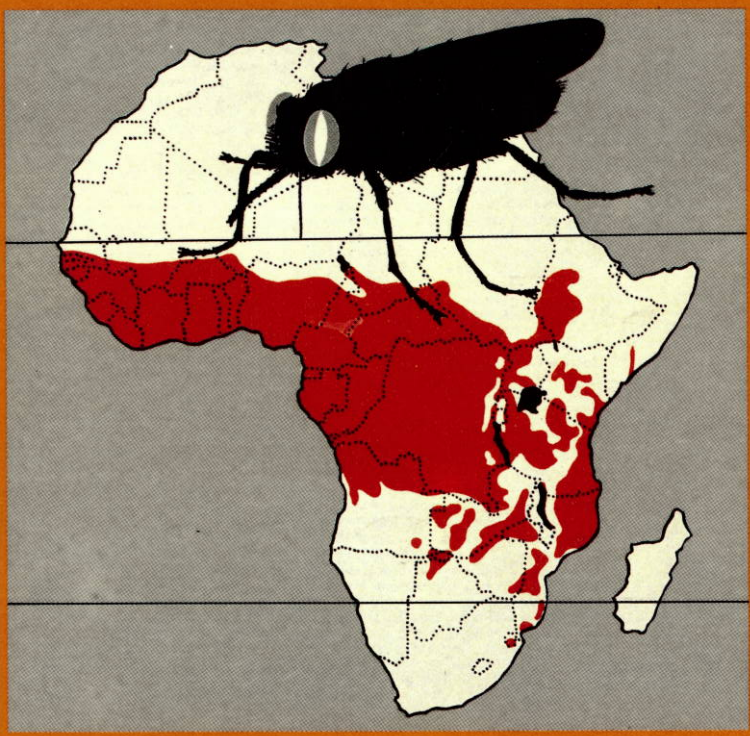
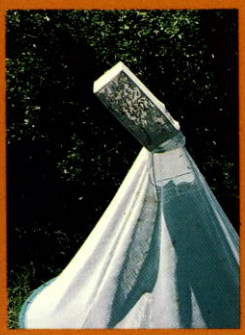
# Sterile Insect Technique for Tsetse Control and Eradication

PROCEEDINGS OF THE FINAL RESEARCH CO-ORDINATION MEETING

VOM
PLATEAU STATE
NIGERIA
6–10 JUNE 1988

ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE





INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1990

# STERILE INSECT TECHNIQUE FOR TSETSE CONTROL AND ERADICATION

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# FOREWORD

Animal trypanosomiasis, transmitted by tsetse flies, is one of the prime limiting factors in African development south of the Sahel. Control or eradication of tsetse flies is one of the two main methods of controlling the disease, the other being drug treatment of livestock.

Tsetse control or eradication depends to a large extent on the use of insecticides. Frequently, these are applied by fixed wing aircraft or helicopters. Ground sprays are used less frequently now than in previous years. Destruction of tsetse habitats by sheer clearing and elimination of wild animals as well as livestock are not commonly used at the present time for tsetse control.

During the past decade the development of the sterile insect technique (SIT) as a means of eradicating tsetse flies has been developed to the point of practical application. Included in this volume are reports of two successful SIT programmes, one in Burkina Faso covering 3000 $km^2$ and another in Nigeria covering 1500 $km^2$. The success of these two programmes clearly demonstrates the efficacy of the technology.

Recent development of the insecticide impregnated screen (frequently called targets) offers a relatively inexpensive and environmentally acceptable method of reducing tsetse populations prior to the release of sterile insects. A combination of the insecticide impregnated screen to reduce the population followed by the release of sterile male tsetse flies is an efficient way of eradicating these pests.

At present, the limiting factor to large SIT projects is the lack of rearing facilities to produce sufficient tsetse flies for radiation sterilization and release. The current system of rearing tsetse flies is labour intensive and research and development are needed to introduce limited automation in order to reduce labour requirements. Other researchable problems which need solutions include methods of shipping tsetse fly pupae long distances and methods of sexing tsetse fly pupae.

The Proceedings include the final reports of scientists co-operating in the 5 year Co-ordinated Research Programme entitled Application of the Sterile Insect Technique for Tsetse Eradication or Control. The work of these scientists contributed directly to the success of the field programmes utilizing SIT for tsetse fly eradication.

The participants in this programme wish to record their appreciation of and admiration for E.D. Offori, who so effectively co-ordinated the programme.

## *EDITORIAL NOTE*

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# REVIEW

# Co-ordinated Research Programme on the Application of the Sterile Insect Technique for Tsetse Eradication or Control

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## 1. INTRODUCTION

In January 1967 a panel of experts convened in Vienna to consider the potential of the sterile male release technique for controlling livestock insect pests. The sterile insect technique (SIT) had at that time recently been successful against the screw-worm fly in the United States of America. The participants recommended that research on SIT be continued and expanded and mention was made of the tsetse fly, *Glossina*, as a pest amenable to control by the technique.

Long before the programme started, research on the biology, behaviour and nutrition of tsetse flies had been in progress at several locations within and outside Africa. In Europe, colonies of tsetse flies had been established in Lisbon, Bristol, Maisons-Alfort, Paris, and Antwerp. The first attempt to rear *Glossina morsitans* and *G. austeni* in the IAEA Laboratory at Seibersdorf was made in 1967, using material provided by the laboratories in Lisbon and Bristol, respectively.

While the primary objectives of the pioneering laboratories were to maintain tsetse colonies for research on their physiology, nutrition and disease transmission, the programme of the Insect and Pest Control Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture aimed at developing techniques and practices for utilizing sterile males in tsetse control/eradication programmes.

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## 2. CO-ORDINATED RESEARCH PROGRAMME ON TSETSE FLIES

In August 1967 the first Co-ordinated Research Programme, entitled Control of Animal Insect Pests by the Sterile Male Technique, was initiated. Under this all embracing title, livestock insect pests such as *Dermatobia, Hypoderma, Rhodnius* and *Glossina* spp. were researched by eight participating laboratories.

In June 1971 a panel of experts was assembled in Paris which included some of the participants of the ongoing Co-ordinated Research Programme. The panel identified five main areas requiring research emphasis for applying the sterility principle in tsetse fly control/eradication campaigns, notably:

(1) Development of tsetse mass rearing technology, including studies of tsetse nutrition and dietary requirements
(2) Irradiation studies and evaluation of the sterility and radiation effects on fly competitiveness
(3) Development of surveillance methods for effective ecological studies as a prerequisite for sterile male release
(4) Development of population suppression techniques
(5) Investigation of the packaging, handling and transport of sterile flies from the producing centre to the release point.

A new programme, entitled Tsetse Fly Control or Eradication by the Sterile Male Technique and initiated in 1974, was the direct outcome of the 1971 meeting in Paris. During the 10 years (1974–1984) of the programme, a total of 21 scientists participated at various times as research contractors and another seven served on a cost free basis. Towards the end of the 10 year period, and especially in support of the field project in Nigeria (the Biological Control of Tsetse (BICOT)), the need arose to elucidate the dietary requirements of tsetse flies in view of the recent advances made in in vitro feeding technology and artificial diet research, and to study trypanosome transmission by released sterile flies. Accordingly, a programme on using isotopes to develop diets and to study disease transmission was introduced in 1981 and ran until 1984, with nine participants from six countries.

The fourth programme, entitled Development of Methodologies for the Application of the Sterile Insect Technique for Tsetse Eradication or Control (1984–1988), resulted in this publication. Its main objectives were:

(a) To provide a research base and support for ongoing and future tsetse control/eradication programmes involving the application of SIT
(b) To develop methods for evaluating and monitoring tsetse SIT campaigns
(c) To develop strategies for incorporating SIT into national and regional (area wide) tsetse and trypanosomiasis control programmes.

Accordingly, emphasis was placed on:

(i) Improvement of in vitro tsetse mass rearing techniques, including the use of synthetic diets
(ii) Development and improvement of tsetse fly traps, attractive devices for population sampling, monitoring and suppression
(iii) Studies of tsetse ecology and population dynamics
(iv) Determination of the vectorial capacity of tsetse species targetted for control by SIT.

A total of 21 scientists from 13 countries participated in the programme at various times.

## 3. SUMMARY OF THE CONTRIBUTIONS

The current publication contains the contributions made by the scientists who participated in the fourth Co-ordinated Research Programme. A range of topics, covering both field and laboratory activities, was addressed.

Information is provided on the crucial steps taken in the planning and implementation stages of successfully completed pilot projects in Nigeria (Paper IAEA/RC/319.3/1) and Burkina Faso (Paper IAEA/RC/319.3/3), with due regard given to the eminent role that insecticide impregnated targets can play in barrier erection and population suppression prior to the release of sterile males. Reference is also made to the technical back-stopping provided by the FAO/IAEA Entomology Unit at Seibersdorf in the process of technology transfer related to tsetse mass rearing, irradiation and monitoring procedures (Paper IAEA/RC/319.2).

Baseline data are given on the population dynamics of *G. tachinoides* and *G. palpalis palpalis* in peridomestic agroecosystems in Nigeria (Paper IAEA/RC/319.3/4) and with seasonal trends in the population dynamics of *G. fuscipes fuscipes* on Buvuma Island, Lake Victoria, Uganda (Paper IAEA/RC/319.3/5). Also reported are the results of a mark–recapture experiment carried out with *G. morsitans morsitans* and *G. pallidipes* on Antelope Island, Zimbabwe (Paper IAEA/RC/319.3/6). Refinement of a mathematical model is proposed that will enable field workers to optimize tsetse population estimates, to assess more accurately the necessary inputs (e.g. the number of targets/traps required per unit area) and to predict the impact of the adopted control/eradication strategy. The paper on surveillance of tsetse fly and cattle populations for trypanosomes in the BICOT Project area (Paper IAEA/RC/319.3/7) refers to the use of sentinel herds for monitoring the progress during control operations and underlines the need for multispecies tsetse control/eradication to solve the trypanosomiasis problem in a geographically defined area.

The fundamental and applied aspects of tsetse fly nutrition are discussed, with special emphasis placed on the nutritional quality of various blood diets, including freeze dried blood, and semi-defined artificial diets (Papers IAEA/RC/319.3/8, 9, 12). The significance of particular individual blood constituents is documented by the comparative observations made on other haematophagous arthropods such as stable flies and ticks. Paper IAEA/RC/319.3/10 deals with the function of the tsetse mycetome and the factors affecting the nature and structure of endosymbionts. Irradiation by gamma rays is reported to produce only slight structural changes and non-significant changes in endosymbiont numbers. Paper IAEA/RC/319.3/11 reports on virus particles infection occurring in a self-supporting colony of *G. pallidipes* reared under semi-natural conditions. Paper IAEA/RC/319.3/13 gives comparative data on the effects of whole blood and artificial diets on trypanosome establishment in the midgut and refers to the effect of lectin secretion in the fly midgut. It is confirmed that feeding flies prior to their release in SIT programmes is effective in reducing the risk of *Trypanosoma brucei* sensu lato and *T. congolense* type infections in tsetse flies. Paper IAEA/RC/319.3/14 elaborates on an appropriate technique for sterilizing natural populations of tsetse flies, making use of juvenile hormone mimics applied to attractive devices. The last contribution (Paper IAEA/RC/319.3/15) highlights the importance of electrophoretic techniques for the understanding of tsetse genetics and the monitoring of genetic variations in laboratory reared and field caught flies of the *morsitans* group. Special considerations are also made regarding hybrid male sterility involving maternally inherited factors.

# ERADICATION OF *Glossina palpalis palpalis* (Robineau-Desvoidy) (DIPTERA: GLOSSINIDAE) FROM AGROPASTORAL LAND IN CENTRAL NIGERIA BY MEANS OF THE STERILE INSECT TECHNIQUE

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## Abstract

ERADICATION OF *Glossina palpalis palpalis* (Robineau-Desvoidy) (DIPTERA: GLOSSINIDAE) FROM AGROPASTORAL LAND IN CENTRAL NIGERIA BY MEANS OF THE STERILE INSECT TECHNIQUE.

A land area of 1500 km$^2$ under an agropastoral development plan in southern Plateau State, Nigeria, was selected to develop and apply the sterile insect technique (SIT) for the eradication of *Glossina palpalis palpalis*. In vitro and in vivo feeding techniques were used to mass breed the species in the laboratory for the production of more than 1.5 million sexually sterile males for release. In the field the initial tsetse fly population could be reduced to less than 10% within 6–12 weeks by continuous trapping and placement of insecticide impregnated targets. Extensive trapping or positioning of targets did not lead to eradication of the species. However, eradication was achieved when sufficient sterile males were released on a weekly basis to maintain a minimal ratio of 10 sterile males to 1 fertile wild male fly for at least three generations. The area was divided into three operational zones and after application of SIT one each was found to be free of *G. p. palpalis* in 1985, 1986 and 1987, respectively. It was further demonstrated that after eradication the entire area of 1500 km$^2$ could be secured against reinvasion by maintaining barriers of insecticide impregnated targets.

## 1. INTRODUCTION

The applicability of the sterile insect technique (SIT) to control or eradicate *Glossina* species has been demonstrated in the past by various experiments and field projects [1–4]. Takken et al. [5] reported on the progress of the Biological Control of Tsetse (BICOT) Project funded by Belgium, the Federal Republic of Germany, Italy, Nigeria, Sweden and the United Kingdom, as well as through the regular budgets of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency. By releasing sexually sterilized *Glossina palpalis palpalis* males from a colony reared at project headquarters, Vom, Plateau State, Nigeria, the target species was eradicated from a 435 $km^2$ pilot area with dense riverine habitats in an extensively cultivated area near Lafia, also in the Plateau State (Figs 1 and 2). During the 3 years of the pilot eradication phase, valuable experience on the application of SIT was gained and efficient prerelease tsetse control strategies were employed in the remaining BICOT field area. Biconical traps and insecticide impregnated targets were used instead of sequential aerosol treatment to reduce the riverine tsetse fly population before the release of sterile males [5, 6]. In the laboratories the mass rearing technology for the support of sterile males that had been developed by the FAO/IAEA Entomology Unit of the IAEA Laboratory at Seibersdorf was transferred to Vom and modified according to the requirements of the project [7, 8].

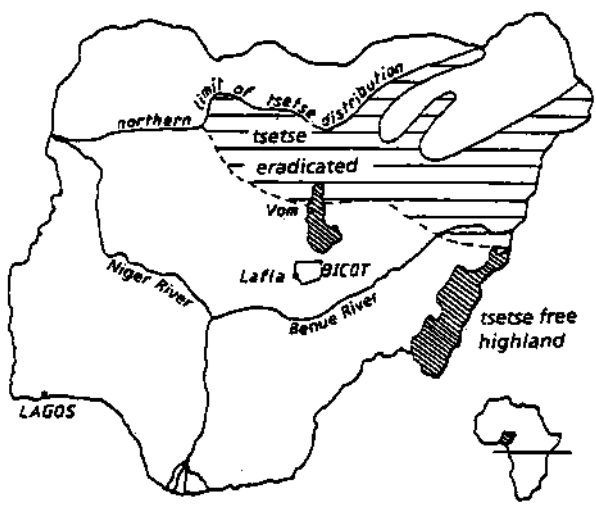


*FIG. 1. Map of Nigeria, indicating present tsetse distribution. Note the position of Vom and the BICOT Project area.*

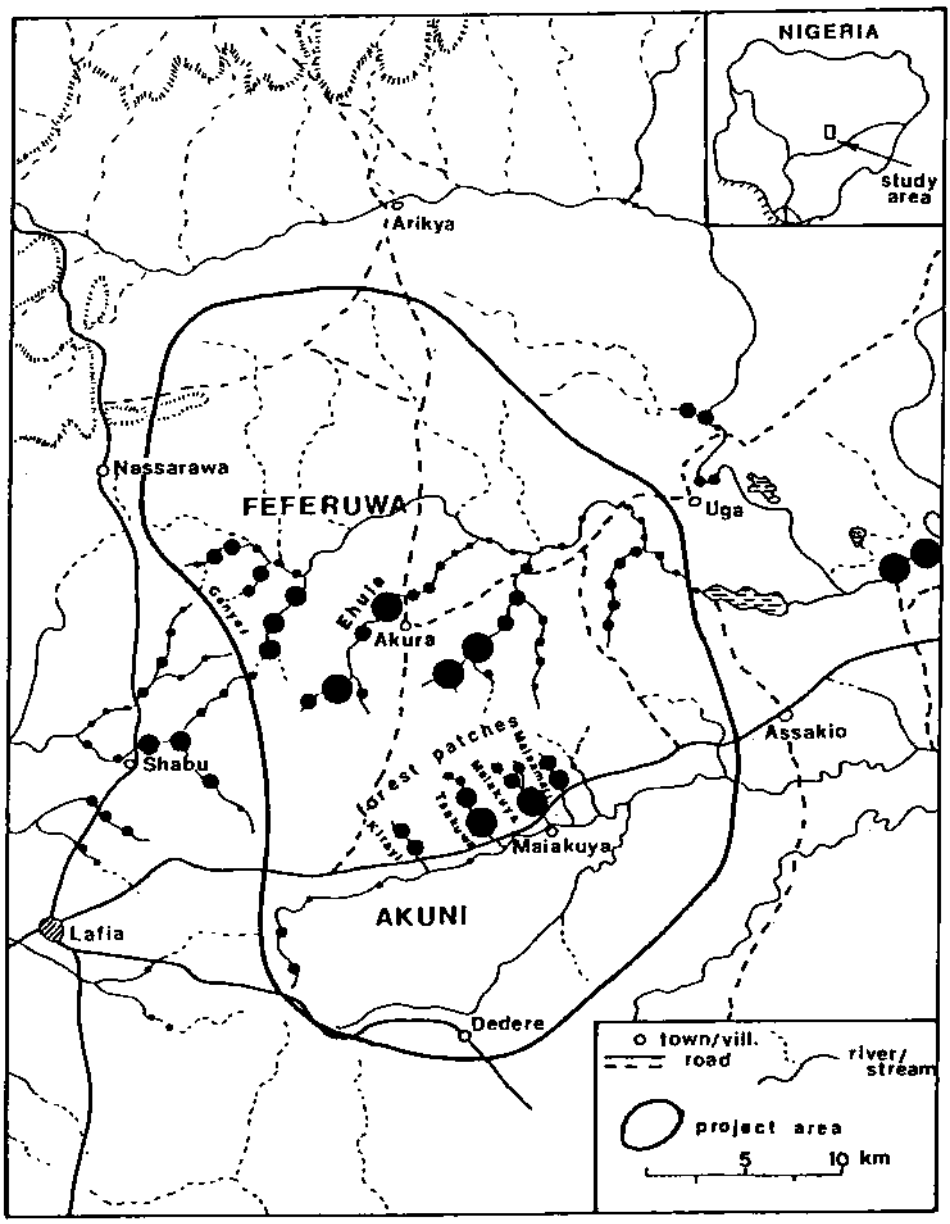


*FIG. 2. BICOT Project area and main foci of* G. p. palpalis *(black dots).*

In addition to support from the donors previously mentioned and the dedication of the BICOT staff, the success of the project was greatly enhanced by co-operation with the National Veterinary Research Institute and the Nigerian Institute for Trypanosomiasis Research (NITR) at Vom, the continuous support of the IAEA Laboratory at Seibersdorf and by other institutes conducting research through the IAEA Co-ordinated Research Programme.

A description is given of the technical activities of the BICOT Project, and the laboratory and field operations.

## 2. LABORATORY OPERATIONS

### 2.1. *G. p. palpalis* mass rearing

Colonization of *G. p. palpalis* was initiated at BICOT, Vom, in November 1979 with pupae from a colony established in 1974 at Seibersdorf. The IAEA colony originated from pupae collected in Nigeria [9]. In the BICOT insectaries the control systems were set to maintain adults and pupae at a constant climate of 24.0 ± 1.5°C and 85 ± 5% relative humidity and a 12 hour photoperiod. Both in vivo and in vitro feeding techniques were applied in different fly colonies to mass rear *G. p. palpalis.*

#### *2.1.1. In vivo rearing*

The size of the in vivo fed *G. p. palpalis* colony was determined by the number of guinea pigs available as host animals and reached its maximum size of 62 637 *G. p. palpalis* females in May 1984; the maximum weekly puparia production (28 214) was achieved in early April 1986. From early 1983 to mid-1987 the average number of *G. p. palpalis* females kept and the number of puparia produced were 44 430 and 15 589, respectively; a total of more than 3.6 million puparia was produced. Between mid-1985 and mid-1987 an average weekly male output of 4147 sterile males was achieved by the in vivo system, of which about 17.8% were 'used' males, i.e. used for mating colony females before being radiosterilized and released.

#### *2.1.2. In vitro rearing*

Initially, lyophilized bovine and porcine blood were imported and reconstituted for in vitro feeding. Use of locally collected fresh frozen bovine blood in early 1984 was a major breakthrough in improving the in vitro colony performance. In mid-1985 the recording, evaluating and reporting system was modified by the installation of electronic data processing. This facilitated detailed analysis of fly colony performance and calculations to predict the weekly sterile male output. The in vitro fed *G. p. palpalis* system became self-supporting for the first time in October 1985.

From early 1983 to mid-1987 the average number of *G. p. palpalis* colony females maintained by the in vitro feeding regimen was 47 542 females, with a maximum of 139 427 females in early March 1986. During the same period approximately 3 million puparia were produced by in vitro fed females. From mid-1985 to mid-1987 the in vitro colonies produced a weekly average of 3574 males for release, including 27% 'used' males.

## 2.2. Maintenance of a host animal colony for in vivo feeding

Maintenance of a healthy and self-sustaining colony of guinea pigs for in vivo feeding of tsetse flies in the laboratory proved to be problematical and expensive, particularly with reference to special vitamin requirements, drugs for medication and a properly prepared feed mixture. For in vivo feeding of *G. p. palpalis* it had been established that when guinea pigs were challenged with 200 or less colony females at a time, they could be used as 'feeders' twice per week. To maintain an in vivo fed *G. p. palpalis* colony of 30 000 females, a host animal colony of 1000 guinea pigs was required. On average, only 40–45% of the animals maintained were at any time used as 'feeders'; the rest were kept in the breeding stock or were below 550 g, the required minimal body weight to be used as feeders (i.e. weaners and sick animals).

Repeatedly, guinea pigs had to be purchased locally to replace those that died in the colony. The colony reached its maximal size of 2313 guinea pigs in May 1984.

## 2.3. Blood collection and in vitro diet preparation

To reduce the overall expense for sterile male production and to keep at least half of the fly rearing operations independent from the maintenance and presence of host animals, development of an in vitro or membrane feeding technique for mass rearing of the target species was made a major objective. In the in vitro feeding technique flies ingest blood, warmed up to body temperature, through a silicone membrane. Initially, lyophilized bovine and porcine blood [10] were obtained from Seibersdorf. The dried blood was reconstituted with water, supplemented with adenosine triphosphate (ATP: $10^{-3}$M) and used as the in vitro diet for feeding. Blood was not obtained locally in order to eliminate the risk of feeding blood contaminated with trypanosomes to colony flies or males that would be released.

After October 1981 the final diet was irradiated in a gamma source (originally $^{137}Cs$, later $^{60}Co$) at 0.5–1.0 kGy in order to reduce microbial contamination, which could cause high mortality in the fly colony [11].

In 1982 the diet of pure lyophilized bovine or porcine blood was changed to a mixture of 50% lyophilized bovine and porcine blood. Tests conducted at Seibersdorf had indicated that the advantages of each, i.e. good fecundity from bovine blood and high offspring weight from porcine blood, could be combined by feeding the

mixture. Starting in January 1983 all equipment used for in vitro feeding was heat sterilized in hot (dry) air ovens in order to reduce even further the risk of microbial contamination.

In early 1984 tests on locally collected fresh bovine blood, done in collaboration with the NITR, demonstrated that a 0.5 kGy treatment reproductively inactivated trypanosomes and that *Trypanosoma vivax, T. congolense* and *T. brucei* in cattle blood were killed by slow freezing (to −20°C) and thawing. Thereafter, local, fresh bovine blood was collected routinely and combined in approximately equal amounts with imported, reconstituted, lyophilized porcine blood. Heparin was added to the fresh blood (800–1000 IU/L) to prevent clotting.

In 1985 a system for routine quality control screening of the in vitro diet was introduced. Screening included a search for bacterial contamination, a routine 25 day feeding test with a small group of *G. p. palpalis* females and, when necessary, dissection of females.

In 1986 collection of 30–50 L per week of porcine blood (1200–1500 IU heparin/L) was initiated from local sources. These quantities were sufficient to maintain the in vitro colonies. From early 1987 all the in vitro fed *G. p. palpalis* colonies were fed a mixture of fresh frozen bovine and fresh frozen porcine blood, and were thereby independent of imported food.

Up to mid-1987 about 5000 L of fresh bovine blood had been collected from the Jos abattoir and about 1000 L of fresh porcine blood had been purchased from a meat processing company in Jos. The average amount of blood that could be collected from one animal during slaughter was 4.5 L for bovines or 2 L for pigs. Usually, less than 10% of the collected blood was rejected because of failure to pass the quality control screening, either due to high microbial contamination or to low nutritional sufficiency, as determined by the 25 day quality control feeding test.

### 2.4. Sterile male handling and release

The project required an efficient procedure for the marking, irradiation, packing, transport and release of male flies in the various sectors of the target eradication area.

For each *G. p. palpalis* colony the distributable excess of males was calculated 2 days before each release, taking into account the colony dynamics and the requirements for colony maintenance. A proportion of the excess males, usually 10–50%, was marked on the thorax with acrylic paint to indicate the date and location of their release.

Usually, the males were exposed in air to 118–120 Gy of gamma radiation (first $^{137}Cs$, later $^{60}Co$) the day before release.

Males, 3–5 days before release, were offered a bloodmeal daily, at least two of which were on guinea pigs: one the day preceding release (after irradiation) and one early in the morning of the release day.

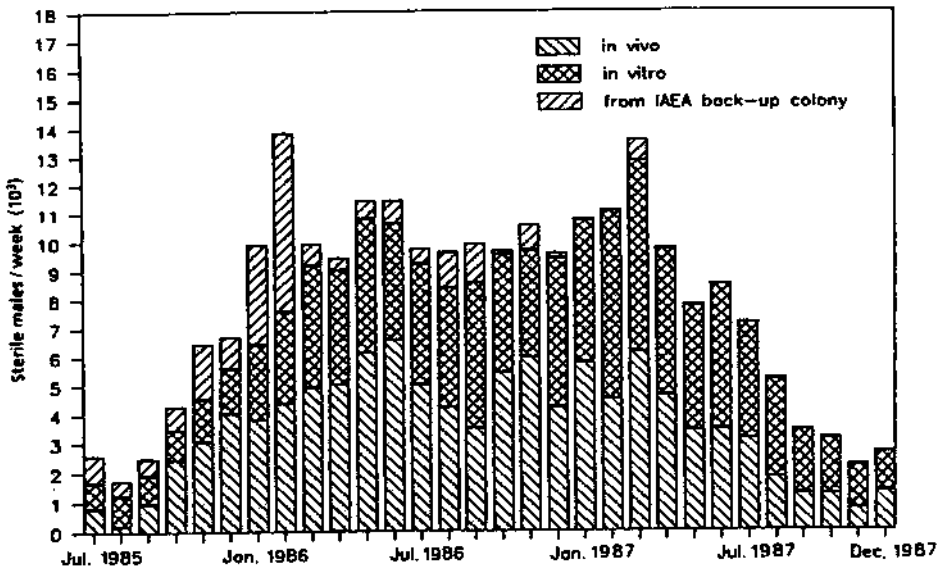


*FIG. 3. Weekly number of sterile males supplied by the BICOT* G. p. palpalis *colonies.*

Up to 1000 males were placed in single 10 L polyethylene containers with mosquito gauze covered windows and a large sliding door. The containers were then placed inside insulated boxes and transported to the field. Provided the males arrived at the release point early enough (before the temperature became too high), more than 95% survived and immediately dispersed from the release container into the target habitat.

Starting in 1985 males that had already been used for mating colony females were also marked, irradiated and released. However, these 'non-virgin' males were probably less viable in the field, as indicated by their reduced recapture rate (about 20–30% of the average recapture rate of the younger 'virgin' males).

A total of about 1.5 million sterile *G. p. palpalis* males had been released up to mid-1987, an average of about 10 658 $\pm$ 2771 males per week between early 1986 and mid-1987. In relation to the number of colony females maintained, or to the number of puparia produced, the sterile male output of the in vivo system was up to 50% higher than that with the in vitro fed colonies: from the same number of puparia, 26% versus 17% from the in vivo and in vitro systems, respectively, were males available for field release. To obtain an equal male output from both systems, the size of the in vitro fed colony had to be increased.

Figure 3 shows the number and origin of males released per week from mid-1985 to mid-1987.

## 2.5. Data evaluation and quality control

To conduct efficient tsetse control and eradication operations, flexibility in applying strategies and making timely decisions are essential. In a SIT programme a large amount of information is collected from the colony production units in the laboratory. Initially, evaluation of all laboratory data was done manually. As of mid-1986, when the fly colonies were expanded, a microcomputer was used to evaluate the performance of *G. p. palpalis* colonies. Thus, means were achieved for rapidly tracing and identifying the source of problems, which in turn enabled corrective measures to be implemented quickly. Furthermore, the sterile male output became predictable.

The BICOT laboratory staff maintained laboratory equipment and delicate biological material for the production and deployment of sterile males. To accomplish this, as in any commercial production, quality specifications were required. Therefore, in addition to the routine checking of equipment and insectary conditions, as well as ensuring continuous supplies such as animal feeds, blood, etc., quality control procedures for the diverse aspects of the project operations were developed and routinely applied.

The acceptable quality specifications established for all aspects of laboratory work with biological materials were as follows:

*Fly colonies*

| | |
|---|---|
| Minimum adult emergence from puparia | 85% |
| Maximum average daily mortality of colony females | 1.2% |
| Maximum portion of 'blood' mortality | 35% |
| Minimum number of puparia deposited per female per 10 days | 0.55 |
| Maximum portion of 'class A' puparia. | 10% |

*In vitro diet*

| | |
|---|---|
| Maximum acceptable microbial contamination of fresh blood after 0.5 kGy irradiation treatment (CFU = colony formation unit) | 30 CFU/mL |
| Minimum production value after 25 day feeding test | 0.65 |
| Maximum acceptable microbial contamination of prepared and proportioned diet (after second irradiation with 100 kGy). | 1 CFU/mL |

*Guinea pig host animal colony*

| | |
|---|---|
| Maximum acceptable weekly mortality | 1% |
| Minimum required female fecundity every 6 weeks | 0.6 |
| Minimum 'feeder' weight | 550 g |
| Minimum offspring weight. | 75 g |

| | |
|---|---|
| *Sterile males* | |
| Age at release: 'virgin' males | 3–5 d |
| Age at release: 'non-virgin' males | 19–25 d |
| Maximum total handling and transport mortality: | |
| 'virgin' males | 5% |
| 'non-virgin' males. | 10% |

## 3. FIELD OPERATIONS

The BICOT field area, situated approximately 200 km by road south of the project laboratories at Vom, is part of the 9400 km$^2$ Lafia Agricultural Development Project, an extensive agricultural development project in southern Plateau State, Nigeria. The BICOT field area covers 1500 km$^2$ and is drained by about 250 linear kilometres of river. It is situated in the subhumid region of Central Nigeria at the transition between the southern Guinea vegetation zone and the derived savannah (Figs 1 and 2). The area has an annual rainfall of about 300 mm, all falling between April and October. It has a high potential for both agriculture and livestock development and comprises the headwaters of the Feferuwa River in the north and the Akuni River in the south. The western boundary zone is that part of the Mada River catchment area from the Akwanga–Lafia road (through Nassurawa and Shabu) in the west to the common watershed with the Ganye Rivers I and III and the Ehula River. The eastern boundary starts at the Feferuwa River in the north and continues to the south via the Alawagana Rivers and Rafin–Gogo to the Akuni River. Most streams within the project area have a well defined and very dense fringe of vegetation (from 40–70 m but up to a maximum of 300 m wide), whereas the upland is under cultivation by subsistence farmers.

By means of aerial surveys the cattle population in the wet and dry seasons was estimated to be less than 40 000 and above 50 000, respectively [12, 13].

The three major river systems in the target area, i.e. the Akuni catchment zone, the Achiba and the Ehula–Ganye River systems, were treated as separate operational areas.

### 3.1. *G. p. palpalis* eradication in the Akuni catchment zone

Originally, it had been planned to reduce the wild tsetse population, prior to the release of sterile males, by aerial application of non-persisting insecticides [14, 15]. Later, it was decided to explore whether other, lower cost and environmentally safer methods such as traps [16] and insecticide impregnated targets [17] could be used instead. Furthermore, to achieve the maximum possible efficiency of the technique, investigations were made on the extent to which the wild fly population could be reduced before sterile male releases, and at what ratio and frequency the sterile

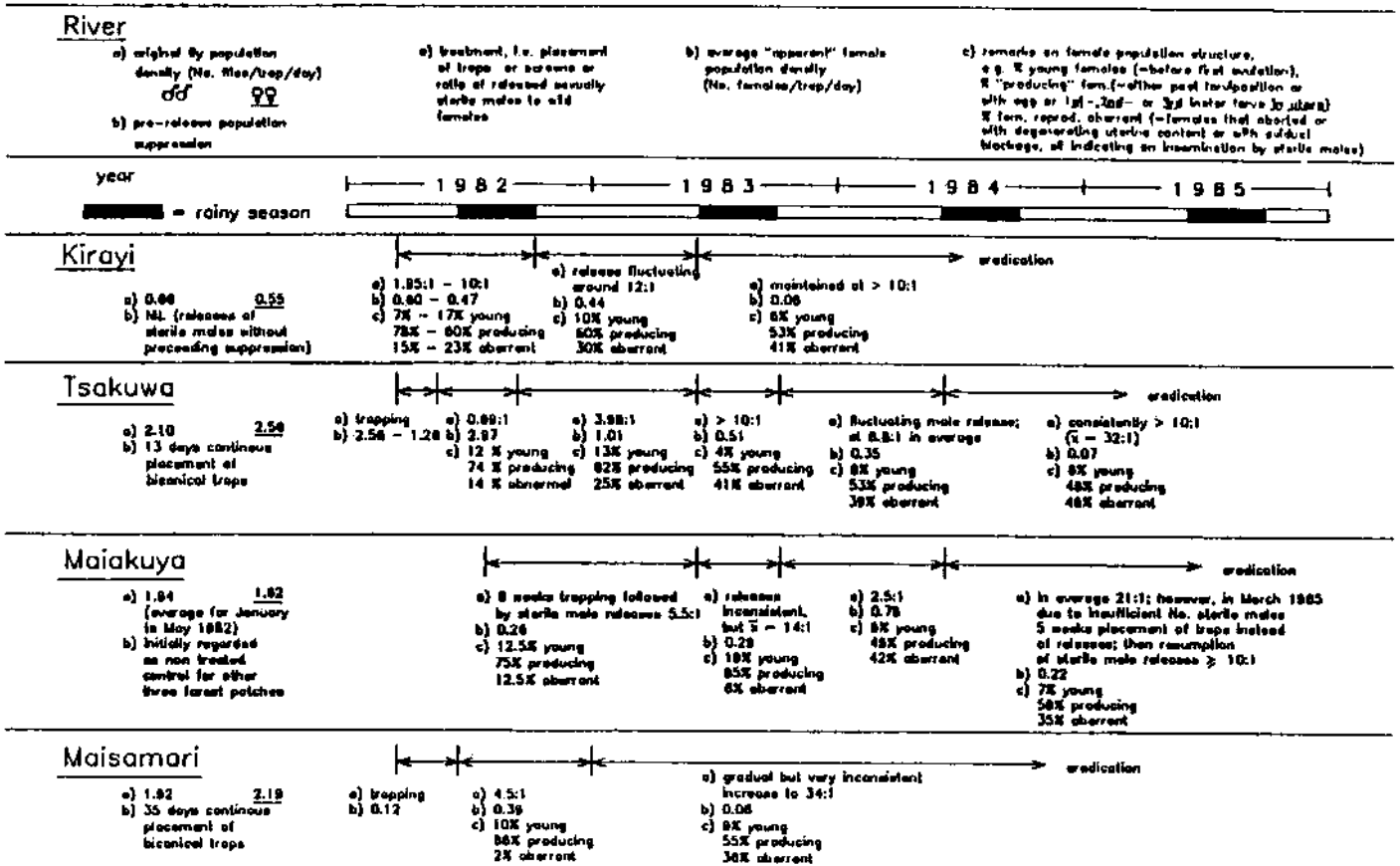


*FIG. 4. Eradication of* G. p. palpalis *in the Akuni catchment area. Four separate forest patches, i.e. Kirayi, Tsakuwa, Maiakuya and Maisamari as experimental release zones, were subjected to different tsetse control/eradication strategies.*

males had to be released in order to maintain an optimal sterile mating pressure on the wild population. The impact of sterile males on the wild target population was studied to gain information on the progress towards eradication.

The Akuni catchment zone, which covers an area of 435 $km^2$ (approximately 30% of the project area) and is drained by four small rivers, i.e. the Kirayi, Tsakuwa, Maisamari and Maiakuya Rivers as well as part of the main Akuni River, was regarded as a pilot area for developing the control, release and eradication strategies. The four forest patches fringing the four small rivers mentioned above had different *G. p. palpalis* densities and were, therefore, subjected to different prerelease population suppression treatments. Figure 4 summarizes the original fly population densities in the four forest patches of the Akuni catchment zone, the tsetse fly eradication and control treatments (including prerelease population suppression) and their effects on the wild female populations.

Two weeks of trapping in the Tsakuwa Forest reduced the average female population density by 50%. After 7 weeks of continuous use of biconical traps in the Maisamari Forest, where the original apparent fly density was slightly lower than that in the Tsakuwa Forest, a reduction of 95% was achieved. During the rainy season releases of sexually sterile males (in mean ratios of 4.5 to 1 wild female in Maisamari and, initially, 1.95 to 1, later 10 to 1, in Kirayi) resulted in a recovery or only in a stabilization of the target tsetse population and had no obvious, or at best only a very small, effect on the composition of the wild female population. However, during the dry season, when tsetse habitats along rivers in the neighbourhood were more isolated from each other as well as from adjacent river systems, a constant ratio of only 3.98 sterile males to 1 wild female in the Tsakuwa Forest was sufficient to establish an increased portion (25%) of females with reproductive aberrancies. Inconsistent releases, even if they periodically led to ratios of above 14:1, proved to be inefficient, particularly in the rainy season (see Fig. 1, treatment of the Maiakuya Forest during the rainy season 1983). Consistent releases that maintained a sterile male to wild female ratio above 10:1 caused more reproductive abnormalities in the female population and resulted in a collapse of the target population. In March 1985 insufficient numbers of sterile males were available for release in the Maiakuya Forest. Instead of continuing sterile male releases at ineffective ratios, biconical traps were placed in the habitat until effective sterile male releases ($>10:1$) could be resumed. Use of traps at a time of advanced tsetse population control (48.8% of the captured females showed reproductive abnormalities at the time the releases were interrupted) did not cause a delay in achieving eradication; *G. p. palpalis* was eradicated from the Maiakuya Forest early in the rainy season of 1985.

### 3.2. *G. p. palpalis* eradication in the Achiba River system

The Achiba River system, which is located north of the Akuni catchment area, drains about 388 $km^2$ of land (26% of the project area). It includes 27 km of unin-

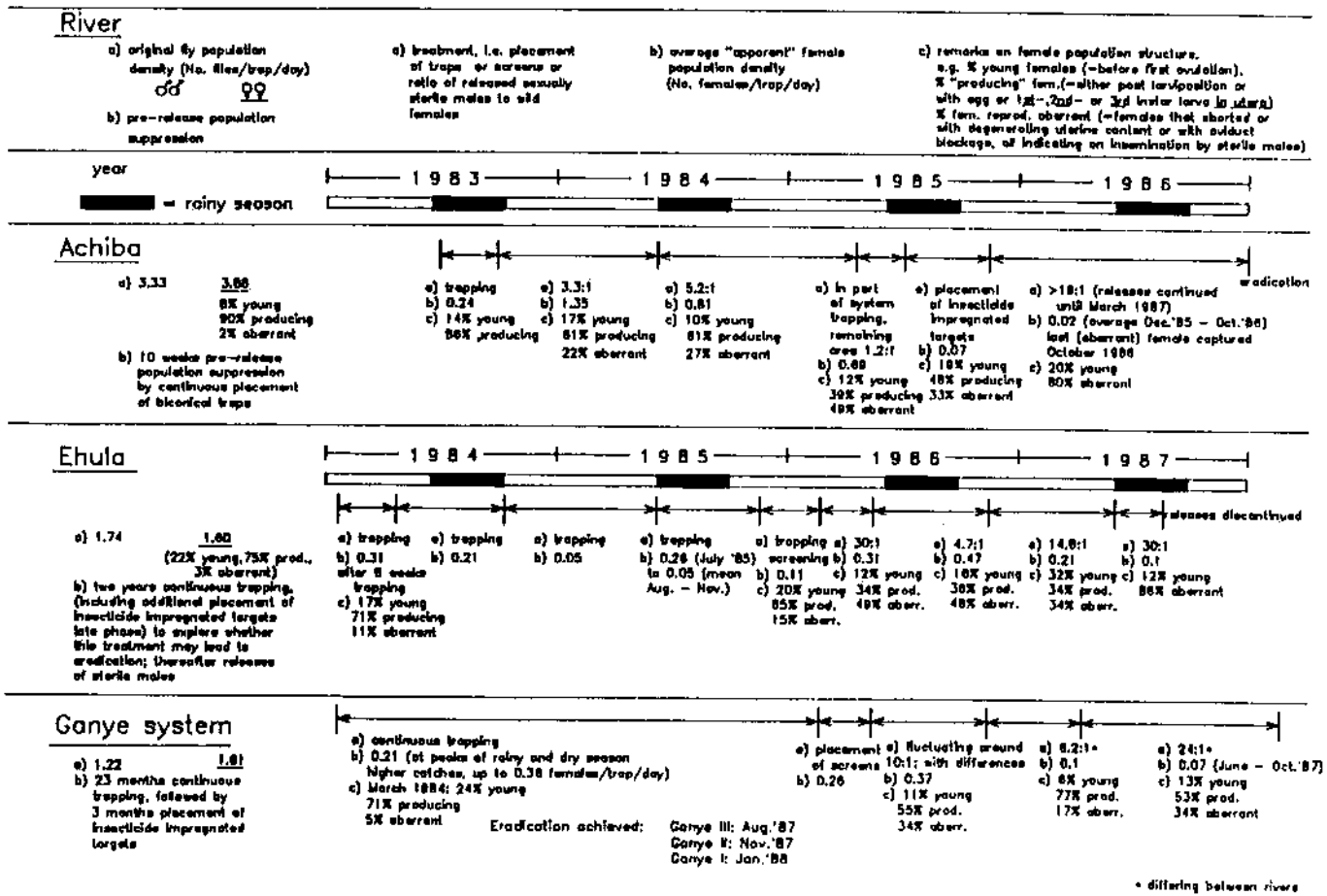


*FIG. 5. Eradication of* G. p. palpalis *in the Achiba, Ehula and Ganye River systems.*

terrupted riverine forest with an average width of 50 m. Figure 5 summarizes the *G. p. palpalis* control and eradication treatments in the fly habitat of the Achiba River system. Continuous placement of biconical traps in the rainy season of 1983 reduced the wild female population density by 94% and caused a shift in the female population structure towards a slightly increased portion of 'young' females (before first ovulation). A sterile male to wild female ratio of only 3.3:1 could be established during the dry season 1983–1984. Although this ratio was insufficient to further reduce or maintain the density of the relic wild fly population, there was an increase in the portion of females with reproductive abnormalities. An increase in the sterile male to wild female average ratio of 5.2:1 for the rainy season of 1984 to March 1985 led to a reduction in the total female population density and in the portion of young females and to an increase in the female portion with reproductive aberrancies. As of March 1985 the release of sterile males had to be discontinued, first in a part of and later in the entire Achiba River system. Instead, traps and later insecticide impregnated targets were placed in the fringing forest. At the time releases were discontinued, 49% of the captured females showed reproductive abnormalities as a result of being inseminated by sterile males. The traps and targets used from the early to the late rainy season of 1985 prevented the *G. p. palpalis* wild population from rebuilding. During the rainy season, when the fly habitat along the river was greatly extended, the apparent density of females in the Achiba Forest was less than during the late dry season; however, the average portion of young females had slightly increased. Except for the Amagara tributary, where no wild females had been captured since the late dry season of 1985, mass releases of sterile males (> 19 sterile males to 1 wild female) were resumed in mid-October 1985. In Amagara, insecticide impregnated targets were kept in position in order to prevent a reinfestation by *G. p. palpalis* from the main portion of the Achiba River. As a result, the relic target population collapsed in late 1985. No more wild females could be captured as of November 1985 and the few unmarked males that were trapped during 1986 were most likely unmarked released sterile males.

### 3.3. *G. p. palpalis* eradication in the Ehula and Ganye River systems

The Ehula and Ganye River systems consist of four main rivers and streams as tributaries (such as the Achiba) of the Feferuwa River, and form the northern boundary of the BICOT Project area. An area of about 675 $km^2$ (45% of the operational area) with good agricultural prospects is drained by these rivers.

In parts of the Akuni catchment area as well as in the tsetse habitat of the Achiba River system, 6–10 weeks of trapping reduced the original fly population density by up to 94%. In February 1984 biconical traps were placed in the Ehula River system to explore whether or not extensive application of this method could eradicate *G. p. palpalis*. Six weeks of continuous trapping reduced the apparent density of *G. p. palpalis* females by 80%. During the remaining period of the dry season

the apparent female density in the Ehula Forest was further reduced and maintained throughout the rainy season of 1984 at 13% of the original population density, i.e. 0.21 females per trap per day. In the dry season of 1984–1985 an average of 0.05 females per trap per day was captured in Ehula. During the rainy season of 1985, in spite of continuous trapping, the apparent female density recovered to the level of the previous rainy season, i.e. 0.26 females per trap per day, and decreased again to 0.05 thereafter. In late 1985 targets were placed between the trap positions before releasing sterile males. However, this did not have any effect on the relic fly population density and after 2 years of continuous trapping plus 1 month of additional suppression measures by insecticide it was concluded that impregnated targets cannot eradicate *G. p. palpalis* in the dense riverine forest of the southern Guinea vegetation zone. Sterile male releases were initiated in early 1986 at an initial ratio of 30 sterile males to 1 wild female. As a result, within 3 months the portion of females with reproductive abnormalities increased to 49%. However, the low apparent wild female density at the beginning of the male releases could not be maintained. In spite of weekly releases of sterile males at the established release sites in the Ehula River system during the rainy season, an average sterile male to wild female ratio of only 4.7:1 could be maintained. Although the average portion of females with reproductive abnormalities remained at 48%, the average apparent female density increased to 0.47 flies per trap per day; the portion of young females also increased. Detailed analysis of the data showed that a relic fly population (about 80% of the total females captured) existed in a relatively large uninterrupted segment of riverine forest (about 300 m wide) insufficiently covered by the sterile male releases. Therefore, in early 1987 releases were intensified in this area. From the late dry season to the early rainy season of 1987, when the released sterile male to wild female ratio was 30:1, on average, only 0.1 females per trap per day were captured and 88% of the trapped females had reproductive abnormalities. The sterile male supply from the fly colonies was sufficient until mid-1987 when, for the first time, no females were captured during monitoring. Usually, releases are continued for an additional few weeks; instead, owing to a shortage of sterile males, the river system was strung with insecticide impregnated targets at 100 m intervals.

In the Ganye River system of three tributaries to the Feferuwa River, continuous trapping was conducted for 23 months, followed by 2 months of treatment with insecticide impregnated targets. The average apparent density of females during the period of continuous trapping was 0.2 females per trap per day (12% of the original fly population density); this included higher catches at the peak of the rainy and dry seasons each year. The placement of insecticide impregnated targets during the mid to late dry season of 1985–1986 did not result in a further reduction in the wild female population density. Release of sterile males was initiated in March 1986. Although the average ratio of sterile male to wild female was 10:1, the wild female density slightly increased initially. During the dry season of 1986–1987 the sterile male to wild female ratios differed between the three Ganye Rivers: in

Ganye I, where most females had been captured, a 16:1 ratio was achieved, whereas in Ganye II and Ganye III the ratios were 3.6:1 and 2:1, respectively. Although the apparent female density could be reduced during this period to 0.10 females per trap per day, the high portion of producing females (77%) confirmed that there were small breeding foci in the Ganye River system that were not sufficiently covered by sterile males. During the mid rainy season of 1987, when the female density had been reduced to 0.07 females per trap per day (average for June to October), the number of sterile males available for release decreased (see Fig. 3). In spite of this, a ratio of 24 sterile males to 1 wild female was achieved because of the small number of wild females remaining. However, the number of males available for release was too low to cover the entire area with an equally high number. Therefore, the distribution of the relic female population had to be analysed very carefully to ensure that the few males available for release were fully utilized at the few locations where captured young and producing females indicated that breeding was still continuing at a low level. This strategy proved successful, since eradication of *G. p. palpalis* was achieved in Ganye III in August 1987; Ganye II and Ganye I followed in November 1987 and January 1988, respectively.

TABLE I. PARTICULARS OF GROUND SPRAYING AND IMPREGNATED SCREENS USED IN THE BICOT PROJECT AREA (DRY SEASON 1983–1984)

| Ground spray (one application only) | | Impregnated screen (one application only) | |
|---|---|---|---|
| Total area (linear km) | 30 km | Total area (linear km) | 165 km |
| Insecticide | Dieldrin (DIELDREX 18) | Insecticide | Deltamethrin (DECIS 0.4% ultra low volume) |
| Concentration | 4% | Concentration | 0.05% |
| | | Total screens | 1200 |
| Amount | 828 kg (4600 L of 18%) | Amount | 144 g (36 L of 0.4%) |
| Insecticide/linear km | 27.6 kg/km | Insecticide/linear km | 0.88 g/km |
| Man-days | 1064 (19 working days) | Man-days | 180 (10 working days) |
| Total staff | 56 | Total staff | 18 |
| Efficiency | 35.5 man·day/linear km | Efficiency | 1.1 man·day/linear km |

### 3.4. Security against reinfestation

The efficacy of using an insecticide impregnated target to intercept the movement of the riverine tsetse species, a low technology and environmentally safe device, was successfully tested in 1984 and has since been established for creating a barrier against reinfestation in the BICOT Project area. In direct comparison, the technique was more cost efficient than the direct application of insecticide to the environment by ground spraying (Table I).

Deltamethrin impregnated targets, totalling about 1080, were set at intervals of 100 m around the periphery of the project area, including the western boundary zone. Targets were reimpregnated every 4 months.

The tightness of the target barrier system was confirmed by releasing about 10 000 marked and sterilized *G. p. palpalis* females at several locations outside the border area. None of these females was captured inside the project area. However, with the increase in human and livestock activities in the area, numerous additional crossing or watering points on the river systems were created. Such points are potential foci for a reintroduction of flies that follow cattle or are passively transported from outside the project area. In view of this threat, additional screens were placed at these points.

Strict surveillance is needed to minimize the rate of screen losses and damage. Up to 80% loss of screens was sometimes recorded within a 4 month reimpregnation period. Because of this, intense efforts were made to involve the local human inhabitants in erecting and maintaining the insecticide impregnated screen barriers.

## 4. DISCUSSION

The different technical activities of the BICOT Project aimed at the development of the SIT methodology and its application to eradicate *G. p. palpalis* from 1500 $km^2$ of land within an area under an agricultural development plan. In the rearing operations valuable experience on the various parameters affecting the sterile male output was gained and incorporated into a system of routine quality control procedures. Although the in vivo fed colony provided more sterile males per producing female, the problems associated with the maintenance of a host animal colony were a challenge for *G. p. palpalis* mass production. Initial difficulties in the in vitro feeding system, especially the need for sufficient amounts of high quality diets, were progressively overcome.

In the dense riverine tsetse habitat of the BICOT Project area efficient, cheap and environmentally safe methods were applied to reduce the *G. p. palpalis* population by more than 90%. Extended application of these methods, however, did not eradicate the insect. The sterile male production units of the project provided sufficient males to eradicate the relic population. The following observations were made with regard to the effect of sterile male releases on the composition and size of the

target population. With weekly releases of sterile males, a consistent ratio of at least 10 sterile males to 1 wild male or female had to be maintained, which is higher than the estimated required 'effective' ratio (a theoretical trend analysis of *Glossina* spp. population reduction using SIT is given in Ref. [18]). Consistent releases below this 10:1 ratio, or inconsistent releases that were, on average, only at or above this minimal ratio, were ineffective in preventing the re-establishment of a breeding population (see the Akuni catchment area, Fig. 4). The experience gained in the Ehula Forest revealed the importance of routinely analysing the distribution of released sterile males and their impact on the density and composition of the wild female population, not only on average for the complete river but also separately for different segments of the entire habitat. Only then could small foci with a breeding population or with fly immigration be identified and appropriately treated, e.g. by creating release points in addition to the routine releases in the vicinity of earlier recognized 'hot spots' such as water crossings.

If insufficient sterile males were available to continue release operations in a river system at an optimal ratio, it proved better to interrupt the releases and place traps or insecticide impregnated targets, or both, in the area rather than to continue with inefficient ratios (Maiakuya, March 1985; Achiba, rainy season 1985).

With the reducing density of the wild population and the consistent number of sterile males released, the average ratio of sterile to wild males or females for the treated river systems is shifted greatly in favour of the released flies. In SIT eradication programmes against other pest insects with much higher original population densities than larviparous tsetse flies with a low reproductive capacity, the number of sterile males released may be reduced according to the diminishing wild population, whereby the ratio of sterile to wild males or females remains constant. In the BICOT Project releases of sterile *G. p. palpalis* males were initiated at a very low density of the target population and it was found that two factors had to be considered to determine the required limit of released sterile males: (1) the minimal ratio of sterile males to wild males or females, and (2) the minimum number of released sterile males to provide a sufficient male distribution to cover the entire fly habitat.

BICOT has demonstrated the efficacy of the sterile male technique by eradicating *G. p. palpalis* from a target area of 1500 $km^2$ in an agroecosystem within the subhumid region of Central Nigeria. However, the problem of animal trypanosomiasis in the area will partially persist unless *G. tachinoides,* which is still present at a few locations of the BICOT Project area, is also eradicated. In spite of this, a very sharp increase in livestock movement in the BICOT area was observed in 1988. An increasing number of cattle owners are taking advantage of *G. p. palpalis* eradication and are settling in the core area of the former eradication activities.

While the screen barriers to prevent *G. p. palpalis* re-immigration are maintained, efforts are being undertaken to explore the possibility of applying SIT to wipe out *G. tachinoides* from the few breeding locations within the area freed from *G. p. palpalis.*

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# RESEARCH AND DEVELOPMENT IN THE IAEA LABORATORY AT SEIBERSDORF IN SUPPORT OF BICOT FOR THE ERADICATION OF *Glossina palpalis palpalis*

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**Abstract**

RESEARCH AND DEVELOPMENT IN THE IAEA LABORATORY AT SEIBERSDORF IN SUPPORT OF BICOT FOR THE ERADICATION OF *Glossina palpalis palpalis*.

A brief summary is given of the major contributions made by the FAO/IAEA Entomology Unit of the IAEA Laboratory at Seibersdorf and the Insect and Pest Control Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in Vienna to the Biological Control of Tsetse (BICOT) Project in Nigeria. Most of the procedures used for the laboratory mass rearing of *Glossina palpalis palpalis* and the field operations in BICOT were based on techniques developed at Seibersdorf.

## 1. INTRODUCTION

The success of the Biological Control of Tsetse (BICOT) Project in Nigeria has derived, in part, from the contributions made by the FAO/IAEA Entomology Unit of the IAEA Laboratory at Seibersdorf. For approximately 4 years (1973–1977), i.e. prior to the project becoming operational, tsetse fly research was undertaken at Seibersdorf specifically in anticipation of BICOT. This preproject phase was supported largely by the Federal Republic of Germany through secondment for 3 years of one full time scientist to Seibersdorf and through provision of equipment for processing blood and developing in vitro rearing techniques.

Participation of the Entomology Unit in BICOT related activities intensified when the project became operational in 1979. Not only was the laboratory involved

in research and development activities to improve tsetse mass rearing, irradiation and quality control of mass produced flies, but it also maintained a back-up colony which produced puparia to support the BICOT fly colony, as required. In addition, staff assisted in the design of the mass rearing facility in Vom, Nigeria, participated in training Nigerian nationals seconded to the project and frequently provided technical advice on the location of laboratory and field operations.

The recently concluded BICOT Project was supported by the Nigerian Government through the provision of personnel and laboratory and office facilities as well as resources for maintaining the project during the 8 years of operation. Throughout the research and development phase and the actual project activities, BICOT received financial support from the following donor countries: Belgium, the Federal Republic of Germany, Italy, Sweden and the United Kingdom.

## 2. REARING OF *Glossina palpalis palpalis*

Laboratory investigations on this species started in July 1974. The founding population, from which production stocks were derived, was supplied by the Nigerian Institute for Trypanosomiasis Research at Kaduna from puparia collected in the field at Abuja. Initially, the fly colony was maintained using guinea pigs as host animals.

### 2.1. In vivo rearing

The BICOT plan of action included the use of live animals as well as artificial feeding techniques to mass rear the target species. In support of this plan, an in vivo system was investigated involving the use of guinea pigs. Research at Seibersdorf emphasized the following:

(1) Design of tsetse fly rearing equipment: chillers, cages, holding and feeding racks, and a control system for temperature and humidity regulation
(2) Determination of the optimal tsetse fly rearing conditions and study of all the aspects relating to reproductive biology: oogenesis, spermatogenesis, spermatophore formation, sperm transfer and insemination, embryogenesis, pregnancy dynamics, larviposition and emergence pattern
(3) Improvement of tsetse fly handling and holding procedures, e.g. sexing, mating, feeding, recording and evaluating fly performance and quality control
(4) Investigations to determine the optimal number of flies per animal per feeding day under conditions of adequate food supply
(5) Investigations on the quality of tsetse puparia, including use of a mechanical sorting device for evaluating the quantitative and qualitative components in a mass rearing operation.

A significant development involved the testing of different types of cages and holding systems. In the standard in vivo tsetse fly rearing system, mated female flies were regrouped in lots of 20–40 individuals per PVC cage. An alternative holding system was later developed in which 200 females were held in a 10 L plastic container. Using the experience gained from these two systems, estimates were made of the requirements for maintaining a colony of 100 000 female *G. p. palpalis* and a production model was eventually developed.

## 2.2. In vitro rearing

During 1976 small scale experimental colonies of *G. p. palpalis* were established on an in vitro system at Seibersdorf from the progeny of females that had been maintained on guinea pigs. The salient steps towards realization of a workable in vitro technology were:

(1) Modification of the original silicone membrane type (used for *G. morsitans*) to meet the requirements of *G. p. palpalis*. By decreasing the quantity of the silicone hardener in the mixture, a membrane was obtained that was readily attractive to *G. p. palpalis*.

(2) Establishment of the criteria for determining the suitability of different types of blood preserved in different ways and supplemented with additives (phagostimulants), including use of defibrinated, heparinized and haemolysed bovine and porcine blood. Adenosine triphosphase was found to be a suitable phagostimulant and was therefore used routinely for in vitro feeding.

(3) Installation of a large capacity lyophilizing machine and testing of various freeze drying programmes. Whole blood and processed defibrinated blood were found to be adequate for tsetse fly rearing.

(4) Determination of optimum ratios of fresh or freeze dried bovine and porcine blood in the adult diet. A 50:50 mixture of bovine and porcine blood produced the best results.

(5) Development of quality control procedures (25 day bioassay) for screening the suitability of processed blood before it is used for stock colony feeding. This procedure ensured that unsuitable blood was not used for fly colony maintenance.

(6) Design of cages enabling an increased fly density per production unit. For operations in BICOT, round cages holding up to 30 flies per cage were found to be ideal for in vitro feeding.

(7) Determination of the effects of dietary changes from an in vivo to an in vitro feeding regimen on the survival and fecundity of newly emerged female flies. The

significance of this procedure lay in the fact that occasional high mortality in the guinea pig colony demanded that the in vivo tsetse fly colony be fed in vitro.

(8) Development of procedures for microbial decontamination of abattoir blood. A dose of 1.0 kGy gamma radiation was found to be bacteriocidal and was recommended for routine treatment of abattoir blood prior to storage. In BICOT freshly collected abattoir blood was treated with 0.5 kGy gamma radiation and deep frozen for several days. Upon thawing, the blood was treated with another 0.5 kGy gamma radiation before being fed to the flies. Tests involving antibiotics and conventional trypanocides showed that these drugs had no deleterious effects on tsetse reproductive biology. They were therefore used routinely for treating blood or the host animal against trypanosome and other infections.

## 3. RADIATION STERILIZATION OF *G. p. palpalis*

Optimal doses of gamma radiation were determined for late stage puparia and young adult flies. New biological evaluation systems related to induced sterility (e.g. embryonic arrest and aberrations in the ovaries) after female and male fly treatment were developed and their relevance tested to measure the progress of eradication. A dose of 120 Gy given in air to young unfed adults caused over 95% sterility in male *G. p. palpalis*. This dosage was recommended for use in BICOT.

## 4. USE OF INSECTICIDES FOR PRIOR SUPPRESSION

In collaboration with the Agrochemicals and Residues Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and its laboratory unit at Seibersdorf, bioassay tests and analytical work were conducted on the various insecticide formulations used for insecticide impregnated targets (blue screens).

## 5. TRAINING

In addition to research and technical support, Seibersdorf provided a total of 23 man-months of training for seven Nigerian staff members of BICOT. Training emphasized all aspects of laboratory and field work for the integrated vector control operations.

## 6. SERVICES

Throughout the implementation phases of BICOT various services were provided by the staff at Seibersdorf. These included:

(1) Maintenance of a back-up colony of *G. p. palpalis* from which hundreds of thousands of surplus puparia were sent to BICOT. At the peak of production the back-up colony contained over 80 000 producing females, which yielded approximately 6000 surplus puparia each week.

(2) Preparation and shipment of freeze dried and quality tested bovine and porcine blood for feeding to colony flies in BICOT. Over 5000 L of freeze dried blood were made available to BICOT between 1981 and 1986.

(3) Procurement of supplies and equipment from foreign sources. The expertise of the entomology staff was used on many occasions by the Procurement Section of the IAEA to identify suitable equipment and supplies for BICOT.

(4) Provision of expert services in support of mass rearing and field operations. At various times staff of the Entomology Unit spent periods from a few days to several months at the BICOT headquarters and field station assisting in the training of local staff or in the designing and running of laboratory and field experiments.

(5) Construction of special equipment. Staff at the Seibersdorf workshop assisted in the design and construction of cool boxes, special racks, trolleys, prototype cages and other equipment for use in BICOT.

# TSETSE FLY ERADICATION IN BURKINA FASO AND EVALUATION OF TRAPS AND TARGETS*

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**Abstract**

TSETSE FLY ERADICATION IN BURKINA FASO AND EVALUATION OF TRAPS AND TARGETS.

Control operations against tsetse flies with the sterile insect technique (SIT) were conducted by the Centre de recherches sur les trypanosomoses animales (CRTA) (Institut d'élevage et de médecine vétérinaire des pays tropicaux/Gesellschaft für Technische Zusammenarbeit (IEMVT/GTZ) Project), Bobo-Dioulasso (Burkina Faso). The project ended in 1984 with the eradication in the Sideradougou pastoral zone of the three tsetse species present there (*Glossina palpalis gambiensis*, *G. tachinoides* and *G. morsitans submorsitans*). Since 1985, besides monitoring of this area, the CRTA oriented its activities towards improving trapping by carrying out research on the form and colour of targets as well as the use of olfactory attractants.

## 1. CONTROL OPERATIONS IN THE SIDERADOUGOU PASTORAL ZONE

### 1.1. Introduction

To permit development of livestock in a 240 000 ha area of the Sideradougou pastoral zone (Fig. 1), the Centre de recherches sur les trypanosomoses animales (CRTA) sought to control tsetse flies in an area of more than 300 000 ha (3000 km$^2$). After a preparatory phase lasting 2 years, in which access roads were built, surveys carried out and barriers constructed, control operations began in 1983 by integrating two non-polluting methods: initially, use of insecticide impregnated screens, followed by release of sterile males.

* Research carried out in association with the IAEA under Research Agreement No. 5491/CF.

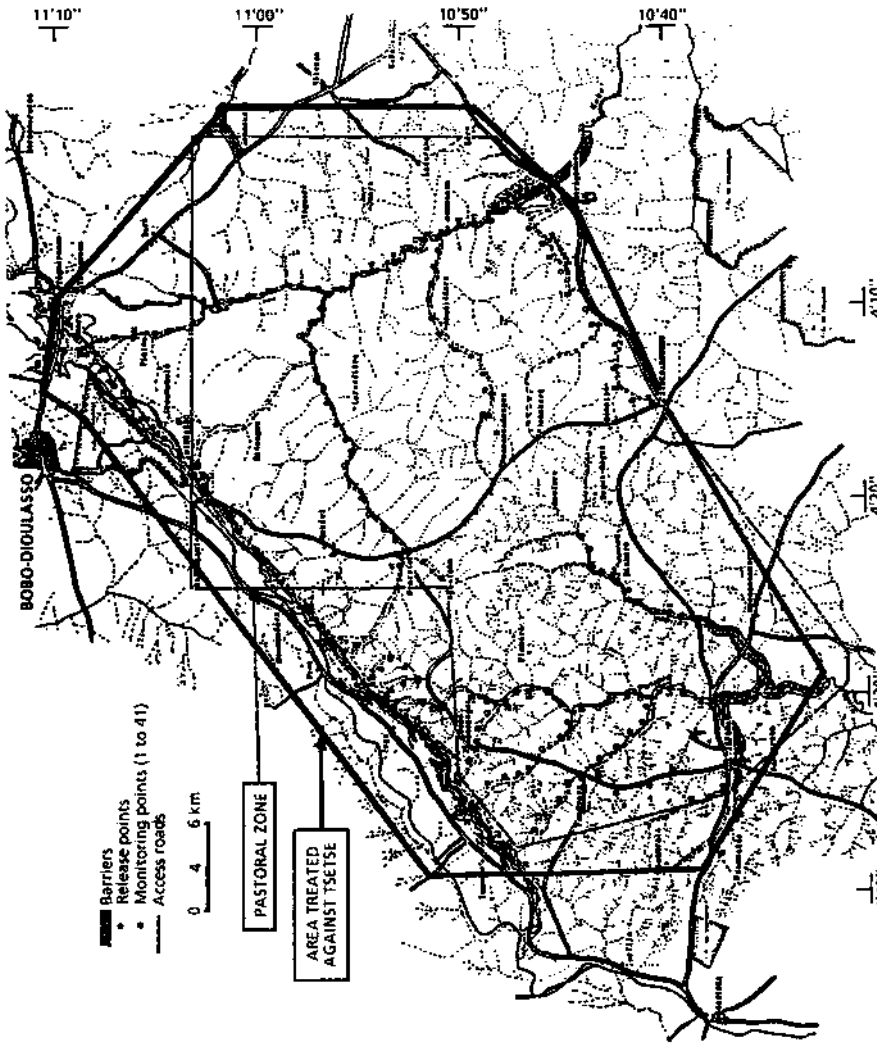



*FIG. 1. The Sideradougou pastoral zone.*

### 1.2. Reduction in the tsetse fly population

To reduce the size of the wild fly population before using the sterile insect technique (SIT), screens were placed along the gallery forests during the dry season at the beginning of 1983. The CRTA placed 7204 blue screens in a linear fashion, 100 m apart, along 600 km of riverine forests to lower the density by trapping [1]. The screens were impregnated with deltamethrin (200 mg per screen), preliminary assays with DDT and dieldrin having shown these insecticides to be less efficient [2–4]. The mean reduction after trapping for *Glossina tachinoides* was 93.0% (85.4–98.2%) and for *G. palpalis gambiensis* 88.1% (74–94.9%) [5].

It is very important in such control operations to obtain the co-operation of the local people. To achieve this, we used various means of communication, including broadcasts, public meetings and posters. As a result of this campaign, loss of targets was maintained at 12%.

### 1.3. Sterile male releases

At the beginning of the rains all the screens, which had been installed for about 3 to 4 months (until May 1983), were removed and the release of males started. Three hundred thousand sterile *G. p. gambiensis* males irradiated with 11 krad (0.11 kGy) and 82 000 *G. tachinoides* irradiated with 10 krad (0.10 kGy) were released along the rivers throughout the rainy season at 2 weekly intervals and 2 km apart.

All these operational factors were fixed during the preliminary surveys carried out between 1976 and 1980. They showed that the sterile males performed as well as wild flies [5–11]; a ratio of 10 sterile males to 1 wild male was found to be most effective [5].

The releases were often carried out on bicycle, following paths along the gallery forest. Use of ultralight aircraft to distribute sterile males also proved very effective. Calculation of the costs of aerial release compared with release on the ground showed that it was cost effective and it may be recommended for use in future SIT projects [12].

In the following year (1984) the area was similarly retreated with screens (3643) and then 467 000 sterile *G. p. gambiensis* and *G. tachinoides* males were released using the same release pattern. Together with the flies released in 1985, a total of nearly 950 000 sterile males were released in the area [13–16].

At the height of the campaign, the colonies of the three tsetse species rose to nearly 330 000 producing females. This was possible because of the many improvements made in the handling, sterilization and transport of flies [17–19]. All the materials used for tsetse breeding were constructed locally. Since 1982 artificial (in vitro) feeding of flies has been carried out using blood from the slaughterhouse at Bobo-Dioulasso that had been sterilized in a caesium irradiator.

### 1.4. Barriers (Fig. 1)

To avoid tsetse reinvasion from the invested areas surrounding the pastoral zone, the CRTA established three barriers of insecticide screens and traps (Challier-Laveissière traps) placed at intervals of 100 m along the river bank. Seven kilometres of river with these targets proved to be an effective barrier. The savannah species *G. morsitans submorsitans*, only found in the south-east limit near the Koba River, was controlled by traps and screens (1760) placed in rows at very close intervals [2–4, 15, 16, 20].

### 1.5. Results of the SIT programme

The efficiency of the barriers and the excellent performance of the irradiated males produced eradication of the tsetse fly population within 2 years. The area is now monitored bimonthly for 2 days by 41 biconical traps placed in the pastoral zone; the barriers are monitored three times a week. Since 1984 not a single fly has been caught in the middle of the area, but some have been found on the borders where supervision is difficult; they are controlled by screens and releases of sterile males.

Recently, a trypanosomiasis survey was carried out both inside and outside the control zone by CRTA [21]. Inside the area *Trypanosoma congolense* has disappeared, while a low level of *T. vivax* still remains. In contrast, outside the control area prevalence is high for both trypanosome species.

## 2. RESEARCH ON TRAPPING

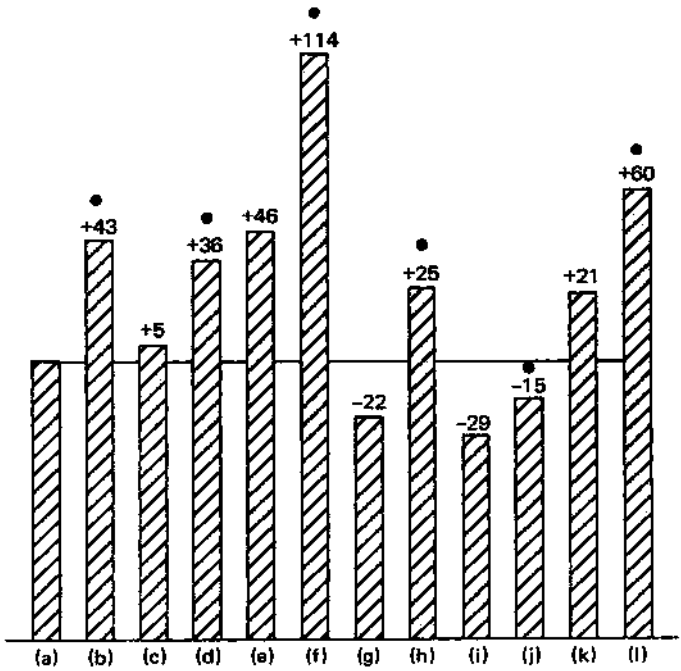
### 2.1. Introduction

Since 1983, with the end of the programme at Sideradougou, the CRTA has engaged in research to increase the efficiency of tsetse control operations by working on the form and colour of traps and targets as well as on the use of olfactory attractants for trapping (mainly for *G. m. submorsitans*).

### 2.2. Results

#### *2.2.1. Studies on the form and colour of targets*

Experiments to study the behaviour of *G. m. submorsitans* towards different coloured cloth screens were carried out using electric traps [22]. The attraction of different coloured screens (blue, white and black) [23, 24], and combinations of these with and without the association of fine black terylene netting, was tested by

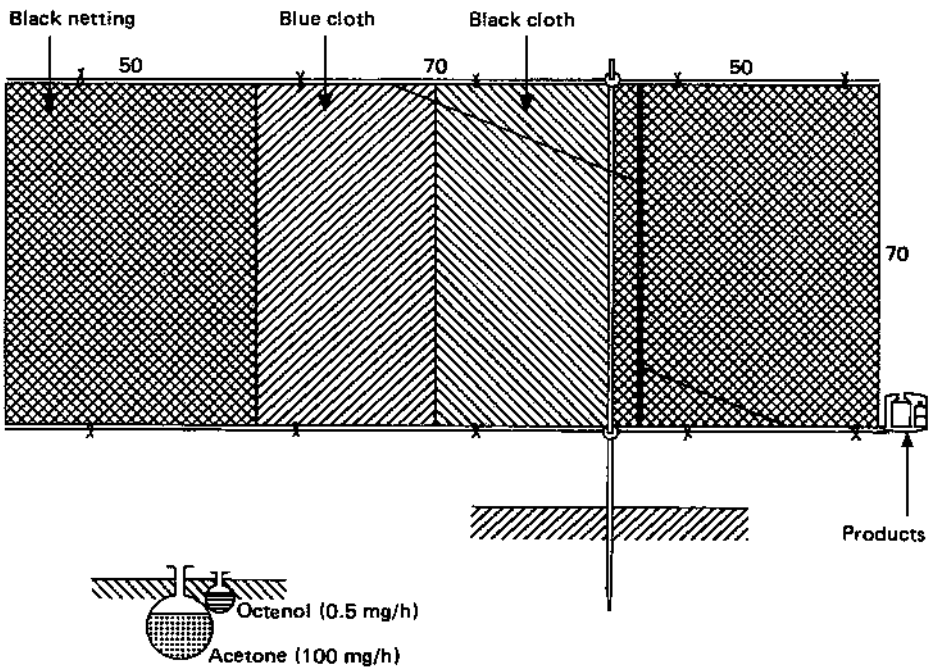



(a) Blue screen (reference)
(b) Blue screen with mosquito net •
(c) Black screen
(d) Black screen with mosquito net •
(e) Blue/black screen
(f) Blue/black screen with mosquito net •
(g) Blue/white screen
(h) Blue/white screen with mosquito net •
(i) Black/white screen
(j) Black/white screen with mosquito net •
(k) Blue/white/black screen
(l) Blue/white/black screen with mosquito net •

*FIG. 2. Schematic comparison of the efficiency of various screens to the blue screen (a) (from Ref. [23]).*

using an electrocuting net covering both sides of the screens. The results showed a clear superiority of blue/black screens compared with all other colours used alone or in various combinations. The black terylene netting increased capture figures in all cases when compared with screens of the same colour without netting (Fig. 2). Blue/black screens with terylene netting were twice as effective as the simple blue screens generally used in West Africa. No differences were found between single colour black or blue screens.

Different trap and screen models were tested to study their efficiency for the control of *G. m. submorsitans*, *G. tachinoides* and *G. p. gambiensis*. The targets were equipped with electrified grids (as described in Ref. [25] and widely used in Zimbabwe [22]), which killed any tsetse fly landing on them, simulating the effect




Acetone: 100 mg/h
Octenol: 0.5 mg/h
The flasks can also be placed on the ground.
Impregnation: deltamethrin 200 mg/screen
Cost: 3500 CFAF
Maintenance: 1500 CFAF (clothes and products)

*FIG. 3. Turning screen, Burkinabe model, CRTA [24]. (CFAF = 1 Communauté financière africaine franc = 0.02 FF).*

of insecticide impregnation. Electrocuted tsetse flies were collected on iron sheets, smeared with insect glue or filled with water and detergent, placed below the targets. A biconical trap without an electrified grid was used as the control.

Ranking of the different targets was the same for the three species studied: a blue cotton screen with a vertical strip of black mosquito netting on both sides was the most efficient, followed by the electrified biconical trap, the simple screen without mosquito netting and, finally, the non-electrified biconical trap. The sex ratios of the captures were variable, depending on the target; the screen mosquito net and the electrified biconical trap caught a considerably greater percentage of females than the simple screen and the non-electrified trap. At the same time the behaviour of *G. p. gambiensis* and *G. m. submorsitans* in relation to the traps and

screens was studied; the differences in the behaviour pattern may explain the differences in the number of flies caught.

### 2.3. Discussion

As a result of this study we recommend the use of the blue screen mosquito netting target for tsetse fly campaigns against the three species prevalent in Burkina Faso because of its very low cost and ease of transport. However, for *G. m. submorsitans* the blue/black screen with black netting is currently the best, since it turns around on its axis, thus avoiding being knocked down by winds (Fig. 3). For the riverine species, the best trap is monoconical, with an internal blue screen in the upper part and a black screen in the lower part. By changing the form and colour of targets it has been possible to reduce the numbers required to control *G. m. submorsitans* from 33/km$^2$ to 5 or 6/km$^2$.

## 3. OLFACTORY ATTRACTANTS

### 3.1. Introduction

The importance of odours in increasing the efficiency of targets for the savannah species of tsetse fly has been demonstrated in Zimbabwe. We have now extended this odour work to the West African species of tsetse.

### 3.2. Materials and methods

All the experiments described used electric grids surrounding the targets to enable the counting of flies coming to land. The targets were either biconical Challier-Laveissière traps, or blue/black cloth screens. To compare the efficiency of the various odours, a series of randomized 4 × 4 Latin squares was used.

The CRTA has a field station near the Komoé River where the trials were carried out on two species (*G. m. submorsitans* and *G. tachinoides*) during the wet and dry seasons. After having tried acetone and octenol, the Overseas Development Natural Resources Institute (ODNRI) sent several samples of various chemicals to be tested in the field in West Africa.

### 3.3. Results

1-octen-3-ol (octenol) and acetone, which had proved to be potent olfactory attractants in Zimbabwe for *G. pallidipes* and *G. m. morsitans*, were the first components to be tried. During the rainy season the catches showed a significant 6.7 fold increase in comparison to non-baited traps. Capture data for males and females

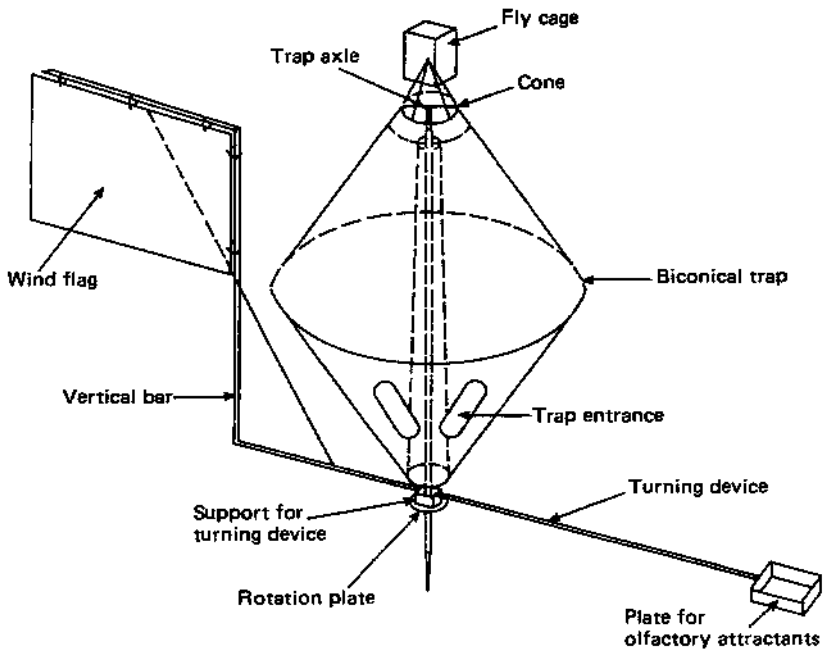



*FIG. 4. Turning biconical trap (from Ref. [31]).*

separately showed a 5.9 fold increase for males and a 7.5 fold increase for females. In the dry season the relative superiority of odour baited traps was reduced, producing only a 2.78 fold increase. This difference is thought to be due to the increased attractiveness of visual traps in the dry season, a factor which is not important in the rainy season [26].

The traps used were biconical with blue lower cones and were equipped with a turning device consisting of a flag on one side and the attractants on the other (Fig. 4) to orientate the odours at a constant 1 m upwind of the traps. As a result of these odour experiments it was possible to reduce the number of traps used in the control operation at Sideradougou against *G. m. submorsitans.*

ODNRI also sent various chemicals to Bobo-Dioulasso for testing, e.g. acids, aldehydes, ketones and alcohols; most were inactive or less active than acetone and octenol [27, 28].

Many urine samples of different animals were also tested; buffalo and cattle urine were both attractive to *G. tachinoides.* Chemical analysis showed that the urine phenolic fraction is the most attractive [29] due to paracresol, metacresol and 3-propylphenol. The effectiveness of these odours was found to vary between tsetse species. Many synthetic products produced by ODNRI were also tested [29–31].

2-methoxy-phenol and acetopherone are slightly repellent to *G. m. submorsitans* and inactive against *G. tachinoides* [30].

## 4. TRIALS OF ODOURS ON RIVERINE FOREST TSETSE FLIES

### 4.1. Introduction

As odour components active with *G. m. morsitans* and *G. m. submorsitans* gave no results with *G. tachinoides*, it was thought that savannah and riverine species react differently, and even that the latter species finds its hosts by vision and not by odour. In collaboration with the ODNRI an experiment was carried out to determine whether these flies are sensitive to olfactory attractants.

### 4.2. Materials and methods

To test the effect of odour alone, pits were constructed to hide the hosts from the flies. Four experimental sites were used along the Komoé River, at least 500 m apart. At each site three pits (2.4 m × 2.4 m × 1.8 m) were dug.

Each pit was exhausted by a battery powered fan placed in a movable PVC plastic tube (6 m) connected to a long fixed tube (20 m, with the same diameter of 20 cm) leading to the trapping place. Both sides of the screen were surrounded by

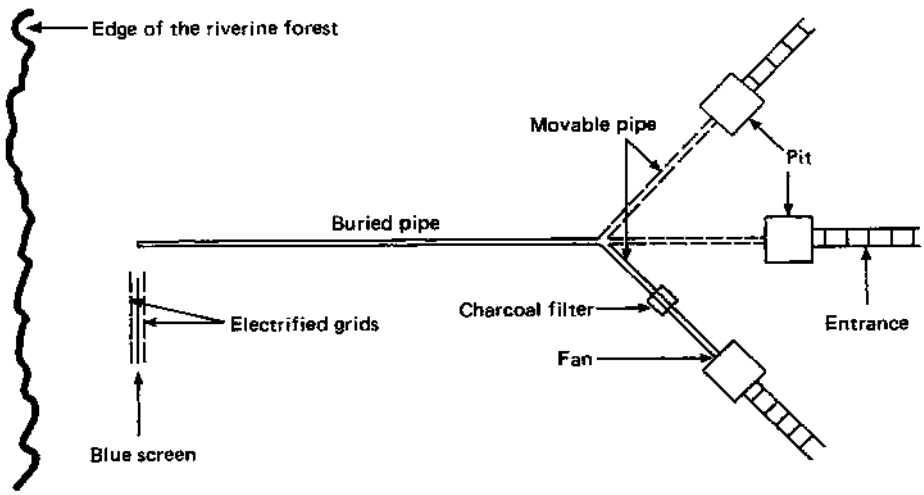



*FIG. 5. Experimental site for the odour of hosts.*

electrified grids (Fig. 5). In the middle of the movable tube a filter was placed containing activated charcoal which allowed only $CO_2$ and very volatile materials to pass through.

Captures were run from 08:00 to 12:00 hours. Odours came from a man, cattle (Baoulé) or a pig placed in the pits.

### 4.3. Results

*G. tachinoides* was found to be attracted by the odours of a man, cattle and a pig, as well as by carbon dioxide. The significant increases relative to the blank treatment were, respectively: 12, 20 and 21%; for *G. m. submorsitans* the figures were 13, 23 and 26% [31–33].

The catches were found to increase when more than one cow was used. Zebu cattle were more attractive (25%) than Baoulé cattle [34].

It was found that active odours are not the same for the tsetse fly species of East and West Africa. For example, in Zimbabwe paracresol is attractive to *G. tachinoides*, but in Burkina Faso it is metacresol that attracts. The urine of some wild hosts did not have the same composition, with more metacresol in West African than in East African species.

## 5. CONCLUSIONS

The research carried out on the action of odours on West African tsetse species provided many practical results. Acetone plus octenol was found to be very attractive to *G. m. submorsitans* in Burkina Faso, but unattractive to *G. tachinoides*. Acetone alone is even a little repellent for this species, while octenol, used with other components, often has a synergistic effect.

The experiments on riverine tsetse with host odours proved, for the first time, that *G. tachinoides* is attracted by odours other than carbon dioxide. *G. p. gambiensis* has now to be tested. These experiments underline the fact that the response to odours is species dependent in tsetse flies and that the correct odour combination has still to be determined for West African species.

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# ECOLOGY OF *Glossina* SPECIES INHABITING PERIDOMESTIC AGROECOSYSTEMS IN RELATION TO OPTIONS FOR TSETSE FLY CONTROL*

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**Abstract**

ECOLOGY OF *Glossina* SPECIES INHABITING PERIDOMESTIC AGROECOSYSTEMS IN RELATION TO OPTIONS FOR TSETSE FLY CONTROL.

Unbaited blue biconical traps were used to sample populations of *Glossina* once a week from April 1984 to March 1988 in three peridomestic agroecosystems of the Nsukka area, Nigeria. Only *Glossina palpalis* and *G. tachinoides* were caught, the latter being more widespread and constituting 99.87% of the 16 862 tsetse flies caught. Serological analysis of 1764 fly midgut contents revealed that *G. tachinoides* had fed on reptiles, birds and mammals, with the domestic pig accounting for 88.08% of the 730 identifiable bloodmeals. The frequency distribution of flies in various stages of the trophic cycle showed that males and females feed at 2.88 ± 0.42 and 2.43 ± 0.44 day intervals, respectively. Of the 10 208 flies examined for trypanosomes, about 1% were infected with *Trypanosoma brucei* or *T. congolense* group trypanosomes, the latter accounting for 53% of the 111 mature infections encountered. The sex ratio in these fly populations was variable, being 1:1 in one agroecosystem but departing significantly from 1:1 in the others, differing markedly between biotopes and seasons. Flies were caught in greater numbers in biotopes containing domestic pigs, while the presence of man depressed trap catches. The larger the pig population in an agroecosystem, the larger the *G. tachinoides* population. However, reduction in the pig population to below five triggered the collapse of one of the *G. tachinoides* populations, which disappeared following the removal of all the pigs. The fly populations exhibited marked seasonal fluctuations in apparent density, largely caused by routine agronomic practices. These density fluctuations undermine recruitment of new adults into the population, especially during the wet season. It is suggested that tsetse populations in this area, already being kept at low density by routine agricultural procedures, could be further reduced by combining insecticide impregnated traps or targets with insect proofing of the piggeries. Methods aimed at undermining the recruitment of young adults into tsetse populations, capitalizing on naturally occurring sex ratio distortion as well as on maintaining populations of preferred hosts of the tsetse fly at low

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levels, should form part of integrated tsetse control packages. Selection of sterile male release sites and the number of sterile males to be released in them during sterile insect technique campaigns should take into account the sex ratio dynamics of target tsetse populations.

## 1. INTRODUCTION

Although a combination of control techniques already in existence could achieve tsetse fly eradication in ecologically isolated ecosystems, few of which exist in Africa, the tsetse fly has already shown signs of resilience to eradication. For example, a number of species in the *palpalis* and *morsitans* groups have already adapted to relatively precarious man dominated ecosystems. While putting land into agropastoral use immediately after tsetse fly control is currently being advocated as the answer to the recolonization of reclaimed areas, some tsetse species are already at home as permanent residents of intensively cultivated agroecosystems with a high human population density and a paucity or absence of wild mammals. Perhaps the best documented of such tsetse populations are the peridomestic *Glossina tachinoides* Westwood and *G. palpalis* (R–D) species of the Nsukka area [1]. To date, no control package has been designed specifically for such tsetse populations.

A summary is given of a 4 year investigation of peridomestic tsetse populations undertaken with the objective of defining appropriate methods for their control.

## 2. MATERIALS AND METHODS

From April 1984 to March 1988 unbaited blue biconical traps [2] were deployed weekly from 06:30 to 18:30 hours in peridomestic agroecosystems located in three villages in the Nsukka area (Orie–Orba, Amesumesu–Edem and Ozalla-Edem) [1, 3, 4]. On each sampling occasion traps were positioned at preselected spots to include different biotopes of the agroecosystem.

All the flies caught were identified by species and sex, counted and then dissected to ascertain the ovarian age structure and the reproductive status of the females [5–7], as well as the trypanosome infection rate [8]. To determine their host spectrum and trophic pattern, the midgut contents of some flies were squashed on Whatman filter paper, sterilized with acetone [9], and forwarded to C. Staack of the Robert Von Ostertag Institute for Veterinary Medicine, Berlin, for serological identification. The feeding interval of the fly was determined surgically [10].

The nature, timing and duration of routine agricultural activities in the study areas were closely monitored. The probable effects of some of these on tsetse populations, especially cultivation and weeding with a hoe, were simulated by burying *G. palpalis* puparia (courtesy of the Biological Control of Tsetse (BICOT) Project in Vom) at depths of 5–50 cm and recording the number of teneral adults emerging into

cages placed on the soil surface. The puparia of *G. tachinoides* were collected from natural sites on farmland and held in the laboratory to determine the emergence, the sex ratio of emergent flies and the rate of puparial parasitization. Pitfall traps were deployed on farmland and all the surface active arthropods caught in them were sent to the Commonwealth Institute of Entomology, London, for definitive identification.

Climatological data for the Nsukka area were obtained from records of the University of Nigeria Agrometeorological Station situated approximately 10 km from the furthest study site.

TABLE I. RELATIVE ABUNDANCE OF *Glossina* SPECIES IN PERIDOMESTIC AGROECOSYSTEMS OF THE NSUKKA AREA, NIGERIA

| Location | Total flies caught | % *G. tachinoides* | % *G. palpalis* |
|---|---|---|---|
| Orie–Orba | 11 825 | 99.83 | 0.17 |
| Amesumesu–Edem | 2 578 | 99.96 | 0.04 |
| Ozalla–Edem | 2 459 | 100 | 0.00 |
| *Total* | 16 862 | 99.87 | 0.12 |

TABLE II. ABUNDANCE OF *G. tachinoides* IN DIFFERENT BIOTOPES OF THE NSUKKA PERIDOMESTIC AGROECOSYSTEMS

| | Flies per trap per year (mean ± SE) | | |
|---|---|---|---|
| Biotope | Orie–Orba | Amesumesu–Edem | Ozalla–Edem |
| Pigsty | 520.58 ± 83.15 | 104.08 ± 20.86 | 199.00 ± 61.61 |
| Farmland near pigsty | 408.00 ± 22.14 | 103.37 ± 13.77 | — |
| Refuse dump | 194.00 ± 37.70 | — | — |
| Farmland distant from pigsty | 38.73 ± 9.05 | — | 22.50 ± 7.50 |

TABLE III. EFFECT OF THE HUMAN PRESENCE ON BICONICAL TRAP CATCHES OF *G. tachinoides* IN BIOTOPES OF THE ORIE–ORBA PERIDOMESTIC AGROECOSYSTEM

| Biotope | Trap No. | No. of flies caught per year (mean ± SE) | | |
|---|---|---|---|---|
| | | Total | Wet season | Dry season |
| Pigsty | 1[a] | 245.50 ± 20.67 | 136.50 ± 19.61 | 109.00 ± 6.53 |
| | 2 | 437.75 ± 33.29 | 172.75 ± 28.50 | 263.00 ± 13.70 |
| | 3 | 880.50 ± 61.00 | 261.25 ± 33.51 | 619.25 ± 53.57 |
| Farmland distant from pigsty | 11 | 128.00 ± 29.00 | 32.50 ± 5.50 | 104.50 ± 34.50 |
| | 12 | 20.50 ± 13.50 | 9.50 ± 5.50 | 20.00 ± 8.00 |
| | 13 | 11.00 ± 6.00 | 2.50 ± 0.5 | 8.50 ± 5.50 |
| | 14 | 7.50 ± 1.50 | 0.50 ± 0.50 | 5.00 ± 1.50 |
| | 15[a] | 11.00 ± 4.00 | 5.50 ± 2.50 | 5.00 ± 1.50 |

[a] Nearest heavy human traffic or concentration.

## 3. RESULTS

Only *G. tachinoides* and *G. palpalis* were encountered in the three agroecosystems. *G. tachinoides* was more widespread and also more abundant than *G. palpalis*, constituting 99.87% of the 16 862 tsetse flies caught (Table I). The total number of flies caught in each agroecosystem appeared to be related to the size and variety of the domestic animal population, which was greatest at Orie–Orba and least, initially, at Amesumesu–Edem but later (as of August 1984) at Ozalla–Edem.

Within each agroecosystem, the size of the catch/trap differed with biotope, being largest around pigsties and smallest on farmland distant from pigsties (Table II). Within biotopes, the presence of man appeared to depress the catch/trap. This was more easily observable at Orie–Orba because of the higher human population density and traffic as well as clearer zonation of its biotopes (Table III). Of the three traps deployed around the pigsty, traps 2 and 3, positioned away from the area of human concentration, caught many more flies than trap 1, which was located nearest to it. Similarly, on farmland distant from the pigsty, the trap located near a main road which habitually experienced heavy human traffic (trap 15) caught fewer flies than its counterpart (trap 11) located near a path which had much lighter human

TABLE IV. SEX RATIO IN POPULATIONS OF *G. tachinoides* IN THE NSUKKA AREA, NIGERIA

| Location | No. of flies caught | % female | Female:male ratio | Significance of deviation from 1:1 ratio |
|---|---|---|---|---|
| Orie–Orba | 11 805 | 43.72 | 0.78 | $P < 0.001$ |
| Amesumesu–Edem | 2 577 | 48.31 | 0.93 | $P > 0.05$ |
| Ozalla–Edem | 2 459 | 53.48 | 1.15 | $P < 0.001$ |

traffic. This effect of man's presence on the size of trap catches of *G. tachinoides* was more pronounced during the dry season when the human traffic and concentration were greater because of the normal increase in trade in the area.

Approximately 81% of the 21 *G. palpalis* and 46% of the 16 841 *G. tachinoides* caught were females. The sex ratios of both tsetse species departed significantly from 1:1 (*G. palpalis:* $\chi^2 = 8.09$, $P < 0.01$; *G. tachinoides:* $\chi^2 = 116.21$, $P < 0.001$). However, this was not the pattern in all three *G. tachinoides* populations; the sex ratio did not depart significantly from 1:1 at Amesumesu–Edem but did in the other locations, being biased in favour of males at Orie–Orba but in favour of females at Ozalla–Edem (Table IV). In addition, within each agroecosystem the sex ratio differed markedly between biotopes (Table V). In biotopes where pigs were permanently present, the sex ratio was always significantly biased in favour of females. On farmland, on the other hand, where pigs were allowed to roam only during part of the dry season (usually between December and March), the sex ratio was invariably biased in favour of males. However, on some parts of the farmland (Table V) the sex ratio did not depart significantly from 1:1, evidently because of the small number of flies usually caught there. Within each biotope where flies were caught in relatively large numbers, the sex ratio underwent marked seasonal fluctuations (Fig. 1), with increasingly more males in catches during the dry season than in the wet season.

The midgut contents of 1764 *G. tachinoides* were sent for serological analysis, but only 730 (41.38%) were in an identifiable condition (Table VI). Of these, 88.08% were from Suidae, 6.17% from Bovidae and 2.60% from primates. The remaining 2.61% were from rodents, birds, reptiles and dogs, in descending order of frequency.

TABLE V. SEX RATIO OF *G. tachinoides* POPULATIONS IN DIFFERENT BIOTOPES OF THE NSUKKA PERIDOMESTIC AGROECOSYSTEMS

| Biotope | Mean ± SE per year | | | | | |
|---|---|---|---|---|---|---|
| | Orie-Orba | | Amesumesu-Edem | | Ozalla-Edem | |
| | % total catch | % females | % total catch | % females | % total catch | % females |
| Pigsty | 55.41 ± 1.58 | 59.32 ± 3.21 | 65.06 ± 4.97 | 63.81 ± 0.8 | 73.35 ± 5.41 | 68.83 ± 6.16 |
| Farmland near pigsty | 28.94 ± 0.51 | 15.28 ± 2.24 | 34.94 ± 4.97 | 20.86 ± 2.94 | — | — |
| Refuse dump | 6.72 ± 0.98 | 48.00 ± 3.38 | — | — | — | — |
| Farmland distant from pigsty | 8.95 ± 2.22 | 44.41 ± 3.14 | — | — | — | 44.50 ± 2.35 |

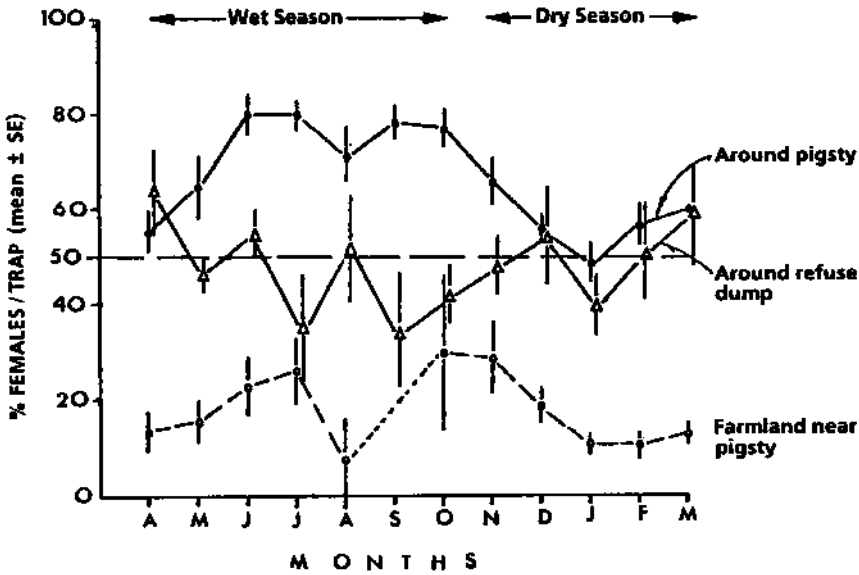


*FIG. 1. Seasonal fluctuations in the sex ratios of* G. tachinoides *populations in different biotopes of peridomestic agroecosystems of the Nsukka area, Nigeria.*

TABLE VI. HOST SPECTRUM AND TROPHIC PATTERN OF *G. tachinoides* IN PERIDOMESTIC AGROECOSYSTEMS OF THE NSUKKA AREA, NIGERIA

| Source of bloodmeal | No. of flies identified | Percentage |
|---|---|---|
| Mammals: | | |
| Suidae | 643 | 88.0 |
| Bovidae: | | |
| Small ruminants | 38 | 5.5 |
| Cattle | 9 | 1.2 |
| Duiker | 2 | 0.2 |
| Primates (man) | 19 | 2.6 |
| Rodents | 11 | 1.5 |
| Canidae (dog) | 2 | 0.2 |
| Monitor lizard | 3 | 0.4 |
| *Total* | 727 | 99.3 |

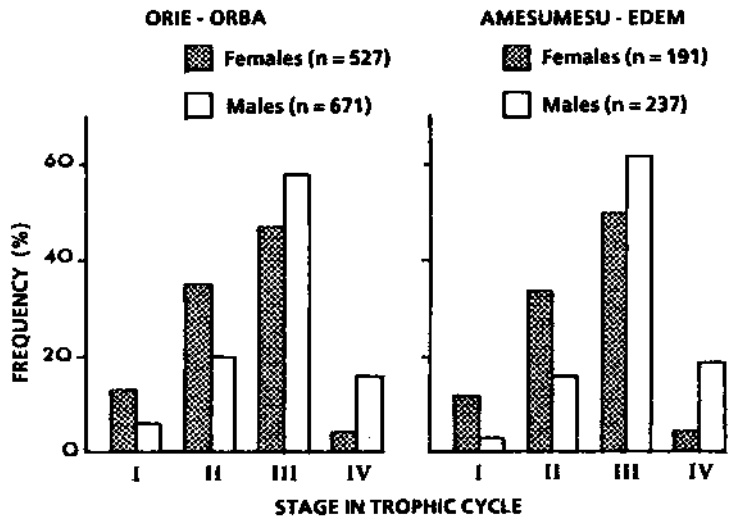


*FIG. 2. Frequency distribution of peridomestic* G. tachinoides *at different stages in the trophic cycle.*

The distribution of flies caught between December 1987 and March 1988 at different stages in the trophic cycle (Fig. 2) showed that females and males were feeding at mean intervals of 2.43 ± 0.44 and 2.85 ± 0.43 days, respectively, at Orie–Orba and 2.45 ± 0.43 and 2.98 ± 0.39 days, respectively, at Amesumesu–Edem.

Of the 10 208 *G. tachinoides* whose midgut, salivary glands and hypopharynx were microscopically examined for trypanosomes, only 2.02% procyclic and 1.09% mature infections were detected. Of the 111 mature infections, 21 (18.29%) were of the *congolense* group, 14 (12.61%) were of the *brucei* group and 38 (34.23%) were mixtures of both trypanosomes. Mature *congolense* group trypanosomes occurred with equal frequency in male and female flies at Orie–Orba and Ozalla–Edem, but more frequently in females than males at Amesumesu–Edem. Mature *brucei* group trypanosomes, on the other hand, were twice as frequent in females, but were not detected in flies of either sex at Ozalla–Edem (Table VII).

The size of all three *G. tachinoides* populations underwent marked seasonal fluctuations, the amplitude differing with location (Fig. 3). During the wet season, the mean daily apparent density of each population decreased steadily from April to July, followed by no further decreases (Amesumesu–Edem), or only slight increases (Orbie–Orba), until October. However, with the onset of the dry season it increased steadily from November to a peak in February, followed by a noticeable

TABLE VII. TRYPANOSOME INFECTION RATES IN *G. tachinoides* INHABITING PERIDOMESTIC AGROECOSYSTEMS OF THE NSUKKA AREA, NIGERIA

| Location | Sex | No. of flies dissected | % trypanosome infection | | |
|---|---|---|---|---|---|
| | | | Procyclic | Mature | |
| | | | | *T. congolense* | *T. brucei* |
| Orie-Orba | Male | 3766 | 1.33 | 0.69 | 0.42 |
| | Female | 3633 | 2.15 | 0.63 | 0.69 |
| Amesumesu-Edem | Male | 924 | 1.83 | 0.22 | 0.33 |
| | Female | 927 | 2.16 | 0.54 | 0.86 |
| Ozalla-Edem | Male | 415 | 4.58 | 0.48 | 0.00 |
| | Female | 541 | 4.07 | 0.37 | 0.00 |
| *Total* | Male | 5107 | 1.68 | 0.59 | 0.37 |
| | Female | 5101 | 2.35 | 0.59 | 0.65 |

decrease from March. The tsetse population at Ozalla-Edem, unlike the others, failed to increase appreciably with the onset of the dry season and completely collapsed following the removal of pigs from the agroecosystem in April 1985. Although three pigs were subsequently reintroduced into Ozalla-Edem in August 1985 (but sold off by the end of February 1986) and one pig in December 1987, between May 1985 and March 1988 only 33 flies (21 females and 12 males) were caught in this agroecosystem. The apparent density was sporadic ($0.40 \pm 0.17$, $0.05 \pm 0.05$ and $0.13 \pm 0.09$ in May, June and August 1985, respectively; $0.15 \pm 0.03$ and $0.05 \pm 0.05$ in January and November 1986, respectively; and $0.20 \pm 0.20$ in March 1987) and never recovered to its predecline level.

The pregnancy rate was evenly high and the abortion rate evenly low during both seasons in each *G. tachinoides* population (Fig. 4). Uninseminated females were not encountered and whenever the pregnancy rate fell below the usual level it

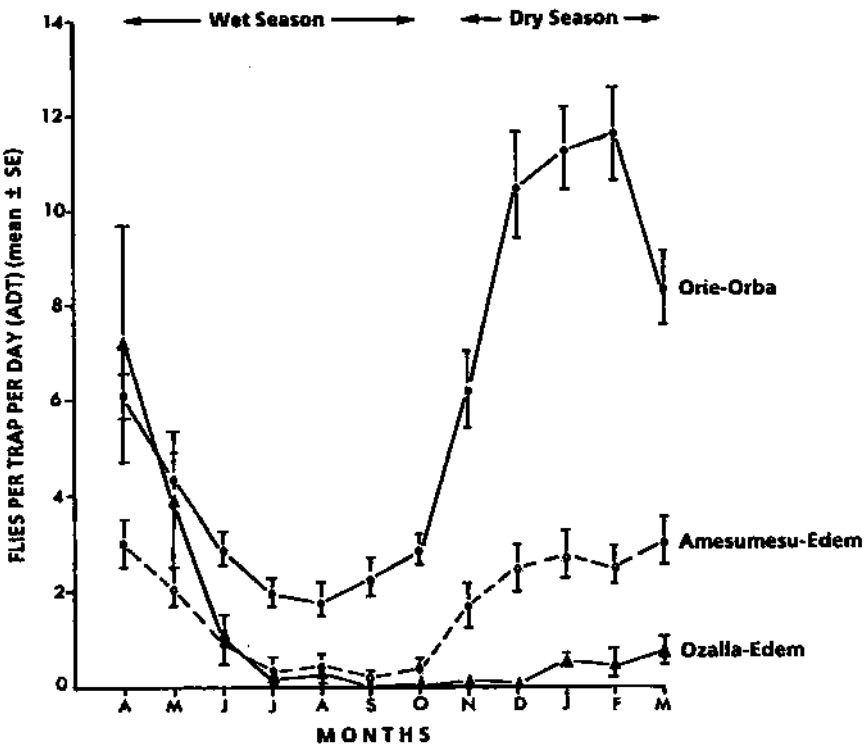


*FIG. 3. Seasonal fluctuations in the apparent density of* G. tachinoides *populations inhabiting peridomestic agroecosystems of the Nsukka area, Nigeria.*

was due to a higher than usual proportion of nulliparous females. Each population comprised mainly old females (66–79%) from the ovarian category 4 + 4n and above (Fig. 5). This age structure prevailed during both seasons (Fig. 6). However, young females (ovarian category 0–3) were better represented in dry season trap catches. During each year, the main bulk of young females (64%) were caught during the dry season, unlike old females, which were caught in virtually equal proportions during both seasons (Fig. 7).

Production of staple root crops in the study areas entailed extensive turning over of the soil up to depths of 60 cm from March to August and less extensively from November to January (Fig. 8). However, the depth at which puparia were buried experimentally had no effect on puparial survival (Table VIII). Nevertheless, none of the teneral adults which emerged at depths greater than 20 cm reached the soil surface. Burial of puparia at depths greater than 20 cm also affected the number of teneral flies which successfully made their way through the soil.

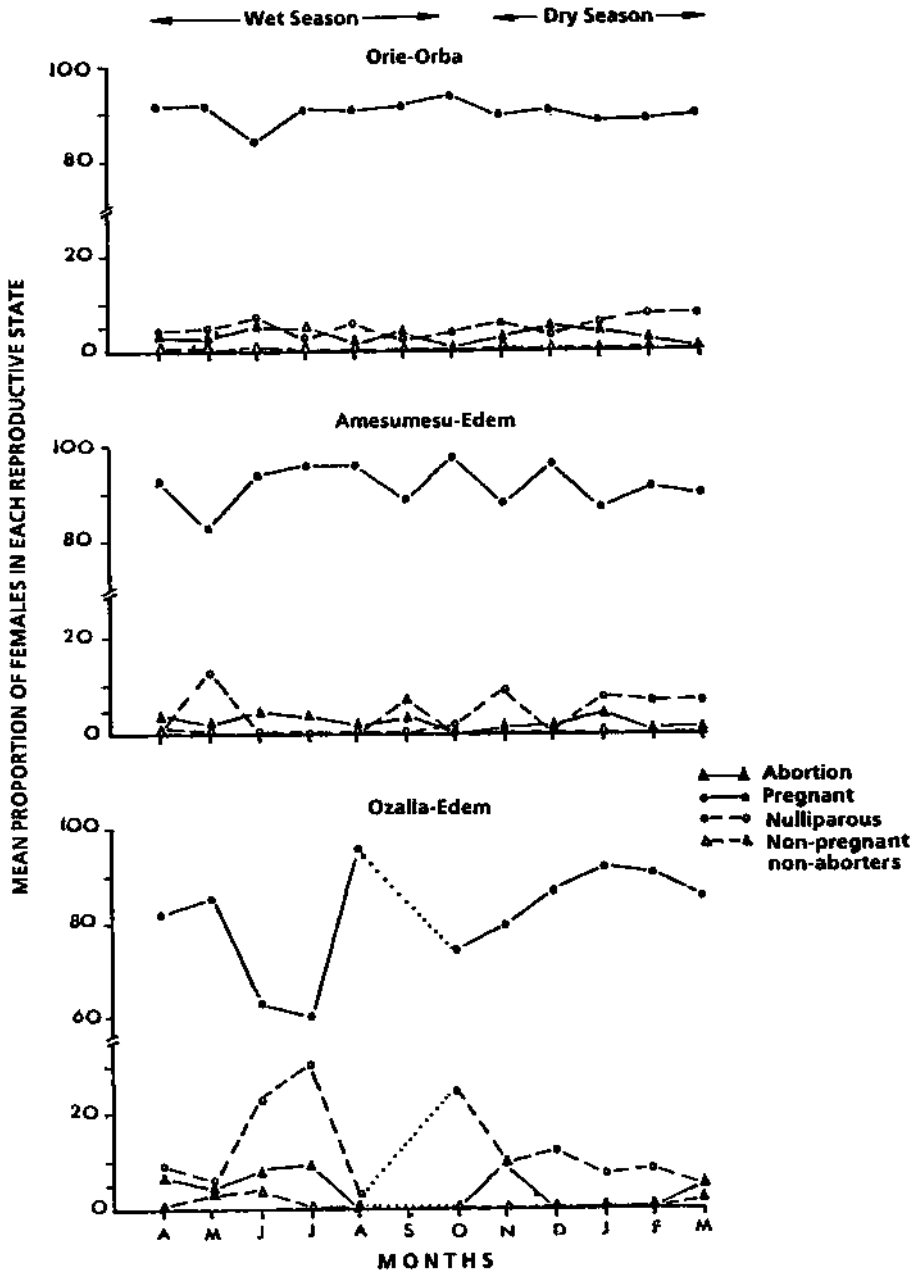


*FIG. 4. Pregnancy and abortion rates in* G. tachinoides *populations inhabiting peridomestic agroecosystems of the Nsukka area, Nigeria.*

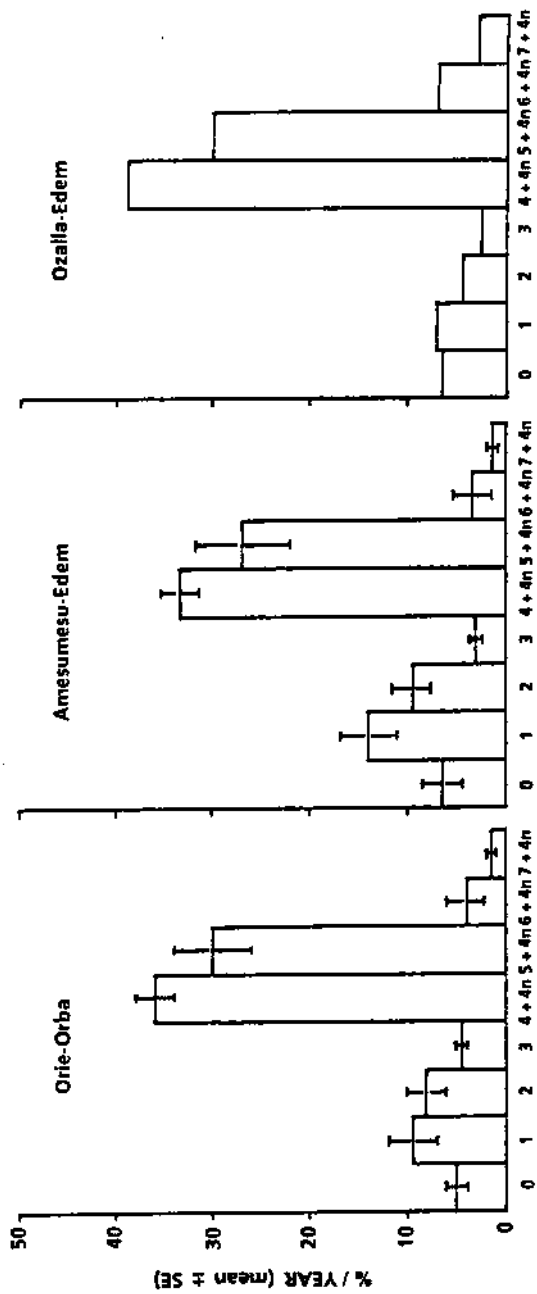


*FIG. 5. Ovarian age structure of* G. tachinoides *populations in different peridomestic agroecosystems of the Nsukka area, Nigeria.*

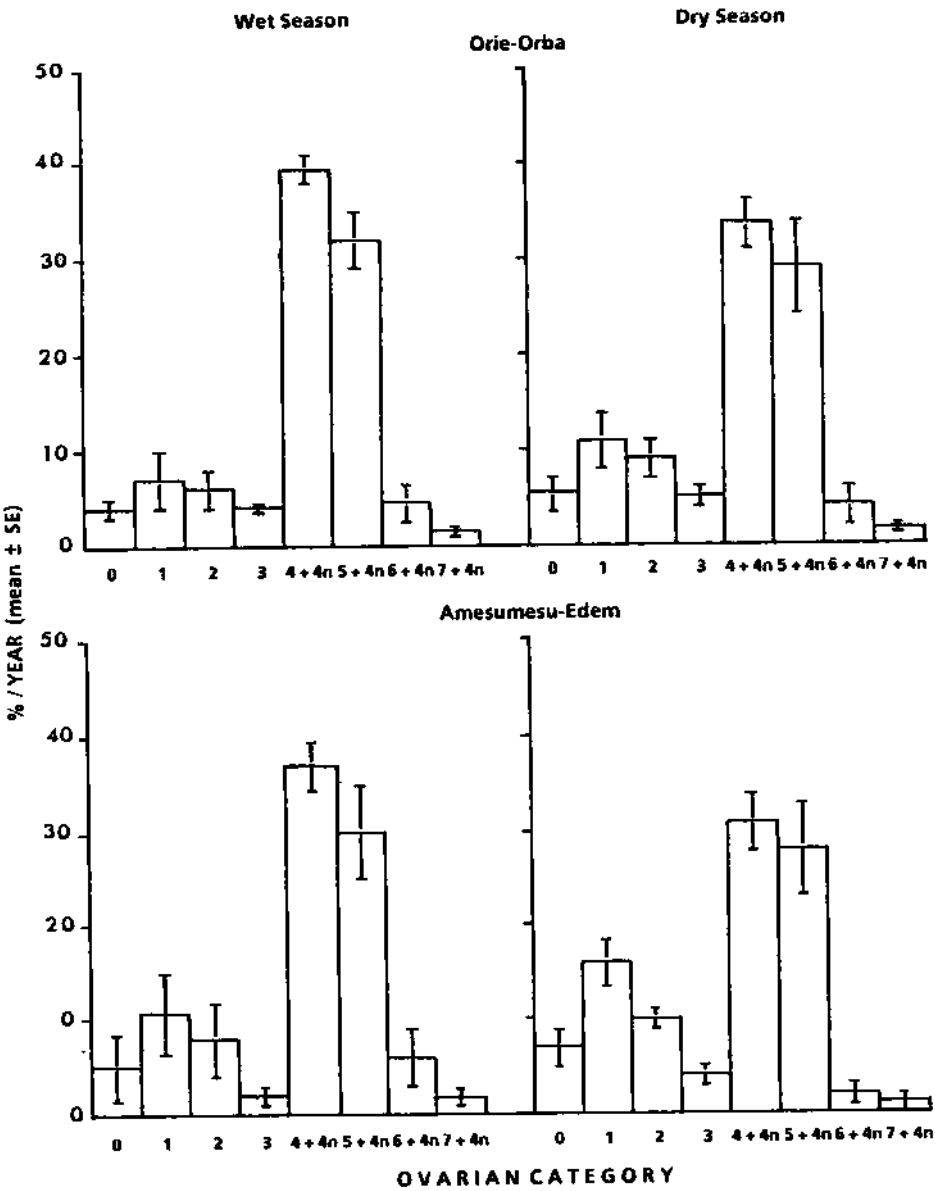


*FIG. 6. Seasonal pattern in the ovarian age structure of* G. tachinoides *populations inhabiting peridomestic agroecosystems of the Nsukka area, Nigeria.*

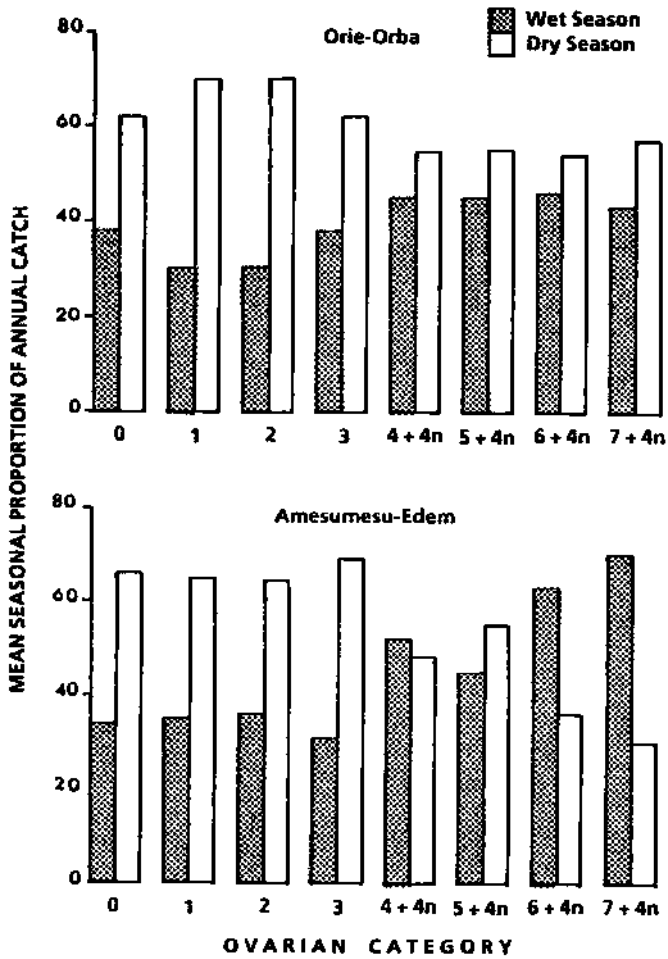


*FIG. 7. Seasonal distribution of peridomestic* G. tachinoides *in various ovarian age categories.*

The surface active arthropod fauna of these farmlands included three predatory genera: *Cylindrothorax* sp. (Coleptera: Meloidae), *Paltothyreus* sp. and *Camponotus* sp. (Hymenoptera: Formicidae). However, only adult tsetse flies emerged from the 116 *G. tachinoides* puparia collected between January and March 1988 from farmland puparial sites and held in the laboratory until eclosion. The emergence rate from these puparia was high (89.65%) and revealed a sex ratio of 1.51 (i.e. 59.61% females).

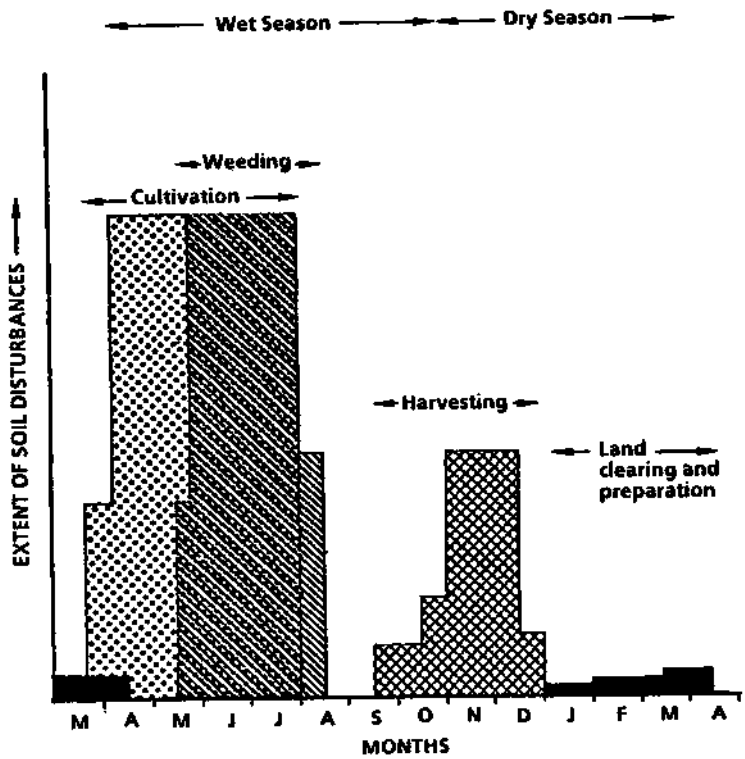


*FIG. 8. Timing and types of agricultural activities in peridomestic agroecosystems of the Nsukka area, Nigeria.*

TABLE VIII. EFFECT OF DEPTH OF PUPARIAL BURIAL ON THE EMERGENCE RATE OF TENERAL *G. tachinoides* FROM THE SOIL

| Depth (cm) | No. of puparia | % eclosion | % surfaced |
|---|---|---|---|
| 5 | 75 | 92.00 | 47.83 |
| 10 | 75 | 89.33 | 35.82 |
| 20 | 75 | 90.67 | 19.12 |
| 30 | 75 | 88.00 | 0 |
| 40 | 75 | 92.00 | 0 |
| 50 | 75 | 89.33 | 0 |

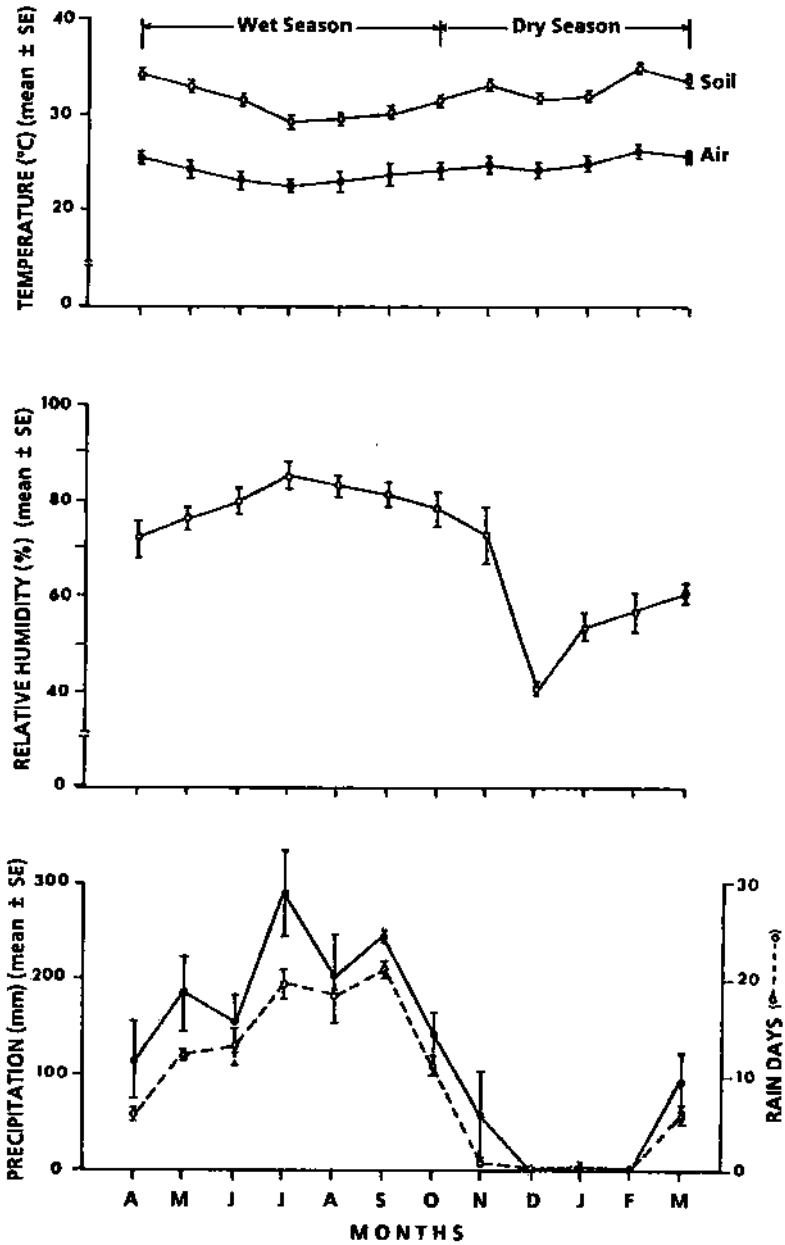


*FIG. 9. Climatological features of the Nsukka area, Nigeria.*

Rainfall increased in amount and frequency from March, reaching a peak in September and then decreasing sharply between October and November, thereby dividing the year into two distinct seasons. This was further characterized by only slight differences in mean monthly air and soil temperatures, but noticeable differences in the mean monthly relative humidity (Fig. 9).

## 4. DISCUSSION

Although the distribution map shows three species of *Glossina* in the Nsukka area (*G. tachinoides, G. palpalis* and *G. longipalpis*) [11], this study found only *G. tachinoides* and *G. palpalis*. When concentrations of domestic pig occur in West African villages both *G. tachinoides* and *G. palpalis* usually become peridomestic [12]; annual average temperatures not exceeding 27°C accompanied by a relative humidity of not less than 60% are also ideal for *G. palpalis* [13], so the scarcity of this species in the Nsukka area is unexpected. The preponderance of *G. tachinoides*, even where the domestic pig population was highest and the diversity of mammalian fauna greatest (i.e. Orie–Orba), suggests that the scarcity of *G. palpalis* in the Nsukka area may largely be due to the absence of other acceptable hosts, e.g. reptiles, wild Suidae or bushbuck [14, 15]. Therefore, although the domestic pig is an important host of the peridomestic tsetse fly in West Africa [12, 16], *G. palpalis* appears to be not as highly dependent on it as *G. tachinoides*. Likewise, the absence of *G. longipalpis* from this area lacking wild Bovidae, especially the bushbuck from which it usually obtains most (about 92%) of its bloodmeals [17], should perhaps be expected.

That *G. tachinoides* also fed on birds and reptiles shows that peridomesticity has not led to a fundamental change in the usual host spectrum of this tsetse species [17], as implied by Baldry [1, 18]. The vulnerability inherent in the observed high dietary dependence of peridomestic *G. tachinoides* on the domestic pig was clearly shown by the collapse of the Ozalla–Edem tsetse population following the removal of all the pigs from that agroecosystem. The collapse was triggered by a reduction in the number of pigs to less than five [19], which clearly suggests that this vulnerability can be exploited for tsetse fly control without totally eliminating the preferred host population. Furthermore, the eventual disappearance of this tsetse population despite the presence of sizeable human and goat populations shows that, at least in Nsukka peridomestic agroecosystems, *G. tachinoides* is a specific rather than an opportunistic feeder and is definitely not anthropophilic; it also has not changed its trophic pattern, as predicted by Baldry [18].

The observed seasonal differences in the sex ratio of these peridomestic *G. tachinoides* populations suggest a need to re-examine the assumption of virtually constant sex ratios in mathematical models of the sterile insect technique (SIT) [20]. Such seasonal differences could periodically upset the ratio of sterile to wild males

in the target population, leading to failure in achieving eradication on schedule without involvement of immigrant wild flies [21]. Furthermore, the marked differences in sex ratio and apparent density between biotopes imply that to achieve and maintain the desired overflooding ratio of sterile to wild males throughout the SIT project area, the number of irradiated males released would need to change with season and biotope.

The observed pattern of seasonal fluctuations in the apparent density of *G. tachinoides* is at variance not only with the findings of Baldry [22] but also with those reported for most tsetse populations [16, 23]. Since the marked seasonal differences in apparent density were not paralleled by comparably marked seasonal climatic differences, the observed pattern could not be explained readily on the usual grounds of temperature and saturation deficit effects on adult natality, mortality and dispersal. However, the incompatibility between the evenly high breeding rate and the paucity of young adults suggests that a substantial proportion of full term pregnancies did not eventually result in the recruitment of new adults into the population, obviously as a result of mortality occurring during the subterranean stages of the developmental cycle. Since the soil temperatures of the Nsukka area were not lethal [23] and no puparial parasitoids or specific predators of either puparia or teneral adults were detected, these potential mortality factors appear incapable of causing such low recruitment of adults. However, since the depth of puparial burial decisively reduced the number of adults reaching the soil surface after eclosion from the puparia, it is evident that routine root crop production activities were principally responsible for the low recruitment of new adults and the rather unusual age structure of *G. tachinoides* in these peridomestic agroecosystems. This conclusion is further supported by the fact that young adults were more poorly represented in samples during the wet season when cultivation and weeding activities were more extensive than during the dry season when harvesting was the only soil disturbing activity and also when the greater bulk of each year's young flies was caught. The effects of land use may partly explain why tsetse populations in villages in West Africa usually occur at lower population densities than those around the fringing cash crop plantations [24]. This suggests that root crop production could be a useful control strategy.

Considering the low apparent density and trophic pattern, the trypanosome species and their very low infection rates in *G. tachinoides* populations, human and livestock trypanosomiases should be minimal in this area. This conclusion is supported by the facts that human sleeping sickness has never been reported in the Nsukka area, the pathogenicity of the prevalent trypanosome species in indigenous West African breeds of domestic animals is low [25] and *palpalis* group tsetse flies have an inherently limited ability to transmit *brucei* and *congolense* group trypanosomes [26–28]. The apparently low economic status of peridomestic *G. tachinoides* obviously makes high financial investment in its eradication unjustifiable. Consequently, it would be prudent to take utmost advantage of the effects of routine

agricultural practices on the fly's abundance and its vulnerable trophic predilection in peridomestic agroecosystems. It seems a viable proposition that further reductions in its apparently low density populations can be accomplished through the combination of insect proofing piggeries in lieu of reducing domestic pig populations and deployment of insecticide impregnated traps or targets which are cheaper and environmentally more acceptable control options than insecticidal spraying.

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# POPULATION DYNAMICS OF *Glossina fuscipes fuscipes* ON BUVUMA ISLAND, LAKE VICTORIA, UGANDA*

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## Abstract

POPULATION DYNAMICS OF *Glossina fuscipes fuscipes* ON BUVUMA ISLAND, LAKE VICTORIA, UGANDA.

A survey made of Buvuma Island to establish the incidence, distribution and population dynamics of tsetse flies showed that *Glossina fuscipes fuscipes* Newstead was the most abundant species. This species occurred in the southeastern, western and northern parts of the island. Although the fly is both riverine and peridomestic in its habits, the survey revealed that its population was concentrated mainly within a distance of about 2 km from the lake shores around houses, in banana plantations, at forest edges and roadsides, in fishing villages and ports of call, on grazing grounds and at water collection points. Studies of the population dynamics revealed seasonal variations in the abundance of the species, with a peak during the main dry season, i.e. in the months of January and February. The breeding peak was reached during the minor rainy season. Trap catches were highest in fishing villages and ports of call, followed in decreasing order by catches at forest edge and roadside locations, at water collection points, around houses, in banana plantations and on grazing grounds. It appears that the fishing villages and ports of call were possibly the most suitable breeding grounds. The efficiency of the traps was reflected in the fact that *G. f. fuscipes* populations were reduced by up to 95% in Tome and 90% in the Bulopa–Walwanda and Lwenyanja villages. Many puparia were also found on the sandy beaches around fishing villages and ports of call. There was evidence of fly movement between the islands and the mainland, indicating that, although geographically isolated, Buvuma Island is not isolated from the mainland in terms of tsetse fly movement.

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## 1. INTRODUCTION

Various control measures such as traps [1–5], mechanical clearing of vegetation, selective game destruction, applications of insecticide from the ground and air, the sterile insect technique (SIT) and other genetic methods [6] have been used, with varying degrees of success, to control tsetse flies. However, host specificity [7] and high labour costs have made game elimination and bush clearing unacceptable. Since the discovery and use of DDT, anti-tsetse fly operations have depended on the application of residual insecticides to known tsetse habitats either from the ground or by helicopter, or non-residual insecticides from fixed wing aircraft or on chemically impregnated traps and screens [8]. Control of *Glossina fuscipes fuscipes* and *G. pallidipes* was attempted in the mid-1950s using dieldrin ground spray along the Nie River in the Central African Republic and in the Nyanza Province of Kenya [9–10]. Similarly, *G. palpalis* populations along the Malawa River, Bukedi [11] and in the Datacha and Jurei areas of West Nile, Uganda [12], were successfully reduced through the ground spraying of DDT.

Recent studies in West Africa have shown that traps and insecticide impregnated screens are effective in reducing populations of riverine tsetse flies. For instance, modified biconical traps, when impregnated with deltamethrin [13], reduced the population of *G. palpalis* by 99% after 3 months and that of *G. tachinoides* by 99.99% along the Leraba River. Since the most effective way of eradicating trypanosomiasis is to eliminate the vector [14], emphasis should be placed on integrated tsetse control and strategies which avoid environmental damage through excessive spraying of insecticides into tsetse habitats. The strong environmental lobby against the use of pesticides in vector control has encouraged many scientists and others involved in vector control to search for inexpensive, cost effective, non-pollutant and environmentally acceptable methods of control such as traps and targets combined with the release of sterile males.

The control of tsetse flies and trypanosomiasis in Uganda was greatly slowed down during the last decade because of civil strife. As a result, the country is currently experiencing a great increase in the incidence of human sleeping sickness [15] and animal trypanosomiasis [16]. The heavily affected areas in Uganda are found in the Busoga region, which includes the Buvuma islands, Lake Victoria shores, northern Uganda, West Nile and southwestern and western Uganda. In an effort to tackle the problem, the Government of Uganda requested assistance from the International Atomic Energy Agency to eradicate tsetse flies on Buvuma Island, using an integrated approach involving SIT. At the same time, the IAEA awarded a research contract, within the Agency's Co-ordinated Research Programme. The work envisaged under the contract aimed at determining the incidence of tsetse flies on the Buvuma islands, studying the distribution of *Glossina* species on the islands, up-dating information on the population dynamics of the tsetse species and determining whether or not Buvuma Island is isolated from the mainland in terms of tsetse fly movement.

## 2. MATERIALS AND METHODS

### 2.1. Study area

Administratively, the Buvuma islands comprise a group of 29 islands, of which only six, namely Buvuma Island proper, Bwema, Buziri, Mpata, Bugaia and Lingira, are large and have settled populations. Buvuma Island proper (219 $km^2$) is the largest and is located in the northern part of Lake Victoria. It lies between 33°12′E to 33°25′E and 0°5′N to 0°20′N. The landscape is characterized by flat topped hills with a fairly gentle gradient ending at the level of Lake Victoria, 1134 m above sea level. Climatically, the Buvuma islands fall under the Lake Victoria zone, where the diurnal variation in temperature is about 7°C and the mean temperature about 24°C most of the year round. There is generally very little variation in temperature and humidity. Rainfall is moderately heavy, about 1125 mm per annum, and is more or less evenly distributed throughout the year, with a major peak in March–April–May and a minor peak in September–November. The major dry season occurs in mid-December–February plus a minor one in June–August.

The vegetation is moist evergreen forest with permanent papyrus forest swamps [17]. The vegetation is primarily short grass on the hill tops and the forests are on the slopes, giving way to extensive inlets of papyrus.

Where there are settlements, mixed farming is practised; farmers grow coffee, bananas and beans, in addition to keeping livestock. The wildlife consists of monkeys, bush pigs, porcupines and other rodents. Spear grass (*Imperata cylindrica*), *Hyparrhenia* species and sedges are the major grasses on the island. The large trees comprise mainly mangoes (*Mangifera indica*), *Maesopsis emini*, *Albizia* species, *Combretum* species, *Polyscias* and *Acacia* species [18, 19].

### 2.2. Trapping

Studies on the incidence, distribution, population dynamics and movement of tsetse flies (*Glossina* species) were started on Buvuma Island in late July 1987. Fly populations were sampled using Lancien pyramidal traps [5] impregnated with deltamethrin (400 mg/trap). A control run of 20 traps was done in August 1987. Subsequently, all the traps used were impregnated with insecticide to improve the efficiency of the catches. However, the non-linear nature of the shoreline, the thick forests and the vegetation layout did not permit placing of the traps at the recommended distance of 100 m apart. The traps were therefore placed in trypanosomiasis high risk areas such as at water collection points, in fishing villages, camps and ports of call, as well as around dwelling houses, in banana plantations, at forest edges and roadsides and on grassland grazing ground in the six villages on the island: Bulopa–Walwanda, Lwenyanja, Kyanamu, Tome, Isiriba and Kachanga.

The traps were laid in the evenings and left on the sites throughout the investigation period. They were only removed every 3 months for reimpregnation with deltamethrin, but were returned the following day. Traps were emptied every morning between 07.00 and 09.00 hours and the catches recorded against the previous day before the traps were reset. Trapping was done for 7 days each month, during which time the biting activity was recorded and the physiological age and trypanosome infection rate in flies were determined by dissection and examination. For the rest of the month trapping was carried out by personnel of the Department of Tsetse Control resident on the island. On average, trapping was carried out for 20 days every month.

### 2.3. Mark–release–recapture

To determine whether or not Buvuma Island is isolated in terms of tsetse fly movements, traps were set at Kiyindi, which is a port of call on the mainland. The traps were emptied daily and the catches recorded. During the main dry season of January and February 1988 flies were trapped, marked with oil paint on the dorsal surface of the thorax and released at the Kyanamu canoe landing point for possible subsequent recapture. During this season human movement greatly increased between the island and the mainland. At the same time it was also observed that flies became active quite early in the morning, presumably because of the high temperatures (25°C) prevalent at this hour.

## 3. RESULTS

### 3.1. Species diversity

It has been suggested in the past that three species of tsetse, *G. brevipalpis*, *G. pallidipes and G. f. fuscipes*, were present on Buvuma Island. However, the current investigation only revealed the presence of *G. f. fuscipes*. The species was encountered in the Kachanga village in the south-east, and in the southern, western and northern parts of the island (Fig. 1). *G. f. fuscipes* must therefore be considered the principal vector of trypanosomiasis on Buvuma Island, and possibly on the adjacent mainland. Distribution of *G. f. fuscipes* was limited to the shorelines and up to about 2 km inland. A total of 6063 flies was caught from Tome, a coastal village on the island. Another 3031 flies were trapped in Bulopa–Walwanda and a further 1856 in Lwenyanja, both of which lie further inland (Fig. 1). The pattern of distribution appeared to follow human settlements, suggesting peridomestic behaviour of *G. f. fuscipes* in the area.

The seasonal variation in the catches (Fig. 2) showed that *G. f. fuscipes* was most abundant during the main dry season, especially in the month of January,

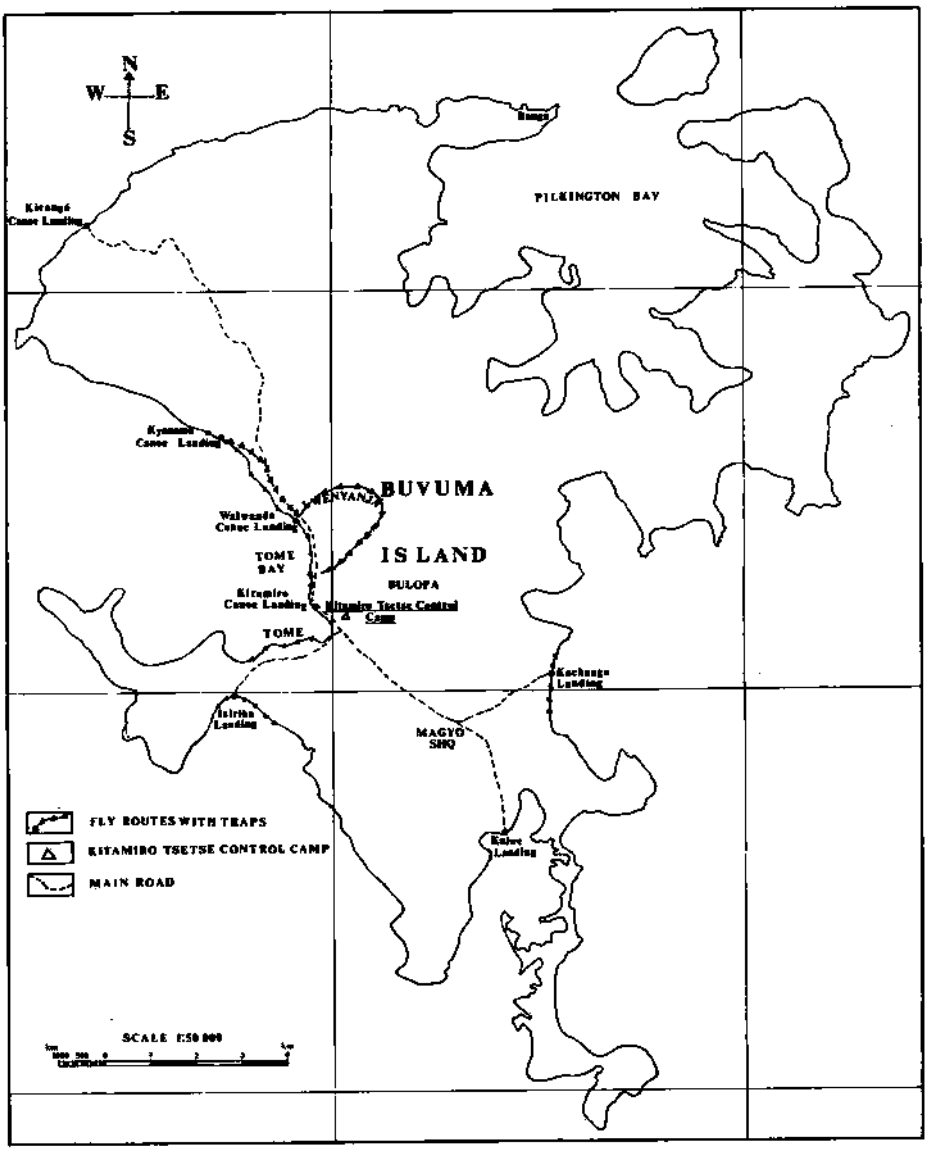


*FIG. 1. Tsetse control trapping sites on the Buvuma islands.*

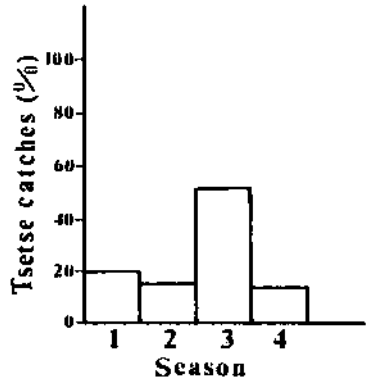


*FIG. 2. Seasonal variation in the catches of* G. f. fuscipes. *Season: 1 = minor dry season (July and August); 2 = minor rainy season (September to December); 3 = main dry season (January and February); 4 = main rainy season (data for March only).*

following the minor rains. This suggested that the flies must have emerged from the larvae deposited in late November or in December.

### 3.2. Population structure

Observations over the entire study period revealed equal ratios of teneral males and teneral females, except in September and March (Table I). The proportions of non-teneral males to non-teneral females followed the same pattern, but with a major departure in March 1988. The ratio of males to females was similar, except again in March. However, the population of non-tenerals showed a continuous decline from July 1987 to March 1988.

The number of flies caught per trap per day also showed variations (Fig. 3), with peak catches in August 1987 and January 1988. The control traps placed in Tome, Bulopa–Walwanda and Kyanamu caught, on average, 22.8 flies per trap per day, which is quite high. Towards the end of the trapping exercise, the population reduction was determined for Tome, Bulopa–Walwanda and Lwenyanja (Fig. 4), which showed a gradual decrease in the population of *G. f. fuscipes*; by January there were very few flies in any of the three villages. Population reduction was more rapid in Tome than in the other two villages, reaching a 95% reduction compared with 90% in the other two villages after 9 months of trapping.

The results summarized in Fig. 5 indicate that tsetse flies were most abundant in fishing villages and ports of call, followed in decreasing order by forest edge and roadside locations, water collection points, human dwellings, banana plantations and grassland grazing grounds.

TABLE I. POPULATION STRUCTURE OF *G. f. fuscipes* ON BUVUMA ISLAND

| | Monthly ratios | | | |
|---|---|---|---|---|
| | Teneral male: teneral female | Non-teneral male: non-teneral female | Male: female | Teneral: non-teneral |
| July | 1:0.92 | 1:0.87 | 1:0.89 | 1:1.53 |
| August | 1:0.76 | 1:0.71 | 1:0.74 | 1:1.06 |
| September | 1:0.59 | 1:0.91 | 1:0.73 | 1:0.91 |
| October | 1:0.98 | 1:1.22 | 1:1.08 | 1:0.82 |
| December | 1:0.90 | 1:0.58 | 1:0.79 | 1:0.40 |
| January | 1:0.81 | 1:0.93 | 1:0.85 | 1:0.51 |
| February | 1:0.97 | 1:1.10 | 1:0.99 | 1:0.27 |
| March | 1:1.41 | 1:2.82 | 1:1.65 | 1:0.34 |

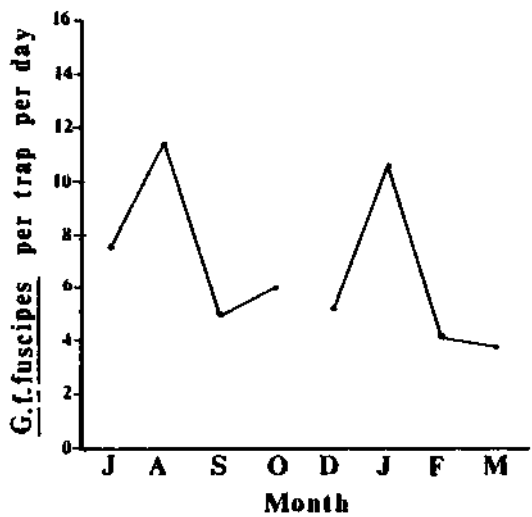


*FIG. 3.* G. f. fuscipes *catches/density on Buvuma Island.*

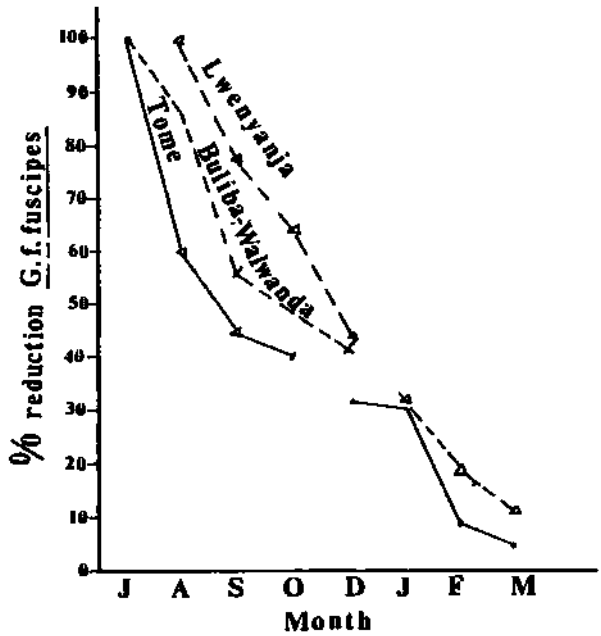


*FIG. 4. Reduction in the* G. f. fuscipes *population.*

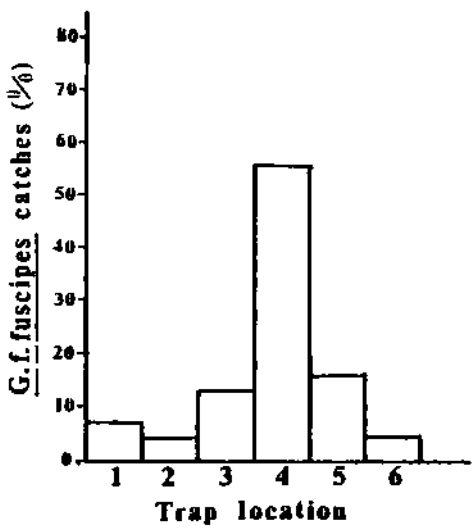


*FIG. 5.* G. f. fuscipes *caught in different locations. Trap locations: 1 = houses; 2 = banana plantations; 3 = water collecting points; 4 = fishing villages and ports of call; 5 = forest edges and roads; 6 = grazing grounds.*

The mark–release–recapture experiment with *G. f. fuscipes* did not yield any meaningful results. None of the marked flies was caught at the Kiyindi trapping site on the mainland. However, flies were seen on canoes travelling from Kyanamu on Buvuma Island to the Rippon Falls landing point near Jinja. Two teneral males and one non-teneral female were caught on one occasion and one teneral male and one teneral female on another journey to Jinja. On several other occasions travellers were bitten, although the tsetse flies escaped being caught. To date, no flies have been caught on the journeys from the mainland to the island. However, there appears to be sufficient evidence for fly movement between the island and the mainland.

## 4. DISCUSSION

The current study has confirmed the presence of *G. f. fuscipes* on Buvuma Island, but this was the only tsetse fly species trapped. As *G. f. fuscipes* is the main vector of human and animal trypanosomiasis on the adjacent Busoga mainland, it is probably also the main vector of the disease on Buvuma Island. Distribution of the fly was along the shorelines, coincidental with human settlements. Human settlements are mostly located about 2 km inland from the lake shore, and although *G. f. fuscipes* appears to display peridomestic behaviour, it seems that the species breeds and feeds mainly at locations on the sandy beaches, as evidenced by the number of adults trapped and the number of puparia collected at landing points.

The seasonal variations observed in *G. f. fuscipes* populations showed that over 50% of the flies were produced during the main dry season in January and February (Fig. 2), suggesting that the main breeding months are late November and December. Most of the flies collected during January and February were teneral and hungry flies, which are potentially dangerous as vectors of trypanosomiases. This contrasts with *G. morsitans*, which has been shown to breed at the peak of the dry season, i.e. just before the onset of the rains, in Zimbabwe [20]. It can be concluded that the risk of trypanosomiasis infection on Buvuma Island is greatest during the main dry season because of the sheer number of flies caught.

It would appear that those sections of the human population at greatest risk are fishermen, traders and women fetching water and, to some extent, travellers between the island and the mainland. Cattle are also at risk, especially at the watering points. The current work has confirmed that *G. f. fuscipes* is peridomestic in its habits on Buvuma Island as well as on the shore areas. Although the percentage of peridomestic flies was low, it should not be ignored because infected fishermen and traders return to their homes, a situation which could easily cause an epidemic if a few fishermen were to be infected.

The Lancien pyramidal traps used in this study reduced the population of *G. f. fuscipes* by about 90% in Bulopa–Walwanda and Lwenyanja and by 95% in Tome. This is comparable to the 99 or 90% recorded in similar trapping operations

[3, 13, 21]. The fly population was reduced to a low level after about 6 months. Nevertheless, it should be remembered that we were not dealing with an isolated population of *Glossina*; infiltration from neighbouring areas into the trapping villages was a probability. The ratio of males to females was about equal, with few exceptions; females lived longer than males and the death of old flies was more than compensated for by the emergence of young flies. The best season for effecting control would be the main dry season.

Buvuma Island is geographically isolated from the mainland and other smaller islands by a vast expanse of water. In terms of tsetse fly movement, however, it is not isolated as canoes have been seen to carry tsetse flies to the mainland; any control measures against tsetse should take this into consideration. It is suggested that an integrated approach involving the use of traps and SIT, coupled with limited use of insecticides, might be the appropriate anti-tsetse fly operation if the animal and human populations are to be protected from trypanosomiasis by environmentally acceptable methods.

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# POPULATION ESTIMATION FROM MARK–RECAPTURE DATA

## *Equations for a pooled mark system and for pooled data, with applications to a study on island populations of tsetse flies in Zimbabwe**

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**Abstract**

POPULATION ESTIMATION FROM MARK–RECAPTURE DATA: EQUATIONS FOR A POOLED MARK SYSTEM AND FOR POOLED DATA, WITH APPLICATIONS TO A STUDY ON ISLAND POPULATIONS OF TSETSE FLIES IN ZIMBABWE.

This study stemmed from a mark–recapture experiment carried out on Antelope Island, Lake Kariba, Zimbabwe, using the tsetse flies *Glossina morsitans morsitans* (Westwood) and *G. pallidipes* (Austen). Batch marking systems were used with a view to applying the Jolly–Seber (J–S) stochastic model for estimation of the fly population levels and the allied vital parameters. Between February and May 1980 a daily batch mark was used; in this way daily J–S estimates could be calculated in the normal way. From October 1980 to April 1984 the population was still sampled daily, but the batch mark was only changed once a week. This change in design means that the J–S ($Q_1$) estimates cannot be used without introducing serious systematic errors. The problem arises, partly, because the apparent marked proportion declines during each week and the 'natural' pooling can underestimate the true value by as much as 50%. This bias is due to the effects of addition by birth of unmarked flies, and to the fact that a fly, once marked in a given week, is ignored until the next week. In addition, a proportion of flies marked in a given week will die before they are eligible for recapture. This tends to cause the marked population to be underestimated and the probability of survival to be overestimated. An approach which overcomes these problems has been developed by considering the further subset of the marked population. For male *G. m. morsitans*, the resulting ($Q_3$) estimates of the marked population and the probability of survival are very similar, on average, to the numerical solution ($Q_2$) estimates, and show considerably less variation. The $Q_3$ estimates indicate that, as expected, the $Q_1$ equations overestimate the probability of survival and severely underestimate the proportion of marked flies in the population. There is no consistent difference between the $Q_1$ and $Q_3$ estimates of the number of births. The above results suggest alternative solutions to the general problem of producing pooled estimates for periods longer than the initial intersample interval. Various series of estimates have been developed and tested on the Antelope Island *daily* marking data.

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## 1. INTRODUCTION

The current research was undertaken over the period 1986–1988. The results presented here centre on the theoretical problem of estimating population parameters, and have been applied to the mark–recapture data set produced by Vale et al. [1] from the Antelope Island mark–recapture study. As such, the study has been largely reliant on computer facilities.

Estimation of the vital parameters of insect populations is made difficult by their small size and, often, their high mobility. Numerous methods have been developed to overcome the problems [2], many of which are based on techniques generically referred to as 'mark–recapture' methods. It is commonplace for the accuracy of such parameter estimates to be rather poor due to problems such as low probability of capture, and the related problems of small sample size and low numbers of marked recaptures. Such problems often lead to 'impossible' parameter estimates (survival values greater than unity and negative recruitment values). It is often necessary to pool data from several consecutive samples in order to increase the accuracy of the estimates, at the expense of losing information on changes (assumed small) in the parameters during the pooling period. Arnason and Baniuk [3] cite three possible ways of approaching the problem:

(1) Reduce the capture history *data* so that one records only whether or not an animal is captured during the chosen pooling period; the estimates are then formed in the usual way. These are called the 'reduced capture history', or RCH estimates.

(2) Pool the *statistics* obtained from the raw data, using appropriate modifications of the Jolly–Seber (J–S) [4] equations to estimate the pooled parameters.

(3) Form weighted or moving averages of the original parameters.

The last method, suggested by Jolly [5], is said by Arnason and Baniuk [3] to give unreliable estimates, but the first two methods have apparently been shown to give similar results, both with substantial improvements in precision [6]. The RCH approach has lately been preferred [7], but it is demonstrated here that this method can, under certain circumstances, lead to substantial errors in parameter estimation. The problem is best approached by considering the experiment which gave rise to it.

In 1979 populations of tsetse flies (*Glossina morsitans morsitans* (Westwood) and *G. pallidipes* (Austen)) were introduced on to a 5 $km^2$ island in Lake Kariba, Zimbabwe [1]. The flies were captured, marked and released, using ox fly rounds [8]. Between February and May 1980 a daily mark was used, but the high probability of recapture made this impractical, and from October 1980 to April 1984 the mark only changed weekly. The two marking techniques referred to above are termed henceforth the 'daily' and 'weekly' marking schemes.

When a fly was caught for the first time in any given week, in the weekly marking scheme, it was given the mark of that week. If, during a fly round, a fly was

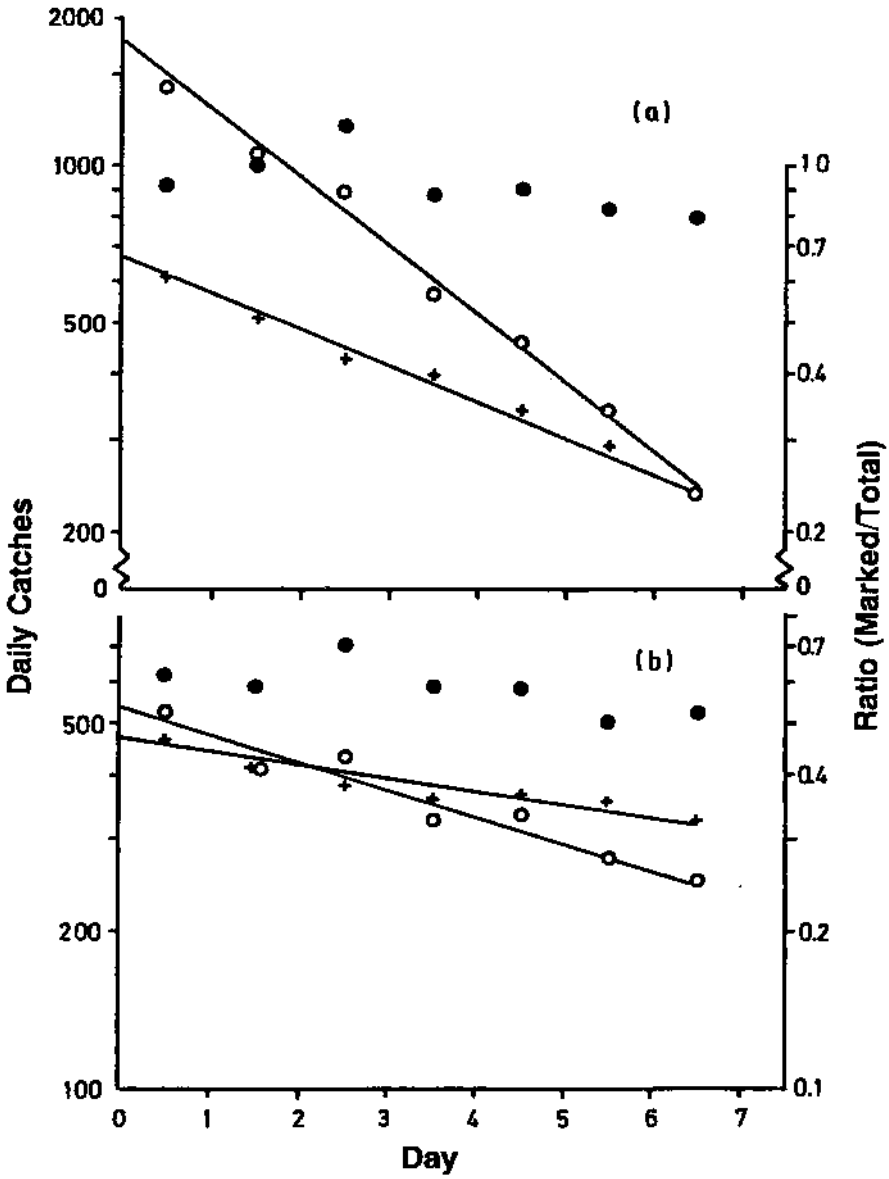


*FIG. 1. Antelope Island mark–recapture results: changes in the daily catches, from ox fly rounds, of* G. morsitans *males (a) and* G. pallidipes *females (b) during a typical week. ((●) = unmarked flies; (○) = marked flies; (+) = ratio of marked to total captures.)*

caught which already bore the mark of the week in question it was released without further marking, but also without being counted again. The 'available' population at any point in a given week consisted of flies which had never been captured (and were thus unmarked), and *marked* flies which had not, however, been captured previously during the week in question. The number of available flies was reduced during any given week, due to deaths and to capture and marking, but was augmented by births in the case of the unmarked population. There was no addition to the *available marked* population during the week. This tended to reduce the number of flies counted, and the proportion of marked recaptures among them, in each successive fly round during the week (Fig. 1).

In the J–S method the proportion of marked flies in each sample is taken as an estimate of the proportion of marked flies ($\alpha_i$) in the population under study, and is used to estimate the parameters of interest. With the experimental design just described, the proportion of marked flies in a sample is not, in general, a good estimate of $\alpha_i$. Thus, for *G. morsitans* males (Fig. 1) the apparent, or measured, marked fraction at the beginning of the week was of the order of 60%. By the end of the week the apparent figure had fallen to about 40%; the 'average' value was about 50%, so that $\alpha_i$ was probably being underestimated by about 20%.

Even when, as in *G. pallidipes*, the apparent marked proportion did not change markedly during the sampling period a further problem exists. This arises from the requirement of the J–S model that all the animals released after a given sample have the same probability of survival and recapture thereafter. However, if the 'sample' is, in reality, a series of samples spread over an appreciable time (relative to the survival probability of the study animal), this requirement will not be satisfied. The paper describes the attempts made to circumvent these problems and applies the results to the experimental data mentioned above. A few points should be noted before analysis is undertaken.

*First*, of note is the chosen design (weekly marking system) performed, at the marking level, the exact pooling of which is achieved by the RCH method [3]. This method will encounter the problems referred to above when applied to data sets similar to ours, but where the individual capture histories are known. *Second*, in the Antelope experiment, once the 'pooled marking' had been performed, neither of the other approaches suggested by Arnason and Baniuk [3] was possible, since the daily parameter estimates and statistics were not recoverable from the data. *Third*, since the experiment included a 16 week period when daily marking *was* carried out, we do have a data set with which we can check the solutions achieved, both against each other and against the pooling techniques suggested previously in the literature. Finally, and by way of justification of the study, it should be noted that this work was motivated by more than a desire to investigate the properties of different 'pooling' techniques as an end in itself. The Antelope Island data set provides the opportunity of obtaining unusually accurate population parameter estimates for about 200 consecutive weeks for *G. morsitans*, and for about 150 weeks for *G. pallidipes*.

The aim of the study is to build a complete mathematical model for both species of tsetse fly which could be of great theoretical and practical benefit. The Antelope Island populations were used as a testing ground for new tsetse control techniques and three different methods were used against the flies during the experiment. It is of great practical importance to be able to determine, as accurately as possible, how these control measures affected the populations. It is thus of practical importance that the best possible estimates are obtained from the original mark–recapture data.

## 2. METHODS

### 2.1. Assumptions, notations and sample i,j

#### *2.1.1. Assumptions*

(1) Animals are sampled during s periods of time, assumed equal in duration for simplicity; during each period K samples are taken. Animals caught in sample j of period i may be killed on capture, or released. In the former case the numbers of unmarked and marked animals (and the marks on them) are noted. In the latter case the animals are categorized and counted in this way, as long as they do not bear the mark of period i; they are then given mark i, and are released. *Animals already bearing mark i on capture, and which are not killed, are ignored, i.e. they are not counted, nor are they categorized, but are simply released.*

(2) Each animal alive in the population during period i has an identical probability $\Phi_i$ of surviving from subsample i,j to i,j + 1 (for all j, j = 1, K − 1).

(3) Other assumptions regarding equality of probability of capture of all animals, mark retention and reporting are as given by Seber [2].

#### *2.1.2. Notations*

| | |
|---|---|
| i | is the time of period i, or the period itself, depending on the context |
| i,j | is the time, assumed instantaneous, of sample j of period i, or the sample itself, depending on the context |
| Mark i | is the mark given to all animals marked during i |
| Sample i,j | is sample j of period i |
| $N_{i,j}$ | is the total population just before sample i,j |
| $M_{i,j}$ | is the marked population at this time |
| $U_{i,j}$ | is the unmarked population at this time. (*Note:* For compactness, we denote the pooled, or averaged, estimates of the total, marked and unmarked populations in period i by $N_i$, $M_i$ and $U_i$, respectively.) |

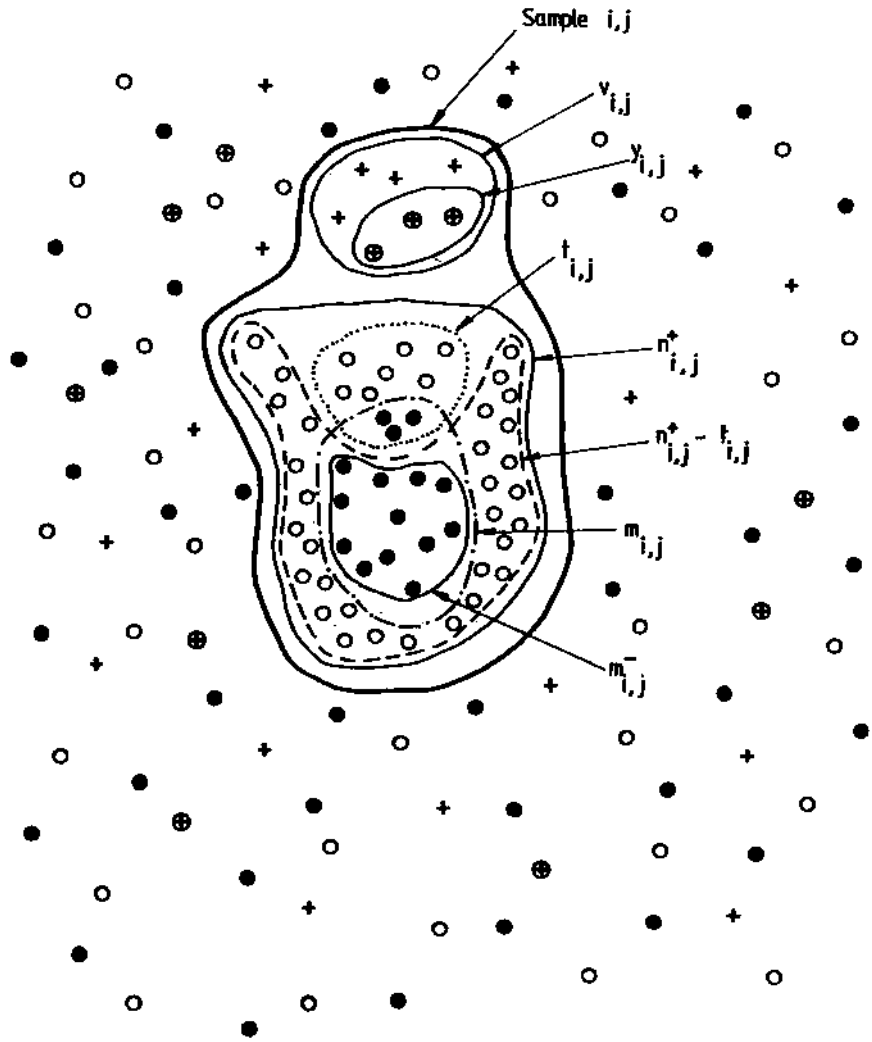


*FIG. 2. A diagrammatic representation of sample j of period i in a mark–recapture experiment. The population consists, at the time of the sample, of unmarked animals (○); animals which have already been caught and marked (for the first time) during period i (+); animals bearing marks from periods prior to i, which have been caught and marked again during i (⊕); and animals bearing marks of periods prior to i which have not yet been caught and marked during period i (●). From sample i,j, a total of $t_{i,j}$ animals which do not already bear mark i, and $v_{i,j}$ which do, are removed from the population. All other animals bearing mark i are 'ignored' (i.e. they are neither recorded nor counted). All other animals* not *bearing mark i are given that mark and released (see text for further explanation).*

*2.1.3. Sample i,j (Fig. 2)*

$n^{+}_{i,j}$ is the total number of animals not bearing mark i

$n_{i,j}$ is the net number of animals given mark i and released

$t_{i,j}$ is the number of members of $n^{+}_{i,j}$ killed on capture

$v_{i,j}$ is the total number of animals killed which already bore mark i on capture

(*Note:* On the basis of the above four definitions $n_{i,j} = n^{+}_{i,j} - t_{i,j} - v_{i,j}$ and $n_{i,j}$ can be negative.)

$y_{i,j}$ is the total number of animals among the $v_{i,j}$ which also bear marks prior to i

$m^{+}_{i,j}$ is the total catch of marked animals

$m_{i,j}$ is the number of members of $m^{+}_{i,j}$ last marked prior to period i (*Note:* $m_{i,j} = m^{+}_{i,j} - t_{i,j}$.)

$m^{-}_{i,j}$ is the number of members of $m^{+}_{i,j}$ which are marked and released

$u^{+}_{i,j}$ is the total catch of unmarked animals

$u_{i,j}$ is the number of members of $u^{+}_{i,j}$ which are given mark i and released

$R_i$ is the number of animals caught, marked and released during period i which survive to the beginning of i + 1

$r_i$ is the number of $R_i$ which are subsequently recaptured at least once

$Z_i$ is the number of animals caught at least once prior to period i, not caught during period i, and which survive to period i + 1

$z_i$ is the number of $Z_i$ which are subsequently captured at least once

$b_i$ is the number of new animals joining the population between samples i,j and i,j + 1, and alive at i,j + 1; $b_i$ is assumed to be independent of j

$B_i$ is the number of new animals joining the population during the whole of period i, and alive at the start of period i + 1

$\Phi_i$ is the probability of an animal surviving from the start of sample i,j to the start of sample i,j + 1; $\Phi_i$ is assumed to be independent of j

$\alpha_{i,j}$ is the proportion of marked animals in the population at time i,j

$p_{i,j}$ is the probability of an animal alive at time i,j being caught in sample i,j

$\hat{}$ is the symbol used to denote estimated values throughout.

The cumbersome notation involving the different subdivisions of sample i,j has apparently been introduced at this point in order to simplify the later development. The basic experimental design, and the ways in which it differs from the typical J–S

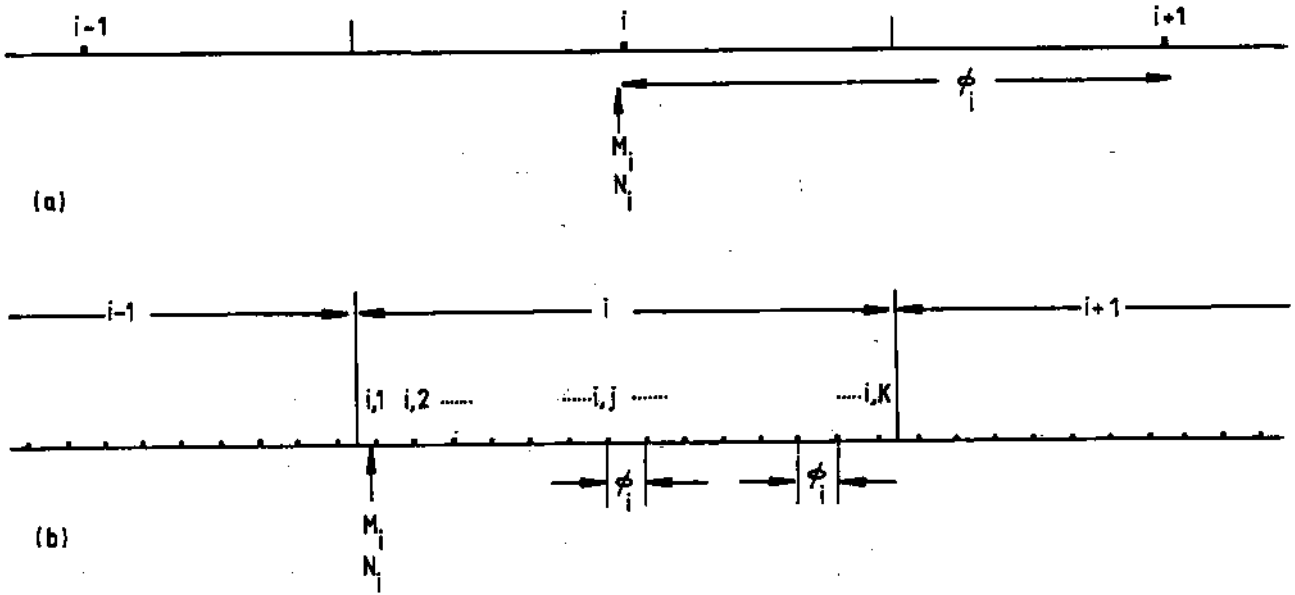


*FIG. 3. (a) Design for a standard J–S mark–recapture experiment. Animals are captured during one or more samples 1,2, ... i, ..., each of which is considered to be of negligible duration. Each animal captured is given a mark corresponding to the sample in which it is captured, unless marks unique to each individual are used. The marked and total populations ($M_i$ and $N_i$) corresponding to period i are defined for the time just before the i-th sample is taken. $\Phi_i$ is the probability that an animal released after sample i will survive, and still be in the population, at the beginning of sample i + 1. The number of additions to the population is similarly defined as the number entering the population between i and i + 1, and which are still alive and in the population at i + 1. (b) A mark–recapture design as used in the Antelope Island experiment. This design differs in that the same mark is applied to animals taken in each of the K samples from each period 1,2, ... i, ... . Again, each of the samples is assumed to be of negligible duration, but the duration of each period is not negligible. Here, the marked and total populations refer to the moment before the first sample of period i is taken. $\Phi_i$ is measured from the end of sample i,j to the beginning of i,j + 1. The number of surviving additions ($b_i$) is measured over the same interval; both are assumed to be independent of j.*

model, are illustrated in Fig. 3; two different pooling techniques are also illustrated. Catches of different categories of animals in a typical sample (i,j) from the Antelope Island 'weekly marking' design are depicted in Fig. 2.

## 3. RESULTS AND DISCUSSION

Jolly [4] and Seber [9] calculated estimates for the required population parameters using a maximum likelihood approach, but an intuitive approach [4] gave identical equations; this approach has been applied to the present problem. For ease of comparison, we now state the J–S estimates relating to sample j of period i, and also the RCH (pooled) estimates (here termed the $Q_1$ estimates) for period i

$$M_{i,j}(J) = (z_{i,j}n_{i,j} + r_{i,j}m_{i,j})/r_{i,j} \quad (1)$$

$$M_i(Q_1) = \Sigma\,(z_i n_{i,j} + r_i m_{i,j})/r_i \quad (2)$$

$$\alpha_{i,j}(J) = m_{i,j}/n^+_{i,j} \quad (3)$$

$$\alpha_i(Q_1) = \Sigma\, m_{i,j}/\Sigma\, n^+_{i,j} \quad (4)$$

$$N_{i,j}(J) = M_{i,j}(J)/\alpha_{i,j}(J) \quad (5)$$

$$N_i(Q_1) = M_i(Q_1)/\alpha_i(Q_1) \quad (6)$$

$$\Phi_{i,j}(J) = M_{i,j+1}(J)/(M_{i,j}(J) - m_{i,j} + n_{i,j}) \quad (7)$$

$$\Phi_i(Q_1) = M_{i+1}(Q_1)/(M_i(Q_1) - \Sigma\, m_{i,j} + \Sigma\, n_{i,j}) \quad (8)$$

$$B_{i,j}(J) = N_{i,j+1}(J) - \Phi_{i,j}(N_{i,j}(J) - t_{i,j} - v_{i,j}) \quad (9)$$

$$B_i(Q_1) = N_{i+1}(Q_1) - \Phi_i(N_i(Q_1) - \Sigma\,(t_{i,j} + v_{i,j})) \quad (10)$$

$$p_{i,j}(J) = n^+_{i,j}/N_{i,j}(J) \quad (11)$$

$$p_i(Q_1) = \Sigma\, n^+_{i,j}/N_i(Q_1) \quad (12)$$

(*Note:* The summations above are for $j = 1,K$ and this will be the case henceforth unless stated otherwise.)

For the J estimates above, note (with reference to Fig. 3) that we do not differentiate between marked animals in sample i,j on the basis of whether that mark was received before or during mark i. We thus take $v_{i,j} = 0$ for all j, in order that the notation makes sense.

### 3.1. Development of new equations for $M_i$ and $\Phi_i$

As with the development of the J–S model we consider the origins of two groups of animals: first, the $R_i$ animals that are alive at the beginning of i + 1, which bear mark i, and $r_i$ of which are recaptured; second, the $Z_i$ animals which were marked prior to i, were not recaptured during i, survived to the beginning of i + 1, and $z_i$ of which are recaptured subsequently. Then

$$R_i = \sum n_{i,j}\Phi_i^{K-j+1} \tag{13}$$

$$Z_i = M_i\Phi_i^K - \sum m_{i,j}\Phi_i^{K-j+1} \tag{14}$$

(*Note:* It should be recalled that all summations are assumed to be over j = 1,K, unless otherwise stated.)

Since every individual of $R_i$ and $Z_i$ is assumed to have the same probability of recapture, and since $r_i$ and $z_i$ of them are recaptured, respectively, we have

$$\frac{r_i}{\sum n_{i,j}\Phi_i^{K-j+1}} = \frac{z_i}{M_i\Phi_i K - \sum m_{i,j}\Phi_i^{K-j+1}} \tag{15}$$

In the classical J–S model this type of development leads to a closed form solution for $M_i$, but here $\Phi_i$ and $M_i$ are not immediately separable. Rearranging Eq. (15) we get the estimate

$$M_i(Q_2) = \left(\sum (z_i n_{i,j} + r_i m_{i,j})\Phi_i^{1-j}(Q_2)\right)/r_i \tag{16}$$

Equation (16) holds for all $2 < i < s$. $M_1$ is clearly zero. For i = 2 we note that $M_2 = R_1$. Also note that when K = 1 Eq. (16) simplifies to give Eq. (1). An alternative relationship between the $M_i$ and $\Phi_i$ is derived by noting that the number of marked animals in the population at the beginning of period i + 1 is the sum of the survivors of animals marked during i, and the survivors of animals marked prior to i, but not captured during i. Thus

$$M_{i+1} = R_i + Z_i \tag{17}$$

so that we can write

$$M_{i+1}(Q_2) = M_i(Q_2)\Phi_i^K(Q_2) + \sum (n_{i,j} - m_{i,j})\Phi_i^{K-j+1}(Q_2) \quad (18)$$

If we take K = 1 in Eq. (18) and rearrange we get Eq. (7). Once estimates for $M_i$ (defined as equal to $M_{i,1}$) and $\Phi_i$ are available (see below), it is then possible to go back and produce estimates for the $M_{i,j}$. Using the same sort of intuitive arguments as above we have, for j = 2,K

$$M_{i,j}(Q_2) = M_i(Q_2)\Phi_i^{j-1}(Q_2) + \sum (n_{i,j} - m_{i,k})\Phi_i^{j-k}(Q_2) \quad (19)$$

where the sum in the second term runs from k = 1, j − 1. It has not proved possible to disentangle $M_i(Q_2)$ and $\Phi_i(Q_2)$, and thereby to produce explicit equations for these parameters; this leads to serious problems in retrieving the estimates. Accordingly, alternative solutions have been sought. Manly and Parr [10] have developed a method for extracting population parameters, from mark–recapture data, similar in principle to the J–S method, using the same categories of marked and unmarked animals, plus a further subset of the marked animals. Thus

$W_i$ is the number of animals captured at least once before period i, captured again during i, and surviving to the start of period i + 1

$w_i$ is the number of $W_i$ subsequently recaptured at least once.

We derive the following equation for the $W_i$

$$W_i = \sum (\bar{m}_{i,j} - y_{i,j})\,\Phi_i^{K-j+1} \quad (20)$$

We now use the assumption of the equality of probability of recapture of all the animals and, in particular, of $W_i$ and $R_i$. From Eqs. (13) and (20)

$$\frac{r_i}{\sum n_{i,j}\Phi_i^{K-j+1}} = \frac{w_i}{\sum (\bar{m}_{i,j} - y_{i,j})\Phi_i^{K-j+1}}$$

from which

$$\sum (w_i n_{i,j} - r_i(\bar{m}_{i,j} - y_{i,j}))\Phi_i^{J-j} = 0 \quad (21)$$

Since $\Phi_i$ is the only unknown in this equation it can, in principle, be calculated by using a numerical technique; the resulting estimates from real data are, however, patently quite absurd. Similarly, attempts to estimate the survival probability from Eq. (16), when apparently reasonable estimates of $M_i$ are available, lead to ridiculous results. The reason is presumably the same — that one is trying to measure

a rate of change over too short an interval. The implicit solution for $\Phi_i$ given by Eq. (21) is thus of no immediate value. However, a useful result can be obtained by recalling Eq. (17) in the form

$$M_i = R_{i-1} + Z_{i-1}$$

and observing that

$$M_i \Phi_i^K = Z_i + W_i \tag{22}$$

Combining Eqs (17) and (22) and assuming the equality of probability of recapture of the two groups of animals, we obtain the following estimate for the survival probability

$$\Phi_i^K(Q_3) = \frac{z_i + w_i}{r_{i-1} + z_{i-1}} \tag{23}$$

(*Note:* The quantities $\Phi_i(Q_3)$ are now used in Eq. (18) to give the $Q_3$ estimates of $M_i$.)

### 3.2. Equations for $N_i$ and $B_i$

In the J–S model, the $N_i$ are calculated directly from the $M_i$, on the assumption that the fraction $(m_i/n_i)$ of marked flies in each sample is identical to the fraction $(\alpha_i)$ of marked flies in the population at large. With the present experimental design this assumption can only be applied to the first sample of each week (because after the first sample some of the marked population are 'ignored'). An approach is now developed which uses the probability of capture of an estimated subclass of the marked population. This probability is used to estimate the *unmarked* population at time i,j and this, when added to the estimate of the number of marked flies, provides an estimate of the total population. For j = 2,K

$$P_{i,j}(Q_3) = \frac{m_{i,j}}{M_{i,j}^{(a)}} \tag{24}$$

where $M_{i,j}^{(a)} = M_{i,j}^{(a)}(Q_3)$

$$= M_i \Phi_i^{(j-k)} - \sum m_{i,k} \Phi_i^{(j-k)} \tag{25}$$

is the estimate of the number of animals in the population bearing marks, other than i, at the start of sample i,j.
(*Note:* The sum in the second term runs from k = 1, j − 1.)

We can use Eq. (24) to estimate the unmarked population ($U_{i,j}$) at time i,j

$$U_{i,j}(Q_3) = \frac{n_{i,j}^{+} - m_{i,j}}{p_{i,j}(Q_3)} \tag{26}$$

(assuming equality of probability of capture of $U_{i,j}$ and $M_{i,j}^{(a)}$). Now, by definition

$$N_{i,j} = M_{i,j} + U_{i,j}$$

so that, using Eqs (19) and (26), we have the approximately unbiased estimate

$$N_{i,j}(Q_3) = \left(\frac{n_{i,j} + 1}{m_{i,j} + 1}\right) M_i \Phi_i^{j-1} + \frac{\sum (n_{i,k}(m_{i,j} + 1) - m_{i,k}(n_{i,j}^{+} + 1))\, \Phi_i^{j-1}}{m_{i,j} + 1} \tag{27}$$

for j = 2,K

$$N_{i,1}(Q_3) = \left(\frac{n_{i,1}^{+} + 1}{m_{i,1} + 1}\right) M_i \tag{28}$$

A point estimate of the population at the mid-point of period i is obtained by calculating the geometric mean of $N_{i,j}$ weighted by $m_{i,j}$. Once $M_i$, $\Phi_i$ and $N_i$ have been estimated, estimates for $B_i$ (the number of births during period i which survive to the end of that period) follow as a natural generalization of the J–S estimates. We have

$$B_i(Q_3) = N_{i+1}(Q_3) - \left(N_i(Q_3)\Phi_i^{K} - \sum (t_{i,j} + v_{i,j})\Phi_i^{j}\right) \tag{29}$$

(*Note:* When K = 1 in this equation we get Eq. (9).)

### 3.3. New pooled estimates for 'batch' or 'unique' mark data sets — analogues of the $Q_2$ and $Q_3$ estimates

Clearly, we can use the same reduced capture histories used for the $Q_1$ estimates to calculate estimates analogous to the $Q_1$, $Q_2$ and $Q_3$ estimates. In other words, we treat the data set as if it were a pooled mark data set. To indicate that we are actually using a reduced data set, we refer to these as the $P_1$, $P_2$ and $P_3$ estimates. The estimating equations are identical, however, to those used for the $Q_1$, $Q_2$ and $Q_3$ estimates and are not repeated.

### 3.4. Pooled estimates of the marked population: $P_4$ estimates — weighted averages

A natural, and simple, way of obtaining a pooled estimate for the marked population ($M_i$) is to take an average of the estimates $M_{i,j}$, weighting each by the inverse of its variance, or by some quantity likely to be proportional to the variance. (The $M_{i,j}$ and their variances are calculated according to the standard J–S equations.) Begon [11] suggests the number of marked animals ($m_{i,j}$), but since the denominator in Eq. (5.7) of Seber [2] for estimating $M_{i,j}$ is the total number of recaptures eventually obtained from release i,j, this value ($r_{i,j}$) would seem to serve at least as well. Furthermore, preliminary work, which is not discussed, shows that $n_{i,j}$ may be an appropriate weighting factor. All of these possibilities are investigated using the data available. It should be noted that, since $M_i$ will most often be exponentially distributed, it is appropriate to perform a log transform on the data prior to calculating the weighted means. We then have the $P_4$ estimates for $M_i$

$$\log_e M_i(P_4) = \frac{\Sigma\, L_{i,j} \log_e M_{i,j}}{\Sigma\, L_{i,j}} \tag{30}$$

where the $L_{i,j}$ are the chosen weights. We shall call all weighted means $P_4$ estimates, differentiating by specifying the weight in each case.

### 3.5. Pooled estimates for $\Phi_i$, $N_i$ and $B_i$ using the $P_4$ estimates of $M_i$ and the modified J–S equations

Once we have good estimates for $M_i$, $\Phi_i$ can be estimated from Eq. (18). The total population ($N_i$) can be estimated using a pooled estimate of the marked fraction ($\alpha_i$), making the assumption that this fraction changes little during any particular pooling period

$$\alpha_i(P_4) = \Sigma\, m_{i,j} / \Sigma\, n_{i,j} \tag{31}$$

$$N_i(P_4) = M_i(P_4) / \alpha_i(P_4) \tag{32}$$

The total births during period i, which survive to the start of period i + 1, can now be calculated from an appropriate analogue of Eq. (29)

$$B_i(P_4) = N_{i+1}(P_4) - N_i(P_4)\Phi_i^K(P_4) + \Sigma\, (t_{i,j} + v_{i,j})\Phi_i^j(P_4) \tag{33}$$

### 3.6. Pooled estimates of $\Phi_i$ obtained directly from the modified Manly and Parr approach: $P_5$ estimates

We have derived pooled estimates of the survival probability ($\Phi_{i,j}$) from pooled estimates of the marked population. The latter may not, however, be a very satisfactory base for estimates of all the other population parameters because the $M_{i,j}$ vary not only with the probability of survival, but also with the sampling effort during period i.

Nevertheless, it is assumed that the survival probabilities *are* independent of the sampling effort and can be estimated without reference to the marked population using Eq. (23). We now use the same approach to obtain the ($P_5$) pooled estimate of $\Phi_i$

$$\Phi_i(P_5) = \frac{\Sigma\ (z_{i,j} + w_{i,j})}{\Sigma\ (r_{i,j-1} + z_{i,j-1})} \tag{34}$$

Having derived pooled estimates of the survival probabilities directly, rather than by way of $M_i$, the approach to estimation of the other parameters can now be somewhat different. Estimates of $\Phi_i$ in Eq. (16) can be used to obtain $M_i$. In so doing, the reduced capture histories must, of course, be used and the information arising from multiple recaptures within the pooling period ignored. It is emphasized, however, that this does *not* make the approach identical to the RCH approach which gives rise to the $P_1$ estimates. The $P_5$ estimates for the fraction marked ($\alpha_i$), the total population ($N_i$) and the number of births ($B_i$) can now be calculated using Eqs (29), (31) and (32) in an appropriate form.

### 3.7. Development of pooled estimates of population parameters from 'pooled mark' data sets

A pooled estimate of the mean survival probability over periods i to i + n, which we call ($\Phi_{i-i+n}$), is obtained using similar arguments to those used above and this leads to an analogue of Eq. (34)

$$\Phi_{i \to i+n}(QP_1) = \frac{\Sigma\ (z_k + w_k)}{\Sigma\ (r_{k-1} + z_{k-1})} \tag{35}$$

where both sums run from k = i, i + n.

The marked population can then be calculated from Eq. (16), bearing in mind that the recapture histories must be reduced such that each animal is recorded only once during the whole pooling period covering periods i to i + n. Pooled estimates of the unmarked (and hence total) population, and of the number of births, are then obtained via suitable analogues of Eqs (26) and (29).

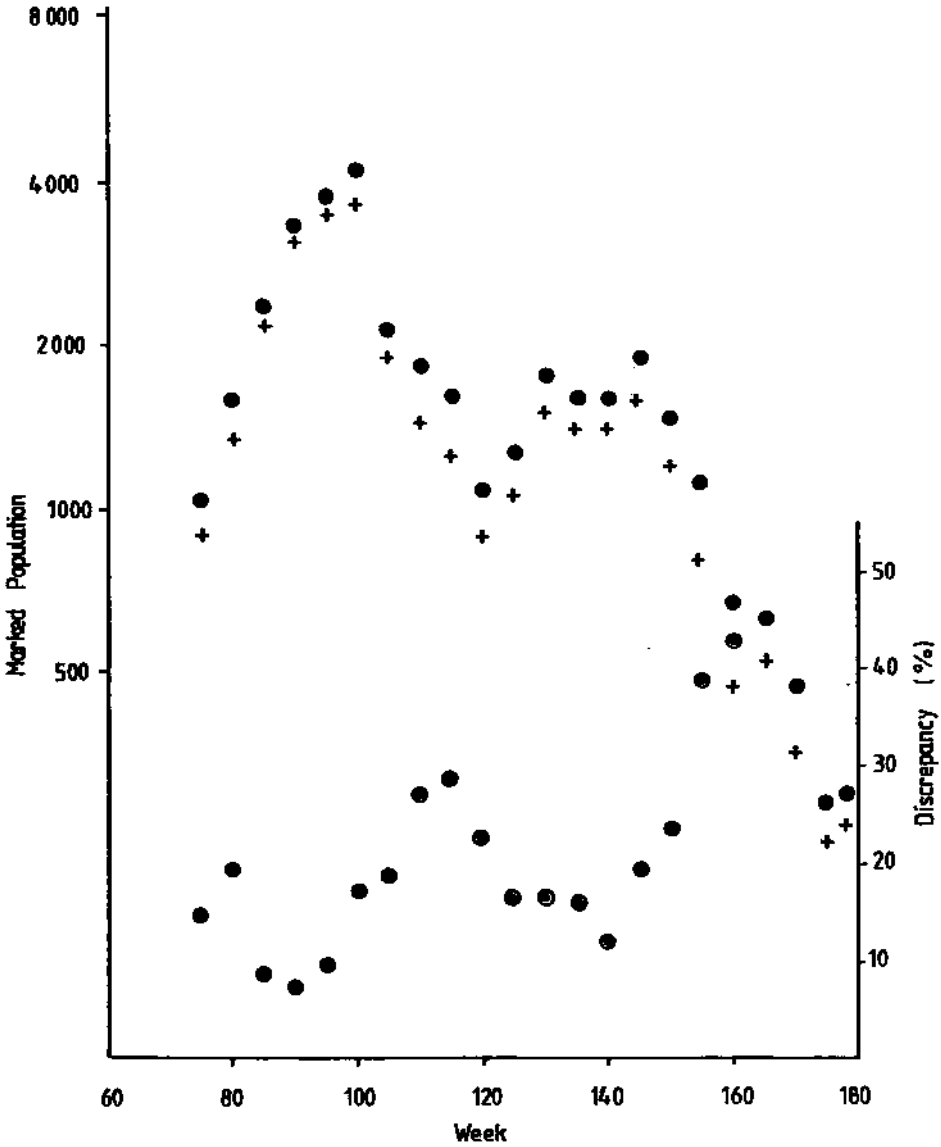


*FIG. 4. Antelope Island: estimates of the marked population of* G. morsitans *males. Five weekly geometric means of weekly estimates; $Q_1$ estimates (+), and $Q_2$ estimates (•) obtained via Eqs (16) and (18), as described in the text. The percentage discrepancy ($\delta_i$) between the two methods (⊙) is shown in the lower graph (right hand scale); $\delta_i$ is defined by $\delta_i = (M_i(Q_2) - M_i(Q_1))\ 100/M_i(Q_2)$.*

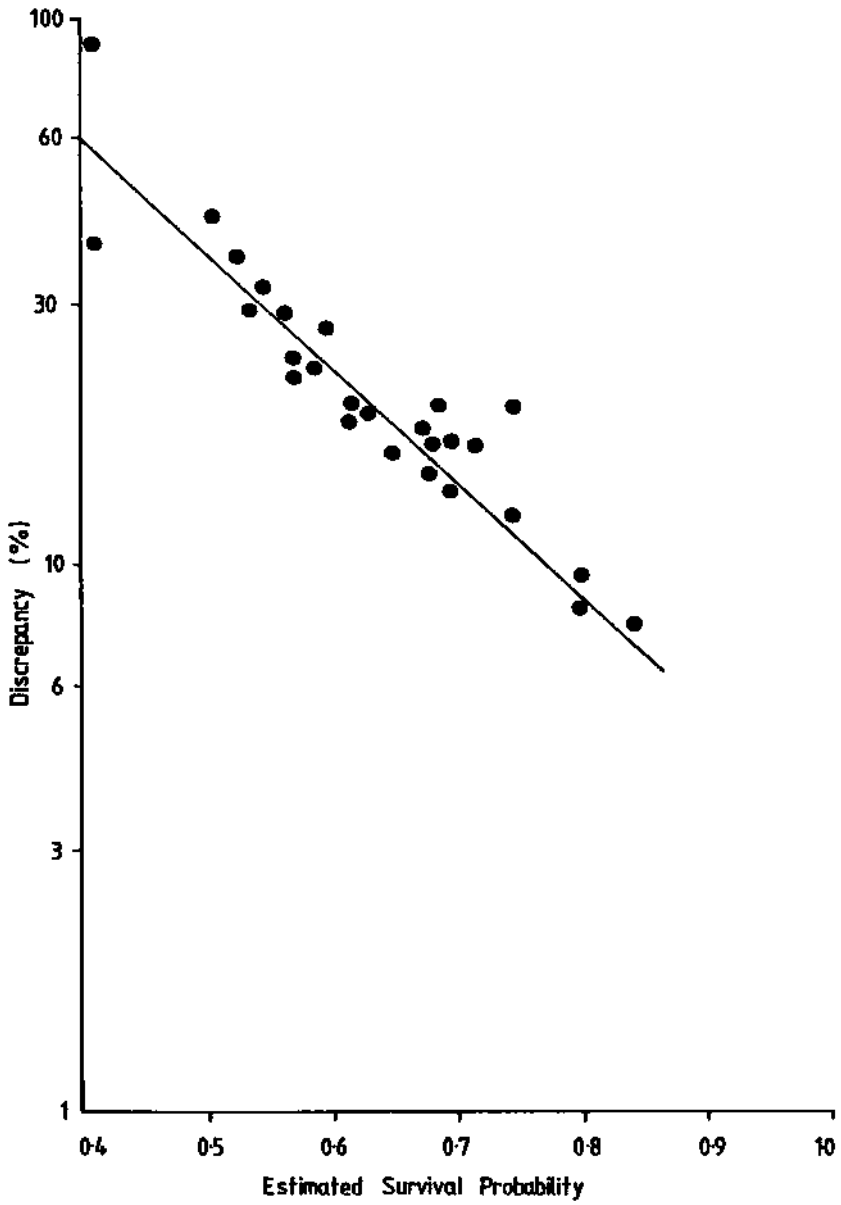


*FIG. 5. The discrepancy ($\delta_j$) between estimates of the marked population of male* G. morsitans *males on Antelope Island plotted against the $Q_j$ estimate of the survival probability (see Fig. 4 for definition of $\delta_j$).*

### 3.8. Estimation of $M_i$ and $\Phi_i$ using the $Q_2$ equations

The modified J–S equations for $M_i$ and $\Phi_i$ do not have an analytical solution, but could be solved numerically. The $Q_1$ equations were used to get a first approximation to $M_i$, which were then used in estimating $\Phi_i$ from Eq. (18). An update of $M_i$ could then be obtained from Eq. (16) and the cycle was repeated until convergence was achieved on the basis of the difference between the values of two consecutive values of $M_i$. This technique was successful (see below) in that $M_i$ did actually converge to generally reasonable values ($Q_2$ estimates). It was noticed, however, that about 10% of the $Q_2$ estimates of $\Phi_i$ were greater than 1, despite the fact that the test data set appeared to be a very good one — in the sense that more than 60% of the population was marked at most times during the experiment, and the population size was generally greater than 1000 (see below). It should be noted that none of the $Q_1$ estimates of $\Phi_i$ obtained using this data set was greater than or equal to unity; in this sense, the $Q_1$ estimates are more 'realistic', even though it is clear from what has been stated in the introduction that the model is inappropriate for the data set in question. It would seem that, although the equations developed here for the $Q_2$ estimates of the population parameters from data sets of the type under consideration are clearly more realistic than those for the $Q_1$ estimates, the method of extracting the $M_i(Q_2)$ and $\Phi_i(Q_2)$ estimates from them is too unstable to be of practical value as it stands.

Although the $\Phi_i(Q_2)$ were unstable, it was found that the $M_i(Q_2)$ were almost always higher than the $M_i(Q_1)$. In addition, if the mean values were taken over 5 week intervals for the whole parameter set, every mean was higher (Fig. 4). Furthermore, the magnitude of the differences changed fairly smoothly with season (Fig. 4, lower plot); it is clear that the underlying cause of the difference is variation in the probability of survival $\Phi_i$ (Fig. 5). This conforms with the theory: using Eqs (2) and (16) we have

$$M_i(Q_2) = M_i(Q_1) + \sum F_{i,j}(\Phi_i^{j-j} - 1) \tag{36}$$

where

$$F_{i,j} = (z_i n_{i,j} + r_i m_{i,j})/r_i > 0$$

and where $\Phi_i^{j-j} > 1$ whenever $j > 1$ and $0 < \Phi_i < 1$. Thus, $M_i(Q_2) > M_i(Q_1)$ and the discrepancy increases with increasing mortality. It also increases with $F_{i,j}$, that is, when the population is being sampled most intensively.

*Text cont. on p. 105.*

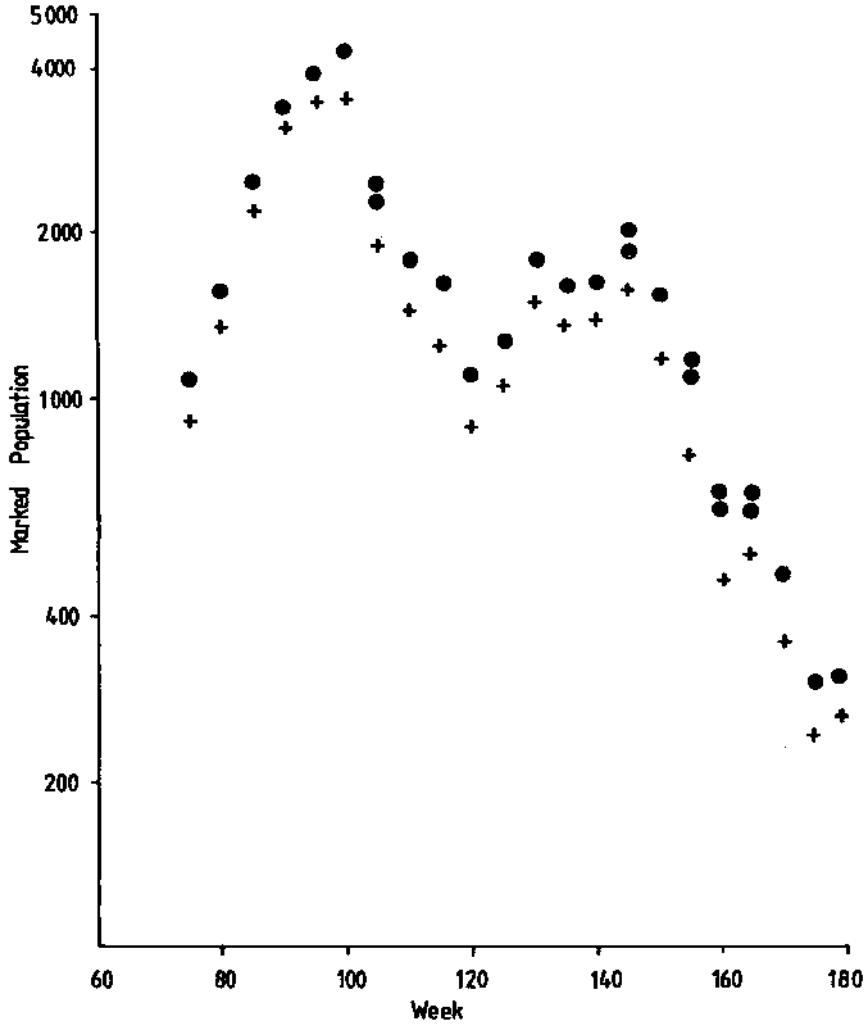


*FIG. 6. Antelope Island: 5 weekly geometric means of weekly estimates of the marked population of* G. morsitans *males. ((+) = $Q_1$ estimates; (•) = $Q_3$ estimates; (⊙) = $Q_2$ estimates (from Eq. (16).) Where the $Q_2$ estimate is not shown, it is graphically indistinguishable from the $Q_3$ estimate.*

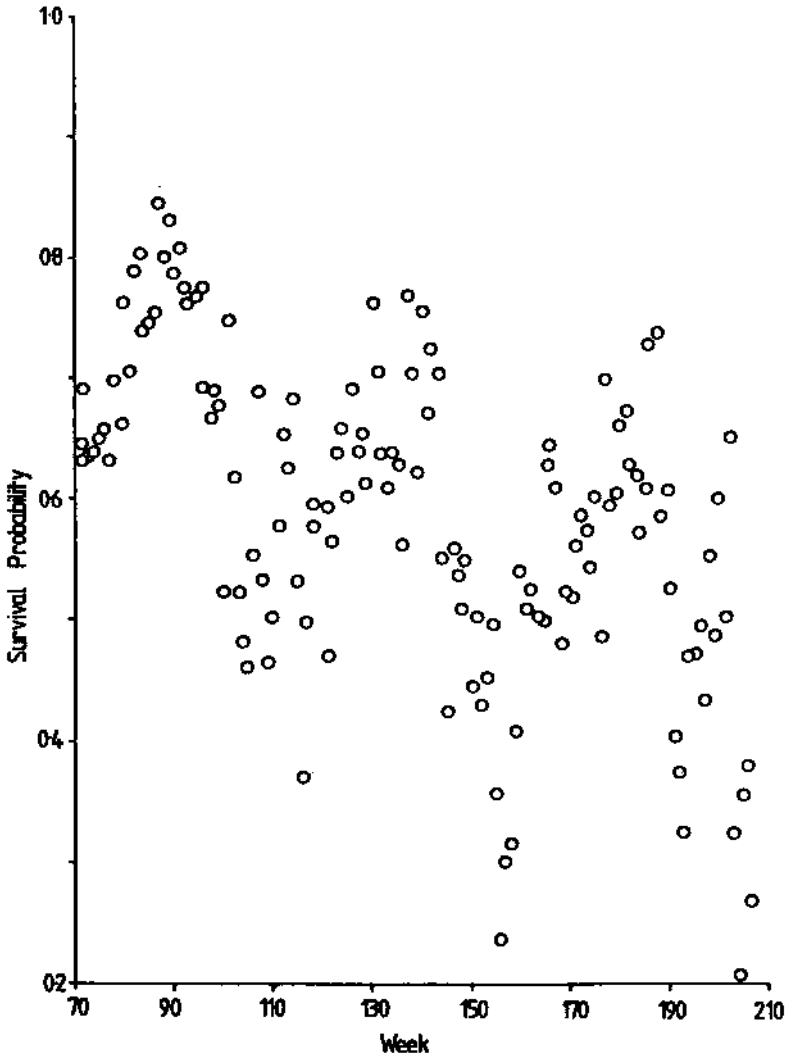


*FIG. 7. Antelope Island: $Q_3$ estimates from Eq. (28) of the weekly survival probability of male* G. morsitans.

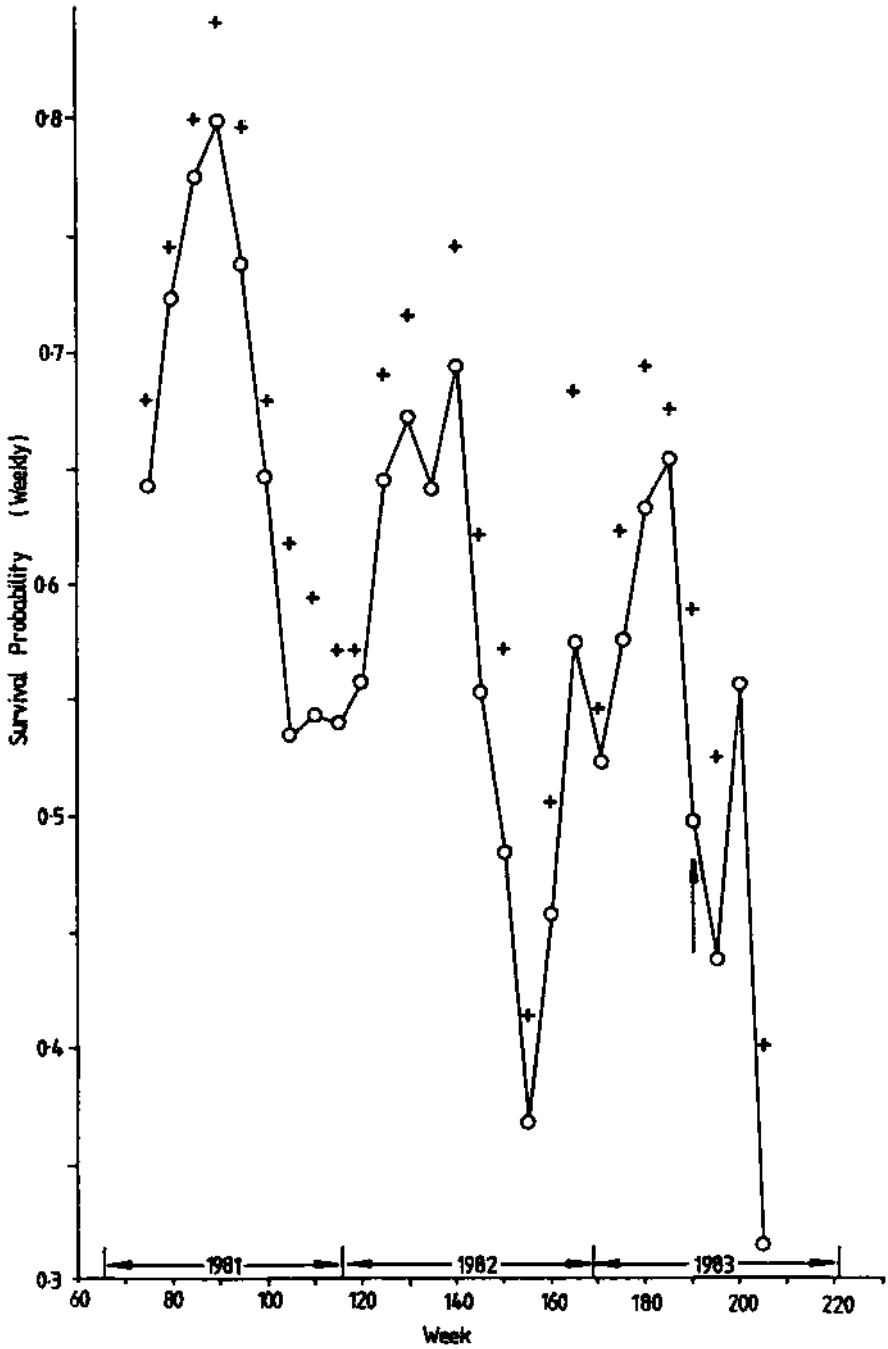


*FIG. 8. Antelope Island: 5 weekly means of the weekly survival probability estimates of male* G. morsitans. *((+) = $Q_1$ estimates; (○) = $Q_3$ estimates (see Fig. 7).)*

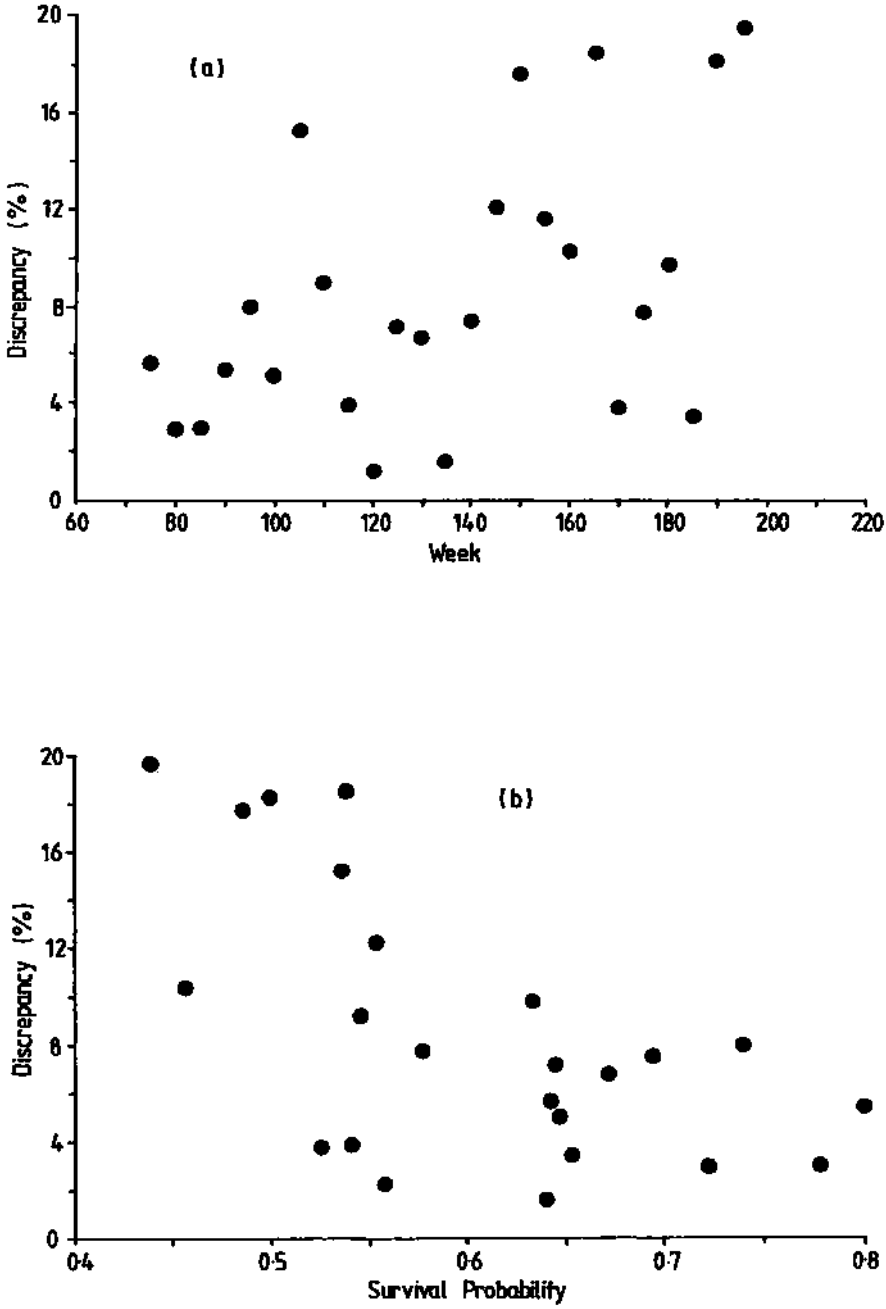


*FIG. 9. Percentage discrepancy (δ) between the 5 weekly means of the $Q_1$ and $Q_3$ estimates of the survival probability (data from Fig. 8), plotted against: (a) week of experiment, and (b) $Q_3$ estimate of the survival probability.*

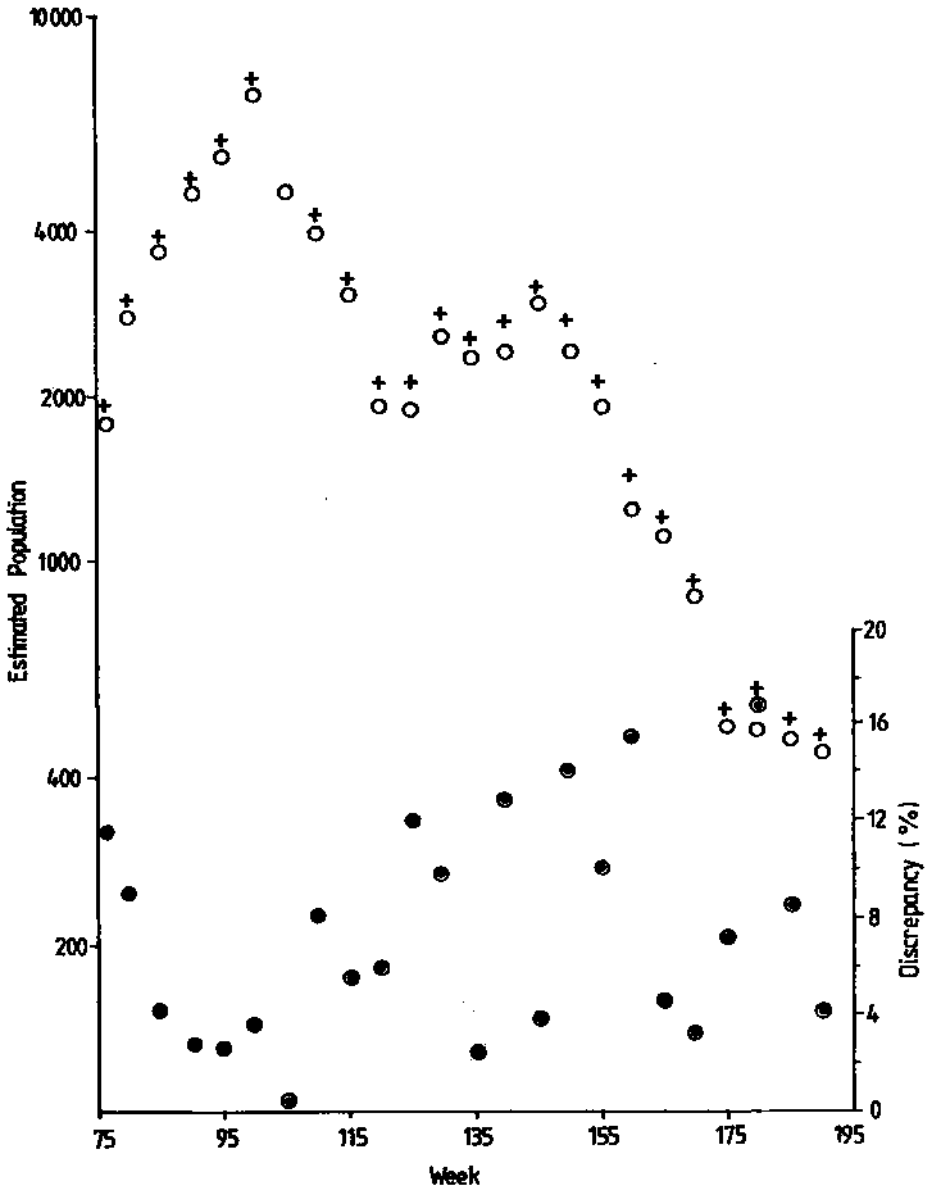


*FIG. 10. Antelope Island: estimates of the total population of male* G. morsitans. *Upper plot: 5 weekly geometric means of the weekly estimates ((+) = $Q_1$ estimates; (o) = $Q_3$ estimates). Lower plot: discrepancy between the $Q_1$ and $Q_3$ estimates.*

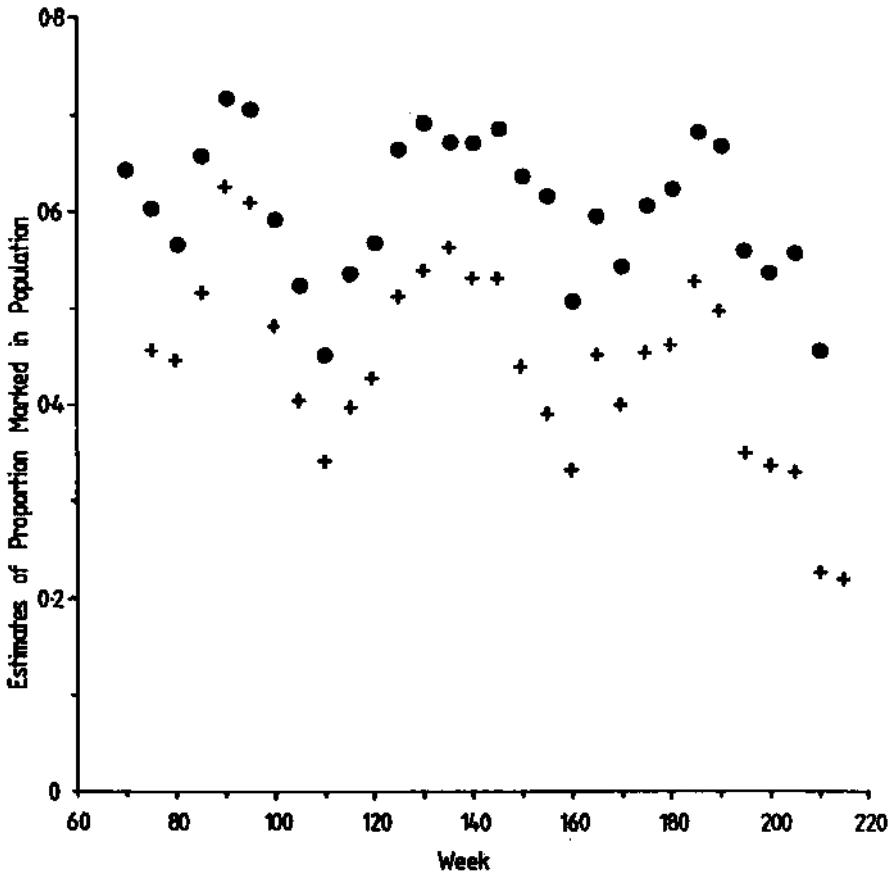


*FIG. 11. Antelope Island: 5 weekly means of the estimates of the proportion of marked flies among the total population of male* G. morsitans. *((+) = $Q_i$ estimates calculated from $\alpha_{i-i+5} = \Sigma\Sigma\, m_{k,j}/\Sigma\Sigma\, n_{k,j}$, where the inner sum is from $j + 1,K$ and the outer from $k = i, i + 5$; (●) = ratio $M_{i-i+5}(Q_3)/N_{i-i+5}(Q_3)$.)*

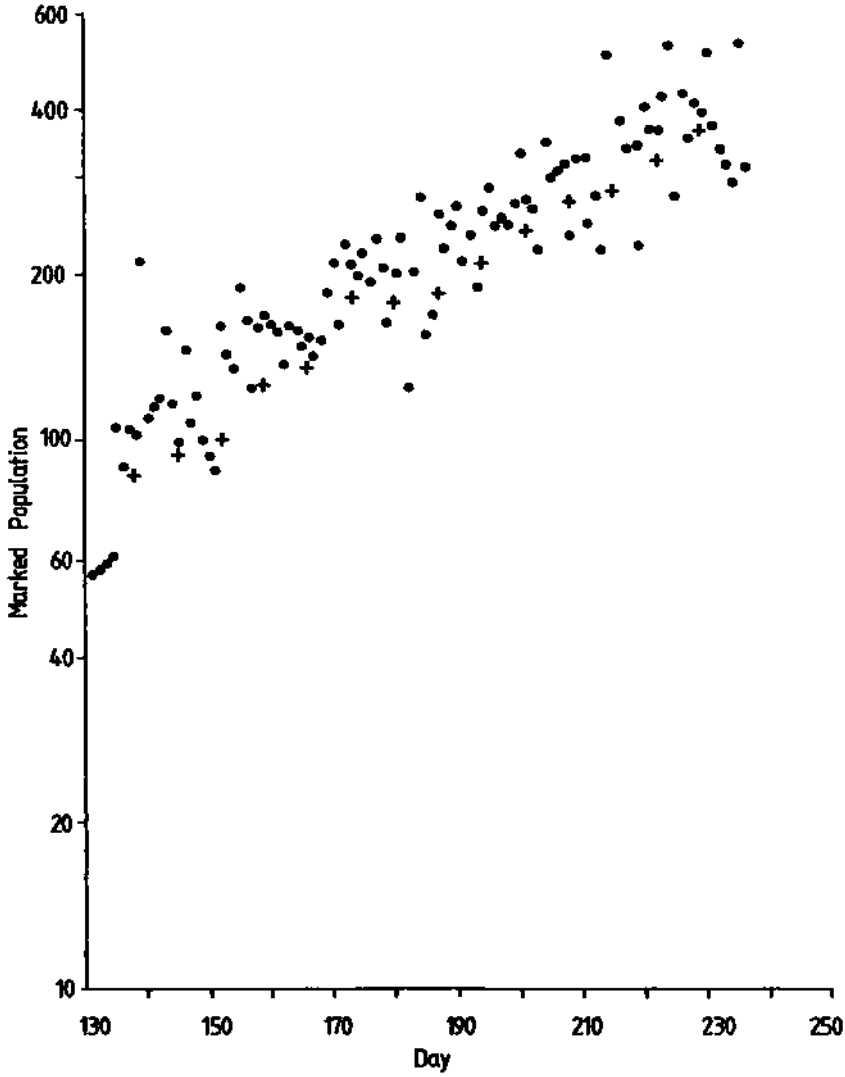


*FIG. 12. Antelope Island: daily marking experiment. Estimates of the marked population of male* G. morsitans. *Daily (J–S) estimates based on original data (•), and $P_1$ weekly pooled estimates (+) based on the reduced capture history.*

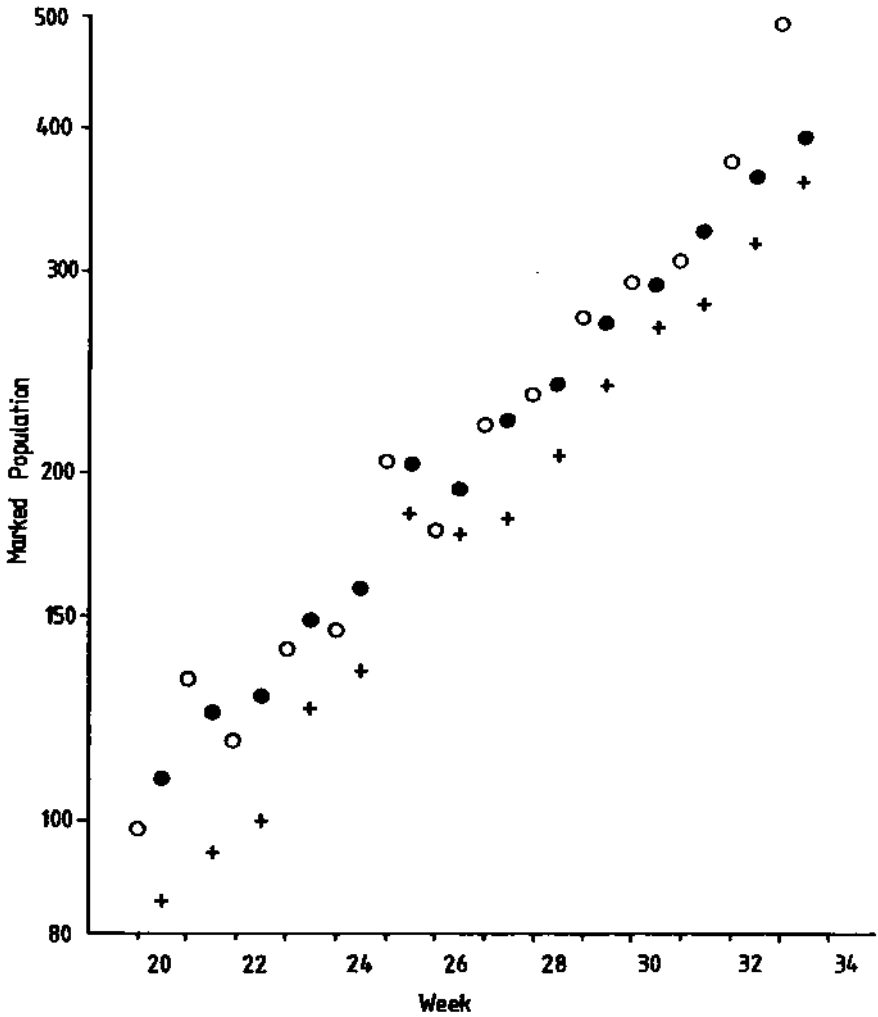


*FIG. 13. Antelope Island: daily marking experiment. Estimates of the marked population of male* G. morsitans. *Weekly pooled values of: $P_4$ estimates (●), i.e. the geometric means weighted by $r_{i,j}$; $P_3$ estimates (○); $P_1$ estimates (+). The latter two estimates are based on the reduced capture history.*

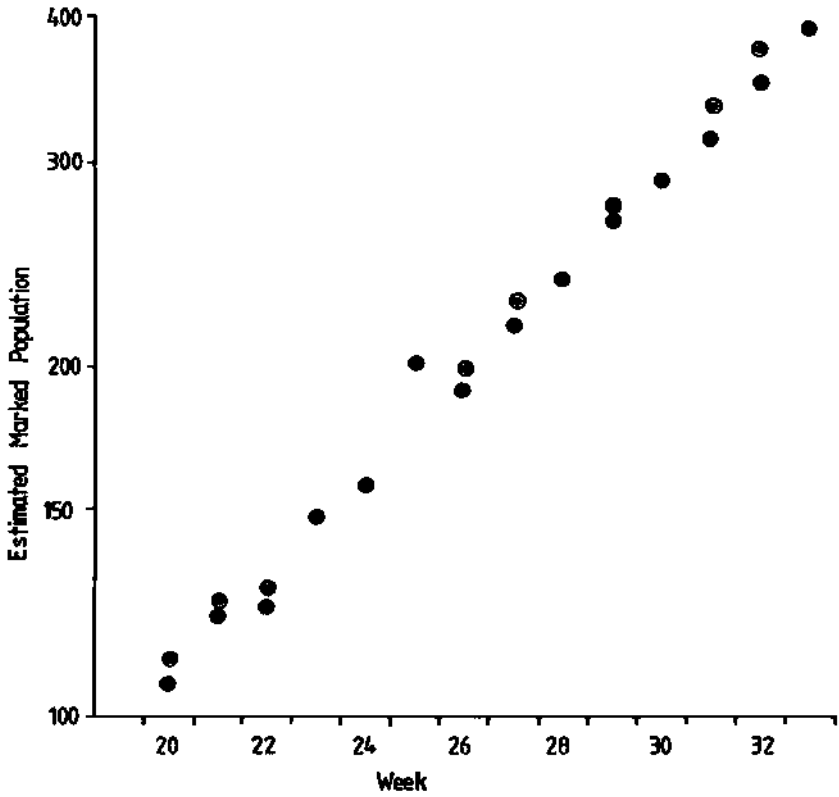


*FIG. 14. Antelope Island: daily marking experiment. Weekly ($P_4$) geometric means of daily estimates of the marked populations of male* G. morsitans *weighted by $r_{i,j}$ (●), or by $m_{i,j}$ (⊙). The difference between weighting by $m_{i,j}$ and $n_{i,j}$ was so small as to be generally graphically indistinguishable; the latter are accordingly not included.*

### 3.9. $Q_3$ estimates of $\Phi_i$ and $M_i$ using Eq. (23)

When Eq. (23) is used, the $Q_3$ estimates of the survival probability are obtained without resorting to iteration, and these can then be inserted into Eq. (16) to generate $M_i(Q_3)$. For the data set under consideration, the resulting estimates of the marked population are very similar to the $Q_2$ estimates, to the extent that the plotted points are generally indistinguishable (Fig. 6). The average (absolute) difference between the two estimates for the 22 points shown in Fig. 6 is of the order of 1%.

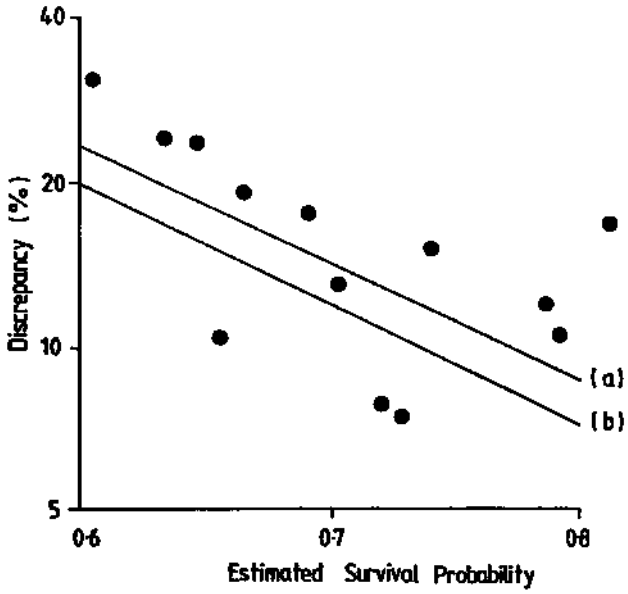


*FIG. 15. Antelope Island: daily marking experiment. Discrepancy between the $P_1$ and $P_3$ estimates of the marked populations of male* G. morsitans *plotted against the $P_3$ estimate of the survival probability. (Discrepancy calculated as in Fig. 4.) Line (a) is that fitted by regression to the data in Fig. 5; line (b) is shifted to take account of the difference between the $P_3$ and the $Q_1$ estimates of the survival probability (see text for further details).*

All of the weekly survival probability estimates obtained from Eq. (23) lie in the interval (0.1) (Fig. 7), unlike the $Q_2$ estimates. On the other hand, the 5 weekly means obtained using the two methods are similar and are both generally lower than the $Q_1$ estimates (Fig. 8). There is no strong trend with time in the discrepancy (Fig. 9(a)) and, while there is a negative correlation between the discrepancy and $\Phi_i(Q_3)$, this is rather weak — compare Fig. 9(b) with Fig. 5. The 5 weekly pooled ($QP_1$) estimate of the weekly survival probability (Eq. (35)), produces estimates which are graphically indistinguishable from the arithmetic means of the $\Phi_i$ over the same period. The difference between the estimates was generally less than 1% and was never greater than 5% for the data set under consideration.

### 3.10. $Q_1$ and $Q_3$ estimates of the total population and the fraction marked in the population

Once the marked population and the survival probabilities have been estimated, the $Q_3$ estimates for the total population can be obtained via Eqs (26)–(28). Since

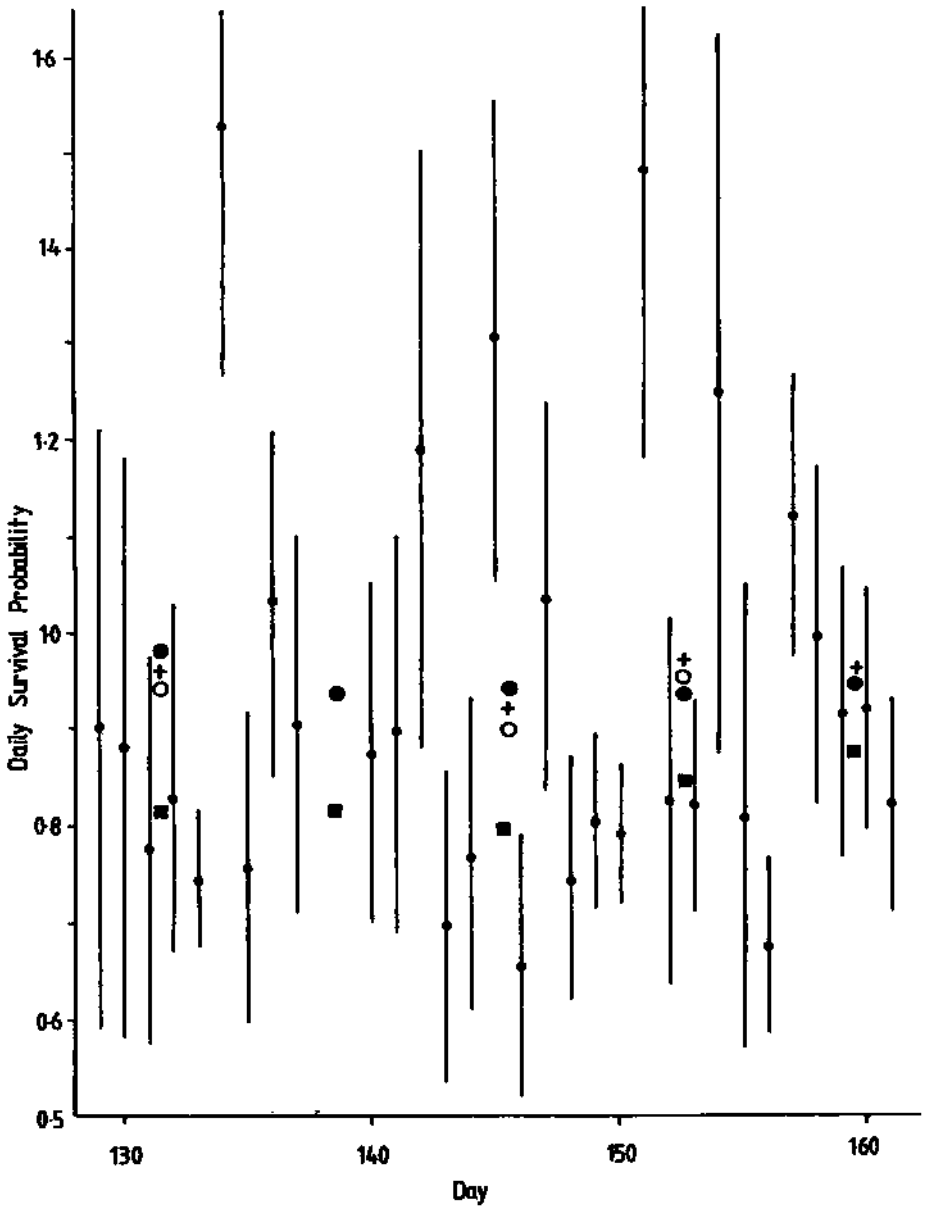


*FIG. 16. Antelope Island: daily marking experiment. Estimates of the daily survival probability of male* G. morsitans *for the first 5 weeks of the experiment. (Daily (J–S) estimates ± one standard error (●) weekly arithmetic means (■); weekly pooled $P_1$ estimates (+); weekly pooled $P_3$ estimates (●).)*

the $Q_1$ estimates of the *marked* population seem to underestimate the true situation (Figs 4–6), it might be expected that the $Q_1$ estimates for the total population might also be too low. In fact, for the data set in question, these estimates are consistently *higher* than the $Q_3$ estimates (Fig. 10). A real overestimate of the total population would result from the $Q_1$ equations if the marked *proportion* was underestimated even more severely than the marked population. This seems quite likely on the basis of the type of results illustrated in Fig. 1; certainly, the $Q_1$ estimates of $\alpha_i$ are consistently lower than the $Q_3$ estimates (Fig. 11), to the extent of 20–50%.

### 3.11. Comparison of various methods of estimating the 'pooled' population parameters using the Antelope Island daily marking data sets for male *G. morsitans*

The daily estimates of the marked population of *G. morsitans* males and the $P_1$ pooled estimates for each week of the experiment are shown in Fig. 12. The daily estimates are sufficiently consistent to make it immediately clear that the $Q_1$ equation underestimates the general level of marked flies in the population at any time. When various pooling techniques are compared it is also clear that the $Q_1$ estimates are too low (Fig. 13). Thus, whereas the $P_3$ and $P_4$ estimates (i.e. the geometric mean of the daily estimates, weighted in this case by $r_{i,j}$) are in good agreement with the $P_3$ estimates, the $P_1$ estimates are generally 10–20% lower. The $r_{i,j}$ weightings have been used for all comparisons with other pooling techniques; the weighting used (the $n_{i,j}$, $m_{i,j}$ or $r_{i,j}$) made little difference to the results (Fig. 14). When the first two were used, the estimates were, on average, 3.2% higher than when the $r_{i,j}$ were used, and they differed from each other by less than 1%.

The magnitude of the discrepancy between the $P_1$ and $P_3$ estimates of the marked population is a function of the survival probability (Fig. 15), as was observed earlier (Fig. 5) for the weekly marking situation. Furthermore, the function relating the two is similar in both cases. The regression line (a) fitted using the weekly marking data from Fig. 5 is included in Fig. 15. It should be recalled that the independent variable in the former case was the $Q_1$ estimate of the survival probability, whereas in the latter case it is the $P_3$ estimate; on the basis of Fig. 9(b), the $P_3$ estimates should be about 5% lower, on average, than the $Q_1$ figures. Line (b) in Fig. 15 is shifted from line (a) by this amount: the difference is not great, and either line produces a reasonable fit to the rather scattered data.

The daily estimates of the survival probability show considerable variability, as witnessed by a plot of the results for the first few weeks of the experiment (Fig. 16); 10 out of the first 32 estimates take values greater than 1.0. This reflects the situation in the data set as a whole, where 33% of the estimates are greater than 1.0. A direct average of these estimates, even when weighted by the inverse of their variances, is unlikely to give a sensible result, given the great variability of the daily estimates. This view seems to be confirmed (Fig. 16). The averages indicate a daily

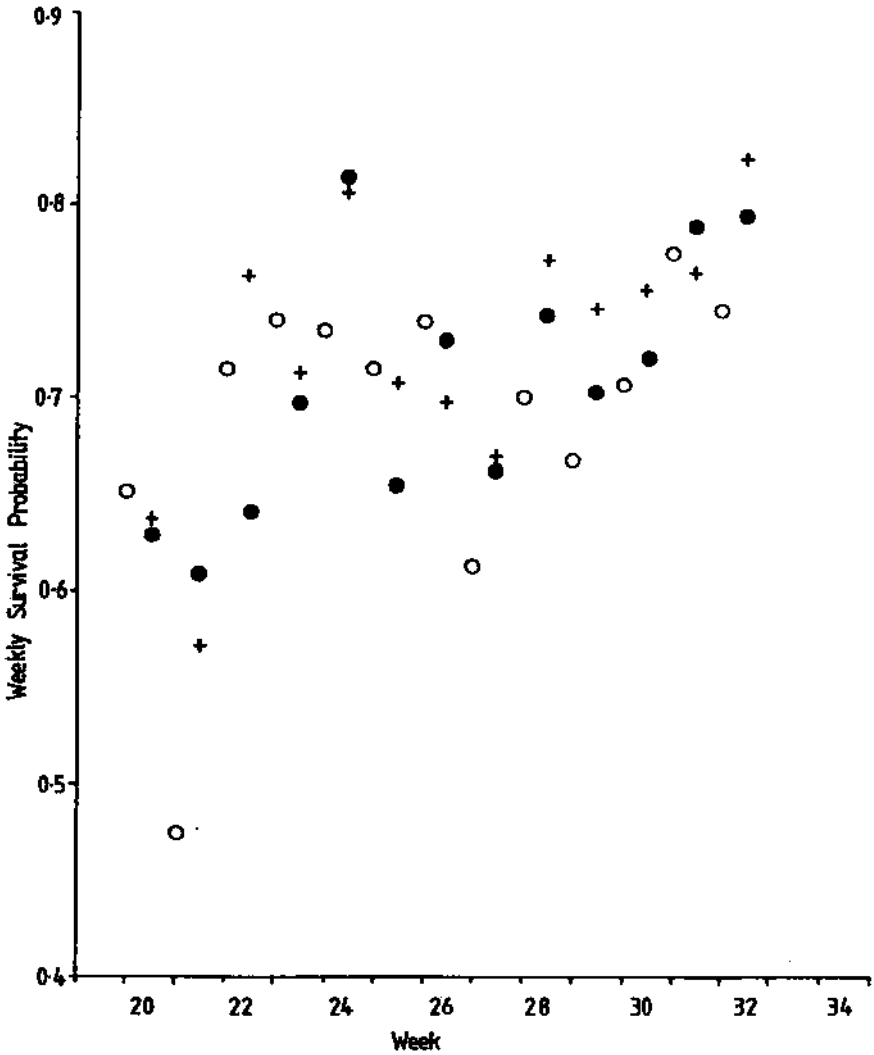


*FIG. 17. Antelope Island: weekly pooled estimates of the daily probability of survival of male* G. morsitans. *($P_4$ estimates calculated, using Eq. (18), from the geometric mean of $M_{i,j}$, weighted by $r_{i,j}$ (●) (see text for further details of calculation). $P_3$ estimates (○) calculated using an analogue of Eq. (23). $P_1$ estimates (+).)*

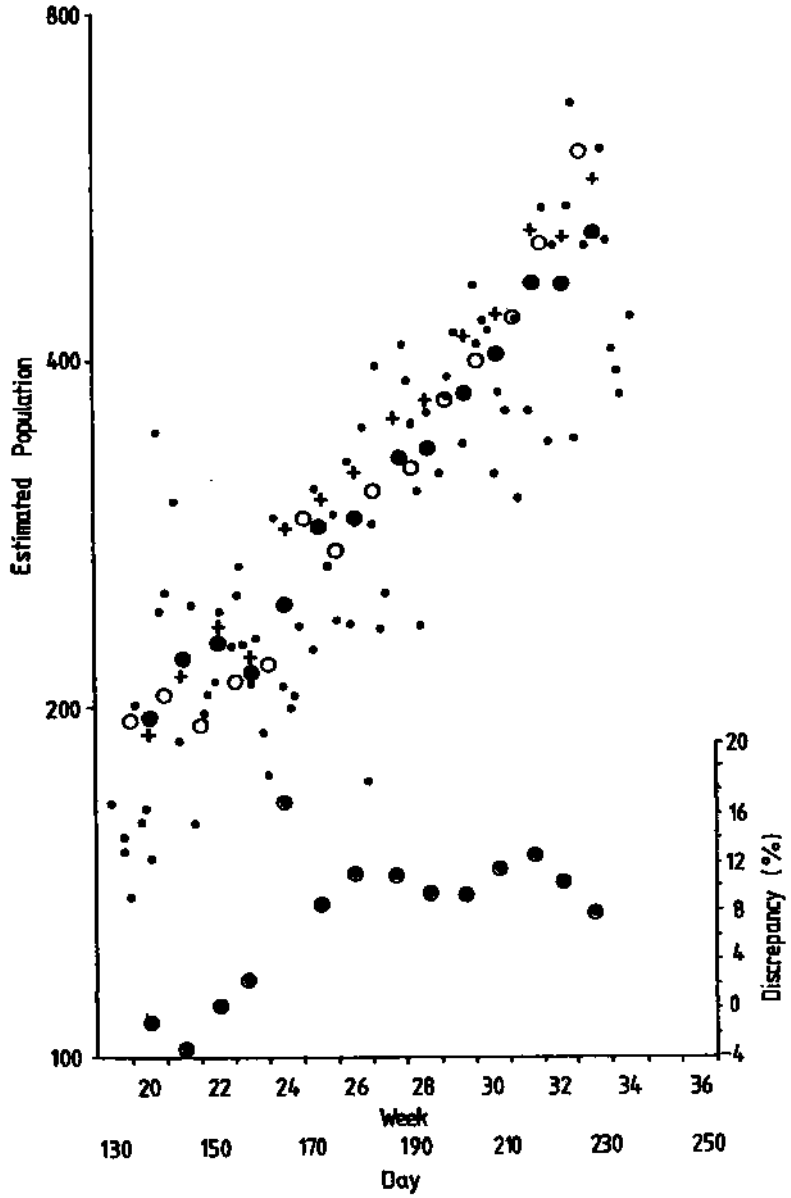


*FIG. 18. Antelope Island: daily marking experiment. Estimates of the total population of male* G. morsitans. *Upper plot: daily and weekly (pooled) estimates of the total population (daily J–S estimates ($N_{i,j}$) calculated from the original data (•); $P_4$ estimates (Eq. (32) obtained using the geometric means of the $M_{i,j}(J)$ weighted by $r_{i,j}$ (●); $P_3$ estimates obtained from the analogue of Eq. (27) (○); $P_1$ estimates (+)). Lower plot: the percentage discrepancy between the $P_1$ and $P_3$ estimates.*

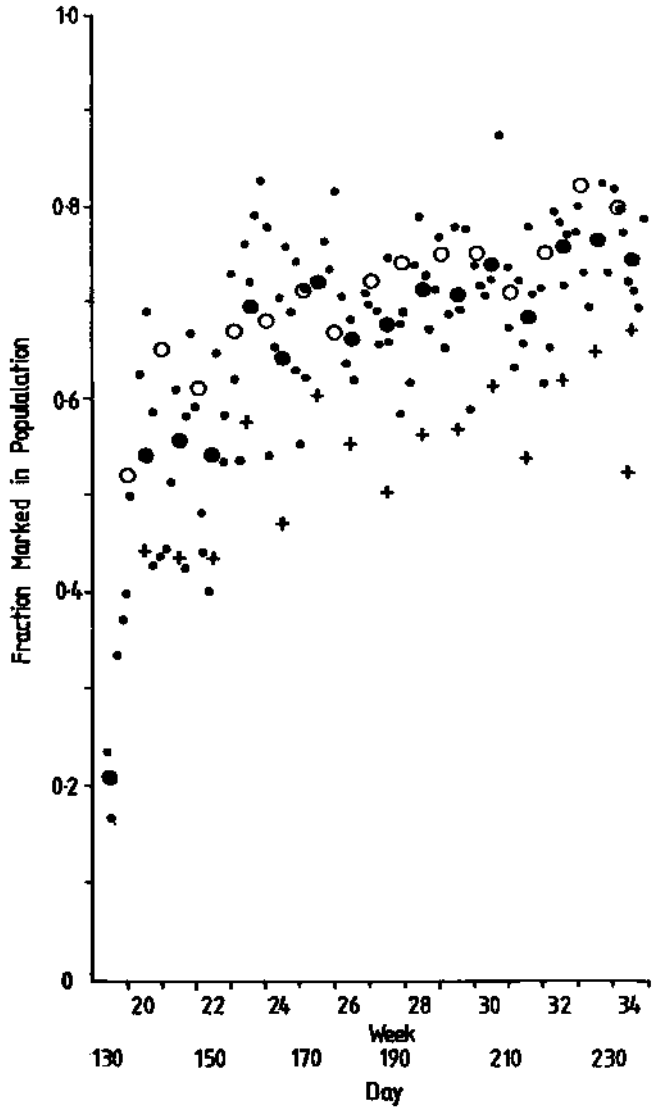


*FIG. 19. Antelope Island: estimates of the fraction of marked flies in the population of male* G. morsitans. *(Daily J–S estimates ($\alpha_{i,j}$) calculated from the original data using Eq. (3) (•); $P_4$ estimates from Eq. (31) (●); $P_3$ estimates from the ratio $M_i(P_3)/N_i(P_3)$.)*

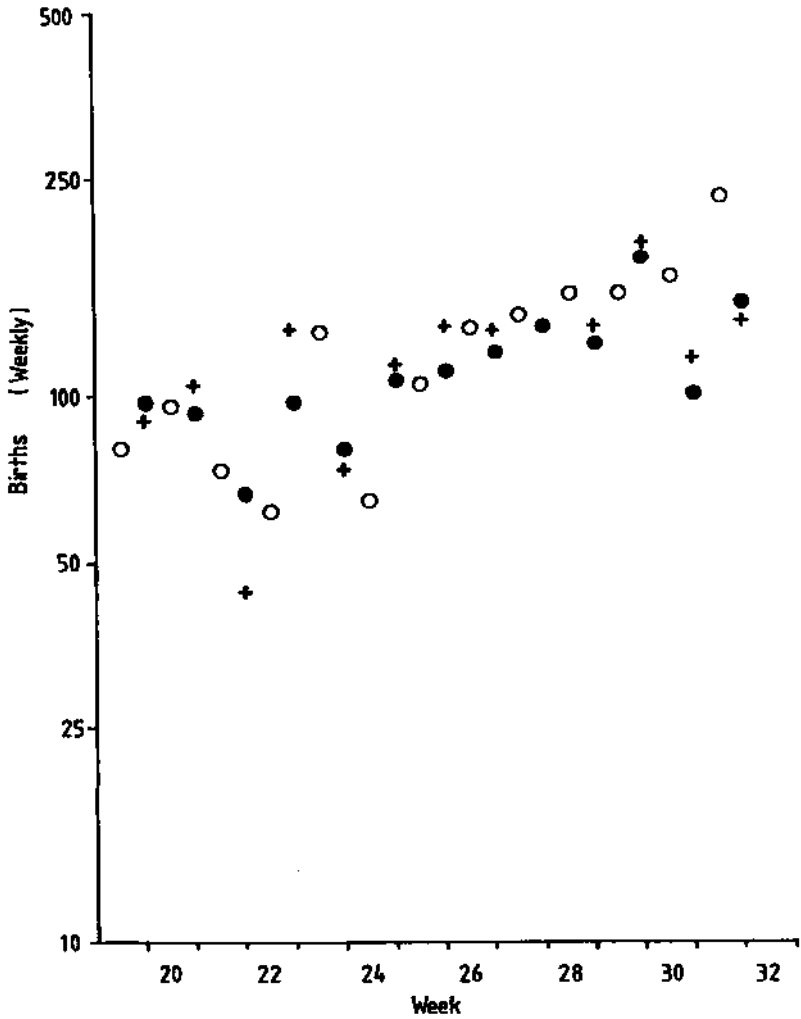


*FIG. 20. Antelope Island: weekly pooled estimates of the numbers of births of male* G. morsitans *during a given week which survive to the beginning of the following week. ($P_4$ estimates (●) and $P_3$ estimates (○) calculated from the analogue of Eq. (29); $P_1$ estimates calculated from the analogue of Eq. (10) (+).)*

death rate of about 20%, which is known to be impossible [12] for a population which is manifestly expanding rapidly [1]. On the other hand, estimates obtained via the $P_1$, $P_3$ and $P_4$ equations (the last using the weighted geometric means of $M_i$ (see Fig. 13) in Eq. (18)), all give broadly similar results (but see next paragraph) in a range acceptable on the basis of the known dynamics of tsetse populations [12].

There is no very clear difference, for the last three pooling methods, between the estimates of the weekly survival probabilities for weeks 19–32 of the experiment (Fig. 17). However, for 9 of the 13 estimates, the $P_t$ estimates are higher than those obtained via the geometric means of $M_{i,j}$, consistent with the view that the former method produces overestimates of the survival probabilities, but that the degree of error involved is a complex function. The $P_3$ estimates are rather variable, but are not consistently higher or lower than the $P_4$ estimates. As with the weekly marking, the $P_1$ estimates of the total population are seen to be consistently higher than the $P_3$ estimates. The latter, in turn, agree well with the $P_4$ estimates and the general level of the daily estimates (Fig. 18). Apart from the first 3 or 4 weeks (when all the estimates tend to be rather unreliable because of the low population levels and the small numbers of marked recaptures), the overestimate using the $P_t$ equation runs at about 10%, which is about the same level as the average (though much more variable) discrepancy between the $Q_1$ and $Q_3$ methods found using the weekly marking data set (see Fig. 10).

As expected, the $P_t$ equation seriously underestimates the fraction of marked flies in the population, as is clear from comparisons with the daily estimates of the marked fraction (Fig. 19). The $P_3$ and $P_4$ estimates, on the other hand, approximate the daily estimates quite well, and are closely similar to each other. As with the weekly marking experiment, there are no consistent differences between the various estimates in terms of the total number of births per week (Fig. 20).

## 4. CONCLUSIONS

Current research has produced methods which should be of general use in producing optimal estimates from mark–recapture data. In particular, it will allow improvements in the estimates already obtained from the Antelope Island data set [1], which will be used to produce a model of the two species. More immediately, improved estimates will be made available of the daily treatment rates as a result of the traps and targets deployed at different times during the experiment. For male *G. morsitans* we know that the population was generally overestimated — by up to 10–15% (see Figs 10 and 18). The proportion of flies being treated daily [1] will thus be underestimated by the same amount. Unpublished results indicate that the error is greater for females, the important sex from the point of view of tsetse fly eradication. Such considerations will affect analysis of the number of targets per unit area required to effect a desired rate of decrease in a tsetse fly population under

specified circumstances. This will be of importance whether targets or traps are being used as a stand alone technique or as an adjunct to some more costly operation, such as aerial spraying or a SIT programme. As such, the current work can be regarded as a contribution towards the optimization of tsetse fly control and eradication campaigns.

## ACKNOWLEDGEMENT

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# SURVEILLANCE OF TSETSE FLY AND CATTLE POPULATIONS FOR TRYPANOSOMES IN THE BICOT AREA DURING THE STERILE INSECT TECHNIQUE CONTROL PROGRAMME*

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**Abstract**

SURVEILLANCE OF TSETSE FLY AND CATTLE POPULATIONS FOR TRYPANOSOMES IN THE BICOT AREA DURING THE STERILE INSECT TECHNIQUE CONTROL PROGRAMME.

Between March 1984 and March 1988 animal surveys for bovine trypanosomiasis were conducted periodically on resident herds in the Biological Control of Tsetse (BICOT) Project area in Lafia, Plateau State, Nigeria, using standard detection methods. Over a specified period sentinel herds were also examined in selected locations within the project area. Fly trapping and dissection were similarly used for trypanosome screening of wild and released flies. In both resident and sentinel herds, infections were detected consistently, showing the persistence of the disease throughout the period of survey. Fly trapping and dissection revealed that the target species, *Glossina palpalis palpalis*, was effectively eliminated from the control zone, or may be persisting only at an undetectable level. However, *G. tachinoides* was present in most of these areas and may therefore have been responsible for the persistence of infection. Infection among resident herds could have also been due to their migratory activities, taking them to areas outside the control project.

## 1. INTRODUCTION

Since trypanosomiasis is endemic in the Biological Control of Tsetse (BICOT) Project area, the persistence of infections is an effective index for determining the success of a control programme. Thus, a continuous study on the incidence of bovine trypanosomiasis in the selected area was accepted as a dependable means of assessing the progress of control. Infection rate studies on tsetse flies captured in the area were also used to monitor the eradication programme.

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## 2. MATERIALS AND METHODS

### 2.1. Diagnostic techniques

The objectives of BICOT were carefully explained to residents of the areas in which sterile tsetse flies were to be released and herdsmen were requested to co-operate with our field survey team by allowing access to their cattle for blood sampling. On this basis, settled herds were selected and during surveys conducted quarterly these herds were sampled for trypanosomiasis.

Standard trypanosomiasis detection methods were used to reveal infection [1]. Blood samples were collected in heparinized vials and stored in a cold box while in transit from the herds to the field laboratory where wet films were examined for trypanosomes. Rats, mice or goats were inoculated with any blood sample showing infection. All samples were also subjected to microhaematocrit centrifugation, buffy coat suspension or capillary examination to detect trypanosomes. Permanent smears were prepared from positive buffy coat mounts for Giemsa staining; these were used later to determine the morphology and identity of isolated parasites. Whenever infectivity was established in rats, mice or goats, laboratory studies were carried out to explore the biology of the parasite. In some cases, drug resistance tests were conducted when relapses occurred after drug treatment of the experimental or sentinel animals.

### 2.2. Use of sentinel herds

In April 1986, 14 sentinel animals were introduced into the project area [2, 3]. These animals, purchased from a tsetse free zone, were transferred to the four preselected areas of the control zone and were checked fortnightly for infectivity. Any infection discovered was treated with a double dose of berenil (diaminzene aceturate). Isolates obtained from relapsed cases were also tested for drug resistance.

### 2.3. Fly trapping and dissection

Challier-Laveissière biconical traps [4] were used to catch the tsetse flies in cleared Akuni forest patches through most of 1987. For control data, fly trapping was also conducted in Kiguna along the Gwayaka River outside the project zone. The BICOT team also maintained regular monitoring studies of flies in the active release areas around the Ehula and Ganye River systems. All flies trapped by this method were dissected and the mouthparts, guts and salivary glands examined for infection [5].

TABLE I. SURVEYS COMPLETED ON SETTLED HERDS (JANUARY 1984–DECEMBER 1985)

| Month of survey | No. of settlements visited[a] | No. of cattle examined | No. of positive trypanosomes | Infection rate (%) | |
|---|---|---|---|---|---|
| *1984* | | | | | |
| March | 2 | 40 | 5 | 12.5 | |
| July | 4 | 64 | 9 | 14.1 | |
| October | 1 | 35 | 14 | 40.0 | 18.5 |
| October | 3 | 46 | 1 | 2.2 | |
| November | 1 | 150 | (Treatment of herd with an infection rate of 40%, October) | | |
| *1985* | | | | | |
| March | 3 | 68 | 1 | 1.5 | 9.7 |
| March | 5 | 45 | 10 | 22.2 | |
| July | 4 | 20 | 2 | 10 | |
| November | 3 | 40 | 1 | 2.5 | |

[a] There are not less than 50 head of cattle per settlement; some are over 250 head.

## 3. RESULTS

Three surveys were completed in 1984 and 1985 (in March, July and November). The infection rates observed were higher (for the same periods) in 1984 than in 1985 (Tables I–III). In these years *Trypanosoma congolense* was more prevalent in the infected animals than either *T. brucei* or *T. vivax*. Initially, only wet film examination was employed in the field laboratory; infected blood was, however, injected into laboratory rats and mice, which were then examined for 30 days. As only few isolates were obtained, even from proven infected blood samples, goat inoculation to recover infective parasites was introduced and proved very sensitive. Similarly, the microhaematocrit centrifugation technique was added to the diagnostic procedures and many more positive cases were detectable (Table IV) [6–9]. A serious outbreak of mixed trypanosome infection was observed in a herd at Kantsakwa in October 1984; three species of trypanosome were detected in different combinations. Since infected flies were not trapped in this area (which was in the sterile insect technique (SIT) control area), these cattle were probably infected

TABLE II. BREAKDOWN OF TRYPANOSOMIASIS PREVALENCE IN SETTLED HERDS IN THE SIT PROJECT AREA (1984)

| Cattle settlement | March | July | October | Annual infection rate (infected/total) |
|---|---|---|---|---|
| Tungan Nupawa | | | | |
| No. of cattle[a] | 11 (1) | — | — | 1/11 |
| Incidence of infection (%) | 9.1 | | | |
| Kantsakwa | | | | |
| No. of cattle[a] | 29 (4) | 19 (1) | 35 (14) | 19/83 |
| Incidence of infection (%) | 13.8 | 5.3 | 40 | |
| Mai-Akuya | | | | |
| No. of cattle[a] | — | 16 (2) | — | 2/16 |
| Incidence of infection (%) | | 12.5 | | |
| Gidan Buba | | | | |
| No. of cattle[a] | — | — | 15 (0) | 0/15 |
| Incidence of infection (%) | | | 0 | |
| Akura | | | | |
| No. of cattle[a] | — | 17 (4) | 3 (0) | 4/20 |
| Incidence of infection (%) | | 23.5 | 0 | |
| Asakio | | | | |
| No. of cattle[a] | — | 16 (2) | — | 2/16 |
| Incidence of infection (%) | | 12.5 | | |
| Ugah | | | | |
| No. of cattle[a] | — | — | 28 (1) | 1/28 |
| Incidence of infection (%) | | | 3.6 | |
| *Total* | 5/40 | 9/64 | 15/81 | 29/185 |
| Infection rate (%) | 12.5 | 14.1 | 18.5 | 15.7 |

[a] Numerals in parentheses represent the total number of animals in the sample with positive trypanosome infection.

TABLE III. BREAKDOWN OF TRYPANOSOMIASIS PREVALENCE IN SETTLED HERDS IN THE SIT PROJECT AREA (1985)

| Cattle settlement | March | July | November | Annual infection rate (infected/total) |
|---|---|---|---|---|
| Kiguna (Gwayaka River south) | | | | |
| No. of cattle[a] | 68 (1) | — | — | 1/68 |
| Incidence of infection (%) | 1.5 | | | |
| Kiguna (Gwayaka River north) | | | | |
| No. of cattle[a] | 45 (10) | — | — | 10/45 |
| Incidence of infection (%) | 22.2 | | | |
| Kantsakwa | | | | |
| No. of cattle[a] | — | 2 (1) | 18 (0) | 1/20 |
| Incidence of infection (%) | | 50? | 0 | |
| Mai-Akuya | | | | |
| No. of cattle[a] | — | 8 (1) | 19 (1) | 2/27 |
| Incidence of infection (%) | | 12.5 | 5.3 | |
| Adogi | | | | |
| No. of cattle[a] | — | 5 (0) | — | 0/5 |
| Incidence of infection (%) | | 0 | | |
| Akura | | | | |
| No. of cattle[a] | — | 5 (0) | — | 0/5 |
| Incidence of infection (%) | | 0 | | |
| Asakio | | | | |
| No. of cattle[a] | — | — | 3 (0) | 0/3 |
| Incidence of infection (%) | | | 0 | |
| *Total* | 11/111 | 2/20 | 1/40 | 14/173 |
| Infection rate (%) | 9.7 | 5.0 | 2.5 | 8.1 |

[a] Numerals in parentheses represent the total number of animals in the sample with positive trypanosome infection.

TABLE IV. COMPARATIVE RESULTS OF THE PARASITOLOGICAL METHODS EMPLOYED IN FIELD DIAGNOSIS OF CATTLE TRYPANOSOMIASIS [6–9]

| Schedule | No. of animals examined (% positive) | No. of positive cases | | | Parasite identification |
|---|---|---|---|---|---|
| | | Rat inoculation | Wet blood film | Microhaematocrit | |
| March 1984 | 40 (12.5) | 0 | 5 | ND | *T. brucei* *T. vivax* |
| July 1984 | 64 (14.1) | 1 | 9 | ND | *T. brucei* *T. vivax* |
| October 1984 | 81 (18.5) | 0 | 6 | 15 | *T. brucei* *T. congolense* *T. vivax* |
| March 1985 | 68 | ND | 0 | 1 | ND |
| | 45 (22.5) | 0 | 4 | 12 | *T. brucei* |
| July 1985 | 20 (10) | 0 | 0 | 2 | ND |
| November 1985 | 40 (2.5) | ND | ND | 1 | ND |

ND = not done.

outside the project area during grazing. This supposition is supported by the observations made in Kiguna in March 1985. The areas surveyed along the Gwayaka River contained temporary settlements of migratory herds and had infection rates as high as 22.5%.

### 3.1. Use of sentinel herds

Tables V and VI show the results obtained in 1986 using sentinel herds. Twelve Zebu cows purchased from a tsetse free zone of Nigeria and shown to be

free of infection by parasitological examination were quartered in selected areas within the project zone:

(1) *Ganye:* An active sterile male release area had two cows grazing around the Ganye I and Ganye II Rivers
(2) *Akura:* Another active release area had three cows grazing in the Ehula forest patches
(3) *Mai–Akuya:* An area within the Akuni forest patches already cleared of *G. p. palpalis;* four cows were quartered in this location
(4) *Kiguna:* An area where no form of tsetse control had been introduced; it was chosen as a control location and three cows were grazed along the banks of the Gwayaka River.

Table V shows that repeated infections were common, even though it could not be established whether such infections were relapses or new infections.

Table VI shows that the infection risk fluctuated seasonally. The infection risk was derived from an equation developed to account for the cattle activities in different locations

$$\text{Transmission rate} = \frac{\text{No. of positive samples}}{\text{No. of samples examined}} \times \frac{1}{\text{No. of animals (per location)}}$$

Thus, Akura, Mai–Akuya and Kiguna showed a persistent high risk of infection throughout the year, whereas at Ganye there were interruptions in May to July and November. August 1986 was significant, since infection was detected at all locations during the month and all animals had to be treated with a double dose (7.0 mg/kg body weight) of berenil.

### 3.2. Isolation of *T. brucei* with potential infectivity for man

The trypanosome isolates obtained from these surveys were propagated in laboratory mice and rats, and stabilates, prepared at peak parasitaemia, were stored in liquid nitrogen for further studies. Clones were prepared from such stabilates. The blood incubation infectivity test [10] was used to screen all *brucei* group isolates for infectivity to man. One of 15 such isolates consistently failed to be inactivated by pooled human serum and was presumed to be man infective (Table VII).

### 3.3. Relapses in sentinel herds and drug sensitivity tests

The sentinel herds were returned to a tsetse free zone at Vom in December 1986. All were treated with 7.0 mg/kg body weight of berenil before the transfer.

TABLE V. INCIDENCE OF TRYPANOSOMIASIS IN SENTINEL HERDS AT DIFFERENT LOCATIONS IN THE BICOT PROJECT AREA (MAY-DECEMBER 1986)

| Location | Cow No. | May | June | | July | | August | | September | | October | | | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 29 | 17 | 26 | 15 | 24 | 7 | 22 | 5 | 18 | 2 | 16 | 30 | 13 | 11 |
| Ganye | 290 | — | — | — | — | — | +ve[a] | — | — | +ve | — | — | — | — | — |
| | 292 | — | — | — | — | — | — | +ve | — | +ve | — | +ve | — | — | +ve |
| Akura | 266 | — | — | — | — | — | — | +ve | — | — | — | — | — | — | — |
| | 267 | — | — | — | — | +ve | — | — | — | — | — | — | — | +ve | — |
| | 268 | — | — | +ve | — | — | — | — | — | — | — | — | — | — | — |
| Mai-Akuya | 285 | +ve | +ve | — | — | — | — | +ve | — | — | — | — | — | — | +ve |
| | 286 | +ve | +ve | — | — | +ve | — | — | +ve | — | — | — | — | — | +ve |
| | 287 | — | — | — | — | — | — | +ve | — | — | — | +ve | — | — | — |
| | 288 | — | — | — | — | +ve | — | — | — | — | — | +ve | — | — | — |
| Kiguna | 278 | — | — | — | — | — | +ve | +ve | — | — | — | — | — | — | — |
| | 279 | — | — | — | — | — | — | +ve | — | — | — | +ve | — | — | — |
| | 280 | +ve | +ve | — | — | — | +ve | +ve | — | — | — | +ve | — | — | +ve |

[a] Positive case.

TABLE VI. TRANSMISSION RATE IN THE PROJECT AREA FROM SENTINEL HERD DATA (MAY–DECEMBER 1986)

| Location | May | June | July | August | September | October | November | December | Annual transmission rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| Ganye | 0 | 0 | 0 | 25 | 25 | 8.3 | 0 | 25 | 10.71 |
| Akura | 0 | 5.56 | 5.56 | 5.56 | 0 | 0 | 11.1 | 0 | 3.17 |
| Mai-Akuya | 25 | 6.25 | 6.25 | 6.25 | 3.13 | 6.25 | 0 | 12.5 | 5.80 |
| Kiguna | 16.7 | 5.56 | 0 | 27.78 | 0 | 5.56 | 0 | 11.1 | 7.94 |
| Monthly transmission rate (%) | 2.08 | 1.39 | 1.04 | 3.47 | 1.04 | 1.16 | 0.69 | 2.78 | 1.54 |

TABLE VII. BLOOD INCUBATION INFECTIVITY TEST ON *brucei* spp. TRYPANOSOMES ISOLATED FROM THE BICOT PROJECT AREA

| Trypanosome strain | Incubation with human serum | | | | Incubation with sheep serum | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of rats | Infected | Prepotency (d) | Longevity (d) | No. of rats | Infected | Prepotency (d) | Longevity (d) |
| Dog isolate *(T. b. brucei)* | 10 | 0 | 0 | 0 | 10 | 9 | 2 | 14 |
| TRIV-39 | 10 | 0 | 0 | 0 | 10 | 7 | 6 | 6 |
| TRIV-40 | 10 | 7 | 8 | 17 | 10 | 10 | 8 | 8 |
| Strain 8/18 *(T. b. brucei)* | 10 | 0 | 0 | 0 | 10 | 10 | 2 | 8 |
| TRIK-1 *(T. b. gambiense)* | 10 | 10 | 2 | 6 | 10 | 8 | 3 | 14 |

TABLE VIII. INFECTIONS OBSERVED IN JULY AND SEPTEMBER TO DECEMBER 1987 IN RESIDENT HERDS IN AREAS CLEARED OF *G. palpalis* (AKUNI), AREAS UNDER THE ACTIVE CONTROL PROGRAMME (EHULA GANYE) AND AREAS IN WHICH NO RELEASE HAD OCCURRED (KIGUNA)

| Survey No. (date) | Location | No. of herds (pens) | No. of samples | No. of positive trypanosomes | | | | Parasite identity by motility | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Wet | Buffy coat/ HCT | Stained thick film | *T. congolense* | *T. brucei* | *T. vivax* |
| 1 (23-7-1987) | Ganye | 4 | 24 | 0[a] | 0 | 0 | 0 | — | — | — |
| 2 (30-9-1987 to 3-10-1987) | Kiguna | 2 | 2 | 0 | 0 | 0 | ND | — | — | — |
| | Akura | 1 | 8 | 0 | 0 | 0 | ND | — | — | — |
| | Adogi | 1 | 5 | 1 | 1 | 1 | ND | 0 | 0 | — |
| | Ganye | 2 | 13 | 1 | 1 | 1 | ND | 0 | 0 | 1 |
| 3 (17-11-1987 to 20-11-1987) | Akura | 3 | 17 | 0 | 0 | 0 | 0 | — | — | — |
| | Adogi | 4 | 15 | 4[b] | 3 | 4 | ND | 0 | 2 | 2 |
| 4 (16-12-1987 to 19-12-1987) | Ehula | 2 | 7 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| | Kiguna | 2 | 15 | 14 | 10 | 14 | 12 | 0 | 2 | 8 |
| | Gidan Gambo | 1 | 5 | 0 | 0 | 2 | 0 | ND | ND | ND |

[a] Microfilariae were detected in four blood samples.

[b] Animals sampled at Adogi were maintained by a cattle dealer who buys his herd also from areas outside the project zone, e.g. Fadama Bauna, off the Gidan Buba zone.

TABLE IX. FLIES TRAPPED (AND DISSECTED) IN THE SAME TIME PERIOD AS THE ANIMAL SURVEY HELD IN 1987

| Date | Location | Total No. of flies caught | | *G. palpalis* | | | *G. tachinoides* | | | *G. longipalpis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | No. of flies infected | Male | Female | No. of flies infected | Male | Female | No. of flies infected |
| 26-9-1987 | Akuni | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| to | Kiguna | 6 | 2 | 5 | 1 | 1 ♀ | 1 | 0 | 0 | 0 | 1 | 1 ♀ |
| 3-10-1987 | | 2 | 6 | 5 | 1 | 1 ♀ | 0 | 0 | 0 | 0 | 3 | 0 |
| | | 1 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-11-1987 | Akuni | 1 | 5 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| to | | 1 | 4 | 0 | 0 | 0 | 1 | 4 | 2 ♀ ♀ | 0 | 0 | 0 |
| 19-11-1987 | | 1 | 4 | 0 | 0 | 0 | 1 | 4 | 1 ♀ | 0 | 0 | 0 |
| 23/24-11-1987 | Kiguna | 13 | 18 | 13 | 16 | 5 ♀ ♀ | 0 | 2 | 0 | 0 | 0 | 0 |
| 24/25-11-1987 | Kiguna | 11 | 12 | 11 | 12 | 2 ♀ ♀ | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-12-1987 | Awuma | 4 | 7 | 3 | 7 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| to | Awuma | 2 | 5 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-12-1987 | Agbabija | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Cattle were checked for infection every week in January, every two weeks in February and March and, thereafter, once every month until December 1987. From these studies, relapses were observed in some cows at different periods. One cow relapsed in January, one in April and three in September. All the cows were treated with the recommended dose of samorin (isomethamidium chloride) at 1 mg/kg body weight. No relapses were observed in December, when the project was discontinued. Blood from the three relapse cases was used to infect goats in order to raise infections for drug sensitivity tests; all the goats remained uninfected. Since there was no resurgence in the animals following samorin treatment, it was assumed that the infection had been cleared and that any drug resistance to berenil acquired by the parasites did not apply to samorin.

### 3.4. Concurrent animal survey and fly trapping (1987)

Following discontinuation of the use of sentinel herds, fly trapping and dissection in selected areas were intensified. These were complemented by a survey of settled herds, as used in 1984 and 1985. Table VIII shows the records made of cattle infections observed in July and September to December 1987.

Infections were not detected in the Ganye and Akura areas, where active sterile male release was being carried out. However, at Adogi in the Akuni area infection was discovered in a herd maintained by a cattle dealer. In the Kiguna area infections were very common, the highest being 14/15 (or 94%) of the sampled herds observed in December. This infection rate in cattle was parallel to the fly infections in Kiguna in November 1987 (Table IX).

## 4. DISCUSSION

The project aimed at assessing the control or eradication of tsetse fly and trypanosomiasis in an area using SIT for the biological control of tsetse flies. We were able to establish that the transmission cycle was not broken, even though *G. p. palpalis* was reduced to below the levels detectable by current methods. Several factors could have contributed to this, including the presence of *G. tachinoides* in the control zone to maintain host to host transmission in cattle, and herds moving in and out of the project zone. The persistence of infection may be enhanced by the presence of drug resistant strains that can be transmitted by available vector species.

Thus, in further extensions of the control programme, a multispecies tsetse control programme will be necessary to eradicate the disease in the area.

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# FREEZE DRIED BLOOD AND DEVELOPMENT OF AN ARTIFICIAL DIET FOR BLOOD FEEDING ARTHROPODS*

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**Abstract**

FREEZE DRIED BLOOD AND DEVELOPMENT OF AN ARTIFICIAL DIET FOR BLOOD FEEDING ARTHROPODS.

The goals of the research were to determine the biochemical differences between freeze dried bovine and porcine blood relative to their nutritional value to *Glossina palpalis palpalis* and *Stomoxys calcitrans* and to develop an artifical diet for mass rearing these flies. Freeze dried bovine and porcine blood were found to differ in their amino acid content; total dietary lipids did not significantly differ, but some notable exceptions were found in fatty acid content. Both sonication and addition of foetal bovine serum to freeze dried bovine blood improved its nutritional value for *G. p. palpalis*. A two component, semi-defined artificial diet was developed for *G. p. palpalis* and *S. calcitrans*. The College Station diet consisted of lipid contaminated bovine haemoglobin (BHb) and bovine serum albumin (BSA). To conduct dietary deletion tests, a process was developed for preparing large quantities of ultrapure lipid free bovine haemoglobin. *S. calcitrans* fed on lipid free BHb plus BSA had zero fecundity. Lipids were re-added to the protein diet in three forms: (1) lipid contaminated BHb, (2) pure erythrocyte ghosts, and (3) pure lipids. It was found that membrane lipid from the erythrocyte is required by *S. calcitrans*. A defined artifical diet consisting of lipid free BHb, BSA, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl serine and cholesterol gave normal adult survival, as well as near normal fecundity and percentage egg hatch for *S. calcitrans*. Knowing the identity of the lipids, it is now possible to prepare dietary formulations to alleviate dependency on the blood proteins BHb and BSA.

* Research carried out in agreement with the IAEA under Research Agreement No. 3108/CF.

† Deceased.

## 1. INTRODUCTION

Research on the evaluation and analytical dissection of freeze dried blood and the subsequent development of a defined diet for stable flies began at College Station in 1979. After working for 7 months in the IAEA Laboratory at Seibersdorf (1980–1981), it was decided to continue the research; this began in March 1982. A substantial portion of the research was funded by the Agency for International Development (1982–1985), the remainder being supported by the Agricultural Research Service of the United States Department of Agriculture. The research was conducted in collaboration with J.P. Kabayo of the IAEA.

The goals of the research at College Station were as follows:

(1) To conduct physical and biochemical measurements of freeze dried bovine and porcine blood to ascertain differences which might be related to the reproductive performance of *Glossina palpalis palpalis* and *Stomoxys calcitrans*
(2) To find additive(s) which might be used with freeze dried bovine blood to improve its nutritional value for *G. p. palpalis*
(3) To determine the major blood constituents in a bloodmeal necessary for normal reproduction in *G. p. palpalis* and *S. calcitrans*
(4) To develop a methodology for the preparation of large quantities of ultrapure lipid free haemoglobin for dietary studies
(5) To determine the importance of lipids in a protein diet for *S. calcitrans*
(6) To reconstitute a defined artificial diet of lipid and protein for use as a substitute bloodmeal for *S. calcitrans*.

In vitro rearing of tsetse flies on a membrane required the use of fresh blood [1–6]. The first successful membrane feeding technique independent of fresh blood was reported by Wetzel and Luger [7]; after successful feeding on blood components that had been stored frozen, Wetzel reported the use of freeze dried bovine blood for feeding *G. p. palpalis* [8]. Later, research at the IAEA led to the establishment of colonies of *G. p. palpalis* on freeze dried blood. However, the reproductive performance of tsetse flies fed on freeze dried blood remained variable [9].

*S. calcitrans* is also an obligate blood feeder and feeds on a variety of hosts [10]. Most laboratory colonies of *S. calcitrans* are fed fresh, non-sterile, citrated bovine blood. No genuine attempts have been made to find alternate sources of bloodmeals because of the ease of maintenance of flies on fresh blood. Stable flies and tsetse flies share some common requirements for a bloodmeal; for normal reproduction both flies require serum and erythrocyte components of blood. Therefore, it was relevant to use stable flies as a preliminary test insect for development of an artificial diet for tsetse flies.

## 2. MATERIALS AND METHODS

### 2.1. Flies

The stable flies used in these studies were taken from a colony maintained in the College Station Laboratory since 1969. The conditions for colony maintenance and egg collection have been described in Ref. [11]. The larval rearing method was modified, as described by Bridges et al. [12].

The tsetse flies (*G. p. palpalis*) used in these studies were from a stock colony maintained at Seibersdorf. The colony was fed exclusively on freeze dried blood through a silicone rubber membrane. All fly handling, maintenance conditions and rearing procedures were as described by Van Der Vloedt [13].

### 2.2. Freeze dried blood

#### *2.2.1. Preparation*

Fresh porcine and bovine blood were obtained in Vienna and freeze dried. Freeze dried blood was either used for feeding tests with *G. p. palpalis* at Seibersdorf or shipped to College Station for analysis and feeding tests with *S. calcitrans*. For comparative purposes, freeze dried porcine and bovine blood were prepared in the College Station Laboratory.

#### *2.2.2. Analysis*

Five randomly selected lots (prepared at the IAEA) of both bovine and porcine blood were used for amino acid analysis. The physiological or free amino acids were analysed according to standard procedures for a Beckman Model 121 amino acid analyser. The total amino acids were also analysed on hydrolysates of the blood. In addition, the haemoglobin concentration, pH and osmotic pressure of reconstituted blood were determined.

Lipid extractions and the chromatographic procedures for lipid separation were carried out as previously described in Ref. [14].

Phospholipid phosphorus was analysed according to the procedures of Duck-Chong [15]. Saponifiable lipids were transesterified as previously described in Ref. [14]. Cholesterol and triglyceride analyses were conducted on a Gilford System 3500 computer directed analyser.

### 2.3. Isolation of haemoglobin

Stroma contaminated bovine haemoglobin (BHb) was prepared from fresh bovine blood as follows: whole blood (lot size: 400 mL) was collected in heparin,

plasma was removed after centrifugation at 1500g for 15 min, and erythrocytes were washed four times in 4 volumes of saline solution (154 mM NaCl). Cells were then diluted twofold in distilled water and subjected to three freeze–thaw cycles to break the cells. The lysed cells were centrifuged at 10 000g for 30 min and the supernatant saved and freeze dried. The dried BHb was stored at ambient temperature until needed for feeding tests [16].

Lipid free or stroma free BHb was isolated by hypotonic hollow fibre dialysis and ultrafiltration of washed bovine erythrocytes. The process has been described extensively in Ref. [17] and used for isolation of BHb, porcine Hb [18] and human Hb [19]. Basically, washed packed erythrocytes are dialysed to a reduced osmotic pressure to make the cells porous without cell lysis. These porous cells are then subjected to 0.1 $\mu$m ultrafiltration in a continuous flow system. The filtrate containing Hb was freeze dried for storage. Haemoglobin prepared in this manner was then subjected to lipid analysis, gel electrophoresis and high performance liquid chromatography analysis for validation of its purity [17]. Commercial BHb was obtained from both the Sigma Chemical Corporation, St. Louis, United States of America, and Serva, Heidelberg, Federal Republic of Germany.

### 2.4. Bovine serum albumin and other dietary constituents

Bovine serum albumin (BSA) (fraction V) or fatty acid free fraction V was obtained from the Sigma Chemical Corporation, as were sphingomyelin (SM) from bovine brain, phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), cholesterol (CH) and choline chloride. All other chemicals were reagent grade or better.

### 2.5. Bovine erythrocyte ghosts

Erythrocytes used for lipid free Hb isolation were used as a source of erythrocyte membranes or erythrocyte ghosts. After extraction of stroma free BHb, as described by DeLoach et al. [17], the retentate from the ultrafiltration process was used as a source of ghosts. Dialysed erythrocytes containing less than 2% of their original Hb were washed three times, each in 4 volumes of hypo-osmotic phosphate buffered saline (15 mM $KH_2PO_4$, 70 mM NaCl, pH 7.2, osmotic pressure 170 mOsm/kg) with centrifugation (10 000g for 30 min) between washes. The resulting pellet of white ghosts was resuspended in 19 volumes of isotonic saline. The ghost cell content of the suspension was determined with a particle counter (Coulter Electronics, Hialeah, USA). With this procedure, the ghost cell count of the suspension was (6–8) $\times$ $10^8$ cells per millilitre.

### 2.6. Diets

Freeze dried bovine blood was reconstituted in distilled water (adenosine triphosphate added to 1 mM final concentration) before feeding to *G. p. palpalis*.

Freeze dried blood was supplemented with foetal bovine serum (10% vol./vol.) for some tests.

Freeze dried bovine blood reconstituted in distilled water was sonicated with a probe type sonicator at 100 W for 3–5 min for each 60 mL of diet [20].

Freeze dried bovine and porcine blood were reconstituted in distilled water and mixed (50:50 vol./vol.) for feeding tests.

BHb (15%) and BSA (6.5%) were combined in distilled water for feeding both *G. p. palpalis* and *S. calcitrans*. Later, diets consisted of combinations of lipid free BHb and BHb reconstituted with 6.5% BSA in distilled water for feeding *S. calcitrans*.

Lipid free BHb was combined with increasing proportions of white ghosts along with 6.5% BSA for reconstituted lipid diets.

Lipid free BHb (12%) plus BSA (6.5%) was reconstituted with the following lipid replacement additives: SM (40 mg); SM (40 mg), PE (7 mg), CH (25 mg); SM (40 mg), PE (7 mg), PS (40 mg), CH (25 mg); PE (7 mg); choline chloride (40 mg), CH (25 mg) per 60 mL of diet.

For each diet tested on *G. p. palpalis*, 15 females were used per cage with 5–7 mature males for mating. Feeding tests were conducted with four cages of flies over a period of 35 days, or through two reproductive cycles. For each diet tested on *S. calcitrans*, 10–15 females were used per cage with five males for mating. With *G. p. palpalis*, the number of pupae, pupal weight and adult survival were monitored. With *S. calcitrans*, eggs collected from days 7–13 were counted, the percentage egg hatch determined and the percentage adult survival recorded.

## 3. RESULTS

Table I summarizes the total amino acids in the freeze dried bovine and porcine blood used for maintenance of *G. p. palpalis* at Seibersdorf. Significant differences ($P = 0.05$) were noted for lysine, histidine, aspartic acid, threonine, serine, glycine, alanine, methionine, isoleucine and phenylalanine. Bovine blood had greater amounts of the essential amino acids phenylalanine and methionine, while porcine blood had a greater amount of isoleucine.

There was little difference in the pH (bovine: 8.2; porcine: 8.0), osmolarity (bovine: 294 mOsm/kg; porcine: 280 mOsm/kg), or the haemoglobin content (bovine: 13.6%; porcine: 13.0%) of the two different animal bloods.

TABLE I. TOTAL AMINO ACIDS IN FREEZE DRIED BOVINE AND PORCINE BLOOD USED FOR MAINTENANCE OF *G. p. palpalis* AT SEIBERSDORF

| Amino acid + ammonia | Concentration (μmol/mL) | |
|---|---|---|
| | Bovine[a] | Porcine[a] |
| Lysine | 114.1 ± 2.0 | 105.4 ± 2.2 |
| Histidine | 67.4 ± 0.8 | 77.3 ± 2.3 |
| Ammonia | 96.6 ± 1.4 | 122.1 ± 2.7 |
| Arginine | 39.2 ± 0.9 | 42.8 ± 1.0 |
| Aspartic acid | 134.7 ± 2.4 | 151.2 ± 3.3 |
| Threonine | 72.2 ± 1.3 | 51.5 ± 1.1 |
| Serine | 88.1 ± 2.8 | 77.7 ± 2.3 |
| Glutamic acid | 105.1 ± 1.5 | 108.8 ± 1.8 |
| Proline | 57.8 ± 0.9 | 59.7 ± 0.9 |
| Glycine | 96.4 ± 2.3 | 108.6 ± 2.7 |
| Alanine | 164.2 ± 2.3 | 156.5 ± 3.0 |
| Half cystine | 9.3 ± 0.7 | 10.1 ± 0.9 |
| Valine | 131.2 ± 2.4 | 123.9 ± 3.1 |
| Methionine | 18.5 ± 0.5 | 9.6 ± 0.5 |
| Isoleucine | 8.9 ± 0.5 | 13.1 ± 0.4 |
| Leucine | 179.9 ± 2.4 | 183.0 ± 2.9 |
| Tyrosine | 30.2 ± 0.6 | 27.0 ± 0.8 |
| Phenylalanine | 77.9 ± 1.2 | 70.3 ± 1.7 |
| *Total protein (%)* | 17.9 ± 0.2 | 17.8 ± 0.4 |

[a] Data are mean ± SD (n = 5).

Particle size analysis of reconstituted freeze dried bovine blood revealed a heterogeneous particle size of 5 to 100 $\mu m^3$. Sonication of these bloods reduced particle size to homogeneity [20]. The results of a feeding test are summarized in Table II. The total production of pupae increased in *G. p. palpalis* fed either sonicated diet. The pupae per mature female-day (PPF) increased from 0.079 to 0.091 for sonicated porcine blood and from 0.075 to 0.090 for sonicated bovine blood. The percentage adult survival was unaffected.

TABLE II. IN VITRO FEEDING TESTS WITH SONICATED FREEZE DRIED PORCINE BLOOD (FDPB) AND BOVINE BLOOD (FDBB)

| | % survival days 18–35[a] | Total pupae by day 35 | Pupae per mature female-day[b, c] | Mean pupal weight (mg) 1st reproductive cycle[c, d] | Mean pupal weight (mg) 2nd reproductive cycle[c, d] |
|---|---|---|---|---|---|
| FDPB: control | 94.7 | 64 | 0.079a | 28.9 ± 4.5a | 30.1 ± 4.9a |
| FDPB: sonicated | 90.2 | 89 | 0.091b | 31.5 ± 3.3b | 31.6 ± 4.6a |
| FDBB: control | 96.5 | 77 | 0.075a | 28.4 ± 3.3a | 26.4 ± 4.2a |
| FDBB: sonicated | 94.2 | 91 | 0.090b | 28.1 ± 2.9a | 28.1 ± 3.3a |

[a] Percentage survival is calculated as follows: (actual mature female-days) divided by (theoretical mature female-days) × 100.

[b] Assuming a 9.5 day reproductive cycle, the theoretical maximum pupae per mature female-day is 0.105.

[c] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).

[d] Data are mean ± SD ($n = 16$).

The *G. p. palpalis* colonies were then fed on freeze dried porcine and bovine blood which had been supplemented with either foetal calf serum, bovine serum, or a 50:50 mixture (Tables III and IV). The addition of foetal calf serum (FCS) to freeze dried bovine blood (FDBB) or freeze dried porcine blood (FDPB) increased the PPF to 0.091 for bovine and 0.098 for porcine. A 50:50 mixture of FDPB and FDBB gave a PPF of 0.084 to 0.100.

The feeding tests on freeze dried bloods with *S. calcitrans* are summarized in Table V; the data are for flies fed for ten consecutive generations on each diet. Stable flies showed significantly ($P < 0.05$) higher reproductive performance when fed on porcine blood diets. These data confirm the general premise that porcine blood is nutritionally superior to bovine blood.

Since it has been established that both serum and erythrocyte components are necessary for a complete diet [9, 21], we conducted feeding and reproductive performance tests with *S. calcitrans* and *G. p. palpalis* on diets consisting of BHb and BSA.

TABLE III. REPRODUCTIVE PERFORMANCE OF *G. p. palpalis* FED ON SUPPLEMENTED FDBB

| Diet | % survival days 18–35[a, b] | Total pupae by day 35 | Pupae per initial female | Pupae per mature female-day[b] | Pupae per mature female-day relative to theoretical maximum | Mean pupal weight (mg) | |
|---|---|---|---|---|---|---|---|
| | | | | | | 1st reproductive cycle[b, c] | 2nd reproductive cycle[b, c] |
| Fresh bovine blood | 94a | 86 | 1.43 | 0.085a | 0.080 | 30.5 ± 4.4a | 31.3 ± 3.9a |
| +FCS | 95a | 85 | 1.42 | 0.083a | 0.079 | 30.6 ± 3.7a | 32.5 ± 3.1a |
| FDBB | 86b | 80 | 1.33 | 0.087a | 0.074 | 26.8 ± 3.6b | 29.7 ± 3.6a |
| FDBB + FCS | 91a | 89 | 1.48 | 0.091b | 0.082 | 28.8 ± 3.0a | 29.7 ± 3.6a |

[a] Percentage survival is calculated as follows: (actual mature female-days) divided by (theoretical mature female-days, or 15 × 18 days) × 100.
[b] Data in the same column followed by a common letter are not significantly different (P = 0.05).
[c] Data are mean ± SD.

TABLE IV. REPRODUCTIVE PERFORMANCE OF *G. p. palpalis* ON FDPB

| Diet | % survival days 18–35[a, b] | Total pupae by day 35 | Pupae per initial female | Pupae per mature female-day[b] | Pupae per mature female-day relative to theoretical maximum | Mean pupal weight (mg) | |
|---|---|---|---|---|---|---|---|
| | | | | | | 1st reproductive cycle[b, c] | 2nd reproductive cycle[b, c] |
| Fresh porcine blood | 98a | 89 | 1.48 | 0.084a | 0.082 | 30.7 ± 3.5a | 30.1 ± 3.7a |
| FDPB | 95a | 87 | 1.45 | 0.085a | 0.081 | 29.8 ± 5.5a | 31.0 ± 5.0a |
| FDPB + FCS | 88b | 93 | 1.55 | 0.098b | 0.086 | 32.2 ± 3.6b | 31.2 ± 4.0a |
| FDPB + FCS sonicated | 96a | 99 | 1.74 | 0.099b | 0.096 | 31.7 ± 4.1ab | 31.0 ± 4.6a |
| FDPB + bovine serum | 94a | 88 | 1.47 | 0.086a | 0.082 | 29.6 ± 3.2ab | 30.2 ± 3.8a |
| FDPB:FDBB 50:50 | 92ab | 84 | 1.40 | 0.084a | 0.078 | 30.2 ± 3.7a | 29.1 ± 4.4a |
| FDPB:FDBB 50:50 sonicated | 98a | 107 | 1.78 | 0.100b | 0.099 | 32.2 ± 4.0b | 32.0 ± 3.8a |

[a] Percentage survival is calculated as follows: (actual mature female-days) divided by (theoretical mature female-days, or 15 × 18 days) × 100.

[b] Data in the same column followed by a common letter are not significantly different (P = 0.05).

[c] Data are mean ± SD.

TABLE V. STATISTICAL ANALYSIS OF FECUNDITY AND EGG HATCH ON FEMALE *S. calcitrans* MAINTAINED FOR TEN CONSECUTIVE GENERATIONS ON RECONSTITUTED, FREEZE DRIED BLOOD OR FRESH HEPARINIZED BLOOD

| Blood meal | Eggs per day per female[a, b] | Egg hatch (%)[a, b] |
|---|---|---|
| FDPB (College Station) | 50.0a | 93.6a |
| FHP | 49.4a | 87.8a |
| FDPB (Vienna) | 48.3a | 91.0a |
| FDBB (Vienna) | 36.8b | 87.1a |
| FDBB (College Station) | 35.4b | 91.2a |
| FHB | 27.1c | 74.2b |

[a] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).

[b] Data are means ($n = 33$).

These two proteins constitute 80% of the total protein in whole blood. The results of these feeding tests are summarized in Tables VI and VII. Stable flies fed on the artificial BHb and BSA (College Station) diet had a reproductive performance equal to that of flies fed on fresh bovine blood. Tsetse flies fed on the College Station diet did not show a reproductive capacity equivalent to that of flies fed on a control diet. However, this was the first time tsetse flies had produced large numbers of puparia on an artificial diet.

Preliminary lipid analysis of BHb prepared in the College Station Laboratory and obtained from a commercial vendor revealed the presence of lipid impurities with BHb [22]. Thus, we began to develop a process for isolating large quantities of lipid free BHb for further dietary studies. A summary of lipid free BHb isolation is given in Table VIII. The percentage yield of Hb was consistently greater than 90%. Of greater importance, lipid analysis of BHb isolated by different techniques revealed several striking features (Table IX). First, BHb prepared by our new process was essentially lipid free. Second, BHb prepared by two methods commonly used by researchers in the field yielded lipid contaminated BHb. High pressure liquid chromatography analysis revealed the lipid free BHb to be 99.9% protein pure [17, 18].

TABLE VI. FECUNDITY, EGG HATCH AND SURVIVAL OF *S. calcitrans* FED ON DIFFERENT BLOOD COMPONENTS

| Diet | Eggs per day[a, b] | Egg hatch (%) | Survival[c] (%) | Relative efficiency of diet[d] (%) |
|---|---|---|---|---|
| Control (test 1) | 40.8a | 98 | 83 | 100 |
| Hb 15% | 5 eggs (total) | — | 0 | 0 |
| BSA 6.5% | 0 | — | 0 | 0 |
| BSA 18% | 0 | — | 0 | 0 |
| Hb 15% + BSA 6.5% (test 1) | 43.8a | 98 | 100 | 107 |
| Control (test 2) | 38.9a | 97 | 92 | 100 |
| Hb 15% + BSA 6.5% (test 2) | 40.2a | 97 | 92 | 103 |

[a] Data are the means of two groups of flies from 7 days of egg collection.
[b] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).
[c] Thirteen day survival of adults.
[d] Relative efficiency of diet = (eggs per day (test)) divided by (eggs per day (control)) × 100.

TABLE VII. RESULTS OF IN VITRO FEEDING TESTS WITH *G. p. palpalis*

| Diet | Survival[a, b] (%) | Pupae per female[b, c] by day 35 | Pupal weight[b, d] (mg) |
|---|---|---|---|
| Hb + BSA | 87a | 1.0a | 24.0 ± 3.2a |
| FDBB[e] | 90a | 1.24b | 27.8 ± 3.4b |

[a] Survival is based on the number of flies surviving after sexual maturity at days 18–35.
[b] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).
[c] Total pupae produced divided by the number of females = pupae per female.
[d] Data are mean ± SD ($n = 6$).
[e] The standard diet used for colony rearing of *G. p. palpalis* at the time these tests were conducted.

TABLE VIII. LIPID FREE HAEMOGLOBIN (LFHb) ISOLATION

| Volume of packed red blood cells (mL) | Starting Hb (g) | Ultrafiltration time (h) | LFHb recovered (g) | Hb yield[a] (%) |
|---|---|---|---|---|
| 275 | 26.7 | 2 | 23.5 | 88.3 |
| 275 | 25.3 | 3 | 20.0 | 90.6 |
| 1680 | 166.3 | 14 | 128.0 | 84.2 |
| 500 | 133.0 | 4 | 117.0 | 89.0 |
| 415 | 95.9 | 5 | 86.4 | 95.5 |
| 669 | 153.7 | 5 | 151.3 | 98.4 |
| 570 | 107.2 | 5 | 85.1 | 91.8 |

[a] Percentage yield of Hb is calculated as follows: (amount of LFHb recovered) divided by (LFHb + Hb remaining in processed red blood cells) × 100. Percentage yield does not take into account loss of cells due to dead volume in apparatus.

TABLE IX. LIPID CONTENT OF BOVINE HAEMOGLOBIN PREPARATIONS

| Hb sample | Cholesterol | $P_i$[a] | SM | PS | PE |
|---|---|---|---|---|---|
| Cells haemolysed in distilled water and processed by ultrafiltration | 7.3[b] | 0.51 | 14.1 | 1.71 | 3.05 |
| Cells haemolysed in distilled water and centrifuged | 2.2 | 0.32 | 3.26 | 0.75 | 1.84 |
| Dialysed cells plus ultrafiltration | 0.0[c] | 0.0 | 0.0 | 0.0 | 0.0 |

[a] $P_i$ = inorganic phosphorus.

[b] Data are means and are expressed as milligram lipid per gram Hb.

[c] There were no detectable lipids from the thin layer chromatography plate.

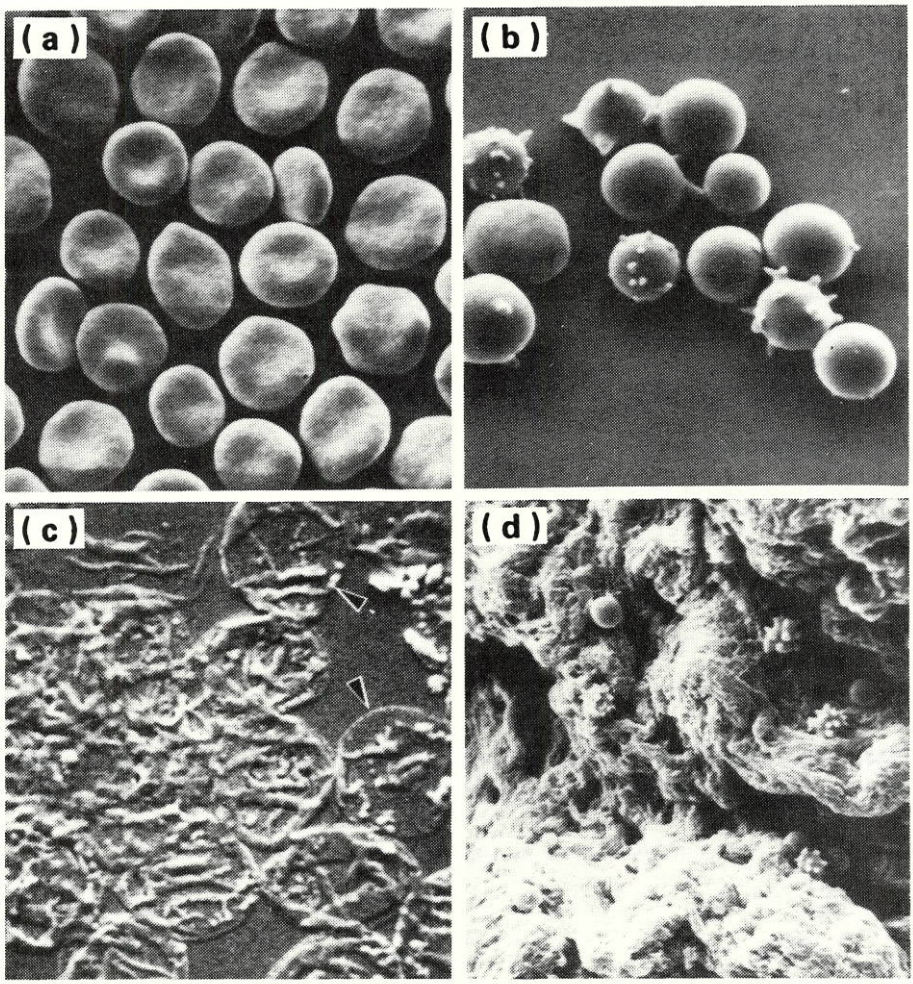


*FIG. 1. Scanning electron microscopy micrographs of red blood cells: (a) normal washed red blood cells; (b) dialysed red blood cells; (c) red blood cell ghosts; (d) stroma from red blood cells.*

Scanning electron microscopy of cells prepared for Hb isolation by an older method or by our new methodology revealed the strikingly different morphological characteristics of the cells (Fig. 1). Dialysed cells were uniformly swollen and no stroma was observed with cells prepared by hypotonic dialysis and ultrafiltraton. However, cells prepared for BHb isolation by the usual methods resulted in an amorphous stroma which contaminated the BHb.

TABLE X. FECUNDITY, EGG HATCH AND SURVIVAL OF *S. calcitrans* FED ON LIPID FREE HAEMOGLOBIN

| Diet | Eggs per female per day[a, b] | Egg hatch[a, b] (%) | Survival[a, b] (%) |
|---|---|---|---|
| FDBB | 69.8a | 97.0a | 95.4a |
| Lipid containing haemoglobin | | | |
| plus BSA | 48.6b | 86.6b | 94.6a |
| plus fatty acid free BSA | 47.2b | 75.1b | 93.0a |
| Lipid free haemoglobin | | | |
| plus BSA | 0c | 0c | 63.2b |
| plus fatty acid free BSA | 0c | 0c | 59.7b |

[a] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).
[b] Data are means ($n = 21$).

TABLE XI. FECUNDITY, EGG HATCH AND SURVIVAL OF *S. calcitrans* FED ON MEMBRANE STROMA AND PROTEIN

| Diet | Eggs per female per day[a, b] | Egg hatch[a, b] (%) | Survival[a, b] (%) |
|---|---|---|---|
| FDBB | 72.6a | 95.3a | 99.7a |
| Lipid containing Hb + BSA | 44.7b | 93.1a | 95.5a |
| Lipid free Hb + BSA | 2.7c | 0b | 86.4b |
| + 1 mL membrane | 12.9d | 1.4c | 91.7b |
| + 5 mL membrane | 44.5b | 45.8b | 99.6b |
| Bovine plasma | 27.5e | 90.1a | 81.2b |
| Plasma + 5 mL membrane | 33.0e | 94.1a | 84.7b |

[a] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).
[b] Data are means ($n = 21$).

TABLE XII. SURVIVAL AND REPRODUCTION IN *S. calcitrans* FED SYNTHETIC DIETS CONTAINING DIFFERENT PROPORTIONS OF HAEMOGLOBIN BOUND LIPIDS

| Diet composition (%)[a] (wt/vol. in distilled water) | | | Hb bound lipid impurities[a] | Survival[b, c] | Eggs per female per day[b, c] | Egg hatch[b, c] |
|---|---|---|---|---|---|---|
| BHb | LFHb | BSA | (%) | (%) | | (%) |
| 12 | 0 | 6.5 | 100 | 97.4 ± 2.6a | 54.5 ± 4.7a | 87.0 ± 6.4a |
| 10 | 2 | 6.5 | 83 | 96.2 ± 4.0a | 73.5 ± 3.4b | 94.8 ± 0.9a |
| 7 | 5 | 6.5 | 58 | 91.4 ± 3.7a | 71.4 ± 3.1b | 87.6 ± 6.5a |
| 5 | 7 | 6.5 | 42 | 98.2 ± 2.0a | 71.4 ± 5.3b | 83.9 ± 6.4a |
| 2 | 10 | 6.5 | 17 | 91.2 ± 4.7a | 43.2 ± 3.3c | 33.5 ± 4.1b |
| 0 | 12 | 6.5 | 0 | 88.9 ± 4.2a | 0.9 ± 0.4d | 2.1 ± 1.5c |

[a] Based on the proportion of lipid free haemoglobin (LFHb) to lipid contaminated haemoglobin.

[b] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).

[c] Data are mean ± SD ($n = 21$).

We then conducted the first feeding experiments with lipid free BHb. *S. calcitrans* fed on FDBB or the College Station diet showed excellent fecundity and survival (Table X). Flies fed on lipid free BHb plus BSA produced no eggs and adult survival was significantly ($P < 0.05$) lower than the control fed flies. These experiments were the first to show the essential dietary nature of an Hb contaminant.

Since lipid free BHb was missing some essential constituents, we utilized white ghosts in a series of feeding tests. Stable flies were fed FDBB (control diet), BHb plus BSA and the lipid free diet combined with white ghosts. To confirm the essential nature of BHb, flies were fed bovine plasma and bovine plasma plus white ghosts (Table XI). Flies had normal fecundity when fed on the College Station diet and only a few eggs were produced with the lipid free BHb diet. Addition of white ghosts restored fecundity to normal; however, the percentage egg hatch remained less than normal. Bovine plasma plus white ghosts failed to meet the full nutritional demand of the flies, thus confirming the essential nature of BHb.

TABLE XIII. EFFECT OF ADDED ERYTHROCYTE GHOST CELL COMPONENTS ON THE NUTRITIONAL VALUE OF A LIPID REDUCED SYNTHETIC DIET TO *S. calcitrans*

| Volume of ghost cell suspension[a] (mL) | Calculated concentration (%) of lipid contaminated Hb (wt/vol.) | Survival[b, c] (%) | Eggs per female per day[b, c] | Egg hatch[b, c] (%) |
|---|---|---|---|---|
| 0 | 0 | 86.7a | 0.9 ± 0.4a | 0a |
| 1 | 0.95 | 77.4a | 5.2 ± 1.1b | 0a |
| 4 | 3.80 | 98.6b | 37.6 ± 4.0c | 12.1 ± 8.2b |
| 10 | 9.50 | 95.2b | 35.8 ± 2.3c | 71.2 ± 6.0c |
| 15 | 14.25 | 95.4b | 54.6 ± 4.4d | 86.4 ± 6.2d |
| 20 | 19.00 | 98.5b | 45.3 ± 3.5d | 91.2 ± 6.0d |

[a] Pellet resulting from centrifugation of indicated volume of ghost cell suspension was resuspended in the lipid free synthetic diet (12% LFHb and 6.5% BSA (wt/vol. in distilled water)).

[b] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).

[c] Data are means and mean ± SD ($n = 21$).

Because BHb appeared to a a good source of lipid, we conducted a series of feeding tests with different proportions of BHb and lipid free BHb. The BSA composition was kept constant at 6.5%, while lipid free BHb varied from 0 to 12%. The fecundity and the percentage egg hatch and adult survival were followed on the stable flies. BHb proved to be a reliable source of lipid for the flies (Table XII). A diet mixture of 12% lipid free BHb and 6.5% BSA resulted in extremely low fecundity (0.9 eggs per female per day). Addition of 2% BHb to 10% lipid free BHb resulted in normal fecundity, but still an abnormal percentage egg hatch. A 5% BHb and 7% lipid free BHb resulted in excellent fecundity and percentage egg hatch.

Because BHb is a mixture of Hb and some erythrocyte membrane constituents, we conducted further feeding tests with varying concentrations of white ghosts and lipid free BHb (BSA was kept constant at 6.5%) (Table XIII). A complex dependency on lipid concentration was observed. While 4 mL of lipid gave high fecundity,

TABLE XIV. CHOLESTEROL AND PHOSPHOLIPID PHOSPHORUS CONTENT OF THE DIETARY CONSTITUENTS

| Diet | Cholesterol[a, b] (mg/dL) | Phospholipid phosphorus[a, b] (mg/dL) |
|---|---|---|
| College Station Hb | 114a | 3.87a |
| Serva Hb | 126a | 3.74a |
| Lipid free Hb | 0b | 0b |
| BSA | 16.6c | 0.05c |
| White ghosts | 124a | 3.62a |
| FDBB | 202d | 7.90d |

[a] Data in the same column followed by a common letter are not significantly different (P = 0.05).

[b] Data are means (n = 6).

the percentage egg hatch was still abnormally low. A 2.5 fold increase in lipid failed to increase fecundity, but improved egg hatch to 71%. A further increase in lipid improved both fecundity and percentage egg hatch.

We then conducted lipid analysis of the different dietary preparations (Table XIV). Lipid analysis corroborated the biological data from feeding tests. The College Station diet, BHb, Serva BHb and an equivalent amount of white ghosts all contained similar amounts of cholesterol and phospholipid. Further analysis into the various classes of phospholipid revealed a similar distribution pattern for the College Station and Serva BHb and a significantly (P = 0.05) different distribution from white ghosts.

On the basis of the lipid analysis data, we conducted feeding tests on diets reconstituted with purified lipids added to lipid free BHb and BSA (Table XV). It appears that SM, PE, PS and CH are all required to some extent by stable flies. A diet consisting of SM and CH, although not totally sufficient for normal fecundity, gave very promising results. Fecundity was 28.3 eggs per female per day, with 82.5% egg hatch. Flies fed on a diet containing SM minus CH produced 25.3 eggs per female per day, but egg hatch was only 1.6%. Thus, CH is required for normal egg hatch and SM is required for egg production.

TABLE XV. EFFECT OF LIPID ADDITIVES ON THE NUTRITIONAL VALUE OF A LIPID FREE SYNTHETIC DIET TO *S. calcitrans*

| Feeding test | Lipid additives (mg/100 mL) (lipid free synthetic diet) | | | | | Survival[a, b] (%) | Eggs per female per day[a, b] | Egg hatch[a, b] (%) |
|---|---|---|---|---|---|---|---|---|
| | SM | PE | PS | CH | Choline chloride | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 78.1 ± 9.2a | 0.4 ± 0.2a | 0a |
| 2 | 40 | 0 | 0 | 0 | 0 | 89.2 ± 3.7a | 25.3 ± 3.3b | 1.6 ± 1.6a |
| 3 | 0 | 7 | 0 | 0 | 0 | 76.3 ± 4.2a | 10.1 ± 1.6c | 0a |
| 4 | 40 | 7 | 0 | 25 | 0 | 93.2 ± 6.0b | 28.3 ± 3.8b | 82.5 ± 2.5b |
| 5 | 0 | 0 | 0 | 25 | 40 | 72.1 ± 3.8a | 26.8 ± 4.2b | 0.9 ± 0.9a |
| 6 | 40 | 7 | 40 | 25 | 0 | 92.1 ± 4.1b | 36.1 ± 5.8d | 73.0 ± 3.0b |

[a] Data in the same column followed by a common letter are not significantly different ($P < 0.05$).

[b] Data are mean ± SD ($n = 6$).

## 4. DISCUSSION

The data presented here are important to the objectives of the IAEA Co-ordinated Research Programme because the goals of the research were developed in close consultation with Agency staff. These goals were twofold: (1) to improve freeze dried bovine blood as a diet for *G. p. palpalis*, and (2) to find a defined artificial diet for blood feeding arthropods.

Amino acid analysis of freeze dried bovine and porcine blood revealed significant differences [23]. However, the results were slightly at variance with those of Moloo and Pimley [24]. Even though the samples analysed were from different lots of blood obtained from the IAEA, there was little variation within each lot of animal blood. These lots of blood were subsequently fed to *S. calcitrans* for ten generations. The results validated the nutritional superiority of freeze dried porcine blood over freeze dried bovine blood [14]. Studies conducted in co-operation with the IAEA on the amino acid contents of the blood revealed several interesting features in both porcine and bovine blood diets that had a high nutritional quality for *G. p. palpalis*. A quantitative analysis of CH, triglycerides, phospholipid phosphorus, phospholipid classes and fatty acid composition was conducted. No correlation was found between fly fecundity and cholesterol content in the range of 90–130 mg/dL of diet [25], nor between triglyceride or total phospholipid content and fecundity. However, the distribution of phospholipids and their location in the blood, i.e. erythrocytes or serum, may be factors contributing to the nutritional quality of a bloodmeal [26]. Subsequent analysis of the diets used to feed *G. p. palpalis* support these conclusions [25].

It is concluded that stable flies are not as discriminating as tsetse flies with respect to fresh or freeze dried blood diets. However, stable flies had a higher fecundity on porcine blood than on bovine blood, much like tsetse flies [26].

A combination of blood components for feeding stable flies and tsetse flies has led to the development of a semi-defined artificial diet for these flies. The College Station diet [16] and the KT-80 diet [27] differ only slightly in their salt content. They both contain essentially the same ingredients obtained from different sources. Lipid analyses of the BHb used in the College Station diet and of the Serva Hb used in the KT-80 diet revealed almost identical lipid contaminants (Table XIV). The significance of these artificial diets is that the bloodmeal has been reduced to two proteins and some contaminants which are likely to be lipids.

Subsequent dietary deletion studies required the preparation of ultrapure lipid free BHb. The methods used by researchers to isolate pure undenatured BHb were unacceptable because organic solvents were used, or because they produced only milligram quantities of protein [17, 18]. We therefore adapted the erythrocyte encapsulation technology [28] developed in the College Station Laboratory to extract pure BHb from erythrocytes. The resulting lipid free BHb (prepared in 100 g quantities) was then used for feeding tests.

Stable flies fed on a lipid free diet produced zero to a few eggs, with zero percentage egg hatch [29]. These data supported our hypothesis that lipids are an essential component in the bloodmeal. Subsequent feeding tests with *G. p. palpalis* confirmed our results with stable flies [30]; tsetse flies fed on a diet consisting of lipid free BHb plus BSA failed to produce a single puparia. With a negative control diet, it was possible to conduct diet reconstitution studies with different sources of animal lipids.

Addition of BHb to lipid free BHb plus BSA restored the nutritional quality to normal. Addition of lipid in the form of white erythrocyte ghosts also restored the nutritional quality to normal [31]. After lipid analysis of the white erythrocyte ghosts, College Station BHb and Serva Hb, we determined the basic phospholipid classes to be the same. Diets consisting of lipid free BHb plus BSA were reconstituted with different lipids. Sphingomyelin and cholesterol were both required, the latter primarily for normal egg hatch. Relative to fecundity, sphingomyelin could be replaced by choline chloride to some extent, but egg hatch was abnormally low. Addition of both PE and PS to the SM and CH diet further improved fecundity.

Sphingomyelin is a minor class of choline containing phospholipids not yet reported in Diptera; the predominant phospholipid in Diptera is phosphatidyl ethanolamine [32]. Recently we have shown that adult stable flies utilize all the lipids in a bloodmeal except for about 80% of the cholesterol [33]. Almost 98% of the sphingomyelin was utilized by the fly, confirming its requirement in the bloodmeal. Although adult flies utilized only 20% of the ingested cholesterol in a bloodmeal, the amount utilized is essential for egg hatch. Newly emerged adult females contain only one-third the amount of cholesterol sequestered in the first egg clutch. Thus, cholesterol in a bloodmeal is required for egg hatch. A substantial portion of the cholesteryl esters found in stable fly eggs contain polyunsaturated fatty acids. Thus, the cholesteryl esters stored in eggs may have a secondary role, that of providing the inset with polyunsaturated fatty acids for normal embryonic development [33].

The identity of long chain fatty acids provided by a bloodmeal and supplied in the form of cholesteryl esters and the phospholipids SM, PE and PS remains to be determined. However, at least one long chain fatty acid has been identified as being required by the mosquito, *Culex pipiens* [34]; addition of arachidonic acid to larval diets of this mosquito substituted for previously used animal derived phospholipids.

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# EFFECTS OF THE NUTRITIONAL QUALITY OF LOCALLY OBTAINED BLOOD DIETS ON THE PERFORMANCE OF *Glossina palpalis palpalis* FED IN VITRO*

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**Abstract**

EFFECTS OF THE NUTRITIONAL QUALITY OF LOCALLY OBTAINED BLOOD DIETS ON THE PERFORMANCE OF *Glossina palpalis palpalis* FED IN VITRO.

Three separate experiments were conducted to evaluate the nutritional quality of locally obtained blood diets on the overall performance of *Glossina palpalis palpalis* fed in vitro. In the first experiment, 30 teneral *G. p. palpalis* females were fed in vitro 6 days a week on a protein deficient bovine blood diet for 25 days. Thirty teneral females fed on a normal bovine blood diet were used as the control. The performance of the test and control groups in terms of survival rate, productivity and puparial weight were observed for 25 days post-emergence. The mean puparial weight of the control flies was significantly greater than that of the test flies. However, the survival rate and fecundity in the two groups did not differ significantly. In the second experiment, batches of female *G. p. palpalis* were similarly fed in vitro on camel or bovine blood diets for 25 days. The survival rate, productivity and puparial weight of the two groups of flies were compared; more puparia of higher weight classes were produced by flies fed on bovine blood than those on camel blood. In a third experiment, six groups of teneral female flies (50 in each group) were maintained on blood diets with different cellular and plasma concentrations. The overall performance of the flies in each group in terms of survival rate, productivity and puparial weight observed over a period of 35 days was similarly evaluated. Flies fed on diets with a packed cell volume (PCV) of 20–30% performed better than those on diets with a PCV of 60% and above. The groups maintained on 100% plasma or 100% cells performed poorly.

## 1. INTRODUCTION

Tsetse flies have been reported to show feeding preferences for certain hosts in the field and the source of blood used to maintain in vitro fed flies has been shown to affect their performance [1]. Recent studies [2, 3] have shown that protein deficient diets affect the fecundity, puparial weight and survival rate of *Glossina* spp.

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An artificial diet consisting of two proteins, haemoglobin and albumin, together with their associated lipids, has been shown to support reproduction in *Stomoxys calcitrans* and *Glossina palpalis palpalis* [4].

The present work examined the effect of feeding protein deficient blood on the survival, productivity and puparial weight of *G. p. palpalis*.

## 2. MATERIALS AND METHODS

### 2.1. Experiment 1

A splenectomized young white Fulani bull was made anaemic by experimental co-infection with *Anaplasma marginale* and *Theileria mutans* blood parasites. The anaemic condition of the bull was determined by the haemocrit (packed cell volume (PCV)) and haemoglobin values and the total serum protein using the Biuret method [5]. The haemoglobin of the blood was evaluated using the Sabli method.

Fresh blood collected under sterile conditions from the anaemic animal was given a routine treatment of 0.5–1 kGy $^{60}$Co gamma radiation to reduce contamination of the blood. The treated blood was then stored frozen at −20°C until required. Before in vitro feeding, the frozen blood was thawed slowly overnight at 4°C and adenosine triphosphate was added at a concentration of 551.15 μg/mL of blood as a phagostimulant. Fresh pooled bovine blood collected from the Jos abattoir and treated as described above served as the control diet.

Two batches of 30 teneral *G. p. palpalis* female flies were fed in vitro 6 days a week on the protein deficient and control diets for 25 days. The flies were kept under controlled insectary conditions. On the second day of their adult life the female flies were mated with mature males (at a ratio of 10 males to 15 females per cage) and then separated after 2 days.

The performance of the test and control groups of flies in terms of survival rate, productivity and puparial weights was observed for 25 days post-emergence. At day 25, the females were dissected to determine the insemination rate and the stage of pregnancy in both groups.

### 2.2. Experiment 2

Fresh heparinized bovine and camel blood collected from a local abattoir were irradiated with a total of 1.5 kGy and stored frozen at −20°C. Batches of female flies were fed in vitro on the camel and bovine blood diets for a 25 day period.

The puparia produced were sorted into different weight classes and the survival rate and productivity expressed by puparia per initial female measured.

The packed cell volume, haemoglobin level, total white blood cells and differential counts and the glucose level of the two blood samples were estimated after collection.

### 2.3. Experiment 3

Three of the six fresh heparinized bovine blood samples collected from the local abattoir were centrifuged and the plasma separated under sterile conditions.

Diets were prepared by adding plasma or cells to the original heparinized bovine blood at the following ratios: 1 part plasma to 9 parts original blood; 1 part plasma to 1 part original blood; 1 part cell to 9 parts original blood; and 1 part cell to 1 part original blood; undiluted plasma and cells were also used as diets.

The six artificially produced diets had the following packed cell volumes: (1) 10% plasma dilution = 36%; (2) 50% plasma dilution = 20%; (3) 100% plasma = 0%; (4) 10% cell concentration = 60%; (5) 50% cell concentration = 70%; and (6) 100% cells = 100%.

A group of 50 female *G. p. palpalis* was maintained on each of these six diets. The experiment was carried out in two replicates and the flies were observed for 35 days, when the survival rate, productivity and mean puparial weight of the flies were measured.

## 3. RESULTS

In the first experiment, the haematocrit values, haemoglobin and total serum protein of the anaemic bovine blood were 18%, 6.4 g/100 mL and 7.2 g/100 mL, respectively, compared with 35%, 9.5 g/100 mL and 8.4 g/100 mL, respectively, for the control blood. The performance of the test and control flies is summarized in Table I. The mean puparial weight of the offspring of the control flies was significantly greater than that of the test groups.

TABLE I. PERFORMANCE OF TEST AND CONTROL FLIES

| Fly performance parameters | Sterile anaemic bovine blood | Pooled abattoir bovine blood (control) |
|---|---|---|
| Pupae per initial female (PPF) at day 25 | 0.63 | 0.7 |
| Mean puparial weight (mg) | 25.8[a] | 27.00[a] |
| Survival rate (%) | 56.7 | 60.0 |
| Insemination rate (%) | 90 | 86.7 |

[a] $P < 0.05$; $t = 2.22$.

TABLE II. RESULTS OF IN VITRO FEEDING OF FEMALE *G. p. palpalis* ON CAMEL AND BOVINE BLOOD

| | Camel blood (mean ± SD) | Bovine blood (mean ± SD) |
|---|---|---|
| Survival rate (%) | 86.5 ± 19.1 | 88 ± 11 |
| Puparia per initial female | 0.67 ± 0.52 | 0.65 ± 0.11 |
| Mean puparial weight (mg) | 25.96 ± 0.85 | 27.88 ± 0.65[a] |
| Productivity value | 0.590 ± 0.14 | 0.585 ± 0.11 |

[a] Significantly different ($P < 0.05$).

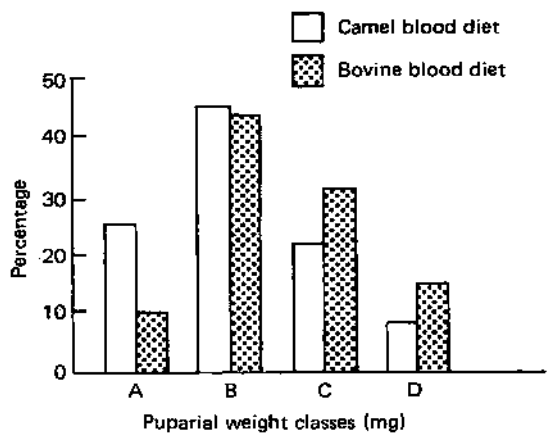


*FIG. 1. Distribution of* G. p. palpalis *puparia into various weight classes. The mean weights of the different puparial classes are: A = 20.05 mg; B = 25.59 mg; C = 30.54 mg; D = 34.24 mg.*

In the second experiment, the percentage survival rates of the flies fed in vitro on bovine and camel blood (86.60 and 88.00%) were not significantly different (Table II). However, the mean puparial weight of females flies fed in vitro on bovine blood was significantly greater ($P < 0.05$) than those fed on camel blood (Table II). The distribution of puparia into the various weight classes is shown in Fig. 1. The flies fed on bovine blood produced more puparia of greater weight (classes C and D) than those fed on camel blood, which produced more puparia of lower weight (classes A and B).

The results of a chemical analysis of bovine and camel blood are shown in Table III. The results of the third experiment are summarized in Table IV.

TABLE III. ANALYSIS OF THE BLOOD COMPONENTS OF CAMEL AND BOVINE BLOOD COLLECTED FROM THE JOS ABATTOIR

| | Camel blood (mean ± SD) | Coefficient of variation (%) | Bovine blood (mean ± SD) | Coefficient of variation (%) |
|---|---|---|---|---|
| Haemoglobin | 7.14 ± 4.44/100 mL | 62.2 | 8.7 ± 3.07/100 mL | 35.29 |
| Total white blood cells | 9021.43 ± 2009.7 | 28.93 | 9800 ± 1137.32 | 1.19 |
| Packed cell volume | 17.67 ± 8.3% | 46.97 | 30.5 ± 8.19% | 26.9 |
| Lymphocyte | 45.75 ± 5.56 | 12.15 | 43.75 ± 23.34 | 53.35 |
| Monocyte | 1.25 ± 1.5 | 120 | 2.5 ± 13.3 | 25.8 |
| Neutrophyl | 48.5 ± 6.055 | 12.5 | 51.5 ± 13.3 | 25.8 |
| Basophyl | ($n$ = 7) | — | ($n$ = 7) | — |
| Eosinophyl | 2 + 73 | 86.00 | 2.25 ± 2.74 | 76.00 |
| Sugar (glucose) | 100 mg/dL | — | 80 mg/dL | — |

TABLE IV. RESULTS OF IN VITRO FEEDING OF *G. p. palpalis* ON SIX DIFFERENT BLOOD DIETS

| Fly group | Blood diet (PCV %) | Survival rate at day 35 (%) | Productivity (PPF) | Mean puparial weight (mg) |
|---|---|---|---|---|
| A | 0 | 38.33 | 0.22 | 23.07 |
| B | 20 | 86.67 | 1.40 | 29.22 |
| C | 36 | 83.33 | 1.42 | 27.81 |
| D | 60 | 88.33 | 1.15 | 27.81 |
| E | 70 | 80.0 | 0.61 | 27.72 |
| F | 100 | 43.33 | 0.31 | 24.63 |

## 4. DISCUSSION

The findings of the study suggest that anaemic protein deficient blood, if fed as diet, significantly affects the mean puparial weight of *G. p. palpalis*. This observation is in agreement with a previous report [6], which stated that reducing the number of erythrocytes leads to a decrease in offspring size in *Glossina morsitans morsitans*.

An artificial diet consisting of two proteins, haemoglobin and albumin, together with their associated lipids, has been shown to support reproduction in *G. p. palpalis* and *S. calcitrans* [4]. Flies fed on serum alone could not obtain enough nutritients to support normal larval growth. Addition of even delipidated haemoglobin to serum supported both ovarian growth and larval development in *G. p. palpalis*. In the present study it has been observed that flies fed on serum alone performed very poorly in terms of survival rate, productivity and mean puparial weight (Figs 2–4).

Recent tests carried out with lipid free haemoglobin (LFHb) demonstrated the importance of erythrocyte lipids in the nutrition of tsetse and stable flies. When flies were fed on LFHb and albumin in place of lipid contaminated haemoglobin and albumin, fecundity was markedly reduced [4].

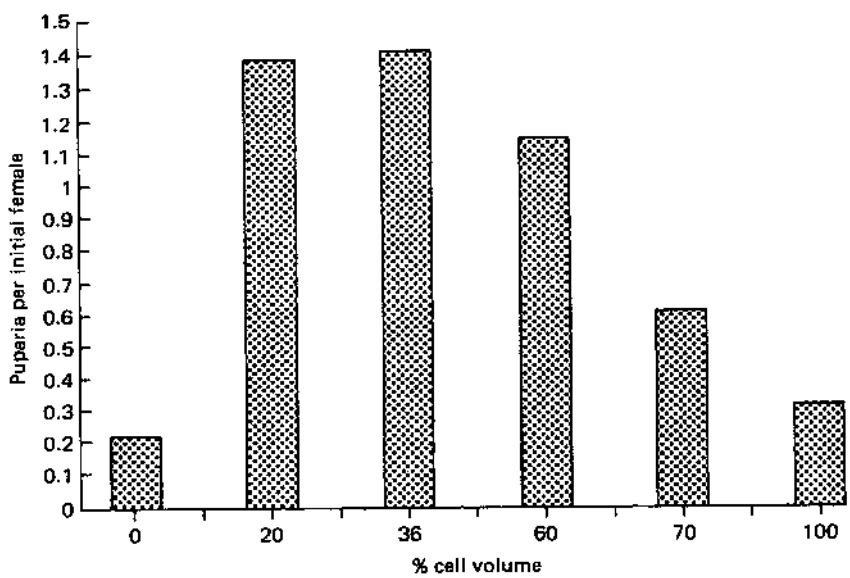


*FIG. 2. Results of in vitro feeding of* G. p. palpalis *(PPF) on six different cellular and plasma dilutions.*

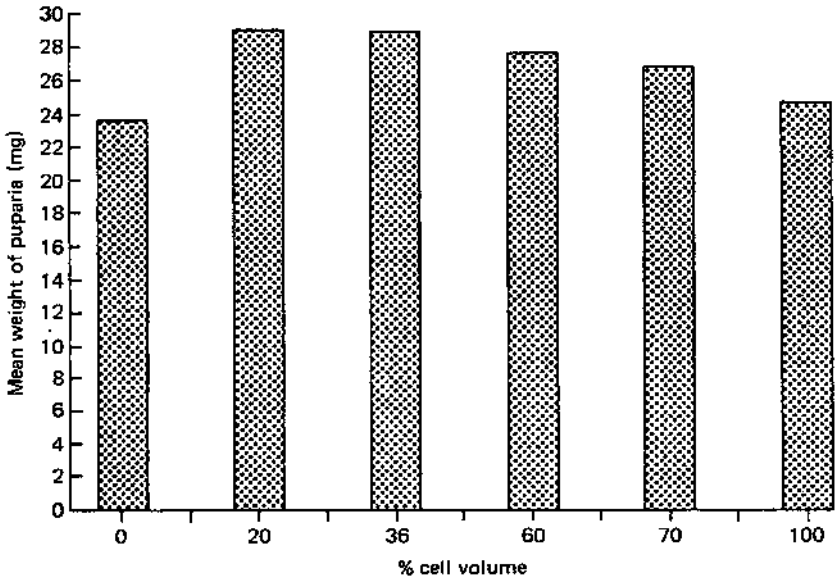


*FIG. 3. Results of in vitro feeding of* G. p. palpalis *(puparial weight) on six different cellular and plasma dilutions.*

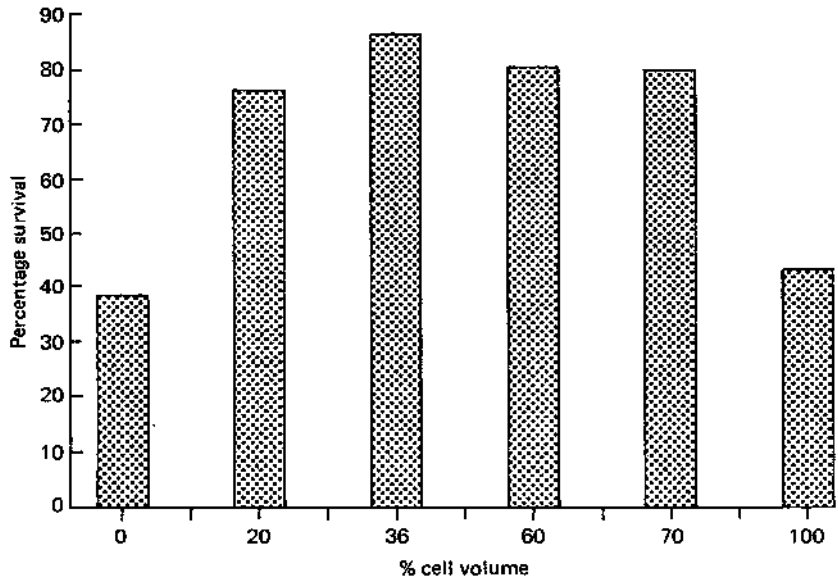


*FIG. 4. Results of in vitro feeding of* G. p. palpalis *(% survival) on six different cellular and plasma dilutions.*

A diet consisting of albumin plus its associated lipids has been found to support reproduction in *G. p. palpalis*, *G. austeni* and *G. m. morsitans* when combined with a suitable salt solution and commercially available or laboratory prepared haemylobin [6].

The in vitro feeding system, although more simple than the in vivo feeding system, could be deficient if certain components of the blood diet such as serum albumin, haemoglobin or the total packed cell volume are low or lacking [4]. The protein content of the blood is utilized by the female fly for the nutrition of intra-uterine larvae. Female tsetse flies do not store protein for larval growth, but it is derived from bloodmeals ingested later in the adult stage [7].

The frequency of feeding, the timing and the size of bloodmeals as well as their composition are all critical for normal reproduction to occur in tsetse flies at the optimal rate [6].

The findings of these experiments suggest that the cellular and plasma components of the blood diet are essential for the normal reproduction and performance of tsetse flies.

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# MYCETOMES AND SYMBIONTS OF TSETSE FLIES MAINTAINED ON A MEMBRANE FEEDING SYSTEM AND THE AGENTS INTERFERING WITH NATURAL REPRODUCTION*

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## Abstract

MYCETOMES AND SYMBIONTS OF TSETSE FLIES MAINTAINED ON A MEMBRANE FEEDING SYSTEM AND THE AGENTS INTERFERING WITH NATURAL REPRODUCTION.

Mycetomes from male and female adults and puparia of *Glossina palpalis palpalis* were dissected from specimens obtained from the IAEA Laboratory at Seibersdorf, Austria. The structure and ultrastructure of the mycetocytes and endosymbionts and their quantitative changes are described and compared with other species of tsetse fly. Pronounced degenerative morphological changes can be caused by, among other factors, starvation. Irradiation by gamma rays produced only slight structural changes and non-significant changes in the endosymbiont number. In organ culture (Leibowitz medium), mycetomes of unfed females release spherical clusters of mycetocytes and lose their dense cover of microvilli. The ultrastructural changes of individual endosymbionts under these conditions are described in detail. The results of basic bioassays showing intensive DNA synthesis and in vitro production of several de novo synthesized proteins of molecular weights of 52–159 kilodalton are given. The possible role of endosymbionts in reproduction and their transmission to the offspring are discussed. While the necessity of a functional mycetome in females can, at least partially, be explained by the production of proteins necessary for reproduction, the role of the mycetome in males remains unclear. The function of the tsetse fly mycetome is shown to be much more complex than earlier supposed and further detailed data on its reproductive function are needed.

## 1. INTRODUCTION

The existence of an intimate relationship between numerous insect species and large numbers of intracellular microorganism like particles has been known for over a century. Basic knowledge and a summary of light microscopical observation of the

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pioneering workers of the 19th and early 20th Centuries [1] on tsetse bacterioid like endosymbionts were presented by Buchner [2] in his extensive monograph.

Since then, numerous studies on the biochemical aspects of the production of some 'essential' substances by mycetomes in vitro and in vivo, the production of aposymbiotic (bacterioid free) flies by antibiotics as well as the ultrastructural changes caused by several physico-chemical factors have been published. Since aposymbiotic tsetse fly females show substantial disorders in their reproduction and fecundity, there is an urgent need to determine the nutritional contribution made by symbiotic bacterioids [3]. However, over the past 20 years knowledge of the true function and especially of the role of the mycetome in tsetse reproduction and gonadal development has not progressed beyond the initial descriptive phase.

The present research project has been carried out since 1 October 1984. This study concentrates mainly on the following: (1) the structural/ultrastructural changes in the mycetome in relation to the age and hunger cycles; (2) the effects of some abiotic factors, especially irradiation; (3) the possibilities of in vitro culture; (4) the basic biochemical characteristics, and (5) the role of the mycetome and its products in reproduction.

## 2. MATERIALS AND METHODS

Adult tsetse flies, *Glossina palpalis palpalis*, of Nigerian origin obtained as puparia from the stock colony of the FAO/IAEA Entomology Unit of the IAEA Laboratory at Seibersdorf, Austria, were maintained under standard conditions [4]. Adults were fed on rabbits 6 times a week; teneral, unfed flies were also used.

Seven day old males, females and puparia were irradiated in air with 14 krad gamma rays from a $^{60}Co$ source at Seibersdorf.[1] A few *G. morsitans*, *G. brevipalpis* and *G. tachinoides* were also studied.

For structural studies and bioassays, the mycetomes were dissected using the technique described by Nogge and Ritz [5]. The ultrastructure of the mycetocytes was studied on both transmission electron microscope and scanning electron microscope preparations. The dissected mycetomes were fixed with 2.5% glutaraldehyde for 20–26 hours at 4°C followed by 1% osmium tetroxide post-fixation (both buffered to pH 7.0 with 0.1M cacodylate buffer); after dehydration they were embedded in Epon Araldite resin. Semi-thin and ultra-thin sections were made using a Reichert Ultracut ultramicrotome. The resin was removed (for details, see Soldan et al. [6]) and the mycetomes were gold coated; they were then observed and photographed under a Philips EM 420 electron microscope and a scanning electron microscope (Texla BS 300) at 15 kV.

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[1] 1 rad = $1.00 \times 10^{-2}$ Gy.

The mycetomes designated for the study of in vitro synthesis of DNA and proteins were superficially sterilized with a solution of antibiotics and incubated in human serum with either 10 $\mu$Ci $^{3}$H-thymidine or 10 $\mu$L of $^{14}$C-leucine (4 MBq/mL). The radioactivity was measured with a Beckmann LS 7000 scintillation spectrophotometer. One dimensional SDS electrophoresis was conducted in 8% running polyacrylamide gel with 2.5% large pore gel on 120 × 120 × 1 mm slabs. Gels were stained with Coomassie brilliant blue R-250 and counterstained with silver. Fluorography was conducted according to the Bonner and Laskey method, and the molecular weight of the proteins was identified according to high and low molecular calibration kits (for details, see Matha et al. [7]).

To establish the organ cultures, the mycetomes were incubated for 5 min in sterile physiological saline, rinsed several times and placed in Leibowitz (L-15) tissue culture medium containing 4% (wt/vol.) tryptose phosphate (10%), foetal serum (20%) and antibiotics (100 IU/mL penicillin and 1 $\mu$g/mL streptomycin). For preparation of the conditioned medium, the mycetomes were carefully cut into pieces and cultivated in a medium with the normal amount of antibiotics, or in a medium which was free of any antibiotics in normal atmosphere (without enrichment of the carbon dioxide or oxygen) at 25 ± 1°C for 1–2 weeks. The developing cultures and individual cells were examined microscopically every 12 hours, and on smears [7, 8].

## 3. RESULTS

### 3.1. Structure and ultrastructure

The mycetome is an elongated organ measuring between 2.4 and 4.5 mm in length and consisting of hypertrophied midgut epithelium cells harbouring endosymbionts — mycetocytes. Mycetocytes are arranged in two longitudinal strips fused together at least at one end and situated in the anterior parts of the midgut. In cross-section, one can see that the double bulge of hypertrophied cells sometimes almost fills up the gut lumen.

Mycetocytes have apparently lost their digestive function, although their inner surface is densely covered with microvilli like other functional epithelial cells. Mycetocytes are cylindrical cells measuring about 50–60 $\mu$m (the nuclei of neighbouring gut cells measure 8–9 $\mu$m in diameter). The nuclei of the mycetocytes are characterized by the absence of a 'nuclear coat'. The nucleolus is clearly visible in the nucleoplasm which contains heterochromatin [4].

Mycetocyte cytoplasm is flocculent and a fairly large number of mitochondria are present. Vesicles, microtubules and free ribosomes are relatively rare and the electron density of cytoplasm in the mycetocytes is low compared with that of normal cytoplasm.

In addition to rare microorganelles, the cytoplasm is filled with rod like endosymbiotic gram negative bacterioids up to 8 μm (usually 4–6 μm) in length and 1.5 μm (usually 1.0–1.4 μm) in width. The bacterial cytoplasm contains ribosome like granules and diffuse filamentous structures which most probably represent the microorganism's DNA. The plasma membrane consists of an outer unit membrane of about 70–90 Å and a poorly contrasted intermediate layer (about 120–160 Å) forming the complete wall of a total thickness of 250–300 Å [9].[2] Microorganisms are oriented in the direction of the longitudinal axis of the mycetocyte, with practically no space between them; the distance between the adjacent endosymbionts is not larger than 110–140 Å [6].

There are no structural differences between the endosymbionts of *G. p. palpalis* and the other species studied, *G. morsitans*, *G. fuscipes* and *G. brevipalpis* [9]. The structure of the mycetome and endosymbionts in *G. tachinoides* seems to be identical.

### 3.2. Structural and quantitative changes

Knowledge of the factors which actually exercise the regulatory function of the number of mycetocyte endosymbionts is very limited. When cultured in vitro, bacterioids show very limited growth in a cell free artificial medium [10]. However, apparent growth occurs in vivo in mycetocytes, especially in larvae from instar 1 [11]. The total number of endosymbionts in the mycetome of teneral *G. m. morsitans* was determined as $(5.86 \pm 1.46) \times 10^5$ in n = 10 flies [5]. The natural changes in endosymbiont number, detectable as changes in the length and dry weight of the mycetome depending on the age and sex of the flies, are described in Ref. [5]. In males it was observed that the mycetome size increased up to day 14 after emergence and then decreased to 1/3–1/4 of the original size in senescent flies. In females, the mycetome volume increases slightly after day 14.

The actual number of endosymbionts is supposed to be regulated by lysozyme, as in cockroaches and aphids. The evidence supporting this type of regulation can be found in the absence of lysozyme at the places bearing mycetocytes, although this protective anti-microorganism factor is present throughout the midgut. However, the mechanism enabling lysozyme access to the endosymbionts remains unknown. Lysozyme itself, when administered by both injection and orally in high concentrations, penetrates the mycetome to destroy the endosymbionts. Principally, there is probably no difference between the decomposition of foreign bacteria and endosymbionts, since both are broken down by lysozyme [5, 12].

Two authors [1, 11] have observed mycetomes bursting and releasing their contents into the gut lumen. As a rule this phenomenon does not occur. Neither Nogge and Ritz [5] studying senescent changes, nor Soldan et al. [6] studying

[2] 1 Å = $10^{-8}$ cm.

changes during starvation, have observed this bursting. Also, hormonal regulation of the mycetome bacterioid number most probably does not take place. In our preliminary experiments ligatured flies (ligature between the thorax and the abdomen) can easily survive 5–6 days without any substantial structural changes in the mycetome.

Our knowledge of the artificial factors affecting the number and structure of endosymbionts and mycetocytes is relatively extensive. Of the physical factors, mycetomes can be destroyed by keeping the flies at a high temperature (35°C) or at a low temperature (below 8°C). We observed some structural changes in flies irradiated with gamma rays, although they were not as pronounced as, for example, those observed after antibiotic treatment. Mycetomes are also affected by specific antibodies against endosymbionts induced in the blood of rabbits [13]. Endosymbionts were experimentally eliminated by various antibiotics (e.g. penicillin, streptomycin, kanamycin, oxytetracycline, chlortetracycline, chloramphenicol, neomycin, gentamycin, etc.) administered in the bloodmeals of flies at concentrations of 0.05–0.1% (chlortetracycline, chloramphenicol) [12, 14, 15]. The same effects were obtained after treatment with sulphonamides such as coccidiostat sulphaquinoxaline [16] or sulphanilamide [12]. However, treatment with antibiotics needs more detailed study, since the flies maintained in stock colonies are currently fed with blood treated with various antibiotics against foreign microorganisms; this treatment has no effect on the endosymbionts. Similarly, mycetomes in organ cultures have been treated repeatedly with antibiotics without any substantial effect on their structure or survival.

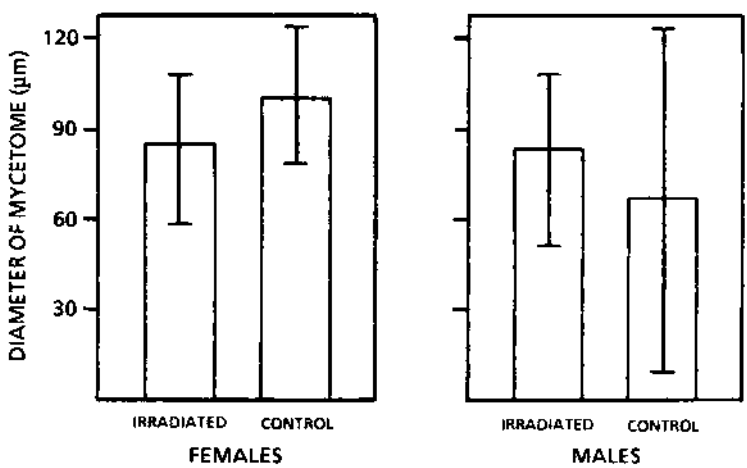


*FIG. 1. Quantitative (diameter) differences in the mycetomes of irradiated and control males and females of different age.*

After antibiotic treatment, the following structural changes take place. One day after application the endosymbionts expand slightly and on subsequent days their cytoplasm disappears from the central area and shrinks to form electron dense chumps attached to envelopes [17]. Elimination of the endosymbionts seems to be dose dependent and time of application dependent, e.g. the later treatment with penicillin or kanamycin begins the smaller the damage to the endosymbionts [12].

Spontaneous destruction of the mycetocytes during starvation differs slightly, since about 1/3–1/2 of the endosymbionts remain unaffected even in 8–9 day old females; in this case, pronounced lysis of the mycetocyte nuclei occurs [6].

Gamma irradiation evidently exerts no quantitative effect on the mycetocytes or the number of endosymbionts. The diameter of the mycetomes measured on semithin sections of 3, 6, 10, 14, 18 and 22 day old teneral males and females of both irradiated and control specimens showed non-significant differences. The cumulative values of 38 irradiated and 56 control specimens are shown in Fig. 1.

## 4. FUNCTIONAL ASPECTS

It is generally thought that the mycetocytes produce vitamins which are not present in vertebrate blood. In *G. m. morsitans* it has been observed that the following vitamins are produced by the endosymbionts: thiamine pantothenic acid, pyridoxine, biotin, folic acid and p-aminobenzoic acid. There is no evidence of the production of riboflavin and nicotinic acid [13, 15]. The ingested blood supplies the demands of the tsetse fly probably for thiamine and biotin, but it is apparently deficient in pantothenic acid, pyridoxine and folic acid. The amount of vitamins needed to produce one 30 mg larva is about 0.018 $\mu$g thiamine, 0.135 $\mu$g pantothenic acid, 0.021 $\mu$g pyridoxine, 0.0015 $\mu$g biotin and 0.018 $\mu$g folic acid, but the actual amount of these vitamins in 200 $\mu$L of blood (amount ingested per larval cycle) is 0.024, 0.060, 0.020, 0.0024 and 0.007 $\mu$g, respectively [13].

Bacterioids are microorganisms using mainly pyruvate and succinate (replaceable by malate) as the main metabolic substrate, but their growth and also their limited motility show the importance of nucleotides, adenosine triphosphate in particular [10]. Laboratory bioassay of $^{3}$H-thymidine [7] showed in vitro that endosymbionts can synthesize DNA as well. The radioactivity measured after 15, 30, 60, 120 and 150 min showed that $^{3}$H-thymidine incorporation into the acid stable fractions of endosymbionts exhibited the highest rate during the first 20 min incubation in human serum. The intensity of incorporation then gradually decreased and ceased between 40 and 60 min (Fig. 2).

Parallel to the study of $^{3}$H-thymidine incorporation, changes in the proteins in mycetome homogenates were followed using $^{14}$C labelled leucine and showed that there are large scale protein fractions, eight detectable by fluorography (Fig. 3). The

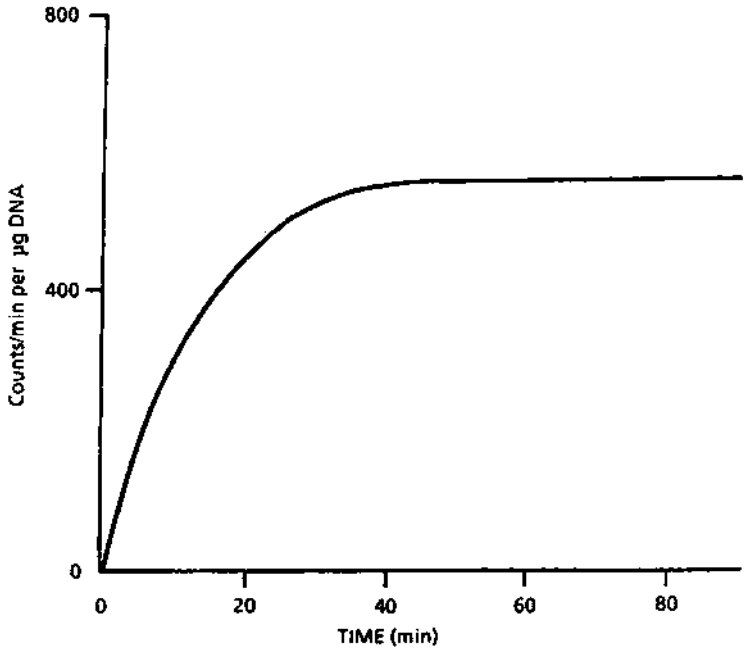


*FIG. 2. Incorporation of $^{3}H$-thymidine into the acid stable fractions of endosymbionts.*

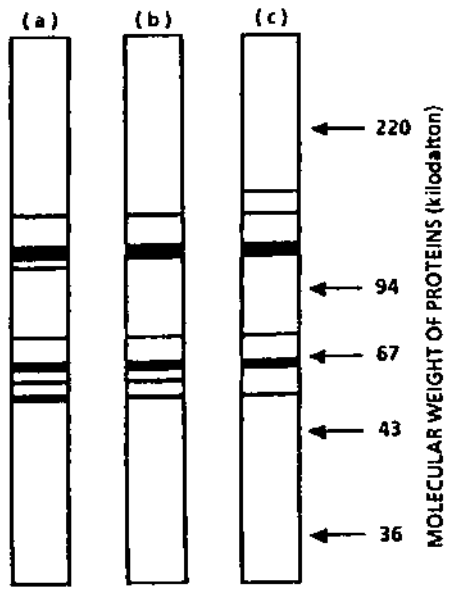


*FIG. 3. Fluorographic positive protein fractions from endosymbionts incubated for: (a) 30 min, (b) 60 min, and (c) 90 min in medium containing $^{14}C$-leucine.*

molecular weights of these fractions were estimated as 150, 130, 110, 100, 70, 60, 57 and 52 kilodaltons [7].[3]

Besides the discovery of symbionin produced de novo in aphid mycetocytes [18], this is the only evidence of protein synthesis in insect mycetomes. Most probably de novo synthesized proteins represent other essential substances which have previously been ignored. However, the production of proteins should be seen in vivo as well. For instance, Ref. [19] showed that in aphids several proteins are produced in vitro, while in vivo symbionin is the only protein synthesized.

Although the role of the mycetome in the development of trypanosomes in tsetse midgut was not investigated in detail, there are some signs of a positive relationship between these flagellates and the mycetome tissues. Compared with some other pathogens, trypanosomes require less energy for their development and, consequently, cause less destruction. There is microscopical evidence of *Trypanosoma brucei* in the connective tissue encasing the mycetome of *Glossina* flies — some chemotropism may lead the flagellates into tissue which is normally not invaded [20].

### 4.1. Organ cultures

Tsetse mycetome cultures showed that this organ can easily be maintained for at least 2 weeks [21]. Some mycetocytes changed their shape from elongated to spherical and migrated individually or in clusters into the medium, while others remained compact in mycetomes in the gut tissue. The free clusters of mycetocytes resembled the 'symbiont balls' found near the posterior egg pole of some Homoptera [22]. These mycetocytes probably do not change their metabolic activity, which is similar to their homogenates incubated in vitro [7].

The results obtained on the behaviour of cultured cells are very similar to those described by Trager [23, 24], who kept small anterior segments of midgut for 3–4 weeks. Ultrastructural studies showed some degenerative changes in the symbionts, but they remained living for a minimum of 2 weeks. The most characteristic ultrastructural feature of symbionts from cultivated mycetomes was the presence of rod like structures composed of plate like subunits. These structures resembled some enzyme or other protein present as crystalline inclusions. One of their possible functions may be as reserve proteins, although this question requires further investigation.

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[3] 1 dalton = 1 u (unified atomic mass unit)
$\simeq 1.66 \times 10^{-27}$ kg.

## 5. DISCUSSION

The important question is if endosymbionts from the adult tsetse fly are able to divide and grow. Nogge and Ritz [5] assumed that symbionts, once they reach their ultimate number in adult fly mycetome, lose their ability to divide quite independently of host influence. Wink [10] observed the limited division and growth of tsetse endosymbionts within the first 3 weeks. After elongation from 8–24 $\mu$m, the endosymbionts divided into single units of 5 $\mu$m length, but no further growth was observed. Analysis of the diameter of symbionts after 2 weeks of cultivation in the present work showed similar results. The average diameter, which is comparatively smaller than the diameter of symbionts from a normal mycetome, revealed wide variation. It may also indicate that a limited number of cellular divisions take place, although there is no direct evidence for this at present. Welburn and Maudlin [25] described divisions of rickettsia like organisms from *Glossina* spp. during in vitro cultivation and it is possible that they may have observed the dividing organisms in their preparation.

On the basis of our results we conclude that mycetocytes can easily be maintained as a primary cell culture for a period of 2–3 weeks. Establishment of long term cell cultures and isolation of cell sublines has not been possible. The tsetse mycetome organ culture described here represents the first basic step in this area. It should provide the necessary means for conducting studies of: (1) the influence of conditioned medium, i.e. containing products of symbionts, on various organs of the tsetse fly; (2) the influence of various compounds added to the cultivation medium on mycetocytes and symbionts, and (3) the influence of induced antibodies added to the cultivation medium on mycetocytes and symbionts.

It is generally well known that aposymbiotic (symbiont free) female tsetse flies usually fail to reproduce [12–14, 16]. High doses of antibiotics such as streptomycin, chloramphenicol or polymixin cause strong morphological disturbances of the endosymbiont after 7 days, accompanied by complete sterility in several weeks. Hill et al. [14] observed the degeneration of egg follicles and germaria within the ovaries in this case. If the ovaries remained morphologically unaffected, the females suffered a series of abortions.

In using high temperature to destroy the mycetomes we observed at least partial sterility and production of abnormal embryos, the development of which ceased during segmentation, or earlier. Malformed embryos (extra embryonal yolk, imperfect dorsal closure, etc.) closely resembled those obtained after copulation with males sterilized by gamma irradiation [26].

On the other hand, loss of endosymbionts does not affect the longevity of flies, as was shown by Nogge and Gerresheim [12] after application of tetracycline, penicillin, kanamycin and lysozyme. However, other antibiotics, e.g. chloramphenicol, rifampicine and streptomycin, do affect the longevity of flies. Small doses

of these antibiotics damaged the symbionts to the extent that flies could produce offspring, but these were symbiont free (and most probably sterile).

It is not known which substance produced by the mycetome is responsible for female fertility. Pell and Southern [16] suggest that folic acid, a precursor for both thymine and purines, is the critical factor. They showed that folic acid is also produced by rickettsia like ovarian symbionts which can, provided they are very abundant, substitute for bacterioid born folic acid from the mycetome and maintain normal fertility. The first sign of sterility attributed to the lack of folic acid is the DNA degeneration apparent in the nurse cell of follicles.

Nogge [13] investigated the effect of omitting 'essential' substances on the production of puparia in *G. morsitans*. Total sterility was achieved using a diet without thiamine, then folic acid, pyridoxine, pantothenic acid, biotin (about 0.1–0.2 puparia per female), and inosit, choline and riboflavin (about 0.3 puparia per female). Surprisingly, the fecundity of flies fed on diets without p-aminobenzoic acid and nicotinic acid (substances probably not produced by tsetse bacterioids) was even higher (0.5–0.6 puparia per female) than that of flies fed on normal, vitamin supplemented blood (about 0.4 puparia per female).

A new opinion on 'essential' substances of microbial origin was introduced by Ishikawa [20], who first found the essential protein in aphids — symbionin. Symbionin is necessary not only for the development of gonads, but also for embryogenesis in parthenogenetic females. However, the function of proteins produced by tsetse fly mycetomes in vitro [8] remains unknown, although their key role in reproduction cannot be excluded.

The role of the mycetome in male tsetse flies remains unclear. There is no doubt that tsetse endosymbionts, unlike those in lice or some nycteribiids, are maintained in the male throughout life, although their number gradually reduces [5]. The fertility of aposymbiotic males has not yet been studied. At least partial sterility of males with damaged mycetomes is very likely and we have observed morphological changes in the mycetomes of sterile, gamma irradiated males. However, the relationship between damaged bacterioids and (damaged?) germ cells is unknown.

Transmission of endosymbionts is not well understood. There is morphological evidence that the eggs are bacterioid free, while bacterioids are harboured by some of the gut cells in larvae from instar 2; also, free endosymbionts are common in gut contents [11]. Bacterioids are transmitted to the offspring via the accessory ('milk') glands of females, where they can be found in gland ducts [1]. It is unclear how they get to the milk glands from the midgut cells (by bursting of mycetomes?). Are endosymbionts in the haemolymph destroyed by host immune reactions? From the purely morphological point of view, there is no further connection between mid-gut and accessory glands, except perhaps through openings in the alimentary tract and gonads.

## 6. CONCLUSIONS

(1) The structure and ultrastructure of *G. p. palpalis* mycetomes, mycetocytes and endosymbionts are described.
(2) Starvation caused progressive degenerative mycetome changes such as in some chemotherapeutics, the extralimital temperatures and immunization.
(3) There are no quantitative and only slight structural changes in the mycetomes of gamma ray irradiated male and female puparia and adults. Irradiation sterility is, therefore, not due to mycetome destruction.
(4) Intensive in vitro DNA synthesis in mycetomes was shown for the first time. In addition to vitamins, mycetomes produce de novo synthesized protein with a molecular weight of 52–159 kilodalton.
(5) The mycetome organ culture was developed as a basis for study of the effect of artificial diets on mycetomes and/or reproduction.
(6) The behaviour and ultrastructural changes of mycetocytes and endosymbionts during the conditioning of organ culture media are described.

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# VIRUS PARTICLES INFECTION IN LABORATORY REARED *Glossina pallidipes* Austen (DIPTERA: GLOSSINIDAE)*

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**Abstract**

VIRUS PARTICLES INFECTION IN LABORATORY REARED *Glossina pallidipes* Austen (DIPTERA: GLOSSINIDAE).

The performance of a self-supporting colony of *Glossina pallidipes* reared under semi-natural conditions at Mbita Point, Kenya, suffers from virus particles infection. *Proteus rettgeri* causes high mortality ('black abdomen') and virus particles infection finds its expression in enlarged salivary glands and sterility.

## 1. INTRODUCTION

Since the pioneering work done by Nash et al. [1] in the early 1960s, a number of *Glossina* species has successfully been reared under laboratory conditions. It is, however, only recently that *Glossina pallidipes* has successfully been reared under controlled laboratory conditions [2]. Otieno et al. [3], on the other hand, have successfully reared a self-supporting colony of *G. pallidipes* under semi-natural conditions. Ochieng et al. [4] have used this approach to mass rear *G. pallidipes*. We took advantage of the simple rearing method to examine whether a laboratory colony of *G. pallidipes* was infected with virus particles and, if so, how the virus affected the colony's growth. Field collected samples have also been found to be infected with virus particles [5]. Data showing that virus particles may affect the performance of laboratory reared *G. pallidipes* are given.

* Research carried out with the support of the IAEA under Research Contract No. 3418/RB.

## 2. MATERIALS AND METHODS

The *G. pallidipes* used in the study were originally collected from the bush around Ruma thicket in the Lambwe Valley, western Kenya. They were maintained in the simple insectary described by Ochieng et al. in Ref. [4]. This insectary provided semi-natural conditions for the flies. The temperature and humidity in the insectary were not regulated so that the environmental conditions and fluctuations would correspond closely to those outside it. The flies were fed on rabbit ears. For routine mating, 9 day old females were mated with 12 day old males in one polyvinyl cage. The ratio of females to males was 3:1.

### 2.1. Records

The recording system used to maintain the colony was adopted from that developed in the IAEA Laboratory at Seibersdorf. The number of puparia produced was recorded daily. After placing the puparia in emergence cages, the number of females, producing females, puparia produced and dead flies was recorded daily. Enlarged salivary glands in *G. pallidipes* have been associated with virus particles infection [6]. Our records also show that when examined under electron microscopy all the flies with enlarged salivary glands have virus particles. We therefore considered flies with such glands to be infected with the virus. A sample of flies was taken from the colony daily. These flies were dissected and their salivary glands examined for hypertrophy. The number of flies in each sample with enlarged salivary glands was recorded.

## 3. RESULTS

The performance of the colony was considered with particular reference to the number of producing females, puparia produced, daily mortality and flies infected with virus particles. The percentage of producing females fluctuated between 70 and 83%; the lowest percentage was recorded in June (Fig. 1(a)). The fecundity of these flies was, however, highest between May and July, after which there was a decline that continued for the rest of the year (Fig. 1(b)). It is interesting to note that there was an inverse relationship between the number of producing females and the number of puparia produced per female (Fig. 2).

The decline perceived at the beginning of these observations coincided with an increased number of deaths. Each of the dead flies had a fully distended black abdomen, suggesting that most of them had failed to digest their bloodmeal. When the gut contents were cultured in nutrient agar plates, pure cultures of bacteria were isolated. The bacteria were later identified as *Proteus rettgeri*.

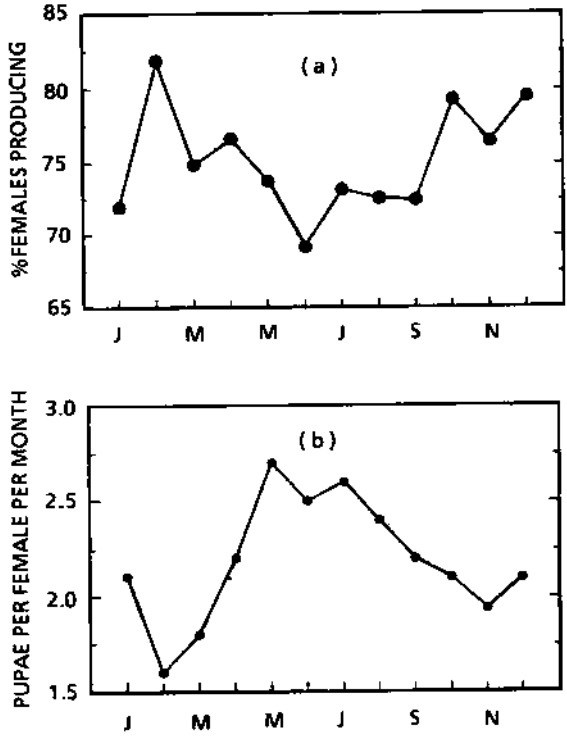


*FIG. 1. Percentage producing females (a) and puparia production (b) of* G. pallidipes *reared under semi-natural conditions at the Mbita Point field station during 1987.*

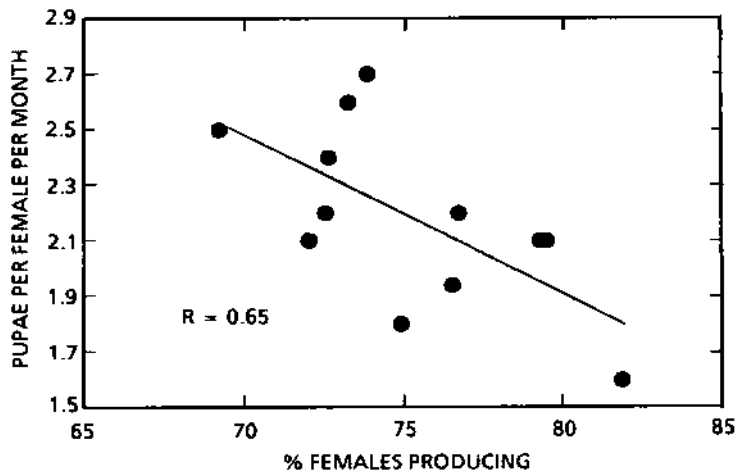


*FIG. 2. Relationship between puparia production and the percentage of producing* G. pallidipes *reared under natural conditions.*

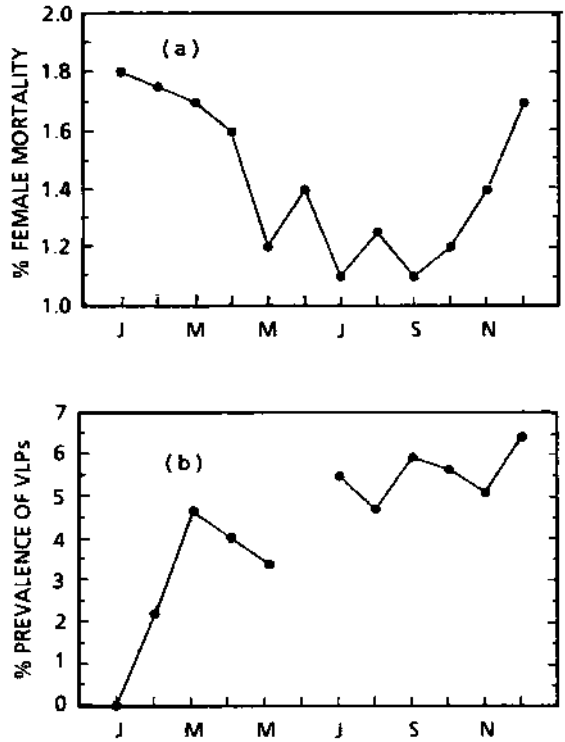


*FIG. 3. Percentage daily mortality (a) and prevalence (b) of* G. pallidipes *reared under semi-natural conditions at the Mbita Point field station.*

Mortality was high when the observations started (Fig. 3(a)), but it then dropped and remained stable for 6 months. Fecundity was highest in these 6 months. There was a sharp increased in mortality from October, an increase which appears to have affected the number of females producing, which in turn affected the colony size.

The prevalence of virus infections in the colony is recorded in Fig. 3(b). The number of flies with virus infections increased quite rapidly (0–4%) between January and March, but slowed down thereafter for the rest of the year. From March to December there was an increase of about 2% in the number of flies affected.

## 4. DISCUSSION

Because the flies used in the study were reared under semi-natural conditions, and in a climate that closely corresponded to that of the field around the insectary,

the fluctuations in the number of producing females observed in the colony over the year may be quite normal. Indeed, Harley had earlier observed (quoted in Ref. [7]) that there was a seasonal production of *G. pallidipes* puparia from Lugala in the Busoga district, southeastern Uganda. He found that the lowest puparia production occurred during the dry season, with the lowest produced during February, the driest month of the year. This February dry spell also affects Mbita Point, south Nyanza, Kenya, where the work was done.

There was an enhanced correlation between the number of producing females and the number of puparia produced per female. A possible explanation is that when a high number of females is producing some may not have started their larviposition, whereas when few females are producing most, if not all, have started their larviposition. This explanation accounts for the apparent high fecundity when female numbers are low and vice versa.

The presence of enlarged salivary glands in field populations of *G. pallidipes* was first noted by Whitnall [8]. More recent studies by Jaenson [6] have shown that the hypertrophy and hyperplasia seen in *G. pallidipes* are associated with virus infection. He also noted that flies with these conditions had reproductive problems; males were often sterile and the ovarioles of some females showed abnormal growth. Odindo [9] later suggested that a high incidence of virus infection in tsetse flies may adversely affect the reproductive potential of tsetse populations and thus make the virus a good candidate for a biological control agent.

Van Etten [10] conducted a series of experiments aimed at creating a laboratory colony of *G. pallidipes* taken from two areas in Kenya. He had limited success with one strain of *G. pallidipes* and failed with the other. He thought that the genetic diversity of the tsetse fly made it possible to rear flies frm Mwalewa (Kenyan coast) and impossible to rear those from Nguruman (Rift Valley). Recently, however, *G. pallidipes* has successfully been reared under laboratory conditions [2] and Ochieng et al. [4] have since shown that it is possible to rear this fly under semi-natural conditions. The studies reported here were carried out with flies reared under semi-natural conditions. Virus particles were detected in the colony towards the end of 1986; their incidence increased during 1987. After the initial sharp rise in incidence, however, the infection virtually stabilized at 4–6%, which was remarkably slow compared with the earlier part of the year. Admittedly, the sample was small and the conclusions drawn need the support of further studies. There seems to be a self-regulating mechanism within the flies that checks the rate at which the organism spreads within the colony. Indeed, this mechanism must be working in field populations, where the prevalence recorded has been between 0.9 and 5.4%. Flies infected with the virus may die after one or two larvipositions, whereas normal flies live much longer and produce more offspring. The findings by Jaenson [11] lend support to this view. He found that of the flies collected from the Kibwezi Forest, Kenya, most with enlarged salivary glands were younger than those with normal salivary glands. From this observation it was inferred that *G. pallidipes* with

enlarged salivary glands had a shorter life span than normal flies. This may account for the overall increase in the number of producing females, albeit at a slow rate. Studies are in progress to see whether experimentally infected flies live as long as normal flies.

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# INFLUENCE OF DIFFERENT NUTRITIONAL SOURCES ON HAEMOLYMPH COMPOSITION AND VITELLOGENESIS IN HAEMATOPHAGOUS ARTHROPODS*

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## Abstract

INFLUENCE OF DIFFERENT NUTRITIONAL SOURCES ON HAEMOLYMPH COMPOSITION AND VITELLOGENESIS IN HAEMATOPHAGOUS ARTHROPODS.

Two laboratory strains of the argasid tick, *Ornithodoros moubata*, were established by feeding them on pig or bovine blood. After three generations, significant differences were observed between identical stages in the unfed body weight of nymphs 2–4 and the female repletion rates, feeding times, number of eggs laid per female, and the pre-oviposition and oviposition periods. Ticks fed on pig blood showed higher absolute values and shorter time periods for these statistics. Females fed on bovine blood had a greater output of eggs and egg mass per milligram of blood ingested. However, no significant variations were noticed in the weight of eggs and larvae between unfed first nymphs and males. The time required for embryogenesis, larval development and premoulting periods, as well as the sex ratio, did not differ between colonies. Regulation of the haemolymph volume during the gonotrophic cycle followed very similar patterns in the two experimental lines. The female tick is able to maintain a constant ratio of about 22% between the haemolymph volume and the body weight throughout the whole oviposition period. Examination of haemolymph during the gonotrophic cycle, as well as investigation of eggs and larvae, revealed a higher amount of total lipid contents and major lipid classes in ticks raised on pig blood. However, the distribution and utilization of lipid components exhibited common characteristics. From these results it is concluded that the variations observed between the laboratory colonies should not be attributed to qualitative differences between pig and bovine blood, but rather to the absolute quantities of blood ingested. In the haemolymph of female *O. moubata* three host serum proteins (albumin, IgG and transferrin) could be demonstrated immediately after feeding and for 4 additional days thereafter. This passage of undigested bloodmeal components is discussed in relation to the concept of a leaky gut.

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## 1. INTRODUCTION

The reproductive success of blood feeding ectoparasites is determined by the suitability of host blood. Under laboratory conditions the choice of blood type is of immense importance for large scale production of blood feeders, e.g. in the sterile insect technique. Numerous investigations have been carried out to develop in vitro feeding systems and artificial diets for haematophagous arthropods [1–6]. Knowledge of the significance of individual blood constituents is needed in order to compose artificial diets which meet the nutritional requirements of individual arthropods. Lipids are a minor fraction in mammalian blood compared with protein content, but the importance of lipids or specific lipid components as essential factors in the diet of blood sucking arthropods is not fully understood. To obtain a better understanding of the influence exerted by dietary lipid we determined the total lipid content and distribution of the major lipid classes in haemolymph, eggs and larvae during the gonotrophic cycle of the argasid tick, *Ornithodoros moubata*, raised on either pig or bovine blood. Additionally, we recorded, for the two laboratory strains, the unfed body weight, the repletion rate and feeding time of all the developmental stages and the change in haemolymph volume of female ticks during the pre-oviposition and oviposition periods.

There is limited information on the significance of undigested bloodmeal components that may enter the haemoceol of the tsetse fly [7]. Despite the fact that the influence of such metabolites on fly physiology still remains unclear, they might have vital implications for the development of artificial diets. To demonstrate the passage of intact blood substances across the midgut barrier of *O. moubata*, the haemolymph of female ticks was tested for the presence of native host serum proteins.

## 2. MATERIALS AND METHODS

### 2.1. Ticks

The tick colony was maintained in an incubator at 28°C and at 35–45% relative humidity. For all feeding purposes, an in vitro feeding system adapted after Tawfik and Guirgis [8] was used (Fig. 1). Parafilm-M was used as a membrane. Two strains have been established over the past years by feeding strictly either pig or bovine blood. The two blood types were obtained from a local slaughterhouse and defibrinated mechanically by shaking the blood in a flask containing glass beads. All the ticks used in the study had fed for at least three generations on one type of blood. They were used in experiments 4–6 weeks after their last moulting period.

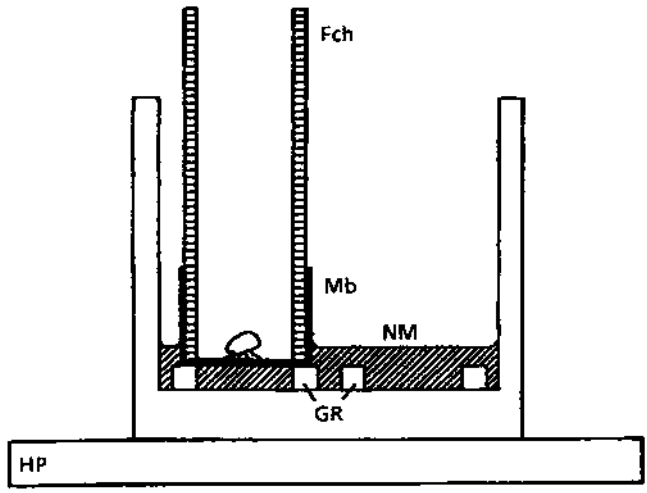


*FIG. 1. Schematic diagram of the in vitro feeding arrangement for* O. moubata. *(Fch = feeding chamber; GR = glass rods; HP = heating plate; Mb = parafilm membrane; NM = nourishment medium).*

## 2.2. Collection of haemolymph and gut contents

To obtain haemolymph and gut contents, the ticks were fixed on to double adhesive paper upside down. After cutting off legs III and IV in the region of the trochanter, the protruding small droplets of haemolymph were collected with a micropipette. By putting slight pressure on the idiosoma, some diverticula of the midgut were forced to stick through the stump of the detached extremities. Contaminating haemolymph was carefully removed from the projecting gut diverticula before the midgut epithelium was sliced. The liberated gut contents were collected with a Pasteur pipette.

## 2.3. Haemolymph volume

To determine the haemolymph volume, the indirect $^{14}$C-inulin dilution method was applied. Ticks were fixed as described above. One microlitre of $^{14}$C-inulin (Amersham and Buchler, with a specific activity of 5.2 mCi/mmol, dissolved in isotonic saline giving an activity of 0.01 mCi/$\mu$L) was injected at the basis of coxa IV into the haemoceol.[1] After an incubation period of 10 min, legs III and

[1] 1 Ci = 3.70 × $10^{10}$ Bq.

IV were amputated and the protruding haemolymph was collected in a calibrated micropipette and mixed with 5 mL scintillation fluid (5.5 g Permablend III in 1 L toluol). The activity was determined with a Packard PLD Tricarb. The haemolymph volume was then calculated on the basis of the measured dilution factor.

### 2.4. Lipid determinations

Eggs were pretreated by washing them several times in chloroform:methanol (2:1; all solvent systems were mixed by volume) to remove the cuticular wax lipids of the egg shell. Fifty microlitres whole pig and bovine blood as well as the gut contents were mixed directly with 3 mL chloroform:methanol (2:1) as well as haemolymph, which had first been centrifuged to remove the cellular components. The eggs, larvae and nymphs were homogenized, then mixed with chloroform:methanol (2:1) and finally sonicated three times for 10 s at 100 W. Lipid extraction was performed after Folch et al. [9]. After the last separation of the solvent systems, the resulting chloroform fraction was divided into two aliquots, one for colorimetric determination of the total lipids and the other for qualitative and quantitative analyses of the different lipid classes. The chloroform was finally evaporated and the samples stored at −4°C before further use.

The total lipids were determined by the sulpho-phospho-vanillin method [10]. A regression plot was made with a standard lipid fraction (Boehringer). To separate the different lipid classes, thin layer chromatography (TLC) was used. The TLC plates (silica gel 60, 25 mm, Merck) were precleaned by development with chloroform:methanol (2:1). Afterwards, they were air dried and then activated at 110°C for at least 2 hours. The lipid extracts were streaked on to the plates and developed consecutively in three different solvent systems (*solvent system 1:* chloroform:methanol:acetic acid (65:25:2); *solvent system 2:* chloroform; and *solvent system 3:* chloroform:hexan (1:3)). Between each development the plates were dried at 70°C. The resulting bands were visualized with iodine vapour and the area occupied by each band was marked for subsequent quantitative analysis. The following standards were used for band identification: monoacylglycerol: Sigma; diacylglycerol: Sigma; tristearin: Fluka; tripalmitin: Fluka; trilinolein: Fluka; triolein: Serva; palmitin: Merck; stearin: Roth; cholesterol: Boehringer; sphingomyelin: Serva; phosphatidylethanolamin: Sigma; phosphatidylcholin: Sigma; phosphatic acid: Sigma. To obtain a regression plot for quantitative analysis, standards were processed as above in appropriate dilutions.

### 2.5. Passage of undigested host proteins

Before being used in this investigation, all the ticks were cleaned with distilled water and dried with a tissue. Collection of haemolymph was performed as described above. Ticks were fixed in the same manner to obtain coxal fluid. The coxal fluid,

which is excreted immediately after feeding via pores at the basis between coxa I and coxa II, was removed with a Pasteur pipette. To raise the concentration of the constituents within a pooled sample of coxal fluid, the sample was freeze dried and finally redissolved in a small volume of distilled water. Grabar-Williams immunoelectrophoresis and rocket-immunoelectrophoresis for identification and quantification of host serum proteins were carried out in 1.3% agarose gels (1 mm) and 0.025M barbituric acid:NaOH buffer (pH 8.6). Antisera against whole bovine serum and bovine serum albumin were purchased from Nordic Immunology. Staining of the gels was achieved with Coomassie brilliant blue (Serva) in ethanol:acetic acid:aqua dest (9:1:9).

## 3. RESULTS

### 3.1. Body weight, feeding time and reproductive performance

There was no significant difference ($P > 0.05$) between the weight of the eggs, the non-feeding larvae, the first nymph and the males (Table I). Beginning with the second nymphal stage, the body weight differed significantly between the two strains. Ticks reared on pig blood had higher weights and the mean factor of weight gain from one stage to the next is 3.9 for pig blood and 3.6 for bovine blood.

TABLE I. BODY WEIGHT OF UNFED STAGES OF *O. moubata* REARED ON TWO DIFFERENT BLOOD TYPES

| Stage | Pig blood strain (mg) | (n)[a] | Bovine blood strain (mg) | (n)[a] |
|---|---|---|---|---|
| Egg[b] | 0.55 ± 0.03 | 800 | 0.54 ± 0.02 | 800 |
| Larvae[b] | 0.44 ± 0.02 | 450 | 0.46 ± 0.02 | 400 |
| Nymph 1[b] | 0.28 ± 0.01 | 500 | 0.26 ± 0.03 | 500 |
| Nymph 2 | 1.19 ± 0.35 | 500 | 0.84 ± 0.11 | 500 |
| Nymph 3 | 2.57 ± 0.82 | 500 | 2.39 ± 0.93 | 500 |
| Nymph 4 | 11.82 ± 2.24 | 450 | 10.13 ± 1.97 | 500 |
| Adult female | 56.25 ± 8.81 | 2000 | 43.92 ± 9.12 | 1450 |
| Adult male[b] | 19.75 ± 1.67 | 250 | 19.23 ± 2.01 | 250 |

[a] n = number of ticks examined.

[b] Not significant: $P > 0.05$ (Student's t-test).

TABLE II. FEEDING TIME AND BODY WEIGHT OF ENGORGED STAGES OF *O. moubata* REARED ON TWO DIFFERENT BLOOD TYPES

| Stage | Pig blood strain | | Bovine blood strain | |
|---|---|---|---|---|
| | Weight[a] (mg) | Feeding time (min) | Weight[a] (mg) | Feeding time (min) |
| Nymph 1 | 1.00 ± 0.03 | 65.0 ± 9.1 | 1.23 ± 0.09 | 50.4 ± 8.4 |
| Nymph 2 | 6.15 ± 1.45 | 40.3 ± 9.4 | 5.31 ± 0.98 | 31.0 ± 7.9 |
| Nymph 3 | 15.52 ± 4.83 | 25.5 ± 6.7 | 12.13 ± 3.56 | 20.3 ± 5.1 |
| Nymph 4 | 38.01 ± 18.65 | 20.2 ± 6.1 | 33.25 ± 17.91 | 25.3 ± 4.7 |
| Adult female | 299.25 ± 16.82 | 43.2 ± 10.2 | 245.15 ± 13.54 | 54.2 ± 9.6 |
| Adult male | 54.85 ± 2.34 | 10.0 ± 1.0[b] | 51.25 ± 1.98 | 10.2 ± 1.0[b] |

[a] Body weight was determined 4 hours after repletion to ensure that primary excretion was completed.

[b] Not significantly different.

Table II shows the feeding time and body weight of engorged ticks. The ticks were measured 4 hours after repletion to ensure that primary excretion was completed. Within the nymphal stages, the feeding time in both strains declined, the males showing the shortest time of all. Female ticks spent more time on the feeding process than other stages, except the first nymph. The engorged body weights of identical stages were significantly different between the two strains ($P < 0.05$). While the first nymph fed on bovine took more blood, all other stages fed on pig blood had higher repletion rates. The differentiation between the two sexes became evident in nymphal stage 4. In both strains the population statistics revealed two distinct peaks of engorged weight (not shown). Consequently, the standard deviation of means in this stage is considerably higher than in other stages (Table II). There was a 90% hatch of males in the group with the smaller weight and a 100% hatch of females in the group with high repletion rates.

The reproductive performance of females is given in Table III. The preoviposition period, which is defined as the time period between the day of repletion and the day that 75% of all the ticks start to lay eggs, and the whole oviposition period were shorter for females fed on pig blood ($P < 0.05$). Furthermore, the number of eggs laid per female was higher in the pig blood strain ($P < 0.05$), whereas the number of eggs per milligram of blood was higher in the bovine line; the ratio of egg mass to blood uptake was about the same in the two strains. Females fed on pig blood laid most of their eggs during the first days of the oviposition period.

TABLE III. REPRODUCTIVE PERFORMANCE OF *O. moubata* REARED ON TWO DIFFERENT BLOOD TYPES

| Blood type | Pre-oviposition period (d) | Oviposition period (d) | Eggs/ female | Eggs/ mg blood | mg egg/ mg blood |
|---|---|---|---|---|---|
| Pig[a] | 9.3 ± 0.7 | 19.5 ± 1.4 | 132.5 ± 13.6 | 0.55 | 0.30 |
| Bovine[b] | 11.0 ± 1.0 | 22.8 ± 1.3 | 125.1 ± 15.7 | 0.62 | 0.34 |

[a] 586 ticks.
[b] 345 ticks.
Mean ± SD for pig and bovine blood females are significantly different.

Females of the bovine blood strain deposited their eggs more regularly. There were no significant differences in the development of the individual stages. The larvae in both lines hatched after 8.25 days, nymph 1 after 5 additional days. The moulting periods, defined here as the time between the uptake of a bloodmeal and the stripping off of the old cuticle, were 8.2 days for nymph 2, 8.4 days for nymph 3, 9.5 days for nymph 4 and 10 days for the adults of both sexes. The sex ratio in the two strains was approximately 1:1.

### 3.2. Haemolymph volume

Females of the two experimental lines show the same pattern of haemolymph volume regulation with differences in absolute values (Figs 2(a) and 2(b)). The sharp increase in haemolymph volume during the first 5 days after feeding is achieved by continuous resorption of water from the stored blood into the midgut lumen. In the pig blood strain the maximum on day 5 had a peak like character, while females of the other strain displayed a more plateau like pattern from days 5–11 (Fig. 2(a)). The following decline in the two lines was more steady in the bovine blood strain. Because of the enormous blood mass imbibed within a relatively short period of time, the haemolymph volume to body weight ratio drops immediately after a bloodmeal to 11.0% (pig) and 9.2% (bovine). However, after repletion a rapid increase occurred in the females of both strains and before the ticks started egg deposition the ratio was regulated between 21 and 23%. This equilibrium was maintained during the whole egg laying period (Fig. 2(b)).

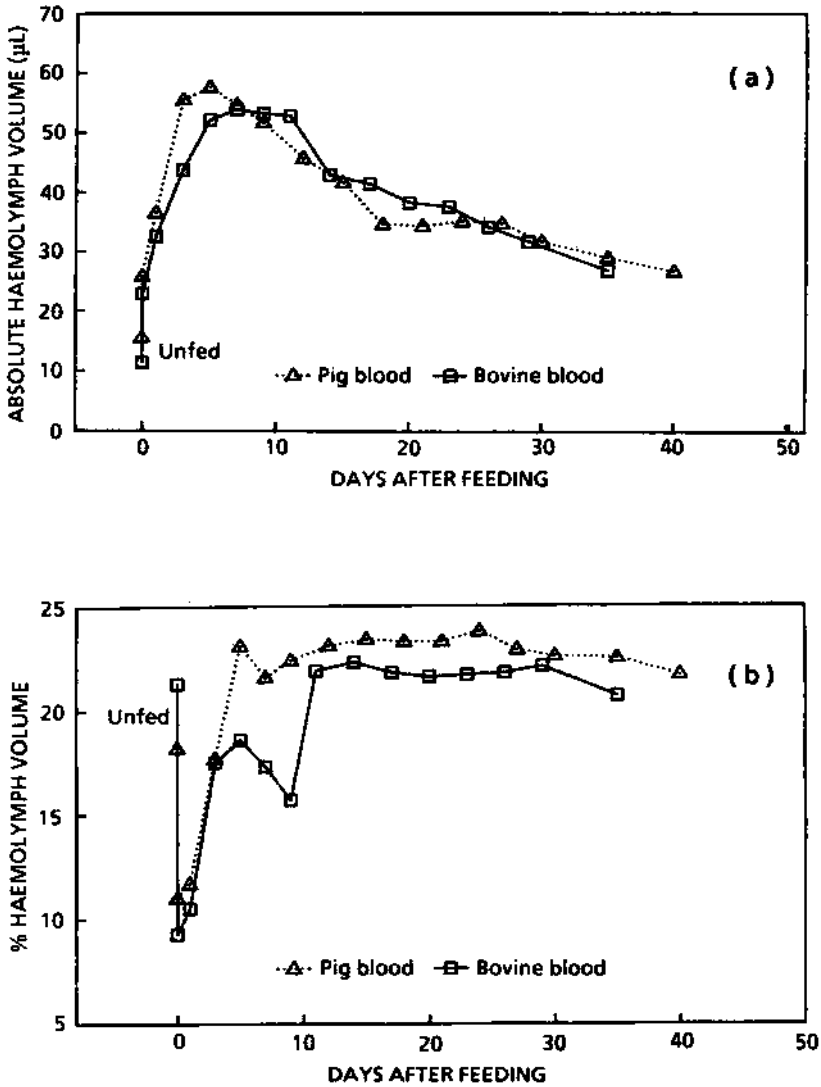


*FIG. 2. Change in the haemolymph volume during the gonotrophic cycle of two laboratory strains of* O. moubata *fed either on pig or bovine blood. (a) Change in absolute haemolymph volume; (b) change in haemolymph volume to body weight ratio.*

TABLE IV. TOTAL LIPID CONTENT AND DISTRIBUTION OF THE MAJOR LIPID CLASSES IN WHOLE PIG AND BOVINE BLOOD USED IN EXPERIMENTS ON THE ANALYSIS OF LIPID METABOLISM DURING THE GONOTROPHIC CYCLE OF *O. moubata*

| Blood type | Total lipids (mg/100 mL) | Distribution of lipid classes (mg/100 mL) | | | |
|---|---|---|---|---|---|
| | | Phospholipids | Cholesterol (free sterols) | Free fatty acids | Triglycerides |
| Pig | 423.5 ± 10.9 | 133.3 ± 9.5 | 85.5 ± 6.1 | 69.5 ± 4.8 | 133.2 ± 31.4 |
| Bovine | 395.3 ± 14.9 | 146.4 ± 4.8 | 79.9 ± 4.8 | 32.5 ± 5.3 | 132.0 ± 9.5 |

Mean ± SD for five samples, with 10 ticks per sample.

### 3.3. Lipids of the gut contents

There were relatively small variations in total lipid content, free sterols and triglycerides in the two blood types used in the study (Table IV). Phospholipids measured in pig blood were about 10% lower than in bovine blood, whereas the level of free fatty acids was 50% higher in pig blood than in bovine blood. Immediately after feeding and even on day 1 after repletion, the pattern of lipid distribution found in the midgut contents represents the same pattern described for the diets (Table V). Owing to the concentrating processes in the midgut, i.e. elimination of surplus water from the bloodmeal, most of the values measured were now higher than in whole blood. This observation does not apply for cholesterol and other sterols. Obviously, there is an immediate reduction in free sterols, which leads to a rapid clearance of this lipid class from the midgut. From day 5 onwards, sterols can no longer be picked up from the gut contents with TLC. This is true for females fed on pig and bovine blood. On day 3 after repletion, the amount of total lipids, phospholipids and triglycerides started to decline. During the pre-oviposition period, their rate of absorption was more intensive than in the following weeks. Phospholipids remain longer than the other lipid fractions in the midgut lumen and are detectable up to 35 days after a bloodmeal. In females fed on pig blood, the level of free fatty acids first drops and then increases again from days 5 to 7. Afterwards, this lipid class disappears, with the beginning of the egg laying period. A similar picture can be seen in females of the bovine blood strain. Examination of unfed 4 week old females revealed no lipid material, even in the postero-lateral gut diverticula. The same result was obtained about 2 months after the ticks had had a bloodmeal.

TABLE V. TOTAL LIPID CONTENT AND DIFFERENT LIPID CLASSES OF THE MIDGUT LUMEN DURING THE GONOTROPHIC CYCLE OF FEMALE *O. moubata*

| Days after feeding | Total lipids (mg/100 mL) | Distribution of lipid classes (mg/100 mL) | | | |
|---|---|---|---|---|---|
| | | Phospholipids | Cholesterol (free sterols) | Free fatty acids | Triglycerides |
| | | **Pig blood strain** | | | |
| 0 | 471.5 ± 14.9 | 150.9 ± 7.6 | 78.4 ± 5.9 | 88.2 ± 5.9 | 150.8 ± 9.5 |
| 1 | 468.7 ± 16.4 | 174.6 ± 6.4 | 68.5 ± 2.6 | 52.1 ± 3.9 | 174.6 ± 6.3 |
| 3 | 365.1 ± 15.6 | 151.0 ± 5.1 | 41.5 ± 3.4 | 43.3 ± 4.9 | 113.0 ± 5.8 |
| 5 | 281.2 ± 18.7 | 133.3 ± 8.0 | | 60.8 ± 6.1 | 88.2 ± 4.2 |
| 7 | 208.1 ± 13.1 | 78.2 ± 2.7 | | 60.7 ± 5.5 | 69.2 ± 7.3 |
| 9 | 164.3 ± 10.4 | 69.5 ± 3.0 | | 43.6 ± 2.5 | 52.1 ± 2.7 |
| 12 | 123.2 ± 9.8 | 69.5 ± 2.9 | | 16.6 ± 1.1 | 34.7 ± 3.1 |
| 15 | 98.7 ± 7.5 | 60.8 ± 2.7 | | | 34.7 ± 4.2 |
| 18 | 82.0 ± 6.9 | 60.1 ± 1.9 | | | 23.4 ± 2.1 |
| 21 | 64.3 ± 6.1 | | | | |
| 24 | 43.4 ± 7.1 | | | | |
| 27 | 35.0 ± 4.1 | 30.7 ± 2.6 | | | |
| 30 | 30.1 ± 3.5 | 30.0 ± 1.2 | | | |
| 35 | 18.2 ± 1.9 | 16.1 ± 2.0 | | | |
| 40 | 7.3 ± 4.9 | | | | |
| | | **Bovine blood strain** | | | |
| 0 | 431.2 ± 21.3 | 170.2 ± 5.3 | 78.0 ± 4.2 | 41.1 ± 3.9 | 150.5 ± 8.1 |
| 1 | 429.5 ± 19.2 | 172.2 ± 3.7 | 69.2 ± 3.6 | 38.7 ± 3.7 | 152.7 ± 7.3 |
| 3 | 408.1 ± 19.1 | 165.1 ± 4.3 | 38.7 ± 2.0 | 41.1 ± 4.1 | 155.5 ± 9.3 |
| 5 | 188.3 ± 9.3 | 105.7 ± 2.6 | | 25.1 ± 2.7 | 54.1 ± 3.1 |
| 7 | 157.9 ± 11.2 | 99.2 ± 3.2 | | 16.7 ± 0.9 | 45.7 ± 3.2 |
| 9 | 129.4 ± 11.5 | 84.1 ± 3.1 | | 9.7 ± 0.9 | 41.1 ± 2.7 |
| 11 | 95.3 ± 9.7 | 62.2 ± 2.7 | | | 37.8 ± 2.4 |
| 14 | 39.7 ± 4.3 | 40.0 ± 2.4 | | | |
| 17 | 37.5 ± 4.9 | 38.7 ± 3.1 | | | |

TABLE V (cont.)

| Days after feeding | Total lipids (mg/100 mL) | Distribution of lipid classes (mg/100 mL) | | | |
|---|---|---|---|---|---|
| | | Phospholipids | Cholesterol (free sterols) | Free fatty acids | Triglycerides |
| | | **Bovine blood strain (cont.)** | | | |
| 20 | 32.2 ± 3.8 | 28.7 ± 4.1 | | | |
| 23 | 27.4 ± 6.1 | | | | |
| 26 | 19.2 ± 6.4 | | | | |
| 29 | 8.3 ± 2.1 | 9.1 ± 1.1 | | | |
| 35 | 2.1 ± 0.4 | 2.0 ± 0.5 | | | |

Mean ± SD for five samples, with 10 ticks per sample.

### 3.4. Lipids of the haemolymph

The haemolymph of unfed females showed a higher value for total lipids, phospholipids and free fatty acids when the ticks were raised on pig blood (Table VI). Triglycerides were higher in the bovine blood strain. Phospholipids were the most abundant lipid class in the unfed female, contributing 57% of the total lipid fraction in the two strains. With an increasing haemolymph volume after engorgement, the concentrations of the haemolymph constituents naturally decreased. On day 7 after a bloodmeal, the amount of total lipids reached a maximum in both lines, but the pig blood strain showed higher absolute values and accumulation rates. The ratio of total lipids in the haemolymph of unfed females to the maximum lipid content was 3.2 for pig blood and 2.4 for bovine blood. The phospholipid content increased in a similar way, with peaks on days 9 and 11, respectively. The decline in total lipids and phospholipids during the oviposition period and afterwards was more pronounced in the bovine blood strain. During the pre-oviposition period the concentration of triglycerides was higher in pig blood, but both lines had a peak on day 7 and between days 15 and 17. Between these two maxima, diglycerides appeared in the haemolymph, with higher levels measured for pig blood ticks. Free fatty acids had a maximum value, together with the overall lipid content, on day 7. They were detectable over a period of 2 weeks in the bovine blood specimens, but up to 5 weeks in the pig blood strain. Free sterols could be discovered only for a relatively short period in the haemolymph and disappeared before day 7. This is true also for sterol esters, which emerged on day 3 and were not noticed in whole blood or in the gut contents.

TABLE VI. TOTAL LIPID CONTENT AND DIFFERENT LIPID CLASSES DURING THE GONOTROPHIC CYCLE IN THE HAEMOLYMPH OF FEMALE *O. moubata*

| Days after feeding | Total lipids (mg/100 mL) | Phospholipids | Distribution of lipid classes (mg/100 mL) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Cholesterol (free sterols) | Free fatty acids | Diglycerides | Triglycerides | Sterolesters |
| | | | | **Pig blood strain** | | | |
| Unfed | 101.7 ± 9.8 | 58.3 ± 3.5 | | 30.1 ± 2.4 | | 19.7 ± 3.1 | |
| 0 | 41.3 ± 10.5 | 19.5 ± 2.1 | | 11.2 ± 1.7 | | 10.3 ± 1.2 | |
| 1 | 38.4 ± 8.1 | 20.6 ± 3.5 | 19.7 ± 1.7 | | | | |
| 3 | 143.4 ± 14.3 | 86.9 ± 4.8 | 20.0 ± 2.2 | 18.6 ± 2.3 | | | 21.1 ± 2.0 |
| 5 | 213.4 ± 11.7 | 104.3 ± 5.7 | 20.2 ± 3.7 | 29.4 ± 3.1 | | 29.7 ± 2.1 | 33.3 ± 1.9 |
| 7 | 330.2 ± 20.1 | 173.9 ± 9.4 | 9.5 ± 1.7 | 30.1 ± 9.1 | 41.5 ± 2.3 | 66.6 ± 3.9 | |
| 9 | 264.5 ± 18.1 | 191.3 ± 8.6 | | 19.7 ± 1.8 | 20.1 ± 7.6 | 41.6 ± 4.1 | |
| 12 | 246.2 ± 16.1 | 173.9 ± 8.4 | | 19.6 ± 2.0 | 20.4 ± 2.5 | 41.6 ± 3.7 | |
| 15 | 234.8 ± 14.9 | 132.3 ± 7.5 | | 19.8 ± 1.7 | 20.1 ± 1.9 | 66.4 ± 4.7 | |
| 18 | 218.1 ± 17.0 | | | | | | |
| 21 | 210.2 ± 13.1 | 131.9 ± 6.8 | | 19.2 ± 2.9 | | 58.8 ± 4.3 | |
| 24 | 203.4 ± 12.9 | 132.7 ± 6.4 | | 20.4 ± 2.7 | | 50.2 ± 3.8 | |
| 27 | 194.3 ± 10.1 | 129.5 ± 5.3 | | 19.2 ± 9.8 | | 45.1 ± 2.5 | |
| 30 | 180.7 ± 8.4 | 126.1 ± 4.2 | | 18.4 ± 3.9 | | 36.3 ± 1.8 | |
| 35 | 163.9 ± 12.2 | 114.7 ± 7.1 | | 10.3 ± 1.1 | | 31.1 ± 18.9 | |
| 40 | 152.7 ± 9.7 | 122.8 ± 8.4 | | | | 29.8 ± 2.1 | |
| 60 | 143.1 ± 11.0 | 141.1 ± 7.8 | | | | | |

| | | | Bovine blood strain | | | | |
|---|---|---|---|---|---|---|---|
| Unfed | 91.5 ± 18.2 | 52.2 ± 2.3 | | 19.7 ± 1.8 | | 23.2 ± 2.1 | |
| 0 | 48.3 ± 6.2 | 25.1 ± 1.2 | | 10.2 ± 1.3 | | 9.1 ± 1.4 | |
| 1 | 57.7 ± 9.6 | 25.4 ± 2.0 | 9.8 ± 1.2 | 10.0 ± 0.9 | | 10.1 ± 1.3 | |
| 3 | 107.3 ± 8.2 | 65.2 ± 2.7 | 10.7 ± 9.9 | 10.2 ± 1.4 | | | 19.7 ± 2.1 |
| 5 | 151.5 ± 12.1 | 91.0 ± 3.6 | 9.8 ± 2.5 | 20.3 ± 2.9 | | 20.0 ± 2.1 | 10.4 ± 1.8 |
| 7 | 219.3 ± 19.7 | 126.2 ± 4.1 | | 24.8 ± 2.4 | 19.7 ± 1.2 | 41.2 ± 1.2 | |
| 9 | 202.7 ± 10.4 | 135.1 ± 3.9 | | 20.2 ± 2.9 | 20.4 ± 2.7 | 24.5 ± 3.1 | |
| 11 | 177.9 ± 14.2 | 142.5 ± 5.7 | | 9.8 ± 2.7 | 9.7 ± 1.9 | 10.1 ± 1.2 | |
| 14 | 153.2 ± 11.9 | 132.1 ± 4.9 | | | | 14.9 ± 2.1 | |
| 17 | 128.6 ± 13.0 | 101.5 ± 4.5 | | | | 24.9 ± 2.3 | |
| 20 | 87.2 ± 8.8 | 70.2 ± 2.9 | | | | 15.0 ± 1.5 | |
| 23 | 76.7 ± 4.1 | 64.5 ± 4.8 | | | | 11.2 ± 2.0 | |
| 26 | 72.6 ± 2.5 | 61.3 ± 3.1 | | | | 10.3 ± 2.0 | |
| 29 | 70.2 ± 1.9 | 64.9 ± 4.9 | | | | 6.2 ± 0.8 | |
| 35 | 64.5 ± 0.9 | 65.1 ± 1.6 | | | | | |
| 60 | 62.4 ± 3.9 | 60.0 ± 2.3 | | | | | |

Mean ± SD for five samples, with 10 ticks per sample.

TABLE VII. TOTAL LIPID CONTENT AND DIFFERENT LIPID CLASSES OF EGG, LARVAE AND FIRST NYMPH OF *O. moubata*

| Days after oviposition | Stage | Total lipids (mg/100 mL) | Distribution of lipid classes (mg/100 mL) | | | |
|---|---|---|---|---|---|---|
| | | | Phospholipids | Cholesterol (free sterols) | Free fatty acids | Triglycerides |
| | | | **Pig blood strain** | | | |
| 0 | Egg | 5.11 ± 0.48 | 1.97 ± 0.30 | 0.71 ± 0.02 | | 2.40 ± 0.28 |
| 1 | Egg | 5.24 ± 0.51 | 2.01 ± 0.41 | 0.71 ± 0.04 | 0.21 ± 0.02 | 2.39 ± 0.13 |
| 2 | Egg | 5.14 ± 0.42 | 1.99 ± 0.23 | 0.64 ± 0.05 | 0.18 ± 0.02 | 2.42 ± 0.09 |
| 3 | Egg | 4.74 ± 0.39 | 2.02 ± 0.24 | 0.41 ± 0.04 | 0.22 ± 0.03 | 2.11 ± 0.10 |
| 4 | Egg | 4.59 ± 0.68 | 2.01 ± 0.31 | 0.37 ± 0.06 | 0.36 ± 0.06 | 1.91 ± 0.07 |
| 5 | Egg | 4.68 ± 0.44 | 1.79 ± 0.21 | 0.25 ± 0.02 | 1.25 ± 0.08 | 1.46 ± 0.09 |
| 6 | Egg | 4.73 ± 0.46 | 1.75 ± 0.15 | 0.21 ± 0.03 | 1.49 ± 0.11 | 1.39 ± 0.12 |
| 7 | Egg | 5.65 ± 0.67 | 1.58 ± 0.23 | 0.11 ± 0.02 | 2.26 ± 0.43 | 1.64 ± 0.23 |
| 8 | Larvae | 4.69 ± 0.71 | 1.60 ± 0.19 | | 1.62 ± 0.32 | 1.34 ± 0.12 |
| 9 | Larvae | 4.33 ± 0.56 | 1.52 ± 0.15 | | 1.58 ± 0.24 | 1.29 ± 0.09 |
| 10 | Larvae | 4.00 ± 0.45 | 1.53 ± 0.21 | | 1.36 ± 0.12 | 1.17 ± 0.11 |
| 11 | Larvae | 2.99 ± 0.21 | 1.51 ± 0.18 | | 0.65 ± 0.03 | 0.86 ± 0.10 |
| 12 | Larvae | 2.75 ± 0.41 | 1.34 ± 0.13 | | 0.74 ± 0.05 | 0.72 ± 0.06 |
| 13 | Nymph 1 | 2.09 ± 0.29 | 1.35 ± 0.08 | | 0.16 ± 0.05 | 0.63 ± 0.08 |
| 14 | Nymph 1 | 2.03 ± 0.15 | 1.36 ± 0.07 | | 0.13 ± 0.02 | 0.50 ± 0.05 |

| | | | Bovine blood strain | | | |
|---|---|---|---|---|---|---|
| 0 | Egg | 4.74 ± 0.61 | 1.81 ± 0.24 | 0.66 ± 0.07 | | 2.21 ± 0.45 |
| 1 | Egg | 4.46 ± 0.41 | 1.78 ± 0.21 | 0.59 ± 0.06 | 0.15 ± 0.03 | 1.98 ± 0.67 |
| 2 | Egg | 4.06 ± 0.39 | 1.62 ± 0.12 | 0.46 ± 0.03 | 0.16 ± 0.03 | 1.78 ± 0.56 |
| 3 | Egg | 3.96 ± 0.32 | 1.59 ± 0.23 | 0.37 ± 0.04 | 0.17 ± 0.05 | 1.78 ± 0.21 |
| 4 | Egg | 3.67 ± 0.44 | 1.54 ± 0.31 | 0.33 ± 0.06 | 0.30 ± 0.04 | 1.45 ± 0.13 |
| 5 | Egg | 4.08 ± 0.38 | 1.49 ± 0.23 | 0.34 ± 0.04 | 1.02 ± 0.02 | 1.28 ± 0.10 |
| 6 | Egg | 4.72 ± 0.41 | 1.48 ± 0.14 | 0.32 ± 0.05 | 1.39 ± 0.11 | 1.56 ± 0.12 |
| 7 | Egg | 5.40 ± 0.65 | 1.50 ± 0.16 | 0.11 ± 0.02 | 2.07 ± 0.24 | 1.69 ± 0.16 |
| 8 | Larvae | 4.11 ± 0.45 | 1.47 ± 0.23 | | 1.47 ± 0.14 | 1.16 ± 0.12 |
| 9 | Larvae | 3.87 ± 0.31 | 1.31 ± 0.18 | | 1.36 ± 0.19 | 1.29 ± 0.09 |
| 10 | Larvae | 3.19 ± 0.23 | 1.08 ± 0.37 | | 1.06 ± 0.08 | 1.02 ± 0.07 |
| 11 | Larvae | 2.86 ± 0.32 | 0.96 ± 0.07 | | 0.85 ± 0.06 | 1.02 ± 0.08 |
| 12 | Larvae | 2.81 ± 0.24 | 0.92 ± 0.11 | | 0.84 ± 0.05 | 1.09 ± 0.06 |
| 13 | Nymph 1 | 1.91 ± 0.34 | 0.84 ± 0.05 | | 0.58 ± 0.05 | 0.51 ± 0.02 |
| 14 | Nymph 1 | 1.84 ± 0.20 | 0.88 ± 0.07 | | 0.52 ± 0.03 | 0.41 ± 0.03 |

### 3.5. Lipids of eggs, larvae and 1st nymph

The total lipid content of the eggs showed a slight decline until day 5 after oviposition, but then a sudden increase over the last 2 days of embryogenesis (Table VII). A similar pattern can be seen with triglycerides, whereas phospholipids and free sterols were decreasing throughout this period. Free fatty acids showed a steady increase during embryogenesis. There were no free sterols detectable during larval development and the first 2 days of nymph 1, but phospholipids, free fatty acids and triglycerides were still present. Along with the total lipid fraction, their concentrations were continuously decreasing.

### 3.6. Passage of undigested host proteins

The data obtained indicate that immediately after feeding on defibrinated bovine blood albumin appears in the haemolymph. The concentration measured 2 hours after repletion ranges from 1.5–15 $\mu$g/mL. Later, the number of ticks positive for albumin declined (Table VIII). About 96 hours after engorgement, and later, albumin was absent from the haemolymph. This protein apparently disappears from the haemoceol within the first 4 days. Examination of starved and freshly moulted ticks failed to show albumin or any other intact meal protein in the haemolymph.

To calculate the total amount of albumin entering the haemoceol, a possible loss via the coxal organs has to be considered. Knowing the total haemolymph volume of ca 10 $\mu$L, the albumin concentration in the haemolymph, as well as the loss via the coxal organs, the amount passing through the gut wall contributes between 0.002 and 0.01% of the total albumin content taken with a bloodmeal.

TABLE VIII. PERCENTAGE OF TICKS POSITIVE FOR SERUM ALBUMIN IN THEIR HAEMOLYMPH AT VARIOUS TIMES AFTER FEEDING

| Hours after feeding | No. of groups[a] | Percentage of ticks | P value (t-test) |
|---|---|---|---|
| 2 | 13 | 98.7 ± 3.3 | |
| 6 | 3 | 70.0 ± 8.7 | < 0.001 |
| 24 | 3 | 44.3 ± 5.2 | < 0.02 |
| 48 | 1 | 24.0 | |
| 92 | 2 | 0.0 | |

[a] Each group contains 5–17 ticks.

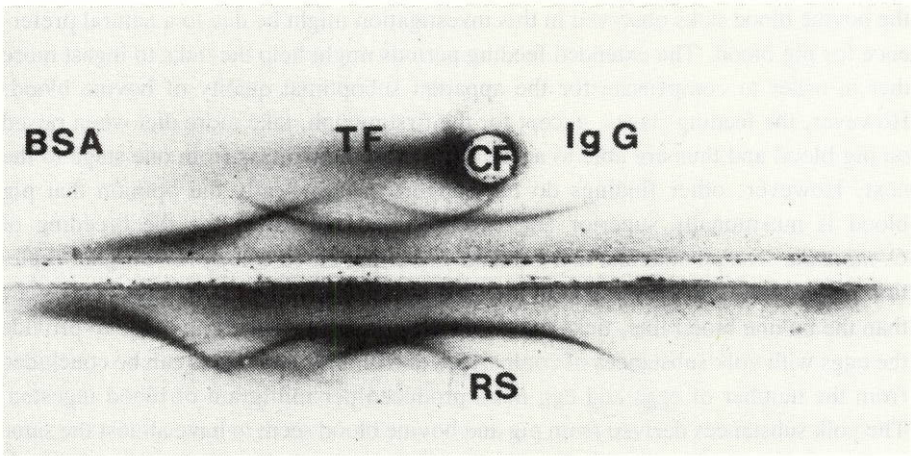


*FIG. 3. Immunoelectrophoretic analysis of concentrated coxal fluid (CF) of female* O. moubata *in comparison to bovine serum (RS). The following serum proteins were detected: serum albumin (BSA), immunoglobulin G (IgG) and transferrin (TF).*

When coxal fluid is concentrated about 50 fold, some meal proteins other than albumin were detected. Figure 3 shows bands corresponding to IgG, transferrin and albumin. Since these three protein fractions are the dominant constituents in mammalian serum, possible minor fractions should be detectable by more sensitive methods. All the serum proteins discovered in the coxal fluid were also present in the haemolymph.

## 4. DISCUSSION

### 4.1. Effect of pig and bovine blood on the body weight, repletion rate, feeding time and haemolymph volume of *O. moubata*

The first successful breeding of *O. moubata* over the complete life-cycle was reported by artificial feeding on pig and bovine blood using bat wing membranes [11]. In contrast to our results, this study showed no significant differences in unfed and engorged weight between females fed on different blood sources. Furthermore, the females of our colonies had considerably higher repletion rates. Dissimilarities also exist in the number of eggs laid per female. Together with data on the percentage of hatchability, it is concluded that bovine blood is nutritionally deficient and not suitable for mass production of *O. moubata* ticks. In fact, bovine species are not natural hosts of this argasid tick [12]. The prolonged feeding times for

the bovine blood ticks observed in this investigation might be due to a natural preference for pig blood. The extended feeding periods might help the ticks to ingest more diet in order to compensate for the apparent suboptimal quality of bovine blood. However, the feeding stages, except for the first nymph, take more diet when raised on pig blood and thus are able to accumulate more body mass from one stage to the next. However, other findings do not support unequivocally the opinion that pig blood is nutritionally superior and bovine blood not suited for the breeding of *O. moubata*. Despite the fact that the ticks of our pig blood colony have higher repletion rates, higher unfed weights in the feeding stages and lay more eggs per female than the bovine blood line, ticks fed on the latter type are apparently able to provide the eggs with yolk substances of comparable nutritional value. This can be concluded from the number of eggs and egg mass produced per milligram of blood ingested. The yolk substances derived from pig and bovine blood seem to have almost the same quality, because there are no significant differences in the egg weight and body weight of larvae and unfed nymph 1. Our laboratory colonies also show no differences in sex ratios, hatching and premoulting periods. Additional nymphal stages 5 and 6 were exceptional in the two strains. The variations observed in the laboratory colonies should therefore not be attributed to the qualitative differences between the diets but rather to the absolute quantities of blood ingested.

### 4.2. Haemolymph volume regulation

The pattern of haemolymph volume regulation during the gonotrophic cycle shows the same characteristics in females fed on either pig or bovine blood. The rapid increase in the absolute volume during the whole pre-oviposition period is achieved by water influx from the midgut lumen. Since the haemolymph of arthropods serves as a very important transport vehicle for nutrients and other essential components, the transport capacity of the haemolymph is expanded with the increasing haemolymph volume, which in turn helps to provide nutrition for egg maturation. At the start of the egg laying period, a constant ratio between body weight and haemolymph volume is maintained throughout the oviposition activities. Preservation of the observed equilibrium is attributed to the simultaneous loss of body weight and water content (haemolymph volume) with egg deposition. This situation might be reflected in the slope of the declining absolute haemolymph volume after oviposition has started. Females fed on pig blood lay most of their eggs within the first 7 days. Correspondingly, the haemolymph volume drops rapidly during this time and is not affected significantly towards the end of the oviposition period. Females fed on bovine blood deposit their eggs more constantly and thus their haemolymph volume is reduced more steadily.

In insects, a ratio of 16–20% between the haemolymph volume and body weight has been suggested [13]. In unfed females of *O. moubata*, the ratio for the pig line is 18.2% and for the bovine line 21.3%, but these do not differ significantly.

During oviposition the ratios are slightly elevated and range between 22 and 23%. For the ixodid tick, *Dermacentor andersoni,* a comparable ratio of 23% has been reported [14].

### 4.3. Lipid metabolism during the gonotrophic cycle of *O. moubata*

The high nutritional requirements during vitellogenesis are demonstrated in the rapid uptake of lipids from the midgut contents within the first week after repletion. Correspondingly, the lipid material in the haemolymph increases. The lipid peak in the haemolymph coincides with the maximal absolute haemolymph volume, indicating that the tick's physiology is directed towards the intensive metabolic processes of egg maturation. In insects, the fat body has an important function as energy depot [15] and thus can be recognized as the ultimate organ where food reserves are accumulated and stored in an appropriate form. For ticks, a fat body like structure is only described for females of some species of ixodid ticks [16]. In argasid ticks, there is at present no evidence of a liver like organ of mesodermal origin such as the fat body in insects. Moreover, soft ticks store food components in their midgut lumen and gut diverticula [17]. Our investigations on *O. moubata* show that in 4 week old unfed females and for more than 40 days after repletion, no lipid material can be found in the midgut lumen and diverticula. This finding suggests that the midgut is not a specific lipid reservoir. The haemolymph seems to be more important as a lipid carrier, since this organ contains a considerable amount of lipids, even in unfed ticks. The distribution of lipid classes in the haemolymph differs from the conditions found in whole blood or in the midgut contents. Phospholipids are the dominant lipid group in the haemolymph of fed and unfed females and might represent, together with triglycerides, the major transport form of lipids and possibly an energy source. The existence of two vitellogenins in the haemolymph of *O. moubata* and the incorporation of these haemolymph precursors into the eggs as vitellins has been demonstrated [18, 19]. This protein contains a lipid fraction of 7.6%, with a high triglyceride content. Diglycerides, which are the primary form of lipid transportation in insects, are obviously not of great significance in *O. moubata.* Free sterols are not present in the haemolymph of unfed ticks, but will appear very rapidly after a bloodmeal. After engorgement, they are absorbed immediately from the midgut lumen, since there is no concentration effect due to water elimination. This observation agrees with the situation in two other soft tick species where sterols are metabolized very quickly [20]. Steroids cannot be synthesized by ticks [21] and this lipid group has to be an essential component in the diet. Interestingly, free fatty acids (FFA) have a peak in the midgut lumen 3–5 days after repletion, especially in females fed on pig blood. A possible explanation would be the liberation of fatty acids from red blood cell haemolysis, but this interpretation needs further investigation. The role of fatty acids as an instant and efficient energy

source might be reflected during embryogenesis. The FFA content of the eggs constantly rises until the larvae hatch. They could be mobilized from phospholipids and/or triglycerides. In contrast to the situation in the haemolymph, triglycerides are the most abundant lipid class in the freshly deposited egg. The fact that phospholipids do not change significantly from the egg to the larvae would favour triglycerides as the main energy donator for the hatching process. The biological significance of the relatively high lipid content of the eggs of *O. moubata* compared with other ticks is simply to ensure the development of the non-feeding larvae and the survival of the first nymph thereafter.

The mode in which the lipid moiety of pig and bovine blood is utilized in the two laboratory strains reveals no prominent differences. Variations are found only in the total amount of lipids or single lipid classes. The higher level of total lipids and FFA in whole pig blood is also maintained in the haemolymph of female ticks, both during embryogenesis and during the larval period. This is not true for phospholipids, which were more abundant in whole bovine blood, but were reduced in the tick compared with the corresponding stage of the pig blood strain. From these results we conclude that qualitative differences between pig and bovine blood do not change the overall pattern of lipid metabolism in *O. moubata*.

### 4.4. Passage of undigested host serum proteins

For *Glossina morsitans morsitans*, it was demonstrated that intact host serum proteins, such as albumin and the $F_{ab}$ fragments of IgG, are capable of passing through the gut wall without being affected by digestive enzymes, thus maintaining their biological properties [7]. In the case of *Stomoxys calcitrans*, serum albumin can also be found in the haemolymph, but there is no evidence of IgG or fragments of IgG [22]. However, the overall amount of blood proteins appearing in the haemolymph is very low and does not exceed 1% of their total uptake. The presence of serum proteins in the coxal fluid of *O. moubata* implies an immediate passage of these substances across the gut barrier, since primary excretion starts at the end of the feeding process. It is known that during and shortly after feeding the midgut epithelium of *O. moubata* shows a profound reorganization of its cellular components [23]. These changes in midgut structure might offer intercellular routes for small amounts of serum fractions to reach the haemolymph. This concept of a 'leaky gut' has also been shown for the mosquito [24] and more recently for the tsetse fly [25], where orally administered peroxidase may enter the haemolymph via intercellular clefts within 2 hours after repletion. The phenomenon of a leaky gut might be a common feature of blood sucking arthropods and the degree of leakage might be dependent on the ultrastructural morphology of the midgut, with its peritrophic membrane layers and its changes during and after feeding. There might be an evolutionary adaptation, as in the case of *Glossina*, where the organism might take advan-

tage of undigested proteins [26]. In *O. moubata*, the importance of intact serum proteins in tick physiology needs further investigation to include components smaller than proteins, e.g. steroids.

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# EFFECT OF REARING DIET ON THE INFECTION RATE IN FLIES RELEASED FOR THE CONTROL OF TSETSE POPULATIONS BY STERILE MALES*

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## Abstract

EFFECT OF REARING DIET ON THE INFECTION RATE IN FLIES RELEASED FOR THE CONTROL OF TSETSE POPULATIONS BY STERILE MALES.

In areas where sleeping sickness is endemic, it is the practice of sterile insect technique (SIT) programmes to give sterilized males a bloodmeal before release into the wild in order to reduce the risk of these released flies acting as disease vectors. This strategy has been adopted because of experimental evidence which showed that it was essential to infect flies at their first feed to establish a *Tripanosoma brucei gambiense* or *T. b rhodesiense* infection in tsetse flies. The aim of the work was to test artificial tsetse diets produced in the IAEA Laboratory at Seibersdorf in order to determine whether they were as effective as whole blood in inihibiting *T. brucei* sensu lato (sl) infections in flies. Seven artificial diets were tested with *T. b. rhodesiense; Glossina morsitans morsitans* males were fed one meal of the diet and then starved for 3 days before the infective feed. None of these diets significantly altered the infection rate of the treated flies and the seven groups produced statistically homogeneous results, with a mean midgut rate of 16% (control flies fed pig blood: 17%). Flies infected as tenerals with the same trypanosome stock produced midgut rates of 61%. Three of the diets were also tested with a *T. congolense* stock. There were no significant differences between flies fed artificial (mean midgut infection rate: 15%) and whole blood diets (19%). *G. m. morsitans* infected as tenerals with this trypanosome stock produced midgut rates of 66%. As with *T. brucei* sl infections, teneral flies were far more likely to develop a *T. congolense* infection than fed flies; this result suggests that all the tsetse flies used in SIT programmes should be fed before release in order to reduce the risk both to man and his livestock. Artificial diets are as effective as whole blood in inhibiting trypanosome infections. The effect of bloodmeal on the fly infection rates is discussed in relation to lectin production in fed flies.

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## 1. INTRODUCTION

Release of sterile males to control natural populations of tsetse fly carries with it the risk of greatly increasing, albeit temporarily, the number of potential disease vectors in the area. Unlike other important disease vectors such as mosquitoes both sexes of the tsetse fly feed on blood and can transmit trypanosomes; this becomes critical when a sterile insect technique (SIT) programme is proposed in an area where sleeping sickness is endemic. However, it has long been appreciated that it is more difficult to infect tsetse flies with sleeping sickness trypanosomes (*Trypanosoma brucei* sensu lato (sl)) if the flies are non-teneral, i.e. if they have already take a bloodmeal [1, 2]. As a result, SIT programmes for tsetse control adopt the strategy of giving flies a bloodmeal before release.

Part of the research programme being carried out in the IAEA Laboratory at Seibersdorf is concerned with the development of a totally artificial diet on which to maintain tsetse flies for SIT programmes as a replacement for the bovine or porcine blood currently in use. The aim of this work was to test the artificial diets being produced at Seibersdorf in order to determine whether they were as effective as whole blood in inihibiting the establishment of *T. brucei* sl infections in flies. These studies were extended to examine the effects of feeding flies before infection with *T. congolense.*

## 2. MATERIALS AND METHODS

(1) **Flies:** *Glossina morsitans morsitans* males were used and maintained on an in vitro feeding system.

(2) **Tripanosomes:** *T. b rhodesiense* EATRO 2340 (see Cornelissen et al. [3] for stock details); *T. congolense* FLY 1/148 (see Young and Godfrey [4] for stock details)

(3) **Infective feeds:** Frozen stabilates of infected mouse blood were used to make up infective feeds in defibrinated pig blood as previously described in Ref. [5]

(4) **Artificial diets:** These were provided by J.P. Kabayo of the IAEA Laboratory at Seibersdorf; diets were freeze dried and made up in saline directly before use.

To simulate field conditions as closely as possible flies were given their first feed on either the experimental diet or normal defibrinated pig blood. Flies were then starved for 3 days. On day 4 the flies were given infective feeds. Flies were then maintained for a further 21 days (*T. congolense* infections) or 28 days (*T. b. rhodesiense* infections) on pig blood and then the midguts, mouthparts and salivary glands (in the case of *T. b. rhodesiense* infections) were dissected and examined for trypanosomes by phase contrast microscopy (× 400). For comparative purposes, groups of teneral flies were infected with the same trypanosome stocks.

TABLE I. MIDGUT AND SALIVARY GLAND INFECTION RATES IN MALE *G. m. morsitans* GIVEN A FIRST, UNINFECTED FEED ON EITHER AN ARTIFICIAL DIET (Nos. JPK5 TO JPK11) OR PIG BLOOD (CONTROLS) AND THEN INFECTED 4 DAYS LATER WITH *T. b. rhodesiense*

*(Flies were dissected 28 days post-infection. Teneral controls were infected at their first feed with the same trypanosome stock on the day of emergence from the puparium)*

| First feed | Infection rate (%) | | Total No. of flies |
|---|---|---|---|
| | Midgut | Salivary glands | |
| JPK5 | 30 | 0 | 41 |
| Control | 21 | 1 | 43 |
| JPK6 | 11 | 0 | 44 |
| Control | 17 | 0 | 46 |
| JPK7 | 18 | 7 | 49 |
| Control | 37 | 16 | 43 |
| JPK8 | 22 | 2 | 46 |
| Control | 10 | 0 | 48 |
| JPK9 | 16 | 0 | 43 |
| Control | 23 | 5 | 43 |
| JPK10 | 8 | 0 | 49 |
| Control | 6 | 2 | 48 |
| JPK11 | 13 | 0 | 45 |
| Control | 12 | 5 | 43 |
| Teneral controls | 61 | 3 | 187 |

## 3. RESULTS

The results of experiments carried out with *T. b. rhodesiense* are presented in Table I. Statistical analysis showed that there was no significant difference in the infection rate between experimental diet and control fed flies, except in the case of diet JPK7, which produced a significantly lower midgut infection rate than the control group ($\chi^2$ 4.1, $0.05 > P > 0.01$). However, this effect was the result of the exceptionally high infection rate of the control flies in this experiment (37%). The

TABLE II. MIDGUT AND HYPOPHARYNGEAL INFECTION RATES IN MALE *G. m. morsitans* GIVEN A FIRST, UNINFECTED FEED ON EITHER AN ARTIFICIAL DIET (Nos. JPK5 TO JPK7) OR PIG BLOOD (CONTROLS) AND THEN INFECTED 4 DAYS LATER WITH *T. congolense*

*(Flies were dissected 21 days post-infection. Teneral controls were infected at their first feed with the same trypanosome stock on the day following the day of emergence from the puparium)*

| First feed | Infection rate (%) | | Total No. of flies |
|---|---|---|---|
| | Midgut | Hypopharynx | |
| JPK5 | 19 | 14 | 36 |
| Control | 19 | 12 | 43 |
| JPK6 | 9 | 9 | 43 |
| Control | 13 | 0 | 39 |
| JPK7 | 17 | 15 | 45 |
| Control | 25 | 23 | 44 |
| Teneral controls | 66 | 32 | 125 |

midgut infection rate of the experimental flies was statistically homogeneous, with a mean rate of 16% compared with a mean midgut rate of 17% in the control flies (heterogeneity in this group was again due to the JPK7 control). The mean salivary gland infection rate was 1.3% (4/317) in the experimental flies, which is significantly less than the 4% of the control flies (13/314). This latter difference was largely due to control data from JPK7, which had a high salivary gland rate of 16%. The teneral control flies had a midgut infection rate (61%) that was more than three times greater than the mean infection rate of the experimental flies infected at their second feed. The results of the *T. congolense* experiments are presented in Table II. There was no statistical difference between the experimental and control groups in the three experiments and the data were statistically homogeneous; the pooled infection rates were: *experimental diets*: 15% midgut, 13% hypopharynx; *control flies*: 19% midgut, 12% hypopharynx. Teneral control flies again had infection rates that were over three times greater (66% midgut, 32% hypopharynx) than those of the experimental flies infected at the second feed.

## 4. DISCUSSION

Development of artifical diets to maintain breeding colonies of tsetse for SIT programmes [6] could lead to cost reductions in fly production. It is the practice of tsetse SIT programmes to feed flies one bloodmeal before release in order to reduce the risk of their becoming vectors of sleeping sickness; it is well established that such a strategy inhibits *T. brucei* sl infections developing in tsetse flies. The purpose of this work was to test some of the artificial diets developed at Seibersdorf in order to determine whether they were as effective as whole blood in inhibiting trypanosome establishment in the fly. The results of experiments with seven artificial diets showed that feeding flies once with these diets was as effective in inhibiting *T. b. rhodesiense* infections in *G. m. morsitans* as feeding whole pig blood. The probability of establishment of a midgut infection in a previously fed fly was about one-third that of a teneral fly. Experiments with *T. congolense* produced remarkably similar results to those obtained with *T. b. rhodesiense*. The three artificial diets tested were again as effective as whole blood in inhibiting establishment of midgut infections. These results also show that there is little difference between *T. congolense* and *T. b. rhodesiense* in response to the fed fly — both infections are equally inhibited, producing about one-third of the teneral infections. Until recently this effect was thought to be limited to *T. brucei* group trypanosomes, but Distelmans et al. [7] using *G. p. palpalis* and Mwangela et al. [8] with *G. m. morsitans* have shown that infecting fed flies with *T. congolense*, as with *T. brucei* sl, is more difficult than with teneral flies. Moloo and Kamunya [9] have recently shown that addition of the drug Samorin to the prerelease feed of sterile males can greatly reduce the subsequent infection rate of tsetse flies infected at later feeds. Addition of Samorin to artificial diets before sterile male release would therefore appear to be an effective strategy.

The mechanism underlying the resistance of fed flies to infection with trypanosomes has been the subject of recent research at the Tsetse Research Laboratory, Bristol. It has been shown by inhibition experiments that tsetse flies secrete lectin in the midgut; the effect of this lectin secretion is to agglutinate procyclic trypanosomes, which leads to cell death [10, 11]. Teneral midguts, however, show little lectin activity and it appears that lectin secretion is a response to some serum factor in the bloodmeal. The removal of serum and, in particular, serum lipids had previously been shown to increase the midgut infection rates [12, 13]. We conclude that the effect of feeding flies before infection is to switch on lectin production, which results in active lectin remaining in the midgut when the trypanosomes enter at the infective feed.

## 5. CONCLUSIONS

Our research has shown that artificial diets are as effective as whole blood in inhibiting the establishment of trypanosome infections in tsetse flies. Feeding flies

such artificial diets greatly reduced the probability of flies establishing a trypanosome infection. The strategy of feeding flies before release in SIT programmes is effective in reducing the risk of development of *T. b. rhodesiense* and *T. congolense* infections in tsetse flies.

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# USE OF JUVENILE HORMONE MIMICS IN THE STERILIZATION OF TSETSE FLIES*

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**Abstract**

USE OF JUVENILE HORMONE MIMICS IN THE STERILIZATION OF TSETSE FLIES.

Three juvenile hormone mimics were tested for their ability to sterilize *Glossina morsitans morsitans*. S-31183 (Sumitomo) was the most effective chemical tested, a topical application of 2 μg being sufficient to sterilize a female for life. Furthermore, application of 20 μg of S-31183 to male *G. m. morsitans* was sufficient to sterilize females mating with such males. These juvenile hormones are currently being tested in the field as a replacement for insecticides used in the baiting of targets and traps for tsetse flies.

## 1. INTRODUCTION

Recent developments in the design of targets and traps for tsetse flies in the field have shown that size, shape [1, 2] and colour [3] are all important in determining the effectiveness of such devices. At least for some of the savannah species of tsetse fly, the combination of a visually stimulating trap and the olfactory stimuli provided by acetone, 1-octen-3-ol, carbon dioxide and various phenolic compounds [4–9] greatly enhances the catch. The effect of odours is much less dramatic when tested on riverine tsetse flies of the *palpalis* group [10].

Use of insecticide baited targets into which tsetse flies blunder or upon which they alight has been shown to be highly effective at suppressing natural populations [11–13]. However, theoretical considerations suggest that under certain circumstances the sterilization of both sexes of fly might be a more effective means of population suppression than killing both sexes [14].

Rearing tsetse flies for the sterilization of males followed by their release into the natural environment has been effective in the suppression of natural populations [15, 16]. However, the technique generally requires a drastic reduction in the tsetse population with insecticide before the release of sterile males commences. The

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technique is costly and cannot rely entirely on the resources of the area involved for its implementation. Isolation of the target population can also be very expensive.

Chemical sterilization of tsetse males as a substitute for radiation sterilization before their release was tested on an island at Lake Kariba, Zimbabwe [17]. Later, the combination of a chemosterilant and the sex pheromone of the species on suitable decoys mounted on targets was advocated as a means of sterilizing *Glossina morsitans* males in a natural population [18]. This was followed by the evaluation of chemosterilant baited traps designed to sterilize both sexes of fly [19, 20].

Recent research has suggested that a substitute for highly toxic and carcinogenic thioaziridine insect chemosterilants might be a juvenile hormone mimic. The move towards simple, cheap and locally manageable techniques for tsetse fly control [11, 13, 21] is gaining impetus and it is against this background that an appropriate technique for sterilizing natural populations of the tsetse fly is being developed.

## 2. MATERIALS AND METHODS

Solutions of the juvenile hormone mimics S-methoprene (Zoecon), S-21149 and S-31183 (Sumitomo) were compared for their ability to arrest metamorphosis following topical application to the puparia of *G. m. morsitans*. Adult females at the start of their second reproductive cycle (immediately following their first larviposition) were similarly treated and all subsequent offspring held to determine their viability.

Radiolabelled S-31183 ($^{14}C$) supplied by Sumitomo was used to determine the rate of cuticle penetration and subsequent distribution of the material within the fly and its offspring. Treated insects were surface washed with hexane before dissection. Various tissues were extracted in chloroform:methanol (2:1, vol./vol.) and all extracts evaporated to dryness before subjecting to scintillation counting. Whole insects were crushed and treated with a tissue solubilizer before similarly subjecting to scintillation counting.

Different formulations of S-31183 were tested on filter paper discs, on black cotton cloth or on black terylene netting for their ability to contaminate adult flies by tarsal contact. Flies were either held in contact with a filter paper disc in a glass tube using a cork plunger, or were anaesthetized with $CO_2$ and placed on the treated cloth or netting surface under a Petri dish. Recovery of the flies was rapid as they stood on the treated surface, being unable to grasp the glass surface of the covering dish.

Male flies were similarly exposed to treated surfaces or received topical doses of candidate compounds before mating to normal vigin females. The offspring of these females were then held to determine their viability; this gave an indication of the amount of material transferred from male to female during copulation.

## 3. RESULTS AND DISCUSSION

Some results have already been published [22] from which it is clear that S-31183 is the most potent of the compounds tested. Development within the puparium was arrested after about 20 days of the normal 30 day intrapuparial period. However, treatment was only effective if undertaken during the first 5 days of puparial life. Normally, such an effect upon the pupae of holometabolous insects would not provide an effective means of control, in that most insect pupae are hidden and not easily treated. Nevertheless, the adenotrophic viviparity of the tsetse fly means that treatment of the adult female could lead to the production of non-viable offspring; this is what we found.

A topical dose of 2 $\mu$g S-31183 to an adult female caused her to produce non-viable offspring for the rest of her life. Treatment with the same dose of S-21149 was only effective in the cycle following treatment, while 2 $\mu$g S-methoprene did not suppress the viability of pupae entirely, even in the cycle following treatment.

Provided mating took place only 1 day after treatment a topical dose of 20 $\mu$g of any of the three compounds on a male was effective in that females mated to such males produced non-viable offspring for the rest of their lives.

Appropriate formulations of S-31183 on black cotton cloth, filter paper discs or on terylene netting have shown that doses as low as 20 ng per female are sufficient to induce production of non-viable offspring for life.

A black cotton cloth target dosed with a small amount of radiolabelled S-31183 and 0.05 mg unlabelled S-31183 per square centimetre, equivalent to 0.5 g/m$^2$, resulted in flies picking up between 5 and 15 ng S-31183 in 1 min. Extending the exposure time to 5 min increased the uptake to between 8 and 65 ng, depending on the formulation used. The higher doses were sufficient to cause total sterility among exposed females, while even the lower doses disrupted reproduction for at least one cycle following treatment.

Males exposed to treated cloths and which picked up the highest dose could transfer sterilizing doses to females during mating only if mating occurred immediately after treatment. However, for the purpose of inducing males to transfer sterilizing doses to females, a system is envisaged where flies are channelled through a trap to make contact with a relatively high concentration of active ingredient. Following this, they would have to mate very soon, otherwise the active material would either be absorbed into the body of the fly or would be lost from its surface by friction. Such devices are currently being tested at the Rekometjie Research Station in the Zambezi Valley and on an island at Lake Kariba, Zimbabwe.

Use of juvenile hormones as 'sterilants' for tsetse flies introduces a new concept for the deployment of targets and traps for tsetse fly control. The compound being tested is very stable and has extremely low toxicity. There is every hope that the techniques being tested will be competitive in cost with the more conventional

insecticidal methods and that they will be environmentally more acceptable in that they are non-polluting and more specific for the target organism.

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# STUDIES OF *Glossina pallidipes* AND *G. morsitans* SUBSPECIES RELATED TO THE GENETIC CONTROL OF TSETSE FLIES*

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**Abstract**

STUDIES OF *Glossina pallidipes* AND *G. morsitans* SUBSPECIES RELATED TO THE GENETIC CONTROL OF TSETSE FLIES.

The amount of genetic variation for three taxa, *Glossina morsitans morsitans*, *G. m. centralis* and *G. pallidipes*, was determined in field collected flies and laboratory reared flies using electrophoretic markers. Since sex ratio distortion, resulting in the production of an excess of females, in colonies of *G. m. submorsitans* is controlled by an X chromosome locus, it was possible to set up a breeding programme that resulted in establishing a colony with 50% males. Preliminary evidence suggests that a relationship exists between sex ratio distortion and an electrophoretically detectable esterase (designated EST-Xnull). Hybridization techniques were used to confirm that a tsetse fly colony maintained by the Zambian National Council for Scientific Research was *G. m. centralis*. Studies of the genetic basis of sterility in hybrid males, produced by crossing *G. m. morsitans* and *G. m. centralis*, have been continued and limitations to the proposed use of maternally inherited sterility factors, as agents for genetic control of *G. m. centralis*, have been uncovered.

## 1. INTRODUCTION

Over the past three decades, electrophoretic techniques have contributed greatly to our ability to measure the amount of genetic diversity in animal populations and have provided a relatively simple, accurate method of recognizing many biochemical markers for studying transmission genetics. Electrophoretic techniques have contributed substantially to our understanding of tsetse fly genetics. For example, in *Glossina morsitans morsitans* Westwood, 11 of the 14 loci that have been mapped [1–3] are for electrophoretically detected traits. Electrophoretic techniques have been used extensively in the work reported on here.

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Some published work is reviewed and new data on the genetics of *G. pallidipes* Austen, *G. m. morsitans*, *G. m. centralis* Machado and *G. m. submorsitans* Newstead carried out since 1 April 1985 are reported.

## 2. MATERIALS AND METHODS

Colonies of *G. m. morsitans*, *G. m. centralis* and *G. m. submorsitans* are maintained in the Department of Entomology, University of Alberta, Edmonton. The histories and maintenance of these colonies have been described previously [4], as have the electrophoretic techniques used [5, 6]. In addition to the above colonies, a small colony (ca 90 breeding females) of *G. m. centralis* originating from the main fly belt west of Mumbwa, Zambia, was established at the University of Alberta in July 1987, starting with puparia provided by F. D'Haeseleer of the Rijksuniversitair Centrum, Antwerp. Field collected *G. m. centralis* from the same area were obtained in March 1987, with the assistance of C. Musama and with the support and facilities of the Zambian Tsetse and Trypanosomiasis Branch, Department of Agriculture and Water Development, Lusaka. *G. pallidipes* adults were obtained from L. Otieno and S.A. Tarimo of the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi. *G. pallidipes* and field collected *G. m. centralis* were transported in dry ice from Africa to Edmonton, where electrophoreses were done subsequently by B.M. Rolseth. Details of some of the breeding experiments are provided in the following sections.

## 3. RESULTS AND DISCUSSION

### 3.1. Comparison of field and laboratory tsetse flies

Much of the current research into the physiology, behaviour, vectorial competence, etc. of tsetse flies employs insectary reared flies. In principle, this may be of concern, since continuous maintenance of closed colonies may result in the selection of laboratory adapted strains that differ significantly from flies in natural populations. For tsetse flies this possibility has been examined by comparing *G. m. morsitans* from the Tsetse Research Laboratory, University of Bristol, with material collected in Zimbabwe [7]. Laboratory reared and field collected *G. m. morsitans* did not differ significantly with regard to the average heterozygosity per locus, the average effective number of alleles per locus, or the number of polymorphic loci detected. No significant differences in allele frequencies occurred at 12 of the 13 loci examined. (The exception was the locus for midgut alkaline phosphatase, *Alkph*.)

A preliminary comparison of field collected and laboratory reared *G. pallidipes* has been made by electrophoretic examination of various loci [8]. The

TABLE I. COMPARISON OF *G. pallidipes* FROM THE ICIPE COLONY AT MBITA POINT WITH THOSE FROM THE LAMBWE VALLEY, KENYA

| Locus[a] | Allele | Mobility[b] | Mbita Point flies | Lambwe Valley flies |
|---|---|---|---|---|
| *Odh* | *s* | 0.67 | 0.52 | 0.63 |
| | *f* | 0.72 | 0.48 | 0.37 |
| | | | (0.499)[c] | (0.466) |
| *Est-1* | *2* | 0.48 | 0.38 | 0.18 |
| | *3* | 0.51 | 0.50 | 0.78 |
| | *4* | 0.55 | 0.12 | 0.04 |
| | | | (0.591) | (0.358) |
| *Me* | *s* | 0.15 | 0.96 | 0.93 |
| | *f* | 0.17 | 0.04 | 0.07 |
| | | | (0.077) | (0.130) |
| Average heterozygosity/locus (H)[d] | | | 0.194 ±0.112 | 0.159 ±0.084 |

[a] Monomorphic loci: *Apk*, $R_f = 0.86$; *Mdh*, $R_f = 0.20$; *Gpd-2*, $R_f = 0.46$.

[b] Electrophoretic mobility relative to the bromophenol blue front ($R_f$) is given for the band produced by the allele indicated.

[c] Values in parentheses are heterozygosity (h), calculated from the formula $h = 1 - (a^2 + b^2 + \ldots)$, where a, b, etc. are the frequencies of the alleles.

[d] H, the average heterozygosity per locus and its standard deviation, was calculated using Eqs (6.5) and (6.6) from Nei [8].

significance of these data is limited by the small number of loci examined. Nonetheless, the data indicated that the ICIPE colony at Mbita Point has slightly more genetic variation than does the field population from which the colony was established (Table I). Although decreases in heterozygosity in laboratory colonies are to be expected, increases have been reported [9]. A more extensive comparison of field and laboratory *G. pallidipes* has been undertaken by S.A. Tarimo and a manuscript in preparation will present evidence that there are significant genetic differences between some field population of *G. pallidipes*, but that the Mbita Point colony is similar to field caught flies from the Lambwe Valley, Kenya.

TABLE II. COMPARISON OF *G. m. centralis* FROM THE UNIVERSITY OF ALBERTA's ZAMBIAN COLONY WITH THOSE FROM THE KAFUE GAME MANAGEMENT AREA, ZAMBIA

| Locus[a] | Allele | Mobility[b] | Colony flies | Field flies |
|---|---|---|---|---|
| *Gopd* | *b* | 0.23 | 0.00 | 0.01 |
| | *c* | 0.24 | 1.00 | 0.99 |
| | | | (0.000)[c] | (0.020) |
| *Est-1* | *c* | 0.51 | 1.00 | 0.99 |
| | *d* | 0.54 | 0.00 | 0.01 |
| | | | (0.000) | (0.020) |
| *Xo* | *z* | 0.20 | 0.00 | 0.02 |
| | *a* | 0.23 | 0.98 | 0.66 |
| | *c* | 0.27 | 0.02 | 0.26 |
| | *d* | 0.30 | 0.00 | 0.06 |
| | | | (0.039) | (0.493) |
| *Ao* | *a* | 0.34 | 0.24 | 0.10 |
| | *c* | 0.38 | 0.76 | 0.81 |
| | *d* | 0.41 | 0.00 | 0.10 |
| | | | (0.365) | (0.324) |
| *Odh* | *c* | 0.48 | 0.00 | 0.03 |
| | *d* | 0.56 | 0.83 | 0.95 |
| | *e* | 0.64 | 0.17 | 0.02 |
| | *f* | 0.69 | 0.00 | 0.01 |
| | | | (0.282) | (0.096) |

TABLE II (cont.)

| Locus[a] | Allele | Mobility[b] | Colony flies | Field flies |
|---|---|---|---|---|
| *Est-2* | *a* | 0.29 | 0.93 | 0.42 |
| | *b* | 0.30 | 0.00 | 0.05 |
| | *c* | 0.32 | 0.07 | 0.35 |
| | *d* | 0.35 | 0.00 | 0.18 |
| | | | (0.130) | (0.666) |
| Average heterozygosity/locus (H)[d] | | | 0.074 ±0.039 | 0.147 ±0.072 |

[a] Monomorphic loci: *Mdh-2*, $R_f = 0.15$; *Apk*, $R_f = 0.86$; *To*, $R_f = 0.40$; *Gpd-2*, $R_f = 0.46$; *Mdh-1*, $R_f = 0.29$.

[b] Electrophoretic mobility relative to the bromophenol blue front is given for the band produced by the allele indicated.

[c] Values in parentheses are heterozygosity (h), calculated from the formula $h = -(a^2 + b^2 + \ldots)$, where a, b, etc. are the frequencies of the alleles.

[d] H, the average heterozygosity per locus and its standard deviation, was calculated using Eqs (6.5) and (6.6) from Nei [8].

*G. m. centralis* collected in the main fly belt west of Mumbwa, Zambia (Table II) [8], show about the same amount of heterozygosity as was seen in *G. m. morsitans* from Rekometjie, Zimbabwe [7], and considerably more variation than was observed in the *G. m. centralis* colony of Zambian origin in Edmonton (Table II). The colonized flies show signs of inbreeding in that they have lost several rare alleles and have a lower heterozygosity than do flies collected in Zambia. Significant differences in the number of gene products observed occurred at the loci for xanthine oxidase (*Xo*), aldehyde oxidase (*Ao*), octanol dehydrogenase (*Odh*) and a thoracic esterase (*Est-2*). This is further indication that genetic drift or selection has taken place in this laboratory colony.

*G. m. centralis* collected by three methods (i.e. in a vehicle, by fly round using a black screen and in F3 traps) in the western fly belt in Zambia did not differ significantly in allele frequencies, nor in the amount of genetic variation in each sample [10]. However, *G. m. centralis* collected from the population at Keembe, Zambia (which is presumably isolated from the western fly belt), had a lower heterozygosity and differed from flies in the western fly belt with respect to allele frequencies at three loci [10].

TABLE III. SELECTION OF A *G. m. submorsitans* COLONY (ORIGINATING FROM BURKINA FASO) FOR A 1:1 SEX RATIO[a]

| Generation | % males | % distorters[b] |
|---|---|---|
| Parental | 38.2 | 24.4 |
| $F_1$ | 37.7 | 23.3 |
| $F_2$ | 43.4 | 11.5 |
| $F_3$ | 45.6 | 3.8 |
| $F_4$ | 45.9 | 0.0 |
| $F_6$ | 48.2 | |
| $F_8$ | 50.8 | |
| $F_{10}$ | 50.8 | |
| $F_{12}$ | 48.8 | |
| $F_{13}$ | 51.0 | |

[a] Selection, as described in text, terminated after $F_4$.
[b] Distorter males were those that sired families with fewer than 20% males.

### 3.2. Sex ratio distortion in *G. m. morsitans*

In most species of tsetse fly, like most other animals, there are approximately equal numbers of males and females. However, in colonies of *G. m. submorsitans* from Burkina Faso [4, 11, 12] and from Nigeria [4, 11–13] there is sex ratio distortion, resulting in an excess of females. Sex ratio distortion appears to be a trait expressed by males. In both colonies, males sire families with an approximately equal number of males and females, or families in which there are only (or nearly only) females [12, 13]. By breeding experiments it was shown that sex ratio distortion is controlled by a locus (or loci) on the X chromosome and a breeding programme for the establishment of a colony of *G. m. submorsitans* with a 1:1 sex ratio was proposed [12]. Table III shows the results of such a selection programme; each generation consisted of about 60 mated females. Flies for the parental generation came from the *G. m. submorsitans* colony originating from Burkina Faso. For the parental generation and the next four generations one male was placed in a cage with five or six virgin females. Puparia were collected and the emerging adults were

sexed. Those families with more than 20% males were retained to establish the subsequent generation. As shown in Table III, the frequency of distorter males declined over the first four generations. Selection was terminated at this point and the colony has been maintained as a closed colony with approximately equal numbers of males and females.

In an independent experiment a colleague, B.M. Rolseth, was selecting a colony for a thoracic esterase (designated EST-Xf, mobility = 0.14 on 9% polyacrylamide gel at pH 8.9) that is apparently controlled by an X chromosome locus. By the time the EST-Xf colony was established it had a 1:1 sex ratio. This suggested an association (or close linkage) between EST-Xf and the ability of males to sire both sons and daughters. We therefore examined *G. m. submorsitans* males from our colony of Nigerian origin for both sex ratio distortion and esterase-X. Each male was allowed to mate with five or six females from the same colony and then was electrophoresed. At least 15 puparia were collected from each 'family' and the emerging adults sexed. Five males that had EST-Xf were able to sire sons and daughters, while 10 males with EST-Xnull (i.e. having little or no staining for EST-X) sired families with fewer than 20% males. Only one male with EST-Xnull sired a significant number of sons (11 sons and 6 daughters), and no males were found having EST-Xf and the distorter trait. Even though the number of flies tested was small, the results show a strong association between EST-Xnull and sex ratio distortion. We are continuing experiments to determine whether the thoracic esterase EST-Xnull and sex ratio distortion are causally linked (i.e. EST-Xnull causes males to sire daughters only, while EST-Xf permits males to sire sons and daughters), or whether the loci for EST-X and sex ratio distortion are simply closely linked on the X chromosome.

### 3.3. Use of hybridization to confirm the taxonomic status of colonized flies

Since the pioneering work of Vanderplank [14], and its confirmation by Curtis [15], it has been known that hybrid males produced by crossing *G. m. morsitans* and *G. m. centralis* are sterile. Use has been made of this phenomenon to confirm the taxonomic identity of *G. m. centralis* maintained by the Zambian National Council for Scientific Research (NCSR), Lusaka.

During a visit to Zambia in March 1987, discussions were held with the staff of the NCSR concerning the identity of one of the tsetse fly colonies being maintained at Chilanga. This colony originated from material collected near Mumbwa and is believed to be *G. m. centralis*, even though the colony is not thriving in a manner typical of *G. m. centralis*. During these discussions it was decided that a simple way of confirming the taxonomic identity of the flies would be to hybridize flies from the Chilanga colony with flies that are known to be *G. m. centralis*.

In March 1987, 104 puparia from the NCSR colony were taken to Antwerp, where part of this study was done at the Prince Leopold Institute for Tropical Medicine. Forty females and 44 males emerged; two adults partially emerged, one

TABLE IV. HYBRIDIZATION TEST OF THE TAXONOMIC IDENTITY OF THE NCSR *G. m. centralis* COLONY

| Cross[a] | No. of females fertile/tested | No. of $F_1$ | | | No. of $F_1$ males | |
|---|---|---|---|---|---|---|
| | | Puparia[b] | Males | Females | Tested | Fertile |
| EDM × EDM | NR 3/15[c] | 67 | NR | NR | 0[d] | — |
| NCSR × NCSR | 0/8[e] | 0 | 0 | 0 | — | — |
| NCSR × RUCA | 2/11 | 6 | 6 | 0 | 5 | 5 |
| EDM × NCSR | NR/37 | 92 | 36 | 30 | 26[f] | 24[f] |

[a] Females are designated first in each cross. See text for origin of the colonies.

[b] Puparial production was over a 7 week period, except for NCSR × RUCA, which were kept for 8 weeks.

[c] NR = not recorded.

[d] No males were tested, but all the males from the EDM colony were fertile when tested with EDM females [4].

[e] Only two females survived for 6 weeks; neither had motile sperm.

[f] Females mated to the two infertile males had no motile sperm in their spermathecae.

fully formed adult died within the puparium and 17 flies died at an early stage in the puparium. Puparia which failed to produce adults were all rather small.

Mated flies were pooled, with others of the same sex, and maintained by feeding daily on rabbits. Early deaths were common among the NCSR flies, but such deaths were not observed among the other *G. m. centralis* maintained in the same insectary. Therefore, it seems that the early deaths were not due to environmental conditions that are uniformly adverse to *G. m. centralis*. The average age, at the time of death, of females that died at an early age was 2.8 days, while for males it was 3.4 days. These results may be slightly biased for a greater age at death among males because males were maintained individually for longer than were females.

Flies from three colonies were used in the hybridization study; these are designated as follows: NCSR = flies from the Chilanga colony of the NCSR referred to above; RUCA = *G. m. centralis* from a colony (of Zambian origin) maintained by F. D'Haeseleer, Rijksuniversitair Centrum, Antwerp; EDM = *G. m. centralis* (of Tanzanian origin) maintained in the Department of Entomology, University of Alberta, Edmonton. $F_1$ males were tested to see whether they were fertile by mating each male with two (occasionally three) EDM females. The females were held until they reached the black lobed stage of the pregnancy cycle. Those females which did

not show signs of being pregnant by 28 days after mating were dissected and the spermathecae examined for motile sperm (at 125 magnifications).

Difficulties were encountered in setting up the NCSR × NCSR cross because of a lack of mature males from this colony at a time when young, receptive females were available. Eighteen NCSR males were mated to a total of 37 EDM females. The puparia were shipped by air to Edmonton, where 26 $F_1$ males were tested.

The fertility of the $F_1$ males (Table IV) indicates that the NCSR colony is *G. m. centralis*. The high mortality among young NCSR flies, when maintained under conditions that were satisfactory for other *G. m. centralis*, and the failure of most of the NCSR females to become pregnant may indicate problems with the health of the NCSR colony. The unexpected sex ratio distortion (6 males:0 females) in the $F_1$ produced by the NCSR × RUCA cross may have resulted from random events, or may indicate a genetic abnormality in one or other of these colonies. The sex ratio of $F_1$ flies from the EDM × NCSR cross (36 males:30 females) was normal. Aside from the abnormal $F_1$ sex ratio mentioned above, the only other anomalous result suggesting differences between the NCSR colony and the EDM colony was the low productivity of the EDM × NSCR cross compared with the productivity of the EDM × EDM cross. Although the difference in productivity is not statistically significant, it is suggestive.

### 3.4. Hybrid sterility

It is well known that hybrid females produced by crossing *G. m. morsitans* and *G. m. centralis* are fertile and may be backcrossed to either subspecies [15–17]. Sterility among the backcross males involves interaction of the X chromosome from one subspecies and either the Y chromosome from the other subspecies [17, 18] or possibly the autosomes from the other subspecies [16]. Some of the grandsons of *G. m. morsitans* females are able to inseminate both *G. m. morsitans* and *G. m. centralis*, but none of the inseminated *G. m. centralis* produces viable offspring [17]. These results were interpreted as indicating that a maternally inherited (i.e. extra chromosomal) factor is passed from *G. m. morsitans* females to their offspring and that this factor prevents backcross males from fertilizing *G. m. centralis*, but not from fertilizing *G. m. morsitans*. It was proposed [17] that if the maternally inherited sterility factor were stable it would be possible to establish an anti-*centralis* colony by crossing *G. m. morsitans* females with *G. m. centralis* and then backcrossing females from subsequent generations to *G. m. centralis*. Within a few generations a colony would be established in which all the chromosomal genes come from *G. m. centralis*, but the maternally inherited sterility factor from *G. m. morsitans* would prevent males in the colony from fertilizing *G. m. centralis*. This colony could then be used as a source of males for the genetic control of *G. m. centralis*.

A preliminary experiment to test whether males from the third backcross generation (of the breeding programme outlined in the preceding paragraph) can sup-

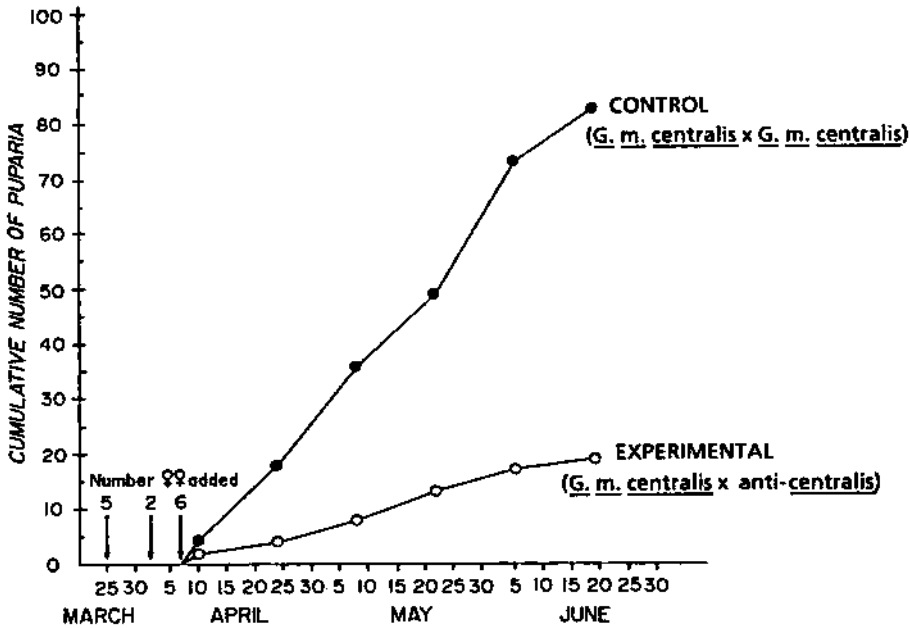


*FIG. 1. Puparial production by* G. m. centralis *females after mating with third generation anti-*centralis *males, or after mating with* G. m. centralis *males.*

press reproduction of *G. m. centralis* was conducted as follows. Thirty males were tested for their ability to inseminate *G. m. centralis*; 17 were non-inseminators. The 13 inseminators were mated a second time with *G. m. centralis*. These females were maintained in the usual manner to see if they became pregnant. One was fertilized and the others were not, indicating that 12 of the 13 males carried the maternally inherited sterility factor. Each male was mated a third time with *G. m. morsitans* and all of these became pregnant.

The fourth mate for each male was a *G. m. centralis* female. Immediately after each pair uncoupled, the female was transferred to a cage with several *G. m. centralis* males and was left with the males for 3–4 weeks. At the same time, a control cage consisting of *G. m. centralis* males and females was set up, using the same procedure.

*G. m. centralis* females that were first mated with third generation anti-*centralis* and then placed with *G. m. centralis* males did not produce as many offspring as did the control females (Fig. 1). It is believed that most of the puparia in the experimental population are from the female that mated with the male who was

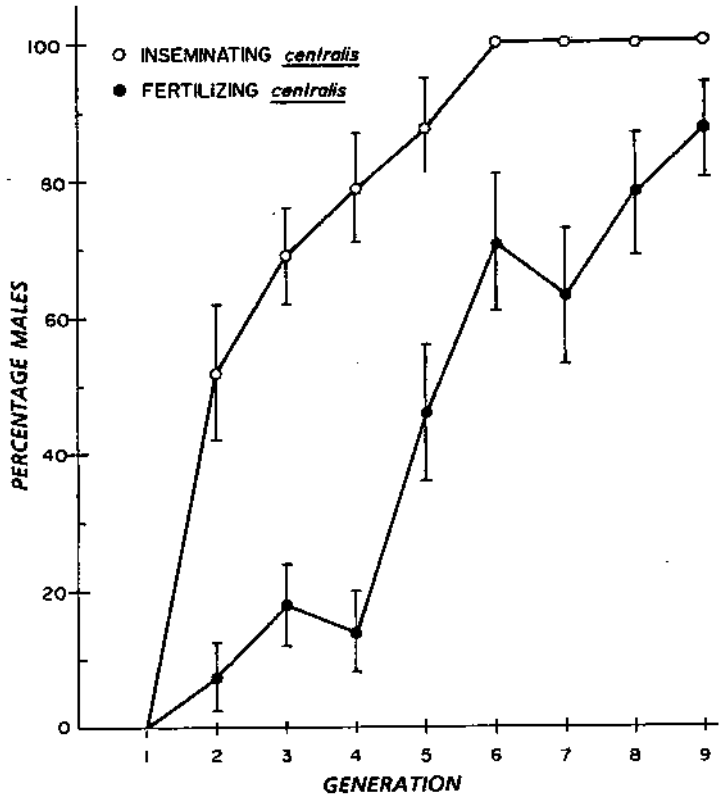


*FIG. 2. Ability of* G. m. morsitans/G. m. centralis *backcross males (from nine generations of recurrent mating to* G. m. centralis*) to inseminate and fertilize* G. m. centralis *females.*

not carrying maternally inherited sterility factors. The results indicated that maternally inherited sterility factors would enable us to create an anti-*centralis* line containing mainly genes from *G. m. centralis* and that males from this line significantly reduce the fecundity of any *G. m. centralis* females with which they mate.

The proposal to establish an anti-*centralis* colony requires that the maternally inherited sterility factor(s) be stable in the presence of *G. m. centralis* chromosomes over many generations. To determine whether this is the situation, the proposed breeding programme was undertaken, beginning with genetically marked *G. m. morsitans* and *G. m. centralis*. The X chromosomes carried markers for both eye and body colour and it was therefore possible, in each generation, to choose males that had an X and a Y chromosome from *G. m. centralis* and to test these males for their ability to inseminate and to fertilize *G. m. centralis*. (This was done by mating each

male with two *G. m. centralis* females and holding each female until she reached the black lobe stage of the pregnancy cycle, or for 28 days, at which time her spermathecae were examined for motile sperm.) The results (Fig. 2) show that the maternally inherited sterility factor was not stable and therefore its potential as a sterilizing agent for use against *G. m. centralis* declined as the females in each generation of the anti-*centralis* colony were backcrossed to *G. m. centralis*.

## 4. CONCLUSIONS

Electrophoretic procedures have been developed for assessing the genetic variability in field and laboratory populations of tsetse flies and for monitoring quality in laboratory colonies. Use of these techniques has been advocated and outlined in several publications [1, 6, 7, 11]. Comparisons of the genetic variation in laboratory and field populations have been conducted for three species (Ref. [17] and Tables I and II in this paper). The results are consistent with the generally accepted view that genetic variation can be maintained at close to normal levels (i.e. those of field populations) in reasonably large colonies.

The Advisory Group [19] recommended applying electrophoretic (and other) techniques to the search for cryptic speciation and assortative mating in natural populations. Electrophoretic techniques have been developed to the point where this is feasible for tsetse flies. A limited study along these lines was conducted with *G. m. centralis* in Zambia and no evidence was found that different sampling methods were collecting flies from different subpopulations [10].

Demonstration that sex ratio distortion in *G. m. submorsitans* is controlled by one or more X chromosome loci [12] led to development of a colony with a stable 1:1 sex ratio rather than an excess of females (Table III). Such a colony ought to be useful in any sterile male release programme directed against *G. m. submorsitans*.

The main objectives of these studies of tsetse genetics are to determine the barriers to gene flow between closely related species and subspecies, and to determine the extent to which genes from one taxon may be incorporated into the genome of another. The work has mainly been carried out with *G. m. morsitans* and *G. m. centralis* (Refs [4, 17] and Figs 1 and 2 in this paper). Limited work also involved hybridizing *G. m. morsitans* and *G. m. submorsitans* (Ref. [4] and unpublished data) and the study has been extended to include hybridization of *G. p. palpalis* Robineau-Desvoidy and *G. p. gambiensis* Vanderplank.

In the *morsitans* group, hybrid male sterility involves both genic [16–18] and maternally inherited factors [17]. The latter seemed to offer the simplest method of developing an anti-*centralis* strain. Third generation males from such a line were capable of suppressing the fecundity of *G. m. centralis* females (Fig. 1). However, the maternally inherited sterility factor appears to be unstable (Fig. 2) and therefore the original proposal to establish an anti-*centralis* strain will need to be modified or abandoned.

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