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Autor: Balogun, R.A.
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Department of Biology, University of Ife, Ile-Ife, Nigeria

Maintenance of *Glossina palpalis* fed through bat's wing membrane on defibrinated blood

R. A. BALOGUN

Summary

Teneral laboratory-bred *Glossina palpalis* flies were successfully fed through bat's wing membrane on defibrinated blood by means of a technique developed in this laboratory. Using this technique almost 100% of the flies engorged blood through the membrane. Ten to fifteen flies were kept in Standard 'Geigy 10' or 'Geigy 15' cages and records were kept of mortality and the weights of the pupae produced; over 78% of the flies were still alive after 60 days of the experiment. Over 75% of the membrane-fed *G. palpalis* females produced pupae in the first 30 days of the females' reproductive life. The mean weight of the pupae produced by the membrane-fed flies was 24.9 mg. The results indicate that membranes made from the African fruit bat wings are usable, and that this type of membrane would be valuable for a medium scale rearing programme for tsetse flies.

Key words: Glossina palpalis; membrane feeding; reproduction rate; lifespan.

Introduction

The membrane feeding technique serves as a useful tool in nutritional and behavioural studies of insects which possess piercing mouth parts. The technique has been used in studies dealing with the transmissions of bacterial, viral and protozoan pathogens by various haematophagous arthropods. It is also being used to study the host-parasite relationship between various *Glossina* spp. and *Trypanosoma* spp. (for references, see Galun and Margalit, 1970). The suitability of a great variety of natural and synthetic membranes for feeding tsetse flies on the blood of different animals was tested over the years (Yorke and Blacklock, 1915; Cockings, 1961; Southon and Cockings, 1963; Rogers,

Correspondence: Prof. R. A. Balogun, Department of Biology, University of Ife, Ile-Ife, Nigeria

1971; Langley, 1972; Balogun, 1974). Recently, the bat wing membrane technique was used to collect saliva of three species of *Glossina* and it has proved to be a very useful method for studying the salivation behaviour of tsetse flies (Youdeowei, 1975).

In recent years there is an increasing awareness of the need to develop economic and efficient techniques for maintaining *Glossina* colonies in the absence of living host animals. This is particularly important when mass rearing of the flies for control by the sterility method is being planned (Galun and Margalit, 1970).

The present paper constitutes the results obtained for the maintenance of *Glossina palpalis* on defibrinated blood fed through wing membranes of the African fruit bat, *Eidolon helvum* Kerr.

Materials and methods

Glossina palpalis puparia used in this study were supplied by the Nigerian Institute for Trypanosomiasis Research, Kaduna and they were reared by a technique routinely used in this laboratory. The pupal jars used were transparent plastic containers. Some clean sand was poured into the containers and the pupae were placed on top of the sand. Not more than 100 pupae were kept in each container which has an internal bottom diameter of 6.5 cm and a height of about 9 cm. The mouth was covered with a circle of voile secured round the neck of the container by a rubber band. The plastic containers were placed on glass slabs about 5 cm above trays of water kept in an incubator at a temperature of 25° C ± 0.5° C and relative humidity of 75 to 80%.

Newly hatched adult *G. palpalis* were removed from the containers 24–28 h after emergence. Normally 10 or 15 female *G. palpalis* were kept in a Standard 'Geigy 10' or 'Geigy 15' cage.

Feeding membranes used were made from bats' wings obtained from populations of the African fruit bat, *Eidolon helvum* inhabiting forests on the University campus at Ile-Ife. The membranes were stretched on rectangular or circular plastic frames, 300 to 350 cm^2 (Balogun, 1975).

The flies were fed on defibrinated bovine blood collected aseptically once weekly from cows slaughtered at the abattoir, Ile-Ife. ATP (0.005 M) was added to blood samples before use in order to guarantee the presence of optimal concentration during feeding (Langley, 1972).

Feeding experiments were carried out by depositing 15 ml defibrinated blood sample on each glass plate (20 × 18 cm) and the plates covered with the appropriate bat's wing membranes. The whole set up was placed on a warm glass plate maintained at 38° C ± 0.5° C with water in a thermostatically-controlled water bath. Standard 'Geigy 10' or 'Geigy 15' cages containing ten or fifteen flies each were allowed to remain in contact with the warmed feeding surface for 15 min. The flies were offered blood daily except Sundays.

In initial feeding experiments high mortality among flies fed through membranes was probably due to bacterial contamination, because bacteria were detected from the intestines of dead flies which were dissected 5 to 10 min after death. This problem was significantly solved in subsequent experiments by autoclaving and heat-sterilising the equipment and other materials used.

Having been offered blood meal, 3 to 4-day-old females were mated 'en masse' in a big cage with netted sides adding about twice as many active males, at least 8 days old. The sexes were left together for 24 to 48 h after which all the males were removed from the cage. The females were then transferred to either 'Geigy 10' or 'Geigy 15' cages plus two males in each cage. The flies were kept at 25° C ± 0.5° C and 75 to 80% relative humidity.

Pupae produced were individually weighed not more than 24 h after their deposition and details of any fly that died were recorded daily.

Table 1. Summary of data from cages of *Glossina palpalis* reared on defibrinated blood through bat's wing membrane

Cage No.	Age of cage (days)	No. of initial female flies	No. of female flies alive	No. of pupae deposited	Mean weight of pupae (mg) ± S. E.
1	60	10	8	32	26.3 ± 0.4
2	60	10	8	30	24.8 ± 0.03
3	60	10	7	28	24.2 ± 0.04
4	60	10	8	30	24.8 ± 0.02
5	62	10	8	32	23.9 ± 0.02
6	62	10	7	30	25.0 ± 0.02
7	62	10	8	32	23.7 ± 0.02
8	62	10	8	34	24.9 ± 0.01
9	65	10	8	28	25.4 ± 0.02
10	65	10	8	32	26.0 ± 0.02
11	60	15	12	34	24.8 ± 0.02
12	60	15	12	36	25.2 ± 0.02
13	62	15	12	38	25.1 ± 0.02
14	62	15	12	34	25.1 ± 0.02
15	65	15	14	40	24.5 ± 0.01
Total	–	175	141	490	–
Mean					24.9 ± 0.18

Results

The results of this study are summarized in the data presented in Table 1. The number and the mean weight of pupae produced by each cage are given in Table 1. The role of ATP as a gorging stimulus in blood ingestion was tested as follows: One group of teneral flies of the same chronological age (24–36 h after emergence) were offered blood with the addition of ATP, through bat's wing membrane while another group of teneral flies (control) were fed on blood without the addition of ATP, through bat's wing membrane. The results showed that about 50% of the flies were stimulated to gorge through bat's wing membrane in the absence of ATP, while more than 95% feeding response was achieved when flies fed on blood containing 10^{-5} M ATP through the membrane. This observation supports the view expressed by Langley (1972) that ATP provides a gorging stimulus for tsetse flies. In the first four reproductive cycles of the female *G. palpalis* majority of the flies (about 80%) produced pupae. Thereafter, productivity fell slightly.

Discussion

The longevity of the membrane-fed *G. palpalis* females is given in Table 1. When the experiment was terminated at the 65th day, about 80% of the flies

were still alive. This suggests that *G. palpalis* flies were provided with adequate nutriment from the defibrinated blood for their basic metabolism to survive during the period of the study. In initial experiments high mortality often occurred in the fly cages and this was associated with blackened, distended abdomen of the flies at death. The reason for this mortality was thought to be due to possible bacterial contamination of the membrane or blood since bacteria were detected in the guts of dead flies. This problem was also reported by Mews and Ruhm (1971) and Langley (1972) in their in vitro feeding experiments for tsetse flies. In this study the problem was solved in subsequent feeding and rearing experiments by aseptic precautions that were taken.

The productivity of the membrane-fed flies indicate that over the first four reproductive cycles the majority of the flies produced a pupa. A decline in pupal production in later cycles was observed and the possible causes for this are under investigation. However, it would seem that increasing reluctance of the flies to feed and a lowered rate of digestion of the ingested defibrinated blood might contribute to the slight decline in pupal production in later cycles. In the later stage of this study, it was sometimes noted that there appeared to be undigested blood showing in the abdomen of the flies 24–36 h after feeding. Also, as there are no standard facilities available yet for rearing tsetse flies in this laboratory the flies were kept in an incubator after feeding. It was observed that pupal productions in the incubator seemed to be higher in early life than in later life. A similar observation was made by Jordan et al. (1968) who reared *G. austeni* Newst. in an incubator with lop-eared rabbits as hosts. It is likely that the temperature in an incubator is higher than in the insectary although in both environments temperature is difficult to measure precisely as it varies with both time and situation.

The results of this study show that it is possible to maintain experimental colonies of the tsetse on defibrinated blood through bat's wing membrane. Good survival can be achieved with rigorous aseptic breeding precautions. Because the bat's wing membrane is easily prepared and keeps well if stored in a refrigerator, it may also be a promising complementary technique for the economical large scale rearing of tsetse flies for control by the sterility method.

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