 female parents) is given in histogram form in Fig. 3.3.2. The mean pupal period of the males is approximately 2 days greater than that of the females. The mean pupal period of the Austeni pupae was for each generation greater than that in the other two colonies. The spread of the pupal period as shown in Fig. 3.3.2 is to some extent due to the fact that the pupae and the emerged flies were only collected once a day, and therefore could have been up to a total of two days older than that recorded.

3.4.1 Non-ecloded pupae

In order to investigate possible reasons for the non-eclosion of some pupae, all pupae that did not eclode after 1 January 1968 were dissected. The results are shown in Table 3.4.1. Exact comparisons between the different colonies were not possible as the total number of pupae that ecloded during the period in question was not recorded. However, it will be noted from Table 3.2.3 that the Austeni emergence rate was lower than the other two colonies.

The pupae were divided into two categories: those which contained a fully or partly developed fly (Group A) and those in which the pupae died early in the pupal development, no recognisable structure being present (Group B). Three-quarters of the Austeni pupae died late in development, while less than a quarter of the Zambesi pupae did so. The difference is significant (X^2 = 80.8: P = 0.005). There is also a significant difference between the Singida and Zambesi pupae (X^2 = 5.9: P = 0.025).

Table 3.3.1 - Mean pupal period

	Gen.	No. of females	Mean female pupal period ± 2 x s.e	No. of males	Mean male pupal period
SINGIDA 37	2	37	32.1 ± 2.7	23	34.8 ± 2.8
	3	29	33.2 ± 3.3	28	35.8 ± 3.0
	4	8	32.4 ± 3.1	13	35.7 ± 3.7
	5	1	28.0	3	32.3 ± 3.2
ZAMBESI 9	2	60	31.5 ± 3.0	44	35.1 ± 3.1
	3	50	33.5 ± 2.7	59	35.8 ± 3.0
	4	30	33.7 ± 3.4	33	34.2 ± 3.3
	5	5	31.4 ± 3.4	4	31.0 ± 1.8
AUSTENI 9	2	40	33.5 ± 3.3	21	35.9 ± 2.7
	3	56	35.0 ± 2.8	34	36.7 ± 2.7
	4	40	34.6 ± 2.7	21	36.4 ± 2.9
	5	10	32.1 ± 3.2	9	33.5 ± 3.5

Note: The generation number refers to that of the female parent.

Fig. 3.3.2 - Distribution of pupal period in Generation 3 of the main colonies

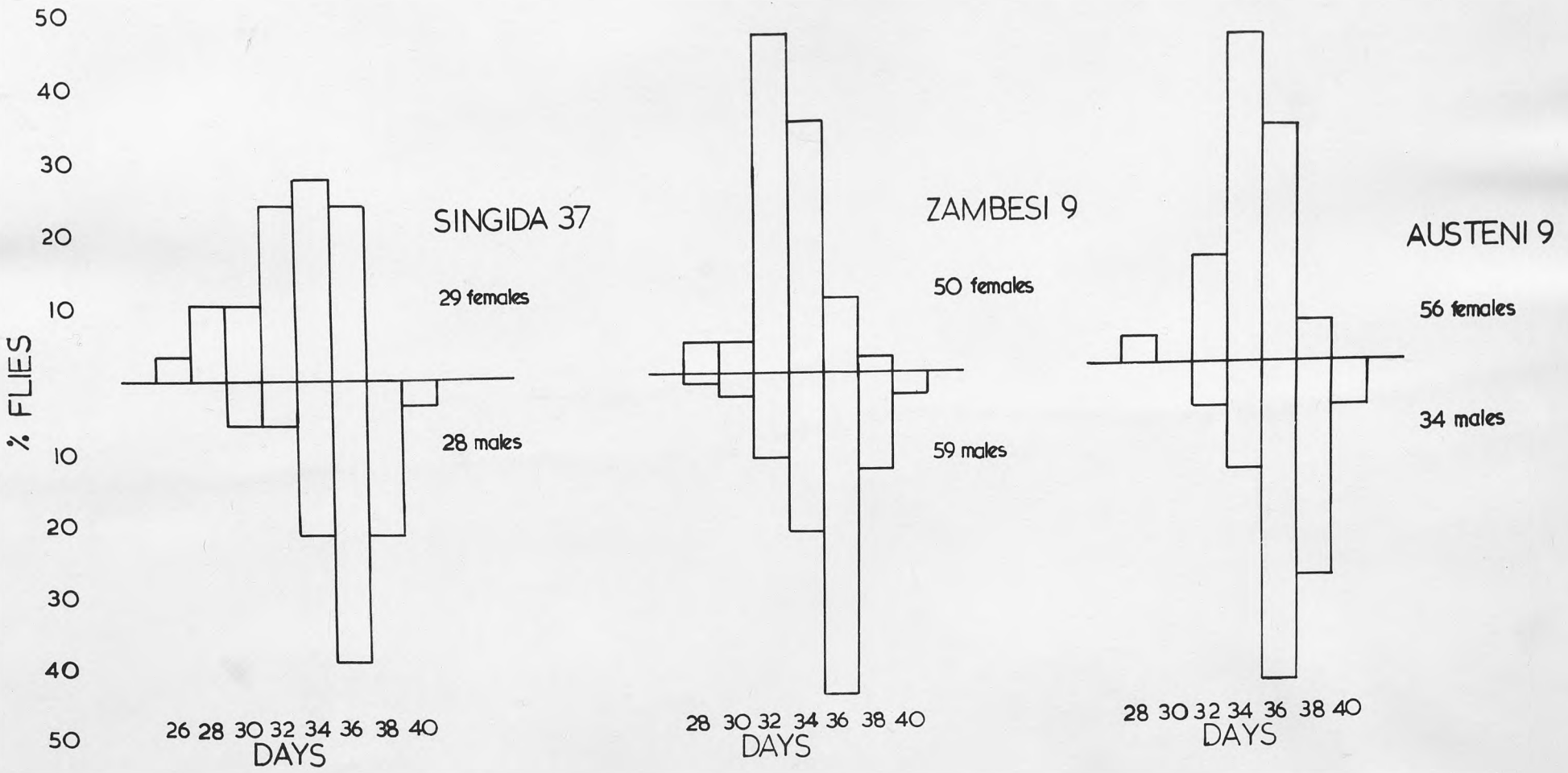

50
40
30
20
10
% FLIES
10
20
30
40
50
26 28 30 32 34 36 38 40
DAYS
SINGIDA 37
29 females
28 males
28 30 32 34 36 38 40
DAYS
ZAMBESI 9
50 females
59 males
28 30 32 34 36 38 40
DAYS
AUSTENI 9
56 females
34 males

Table 3.4.1 - Total non-ecloded pupae (from 1 January 1968 - 3 August 1968)

	No. non ecloded pupae	A	B	
Singida	91	7(7.7%)	84(92.3%)	$\chi_2 = 5.85$, $P < 0.025$
Zambesi	99	21(21.2%)	78(78.8%)	$\chi_2 = 80.83$, $P < 0.005$
Austeni	179	139(77.6%)	40(22.4%)	
Total	369	167(45.3%)	202(54.7%)	

Key

A contained recognisable adult structures and therefore died late in development

B contained no recognisable adult structures and therefore died early in development

Table 3.4.2 - Total non-expanded flies (from 5 November 1967 - 3 August 1968)

	Male	Female
Singida	6 (54.5%)	5 (45.5%)
Zambesi	4 (33.3%)	8 (66.7%)
Austeni	20 (24.7%)	61 (75.3%)

3.4.2 Non-expanded flies (flies that emerged but did not fully expand)

Non-expanded flies were recorded during the period 5 November 1967 and 3 August 1968. For similar reasons to those given in section 3.4.1 above, it was not possible to compare the proportion of non-expanded flies in each colony. However, as the colony sizes were kept at approximately the same level, it can be deduced from table 3.4.2. that there was a greater proportion of non-expanded flies in the Austeni colony than in the other two colonies. Three-quarters of the Austeni flies that failed to expand were female.

3.5 Sex Ratio

The sex ratio in each generation is shown in Table 3.5.1. In the case of Singida 37 and Austeni 9 not all males that ecloded in Generation 1 were recorded, and this accounts for the discrepancy in the sex ratio in these groups. With the exception of Generation 1 (for reasons given above) although more females emerged than males, the difference was not significant.

3.6 Mating

No direct check was made of the success of mating of individual females. However, an examination was made of those females that lived more than 20 days without producing a larva. For instance in Austeni 9, generation 2, there were 51 productive flies. Of the 9 non-productive females, four of the matings were by males that were only used once. One male was used twice, both times unsuccessfully, but the remaining four males were used successfully for mating on at least one other occasion. Out of a total of 16 matings by the 9 males concerned, 5 were successful and

Table 3.5.1 - Sex Ratios

SINGIDA 37						
Generation	1	2	3	4	5	6
	(72 flies)	(145 flies)	(355 flies)	(359 flies)	(98 flies)	(8 flies)
Males	31 (43.1%)	70(48.3%)	165(49.3%)	175 (48.8%)	47 (48.0%)	3 (37.5%)
Females	41 (56.9%)	75 (51.7%)	170 (50.7%)	184 (51.2%)	51 (52.0%)	5 (62.5%)
ZAMBESI 9						
Generation	1	2	3	4	5	6
	(147 flies)	(344 flies)	(737 flies)	(625 flies)	(156 flies)	-
Males	75 (51.0%)	165 (48.0%)	363 (49.3%)	294 (47.0%)	87 (55.8%)	-
Females	72 (49.0%)	179 (52.0%)	374 (50.7%)	331 (53.0%)	69 (44.2%)	-
AUSTENI 9						
Generation	1	2	3	4	5	6
	(41 flies)	(132 flies)	(340 flies)	(408 flies)	(190 flies)	(21 flies)
Males	15 (36.6%)	60 (45.5%)	173 (50.9%)	196 (48.0%)	91 (47.9%)	9 (42.9%)
Females	26 (63.4%)	72 (54.5%)	167 (49.1%)	212 (52.0%)	99 (52.1%)	12 (57.1%)

11 unsuccessful. However, of the 11 unsuccessful matings, 4 of the females died only shortly after the 20 day limit, and it is possible that had they lived longer they would have proved to be fertile.

From the results it seemed likely that at least some of the males used were infertile so an examination was also made of the mating performance of the 38 males used in Generation 2 of Austeni 9 (Table 3.6.1). Of a total of 65 matings that took place, 13 were unsuccessful; in three cases the female died before reaching 20 days old; and 49 of the matings were successful. The 13 unsuccessful matings were spread among 10 males of which 3 were only used once. One male was used unsuccessfully twice, but the remaining 6 males had at least one successful mating.

From these results it would appear that the cause of the non-productivity amongst females was caused not by sterility of the males, but more probably by some factor such as the state of nutrition or unattractiveness of the female at the time of mating.

3.7 Longevity

3.7.1 Distribution of longevity

The distribution of longevity of those flies dying naturally in the first two generations is given in Table 3.7.1. Once again, direct comparison, except in the case of Generation 1 is complicated by the number of flies culled in the second and subsequent generations. This difficulty is well shown in generation 2 of Zambesi 9, where because 40.2% of the females in that generation were culled (Table 3.14.1) and 10.2% were still living at the time of analysis, the mean longevity is greatly/

Table 3.6.1 - Austeni 9 - Generatio 2: Mating success of males measured by the number of productive (1), non-productive (X) and doubtful (0) females mated with.

No. of males	Matings record 1st	2nd	3rd	
3	X			Completely unsuccessful matings (4 males)
1	X	X		
1	1	X		Partially unsuccessful (6 males)
2	X	1		
1	X	1	1	
1	X	1	X	
1	X	X	1	
1	1	1	0	Successful and doubtful (3 males)
2	0	1		
15	1			Completely successful (25 males)
7	1	1		
3	1	1	1	

Note: The mating was considered doubtful where the female died before reaching 20 days old.

Table 3.7.1 - Distribution of female longevity

Age at death (days)	Singida 37		Zambesi 9		Austeni 9	
	Gen 1 (39 flies)	Gen 2 (37 flies)	Gen 1 (66 flies)	Gen 2 (60 flies)	Gen 1 (26 flies)	Gen 2 (40 flies)
0 - 4	1 (2.6%)	2 (5.4%)	2 (3.0%)	11 (18.3%)	1 (3.9%)	5 (12.5%)
5 - 9	1 (2.6%)	2 (5.4%)	5 (7.6%)	7 (11.7%)	1 (3.9%)	0
10 - 19	1 (2.6%)	2 (5.4%)	4 (6.1%)	4 (6.7%)	1 (3.9%)	1 (2.5%)
20 - 39	4 (10.3%)	1 (2.7%)	14 (21.2%)	6 (10.0%)	5 (19.2%)	7 (17.5%)
40 - 59	2 (5.1%)	5 (13.5%)	1 (1.5%)	8 (13.3%)	1 (3.9%)	6 (15.0%)
60 - 79	7 (18.0%)	3 (8.1%)	8 (12.1%)	6 (10.0%)	4 (15.4%)	5 (12.5%)
80 - 99	4 (10.3%)	3 (8.1%)	3 (4.6 %)	2 (3.3%)	2 (7.7%)	3 (7.5%)
100 - 119	4 (10.3%)	2 (5.4%)	3 (4.6%)	4 (6.7%)	3 (11.5%)	2 (5.0%)
120 - 139	3 (7.7%)	4 (10.8%)	4 (6.1%)	3 (5.0%)	1 (3.9%)	1 (2.5%)
140 - 159	3 (7.7%)	2 (5.4%)	2 (3.0%)	3 (5.0%)	0	4 (10.0%)
160 - 179	1 (2.6%)	4 (10.8%)	2 (3.0%)	3 (5.0%)	0	2 (5.0%)
180 - 199	0	3 (8.1%)	6 (9.1%)	1 (1.7%)	3 (11.5%)	2 (5.0%)
200 - 219	3 (7.7%)	2 (5.4%)	3 (4.6%)	1 (1.7%)	1 (3.9%)	2 (5.0%)
220 - 239	2 (5.1%)	2 (5.4%)	2 (3.0%)	1 (1.7%)	1 (3.9%)	0
240 - 259	2 (5.1%)	0	3 (4.6%)	0	0	0
260 - 279	1 (2.6%)	0	1 (1.5%)	0	2 (2.7%)	0
280 - 299	0	0	1 (1.5%)	0	0	0
300 - 319	0	0	0	0	0	0
320 - 339	0	0	2 (3.0%)	0	0	0
Mean longevity (± 2 x s.e)	112.4 ± 17.3	109.9 ± 16.6	108.5 ± 19.2	63.8 ± 15.8	101.8 ± 18.4	78.8 ± 15.9

greatly depressed.

3.7.2 Survival curves

Figs. 3.7.2 - 3.7.4 give the survival curves for Generations 1 and 2 of the three main colonies.

The few flies that were culled in the first generation were ignored, but in the second generation, the culled flies were distributed amongst those dying naturally. For instance, those flies that were culled at 40 days old were distributed amongst the flies dying naturally that had survived longer than 40 days, in direct proportion to the number that died in any one period. Those second generation flies that were living at the time of the analysis were also ignored, and this factor was largely responsible for the shorter maximum longevity seen in Generation 2. From the graphs it will be seen that the survival curves for each species and generation had similar characteristics with a relatively high proportion dying within the first few days of life, followed by a steady rate of mortality, with the last fly dying at between 280 and 340 days.

Since males were culled after being used once or twice as mates, it was not possible to draw their survival curves.

3.8 State of nutrition at death

Table 3.8.1 gives the distribution of the state of nutrition at death of flies in the three main colonies. The majority of the flies of both sexes died in a starving condition. The cause of death of those flies that died in an apparently engorged condition was due, in most cases/

Fig. 3.7.2 - Singida 37 - Survival curve for Generations 1 and 2

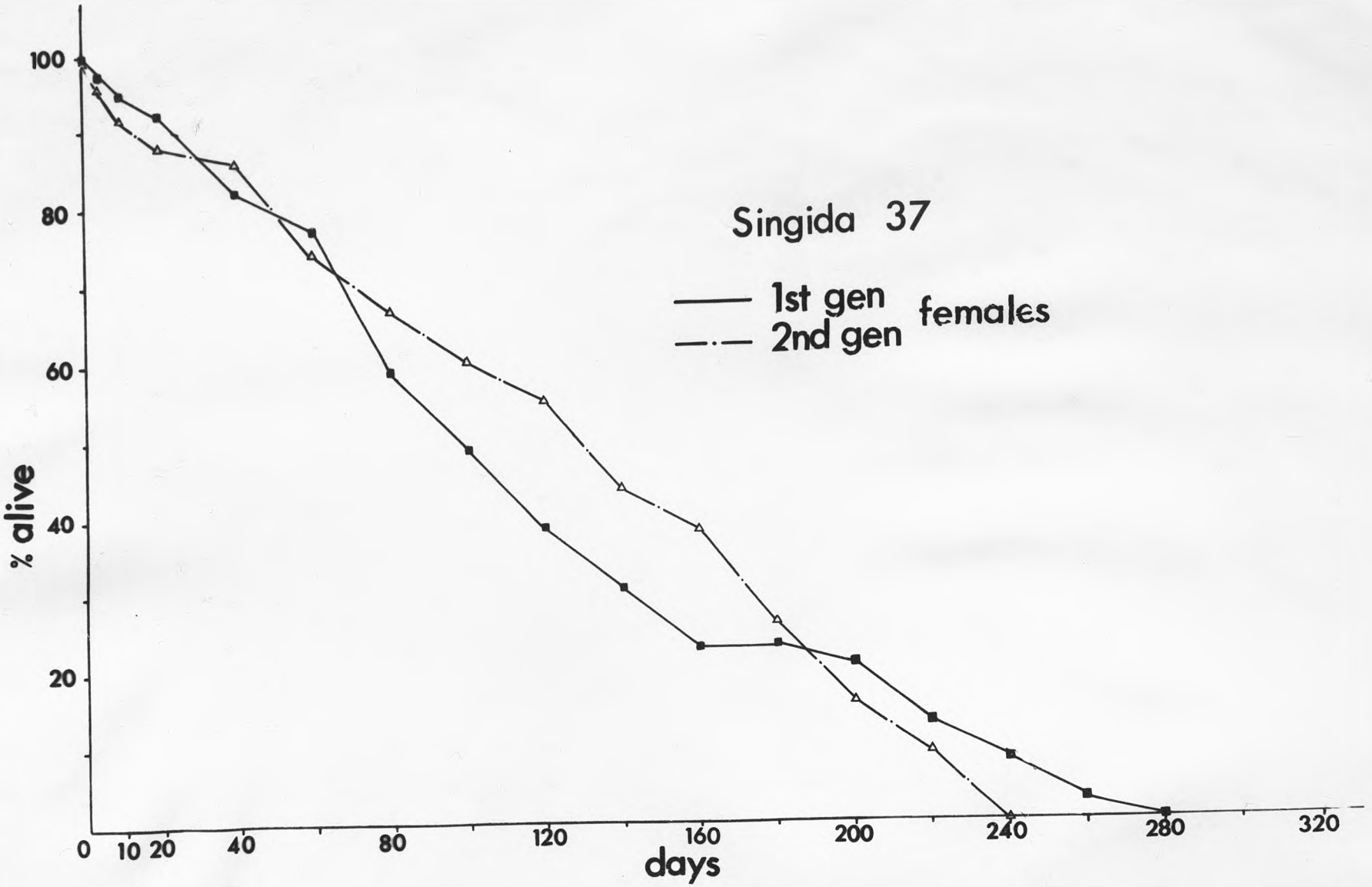

Singida 37
1st gen
2nd gen
females
% alive
100
80
60
40
20
0
10
20
40
80
120
160
200
240
280
320
days

Fig. 3.7.3 - Zambesi 9 - Survival curve for Generations 1 and 2

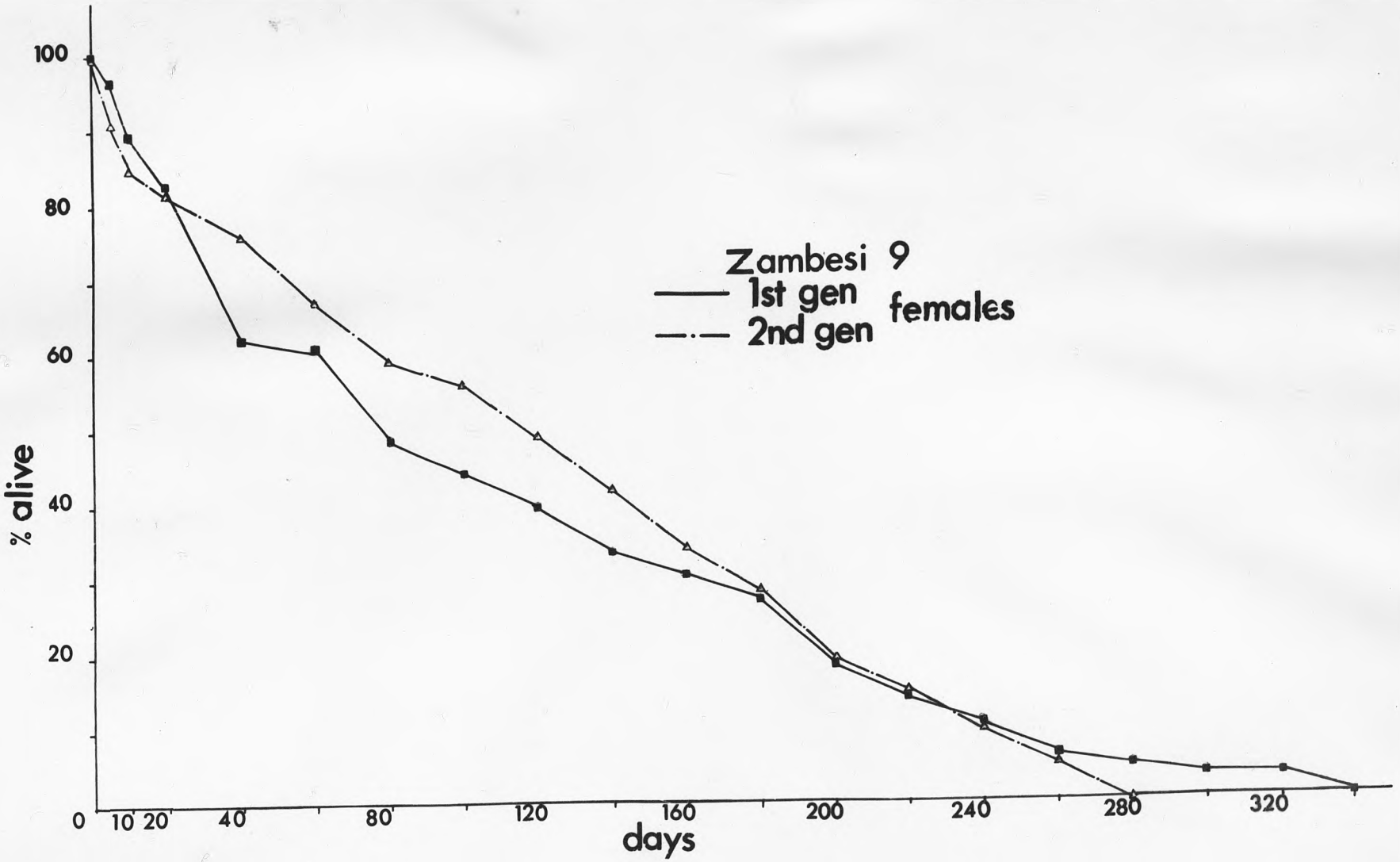

Zambesi 9
1st gen
2nd gen
females
% alive
days
100
80
60
40
20
0
10
20
40
80
120
160
200
240
280
320

Fig. 3.7.4 - Austeni 9 - Survival curves for Generations 1 and 2

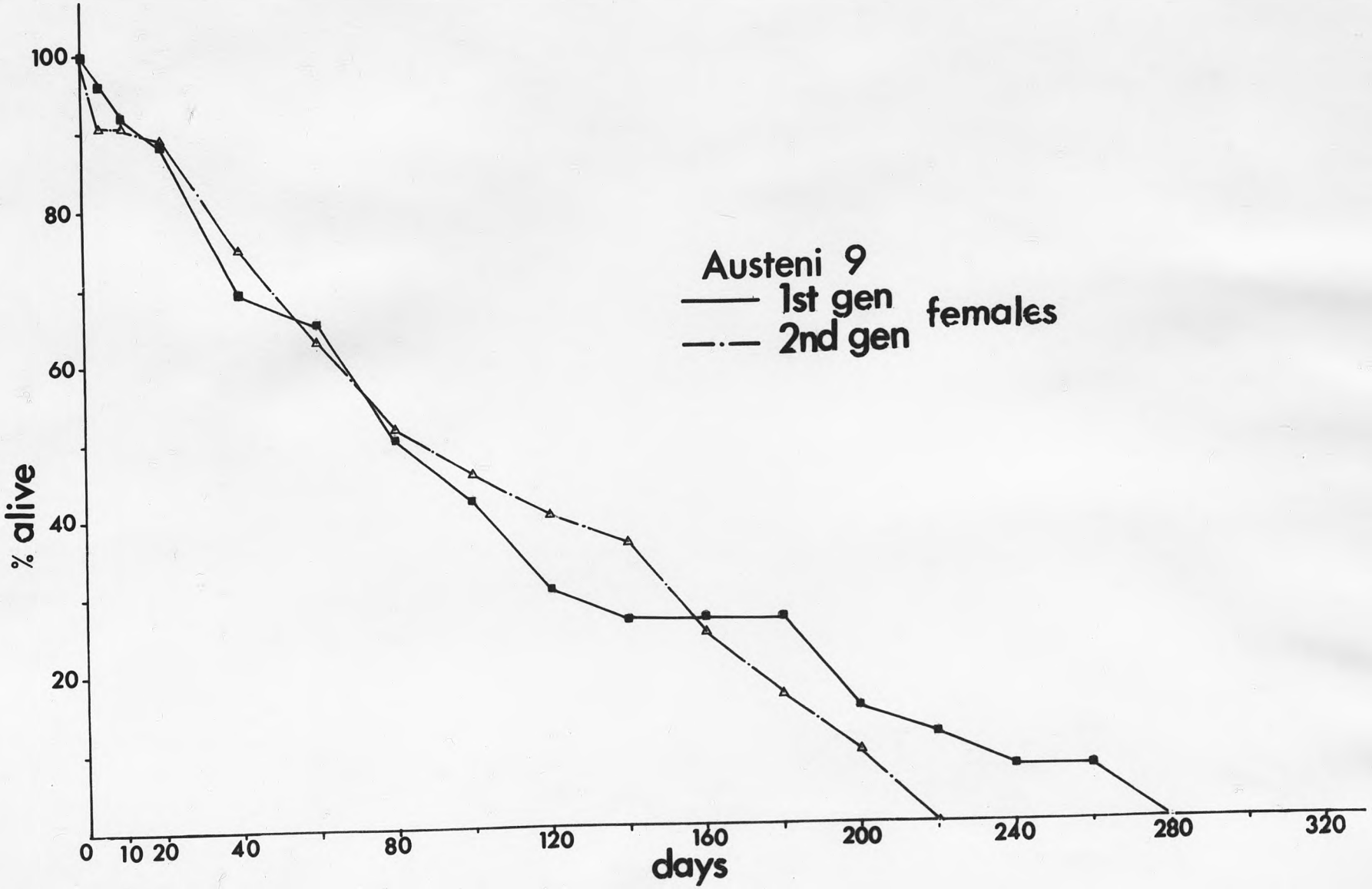

Austeni 9
1st gen
2nd gen
females
% alive
100
80
60
40
20
0
10
20
40
80
120
160
200
240
280
320
days

Table 3.8.1 - State of nutrition at death

		Males	Females
SINGIDA 37			
(199 flies)		(85 flies)	(114 flies)
	A	80 (94.0%)	88 (77.2%)
	B	4 (4.7%)	3 (2.6%)
	C	1 (1.2%)	4 (3.5%)
	D	0	19 (16.7%)
ZAMBESI 9		Males	Females
(395 flies)		(184 flies)	(211 flies)
	A	158 (85.9%)	152 (72.0%)
	B	12 (6.5%)	11 (5.2%)
	C	9 (4.9%)	14 (6.6%)
	D	5 (2.7%)	34 (16.1%)
AUSTENI 9		Males	Females
(263 flies)		(91 flies)	(172 flies)
	A	91 (100%)	140 (81.4%)
	B	0	6 (3.5%)
	C	0	7 (4.1%)
	D	0	19 (11.0%)

A - Starving
B - Good state of nutrition but no blood showing on ventral surface of abdomen
C - Good state of nutrition with some blood showing on ventral surface of abdomen, but not engorged
D - Engorged or apparently engorged, with greater part of ventral surface of abdomen red or black

cases, to a burst crop. In some instances, especially in the Austeni colony, 'full-term' larvae pupated in-utero, and although the fly often lived for some days or weeks after this, the mechanical blockage obviously interfered with the intake and digestion of the blood meal. Very few males died in an engorged condition.

3.9 Age specific fecundity

3.9.1 Interlarval period and number of days to first larviposition

Table 3.9.1 and Fig. 3.9.2 show the distribution of the mean interlarval period in the three main colonies. The theoretical interlarval period is approximately ten days at 25°C (Glasgow, 1963). It will be seen that only Austeni 9 approached the theoretical value with a mean I.L.P. of 10.1 days in the first generation, and only one fly in the first 2 generations of this colony had a mean interlarval period of more than 14 days. In the other two colonies, not only was the mean much higher, but there was a much greater spread with the mode at between 12 and 14 days. It is not known whether this was due to a slower rate of ovulation in the G. morsitans colonies, or a greater number of undetected abortions. Table 3.9.3 and Fig 3.9.4 give the distribution of the number of days after eclosion before the deposition of the first larva. If the first two generations are combined, the mode in each colony is between 18 and 20 days, but the variation is considerable, especially in the two G. morsitans colonies. Those flies which deposited for the first time at about 30 and 40 days old probably missed one or two complete reproductive cycles respectively. Those taking an intermdiate number of days may have also missed one or more cycles, but due to the early death of the larva in utero the cycles may have been speeded up. Only

in/

Table 3.9.1 - Distribution of mean interlarval period

Mean interlarval period (days)	Singida 37		Zambesi 9		Austeni 9	
	Gen 1 (23 flies)	Gen 2 (27 flies)	Gen 1 (36 flies)	Gen 2 (27 flies)	Gen 1 (17 flies)	Gen 2 (25 flies)
8	0	0	2 (5.6%)	0	3 (17.7%)	0
9	0	2 (7.4%)	1 (2.8%)	2 (7.4%)	3 (17.7%)	10 (40.0%)
10	1 (4.4%)	5 (18.5%)	2 (5.6%)	3 (11.1%)	5 (29.4%)	5 (20.0%)
11	4 (17.4%)	0	2 (5.6%)	2 (7.4%)	5 (29.4%)	4 (16.0%)
12	1 (4.4%)	2 (7.4%)	6 (16.7%)	6 (22.2%)	1 (5.6%)	2 (8.0%)
13	4 (17.4%)	4 (14.8%)	8 (22.2%)	0	0	1 (4.0%)
14	5 (21.7%)	3 (11.1%)	8 (22.2%)	2 (7.4%)	0	2 (8.0%)
15	0	2 (7.4%)	2 (5.6%)	4 (14.8%)	0	0
16	3 (13.0%)	3 (11.1%)	1 (2.8%)	2 (7.4%)	0	0
17	1 (4.4%)	0	0	3 (11.1%)	0	0
18	1 (4.4%)	0	1 (2.8%)	1 (3.7%)	0	0
19	0	0	1 (2.8%)	0	0	0
20 - 29	3 (13.0%)	4 (14.8%)	1 (2.8%)	2 (7.4%)	0	0
30 - 39	0	0	1 (2.8%)	0	0	0
40 - 99	0	2 (7.4%)	0	0	0	0
Overall mean interlarval period ($\pm$ 2 x s.e)	15.0 $\pm$ 1.6	17.7 $\pm$ 4.8	13.8 $\pm$ 1.4	14.2 $\pm$ 1.3	10.0 $\pm$ 0.9	11.5 $\pm$ 1.5

Table 3.9.1.1 - Lisbon 1 (Generations 1 and 2) - Pupal production and mean interlarval period

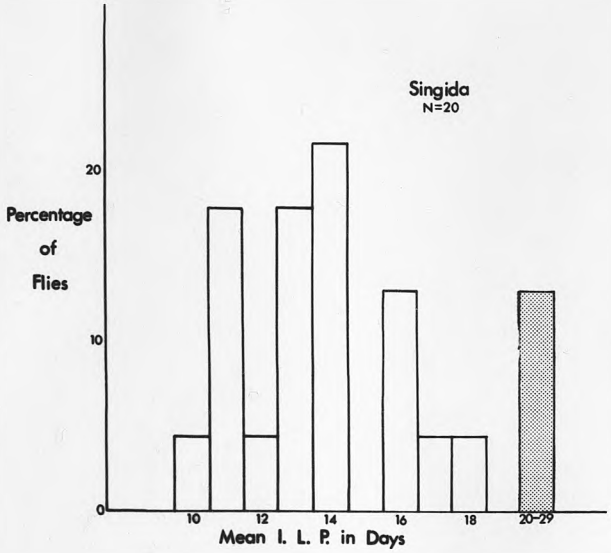
	Fly no.	No. of pupae	Mean I.L.P.	
Generation 1 (10 females)	978	7	9.3	Overall mean I.L.P. = 11.4 days No. pupae deposited = 46 No. abortions = 6
	988	4	9.3	
	992	2	9.0	
	994	3	10.5	
	996	7 (2)	14.5	
	1003	2	12.0	
	1029	9 (1)	13.2	
	1030	5 (2)	9.8	
	1060	4 (1)	16.3	
	1061	3	10.0	
Generation 2 (8 females)	1506	3	11.0	Overall mean I.L.P. = 12.5 days No. pupae deposited = 35 No. abortions = 4
	1536	2	11.0	
	1539	4	10.3	
	1553	5 (1)	12.2	
	1623	4	14.0	
	1788	3 (1)	20.0	
	1817	8 (2)	12.0	
	2187	6	9.6	

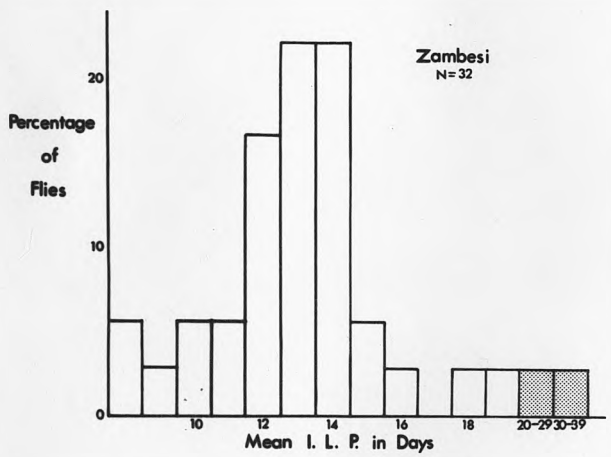
Note: The figures in brackets indicate the number of abortions deposited by each fly.

Fig. 3.9.2 - Distribution of mean interlarval period of Generation 1 Flies in three main colonies

N = no. of flies considered

Flies with a mean interlarval period in excess of 20 days are grouped in periods of 10 days (stippled columns)


Singida
N=20
Percentage
of
Flies
20
10
0
10
12
14
16
18
20-29
Mean I. L. P. in Days


Zambesi
N=32
Percentage
of
Flies
20
10
0
10
12
14
16
18
20-29
30-39
Mean I. L. P. in Days

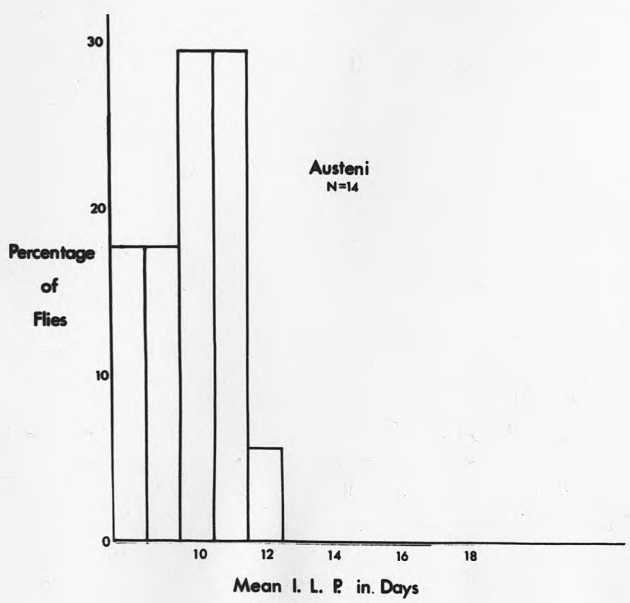

Austeni
N=14
Percentage
of
Flies
30
20
10
0
10
12
14
16
18
Mean I. L. P. in. Days

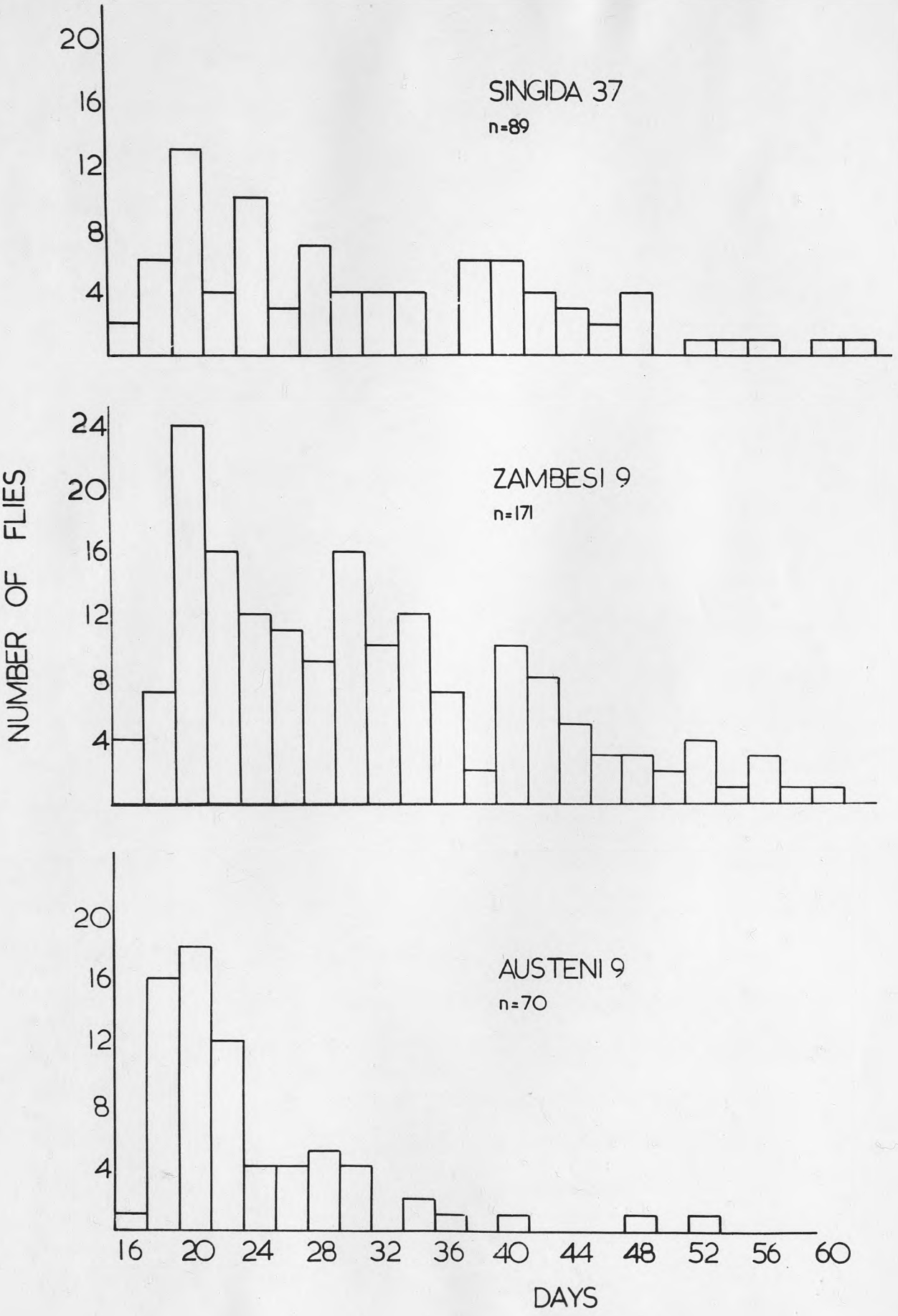
Table 3.9.3

Distribution of number of days from eclosion to first larviposition

Days from eclosion to 1st larva	Singida 37		Zambesi 9		Austeni 9	
	Gen 1 (27 flies)	Gen 2 (62 flies)	Gen 1 (46 flies)	Gen 2 (125 flies)	Gen 1 (19 flies)	Gen 2 (51 flies)
15	-	-	-	2	-	-
16	-	2	-	2	-	1
17	1	2	-	2	-	7
18	-	3	4	1	-	9
19	1	5	5	6	3	7
20	-	7	5	8	2	6
21	1	-	3	7	2	5
22	-	3	1	5	2	3
23	2	5	2	6	1	1
24	2	1	1	3	2	-
25 - 26	1	2	1	10	1	3
27 - 28	1	6	1	8	1	4
29 - 30	-	4	5	11	4	-
31 - 32	2	2	1	9	-	-
33 - 34	1	3	2	10	1	1
35 - 36	1	-	-	7	-	1
37 - 38	4	2	1	1	-	-
39 - 40	1	5	2	8	-	1
41 - 42	4	-	4	4	-	-
43 - 44	1	2	2	3	-	-
45 - 46	-	2	2	1	-	-
47 - 48	2	2	-	3	-	1
49 - 50	1	-	1	1	-	-
51 - 52	1	1	2	2	-	1
53 - 54	-	1	-	1	-	-
55 - 56	-	-	1	2	-	-
57 - 58	-	1	-	1	-	-
59 - 60	-	1	-	1	-	-
61 - 62	-	-	-	-	-	-
Mean (days)	34.7	29.5	29.4	30.3	24.2	22.3
Median (days)	37 - 38	27 - 28	29 - 30	27 - 28	23	20

Fig. 3.9.4 - Distribution of the number of days from eclosion to the deposition of the first larva

1st and 2nd generations combined. N = number of flies considered


SINGIDA 37
n=89
ZAMBESI 9
n=171
AUSTENI 9
n=70
NUMBER OF FLIES
DAYS
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60

in the 1st generation of the Austeni colony had the majority of larvipositions occurred by 20 days: the theoretical time given in the model described in Section 1.2

3.9.1.1 Pupal production and mean interlarval period - Lisbon 1 colony

Table 3.9.1.1 gives the individual and total pupal production and the individual and overall mean interlarval periods of the first two generations of Lisbon 1. Further reference to these figures will be made in the Discussion (Section 4.7.3).

3.9.2 Pupal production

Table 3.9.5 gives the distribution of pupal production of those flies dying naturally in Generations 1 and 2 of the three main colonies. The table shows that a high proportion of flies in each generation did not produce any pupae at all, while a large proportion of the total number of pupae were produced by a relatively small proportion of the total number of flies. For example, in generation 1 of the Zambesi Colony, while 25 flies produced no pupae at all, 6 flies produced a total of 60 pupae which was nearly half the total required to maintain the size of the colony in the next generation. The point at which the flies had produced twice the number of pupae as there were female flies in that generation, thereby replacing their parents, was taken as the'break-even point.' Any pupae in excess of this will give rise to an increase in the size of the next generation, providing that all pupae will emerge and that there will be a 1:1 sex ratio. Since the eclosion rate is rarely 100%, the break-even point is obviously greater than 2.

This break-even point was examined from two angles:

a/

Table 3.9.5 - Pupal production of flies dying naturally

No. of pupae produced	Singida 37		Zambesi 9		Austeni 9	
	Gen 1 (39 flies)	Gen 2 (37 flies)	Gen 1 (66 flies)	Gen 2 (60 flies)	Gen 1 (26 flies)	Gen 2 (40 flies)
0	14 (35.9%)	7 (18.9%)	25 (37.9%)	32 (53.3%)	8 (30.8%)	13 (32.5%)
1	3 (7.7%)	5 (13.5%)	6 (9.1%)	6 (10.0%)	1 (3.9%)	3 (7.5%)
2	4 (10.3%)	2 (5.4%)	1 (1.5%)	3 (5.0%)	2 (7.7%)	2 (5.0%)
3	4 (10.3%)	4 (10.8%)	3 (4.6%)	3 (5.0%)	0	1 (2.5%)
4	1 (2.6%)	2 (5.4%)	2 (3.0%)	2 (3.3%)	1 (3.9%)	5 (12.5%)
5	2 (5.1%)	2 (5.4%)	7 (10.6%)	2 (3.3%)	1 (3.9%)	3 (7.5%)
6	1 (2.6%)	1 (2.7%)	0	5 (8.3%)	4 (15.4%)	1 (2.5%)
7	2 (5.1%)	1 (2.7%)	1 (1.5%)	1 (1.7%)	1 (3.9%)	2 (5.0%)
8	2 (5.1%)	2 (5.4%)	2 (3.0%)	3 (5.0%)	1 (3.9%)	0
9	1 (2.6%)	4 (10.8%)	4 (6.1%)	0	0	1 (2.5%)
10	0	2 (5.4%)	6 (9.1%)	3 (5.0%)	0	0
11	0	2 (5.4%)	4 (6.1%)	0	0	4 (10.0%)
12	2 (5.1%)	1 (2.7%)	2 (3.0%)	0	0	1 (2.5%)
13	2 (5.1%)	1 (2.7%)	0	0	1 (3.9%)	0
14	0	1 (2.7%)	1 (1.5%)	0	0	2 (5.0%)
15	0	0	1 (1.5%)	0	4 (15.4%)	0
16	0	0	0	0	0	2 (5.0%)
17	1 (2.6%)	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	1 (1.5%)	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	1 (3.9%)	0
22	0	0	0	0	1 (3.9%)	0
23	0	0	0	0	0	0
24	0	0	0	0	0	0
Total pupae produced	147	185	295	130	169	190

a by calculating the proportion of the total number of flies in a generation that would be required to maintain the same size of population in the next generation (i.e. to reach the break-even point of two pupae per female).

Fig. 3.9.6 shows the contribution of each fly in the first generation to the total pupal production of that generation. The pupal production is plotted along the horizontal axis and is grouped in categories. The top histogram in each case shows the percentage of the total pupae produced and the inverted one the percentage of the total number of flies, both plotted against the pupal production category in which they fell. The shaded part of each histogram represents the percentage of flies in pupal category one that produced no pupae at all. The dotted line represents the break-even point in each generation. This was taken as the point at which the lowest producing flies had produced twice the number of pupae as there were female flies in that generation, thereby replacing their parents. Thus, pupae and flies to the left of the dotted line can be regarded as the number of females and pupae that would be necessary to maintain the size of the colony at the same level from one generation to the next. Correspondingly, those to the right of the dotted line can be regarded as the female flies and pupae which are responsible for the increase in the size of the colony from one generation to the next.

For instance, in the case of the Singida colony, the 13% of the flies to the right of the dotted line were responsible for 45% of the total pupal production and these pupae gave rise to an 80% increase in the number/

Fig. 3.9.6

Contribution of each fly in first generation to the total pupal production of that generation

Horizontal axis (A):

Pupal production category	1	2	3	4	5	6	7	8	9	10	11	12
No. of pupae produced by female fly	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18 -19	20 -21	22 - 23

Vertical axis (B): Percentage of total number of pupae produced in each pupal production category

Vertical axis (C): Percentage of total number of female flies in each pupal production category

The shaded portions of the histograms represent the percentage of the total number of flies that were non-productive.

For further explanation - see text.

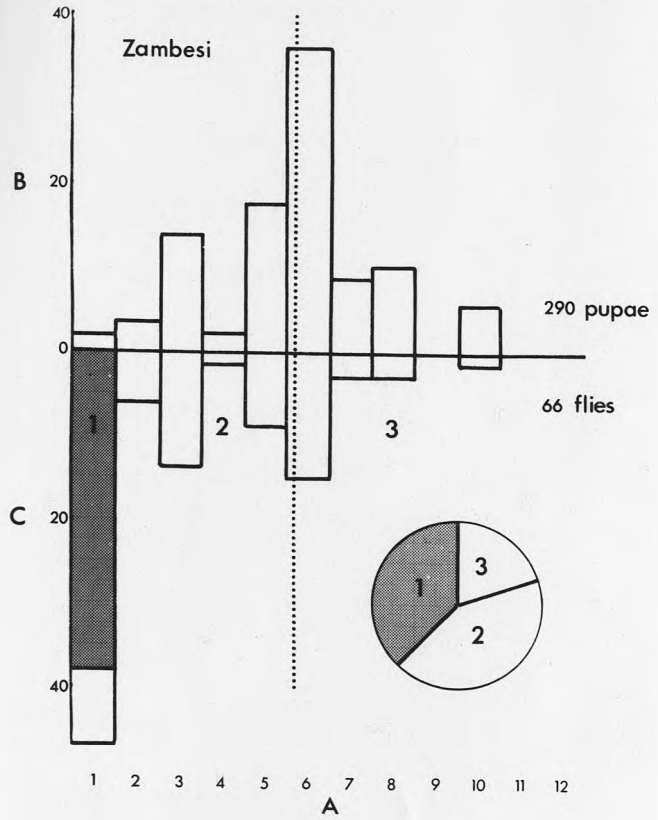
The circle beside each histogram corresponds to the following groups of flies in each figure:

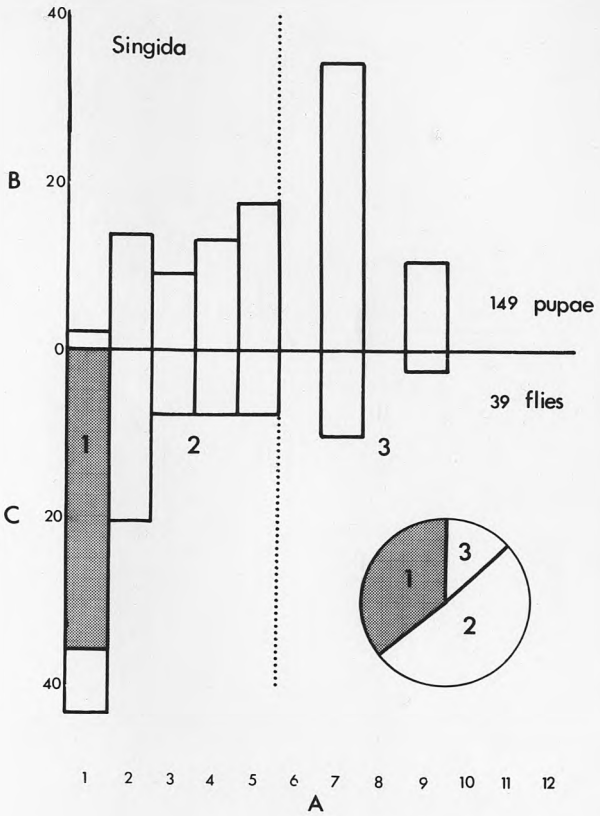
1 Stippled. Proportion of non-productive flies.

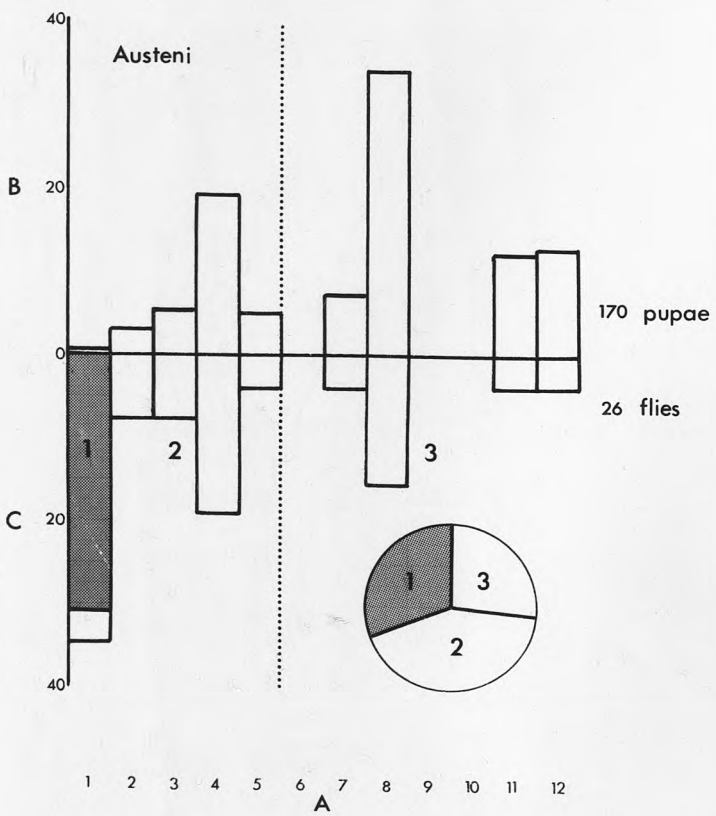
2 The proportion of productive flies required to maintain the size of the colony in the next generation. (i.e. those to the left of the dotted line, excluding non-productive flies).

3 The proportion of the productive flies that gave rise to the increase in size of the next generation. (i.e. those to the right of the dotted line).

The exact percentages in each case are given in Table 3.9.5.


Zambesi
40
B 20
0
C 20
40
1
2
3
290 pupae
66 flies
1 2 3 4 5 6 7 8 9 10 11 12
A


Singida
40
B 20
0
C 20
40
1
2
3
149 pupae
39 flies
1 2 3 4 5 6 7 8 9 10 11 12
A


Austeni
40
B 20
0
C 20
40
1
2
3
170 pupae
26 flies
1 2 3 4 5 6 7 8 9 10 11 12
A

number of female flies in the next generation (See Table 3.13.1. The exact percentages in each case are given in Table 3.9.7.

b The break-even point was also examined by determining the mean number of pupae per productive female in Generation 1 of each colony that were required to maintain the overall size of each colony in Generation 2. From Table 3.9.8 it will be seen that only the fourth and subsequent pupae deposited by a fly contributed to the increase in size of the next generation. The actual break-even point is thus greater than two because non-productive flies have been included in the the calculation, and of course, not all the progeny survived.

If only the flies that produced one or more pupae are considered, the mean number of pupae per fly is considerably increased, and this is also shown in Table 3.9.8. The Austeni colony produced nearly twice as many pupae per productive female as the Singida colony, while the production of the Zambesi colony was midway between them.

3.9.3 Abortions

The distribution of abortions deposited by those flies which died naturally is given in Table 3.9.9. No abortions were recorded from over half the flies in each colony. The highest number of abortions per pupae produced occurred in Generation 2 of Zambesi 9 where there were three abortions for every ten pupae produced. Only one abortion was recorded for every ten pupae in the Austeni colony.

3.9.4 Pupal weight

Only/

Table 3.9.7 - Productivity of flies (Gen.1)

	Singida 37 (39 flies)	Zambesi 9 (66 flies)	Austeni 9 (26 flies)
Non-productive flies	14 (35.9%)	25 (37.9%)	8 (30.8%)
Productive flies required to replace parents	20 (51.3%)	28 (42.4%)	11 (42.3%)
Productive flies to give increase in size of next generation	5 (12.8%)	13 (19.7%)	7 (26.9%)

Table 3.9.8 - Mean number of pupae from productive flies required to maintain the size of the population in the next generation.

	Singida 37 Gen 1	Zambesi 9 Gen 1	Austeni 9 Gen 1
No. of female flies	39	66	26
No. of non-productive flies	14 (35.9%)	25 (37.9%)	8 (30.8%)
No. of productive flies	25 (64.1%)	41 (60.1%)	18 (69.2%)
Total no. of pupae produced	147	295	169
Mean no. of pupae per female	3.8	4.5	6.5
Mean no. of pupae per productive female	5.9	7.2	10.6
Total pupal production required to maintain the size of each colony in next generation	78	132	52
Mean no. of pupae per productive female to maintain size of colony in next generation	3.1	3.2	2.9

Table 3.9.9 - Abortions of flies dying naturally

No. of abortions	Singida 37		Zambesi 9		Austeni 9	
	Gen 1 (39 flies)	Gen 2 (37 flies)	Gen 1 (66 flies)	Gen 2 (60 flies)	Gen 1 (26 flies)	Gen 2 (40 flies)
0	24 (61.5%)	19 (51.4%)	41 (62.1%)	42 (70.0%)	15 (57.7%)	27 (67.5%)
1	5 (12.8%)	13 (35.1%)	12 (18.2%)	7 (11.7%)	6 (23.1%)	9 (22.5%)
2	6 (15.4%)	1 (2.7%)	9 (13.6%)	7 (11.7%)	4 (15.4%)	3 (7.5%)
3	4 (10.3%)	4 (10.8%)	1 (1.5%)	3 (5.0%)	1 (3.9%)	1 (2.5%)
4	0	0	3 (4.6%)	0	0	0
5	0	0	0	0	0	0
6	0	0	0	1 (1.7%)	0	0
Total no. of abortions	29	27	45	36	17	18
Total no. of pupae (from Table 3.9.3)	147	185	295	130	169	190
Abortions : pupae ratio	0.2 : 1	0.15:1	0.15:1	0.3:1	0.1:1	0.1:1

Only the pupal weights of those flies that emerged were considered.

Table 3.9.10 shows the distribution of the pupal weights of female flies in Generations 1 to 3, in the three main colonies. The mean weight is shown at the foot of the table. The pupal weight distribution of Generations 1 and 2 is also shown in Fig. 3.9.11.

Both table and figure show that in all colonies the pupal weight showed a much greater variability in Generation 2 than in Generation 1. For instance, in Generation 1 of the Austeni colony the pupal weight varied between the second and fifth weight categories (17 - 28 mgs.) while in Generation 2 the spread was from the first to the seventh categories (14 - 35 mgs.). There may have been some selection of pupae before they were despatched and this may have influenced the result. Comparisons between the pupal weights of the flies in the first generation and those in subsequent generations are however complicated by loss in weight that occurs during the pupal development (Bursell, 1960: Nash and Kernaghan, 1965). While those pupae that were deposited in the laboratory were weighed within 24 hours of deposition, the pupae received from Africa or other laboratories were weighed at varying times during the pupal period of development.

The greater spread of the weights of pupae in the second and subsequent generations in reflected in the increased standard error about the mean. The depressed weight of Generation 2 pupae was, at least in part, due to the torsion balance being inaccurate for a short period, the recorded weights being 10 - 15% lower than the actual weights.

Table 3.9.10 - Pupal weights of flies that emerged

Weight in mgs.	Singida 37		
	Gen 1 (72 flies)	Gen 2 (145 flies)	Gen 3 (334 flies)
14 - 15	0	3 (2.1%)	1 (0.3%)
16 - 17	0	0	0
18 - 19	0	7 (4.8%)	3 (0.9%)
20 - 21	0	24 (16.6%)	8 (2.4%)
22 - 23	0	13 (9.0%)	21 (6.3%)
24 - 25	2 (2.8%)	19 (13.1%)	34 (10.2%)
26 - 27	12 (16.7%)	28 (19.3%)	46 (13.8%)
28 - 29	21 (29.2%)	23 (15.9%)	76 (22.8%)
30 - 31	20 (27.8%)	16 (11.0%)	50 (15.0%)
32 - 33	13 (18.1%)	5 (3.4%)	49 (14.7%)
34 - 35	4 (5.6%)	6 (4.1%)	22 (6.6%)
36 - 37	0	1 (0.7%)	17 (5.1%)
38 - 39	0	0	6 (1.8%)
40 - 41	0	0	1 (0.3%)
42 - 43	0	0	0
Mean weight ($\pm$ 2 x s.e)	30 $\pm$ 0.5	25.9 $\pm$ 1.4	29.3 $\pm$ 1.4

Table 3.9.10 - Pupal weights of flies that emerged (cont'd)

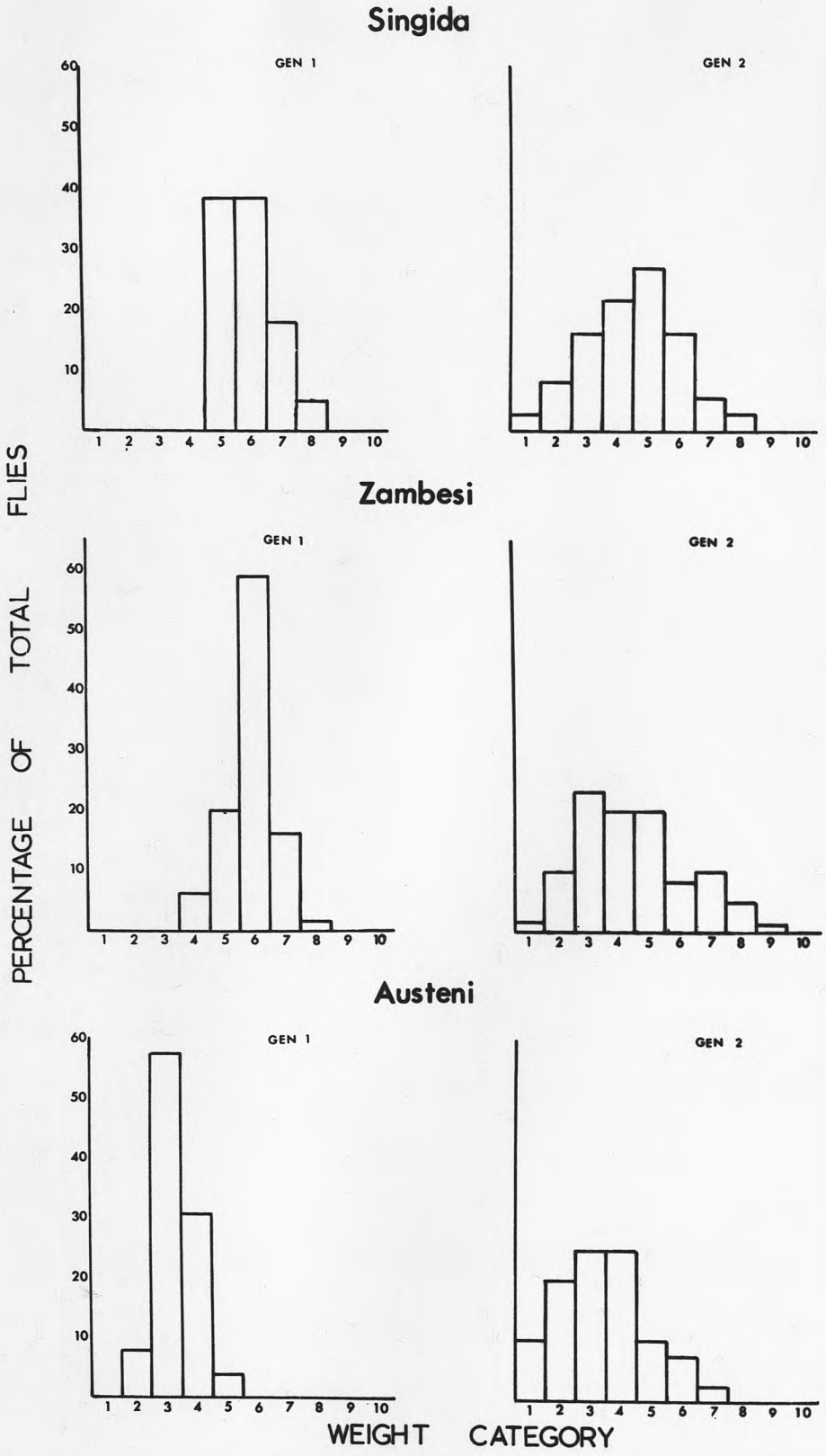
Weight in mgs.	Zambesi 9		
	Gen 1 (147 flies)	Gen 2 (344 flies)	Gen 3 (737 flies)
14 - 15	0	2 (0.6%)	1 (0.1%)
16 - 17	0	10 (2.9%)	1 (0.1%)
18 - 19	0	19 (5.5%)	8 (1.1%)
20 - 21	0	24 (7.0%)	13 (1.8%)
22 - 23	0	58 (16.9%)	46 (6.2%)
24 - 25	8 (5.4%)	50 (14.5%)	90 (12.2%)
26 - 27	25 (17.0%)	50 (14.5%)	117 (15.9%)
28 - 29	54 (36.7%)	39 (11.3%)	123 (16.7%)
30 - 31	46 (31.3%)	33 (9.6%)	132 (17.9%)
32 - 33	11 (7.5%)	34 (10.0%)	101 (13.7%)
34 - 35	3 (2.0%)	14 (4.1%)	64 (8.7%)
36 - 37	0	8 (2.3%)	25 (3.4%)
38 - 39	0	2 (0.6%)	14 (1.9%)
40 - 41	0	1 (0.3%)	2 (0.3%)
42 - 43	0	0	0
Mean weight (± 2 x s.e)	29.9 ± 0.7	25.9 ± 1.6	29.6 ± 1.4

Table 3.9.10 - Pupal weights of flies that emerged (cont'd)

Weight in mgs.	Austeni 9		
	Gen 1 (41 flies)	Gen 2 (131 flies)	Gen 3 (340 flies)
14 - 15	0	5 (3.8%)	8 (2.4%)
16 - 17	0	13 (9.9%)	8 (2.4%)
18 - 19	5 (12.2%)	18 (13.7%)	26 (7.7%)
20 - 21	13 (31.7%)	21 (16.0%)	45 (13.2%)
22 - 23	16 (39.0%)	25 (19.1%)	98 (28.8%)
24 - 25	6 (14.6%)	16 (12.2%)	73 (21.5%)
26 - 27	1 (2.4%)	14 (10.7%)	50 (14.7%)
28 - 29	0	15 (11.5%)	24 (7.1%)
30 - 31	0	3 (2.3%)	7 (2.1%)
32 - 33	0	1 (0.8%)	1 (0.3%)
34 - 35	0	0	0
36 - 37	0	0	0
38 - 39	0	0	0
40 - 41	0	0	0
42 - 43	0	0	0
Mean weight ($\pm$ 2 x s.e)	22.7 $\pm$ 0.6	22.7 $\pm$ 1.3	24.1 $\pm$ 0.7

Fig. 3.9.11 - Percentage of total number of pupae that emerged as female flies in each weight category

Wt. category	1	2	3	4	5
Wt. in mgs.	14.0-16.9	17.0-19.9	20.0-22.9	23.0-25.9	26.0-28.9
Wt. category	6	7	8	9	10
Wt. in mgs.	29.0-31.9	32.0-34.9	35.0-37.9	38.0-40.9	41.0 -42.9


Singida
GEN 1
GEN 2
Zambesi
GEN 1
GEN 2
Austeni
GEN 1
GEN 2
PERCENTAGE OF TOTAL FLIES
WEIGHT CATEGORY

3.9.5 Correlation of pupal weight with pupal production and longevity in the Zambesi colony

Figs. 3.9.12 - 13 show that there is no correlation between the weight as a pupa of a female and either its subsequent longevity or the number of pupae it produced.

3.9.6 Correlation between pupal production and age of fly

The number of pupae deposited by a fly is obviously related to its age. However, in order to investigate whether the rate of pupal production fell off with age, and to see how much it deviated from the theoretical model (Section 1.2) the productivity of flies culled from the colonies was plotted against their age at culling. The results are shown in Figs. 3.9.14 - 16. The broken line in each case represents the theoretical pupal production assuming that the first pupa was deposited at 20 days, and that the pupae were deposited at 10 day intervals thereafter. The best fit for the line through the points was calculated mathematically, by computer.

From the graphs it will be seen that the mean pupal production for each age group was very much closer to the theoretical figure in the case of the Austeni colony than in the other two colonies. The solid line gives the predicted value for the number of pupae that would have been produced by a fly of a given age.

In the case of the Singida and Austeni colonies the number of pupae produced was not affected by the age of the fly, but the best fit for the Zambesi colony was not a straight line, and showed a fall off in/

Fig. 3.9.12 - Zambesi 9 - Correlation of weight as a pupa of each female (mgs.) with its subsequent longevity (days) All generations combined.

ZAMBESI 9

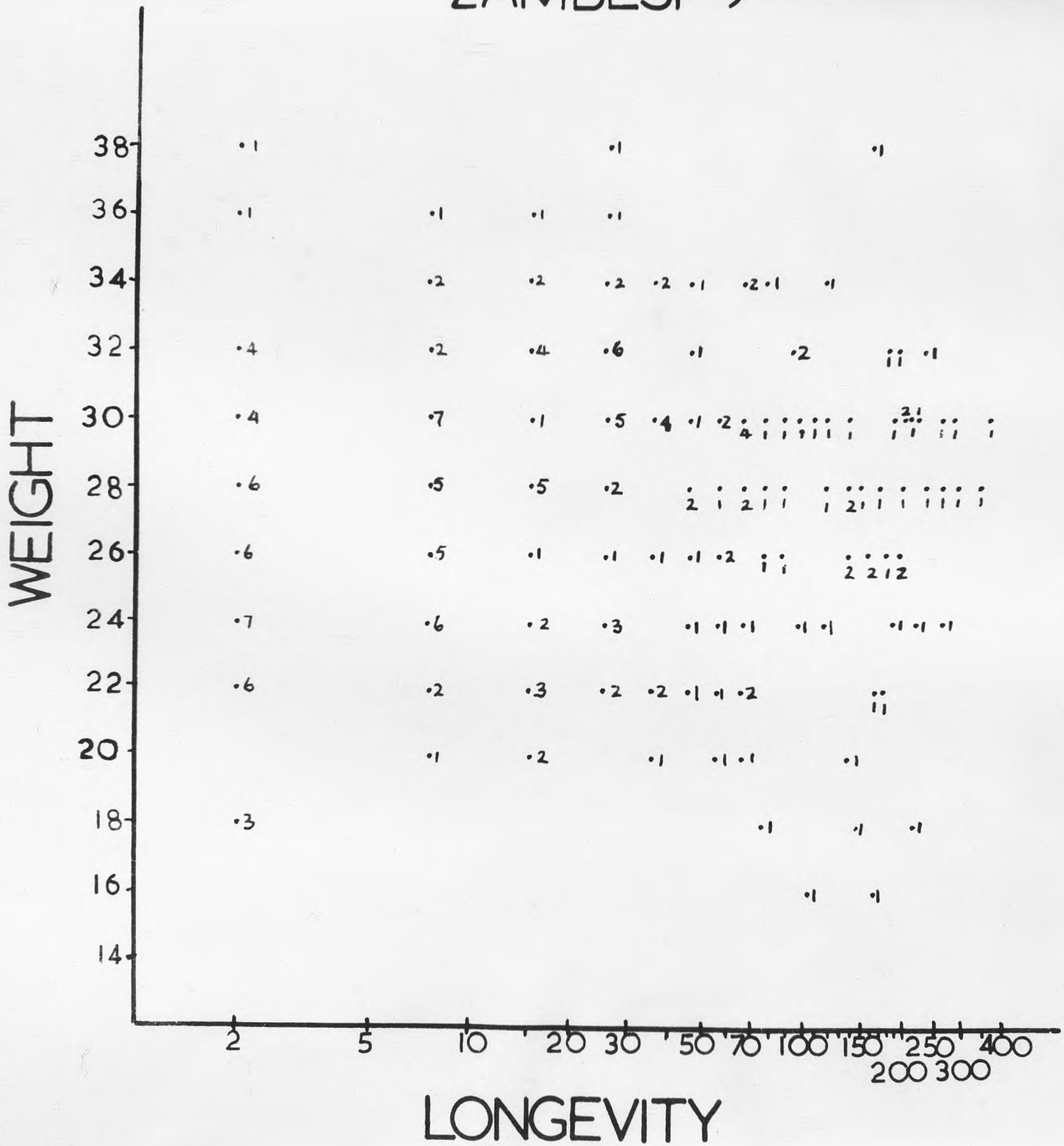

WEIGHT
38
36
34
32
30
28
26
24
22
20
18
16
14
2
5
10
20
30
50
70
100
150
200
250
300
400
LONGEVITY

Fig. 3.9.13 - Zambesi 9 - Correlation of weight as a pupa of each female (mgs.) with the number of pupae it produced. All generations combined.

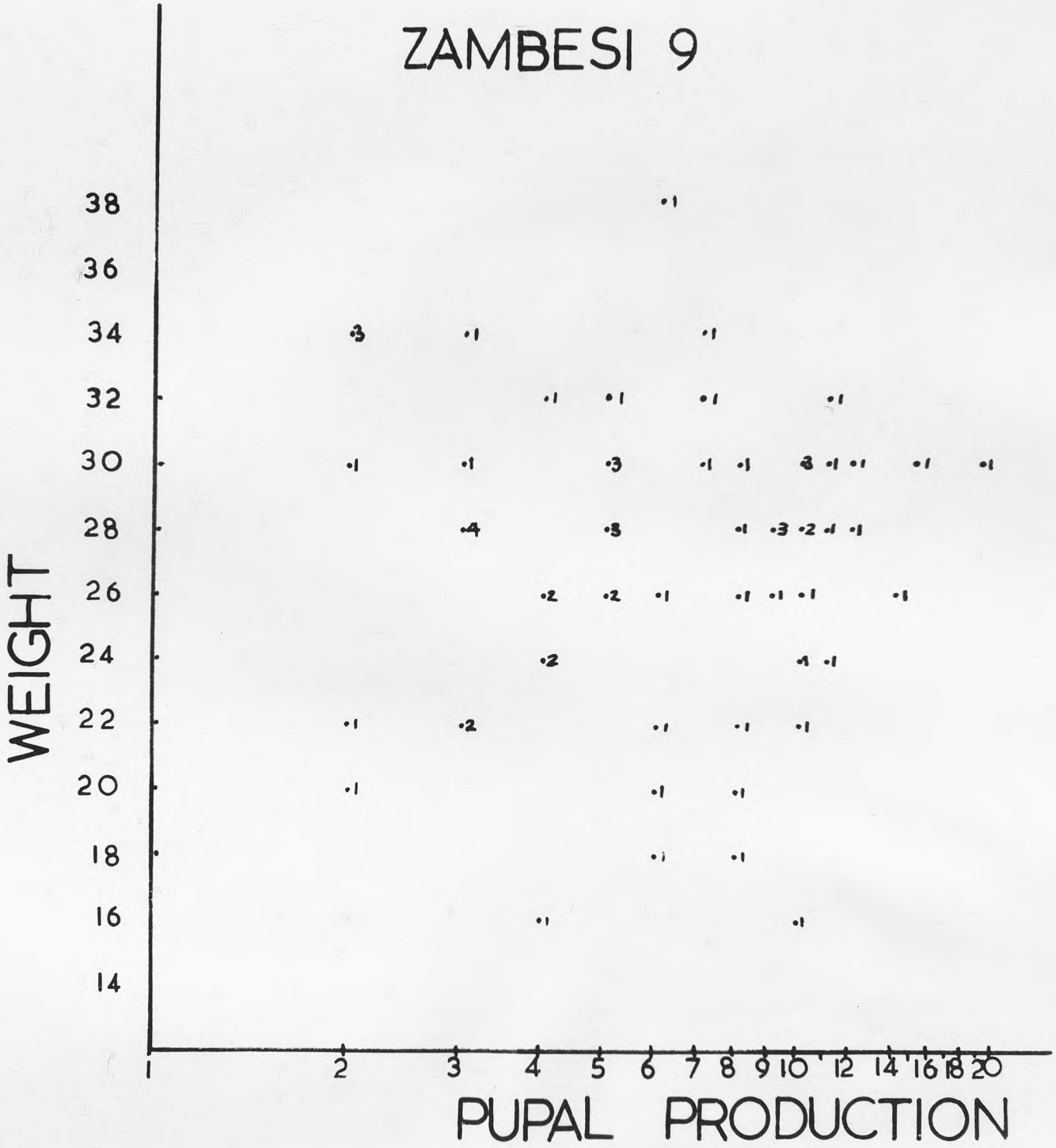

ZAMBESI 9
WEIGHT
38
36
34
32
30
28
26
24
22
20
18
16
14
1
2
3
4
5
6
7
8
9
10
12
14
16
18
20
PUPAL PRODUCTION

Fig. 3.9.14 - Singida 37 - Correlation between pupal production and age of fly

Culled flies only

n = number of flies considered

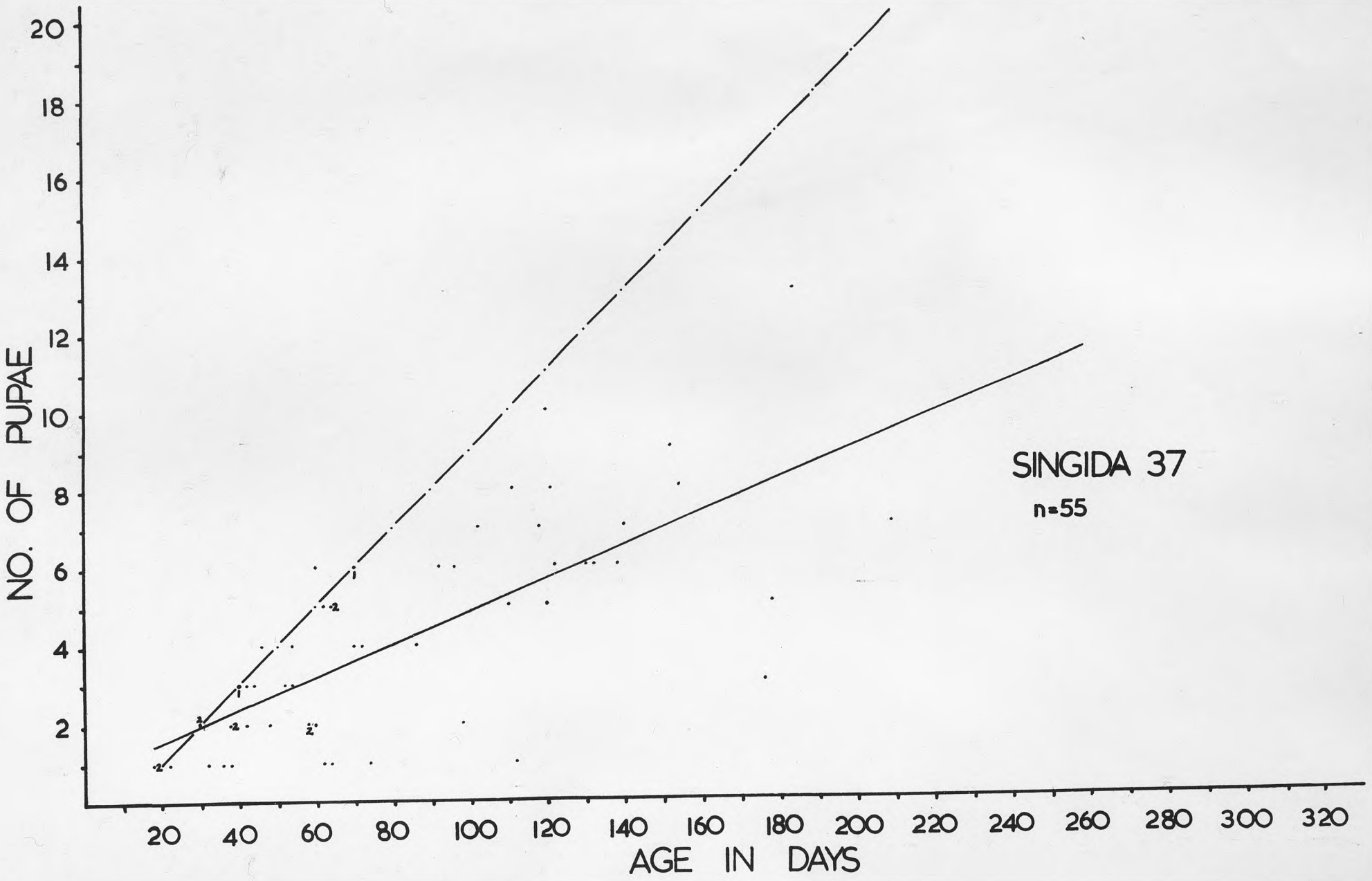

NO. OF PUPAE
20
18
16
14
12
10
8
6
4
2
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
AGE IN DAYS
SINGIDA 37
n=55

Fig. 3.9.15 - Zambesi 9 - Correlation between pupal production and age of fly

Culled flies only

n = number of flies considered

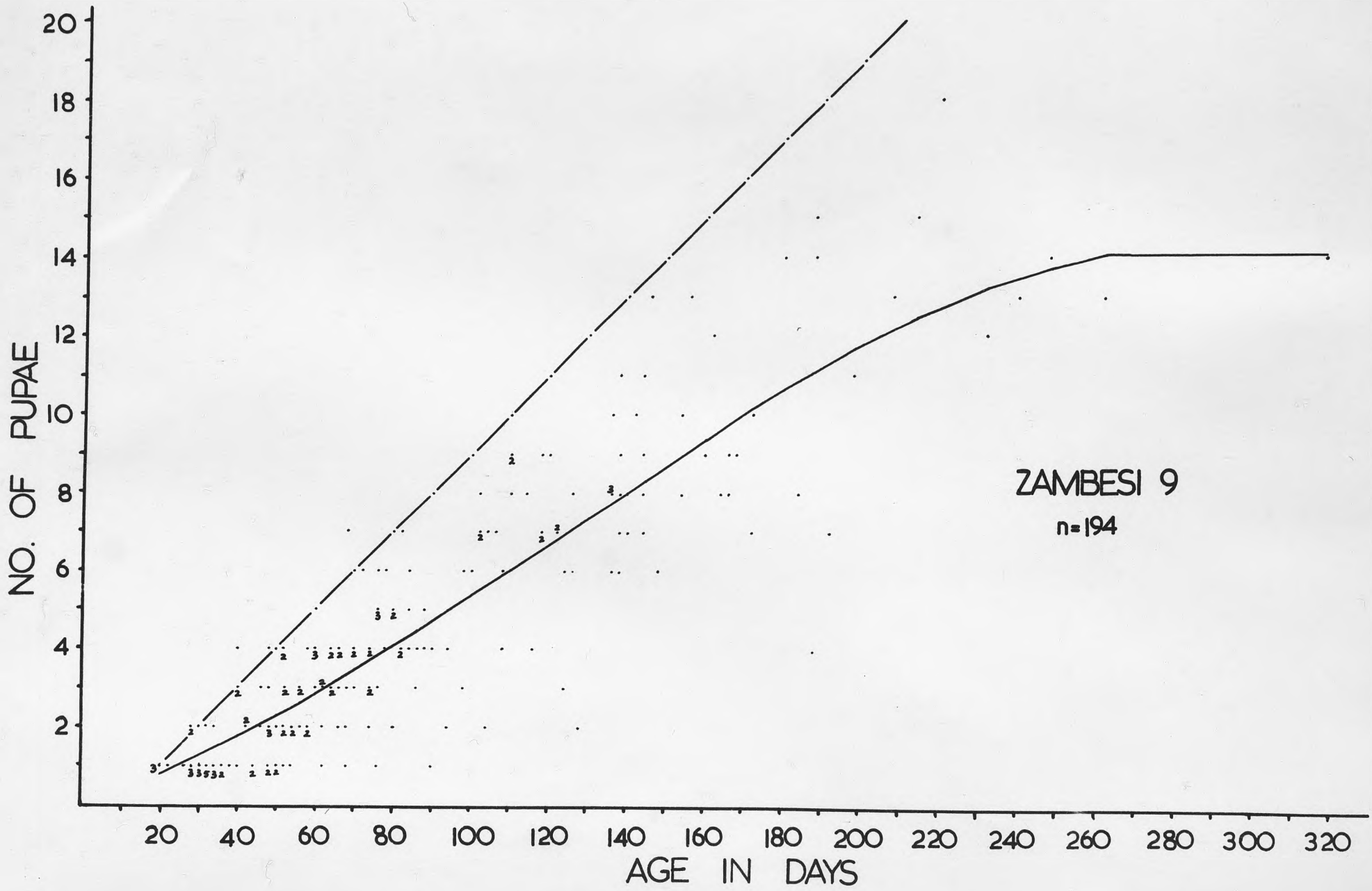
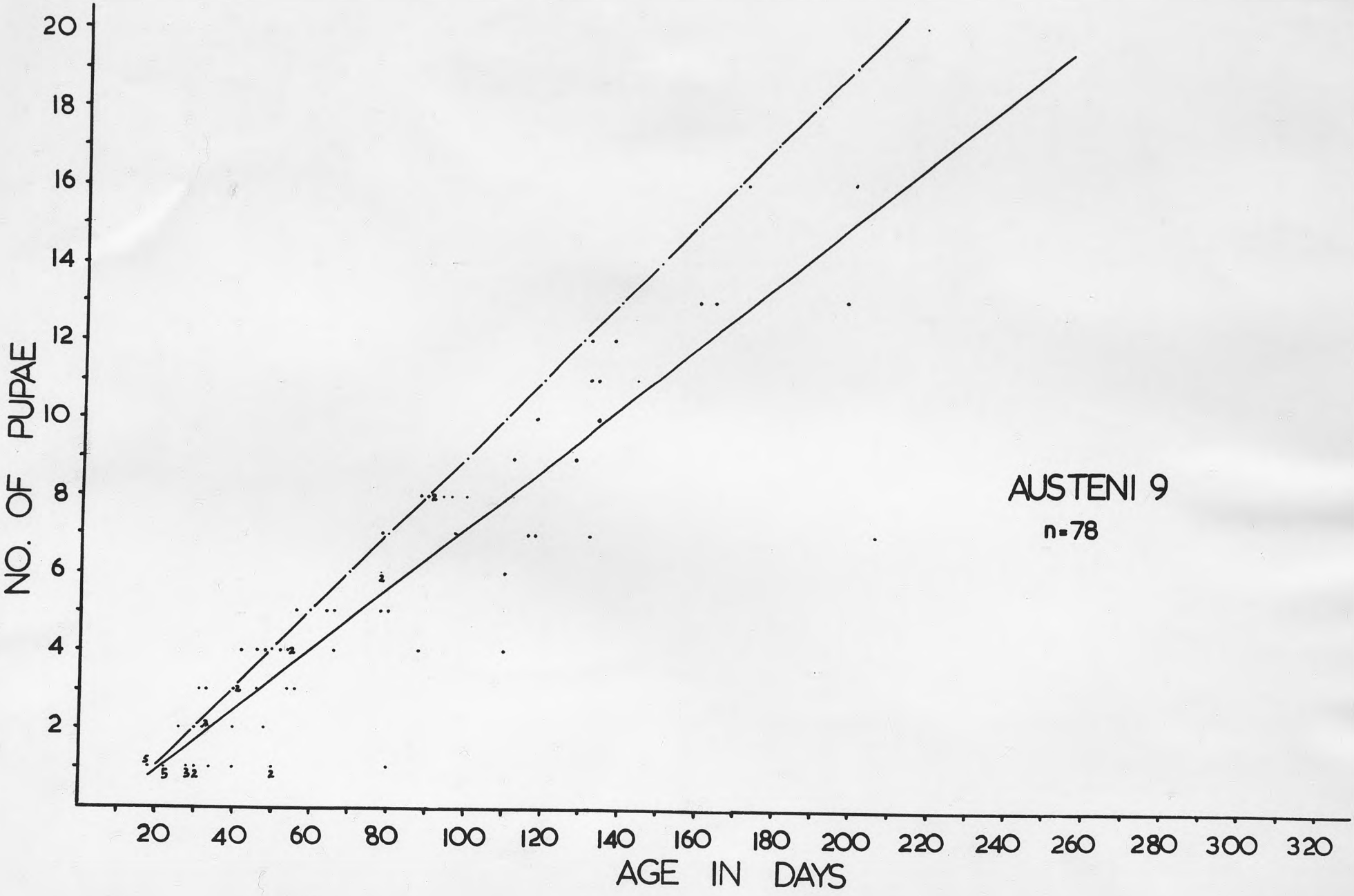

NO. OF PUPAE
20
18
16
14
12
10
8
6
4
2
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
AGE IN DAYS
ZAMBESI 9
n=194

Fig. 3.9.16 - Austeni 9 - Correlation between pupal production and age of fly

Culled flies only

n = number of flies considered


NO. OF PUPAE
20
18
16
14
12
10
8
6
4
2
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
AGE IN DAYS
AUSTENI 9
n=78

in pupal production towards the end of the reproductive life, However, the last part of the curve was based on the performance of only a few flies, and not too much weight should be attached to it.

The number of pupae that a fly could be expected to have produced at a particular age is given in Table 3.9.17. Only the Austeni colony approached the theoretical maximum given in the model in Section 1.2.

Factors affecting succeeding generations

3.10 Generation interval

Column 4 of Tables 3.10.1 to 3.10.5 gives the generation intervals of the colonies considered. The generation interval is defined as the time between the start of successive generations (i.e. between the emergence of the first fly in one generation and the emergence of the first of their progeny). If at 25°C the first reproductive cycle takes 20 days and the pupal development 30 days, then the generation interval for one fly will be about 50 days providing that the first offspring is a female.

Results show that this interval tended to be larger than the theoretical value when the generation was small unless the first female was a regular breeder. An example of this is shown in Generations 5 and 6 of Lisbon 1 (Table 3.10.4) where there was only one reproductive female.

A high proportion of the later generations were culled and this tended to distort the results for the generation interval between these generations/

Table 3.9.7 - Pupal production calculated from slope of graph in Figs. 3.9.14 - 16

	Number of pupae per female by day:			
	58	100	150	200
Singida 37	3.1	4.8	6.9	9.0
Zambesi 9	2.7	5.4	8.7	11.8
Austeni 9	3.9	7.1	11.0	15.0
Theoretical maximum (See Section 1.2)	4.8	9.0	14.0	19.0

Table 3.10.1 - Singida 37 - Generation Intervals

1	2	3	4	5	6	7	8
Generation	Date of eclosion of 1st fly	Date of eclosion of last fly	Generation interval	Interval between 1st & last eclosion (days)	Date of death of last fly	Length of Generation (days)	No. in Generation
1	C 229	C 251	-	22	D 146	282	72
2	C 278	D 157	49	244	(D 196)	(283)	145
3	C 330	(D 196)	52	(231)	(D 196)	(231)	(340)
4	D 028	(D 196)	53	(168)	(D 196)	(168)	(381)
5	D 109	(D 196)	71	(87)	(D 196)	(87)	(120)
6	D 157	(D 186)	48	(29)	(D 196)	(39)	(8)

Mean generation interval - 54.6 days

Note: Figures in brackets indicate that the generation is not complete

Table 3.10.2 - Zambesi 9 - Generation Intervals

1	2	3	4	5	6	7	8
Generation	Date of eclosion of 1st fly	Date of eclosion of last fly	Generation interval	Interval between 1st & last eclosion (days)	Date of death of last fly	Length of Generation (days)	No. in Generation
1	C 126	C 243	-	27	D 195	344	147
2	C 269	D 172	53	268	(D 196)	(292)	344
3	C 319	(D 196)	50	(242)	(D 196)	(242)	(759)
4	D 025	(D 196)	71	(171)	(D 196)	(171)	(718)
5	D 097	(D 196)	72	(101)	(D 196)	(101)	(213)
6	D 190	(D 192)	93	(2)	(D 196)	(6)	(2)

Mean Generation Interval - 67.8 days

Note: Figures in brackets indicate that the generation is not complete

Table 3.10.3 - Austeni 9 - Generation Intervals

1 Generation	2 Date of eclosion of 1st fly	3 Date of eclosion of last fly	4 Generation interval	5 Interval between 1st & last eclosion (days)	6 Date of death of last fly	7 Length of Generation (days)	8 No. in Generation
1	C 236	C 241	-	5	D 149	278	41
2	C 286	D 171	50	250	(D 196)	(275)	132
3	C 337	(D 196)	51	(224)	(D 196)	(224)	(351)
4	D 029	(D 196)	57	(167)	(D 196)	(167)	(439)
5	D 100	(D 196)	67	(96)	(D196)	(96)	(219)
6	D 157	(D 195)	57	(38)	(D 196)	(39)	(31)

Mean generation interval - 56.4 days

Note: Figures in brackets indicate that the generation is not complete

Table 3.10.4 - Lisbon 1 - Generation Intervals

1 Generation	2 Date of eclosion of 1st fly	3 Date of eclosion of last fly	4 Generation interval	5 Interval between 1st & last eclosion (days)	6 Date of death of last fly	7 Length of Generation (days)	8 No. in Generation
1	B 227	B 237	-	10	C 010	138	45
2	B 278	C 019	51	147	C 109	196	27
3	B 330	C 094	52	129	C 128	163	29
4	C 015	C 145	50	130	C 206	191	13
5	C 061	C 190	46	129	C 233	172	10
6	C 201	C 222	140	21	D 069	233	3
7	C 334	D 021	133	52	(D 196)	(227)	4
8	D 058	(D 193)	89	(135)	(D 196)	(138)	(9)
9	D 137	(D 193)	79	(56)	(D 196)	(59)	(2)

Mean generation interval - 80.0 days

Note: Figures in brackets indicate that the generation is not complete

Table 3.10.5 - Singida 24 - Generation Intervals

1 Generation	2 Date of eclosion of 1st fly	3 Date of eclosion of last fly	4 Generation interval	5 Interval between 1st & last eclosion (days)	6 Date of death of last fly	7 Length of Generation (days)	8 No. in Generation
1	B 342	B 356	-	14	C 109	132	50
2	C 027	C 112	50	85	C 133	106	26
3	C 086	C 120	59	34	C 147	61	9
4	C 138	C 152	52	14	C 200	62	2

Mean generation interval - 53.7 days

generations (e.g. between generations 5 and 6 in Zambesi 9(Table 3.10.2).

3.11 Length of generations

This is defined as the number of days from the eclosion of the first fly in a generation to the death of the last. Column 7 of Tables 3.10.1 to 3.10.5 gives the length of each generation. Brackets round the figures indicate that the generations are not yet complete. The length of the generation depends on the longevity of the flies in that generation together with the longevity and pupal production of the females of the previous generation. In the three main colonies (Tables 3.10.1 to 3.10.3) the length of the only complete generation (Generation 1) exceeded 250 days in each case and in Zambesi 9 was nearly 350 days. As the theoretical generation interval is only about 50 days, each succeeding generation will last considerably longer than the previous one, thereby causing an increasing overlap.

3.12 Population increase

The number of females in each generation at the time of the analysis is shown in Table 3.12.1. As there were very few flies alive in Generation 1 of the three main colonies (See Table 3.14.1) there was unlikely to be any further increase in the size of Generation 2. However, Generation 3 *et seq.* were still expanding in size at the time of the analysis and in order to indicate the size of successive generations assuming the same rate of increase that occurred between Generations 1 and 2, Table 3.12.2 have been constructed. This shows the theoretical increase in the number of females in Generations 3 and 4.

The size of Generation 3 *et seq.* were distorted by the number of flies/

Table 3.12.1 - Increase in size of generations (females only)

Generation	Singida 37		Zambesi 9		Austeni 9		Lisbon 1		Singida 24	
	No in gen	% increase	No in gen	% increase	No in gen	% increase	No in gen	% increase	No in gen	% increase
1	41	-	72	-	26	-	29	-	23	-
2	75	83	179	149	72	176	15	-48	16	-30
3	(170)	127	(374)	109	(167)	132	19	34	6	-62
4	(184)	8	(331)	-11	(212)	27	9	-53	1	-83
5	(51)	-	-	-	(99)	-	6	-33	Died	out
6	(5)	-	-	-	(12)	-	3	-50		
7	-	-	-	-	-	-	1	-67		
8	-	-	-	-	-	-	(4)	300		

Note: the figures in brackets indicate that the generation is not complete

Table 3.12.2 - Theoretical increase in population size of generations 3 and 4 as calculated from the percentage increase between generations 1 and 2 (females only)

	Generation	Singida 37		Zambesi 9		Austeni 9	
Actual size of Generations 1 and 2	1	41	% increase = 83%	72	% increase = 149%	26	% increase = 176%
	2	75		179		72	
Theoretical size of Generations 3 and 4	3	137		446		199	
	4	251		844		549	

flies culled (See Section 3.13), but as Generation 3 was still expanding at the time of the analysis, it is likely that the percentage increase between Generation 2 and 3 would have been greater than that which occurred between Generation 1 and 2.

With those flies that were fed on guinea-pigs (e.g. Singida 24 and the early generations of Lisbon 1 - Table 3.12.1) there was a steady decrease in the size of successive generations, and Singida 24 died out completely by the 5th generation, and Lisbon 1 almost did so. With the advent of the use of rabbits' ears, the latter colony began to increase in size in the 8th Generation.

Fig. 3.12.3 shows the population increase of the 3 main colonies in graphical form. Only the first 4 generations are shown, but from Table 3.12.1 it will be seen that generation 6 had already begun by the time the results were analysed, while generation 1 had only just been completed.

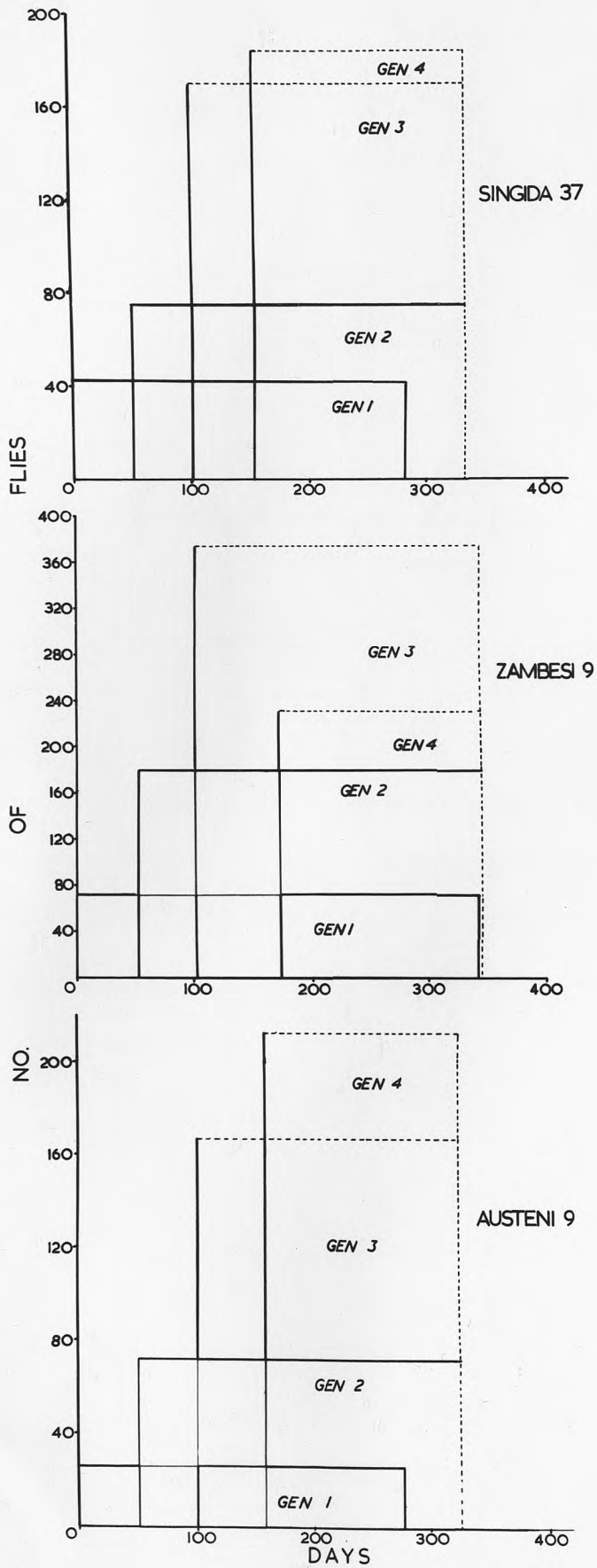
From Table 3.12.1 it will be seen that the rate of increase between Generations 1 and 2 was greater in the Austeni colony than in the two G. morsitans colonies. The subsequent rates of increase are difficult to interpret because of the number of flies culled.

3.13 Numbers of flies culled

In order to keep the size of the colonies within manageable proportions the colonies had to be drastically culled. This was done by removing complete lines of females, i.e. those originating from a single female parent/

Fig. 3.12.3 - Population Increase

The height and width of each histogram represents the size and the beginning and end respectively, of each generation at the time of the analysis. Dotted lines are shown where the generation is still increasing in size, or has not yet been completed.


200
160
120
80
40
0
100
200
300
400
FLIES
GEN 4
GEN 3
GEN 2
GEN 1
SINGIDA 37
400
360
320
280
240
200
160
120
80
40
0
100
200
300
400
OF
GEN 3
GEN 4
GEN 2
GEN 1
ZAMBESI 9
NO.
200
160
120
80
40
0
100
200
300
400
DAYS
GEN 4
GEN 3
GEN 2
GEN 1
AUSTENI 9

parent in generation 1. Table 3.13.1 gives the proportion of flies in each generation that had been culled at the time of the analysis of the data. All males surplus to requirements were culled, and this accounts for the higher proportion of culled males than females. The table shows than an increasing proportion of each generation was culled. As the size of the Austeni 9 colony was originally small, a lower percentage of flies were culled from the early generations of this colony.

Table 3.13.2 shows the distribution of the age at which flies were culled. If the flies that were culled on emergence are ignored, the distribution of age at culling was approximately normal.

3.14 Number of flies alive at time of analysis

This is given in Table 3.14.1. While there were no flies alive in Generation 1, there was still a considerable proportion of Generation 2 flies still alive. The largest number of flies still alive was in Generation 4, and Generations 5 and 6 were just beginning.

Table 3.13.1 - Number of flies culled per generation

Generation		Singida 37		Zambesi 9		Austeni 9	
		Males	Females	Males	Females	Males	Females
1	No in gen	31	41	75	72	15	26
	No culled	10 (32.3%)	1 (2.4%)	31 (41.3%)	2 (2.8%)	9 (60.0%)	0
2	No in gen	70	75	165	179	60	72
	No culled	30 (42.9%)	11 (14.7%)	92 (55.8%)	72 (40.2%)	28 (46.7%)	16 (22.2%)
3	No in gen	165	170	363	374	173	167
	No culled	112 (67.9%)	58 (34.1%)	236 (65.0%)	184 (49.2%)	108 (62.4%)	57 (34.1%)
4	No in gen	175	184	294	231	196	212
	No culled	123 (70.3%)	99 (53.8%)	207 (70.4%)	187 (81.0%)	138 (70.4%)	103 (48.6%)
5	No in gen	47	51	87	69	91	99
	No culled	34 (72.3%)	40 (78.4%)	64 (73.6%)	57 (82.6%)	61 (67.0%)	54 (54.5%)
6	No in gen	3	5	0	0	9	12
	No culled	2 (66.7%)	5 (100 %)			8 (88.9%)	12 (100 %)
Total no of flies		491	526	984	1025	544	588
Total no culled		311 (63.3%)	214 (40.7%)	630 (64.0%)	502 (49.0%)	352 (64.7%)	242 (41.2%)

Note: Generations 2 - 6 were not complete at the time of the analysis

Table 3.13.2 - Distribution of age of female flies at culling

Age at culling (days)	Zambesi 9 No of flies culled				Singida 37 No of flies culled				Austeni 9 No of flies culled			
	Gen 1	Gen 2	Gen 3	Gen 4	Gen 1	Gen 2	Gen 3	Gen 4	Gen 1	Gen 2	Gen 3	Gen 4
0 - 4		5	63	106			26	62		1	26	59
5 - 9		0	7	13			5	3		1	2	10
10 - 19		0	10	17			4	8		0	0	11
20 - 39		6	15	20	1	2	3	7		2	6	9
40 - 59		8	26	11		2	4	8		0	4	7
60 - 79		7	22	14		0	4	6		1	5	2
80 - 99		5	12	4		0	1	3		2	4	2
100 - 119	1	8	9	0		2	3	2		2	3	1
120 - 139		11	9	1		2	5			2	3	2
140 - 159		3	7	1		0	2			1	1	
160 - 179		8	1			1	1			3	0	
180 - 199		5	1			1				0	2	
200 - 219		3	0			1				1	1	
220 - 239		1	1									
240 - 259		1										
260 - 279		1										
280 - 299												
300 - 319	1											
320 - 339												
Total	2	72	183	187	1	11	58	99	0	16	57	103
Mean age at culling (days)	216	111	45	16	22	110	38	17	-	104	47	15

Table 3.14.1 - Flies alive at time of analysis

Source	Gen	Males			Females		
		No in gen	No alive	% of total alive	No in gen	No alive	% of total alive
Singida 37	1	31	0	-	41	0	-
	2	70	18	9%	75	27	8%
	3	165	54	26%	170	108	35%
	4	175	97	46%	184	132	43%
	5	47	38	18%	51	40	13%
	6	3	3	1%	5	3	1%
Totals		491	210		526	310	
Zambesi 9	1	75	0	-	72	0	-
	2	165	33	8%	179	52	10%
	3	363	139	36%	374	198	39%
	4	294	156	40%	331	216	43%
	5	87	62	16%	69	43	8%
	6	0	0	-	0	0	-
Totals		984	390		1025	509	
Austeni 9	1	15	0	-	26	0	-
	2	60	15	8%	72	19	6%
	3	173	48	25%	167	81	27%
	4	196	76	39%	212	123	40%
	5	91	46	23%	99	73	24%
	6	9	9	5%	12	9	3%
Totals		544	194		588	305	

4. Discussion

4.1.1 Controlled environment fly room

Little has been published on the design of insectaries. Bertram and Gordon (1939) gave an account of the adaptation of a small room into an insectary, and the design of both Azevedo's and Nash's constant temperature rooms were examined during visits to these establishments. However, for reasons of cost, the design of the building to be adapted, and the climate of the country concerned, these were of little help in the design of the constant temperature room described in this thesis.

The temperature and humidity requirements were similar to those in the Lisbon fly-room, as was the number of air changes per hour. It was the latter requirement which caused much of the difficulty in the design of the apparatus, but it was considered important in order to keep down smells in the room which might excite the flies and also to improve the working conditions.

The final design for the fly room (Section 2.1) proved excellent, once certain initial difficulties had been solved. The first of these was a "layering" of warm and cool air in the room due to inadequate air speed from the vents in the ducting. This was eliminated by increasing the fan speed and removing the ducting. The second problem which proved to be more difficult to eliminate was the deposition of large quantities of dust which adhered to all shiny surfaces in the room. At first this was thought to be due to the oxidation of the galvanised iron ducting, but was eventually traced to the water supply which although containing only 30 parts per million of dissolved solids, proved/

proved to be enough to cause the trouble.

A further problem was the abundant growth of algae, fungi and bacteria in the humidifier itself. Both these problems were eventually rectified by the installation of a bacterial filter and a deioniser in the water supply to the humidifier.

The intake of air from the outside ensured a slight positive pressure in the room which effectively reduced any fluctuation in temperature when the door was opened. The conditions were further improved by the provision of an additional room next door to the fly room which acted as a buffer against the entry of cold air.

4.1.2 Temperature

Although fluctuations of temperature within the tolerated range are obviously not harmful to Glossina, for experimental purposes it is essential to maintain as constant a temperature as possible. The limiting factor at the lower end of the temperature range is the lowered rate of pupal production, and also the lowered stimulus to feed (McDonald, 1960).

At the upper end of the temperature range, the limiting factor is the reduced longevity, and although larval development can continue (MellanbyH, 1937) a continuous high temperature above 29°C appears to be lethal for embryonic development (Roubaud and Colas-Belcour, 1936; Mellanby H, 1937). It is possible that pupae can stand a higher developmental temperature than adult flies, for Bursell (1960c) found/

found that the upper limit for the successful development of G. morsitans pupae was 32°C; although he considered that the optimum temperature was 22 - 24°C, for at this temperature the emerging adults had maximum fat reserves.

Thus in the laboratory where a constant temperature is being employed, it appears that in order to speed up the life-cycle, a higher pupal than adult fly temperature could be maintained. However, when pupae and flies have to be kept in the same room, this is not possible, and in this laboratory a temperature of 25±0.5°C was chosen as being midway between most of the temperatures used previously (See Section 1.4.3) and close to the observed temperatures in Lisbon.

4.1.3 Water relations

It would seem unlikely that water balance in adult flies would ever become a critical factor because in well fed individuals the elimination of excess water gained in the fluid diet is the major problem. The adult fly is able to control the rate of water loss by regulation of the spiracles, by primary excretion and by regulating the water content of the faeces (Bursell, 1960a). Even in teneral flies the fat reserves are likely to become exhausted before the flies become desiccated (Bursell, 1959).

Although no fat estimations were done on flies that died, the probability is that the majority of those thad died in a starving condition (Table 3.8.1, Category A), had exhausted their fat reserves. It was observed that those teneral flies that did not feed, either made no attempt, even/

even though apparently starving, or were unable to pierce the skin, presumably due to some weakness in the proboscis. Older flies which died in a starving condition often probed repeatedly, but were unable to withdraw any blood. This may have indicated a blockage of the proboscis or oesophagus.

The water relations of tsetse pupae are far more critical, and while some species such as G. morsitans are able to complete their development at relative humidities as low as 10%, in others such as G. brevipalpis it may be as high as 80%. There is also a close correlation between resistance to desiccation of the pupae of different species and the habitat in which they occur. Thus in the field, water balance may be a limiting factor in the invasion of tsetse in marginal areas (Bursell, 1958).

In the laboratory, the pupal environment is very important, but when Azevedo and Pinhao's technique (Section 1.4.4) of burying the pupae in damp sand was followed in preliminary experiments here, it was found that within a week the sand had dried out leaving a crust on top. It was also observed that a high proportion of the emerging flies were crippled. Thereafter the pupae were buried in dry sand and, apart from G. austeni, the number of crippled flies was negligible (Table 3.5.1). Presumably, the relatively high proportion of crippled G. austeni is associated with an unsuitable pupal environment since in nature their normal habitat is more humid than G. morsitans. This would also explain the higher proportion of non-emerged pupae in the Austeni colonly (Table 3.3.1) particularly as more G. austeni pupae died late in development than in the other two colonies (Table 3.4.1). This may indicate/

indicate that the developing imagines which died late in development did so due to desiccation, as the rate of water loss in the last few days of pupal life is very high (Bursell, 1958). Similarly, the emerging flies require body fluids to expand the cuticle (Wigglesworth, 1954) and those that were crippled may have been unable to do so because of insufficient haemolymph. However, Nash and Kernaghan (1965) found that the percentage of non-viable emergents (including crippled flies) was not affected by any of the media in which the pupae were placed. Nash and Jordan (1969) however did report a high proportion of crippled flies due to overcrowding of the pupae and consequent mutual disturbance of the flies when they emerged. In this laboratory the pupae were kept singly in tubes, so overcrowding could not have been the factor involved.

The emergence rate of the G. austeni pupae that were deposited in this laboratory (Table 3.2.3) is disappointing when compared with the 96.4% obtained by Nash and Kernaghan (1965a) who buried pupae in dry sand suspended over wet sand. The results are not explained fully by the difference in the method of maintenance of the pupae, as the emergence rates of the batches of G. austeni pupae received from Bristol (Fig. 3.2.2) were as good or better than those obtained by Nash and Kernaghan (1965a). These pupae spent some half to three-quarters of their development in dry sand suspended over wet sand, and after being unpacked and weighed in this laboratory spent the remainder of the period in individual tubes as described in Section 2.3.1. The stage at which these non-emerging pupae died is not known. However, in all other aspects such as weight (Section 3.9.4) the pupae appeared to be similar, so it can only be surmised that the effects of the methods of pupal

maintenance/

maintenance used in Bristol were sufficient to overcome the effects of the journey and the period spent in less favourable conditions in this laboratory.

The emergence rate of the pupae deposited by Generation 2 flies (Table 3.3.1) were slightly depressed because a few pupae were still developing at the time of the analysis. However, the pupal mortality of the two G. morsitans colonies compared favourably with the 6.6% mortality obtained by McDonald (1960) from pupae which were buried in dry sand suspended over wet sand. Deaths that occurred early in development may have been due to trauma caused when the pupae were handled immediately after deposition. For instance Nash and Kernaghan (1965a) obtained a pupal mortality of only 1 (0.37%) out of 270 pupae that were allowed to pupate in dry sand and then left undisturbed for 9 - 16 days before weighing and reburyings.

4.2 Hosts

Although Azevedo and Pinhao (1964, 1968) and Itard and Maillot (1966) successfully used guinea-pigs as hosts for feeding G. morsitans, previous workers including Roubaud (1915, 1917) Geigy (1948), Willett (1953) and Foster (1957) had little success using this species.

Attempts in this laboratory to repeat Azevedo's success with guinea-pigs failed, and the colonies maintained on this host died out before reaching the fifth generation (Table 3.10.5).

The problem of providing the right conditions together with a suitable host to stimulate the fly to probe and obtain sufficient blood during the

the short period each day when it is is offered is of considerable importance. Three separate but related factors may be considered:

4.2.1 The blood type

There is little evidence that the blood of a particular species of animal is more attractive than another, or that a monotonous diet of blood from one species of animal has any detrimental effect on the fly. For instance, Langley (1966a) found that when laboratory-reared males were fed on six different mammals, including ox, the rate of digestion, as reflected by the rate of excretion did not differ significantly from one to another. He did however find that males caught in the field after feeding on a bait oxen digested their meals significantly faster than any flies that were fed on guinea-pigs in the laboratory. This showed firstly that the blood type did not affect the rate of digestion of a blood meal, and secondly that the conditions provided in the laboratory were not as ideal as those in the field. Langley suggested that this was due to pre-feeding behavioural differences which led to a metabolic uncoupling in the laboratory-fed flies, but the rapid digestion that occurs in flies kept in small cages and fed on rabbits'ears, together with high pupal production and excellent longevity (Nash et al 1967b) tends to contradict this theory.

4.2.2 Fly cage design, and application of cage to host

Putting an animal inside the fly cage has not proved successful, (Mellanby and Mellanby, 1937), so alternatively the fly cage has to be applied to the animal. As the fly has therefore to feed through the mesh of the cage, it has to be able to obtain sufficient purchase

to get through the flattened hair and to pierce the skin of the host. In addition, if the cage moves relative to the host, the fly will be forced to remove its proboscis, and to reprobe. When guinea-pigs were used the flanks had to be shaved, and it proved difficult to prevent the animals' movement. With lop-eared rabbits, however, the ears could more easily be prevented from moving and shaving was never necessary because the hair on the ear was so sparce.

It was decided at the start of the project to keep the flies singly in cages partly because of the suggestion by Azevedo and Pinhao (1964) that this might have been a factor in the success of their colony, but also because it would allow the accurate recording of the life history of individual flies.

Originally, it was intended to use the "Geigy cage" described by Roubaud (1917) and Geigy (1948) and used by Azevedo and Pinhao (1964), Itard and Maillot (1966) and, with modifications, by Nash et al (1966b, c, 1967b) and Azevedo and Pinhao (1968). However, because of the cost of making this type of cage an alternative was looked for, and the 'tin cage' was developed.

The advantage of this design lay in its cheapness, and the ease with which it was made from the original tin. It was easy to handle, and the netting was automatically tightened over the open end when the rim was forced on. No loose ends of netting were left inside the cage to entangle the fly, which was a common fault in the Geigy cage (Pinhao, personal communication). Furthermore, two cages fitted into the/

the feeding rack along each flank of the guinea-pig as opposed to only one Geigy cage. The tin cage was easily cleaned, and the netting quickly replaced should this become necessary. In addition, the record card for each fly could be attached to cage with an elastic band, allowing easy recognition of the fly, together with details of its life history.

With the changeover from guinea-pigs to rabbits ears for feeding, a smaller cage became necessary. Both Nash et al (1967b) and Azevedo and Pinhao (1968) used smaller versions of the Geigy cage, but once again these were very expensive to make, and in addition, both suffered from three further disadvantages. Firstly, it was a tedious process transferring the flies from one cage to another. Secondly, only a small label attached by a piece of string could be used, and thirdly the application of the cages to the ear was difficult.

The design of the polystyrene cage overcame these disadvantages and was very cheap and easy to make. From the feeding point of view it is desirable to have a shape of cage such that when the fly is offered food, it can never be so far away from the host that the scent and temperature gradient does not attract it towards the host. Nash et al (1966b) suggested that their new "Geigy 25" (an enlarged Geigy cage) was superior to their P.N.M. cage because (1) a greater surface area was in contact with the host; (2) a fly at its furthest point from the host was only half the distance away (2 inches), and (3) that the open structure of the Geigy cage allowed the formation of a well/

well defined scent gradient which facilitated the location of the food source.

Any size of polystyrene cage could be used, and the size decided upon was a compromise. On the one hand, the size was dictated by what was available at the time, the need to fit as many cages as possible onto a rabbit's ear for feeding, and a reasonable depth for ease of manipulating the flies into and out of the cage. On the other hand, in Nash's opinion, 64 cc. (4 cu. ins) is the minimum volume that should be allowed for each fly (Nash et al 1966c). Also the cage should be shallow so that the fly is never too far away from the host's skin, and the maximum surface area is in contact with it.

With the size of the cage used, the fly was never more than 5 cm. away from the host when offered food, and the area in contact with the rabbits ear was 11.0 $cm.^2$ This compared with 3.8 cm. and 19 $cm.^2$ respectively, in the case of Nash's small Geigy cage. Thus, measured from these two points of view, Nash's cage was marginally superior. The polystyrene cage was also slightly smaller (55.2 cc.) than the minimum volume of 64 cc. recommended by Nash et al (1966c), and it had solid sides which the same authors (1967b) considered to be a disadvantage. Nevertheless, the feeding of the flies kept in the polystyrene cages was satisfactory, as was the pupal weights of the pupae produced (see below). It would therefore seem that cage requirements are less rigorous than Nash et al supposed, and that furthermore, the polystyrene cage used here had significant advantages in the handling of the cages, manipulation and feeding of the flies as/

as well as the recording of the data in the card attached to the cage.

4.2.3 The availability of the blood source

Having found the host, the fly must be stimulated to probe the skin and feed as rapidly as possible. Nash (1955) reported that if goats were used too frequently, the flanks became dry, scaly and oedematous, and the flies became reluctant to feed. The use of fresh goats promptly resulted in most of the flies feeding. Nash et al (1965) overcame the problem by slowly increasing the number of flies applied each day, and also by only using the goats twice weekly.

In this laboratory, both the guinea-pigs and rabbits were normally used every third day, although in the case of the latter, occasionally they had to be used every second day owing to a shortage of rabbits. However, no sensitisation was observed in either the guinea-pigs or rabbits following the feeding of the flies, and this agrees with the observations of Gordon and Crewe (1948). Some bruising of the rabbits ears was occasionally observed, but this seldom persisted for more than a few days.

The blood supply to the guinea-pig flank and the rabbit's ear are however very different. The latter is used by the rabbit as a means of losing heat, and is highly vascularised. The skin temperature is therefore higher than that of the guinea-pig flank, thus steepening the temperature gradient between the top of the cage and the feeding surface. A high skin temperature may also encourage the fly to probe more readily.

Having/

Having probed, the fly has a much greater chance of finding a blood capillary in the rabbit's ear than the guinea-pig flank, and it was regularly observed that a fly had completed feeding within a minute of the cage being applied to the rabbit's ear.

There is no doubt that much of the success in establishing a closed colony of Glossina in this laboratory was due to the adoption of the rabbit ear technique. The flies were able to locate the skin, probe and feed far more rapidly than was thecase with guinea-pigs.

4.3 Mating

The mating of tsetse in the laboratory is of critical importance, and not enough attention was paid to it in this project for some time. Several factors have to be taken into consideration:

4.3.1 Age of male and female at mating

Nash (1963) considered that for each species of Glossina it was essential to know the age when the female is most willing to mate, and the age at which the male becomes fully potent. Squire (1951) considered that in the wild mating takes place with G. palpalis within a day or two of emergence, and this was confirmed by Saunders (1962) who found that G. fuscipes (previously named G. palpalis fuscipes) were nearly always mated before they took their first blood meal. On the other hand, G. pallidipes females were mated during or after the first blood meal.

In the wild, mating is extremely successful and for instance Vanderplank (1947)/

(1947) found that of over 10,000 wild G, swynnertoni and 2,000 wild G. pallidipes dissected, all non-teneral and one third of the teneral females had been inseminated. Even in populations of extremely low density, Teesdale(1940) found no evidence to suggest that the insemination rate of G. palpalis was any lower than in more densely populated areas.

Thus in the wild, the method by which males meet and mate with young females is extremely efficient. Swynnerton (1936) stated that this was done by means of a ' following swarm' of males. Bursell (1961) suggested that such males, which had abundant fat reserves and which followed moving objects were in an 'sexually appetitive' phase.

The results from laboratory experiments are more difficult to interpret. Mellanby K. (1936) found that G. fuscipes males were not potent before they were six days old and only 20 - 40% of the females were fertilised when they were less than 4 days old. This rose to 75 - 90% of the females when they were 5 - 8 days old. He found that females older than 8 days became less attractive to the males. This is not consistant with the field observation described above, which showed that practically all females of G. fuscipes were inseminated when seeking their first blood meal. Furthermore, Nash (1955) found that with G. palpalis, fertilisation was greatest when the females were three days old, but that two day old females were difficult to fertilise. He found that as a group, 1 - 5 day old females were more readily fertilised than those 6 - 10 days old.

The results of Southon and Cockings (1963b) with G. morsitans were

less/

less conclusive so far as the age range of the females was concerned, as only those 1 - 4 days old were considered. However, the degree of insemination showed a progressive increase with the age of the female.

The age of the males used for mating also appears to be important. Mellanby K, (1936) found that no males of G. fuscipes under five days old fertilised any females, but all those six days and older were fully potent. It would appear that the position is not quite so clear cut as this for Nash (1955) found that a small percentage of males of G. palpalis were fertile shortly after emergence. However, he found that older males were much more aggressive than males under seven days old, and that the latter were often knocked off the females by the older males.

Southon and Cockings (loc cit) found that with G. morsitans, ten and fifteen day old males gave a significant increase in the insemination percentage over five day old males, and in some cases a 100% rate of insemination occurred in females only one day old when mated with five or ten day old males.

Mellanby and Mellanby (1937) considered that a female tsetse was mated only once during her life time, and Mellanby K (1936) found that the spermathecae were still full of living sperm 200 days after fertilisation. When Nash (1955) attempted to mate females aged from 65 - 123 days old with 7 day old males he obtained variable results with a maximum of 20% in which copulation took place.

From/

From the above work, it will be seen that most authorities agree that older (6 - 15 day old) males should be mated with younger (1 - 4 day old) females, and this procedure was followed by Itard and Maillot (1966) with G. morsitans and G. tachinoides and Nash et al. (1966c) with G. austeni. A similar method was used in this laboratory and males were at least seven days old when used for mating with 2 - 4 day old females. Pairing took place immediately in nearly all cases. Where the male appeared to be reluctant to mate, it was always replaced by another (see below).

4.3.2 Nutritional state of male and female

Mellanby K. (1936) and Hoffmann (1954) found that in the laboratory G. fuscipes females were more attractive after they had fed, and Nash et al (1966c) did not mate females of G. austeni unless they had fed on the second or third day. Less attention appears to have been given to the nutritional state of the male, but it was found in this laboratory that recently fed males found it physically difficult to mount the female and engage the claspers around the tip of the abdomen. On the other hand, starving or weak males were sometimes reluctant to mate. It was often noticed, however, that the sexual drive to mate with a female quickly overcame hunger and, although vigorously probing one moment, as soon as a female was introduced into its cage it stopped and immediately mounted the female. The female often had no such inhibitions however, and if hungry and given the opportunity, it would attempt and often succeed in feeding whilst being mated. It was noticed too that the female was far more receptive when fully engorged than when hungry, and/

and a female that had been placed with a male in the same cage for twelve hours or more sometimes allowed the male to re-mount after it had engorged, whereas before feeding it had refused.

4.3.3. Duration of mating period

Mellanby K (1936) stated that copulation in tsetse lasted from half an hour to two hours, and those that separated after a few minutes were not fertilised. Hoffmann (1954) found that copulation in G. palpalis seldom lasted more than four hours, the flies usually separating after two or three hours, and Nash (1955) after finding that mating in G. palpalis was complete in 24 hours separated the flies after 24 hours instead of 48 hours. Nash et al (1966c) used a similar procedure with G. austeni and achieved a 95% insemination rate. Other workers have however left the flies together for longer periods: for instance Azevedo and Pinhao (1964) left the male and female together for 2 - 3 days while Itard and Maillot (1966) separated them after 3 - 4 days. In view of the varying states of receptivity of the female, and in spite of Nash's excellent results, it would seem wiser to leave the flies together for a period in excess of 24 hours so that the female is given an opportunity to feed with the male in the cage, so that remating could take place if the feamle again became receptive.

In this laboratory, for a greater proportion of the period under review, the flies were generally separated after 24 hours and this may have been one of the factors affecting the number of females that died without producing a pupa (see below). When the female did feed with the/

the male still in the cage it was often observed that remating did take place.

4.3.4 Mating procedure

Flies can be mated singly or in a group. If mated singly there is always a chance that the male although potent might not be fully fertile and, even if copulation has taken place, the female might remain uninseminated. However, with this method, the amount of disturbance is reduced and it is possible to make sure that both male and female are in a satisfactory nutritive state, and that copulation is achieved in each case.

Nash et al (1966c) used this method, and after being mated singly in a glass tube, the females were held together in a 'mass' cage. Their excellent results are commented upon above. On the other hand, the females may be mated in bulk (i.e. in a mass cage) by introducing an appropriate number of males into a mass cage. With this method there is the possibility that the least attractive females will remain uninseminated, and that disturbance may cause the flies to separate before insemination has been completed. However, provided the flies are left together long enough, any females that have not been satisfactorily inseminated will remain receptive and will be remated by another male.

For this reason Nash (1955) used a ratio of males to females of either 2:1 or 57:43 although it is not clear why these particular ratios were chosen. Southon and Cockings (1963b) found that the ratio of males and females of G. morsitans was critical, and obtained best results when/

when the ratio was 2:1 in favour of the males. These results conflicted with those of McDonald (1960) who, using G. morsitans, in a 1:1 ratio and separating the flies after less than 24 hours achieved a 97% insemination rate.

A further safeguard against any females remaining uninseminated would be to leave one or more males in the cage after separation. Nash (1955) added five young males to each cage of 20 females after separation but was not able to determine the effect of the procedure. Nash et al (1966a) used a similar method with G. austeni but left only one male per cage of 25 females.

4.3.5 Number of times a male may be used

It is not known how soon a male having mated is ready to mate again (Glasgow, 1963) or whether there is any regeneration of sperm in the testes. Glasgow (loc cit) assumed that a male mated every sixth day, and assuming a Poisson distribution for the number of times the male copulated with a female, he calculated that a mean life of only nine days would be enough to ensure the fertilisation of every female. Dame (1968) found that in multiple mating experiments, G. morsitans females mated with more than one male, but although males mated several times, they tended to become infertile after the fourth or fifth mating.

In summary, it appears from results published so far that so long as the male and female are of a suitable age and physiological state, the/

the method of mating does not influence the degree of success. However, great care must be taken as the requirements for different species undoubtedly vary. For instance, G. pallidipes has proved difficult to mate in the laboratory (Willett, 1953; Cockings, 1959). Odhiambo (1968) found that no matings were observed with this species in flies less than six days old, and that the mean age of females was 14.5 days. However, the interpretation of his results is difficult as the number of flies used was small and full details were not given.

No direct check was made in this laboratory of the insemination percentage achieved, but it seems likely from the number of non-productive flies (see Tables 3.6.1 and 3.6.2) that the percentage insemination was lower than that achieved by other workers (see above).

For instance, in the Austeni colony 31% and 32% of the females in the 1st and 2nd generations produced no pupae at all (Table 3.9.3). If the flies that died before reaching the reproductive age (taken as 20 days) are subtracted from these figures (see Table 3.7.1) it will be seen that of the total number of females in each generation, 19% and 17% respectively, died when over 20 days having produced no pupae at all. This was not due to the pair failing to copulate for all the cages were checked shortly after completing the mating routine (Section 2.3.2.3) and any females that had not paired were mated with a fresh male.

An analysis of the performance of 2nd generation G. austeni males

(Table/

(Table 3.6.2) shows that it is unlikely that it is the sterility of the male that is at fault. Similarly, with second generation G. austeni females (Table 3.6.1) no discernible pattern emerges, and the age of the male at mating did not seem to affect the results. It is possible that the flies were separated too soon and that if they had been left longer, re-pairing might have taken place after the female had taken another blood meal. However, in view of the results of Nash et al (1966c) there is little evidence to support this. Nash and Kernaghan (1965b) did report that a high proportion of females dying in the first 14 days after mating were uninseminated and they suggested that this was due to those females with a poor life expectancy being unattractive to the males, but at the time the paper was written, they had not succeeded in establishing a closed colony and their results were poor compared with those achieved later (Jordan et al, 1967).

It is possible that the apparent sterility of some females may not have been due to their not being inseminated but due to some other factor such as poor nutrition and undetected abortion.

4.4 Data recording and analysis

When, before this project began, the author spent some time working in Azevedo's laboratory in Lisbon, it was apparent that the accurate recording of the results from a colony of tsetse was an extremely important but complex process. The analysis by Pinhao (1966) of the first 20 generations of Azevedo and Pinhao's colony of G. morsitans in/

in Lisbon proved to be a mammoth task (Pinhao, personal communication) even though the colony was small and had not by then started to expand rapidly (see Table 4.4.1).

Little detailed analysis of colonies of tsetse had been attempted before, and the system devised had to be both simple to operate and as flexible as possible so that it could be adapted to take in any circumstance that might arise.

The system finally decided upon was based on the recording of the various events in the life history of a fly on one of a number of daily registers, which were then used to set up, and update where necessary the individual fly records which were to be held on magnetic tape. It was hoped that in this way the problem of the permanent storage of, and easy access to, large amounts of data would be overcome.

Because of the possibility that Azevedo and Pinhao's success might have been due to some genetic adaptation of their flies to the laboratory as suggested by Willett (personal communication) it was also decided to link flies of the same generation and family together so that any such adaptation could be examined.

The University acquired its own computer shortly after this project began, and the development of the programs to record and analyse the data was dependent to a considerable extent on the development of the software for the computer. Of the drawbacks to the system the most important/

Table 4.4.1 G. morsitans Data for 1st 20 generations extracted from Pinhao (1966)

Gen	Gen length	No females	Mean female longvt (days)	% females dead at 22 days	% productive females	Mean no pupae per female	Mean no pupae per reproductive female (P.R.F.)	Max no pupae per female	Mean time to first larviposition	Mean ILP
1	205	22	87.4	18.1	77.0	3.7	4.8	10	17.4	12.4
2	280	44	70.5	22.5	58.1	3.4	5.2	14	17.8	10.9
3	253	58	60.0	28.8	57.1	1.9	3.5	10	20.8	13.1
4	308	51	53.9	38.0	53.2	2.4	4.4	11	17.8	10.7
5	258	53	42.5	41.5	61.5	1.7	2.8	10	20.5	13.8
6	356	30	49.7	43.4	50.0	1.6	3.1	9	18.3	11.2
7	379	17	41.5	56.3	43.8	2.4	5.6	10	20.6	10.6
8	260	14	77.1	35.7	64.3	5.2	8.1	17	17.2	10.2
9	281	34	68.1	24.3	54.5	2.6	4.8	12	16.4	10.9
10	256	35	44.7	58.8	23.5	1.8	7.6	20	15.3	10.0
11	372	24	60.8	41.6	37.5	1.9	5.1	11	15.7	10.6
12	316	22	65.1	38.2	38.1	2.4	6.3	15	19.5	8.9
13	305	20	80.8	15.0	45.0	2.5	5.4	13	19.9	10.9
14	300	23	56.1	26.1	69.6	2.6	3.8	15	18.5	9.6
15	479	26	39.7	57.8	42.3	2.0	4.3	14	21.4	9.4
16	561	23	62.2	47.8	47.8	4.0	8.3	14	17.0	9.6
17	616	33	66.4	31.3	64.5	3.6	6.1	13	16.9	11.0
18	706	50	54.1	40.4	57.1	2.2	3.9	14	17.6	11.4
19	690	44	79.0	27.9	62.8	4.8	7.7	15	19.0	9.9
20	768	83	98.8	17.1	80.7	5.6	6.9	15	17.6	10.4

important proved to be the relatively small size of the computer which restricted the amount of data that could be processed at any one time. Also, because magnetic tape and not disc was used to store the data, much of the computing time was spent in winding the tape backwards and forwards in order to gain access to the records, as opposed to the extremely fast speed at which the actual computing was done.

The potential rate of increase of a colony once it began to expand was not fully appreciated at the beginning of the project. The task of feeding the flies proved to be the most difficult, and the size of the population was limited by the number that could be fed each day by the staff available. This necessitated the culling of large numbers of flies which in turn affected the interpretation of the results. It is hoped that by development of techniques for analysing the results that these difficulties can to a large extent be overcome, but these were not available at the time this thesis was written.

4.5 Eclosion rate / Pupal mortality

This has been discussed fully in Section 4.1.3.

4.6 Longevity / Causes of death

4.6.1 Introduction

As the innate capacity for increase in *Glossina* may be less than that of some small mammals such as *Microtus agrestis* or *Rattus natalensis* (see/

(see Glasgow, 1963), the longevity of a population is of fundamental importance. A female must live a minimum of 30 days before it can produce two pupae to replace itself and the male with which it was mated. In many previous attempts to establish a colony, poor longevity was one of the primary causes of failure. For instance, Foster (1957) found that 67% of the third generation of his G. morsitans colony had died before reaching 31 days old. Willett (1953) found that the mean longevity of female G morsitans fed on guinea-pigs decreased from 57 days in the second generation to 39 days in the fourth generation. The mean longevity obtained by Pinhao (1966) varied from 39.7 days in generation 15 to 98.8 days in generation 20 (see Table 4.4.1), but the number of flies in each generation were small, and the poor longevity of a few flies adversely affected the mean.

Nash et al (1958) considered that when the daily mortality amongst the fertilised female stock was below 2%, the population was doing well, and if it rose above this figure for more than a day or two, something was radically wrong.

4.6.2 Male longevity

It is presumed that females live considerably longer than males under natural conditions, and this is usually put down to the males' greater activity and their suffering heavier losses by enemies (Foster 1957). The same holds true in the laboratory, and for instance both Foster (loc cit) and Willett (1953) found that females lived considerably longer/

longer than males. Foster found that cause of death in males was often due to feeding difficulties, and they often died after refusing food for ten to twenty days. A similar picture was also observed in this laboratory, and 85% to 100% of the males in the three main colonies died of starvation (Table 3.9.1).

So long as the males in a colony live long enough to fertilise the females, the longevity of the male is irrelevant, and as the number of males in the colonies were kept to the minimum, no figures for the male longevity have been given.

4.6.3 Female longevity

The causes of female mortality are more various, and may be related to starvation caused by feeding or other difficulties, or reproductive complications. The causes of death in the female population may conveniently be divided into those that died before reaching 20 days old, and those that died over 20 days old, by which time they should have produced their first larva.

4.6.4 Early mortality

It is not known whether the proportion of fat in the newly deposited pupa varies to any great extent under different conditions but from indirect evidence it seems likely that it will vary according to the nutritional state of the female parent, and to a lesser extent on the amount of energy expended by the larva crawling around before pupating. Buxton and Lewis (1934) considered fat to be the main food/

food reserve of tsetse pupae and in addition substantial amounts of water are produced by the oxidation of fat (Bursell 1958). Bursell found that at 25° ± 0.8°C and 98% R.H. the fat content fell from 31.9% shortly after deposition to 18.2% at 24 days old, when measured as the percentage of the total dry weight. When the pupae were kept at 0% R.H. the fat content fell to 14.6% indicating that more fat had been oxidised to produce metabolic water.

It seems likely that the weakness of newly emerged laboratory-reared flies that was a feature when guinea-pigs were used as hosts in this laboratory may have been due to poor fat reserves on eclosion.
A similar problem was encountered by Foster (1957) who found difficulty in persuading newly emerged flies to feed, and which led to a high mortality amongst young flies. In the first and second generations 25% of the flies died in the first 10 days of life, while in the third generation this figure rose to 50%. In the three main colonies studied in this project, mortality in the first nine days of life varied in the first generation from 3.9% in the Austeni colony to 10.6% in the Zambesi colony (Table 3.7.1).

The mortality in the first nine days apparently rose in the second generation, but the figures were greatly distorted by the number of flies still alive (Table 3.15.1) and the number culled (Table 3.14.2). This is particularly so in the case of the Zambesi colony, when if both culled and living flies are taken into account the female mortality in the first nine days falls from 30% to 9.8%.

When/

When the colonies were maintained on rabbits' ears, it was often found that the newly emerged flies would not feed on the day of emergence even though they may have emerged up to 24 hours earlier, and in these cases the abdomens appeared rounded and full of fat.

The number of flies that were very weak on emergence was small in the rabbit fed colonies when compared with those fed on guinea-pigs and they occurred most often amongst very small flies. Azevedo and Pinhao (1968) found that in those flies that died early, death occurred after a period of some days during which the flies did not feed spontaneously and they regarded this as being due to a congenital debility. A similar picture was observed in this laboratory and the flies were often unable to probe the skin to feed and died after a few days.

A further cause of death in young flies which was not recorded separately, was the escape of ingested blood (nearly always at the first blood meal) into the haemocoel, due to a rupture of some part of the alimentary tract. This caused the thorax and legs of the fly to turn pink and death normally followed within a day or two. These flies never appeared to be engorged, and can be separated from the small proportion of young flies that died in an engorged condition. The cause of death of the latter group was not certain, but was also noted by Nash et al (1966b), and may have been due in some instances to a blockage of the alimentary tract. These flies looked perfectly normal however, and could easily be distinguished from the older flies that died in an engorged condition (see below).

The/

The degree of crippling of flies on emergence varied from complete non-expansion to deformity of only one or both wings. In the case of the totally non-expanded flies, these were obviously unable to feed, but even those flies which appeared normal except for some crippling of the wings were often also unable to feed and they rarely lived more than a day or two. Crippling was a major cause of early mortality in the Austeni colony but occurred much less often in the other colonies (Table 3.4.2).

It was noted in this laboratory that the rate of digestion in infertile flies slowed down after about the 15th day of life, and that after this time they began to feed much less often and build up large fat reserves. It is possible that the poor digestion of these flies may have lowered their longevity. Nash and Kernaghan (1965b) recorded that a high proportion of the G. austeni females that died early were uninseminated and they suggested that this may have been due to the weaker flies being less attractive to the males. In view of the experience in this laboratory, it is possible to interpret their results the other way round: for whatever reason the flies were uninseminated their digestion nevertheless became impaired, and this may have lowered the longevity of these flies.

The comparison of the rate of early death with, for instance, the results given by Jordan et al (1967) is made more difficult by the fact that they measured longevity from after mating, and not from the/

the date of eclosion. However, Nash et al (1969b) stated that 10.9% of the newly emerged flies failed to develop their wings during this period, and as this was not taken into account in the paper by Jordan et al (1967) the survival curve which they drew gave a somewhat false picture of the early mortality in their population. When the 6.2% mortality calculated from the survival curve of Jordan et al is added to the 10.9% mortality due to crippling, this gives an effective mortality of 17.1% for the first 20 days of adult life. This compares with approximately 14.3% calculated from the data given by Jordan and Curtis (1968) assuming a similar pupal mortality. These figures compare favourably with the 11.7% mortality in the first 19 days of Generation 1 of the Austeni colony, and 7.8% and 16.7% in the Singida and Zambesi colonies respectively.

While the conditions under which G. austeni were kept were sufficiently similar to those of Nash, Jordan and Curtis quoted above, to allow direct comparison, there have been no published results from G. morsitans populations maintained on rabbits' ears. The colonies described by Itard and Maillot (1966) and Pinhao (1966) were maintained on guinea-pigs and were only slowly increasing. While the mean longevity of these colonies was shorter than that obtained with rabbits in this laboratory, the mortality of Itard and Maillot's two colonies of G. morsitans in the first 20 days was between 15% and 25%, a figure only slightly greater than the results obtained here.

The early mortality in the first 20 generations of the colony described/

described by Pinhao (1966) increased from 18.1% in the first generation to 58.8% in the tenth. Thereafter the mortality figures for the first 22 days varied greatly from 15% in the thirteenth to 57.8% in the fifteenth generation. Unfortunately, no comparable figures are given by Azevedo and Pinhao (1964) for the same colony in the rapidly expanding phase which started shortly after the period described by Pinhao (1966).

The first 20 days are the most critical period in the adult fly's life. During this period it has to face the hazards of eclosion, obtaining its first blood meal, mating successfully and the nourishment, growth and deposition of its first larva. The prevention of a high mortality during this period has proved to be one of the most difficult problems to overcome.

4.6.5 Late mortality

The causes of death after the first twenty days of life are more varied and less certain. Two broad groupings may be made: (a) those concerned with feeding and digestion, and (b) reproductive disorders.

(a) Feeding and digestive disorders

From 72% to 88% or all females in the three main colonies (Table 3.8.1) died in a starving condition. It was noted that as a general rule, as the females got older they tended to feed much less readily and often than when they were younger. Failure to feed when the fly was apparently hungry was sometimes due to a reluctance to probe, or more often, having probed, the inability to/

to inbibe any blood. The latter was possibly due to a blockage in the mouthparts or oesophagus, presumably due to clotted blood, but this was not investigated. It was often not possible to be certain of the cause of death of those flies that died in the intermediate B and C categories (Table 3.8.1) but a significant proportion of females died in an apparently engorged condition (Category D). Nash et al (1966b) considered that a high proportion of flies dying in an engorged condition was indicative of a good nutritional state amongst the population. The proportion of flies dying in a gorged condition was similar, at 16%, in the two G. morsitans colonies, but was only 11% in the Austeni colony.

(b) Reproductive disorders

Although this was a much less common cause of death, a significant number of females in the Austeni colony died with a larva that had pupated in utero, as described by Robinson (1964). Nast et al (1967b) found that about 5% of their G. austeni colony that were fed on rabbits died in this state, and considered that it may have been caused by the fly feeding shortly before larviposition was due to take place, so pinning the larva in the uterus. This condition was much less common in the two G. morsitans colonies kept in this laboratory.

A comparison of the survival curve in the paper by Jordan et al (1967) with that of Generation 1 of the Austeni colony in this laboratory reveals that while only 4% of the Bristol colony died between 20 and

80/

80 days old, 38% of the Austeni colony in this laboratory died. Thereafter the proportion of flies dying in the latter group was less. The maximum longevity was similar, with the last flies dying at about 280 days old. Although the numbers were small, the mortality of the flies in the 20 to 80 day age group was thus disappointing compared with the results of the Bristol population. The reason for this could not be ascertained.

The survival curves of the Zambesi and Singida colonies were superior to those published for G. morsitans by Itard and Maillot (1966) using guinea-pigs as hosts, and this was mainly due to a much lower maximum longevity in the latter colonies.

The mean longevities of the 1st generation of all three colonies exceeded 100 days which although less than the 139 days recorded by Jordan et al (1967) for G. austeni is far superior to that obtained by Itard and Maillot (1966) and Pinhao (1966) (see Table 4.4.1).

The mean longevity of the second generation was depressed for reasons already given above, but if both culled and living flies were to be included, the longevity would be similar to that of the 1st generation.

4.7 Age specific fecundity

4.7.1 Introduction

In section 1.2 a hypothetical model was given of the potential rate of increase in a population of Glossina, and the actual rate of increase/

increase achieved will be discussed in relation to the model. Glasgow (1963) gave figures based on data from Nash (1955) for the innate capacity for the increase in numbers in tsetse based on a general model quoted by Andrewatha and Birch (1954), but Jordan and Curtis (1968) showed that the rate of increase of a population of G. austeni was higher.

Unfortunately, at the time that this thesis was written, computer programs to convert the data to a format that could make use of Glasgow's model were not available. The other serious difficulty in a critical analysis of the data was the small number of flies in each generation and the fact that only the 1st generation was complete at the time of analysis.

The number of pupae produced by a female is a function of longevity, the number of days taken to produce the 1st larva, and the interlarval periods thereafter. Longevity has already been discussed (section 4.6) and each of the other factors concerned are reviewed below.

4.7.2 Time to 1st pupa

In G. morsitans maintained at 26°C the first mature egg is ovulated on the eighth or ninth day of imaginal life, Saunders (1960b), so at 25°C this time will be slightly longer. With flies maintained at the latter temperature, providing the females are mated before the ovulation of the first egg, and that no abortions occur, the first larva should be deposited by about the 20th day. Pinhao (1966) recorded/

recorded mean times of between 15.3 and 20.8 days for the 1st twenty generations of G. morsitans (see Table 4.4.1), but his figures must be interpreted with caution as some of the recorded periods for the deposition of the first larva are as low as six days which is clearly impossible. Roubaud (1917) who maintained his colony of G. morsitans at 25 - 27°C gave the dates of eclosion and successive larvipositions of 15 females over a period of three years. The time to first larviposition in 13 of these flies varied between 15 and 35 days with a mean of 20.5 days. A similar pattern occurred in the two G. morsitans colonies maintained in this laboratory, and the number of days before the deposition of the first larva was extremely variable (Table 3.9.3 and fig. 3.9.4).

When flies are kept in mass cages, the mean time to first larviposition is more difficult to calculate as the pupae of flies that failed to deposit a larva in the first reproductive cycle will be masked by the pupae produced in the second and subsequent cycles.

The number of pupae produced per day at 25.5°C by 160 original G. austeni females for the 1st three reproductive cycles has been extracted from data given by Jordan et al (1967) and is given in Table 4.7.1.

Allowing an interlarval period of 9 days, the mean time to first larviposition is 20.6 days. This compares with 24.2 and 22.3 days for the first two generations of the Austeni colony. The mode at

19/

Table 4.7.1

G. austeni

Number of pupae produced per day by 160 original females for the first three reproductive cycles. Data extracted from Jordan et al (1967)

1st cycle		2nd cycle		3rd cycle	
Days from eclosion	No. of pupae	Days from eclosion	No. of pupae	Days from eclosion	No. of pupae
17	3	26	11	35	16
18	13	27	37	36	21
19	41	28	38	37	44
20	22	29	7	38	15
21	26	30	19	39	21
22	16	31	7	40	9
23	2	32	1	41	2
24	2	33	2	42	4
25	6	34	3	43	4
Total no. of pupae per 9 day period	131		125		136
Mean number of days to first larviposition = 20.2 days (calculated from columns 1 and 2)					

19 days in Jordan's colony is similar to the 19 to 20 days recorded for the first two generations combined in all three main colonies maintained in this laboratory (Fig. 3.9.4).

4.7.3 Interlarval period

The mean interlarval period of G. morsitans was stated by Saunders (1960) to be about 10 days at 26°C, but Nash et al (1967b) found that the period for G. austeni was one day less than this at 25.5°C. The mean interlarval periods obtained in this laboratory were very variable. The period for the Austeni colony proved to be much more regular than that of either the Zambesi or Singida colonies, and over 75% of the flies in generations 1 and 2 of the former colony had mean interlarval periods of between 9 and 11 days (Table 3.9.1).

Apart from temperature, the factor most likely to shorten the mean interlarval period is the acceleration of the reproductive cycles due to abortions. (The deposition of abortions was included in the calculation of the mean interlarval period.)

The factors tending to lengthen the mean interlarval period are much more various. Nash et al (1967b) found that the interlarval periods of some flies were less regular than others and out of eight G. austeni females maintained singly, four had regular interlarval periods, one had regular interlarval periods until the last pupa produced, and three were more or less irregular. However, only ten out of the 96 interlarval periods were of more than ten days, and only/

only one fly had a overal mean in excess of ten.

At the time that this thesis was written, only the overall mean interlarval periods could be calculated, and information about successive interlarval periods was not available. However, bearing in mind that the temperature at which Nash kept his flies was slightly higher than in this laboratory, the mean interlarval periods of females in the Austeni colony were similar to those obtained by Nash. It seems likely, therefore, at least under the conditions provided, that G. austeni is a more regular breeder than G. morsitans as the mean interlarval period of the Zambesi and Singida colonies showed a much greater variability than that of the Austeni colony. The mode of generation 1 of both the former colonies was 13 to 14 days, and in the Zambesi colony 61% of the females had a mean interlarval period of between 12 and 14 days.

These results are in contrast to those obtained for G. morsitans by Pinhao (1966), where the overall mean for the first 20 generations was between 8.9 and 13.8 (see Table 4.4.1). Many of the interlarval periods that Pinhao recorded were so low as to be suspect, but nevertheless it would appear that even though the overall results from his colony were poor, the overall mean interlarval period was not affected. This agrees with the results of the first two generations of Lisbon 1 (Table 3.9.1.1) in which the overall mean was 11.4 and 12.5 days respectively even though the Lisbon colony at that stage being fed on guinea-pigs was showing very poor results.

Although/

Although the results from the Lisbon colony maintained in this laboratory are scanty it is possible that the flies from Azevedo's Lisbon colony have an inherently shorter interlarval period than either of the other two G. morsitans colonies kept in this laboratory. This is surprising especially in the case of the Zambesi colony which although it originated from a different area, is of the same sub-species as the Lisbon colony.

4.7.4 Pupal production

The number of pupae produced is a function of the number of days before the deposition of the 1st larva, the interlarval period and the longevity. The overall mean number of pupae produced per female is greatly affected by early mortality and uninseminated females, and it was for this reason that Jordan et al (1967) used the term pupae per reproductive female (P.R.F.) so as to isolate the rate of reproduction from the other factors.

The high proportion of non-productive females (Table 3.9.3) greatly reduced the mean number of pupae per female produced. For instance, in Generation 1 of the Austeni colony the mean number of pupae per female was 6.5, while the P.R.F. was 10.6 (Table 3.9.8). The results obtained by Pinhao (1966) also showed this difficulty and while the mean number of pupae per female in the first 20 generations varied from 1.6 to 5.6, the P.R.F. was 2.8 to 8.3 (Table 4.1.1). The results of Foster (1957) are even more marked, and of 280 female 1st generation G. morsitans, 51% died before reaching the reproductive age/

age and a further 22% were non-productive. The remaining 27% of the flies produced 349 pupae giving a P.R.F. of 4.65 against a mean number of pupae per female of 1.25.

It was the success achieved by Jordan et al (1967) in cutting down the pre-reproductive losses that contributed to the extremely high figures of 11.4 pupae per female. Although if the deaths of females that occurred before the end of mating had been included the figure would have been lower, these workers achieved a yield of 4 pupae per female against a P.R.F. of 4.3 by day 58. The maximum number of pupae that could have been produced is 5 (see Section 1.2).

It was not possible at the time this thesis was written to examine the age at which successive pupae were produced. The nearest approximation that could be obtained was achieved by finding the number of pupae that had been produced by flies that were culled at varying ages. This was examined in Section 3.9.6 (Figs 3.9.14 to 16 and Table 3.9.17). Only the Austeni colony approached the P.R.F. of 4.3 at day 58 achieved by Jordan et al (1967). It is interesting to note that when measured in this way, the pupal production in the Zambesi colony fell off with age, and this is contrary to what was reported by Azevedo and Pinhao (1968). It is possible that had more flies been considered the same trend would have been established in the other colonies.

4.7.5 Pupal weight

The weight of pupae shortly after deposition has often been used to give/

give an indication of how well a laboratory population is doing. For instance, both Willett (1953) and Foster (1957) found that the mean weight of pupae decreased from generation to generation, and the latter author excluded all emergent G. morsitans flies that had an initial pupal weight of below 24 mg. Nash and Kernaghan (1965a) laid great emphasis on the pupal weight, and discarded all G. austeni pupae below 15 mg.

When the colonies were maintained on guinea-pigs in this laboratory, the situation was similar, and few G. morsitans flies that had an initial pupal weight of under 20 mg. lived for more than a few days. Nash and Kernaghan (loc cit) estimated that the initial weight of their G. austeni pupae was about 4 mg. below that in the field, and they considered that this deficiency was a major factor in the poor performance of the colony. Later, when they got over their initial problems, the pupal weights increased to close to the estimated weight of wild pupae (Jordan et al, 1967).

The mean weight of pupae deposited by flies in the Austeni colony that were maintained on rabbits' ears compared well with those obtained by Jordan et al (loc cit), and those of the two G. morsitans colonies were also about the same as the estimated weight of 'wild' pupae. (Willett, 1953.)

However, with the increase in mean pupal weight, although there were fewer smaller pupae deposited, there was no correlation between the initial/

initial weight of the female fly as a pupa and either its longevity or pupal production.

An example of this is given in figs. 3.9.12 - 13 in which the initial pupal weights of the Zambesi colony were plotted against longevity and pupal production respectively.

Newly emerged flies from the colonies maintained on guinea-pigs were nearly always hungry within a few hours of emergence, while those from the rabbit maintained colony frequently did not feed for 24 hours or more after emergence. Although no measurements were taken, it is presumed that the fat reserves of the newly emerged flies in the former colonies were much lower than those from the colonies maintained on rabbits' ears. Moreover, it appears that so long as the newly emerged flies have sufficient fat reserves to last until their first blood meal, the initial pupal weight on which so much emphasis has been placed by many workers, is not so important as had been presumed previously.

4.8 Population increase

The colony of Azevedo and Pinhao (1964) underwent little expansion in the first 20 generations, and Pinhao (1966) was able to keep records of and analyse the data from every fly in the colony up to that period. The reason for the rapid expansion in the size of their colony thereafter is impossible to ascertain with certainty, but as their colony of G. morsitans orientalis from Rhodesia (from the/

the same area as the Zambesi colony was derived) underwent a similar evolution (Azevedo and Pinhao 1968), it is possible that their regime for the maintenance forced a much greater selection pressure on the colony than was the case in this laboratory when, using rabbits' ears, there was an immediate and rapid population increase. It is interesting to note that the Lisbon colony (~~Table 3.10.4~~) survived (although only just: Table 3.10.4), while Singida 24 (Table 3.10.5) decreased steadily in numbers until it had died out by the fifth generation.

As soon as the colonies began to expand, the number of flies was kept stable by removing whole 'lines' of flies descended from a single female parent in generation 1. Although the figures are not given, it was evident that certain lines of flies increased in numbers far more quickly than others, which in some instances died out. Thus a similar selection pressure to that which may have occurred in Lisbon may have been operating, but was masked by the overall rapid rate of increase of the colonies.

This view is supported by the results shown in fig. 3.9.6, where it will be seen that only a small proportion of the female flies were responsible for the rapid rate of increase in the overall size of the population.

4.9 Conclusions

When this project was started in 1965, the only colony being successfully maintained was that of Azeveo and Pinhao (1964). Partly because of this previous lack of success, much of the laboratory work on the colonisation of tsetse has tended to be subjective. At least three other laboratories, including Edinburgh, have now succeeded in establishing closed colonies of a number of species, and there has also been a large increase in the amount of research work done on various aspects of the fly.

However, no detailed analysis based on the records of individual flies, or rapidly increasing populations of tsetse, have been attempted previously. The analysis of the colonies that has been presented in this thesis represents the first step in a much more detailed examination of the results from the same colonies maintained in the laboratory over a period of about two years. In this analysis it is hoped to overcome many of the difficulties which were associated with the necessity of culling large numbers of flies from the colonies in order to keep the population size within limits. The drawing up of life tables and the analysis of the fly lines are both being undertaken.

Due to the selective culling of certain 'lines' all four populations of flies in this laboratory were descended from a very small number of original females, and there was therefore a high degree of inbreeding in the colonies.

There/

There is some recent evidence from other work (Jordan, personal communication) that inbreeding of flies in the laboratory may produce inbreeding depression, and comparison of inbred and outbred lines of flies would be valuable in assessing the danger of such an occurrence happening in the mass rearing of flies.

The taking of regular blood meals by the flies together with their digestion has been one of the major problems in this project, and much work needs to be done on the mechanisms of the motivation of the fly to feed, together with a study of the correlation of the frequency of feeding with pupal production. Both these lines of investigation have been started. Following on from this, one of the most important problems still to be overcome is the successful maintenance of a colony using a membrane feeding technique, and some progress has been made in devising a method for doing this.

5 Appendix

Data recording and analysis

5.1 Introduction

Brief details of the method of data recording and analysis is given in section 2.4. This appendix gives further details of the methods used.

A fly or pupa was identified by a number which was recorded on a card on which brief details of the life history were also recorded. The card was attached to the appropriate fly cage or pupa tube.

As each event in the life history of a fly or pupa occurred, it was recorded on the appropriate daily register. These registers were transferred onto punched cards which were then used to set up or update the individual fly or pupa records, which were held on magnetic tape.

An up-to-date record of the life history of any fly or pupa could thus be obtained at any time, and in addition, using standard statistical routines already available, the fly records could be grouped together in any way desired, and then analysed.

Details of the recording procedure, and the methods used to set up, update and analyse the fly and pupa records are described below.

5.2 Daily registers (see plate 2.4.2)

On emergence, each fly was allocated a number prefixed by the letter 'M' or 'F' according to its sex. Each pupa (or abortion) was likewise given a number prefixed by the letter 'P'. Events in the life history of a fly or pupa were recorded daily as they occurred on one of the

five different coloured registers.

5.2.1 Eclosion register (pink)

The items recorded were:

Column 1 - the new number of the fly, prefixed by the letter 'M' or 'F' according to its sex.

Column 2 - the pupa number of the pupa from which the fly emerged, prefixed by the letter 'P'.

Column 3 - the fly number of the female parent of the fly (if this was unknown, 'F 0' was entered in this column).

5.2.2 Mating register (green)

column 1 - the female fly number

Column 2 - the male fly number

Column 3 - the letter 'U' if the mating was thought to be unsuccessful. Otherwise this column was left blank.

5.2.3 Pupa register (blue)

Column 1 - the new pupa number prefixed by the letter 'P'.

Column 2 - the fly number of the female parent fly.

Column 3 - the weight of the pupa in milligrams prefixed by the letter 'W' or if an abortion, its weight prefixed by the letter 'A'. In cases of those abortions too small to weigh, 'A 0' was recorded. Pupae that were damaged, and for this reason would not have emerged, were also recorded as abortions.

5.2.4 Deaths register (yellow)

Column 1 - the fly number (together with its sex).

Column 2 - the nutritional state of the fly at death. This entry consisted of a letter corresponding to the nutritional state:

'A' - fly in poor nutritional state

'B' - no blood showing in abdomen, but the fly in a good nutritional state

'C' - some blood showing in abdomen - but not engorged.

'D' - engorged or apparantly engorged.

The appropriate letter was followed by a figure corresponding to the number of days before dying that the fly had not fed. This was calculated from the number of card slips attached to the cage (see feeding routine).

5.2.5 Batch pupa register (white)

This register was used to record pupae received in bulk either from the field ('wild' pupae) or from another laboratory, and whose parentage was therefore not known:

Column 1 - Pupa number (as in the pupa register).

Column 2 - 'F 0' (i.e. parent female unknown).

Column 3 - weight of the pupa prefixed by the letter 'W' (as in the pupa register).

This register was headed by the words 'Batch pupa register' followed by the date (see below) and then the source and batch number. When fed into the computer, the source and batch number together with the first pupa in the batch were automatically recorded in the dictionary block (see below).

The sources recorded were:

Source 1 SINGIDA G. morsitans morsitans ('wild' pupae) from Singida in Tanzania.

Source 2 ZAMBESI G. morsitans orentalis ('wild' pupae) from the Zambesi valley in Rhodesia.

Source 3 LISBON G. morsitans morsitans (laboratory pupae) from the Institute of Tropical Medicine, Lisbon, Portugal.

Source 4 EDINBURGH G. morsitans morsitans that originated from early batches of 'wild' pupae sent from Singida, but which were not recorded on the computer.

Source 5 AUSTENI G. austeni (laboratory pupae) from the Tsetse Research Laboratory, Bristol.

Source 6 PALPALIS G. palpalis ('wild' pupae) from the Nigerian Institute for Trypanosomiasis Research, Nigeria. (Only one batch of this species was received, of which only very few emerged.)

Each daily register was headed by the name of the register (e.g. Pupa Register, Eclosion Register, etc.) followed by its date.

The dates, for ease of subsequent analysis, were calculated from 1 January each year and were prefixed by a letter corresponding to the year: 'A' - 1965, 'B' - 1966, 'C' - 1967, etc. For instance, 'A' 15 corresponded to January 15, 1965, 'B' 101 to April 11, 1966, and 'C' 365 to December 31, 1967.

5.3 Identification of flies and pupae in the laboratory

5.3.1 Flies: the sex and source of each fly was identified by the colour of the card attached to its cage. The layout and colour coding of these cards is shown in Fig. 5.3.1. The date of deposition of each pupa was recorded down the left-hand side of the fly card.

5.3.2 Pupae: a description of the method of collection and weighing of the pupae is given in section 2.3.1. After weighing, each pupa was placed in a polystyrene tube and a pupa card with the following information on it, attached by an elastic band to the tube:

1. the pupa number
2. the female parent number
3. its weight in milligrams
4. the source batch and generation number of the female parent.

Items 2 and 4 were obtained by reference to the pupa tray.

The fly and pupa cards were made out at the same time as the respective registers, using automatic numbering machines for the new pupa and fly numbers. On emergence of the fly, its pupa card was stapled behind the fly card. On the death of the fly, its card with the pupa card attached was filed in numerical order.

5.4 Transfer of registers to punched cards

Each daily register was punched onto similarly coloured cards. The date for each fly or pupa was punched on a separate card. A 'Header card' recorded the register type and date. (In the case of a batch

pupa register, a second header card recorded the source and batch number.) The end of each register was signified by a card punched with two asterisks ('tail card').

5.5 Checking of punched cards against original register (Program T.F.3)

In order to eliminate mistakes in transfering the register onto punched cards, a checking program was run (Code T.F.3).

This program, which was run weekly, inspected the punched cards and produced a print-out of the registers. It also checked for duplicate pupa or female parent numbers in each register, and if any occurred they were captioned at the foot of the register. The program checked the layout of each card and if faulty it was placed in the 'rejected' column in the print-out.

The print-out of each register was then visually compared with the original register sheets, and any cards that were found to be incorrect were re-punched. The print-out of each register was stapled to the original register sheet.

5.6 Layout of records on the magnetic tape

A magnetic tape is divided up into a number of sections, each section being subdivided into 512 'words'. The records of each fly or pupa were recorded in a standard format, seven words being used for a fly record, and three words for a pupa record. The fly and pupa records were held together in groups termed fly or pupa blocks respectively. Each section of the magnetic tape was used

to store one block, which held either 65 fly records or 128 pupa records. The letter 'F' or 'P' in the first word of the block designated as a fly or pupa block respectively.

The layout of the block headings and the fly and pupa records are given in Figs. 5.6.1.a and b, and 5.6.2.

It will be seen that the layout of the fly record varied according to the sex of the fly. The type of layout was determined by the prefix 'F' or 'M' before each fly number in the eclosion register. Female numbers were recorded in Y 1 of the fly record as a positive number, and males as a negative number (Figs. 5.6.1.a and b).

5.7 Whereabouts of records on magnetic tape

In order to keep a record of the type of block that had been allocated to each particular section on the magnetic tape, and also the whereabouts of any fly or pupa record, three further blocks were kept at the beginning of the magnetic tape.

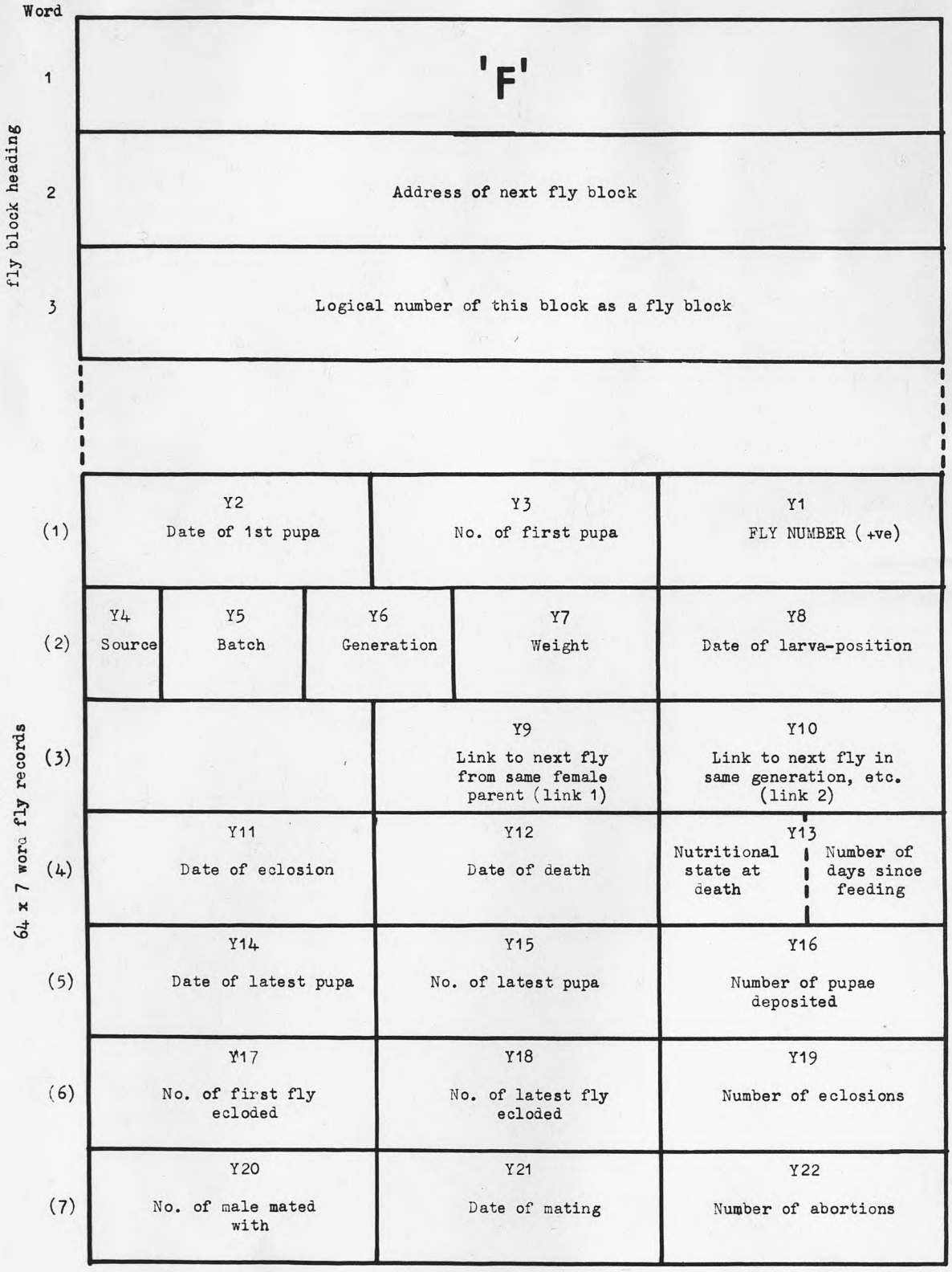
5.7.1 Dictionary Block (Fig. 5.6.3)

This block had three functions:

(a) General Dictionary: this indicated the address of the first and latest fly, pupa and generation blocks (see below) together with the next available free sections. When a new block was required, the dictionary was 'asked' where the next available section was, and this was then allocated to it, linking it to the previous block as described above.

Fig. 5.6.1.a

Magnetic tape layout of a fly block heading and a female fly record.



FLY BLOCK, heading and a 7 word fly record (female)

Fig. 5.6.1.b

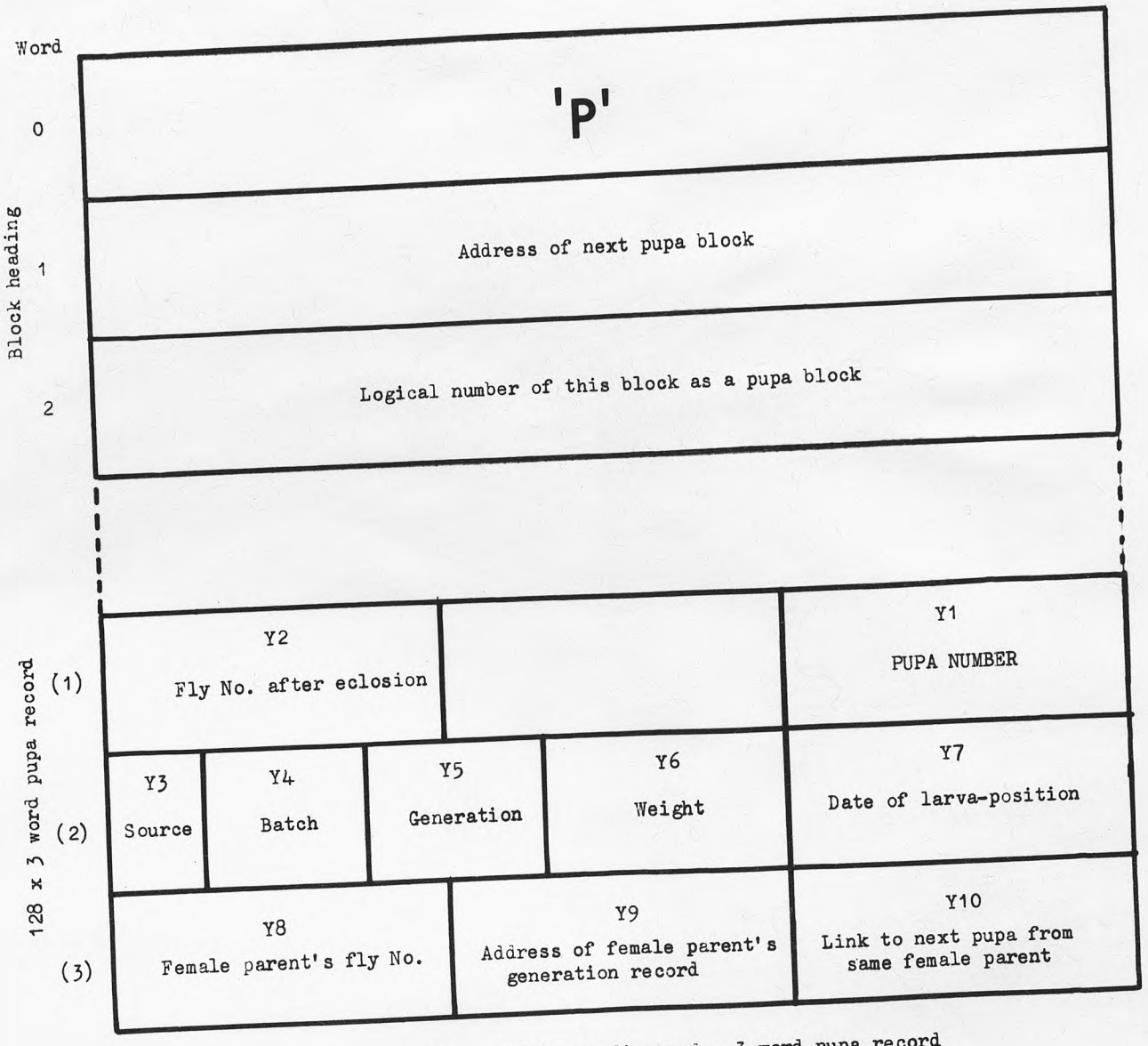
Magnetic tape layout of male fly record.

Word					
1					Y1 FLY NUMBER (-Ve)
2	Y4 Source	Y5 Batch	Y6 Generation	Y7 Weight	Y8 Date of larva-position
3			Y9 Link to next fly from same female parent (link 1)		Y10 Link to next fly in same generation etc. (link 2)
4	Y11 Date of eclosion		Y12 Date of death		Y13 Nutritional state at death | Number of days since feeding
5	Y14 No. of 1st female mated with		Y15 No. of 2nd female mated with		Y16 No. of 3rd female mated with
6	Y17 No. of 4th female mated with		Y18 No. of 5th female mated with		Y19 No. of 6th female mated with
7					Y22 Number of matings

7 word male fly record

Fig. 5.6.2

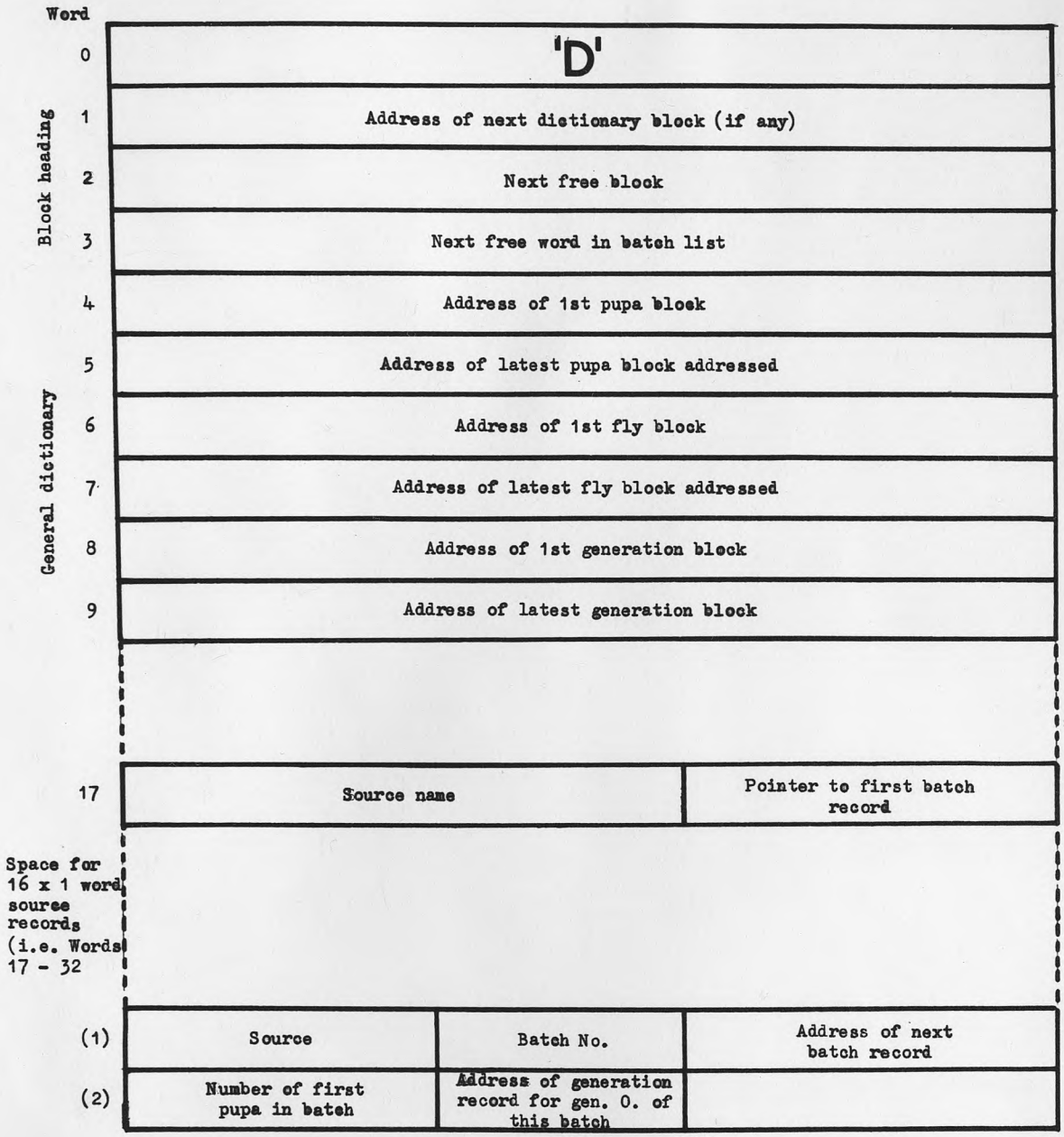
Magnetic tape layout of a pupa block heading and a pupa record.



PUPA BLOCK Heading and a 3 word pupa record

Fig. 5.6.3

Dictionary block heading, general dictionary and source and batch records.



DICTIONARY BLOCK Heading, general dictionary, and source and batch records

(b) Source Records: the dictionary block was also used to record the names allocated to the particular source from which the original pupae were obtained, together with a pointer to the whereabouts of the first batch record stored further in the dictionary block. (See below.)

(c) Batch records: the last part of the dictionary block was used to record the batch numbers from each source. The source and batch number of the first pupa in the batch and the whereabouts of the generation record (see below) of the batch were also recorded.

5.7.2 Index Block (Fig. 5.6.4)

The index block recorded the number of the section (i.e. the absolute address) that each block was stored on the magnetic tape.

5.7.3 Generation Block (Fig. 5.6.5)

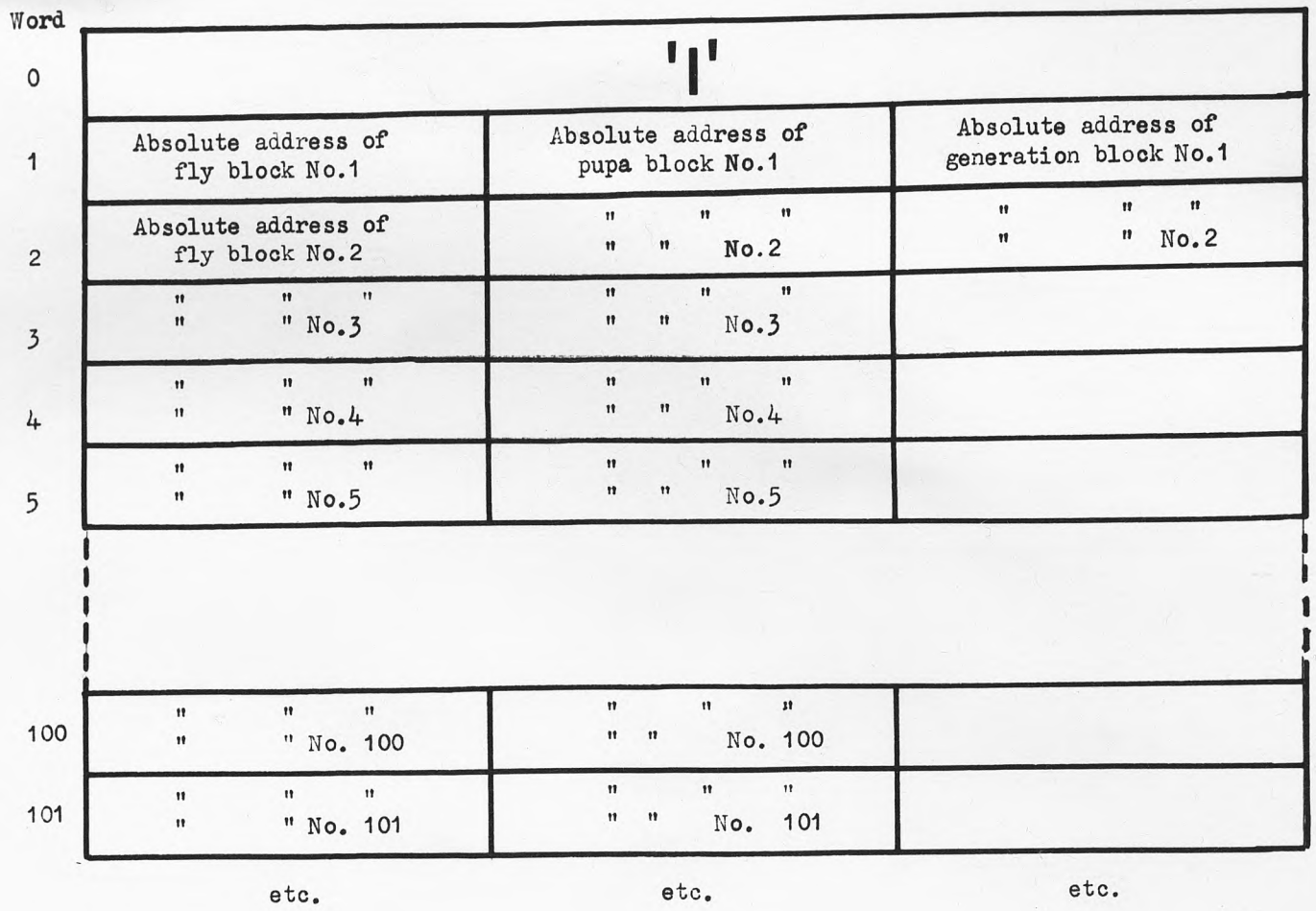
As described above, each batch of pupae that arrived were allocated a source and batch number, the offspring in subsequent generations keeping the same source and batch number.

When the first fly of a new generation of a particular batch emerged, a new generation record was set up, and the previous generation record linked to it.

The generation record also recorded the latest fly from the same source and batch, and as each subsequent fly of the same generation

Fig. 5.6.4

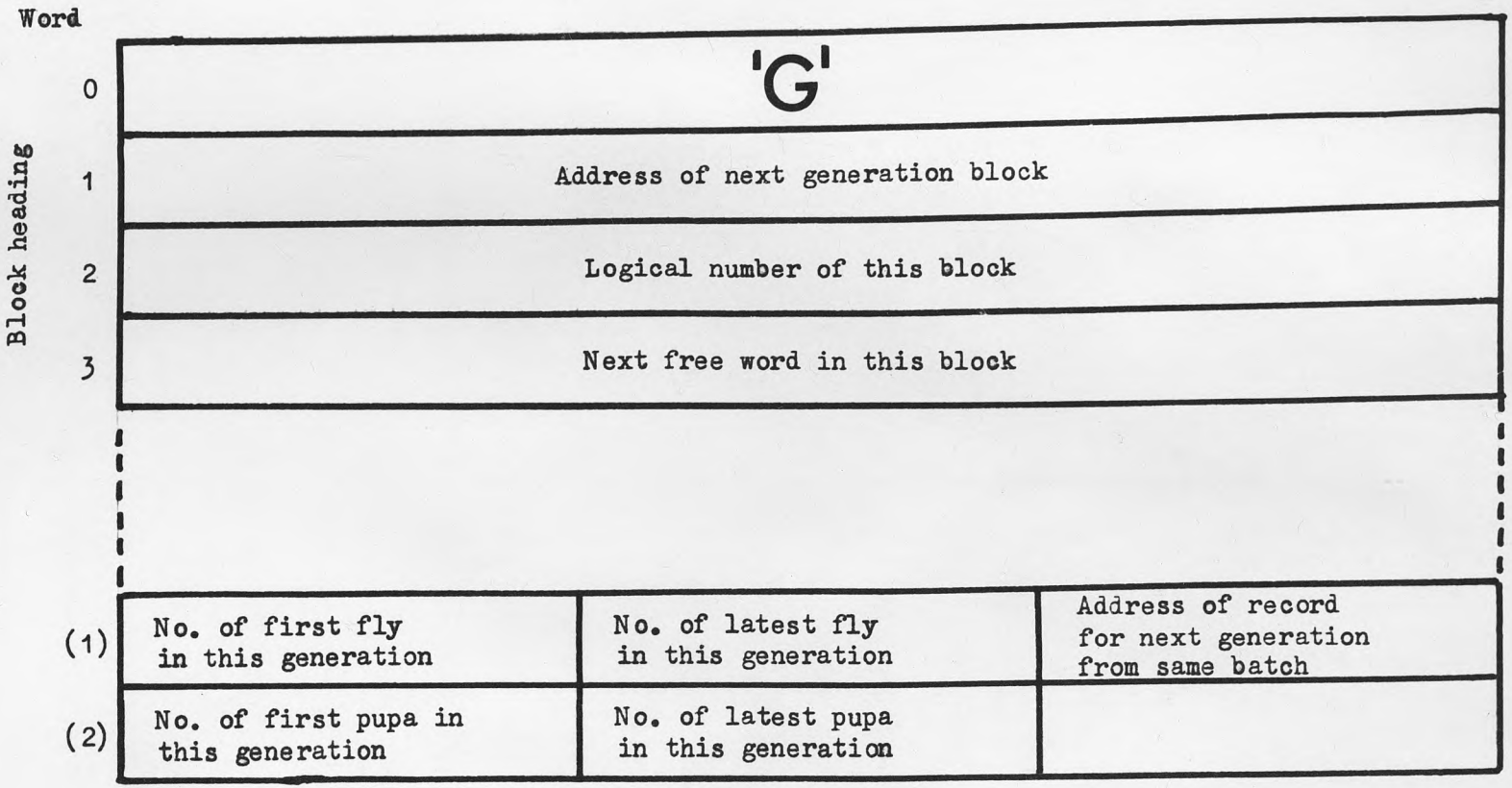
Index block.



INDEX BLOCK, showing whereabouts on the magnetic tape (i.e. the absolute address) of each fly, pupa and generation block.
(The word No. coincides with the 'logical No.' of each fly, pupa and generation block held on the magnetic tape).

Fig. 5.6.5

Generation block.



2 word generation records

GENERATION BLOCK, heading, and generation records

was recorded it displaced the number of the fly previously recorded with its own number. Flies of the same source, batch and generation were linked with each other in word 3 (Y 10) or each fly record (Fig. 5.6.1.a, b).

The same process occurred for each pupa record, the pupa taking on the same generation number as its female parent. Pupae of the same source, batch and generation were not, however, linked together.

Identification of source, batch and generation of the pupae and flies in the computer.

The source, batch and generation of each pupa was identified by reference to those details in the female parent's record during the updating process in Cycle 1 (pupa records) described below. The source batch and generation of the fly were similarly obtained by reference to its record as a pupa during Cycle 1 (fly records). For ease of computation, two rules were followed:

(a) 'Wild' or laboratory batch pupae were called Generation 0, and the eclosed flies from these pupae, Generation 1.

(b) A pupa deposited in the laboratory took on the same generation number as its female parent. On eclosion, the generation of the fly was recorded as one more than its generation as a pupa.

5.8 Setting up and updating records (Program T.F. 4)

Having been checked, the daily registers were again read into the computer and the information used for setting up or updating the

records. The next register was then read in, and the process repeated. The particular kind and date of the register being read in was identified by the 'Reader card' and the end of the register by the 'tail card'. (See section 5.4 above.)

5.8.1 Pupa register and batch pupa register (see fig. 5.8.1)

Four separate operations (termed 'cycles') were performed:

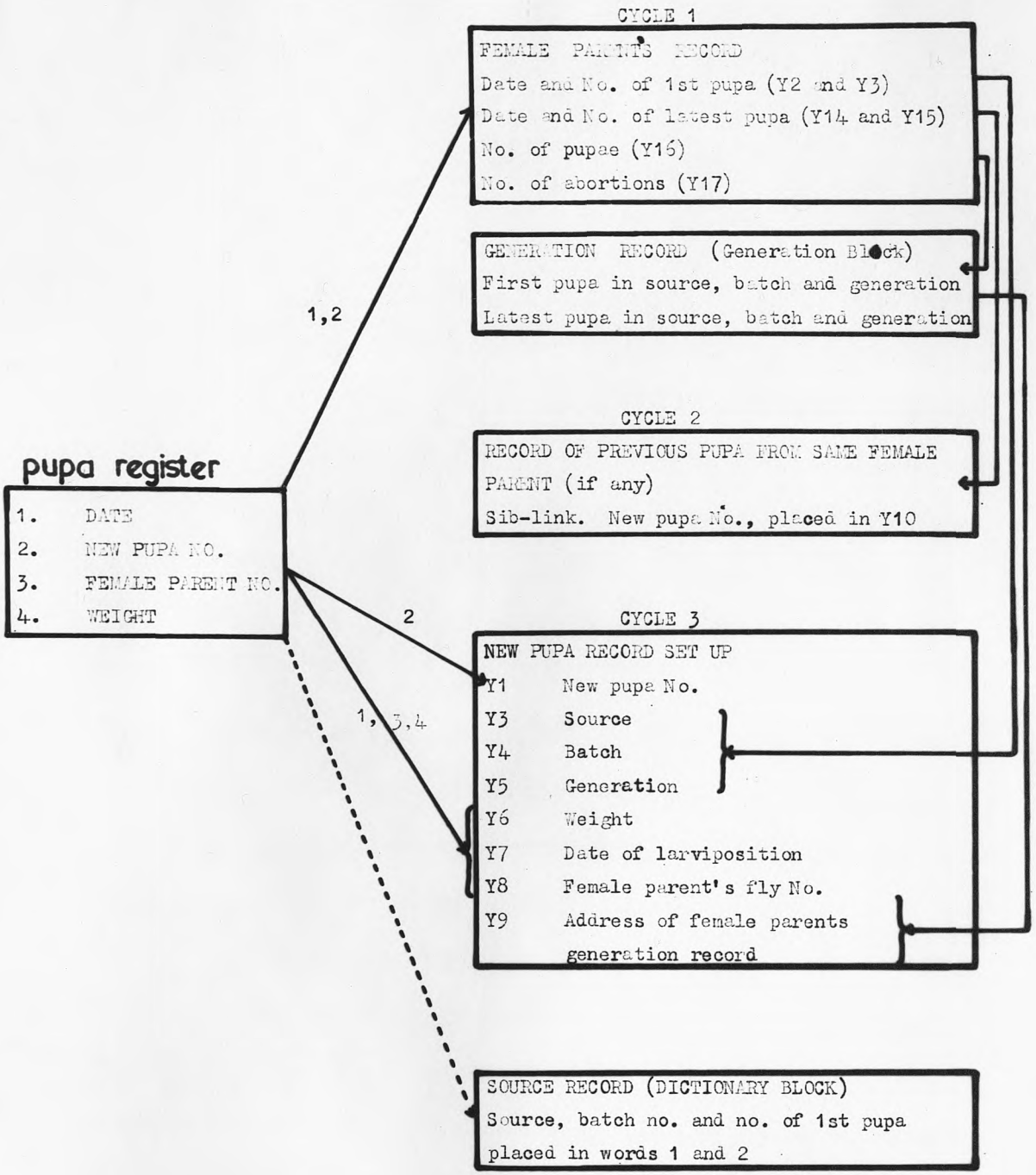
(a) Preliminary cycle: a check was made in the appropriate pupa block to make sure that the pupa numbers had not already been set up.

(b) Cycle 1: each female parent's fly record was updated with the new pupa number and the date of larviposition. In the case of it being its first pupa, Y2 and 3 (the date of number of the first pupa respectively) and Y 14 and 15 (the date and number of the latest pupa) were completed. Subsequent pupae from the same female parent displaced the number and date of larviposition of the latest pupa (Y 14 and 15). The number of pupae (Y 16), or in the case of an abortion, the number of abortions (Y 22), were also amended. In the case of a batch pupa record, the female parent number was unknown, and this part of Cycle 1 was omitted. Lastly, in Cycle 1, the latest pupa record for the particular source and batch, which was held in the generation block, was brought up to date.

(c) Cycle 2: linked the latest sibling pupa or abortion with the previous one, (if any), from the same female parent and which had been noted during Cycle 1. This was done by fetching the previous sibling's record and placing the most recent sibling's

Fig. 5.8.1

Setting up a new pupa record and updating related fly and pupa records following the deposition of a pupa, or the arrival of a new pupa batch.


CYCLE 1
FEMALE PARENT'S RECORD
Date and No. of 1st pupa (Y2 and Y3)
Date and No. of latest pupa (Y14 and Y15)
No. of pupae (Y16)
No. of abortions (Y17)
GENERATION RECORD (Generation Block)
First pupa in source, batch and generation
Latest pupa in source, batch and generation
CYCLE 2
RECORD OF PREVIOUS PUPA FROM SAME FEMALE PARENT (if any)
Sib-link. New pupa No., placed in Y10
pupa register
1. DATE
2. NEW PUPA NO.
3. FEMALE PARENT NO.
4. WEIGHT
1,2
2
1, 3, 4
CYCLE 3
NEW PUPA RECORD SET UP
Y1 New pupa No.
Y3 Source
Y4 Batch
Y5 Generation
Y6 Weight
Y7 Date of larviposition
Y8 Female parent's fly No.
Y9 Address of female parents generation record
SOURCE RECORD (DICTIONARY BLOCK)
Source, batch no. and no. of 1st pupa placed in words 1 and 2

number in it in Y 10.

(d) Finally, Cycle 3: this set up a new pupa record for the pupa or abortion, completing Y 1 and Y 3 to Y 9.

5.8.3 Eclosion Register (see Fig. 5.8.2)

(a) Preliminary cycle (as above).

(b) Cycle 1 fetched the pupa record of the fly before eclosion and placed the new fly number in Y 2.

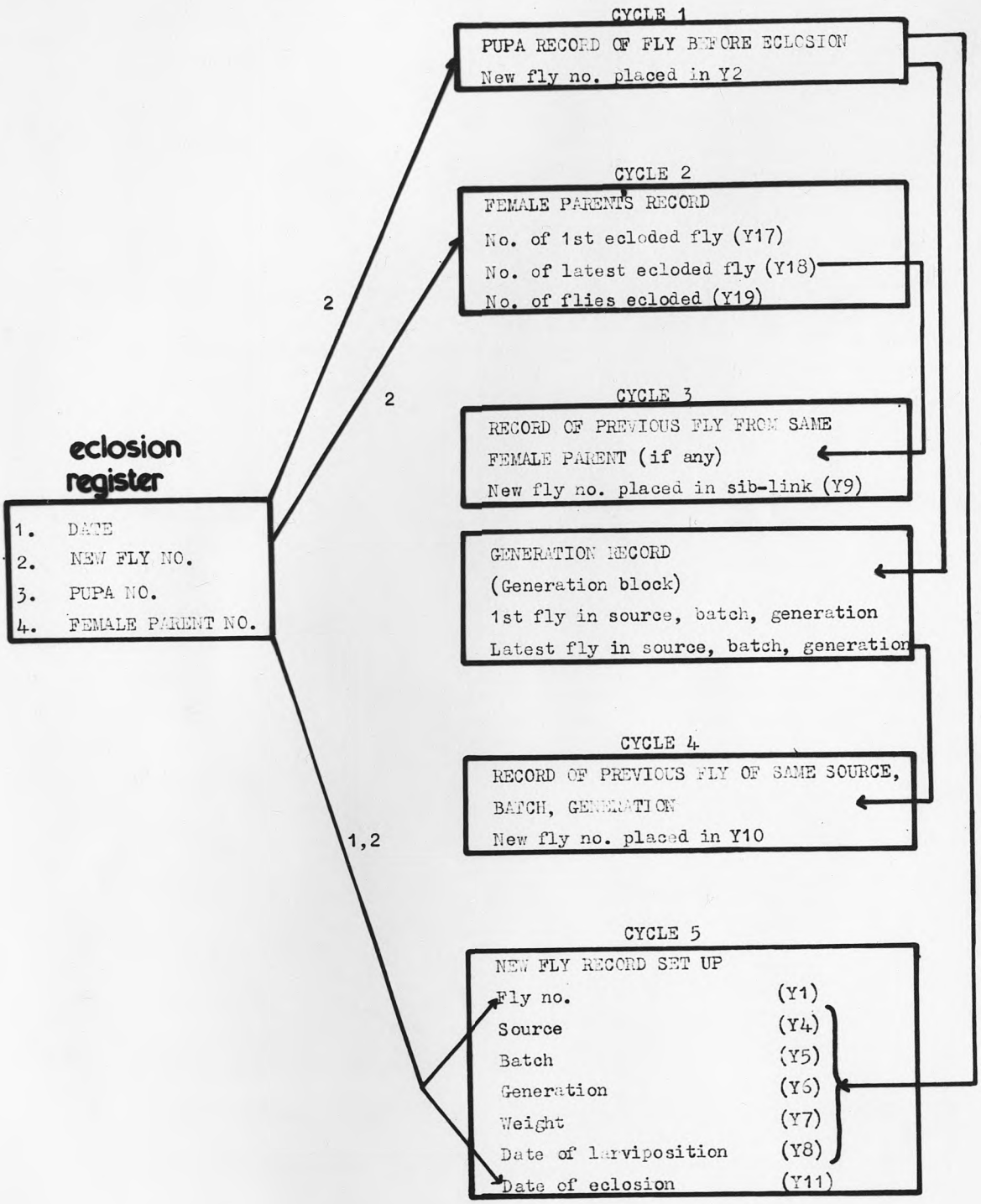
(c) Cycle 2 fetched the female parent's fly record. In the case of it being its first offspring (fly) to eclode, both Y 17 and 18 were completed. Subsequent flies to eclode displaced the number of the previous fly contained in Y 18. The number of flies that ecloded from pupae deposited by the female parent (Y 19) was also amended. Cycle 2 was not attempted in the case of generation 1 flies (i.e. 'wild' flies) whose female parent's number was not known.

(d) Cycle 3 was concerned with the linking of the latest sibling fly to eclode with the previous one (if any) from the same female parent. (The number of the fly had been noted during Cycle 2.) The fly record of the previous sibling was fetched and the most recent fly sibling's number placed in Y 9 (Link 1).

(e) Cycle 4 linked the previous latest fly of the same source, batch and generation with the latest fly. This was done by referring to the particular generation record in the generation block and fetching the previous latest fly's record and placing the most recent fly's number in it in Y 10 (Link 2). The 'latest fly' in the generation record was also amended. In the case of it

Fig. 5.8.2

Setting up a new fly record and updating related fly and pupa records following the eclosion of a fly.


eclosion register
1. DATE
2. NEW FLY NO.
3. PUPA NO.
4. FEMALE PARENT NO.
2
2
1,2
CYCLE 1
PUPA RECORD OF FLY BEFORE ECLOSION
New fly no. placed in Y2
CYCLE 2
FEMALE PARENT'S RECORD
No. of 1st ecloded fly (Y17)
No. of latest ecloded fly (Y18)
No. of flies ecloded (Y19)
CYCLE 3
RECORD OF PREVIOUS FLY FROM SAME
FEMALE PARENT (if any)
New fly no. placed in sib-link (Y9)
GENERATION RECORD
(Generation block)
1st fly in source, batch, generation
Latest fly in source, batch, generation
CYCLE 4
RECORD OF PREVIOUS FLY OF SAME SOURCE,
BATCH, GENERATION
New fly no. placed in Y10
CYCLE 5
NEW FLY RECORD SET UP
Fly no. (Y1)
Source (Y4)
Batch (Y5)
Generation (Y6)
Weight (Y7)
Date of larviposition (Y8)
Date of eclosion (Y11)

being the first fly of a new generation, a new generation record was set up, linking it with the previous one.

(f) Cycle 5 set up a new fly record for the fly, completing Y 1, Y 4 to 8 and Y 11.

5.8.4 Mating Register

This register was used to update the mating records of both female and male.

The female fly record was fetched and the number of the male it was mated with together with the date of mating were placed in Y 20 and 21. The male fly record was then fetched and if it was its first mating the female's number was placed in Y 14. The second to the sixth matings were placed in Y 15 to 19 respectively. The total number of matings were recorded in Y 22. An unsuccessful mating was signified by a negative sign in front of the fly with which it was mated.

5.8.5 Deaths Register

The dead fly's record was fetched, the date of death entered in Y 12. The nutritional state at death, and the number of days since feeding were entered in Y 13.

5.8.6 Checks against errors in data

During the setting up and updating of the records, a number of errors were checked for, and if found, the error was printed out and the updating process on the particular fly or pupa record suspended.

If, on investigation, the error was found to be in an entry in the register being updated, the entry was repunched, and put in as a new register header and tail cards. If any of the records on the magnetic tape were found to be faulty, a correction program was run to correct the entry (T.F. 6, see below).

5.9 Access to records on magnetic tape (Program T.F. 5)

This program produced a print-out of the contents of any desired section of the magnetic tape. The section required could be called for by referring either to its section number (absolute address) or to the type and number of the block in that section (i.e. the logical number). The Dictionary, Index and Generation blocks were called for by reference to the code letters, D, I and G respectively.

5.10 Correction of errors on magnetic tape (Program T.F. 6)

This program was used to correct any errors in the records on the magnetic tape. In the case of fly or pupa records, the program 'fetched' the required record, corrected the incorrect parts of it by reference to the 'Y' number concerned, and then packed the corrected record back onto the magnetic tape. In the case of the Dictionary, Index or Generation records, the word number concerned was corrected in a similar manner.

5.11 Security of records

The same magnetic tape (RD ZOO 003) was normally used for running T.F.s 4, 5 and 7 (see below). To guard against computer failure

during the updating process, or the magnetic tape being damaged, three further magnetic tapes RD Z00 001, RD Z00 002 and RD Z00 005 were kept. At the end of each weekly run of T.F. 4, the records on RD Z00 003 were copied onto one of the three spare tapes in rotation. In the event of a failure, the latest correct tape was copied back onto RD Z00 003 and the updating process restarted from the appropriate point.

5.12 Analysis of records

The records held on magnetic tape were first transformed into parameters that could most easily be analysied by using standard statistical programs already available.

The eighteen parameters chosen are given in table 5.12.1. The statistical programs were then used to group the records together according to the source, batch and generation of the flies and then perform the analysis required.

5.13 Fly 'lines'

In order both to keep the colonies within the size that could conveniently be handled, and also to analyse the performance of the individual fly and its descendants, a number of programs were developed to allow a fly's descendants to be identified. Each original female parent of a particular source and batch in Generation 1 was given a line number, its descendants (both pupae and flies) being given the same number. The linkages between each female parent in turn, and its offspring are given in Fig. 5.13.1. From this it was

Table 5.12.1 Parameters used for analysis of data

X(1) = Fly number (Y1)

X(2) = Sex 0 = unknown
1 = male
2 = female

X(3) = Source (Y4)

X(4) = Batch (Y5)

X(5) = Generation (Y6)

X(6) = Weight in mgs. (Y7)

X(7) = pupal period (Y11 - Y8)

X(8) = Mating (females only) 1 = mated
2 = not mated

X(9) = Longevity (Y12 - Y11)

X(10) = Days since feeding (Y13)

X(11) = State of nutrition (Y13)

X(12) = Number of viable pupae (i.e. less abortions) (Y16 - Y22)

X(13) = Number of abortions (Y22)

X(14) = Mean interlarval period = $\frac{Y14 - Y2}{Y16 - 1}$

X(15) = Number of eclosions (Y19)

X(16) = % eclosions = $\frac{Y19 \times 100}{Y16 - Y22}$

X(17) = Siblink (Y9)

X(18) = Generation link (Y10)

Note The 'Y' number in brackets after the parameter refers to the fly record (Fig. 5.6.1 a and b).

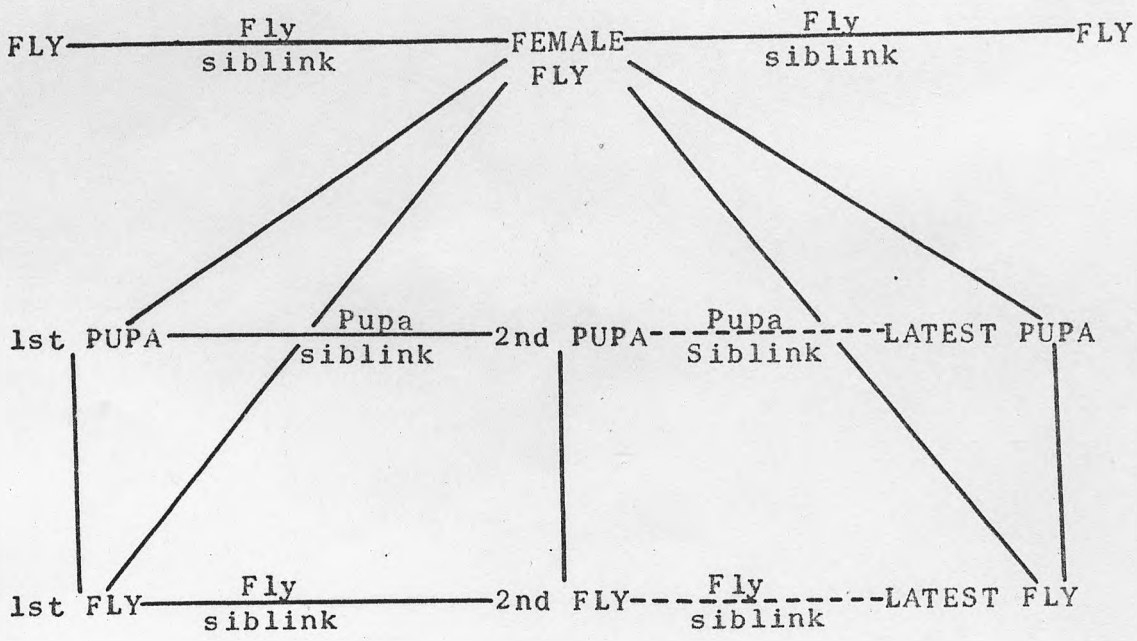


Fig. 5.13.1

LINKAGE OF FLY LINE

possible to build up life tables. Further developments in the updating program (T.F. 4) now allows this to be done routinely.

Acknowledgements

This project was done under the tenure of a Wellcome Research Fellowship from the Animal Health Trust, and I am most grateful to it and to the Wellcome Trust for their generous financial support.

My thanks are due to my supervisor, Dr D S Saunders, and to Dr J A Campbell for much helpful advice and criticism.

Without the help of the team who have been responsible for much of the work of maintenance of the colonies, this project could not have been attempted. I should like particularly to thank Mrs Pat Smith, Mrs Helen Johnstone and Mrs Isobel Burden.

The staff of the Regional Computing Centre and particularly Miss Marion Fisher have been of great assistance in all aspects of the recording and analysis of the date.

I am grateful to Mr Neil Mackay and Mr Ronnie McGowan of the engineering workshop for their technical assistance, to the Librarian, Miss Anne Stark, for all her help, and to Mr Dennis Cremer and Miss Morag Henderson for the photographs.

Miss Sarah Kirkup and Mrs Anna Young typed the manuscript most efficiently and quickly.

7. References

Azevedo, J.F. de and Pinhão, R. da C., (1964)
The maintenance in the laboratory of a colony of G. morsitans (Diptera) since 1959.
Bull. Wld. Hlth. Org. 31, 835 - 841.

_____ and _____, (1968)
Prospects offered by the laboratory breeding of Glossina morsitans in the laboratory.
Proceedings of a panel on the control of livestock insect pests by the sterile male technique, organised by the Joint F.A.O./I.A.E.A. division of Atomic Energy in food and agriculture. Vienna 1967 pp 5 - 18.

_____, _____, Abreu, M.M.A., and Dias, J.A.T.S., (1960)
Criação de Glossima morsitans West. no laboratorio.
Anais. Inst. Med. trop. 17, 5 - 35.

Agricultural Research Council of Central Africa (1964). Annual Report.
Salisbury, Rhodesia.

Andrewatha, H.G. and Birch, L.C., (1954)
The Distribution and Abundance of Animals.
Chicago, University of Chicago Press.

Bertram, D.S. and Gordon, R.M., (1939)
An insectarium with constant temperature and humidity control; together with a description of a simplified technique for the rearing of Anopheles maculipennis var atroparvus.
Ann. trop. Med. Parasit., 33, 279 - 288.

Bursell, E., (1957)

Spiracular control of water loss in the tsetse fly.

Proc. R. ent. Soc. Lond. (A), 32, 21 - 29.

_____, (1958)

The water balance of tsetse pupae.

Phil. Trans. R. Soc. Ser. B., 241, 179 - 210.

_____, (1959)

The water balance of tsetse flies.

Trans. R. ent. Soc. Lond., 111, 205 - 235.

_____, (1960a)

Loss of water by excretion and defaecation in the tsetse fly.

J. exp. Biol., 37, 689 - 697.

_____, (1960b)

Free amino acids of the tsetse fly (Glossina).

Nature, 187, 778.

_____, (1960c)

The effect of temperature on the consumption of fat during pupal development in Glossina.

Bull. ent. Res., 51, 583 - 598.

_____, (1961)

The behaviour of tsetse flies (Glossina swynnertoni Austen) in relation to sampling.

Proc. R. ent. Soc. Lond. (A), 36, 9 - 20.

Buxton, P.A. and Lewis, D.J., (1934)

Climate and tsetse flies: Laboratory studies upon Glossina submorsitans and tachinoides.

Phil. Trans. R. Soc. Ser. B., 224, 175 - 240.

Buxton, P.A., (1955)

"The Natural History of Tsetse Flies."

London School of Hygeine and Tropical Medicine, Memoir No. 10.

BUXTON, P.A. and MELLANBY, K., (1934). The measurement and control of humidity. Bull. ent. Res. 25, 171-75.

Cockings, K.L., (1959)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1958, pp 26 - 31, Govt. Printer, Nairobi.

_____, (1960)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1959, pp 13 - 18, Govt. Printer, Nairobi.

_____, (1961)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1960, pp 32 - 33, Govt. Printer, Nairobi.

_____, (1961)

The behaviour of tsetse flies (Glossina swynnertoni Austen) in relation to sampling.

Proc. R. ent. Soc. Lond. (A), 36, 9 - 20.

Buxton, P.A. and Lewis, D.J., (1934)

Climate and tsetse flies: Laboratory studies upon Glossina submorsitans and tachinoides.

Phil. Trans. R. Soc. Ser. B., 224, 175 - 240.

Buxton, P.A., (1955)

"The Natural History of Tsetse Flies."

London School of Hygeine and Tropical Medicine, Memoir No. 10.

Lewis, London.

Cockings, K.L., (1959)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1958, pp 26 - 31, Govt. Printer, Nairobi.

_____, (1960)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1959, pp 13 - 18, Govt. Printer, Nairobi.

_____, (1961)

Laboratory maintenance of Glossina pallidipes.

E. Afr. Tryp. Res. Org. Rept. 1960, pp 32 - 33, Govt. Printer, Nairobi.

Dame, D.A., (1968)
Chemosterilisation, rearing and ecological studies of Glossina.
Proceedings of a panel on the control of livestock insect pests, by the sterile male technique, organised by the joint F.A.O./I.A.E.A. division of Atomic Energy in Food and Agriculture.
Vienna 1967, pp 23 - 24.

East African Trypanosomiasis Research Organisation Annual reports, 1958, 1959, 1960, 1961, 1963, 1967.
Government Printer, Nairobi.

Edney, E.B. and Barras, R., (1962)
The body temperature of the tsetse fly Glossina morsitans.
Westwood (Diptera, Muscidae).
J. Insect Physiol., 8, 469 - 481.

Foster, R., (1957)
Observations on laboratory colonies of the tsetse flies Glossina morsitans West. and Glossina austeni Newstead.
Parasitology, 47, 361 - 374.

Geigy, R., (1948)
Elevage de Glossina palpalis.
Acta. Trop., 5, 201 - 218.

Glasgow, J.P., (1961)
The feeding habits of Glossina swynnertoni Austen.
J. Anim. Ecol., 30, 77 - 85.

_____, (1963)

"The Distribution and Abundance of Tsetse."

Pergamon Press, Oxford.

Gordon, R.M. and Crewe, W., (1948)

The mechanisms by which mosquitoes and tsetse flies obtain their blood meal, the histology of the lesions produced, and the subsequent reactions of the mammalian host; together with some observations on the feeding of Chrysops and Cimex.

Ann. Trop. Med. Parasit., 42, 334 - 63.

_____, _____ and Willett, K.C., (1956)

Studies on the deposition, migration and development to the blood forms of trypanosomes belonging to the Trypanosoma brucei group. 1. An account of the process of feeding adopted by the tsetse fly when obtaining a blood meal from the mammalian host, with special reference to the ejection of saliva and the relationship of the feeding process to the deposition of metacyclic trypanosomes.

Ann. Trop. Med. Parasit., 50, 426 - 437.

Hagan, H.R., (1951)

"Embryology of the Viviparous Insects."

Ronald Press, New York.

Hoffmann, R., (1954)

Zur Fortplanzungsbiologie und zur intrauterinen Entwicklung von Glossina palpalis.

Acta. Trop. 11, 1 - 57.

Howard, L.O., (1911)

The House Fly. Disease Carrier. An account of its dangerous activities and of means of destroying it.

F.A. Stokes. New York.

Itard, J., (1968)

Resultats recents d'un elevage de Glossina tachinoides West. entrepris a Maisons-Alfort.

Proceedings of a panel on the control of livestock and insect pests by the sterile male technique organised by the joint F.A.O./I.A.E.A. division of Atomic Energy in Food and Agriculture.

Vienna 1967, pp 71 - 74.

_____ and Maillot, L., (1966)

Notes sur un elevage de Glossines (Diptera-Muscidae) entrepris, a partir de pupes expediees d'Afrique, a Maisons-Alfort (France).

Revue. Elev. Med. Vet. Pays. trop., 19, 29 - 44.

Jack, R.W., (1939)

Studies in the physiology and behaviour of Glossina morsitans Westw.

Southern Rhodesia, Mem. Dept. Agr., 1, 1 - 203.

Jackson, C.H.N., (1937)

Some new methods in the study of Glossina morsitans.

Proc. Zool. Soc. Lond., 1936, 811 - 96.

Jacquemin, P., (1956)

Un dispotif pratique pour nourrir les arthropodes hematophages.

Ann. Parasit. Hum. Comp., 31, 476 - 479.

Jordan, A.M., (1962)

The pregnancy rate in Glossina palpalis.

Bull. ent. Res., 53, 387 - 93.

_____ and Curtis, C.F., (1968)

Productivity of Glossina austeni Newst. maintained on lop-eared rabbits.

Bull. ent. Res., 58, 399 - 410.

_____, Nash, T.A.M. and Boyle, J.A., (1966)

The large scale rearing of G. austeni in the laboratory. II. The use of calves as hosts on seven days of the week.

Ann. trop. Med. Parasit., 60, 461 - 68.

_____, _____ and _____ (1967)

The rearing of G. austeni with lop-eared rabbits as hosts. I. Efficacy of the method.

Ann. trop. Med. Parasit., 61, 182 - 188.

Kernaghan, R.J. and Nash, T.A.M., (1964)

A technique for the despatch of pupae of Glossina and other insects by air from the tropics.

Ann. Trop. Med. Parasit., 58, 355 - 358.

Langley, P.A., (1966a)
The effect of environment and host type on the rate of digestion in the tsetse fly Glossina morsitans Westw.
Bull. ent. Res., 57, 39 - 48.

_____, (1966b)
The control of digestion in the tsetse fly, Glossina morsitans. Enzyme activity in relation to the size and nature of the blood meal.
J. Insect. Physiol., 12, 439 - 448.

_____, (1967)
The control of digestion in the tsetse fly Glossina morsitans: A comparison between field flies and flies reared in captivity.
J. Insect. Physiol., 13, 477 - 486.

Lumsden, W.H.R. and Saunders, D.S., (1966)
Tsetse Flies.
"Insect colonisation and Mass production."
Chapter 11, pp 153 - 174. Academic Press, New York.

Matheson, R., (1950)
"Medical Entomology."
Constable, London.

McDonald, W.A., (1960)
The laboratory rearing of Glossina morsitans submorsitans Newst. in Northern Nigeria.
8th Intern. Sci. Comm. Trypanosomiasis Res., p 247.
Commission for Technical Cooperation in Africa South of the Sahara, London.

Mellanby, H., (1937)
Experimental work on reproduction in the tsetse fly Glossina palpalis.
Parasitology, 29, 131 - 141.

_____ and Mellanby, K., (1937)
Rearing tsetse flies in captivity.
Proc. R. ent. Soc. London (A), 12, 1 - 3.

Mellanby, K., (1936)
Experimental work with the tsetse fly, Glossina palpalis, in Uganda.
Bull. ent. Res., 27, 611 - 633.

Nash, T.A.M., (1955)
Laboratory rearing of G. palpalis.
Ann. Rep. W. Afr. Inst. Tryp. Res. 1954, 30.

_____, (1963)
Progress and problems in the establishment and maintenance of laboratory colonies of Tsetse flies.
Bull. Wld. Hlth. Org., 28, 831 - 836.

_____ and Kernaghan, R.J., (1965a)
The weight and viability of puparia of G. austeni in relation to the conditions provided for pupal development.
Ann. trop. Med. Parasit., 59, 226 - 234.

_____ and _____, (1965b)

A high proportion of early deaths among uninseminated females of G. austeni.

Bull. ent. Res., 56, 65 - 66.

_____, _____ and Wright, A.I., (1965c)

A method for the prevention of skin reactions in goats used for feeding tsetse flies.

Ann. trop. Med. Parasit., 59, 88 - 94.

_____, _____ and Boyle, J.A., (1966a)

The large scale rearing of G. austeni in the laboratory. 1. The use of pregnant and non-pregnant goats as hosts.

Ann. trop. Med. Parasit., 60, 39 - 47.

_____, Jordan, A.M. and Boyle, J.A., (1966b)

The large scale rearing of G. austeni in the laboratory. III. Confirmation of the value of pregnant goats as hosts.

Ann. trop. Med. Parasit., 60, 469 - 481.

_____, _____ and _____, (1966c)

A promising method for rearing G. austeni on a small scale, based on the use of rabbits ears for feeding.

Trans. R. Soc. trop. Med. Hyg., 60, 183 - 88.

_____, _____ and _____, (1967a)

Retraction of the claim that host pregnancy affects pupal production by the tsetse fly.

Nature 216, 163 - 64.

_____, _____ and _____, (1967b)

A method of maintaining G. austeni singly, and a study of the feeding habits of the female in relation to larviposition and pupal weight.

Bull. ent. Res., 57, 327 - 336.

Nash, T.A.M. and Jordan, A.M., (1969a)

Further progress in the rearing of Glossina spp. at Langford near Bristol.

Trans. R. Soc. Trop. Med. Hyg., 63, 127 - 28.

_____, _____ and Boyle, J.A., (1969b)

The large scale rearing of Glossina austeni in the laboratory. iv. The final technique.

Ann. trop. Med. Parasit., 62, 336 - 341.

Parker, A.H., (1956)

Experiments on the behaviour of Glossina palpalis larvae together with observations on the natural breeding places of the species during the wet season.

Ann. trop. Med. Parasit., 50, 69 - 74.

Peterson, A., (1955)

A manual of entomological techniques.

Ohio.

Pinhão, R. de C., (1966)

Contribuição para o estudo da reproducao e ciclo evolutivo da Glossina morsitans West.

Anais. Inst. Med. trop., 23, 311 - 450.

Robinson, C.G., (1964)
Abnormality in the tsetse fly.
Trans. R. Soc. trop. Med. Hyg., 58, 579.

Rodhain, J. and Van Hoof, M.J., (1944)
Au sujet d'un elevage de Glossina palpalis en Europe et de quelques essais d'evolution chez cette glossine des Trypanosoma lewisi et cruzi.
Ann. Soc. Belge. Med. trop., 24, 54 - 57.

Roubaud, E., (1915)
Sur un essai d'elevage de Glossines dans les laboratoires d'Europe.
Bull. Soc. Path. exot., 8, 34 - 36.

_____, (1917)
Histoire d'un elevage de Glossina morsitans a 1'Institute Pasteur de Paris.
Bull. Soc. Path. exot., 10, 629 - 640.

_____ and Colas-Belcour, J., (1936)
Observations biologiques sur les Glossines (G. palpalis, G. morsitans).
Bull. Soc. Path. exot., 29, 691 - 696.

Saunders, D.S., (1960a)
Ovaries of Glossina morsitans.
Nature, 185, 121 - 122.

_____, (1960b)
The ovulation cycle in Glossina morsitans West. (Diptera: Muscidae) and a possible method of age determination for female tsetse flies by examination

of their ovaries.
Trans. R. ent. Soc. Lond., 112, 221 - 238.

_____, (1962)
Age determination for female tsetse flies and the age composition of samples of Glossina pallidipes, Aust., G. palpalis fuscipes Newst. and G. brevipalpis Newst.
Bull. ent. Res., 53, 579 - 595.

Southon, H.A.W. and Cockings, K.L., (1963a)
Laboratory maintenance of Glossina.
E. African Trypanosomiasis Res. Org. Rept. 1961, pp 30 - 33, Govt. Printer Nairobi.

_____ and _____, (1963b)
Fertilisation of G. morsitans in the laboratory.
E. African Trypanosomiasis Res. Org. Rept., 1962 - 63, pp 33 - 37, Govt. Printer, Nairobi.

Squire, F.A., (1951)
Observations on mating scars in Glossina palpalis.
Bull. ent. Res., 42, 601 - 604.

Swynnerton, C.F.M., (1936)
The Tsetse flies of East Africa. A first study of their ecology with a view to their control.
Trans. R. ent. Soc. Lond., 84, 1 - 579.

Teesdale, C., (1940)

Fertilisation in the tsetse fly, Glossina palpalis, in a population of low density.

J. Anim. Ecol., 9, 24 - 26.

Thomson, W.R., (1931)

On the reproduction of organisms with overlapping generations.

Bull. ent. Res., 22, 147 - 172.

Vanderplank, F.L., (1947)

Experiments in the hybridisation of tsetse flies (Glossina, Diptera) and the possibility of a new method of control.

Trans. R. ent. Soc. Lond., 98, 1 - 18.

_____, (1948)

Studies of the behaviour of the tsetse fly G. pallidipes in the field: influence of climatic factors on activity.

J. anim. Ecol. 17, 245 - 60.

Wigglesworth, V.B., (1954)

The physiology of Insect Metamorphosis.

Cambridge University Press.

Willett, K.C., (1953)

The laboratory maintenance of Glossina. I.

Parasitology, 43, 110 - 130.