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NOTES TECHNIQUES

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A simple and cheap method for breeding of tsetse flies

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Résumé

The authors give a description of a simple breeding colony of tsetse flies which was established with a minimum of cost. It provides regular supply of flies for laboratory research and training purposes.

Summary

Les auteurs donnent la description d'une colonie de mouches tsétsé qu'ils ont établie de façon artisanale avec un minimum de frais. Cette colonie leur fournit régulièrement des mouches pour les recherches de laboratoire et pour l'enseignement.

Introduction

Breeding of tsetse flies has been a concern of many investigators dealing with african trypanosomiasis and its vector. Nowadays it may look very surprising how difficult it was to realise reproduction of tsetse flies under laboratory conditions fifty or more years ago. Roubaud (8) was the first to deal with successful experiments outside Africa, and maintained a *Glossina morsitans* strain for three years in Paris. Subsequently the Mellanby's (6) reared *Glossina palpalis* in London. At the Institute of Tropical Medicine of Antwerp Rodhain and Van Hoof (7) repeated their previous experiments from the Shaba province (Zaire) with *Glossina palpalis*. It is surprising to learn that these scientists usually worked with flies kept individually or in small numbers.

Geigy (1) reported in detail in 1948 on his large scale experiments at the Swiss Tropical Institute in Basle where a population of *Glossina palpalis* was reared and maintained for laboratory investigations. The real mass- rearing of tsetse flies started with the prospect for controlling tsetse flies by releasing irradiated males.

Hence, several large breeding colonies of tsetse flies have been set up in Africa and in Europe, harbouring tens of thousands of breeding females: Bobo Dioulasso (Burkina Faso), Vom (Nigeria), Tanga (Tanzania), Nairobi (Kenya), Maison-Alfort (France), Bristol (United Kingdom), Vienna (Austria), Antwerp (Belgium). Intensive tsetse fly research has been organized thanks to these large scale colonies; important field trials of release of sterile males in order to control or even eradicate the fly have been set up.

In vivo feeding on guinea-pigs or rabbits is successfully replaced by *in vitro* feeding- techniques through artificial membranes; artificial diets can be substituted to blood meals.

In view of our research and teaching programs a small breeding unit was established requiring a strict minimum of investment and maintenance to provide simple training facilities and a regular supply of tsetse flies for research purposes, of which the description is herewith given.

Material and Methods

In general all species of tsetse flies tolerate temperatures between 24-26° C but show marked differences in their humidity requirements. The optimal environmental conditions for the riverine species (*Palpalis*-group) are a relative humidity (RH) of 75-90 % whereas savannah species (*Morsitans* group) prefer lower RH (60-70 %). Optimal degree of humidity can be obtained by special sophisticated equipment, but in our experience, a simple reservoir filled with water may sometimes be sufficient. In order to prevent excessive fly activity a 12-hour day-length is recommended.

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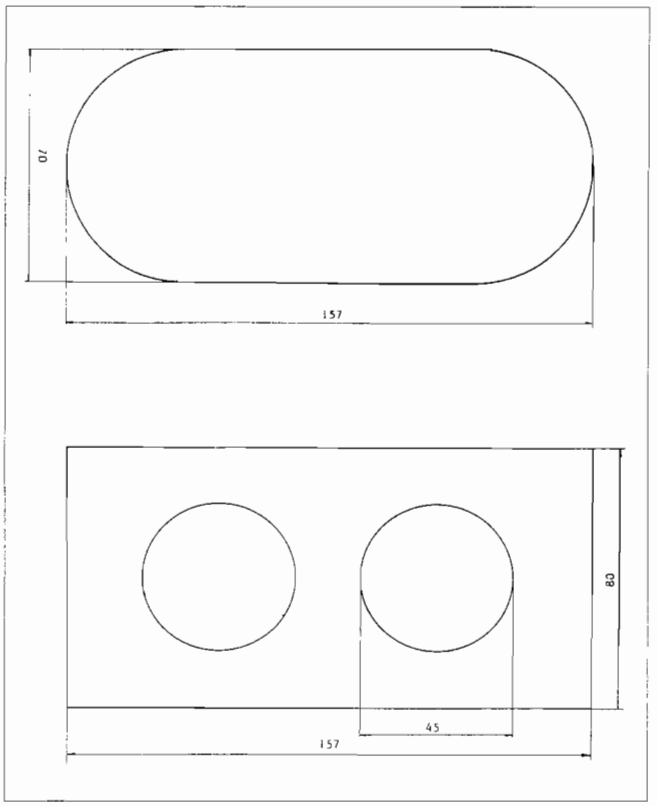

Figure 1 · Upper side view of a female tsetse fly cage (measurements in mm.).

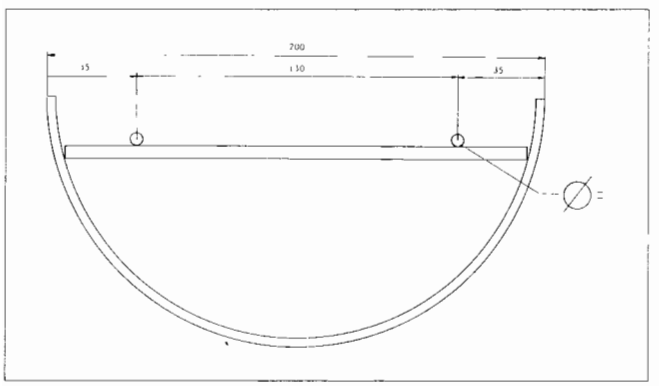

Figure 2: Cross-section through a gutter in which the produced larvae can be collected (measurements in mm.).

About 20 flies are kept in standard cages. For the construction of these cages factors such as : durability, cost, easy cleaning, easy manufacturing, adaptability to feeding surface, etc. must be taken into account. Polyvinyl chloride (PVC) rain-pipes with a diameter of 125 mm are cut in pieces of 8 cm or 4 cm for the cages for females and for males respectively. These PVC rings, are heated in hot water (70-80° C) and shaped into an elongated fly cage using a wooden mall (Fig. 1)

The flies are brought into, or removed from the cages through a hole closed with a cork. They have a tightly-streched non absorbant terylene netting (diameter 3 mm) on the open sides and are placed on bars, above a gutter in which the produced larvae can be collected (Fig. 2).

Tsetse flies can be immobilized by means of nitrogen gas or by chilling. At 1-4° C the flies are quickly immobilized with little or no influence on reproduction and survival (5). Obviously, these methods need some special equipment as a source of nitrogen or a small deepfreezer. On the other hand one can also use a transparent plastic tube with a diameter a little smaller than the hole of the cage and closed at one side with a gauze. When such a tube is used it is essential not to put too many flies in one cage. As female flies need to copulate only once in their life and to prevent excessive mating, males 6-8 days old, are put in a cage containing 2-3 days old female flies. They are kept together for 48-72 hours. Males are kept in the colony because they are capable of successfully inseminating up to six females. Normally it takes about 20 days to produce a first larva.

Every morning, pupae are collected and kept in the fly room during the pupal period (about 30 days). Puparia may be maintained at a slightly lower temperature and at a somewhat higher relative humidity than adult flies. After dropping their first larva mature females continue to produce almost every ten to twelve days. The first larval period, the inter-larval period and the pupal period are temperature dependent and can be calculated (25 days after larviposition the pupae are transfered to the eclosing cages. These are made out of rectangular PVC-tubes (20 x 10 cm), cut in 10-15 cm sections.

A hole, slightly bigger than a small Petri-dish (diameter = 6 cm) is made in one of the large sides (bottom).

A revolving or sliding door is made to cover this hole. A hole (same size as in normal cage) is made in one of the opposite corners (top), in order to remove flies.

Open sides are covered with terylene netting (1 mm).

To avoid premature mating (female tsetse tend to refuse a second copulation and teneral males are incapable of successfully inseminating a female), the pupae produced in one day are left together in the same eclosion cages. Female flies emerge earlier than males and are transfered to adult cages before the latter emerge.

Tsetse flies can be fed in two ways · *in vivo* and *in vitro*. For the *in vitro* feding of the flies special equipment and precautions for collecting aseptic blood and sterility (irradiation) are necessary *In vivo* feeding method on the other hand is very simple and better suited for a small colony of flies. It is usually performed on either piglets, goats, cattle, guinea-pigs, and lop-eared rabbits (3).

These animals must be kept under special conditions. The use of insecticides on the host and in the fly room must be STRICTLY prohibited. Treating host-animals, with antibiotics may also cause a high mortality and a reduction of reproduction (9).

Our flies are fed for 15 minutes on the flanks of these guinea-pigs. The guinea-pigs, not shaved or washed, are stocked in such a way that the cages, containing the flies, can be placed between them. On each guinea-pig about 250 flies a day can be fed for 2 days a week. In most laboratories tsetse flies are fed every day except sunday. Our flies are fed every morning except on friday, in the afternoon, and left unfed over the week-end.

Results

The I.T.M. Veterinary Department has a tsetse fly colony with 5 species: *G. tachnoïdes, G. palpalis palpalis, G. palpalis gambiensis, G. fuscipes fuscipes* and *G. morsitans centralis.* All these flies are kept under the same conditions (25° C, RH 75-80%) in a small room of about 30 m³. To monitor the colony, parameters such as female mortality, mortality after copulation, date of copulation, date of separation, eclosion percentage as well as number and sex of the emerged flies are daily recorded. The emerged flies are separated and put in adult cages. To have an idea of the age of the flies, the colony is divided in age groups of ten days containing flies with the same ovarial configuration.

According to Itard and al. (3) satisfactory reproduction has been obtained when daily female mortality is $\leq 2\%$, and when at least 1.8 pupae per female per 30 days are produced, with an eclosion percentage $\geq 85\%$.

Because of the 5 days feeding regime, we have a higher mortality after a week-end. This can be prevented by feeding on saturday. Our colony is in expansion phase, this means that all the offspring produced are reintroduced. Its growth rate is represented by the formula: $N_t = N_o\, e^{rmt}$ in which:

t = time interval in days
N_o = number of females at time O
N_t = number of females at time T
rm = Coefficient of natural growth. This value is characteristic of the species considered is function of the conditions under which the population is raised and can be calculated (4).

Conclusions

Although up to 20 years ago rearing glossina was difficult, we now experienced that it can be done in a simple and cheap way. A minimum of equipment for climatization is necessary; the environmental conditions for all species should be maintained at the optimum level and variations should be minimized. The flies have to be fed in a precise way *in vivo* on guinea-pigs or other hosts 5 ot 6 times a week.

A tsetse fly colony must be kept out of any contact with insecticides. Footwear as well as clothes are a known source of insecticide contamination and measures must be taken to prevent this danger. We realize that it would be to labour-intensive to built up a large colony in this artisanal way, but a population of several hundreds female flies can be kept in such a simple and cheap way, giving ample opportunity to provide regular supply of tsetse flies for laboratory research or training purposes.

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Literature

1 Geigy R., 1948. Elevage de *Glossina palpalis.* Acta Tropica, **5**, 201-218.

2. Glasgow J.P., 1970. The Glossina community, pp. 348-381. in: H.W. Mullighan, The African *Trypanosomiasis,* London.

3. Itard J., Bauer B., 1984. Elevage des glossines. Synthèse. Rev. Elev. Méd. Vét. Pays trop., **37**, 143-175.

4. Jordan A.M., Curtis C.F., 1968. Productivity of *Glossina austeni Newst.* maintained on lop eared rabbits. Bull. ent. Res., **58**, 399-410.

5. Maillot L., 1970. Influence du froid sur les tsé-tsé et ses indications. Rev. Elev. Méd. Vét. Pays trop., **23**, 327-331

6. Mellanby H. and Mellanby K., 1937. Rearing tsetse flies in captivity. Proc. R. Entomol. Soc. London., **12**, 1-3.

7 Rodhain J., Van Hoof M.T., 1944. Au sujet d'un élevage de *Glossina palpalis* en Europe et de quelques essais d'évolution chez cette glossine de *Trypanosoma lewisi* et *cruzi.* Ann. Soc. Belge Méd. Trop., **24**, 54-57.

8. Roubaud E., 1917. Histoire d'un élevage de *Glossina morsitans.* Bull. Soc. Pathol. Exot., **1**, 629- 640.

9. Sellin E., Boudoiseau G., Cuissance D., 1979. Effets sur *Glossina palpalis gambiensis* de substances médicamenteuses administrées au lapin hôte-nourricier. Rev. Elev. Méd. Vét. Pays trop., **32**, 267-275.

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