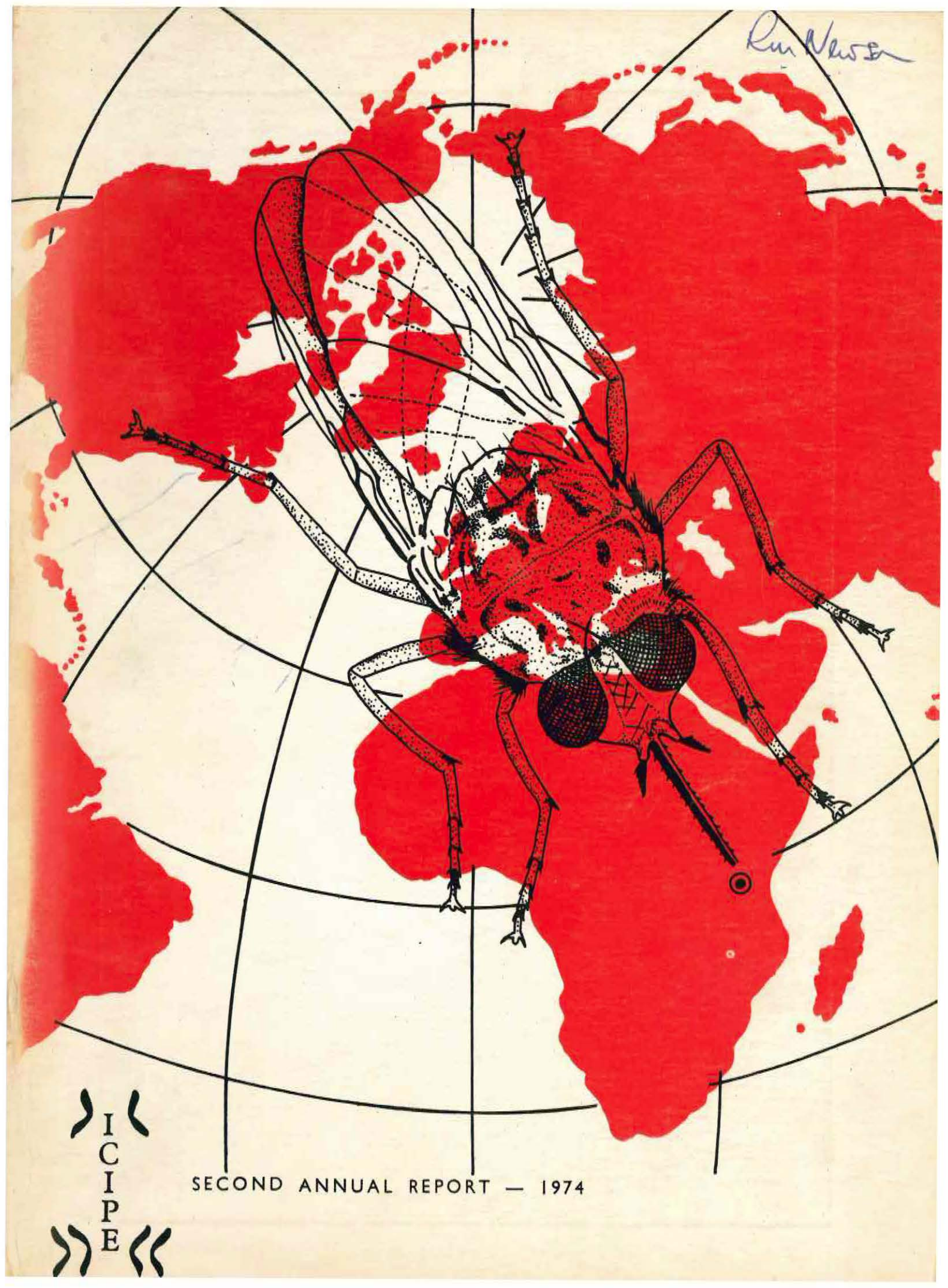

ICIPE

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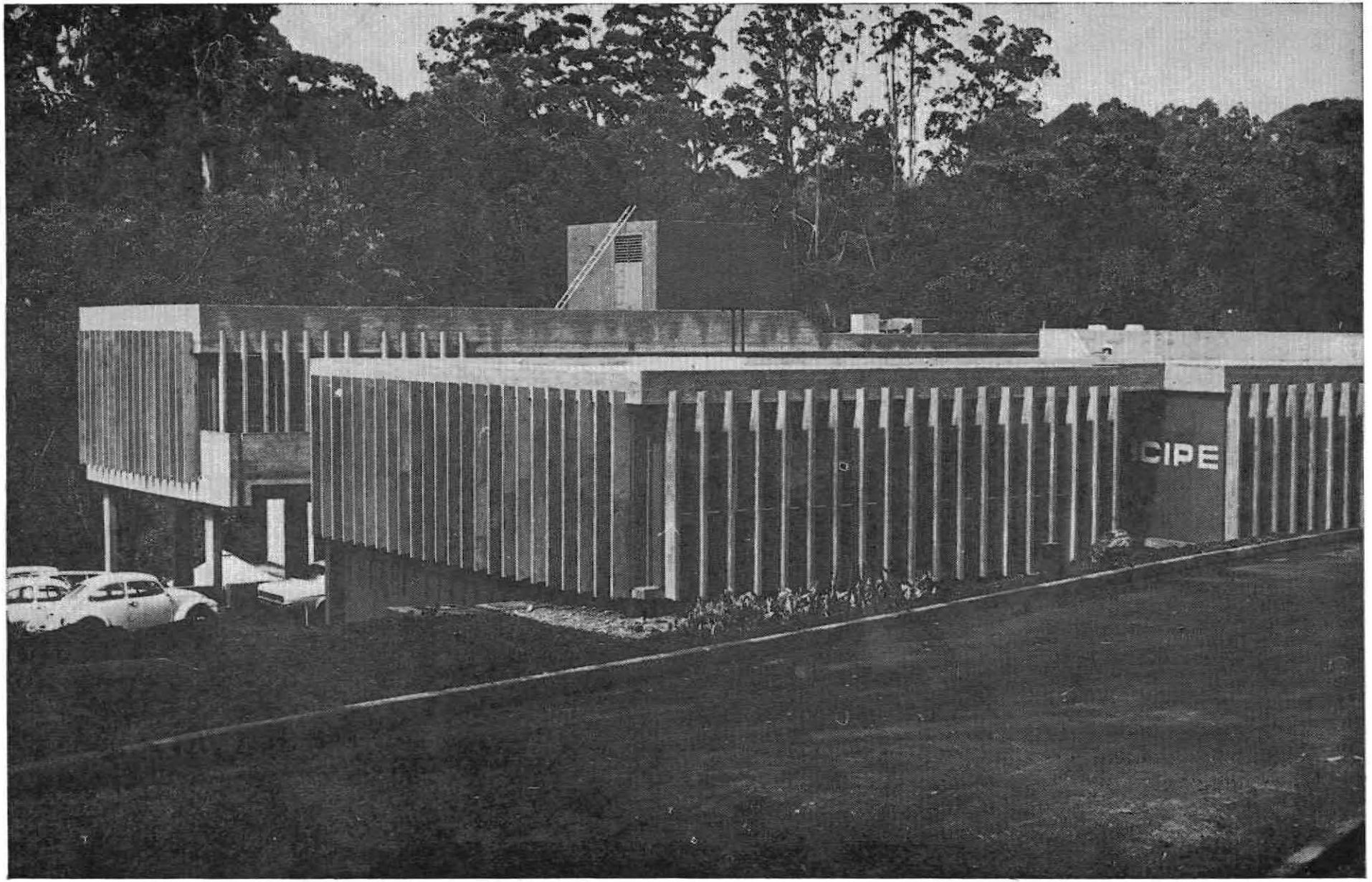
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THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

SECOND ANNUAL REPORT

1974

Nairobi, October 1974

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Dr. Mohammed Chaudhury	Bagladesh	1.3.74	Insect Development and Reproductive Physiology
Dr. David Denlinger	U.S.A.	1.3.72–30.6.74	Aestivation-Diapause
Dr. Dean Fanara	U.S.A.	1.12.71–30.11.73	Mosquito Biology Unit
Dr. Walter Hausermann	Swiss	1.1.71–31.8.74	Mosquito Biology Unit
Dr. Tawfik Hefnawy	Egyptian	12.4.74	Tick
Dr. Christopher J. Heather	British	9.2.74	Fine Structure
Dr. Manfred Kaib	German	1.9.73	Electrophysiology
Dr. George Karuhize	Ugandan	16.6.72	Electrophysiology
Dr. Syed Khasimuddin	Indian	1.12.73	Armyworm
Dr. Isao Kubo	Japanese	17.5.74	Chemistry
Dr. Reinhard Leuthold	Swiss	1.10.71–30.8.74	Termite
Dr. Nancy L. Lorimer	U.S.A.	1.6.74	Mosquito Biology Unit
Dr. Philip Lounibos	U.S.A.	15.7.74	Mosquito Biology Unit
Dr. Frederick Mathez	Swiss	28.1.73	Termite
Dr. Asafu Maradufu	Tanzanian	1.3.74	Chemistry
Dr. Wei Chun Ma	Dutch	1.5.72	Electrophysiology
Dr. Paul McDonald	U.S.A.	1.9.71–23.5.74	Mosquito Biology Unit
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Dr. Fidelis Ogah	Nigerian	1.10.73	Genetic Variability
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Ir. Paul Scheltes	Dutch	1.10.71	Insect Development and Reproductive Physiology
Dr. Julian Shepherd	U.S.A.	1.2.73–31.8.74	Insect Development and Reproductive Physiology
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Sister Mary Leahy	U.S.A.	1.10.73–31.5.74	Tick
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Professor M. P. Pener	Israel	13.2.73–15.11.73	
Dr. Athony Youdeowei	Nigeria	1.7.74–30.9.74	Salivary Gland Physiology
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Mr. John Kawooya	Ugandan	1.9.73	Insect Development and Reproductive Physiology
Mrs. Christine K. A. Mango	Kenyan	1.1.71	Tick
Mr. Kenuel Ogwaro	Ugandan	1.9.73	Insect Development and Reproductive Physiology
Mr. Joseph Owor	Ugandan	1.12.73	Fine Structure
Mr. Daniel Punyua	Kenyan	1.9.73	Tick
Miss Lina Sequeira	Kenyan	1.2.72	Fine Structure
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RELEVANT RESEARCH

1974 can be regarded as the year in which the ICIPE Research Centre reached its critical mass, after two years of steadily building up the scientific, technical, and other supporting staff. It can also be regarded as the year in which the ICIPE began to make a real impact on the major objective we set ourselves — to identify vital gaps in our knowledge and make new discoveries that might lead eventually to novel, ecologically acceptable methods of insect pest control. In at least three areas of our major research programmes — on ticks, tsetse, and termites — we have pushed back the boundaries of our ignorance to a considerable extent. It is our intent to pursue these avenues of research, and to open other avenues in other research programmes, in the coming year.

A significant development in the year under review is the decision to mount a new programme on the Sorghum Shootfly. The decision to do so was taken by the Governing Board in April 1974, as a result of the strong recommendations of the Policy Advisory Committee. The decision is a significant one in three main respects: firstly, this is the first new programme to be adopted since the operational establishment of the Centre three years ago; secondly, the impetus for the decision came from a sister organization, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), based in Hyderabad, India, and not from within the ICIPE itself; and, finally, this particular target insect pest is of much more economic significance in India and other countries than in Africa itself (although it is important here also). This new commitment has therefore demonstrated the internationality of the ICIPE and its readiness to carry out periodic reviews of its programmes.

The International Committee took the initiative to have the research and training programmes of the ICIPE reviewed by an independent international review panel. The panel started its work in October 1974; and its final report, containing its recommendations, will be considered in June 1975. This review is the first of such critical reviews the ICIPE intends to invite from time to time. This initial event is therefore being awaited with great anticipation, and it is likely to signal the beginning of the maturation process of the ICIPE Research Centre.

Thomas R. Odhiambo

ICIPE Research Centre,
Nairobi,
17th April 1975.

2

CENTRAL ADMINISTRATION

The period between September 1973 and September 1974 has been packed with activities. The ICIPE acquired the services of Mr. J. H. Jivanjee who was appointed Financial Manager in December 1973. The Deputy Director, Science, Dr. J. Strangways-Dixon was appointed in September 1974.

During the same period the building contractors moved onto the site at Chiromo to start work on "The Northern Star" Building. Due to world-wide shortage of steel the work was delayed and the estimated date of completion, that is May 1974 was not achieved. Nevertheless the rented accommodation in Siaya (formerly Surrey) Road was vacated at the end of May, and the ICIPE Staff moved back to Chiromo. The Administration moved into block one of the new building and for the first time in two years the Administration and the Scientific Staff were reunited at the ICIPE Headquarters. The builders continued to work around the staff — painting, chipping and fixing things.

The Government of Kenya granted four hectares out of forest land at Langata to the ICIPE and negotiations for land at Kajiado for the establishment of a field station resulted in a ten acre plot being included (for ICIPE) in the Kajiado development plan. Accommodation for a scientist working on the Sorghum Shootfly was arranged with the Cotton Research and Sugar Cane Research Officers at Kibos, near Lake Victoria.

A number of important meetings took place:

(a) *Governing Board Meetings:* The Governing Board held its 20th (Special) Meeting on 15th January 1974 at the Centre and the 21st Meeting was held in Nairobi on 21st April 1974. The 22nd Meeting was held on 6th and 7th September 1974 in Munich.

(b) *Policy Advisory Committee:* The Policy Advisory Committee held its second meeting at Mombasa where the Mosquito Biology Programme was closely scrutinised, from 19th to 20th April 1974.

(c) *African Committee:* The African Committee held its 5th Meeting at Ibadan, Nigeria on 28th April 1974. The Second ICIPE Scientific Meeting organised jointly by the African Committee and the Agricultural Research Council of Nigeria (A.R.C.N.) was held at the A.R.C.N. Headquarters (Moore Plantation) from 25th to 29th April and was officially opened by the Honourable Commissioner for Agriculture and Natural Resources (Western State) on the 26th April 1974.

(d) *The International Committee:* The International Committee held its 4th meeting on 7th and 8th September 1973 at the Royal Danish Academy of Letters and Science, Copenhagen and the 5th meeting on 5th and 6th September 1974 in Munich.

(e) *The Long-Range Finance Committee:* The Long-Range Finance Committee held its 1st meeting in Nairobi on 18th April 1974 and its 2nd meeting in Vienna on 30th August 1974.

(f) *Resident Science Council:* This Council continued to meet monthly and throughout the period of reporting twelve meetings were held.

(g) *Miscellaneous:* During the period 7 Research Scientists, 3 Experimental Officers joined the ICIPE while 6 Scientists left after the completion of their assignments. The ICIPE said farewell to Mrs. L. de Graft after a distinguished service of nearly five years. The Clerk of Works, Mr. Berry and the Communications Officer Mrs. Nancy Donovan also left. At least three hundred persons visited the Centre.

The period may well be described as being one of growth and consolidation. The spirit of togetherness continued to characterise the Icipeism.

AFRICAN COMMITTEE OF I.C.I.P.E.

The African Committee of I.C.I.P.E. in reviewing its role in the context of the overall objectives of the I.C.I.P.E., noted that the principal objectives of the I.C.I.P.E. in the field of research and education are as follows:

(i) To promote and carry out high quality and advanced research in various fields of insect science,
(ii) To provide advanced training in research,
(iii) To provide an international forum for the discussion and exchange of knowledge in insect science, and
(iv) To foster and promote the growth of the scientific community in tropical Africa.

The African Committee, in considering its role in the execution of these broad objectives appears to wish to concentrate on the activities that would ensure the relevance of the philosophy and objectives of I.C.I.P.E. to African problems. It was considered that while I.C.I.P.E. would remain an internationally reputable multidisciplinary research centre where scientists from different parts of the world could interact in the search for knowledge, in the long run its future development and significance of its contribution must be firmly rooted in Africa. The Committee feels that a good beginning has been made in the selection of target insect species which are of continental and considerable economic significance in human health and welfare. But considerable emphasis must be given to the contemporaneous development of the expertise that would provide continuity in Africa and relate programmes to Africa in a proper ecological context. The Committee considered that one of the ways in which this can be achieved is by the designation or appointment of African Directors of Research and the establishment of outreach programmes in different parts of the continent.

It is obvious that such African Directors of Research would not have funds of their own to organise and execute research programmes. It is, therefore, important that I.C.I.P.E. should seek funds to support such research which may not necessarily be based in Nairobi but which would have relevance to the understanding of the ecology of the target species in other parts of the continent. Programmes which appear amenable to such arrangements include:

(i) Studies on the ecology of tsetseflies in the Senegal, Niger and Benue valleys and in the savanna of West Africa,
(ii) Studies on the ecology and behaviour of the armyworm and stemborers in Central and Western Africa, and
(iii) Studies on the ecology of termites in other types of agro-ecosystems in the derived savanna and true savanna areas of the continent.

African Directors of Research could also concentrate on areas of applied entomology research and in such cases provide a vital link between the development of species—specific, non-toxic and non-persistent methods of pest management from physiological and behavioural research and the feasibility and application studies in the various ecological zones of the continent. The laboratories and field facilities of such Directors could become the nucleus of sub-centres where meaningful training and research for young African scientists could also take place, and from where qualified research workers could be nominated for periods of service at I.C.I.P.E. In all, it would seem desirable that continuity in research be maintained in preference to piece-meal contributions to continental problems.

The Committee was of the opinion that it should provide the main vehicle for the growth and development of a scientific community in Africa. It proposes to achieve this by forging closer links between young associations, academies or bodies in different regions of Africa and by organising review meetings, symposia and workshops that would focus attention on continental or regional problems in insect science and recommend a framework for collaborative effort in solving such problems through the application of science and technology.

The Committee is of the opinion that effective working links should be established with existing institutions in different parts of Africa. Such links would stimulate the interest of young African scientists in the programmes of I.C.I.P.E. and facilitate collaborative effort in research. I.C.I.P.E should take the initiative in this regard. African Scientific Organisations, even those not yet organised as academies, should also become associated with I.C.I.P.E. and function by stimulating African scientific interest in the Centre and by promoting national and international cooperation with regard to the programmes and Finances of the Centre.

T. Ajibola Taylor,
Chairman, African Committee.

28th November, 1974.

4

TRAINING

During the period of reporting a certain amount of training work was undertaken, both locally and overseas. A number of Technicians, and Experimental Officers were awarded training fellowships to enable them to acquire skills in laboratories abroad. A number of Technicians and Junior Technicians enrolled with the Kenya Polytechnic in Nairobi.

There was collaboration with a number of Directors of Research who offered training facilities for ICIPE staff in their own laboratories for periods varying from one month to one year.

The Centre also received and trained a Technician from Zambia and a scientist from Ibadan in tsetse rearing techniques. The University of Nairobi accepted and registered one of the ICIPE staff for a Ph.D. course.

It is expected that the training programme will be intensified and that the ICIPE will play a much bigger role in the next few years.

5

AFRICAN ARMYWORM RESEARCH

Spodoptera exempta

Director of Research:
Professor J. W. S. Pringle (1970)

Scientists:
Dr. D. J. Aidley (1973–1974)
Dr. S. Khasimuddin (1973)

Collaborators:
Dr. Wei-Chun Ma (1972)
Electrophysiology
Dr. A. Maradufu (1974)
Chemistry
Dr. S. Takahashi (1973–1974)
Chemistry
Miss M. L. Sequeira (1970)
Fine Structure

Abstract

The African armyworm, *Spodoptera exempta*. has long been known as a major pest of graminaceous crops and pasture grasses in Eastern Africa, ranging from South Africa to Northern Ethiopia and even, in some years, into the Yemen. It has also been recorded from other parts of Africa, from Europe and across Asia and Pacific to Hawaii. Its widespread geographical distribution, its sporadicity of occurrence in space and time but with a distinct south to north trend following the tropical rain fronts, its existence in two forms, gregarious and solitary, all led to early theories of migration. However, the occurrence of small numbers of adults in light traps throughout the year in many parts of its range supported the alternative hypothesis of outbreaks arising from resident populations of the solitary form when favourable environmental conditions prevailed. The operation by the East African Agriculture and Forestry Research Organization (EAAFRO) of a network of light traps throughout Eastern Africa, coordinated by Mr. Eric Brown from 1960 onwards, with results analysed in conjunction with meteorological data by Rainey and Betts, virtually confirm the migration theory, although the role of resident populations still needs elucidation.

Research on this species at ICIPE started in 1973 with the dual objective of obtaining further information which would assist and improve the accuracy of the warning service operated by (EAAFRO) at Muguga and of elucidating in more detail the behaviour of the insects with particular reference to feeding, growth, flight capacity and reproduction, in order to identify features of its life history which might be susceptible to forms of control other than chemical spraying. This work has achieved the following preliminary results:

1. The female moth is non-selective with regard to the species of plant on which she oviposits.
2. Only plants of the families Graminaceae and Cyperaceae are acceptable as food for the larval stages. The neonate larvae escape from unsuitable substrates by hanging from a silken thread which, after breaking gives them a certain buoyancy in air; in the typical range-land habitats of the armyworm, larvae have a good chance of landing on wild grasses that form their natural food.
3. The later (fifth and sixth instar) larvae have a certain mobility and may invade plots of maize and other cereal crops. Contact chemoreception plays a major role in food selection.
4. Chemosensitive receptor organs (of known external structure) are present on the mouth-parts with some of the receptors showing preferential sensitivity to stimulation by sucrose and others possessing a high sensitivity to meso-inositol. Feeding tests using artificial diets show a positive correlation between sugar receptor specificity and ingestion of these sugars, but the behavioural role of the inositol receptor has not been clarified.
5. The effect of environmental temperature on the development rate of the egg, larva and pupa has been measured accurately and simple morphological criteria developed for determining the age of larvae.
6. In the laboratory stock, release of pheromone by the adult female and response of the male start only after 2–3 days. Reproductive behaviour is maximal between midnight and 3.30 a.m. and almost zero in the daytime. The behavioural response of the male has been quantified to the point where it can be used as a bioassay for chemical studies of the nature of the pheromone.
7. At outbreaks in the field, adult emergence takes place between sunset (6.45 p.m.) and midnight, with a peak about 9.0 p.m. No moths are caught by pheromone traps in the emergence area.
8. The insects (larvae and adults) at outbreaks are not always homogeneous in age. This, taken with the irregularity of flight behaviour, suggests that adult migration is not the only source of outbreaks. There is local evidence for resident populations.
9. Both sexes of the adult moth are capable of flying for a sufficient time to account for the migratory range indicated by earlier evidence from the light-trap network.
10. Flight stops at temperatures below 11.3°C and does not start below 13.8°C.
11. Respiration and wet and dry weights have been measured in eggs, larvae, pupae and adults.

12. The duration of life of adult moths is doubled if they have access to water and increased by a further 50% if they can drink sugar solutions. Fecundity is not increased by feeding, but the peak of egg-laying is delayed, making possible a longer period of flight.

PHYSIOLOGY

Flight Capability

Study of temporal changes in the distribution of *Spodoptera exempta* in East Africa has led to the conclusion that the moth undertakes migratory flight over a considerable distance. The question arises as to whether the moth is physiologically capable of the prolonged flight that would be necessary for such migration.

Moths were flown on a flight mill consisting of a counter-balanced steel strip, radius 20 cm, with a glass pivot. They were suspended from the arm by a piece of aluminium foil attached to the dorsal thoracic cuticle with Evostick Impact adhesive. Revolutions of the arm were counted using a light beam and a photo-electric cell; flight times were measured with a stopwatch.

Of the first 15 moths investigated, six flew for more than two hours. Details of their individual flights are given below (Table 1).

Table 1.

Sex	Age (days)	Duration (hr)	Distance (Km)
F	2	5.1	13.0
M	5	19.2	49.4
F	2	19.3	67.8
F	3	5.7	16.3
M	2	11.0	39.4
M	2	24.7	116.3
Means		14.2	50.4

Under natural conditions the flight speed will be higher since the moth will not have to overcome the drag of the mill. Assuming that power output on the mill is equal to that in free flight, it is calculated that the average equivalent free flight speed is 4.8 km/hr.

These results show that some armyworm moths of both sexes are physiologically capable of flying continuously throughout the night. During a ten-hour flight at 5 km/hr a moth would travel 50 km in still air. In an 8 km/hr wind, a moth flying at random with frequent changes in direction would travel 80 km in ten hours. If the flight were oriented downwind the distance would be 130 km.

The East African Agriculture and Forestry Research Organization produces weekly forecasts of the probability of larval armyworm outbreaks in East Africa. The results described here should assist in the preparation of these forecasts.

Effect of temperature on flight

Moths on the flight mill were placed in a "Hot-pack" refrigerated incubator cooled until flight stopped and then re-heated until it started again. Flight stopped at an average temperature of 11.3°C (S.D.±1.8°) and started at an average of 13.8° (S.D.±3.4°).

Flight speeds were low at temperatures just above threshold, increasing up to about 20°C, and more or less independent of temperature above this level.

These results are relevant to the migratory flight performance of moths in airstreams of different temperatures.

Respiration during the life cycle

Oxygen consumption and (in some cases) carbon dioxide output have been measured using Warburg manometry. Some results are given in table 2.

Table 2.

Stage	O_2 uptake (µl/g. hr) at 25°C	RQ
Egg	478–707	0.86
Larva V	714	
VI	702	
VI (prepupa)	518	
Early pupa	134	3.02
Late pupa	704	0.82
Adult at rest	1214	1.14

Results such as these are to be combined with measurements of food consumption and growth to provide an energy budget for the life cycle.

The pupal stage lasts 10 days at 25°C. During this time the mean total O_2 consumption is 11.4 ml., this would correspond to a fat consumption of 5.6. mg., i.e. 12% of the initial dry weight.

When the larva is feeding, oxygen consumption is approximately doubled. The extra oxygen consumed is of the order of 7 µl per mg (fresh weight) of leaf eaten.

Weight changes during the life cycle

The new-laid egg usually weighs about 80 µg and its dry weight is 21% of this. During embryonic development the weight falls by about 15%. A few hours after hatching the first instar larva begins to feed. This leads to an increase in water content, such that the dry weight of the larval stages is 12–16% of the fresh weight. The stage VI larva weighs about half a gram at its maximum, at which time it ceases to feed. During the next four days a large quantity of water is secreted whereas there is very little fall in dry weight. The pupal fresh weight is usually in the range 100 to 200 mg and the dry weight is 30 to 35% of this. The adult moth voids about 20 mg of meconium from the gut soon after emergence, and a further 12 mg are lost during the first day of adult life; the dry weight is then about 40% of the fresh weight. Weight loss usually continues at a mean rate of 1 to 5 mg/day, except that a few moths gain weight when fed on sugar solutions. A female moth produces 300 to 1000 eggs during her life, weighing 24 to 80 mg in all.

Drinking, longevity and fecundity in the adult moth

The adult moth possesses a functional proboscis and will drink water or sugar solutions. A few occasions of drinking nectar or honeydew in the field have been reported. Clearly it would be desirable to have some information on the importance of drinking in the life of the moth.

Moths were kept in 100-ml plastic tubes and weighed daily. Two series of experiments were performed. In the first series, small foil trays with wetted cotton wool were always present in the tubes. In the second series, the moths were only allowed to drink once per day and were weighed before and after each drinking session. Half of the moths were allowed to drink water, and half sucrose solution (20% in the first series, 10% in the second).

Moths which were not supplied with water died within 4 to 6 days of emergence.

The mean lengths of adult life for moths fed on water were 10 days for males and 9 days for females. Access to sugar increased the mean length of life to 16 days for males and 13 days for females. The maximum life on water diet was 13 days. Variability in life span was much greater for sugar-fed moths, maxima being 22 days for a female and 30 days for a male.

Fecundity was not affected by diet; water-fed moths produced just as many eggs as sugar-fed ones. However the timing of egg production was somewhat delayed in sugar-fed females. Of all the eggs laid in each class, three-quarters of them were laid by day 6 for water-fed females, whereas it was not until day 12 that this figure was passed for sugar-fed moths.

The amounts taken at each drink were very variable, but the overall mean value for sugar (19.8 mg) was nearly double that for water (10.4 mg). There is some suggestion in the data that males may drink more than females. The largest drink observed was by a four-day old male moth which drank 75.7 mg of 10% sugar solution at one session, thereby more than doubling its weight.

FOOD SELECTION

INTRODUCTION

The female armyworm moth contributes little or nothing to the determination of the type of food used by the next larval generation. According to Hattingh (1941) and personal observations, the female is unselective with regard to the species of plant on which she oviposits; eggs are deposited on any kind of substrate having a dry and smooth surface. Conversely, only plants belonging to the families of Graminaceae and Cyperaceae are acceptable as food for the larval stages (see review by Brown, 1962). The neonate larvae escape from unsuitable substrates by hanging from a silken thread, which, after breaking gives them a certain buoyance in air. In the typical range-land habitats of the armyworm the wind-borne larvae have a good chance of landing on the wild grasses that form their natural food.

The latter (fifth and sixth) instar larvae have a certain mobility and, apart from damaging rangeland, may invade plots of maize and other cereal crops. During locomotory activity in the field the larvae are commonly observed probing on forbs and weeds that they encounter. The evidence indicates therefore that olfaction or visual stimuli play no significant part in orientation to food. Yet it should be borne in mind that these sensory modalities might well be involved together with taste during actual feeding. Taste perception and olfaction are probably of crucial significance in determining the strictly graminivorous behaviour of the armyworm and it is for this reason that one of the objectives of the armyworm project is to understand the sensory physiological basis of chemo-perception in the armyworm larva.

RESULTS

Taste perception in the armyworm: sensory physiology

Apparatus for recording and analyzing electrical impulses generated by adequately stimulated taste receptors has been installed in the ICIPE Electrophysiology laboratory. The different types of sensory organs on which our attention will or has been focused are shown in Figs. 1 and 2. Organs of interest are: (a) the medial and lateral styloconic sensilla on the maxillary galeae; (b) the eight pegs at the tip of the maxillary palpi; (c) the pit-like organs on the epipharyngeal surface of the labrum, and (d) the pegs at the tip of the antennae.

Electrophysiological examination, employing the tip recording technique, of the medial and lateral styloconic sensilla has yielded preliminary information indicating the existence of salt- and sugar-sensitive receptor cells in these organs. Stimulation with a range of mono-, di-, and tri-saccharides showed that sucrose is by far the most effective stimuli for the sugar-sensitive receptors in the medial and lateral sensilla. Compounds examined were as listed in Table 1. Some recordings are shown in Figs. 3 and 4.

The sucrose-sensitive receptor contained in the medial sensilla also responds to stimulation by inositol (a cyclohexitol naturally occurring in plants) at concentrations as low as 10^{-5} moles/litre and as such shows a much geater sensitivity to inositol than to sucrose. A concentration range of 10^{-5}—10^{-1} moles/litre induced only a small increase in the firing frequency of action potentials in the lateral sensilla (Fig. 5). Stimulation of both types of sugar receptors with the water-soluble fraction of fresh maize leaves showed that both receptors are activated to a considerable extent. A representative example of the rates of adaptation of the sugar- and inositol-sensitive receptors in both the medial and the lateral styloconic sensilla is shown in Fig. 6.

Trials with chemical compounds which according to literature occur in grasses have so far failed to give any positive results. Compounds which have been tested are:

(1) coumarin, in diluted and saturated solutions and (2) a range of naturally occurring free amino-acids. Further progress in the electrophysiological study of phagostimulatory active compounds will largely depend on the results of the behavioural investigations (see below)

Table 1. Feeding stimulative effectiveness for *S. exempta* of sugars relative to the effect of sucrose. C.I.= consumption index; s.e. = standard error of the mean. C.I.< 1 signifies the per cent larvae showing a consumption index smaller than one. See text.

	Day 1		*Day 2*		*Day 3*		
	(2) C.I.±s.e.	C.I.< 1 (%)	C.I.±s.e.	C.I.< 1 (%)	C.I.±s.e.	C.I.< 1 (%)	N
Sucrose 1	100.00	0	100.00+	0	100.00	00	
Fructose	112.23±13.96	0	135.03±14.88	0	123.24±10.12	00	21
Raffinose	33.84± 8.34	32	95.51±16.21	0	101.61±15.05	55	19
Maltose	13.10± 3.09	26	51.45± 7.94	11	80.94± 8.84	0	19
Glucose	13.67± 5.20	54	63.45±10.27	14	69.78±11.72	18	28
Melibiose	4.89± 1.42	50	18.19± 3.64	19	34.77± 7.38	25	25
Galactose	5.68± 1.94	40	17.87± 4.15	20	28.98± 6.21	20	15
Rhamnose	4.46± 1.49	53	4.73± 2.14	53	5.76± 2.76	67	15
Inositol	5.01± 3.73	70	5.86± 1.93	50	8.57± 3.25	55	20

(1) Mean faecal production per larva (a) on basic diet + sucrose: 10.40 mg (day 1); 7.16 mg (day 2) and 7.92 mg (day 3); N=180 (b) basic diet without sugars added: 1.76 mg (day 1); 1.37 mg (day 2); 0.84 mg (day 3); N=39.

(2) C.I. = mg. dry faecal pellets $\frac{\textit{test compound—basic diet}}{\text{sucrose—basic diet}} \times 100$;

a sucrose control and basic diet control was run with each test.

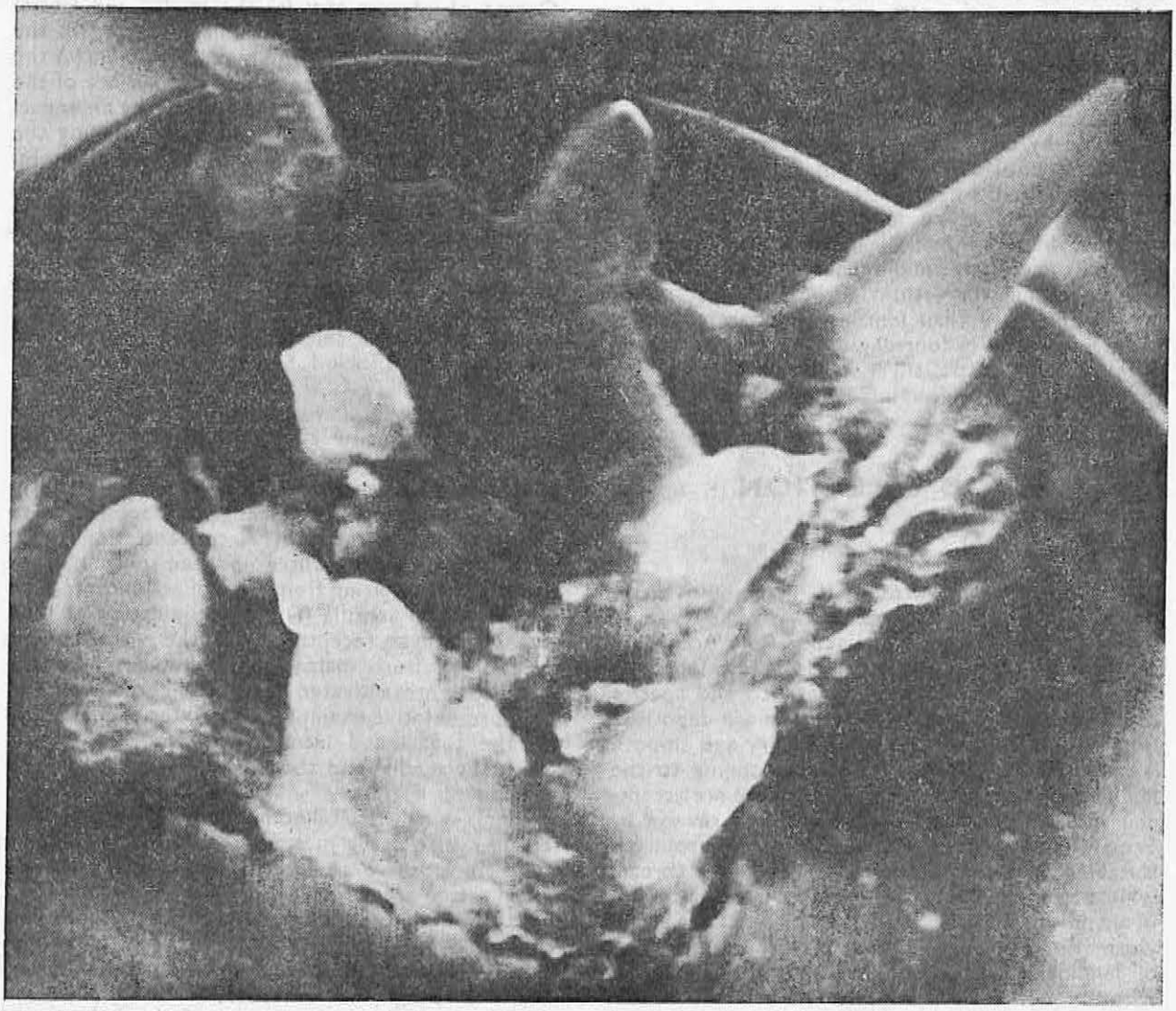

Fig. 1a. Scanning electronmicrograph of the tip region of the maxillary palp of the armyworm larva showing eight sensilla;.

Fig. 1b. Scanning electronmicrograph of a unilateral pair of sensilla styloconica on the maxillary galea.

Fig. 1c. Scanning electronmicrogaph of a sensillum coeloconicum on the epipharyngeal surface of the labrum.

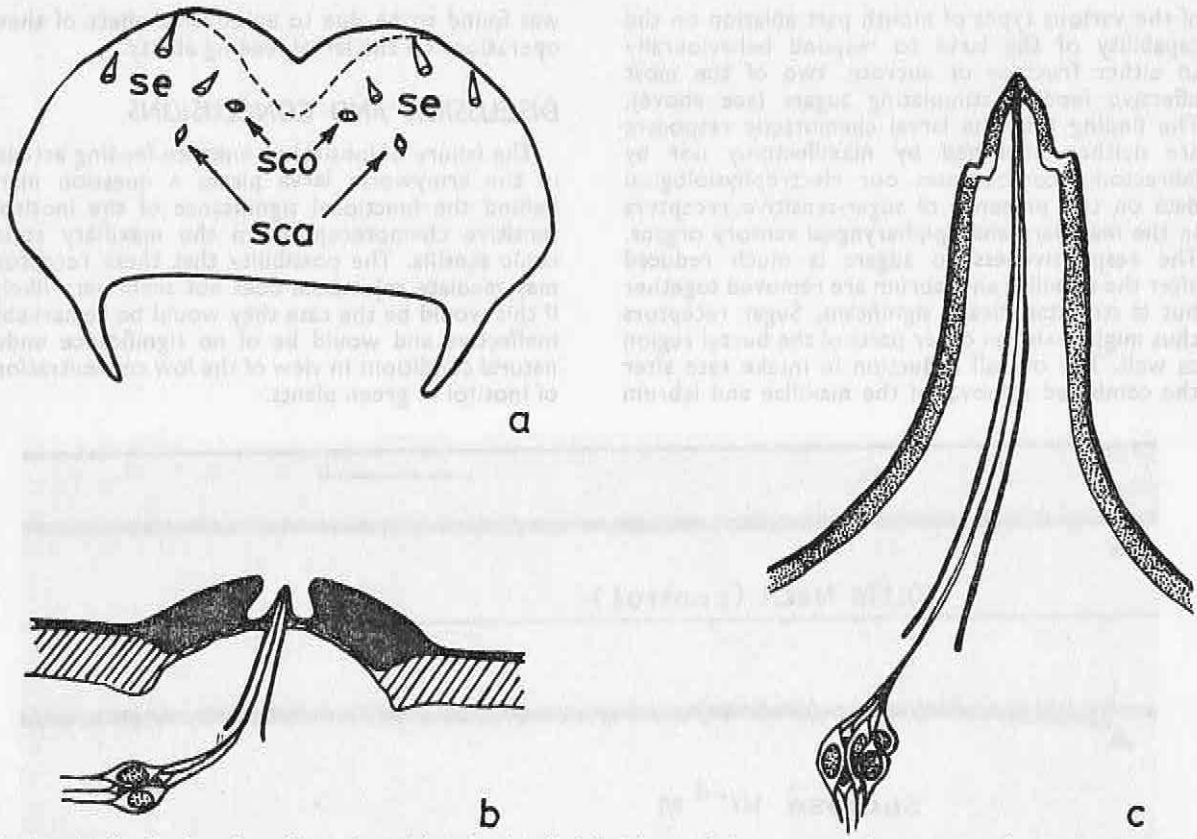

Fig. 2. (a) distribution of sensilla on the epipharyngeal wall of the labrum of the armyworm larva: se=tactile setae; sco=sensilla coeloconica; sca=sensilla campaniformia.
(b) schematic representation of a sensillum coeloconicum.
and
(c) sensillum styloconicum.

Other organs of interest are the pit-like organs on the epipharyngeal surface of the labrum (Figs. 1 and 2). Scanning EM observations confirmed our light microscopic findings that the central papillum is surrounded by a semi-circular wall. Tip recording from the papilla is rendered difficult by its extremely small size, but the preliminary results suggest that these sensilla also are salt- and sugar-sensitive, but, unlike the styloconic sensilla, are inositol-insensitive.

Behavioural studies: phagostimulation by sugars

The behavioural significance of the electrophysiological results on sugar receptors described above was investigated by means of feeding experiments conducted with standardized sixth instar larvae. The assay method used consisted essentially of measuring the dry weight faecal pellets per larva when feeding on an agar-cellulose diet fortified with the test compound (sugars). 0.1 M concentrations of the test compound were used in the initial experimental series; this concentration is approximately that of sucrose in most grasses (Waite and Boyd, 1953). Two control tests were run concurrently with each experiment; one consisting of measuring the response to basic diet without sugar and the other measuring the response on diet containing 0.1 M sucrose. Feeding activities were followed for three consecutive days at a constant temperature of 30°C.

The results are summarized in Table 1. The values given are those relative to the effects of both control tests (expressed in a consumption index or C.I.). Values of C.I. equal or greater than unity are considered as positive responses. From the Table it can be seen that highest average feeding rates are induced by fructose and sucrose where all test larvae show a positive reaction. Furthermore, on sucrose the average daily intake is relatively high over the whole experimental period of three days. On the moderately active sugars (fructose, raffinose, maltose, glucose), as well as on the weakly active sugars (melibiose, galactose) the intake is initially low but increases 3 to 5 fold by the third day; the percentage larvae rejecting generally decreases with increasing average feeding rate. Rhamnose and inositol both appeared to be inactive compounds. The results of subsequent experiments indicate that these two compounds actually are capable of inhibiting the responses to sucrose. Concentrations as low as 0.004 M of sucrose have been shown to induce a significantly positive feeding response but this is inhibited slightly yet significantly by the addition of either rhamnose or inositol at a relatively high concentration (0.1 M).

Behavioural studies: ablation experiments

Evidence for the existence of sugar-sensitive sites on the maxillae and epipharyngeal surface of the armyworm could be obtained through ablation experiments. Fig. 7 summarizes the effect

of the various types of mouth part ablation on the capability of the larva to respond behaviourally to either fructose or sucrose, two of the most effective feeding stimulating sugars (see above). The finding that the larval chemotactic responses are neither abolished by maxillectomy nor by labrectomy corroborates our electrophysiological data on the presence of sugar-sensitive receptors in the maxillary and epipharyngeal sensory organs. The responsiveness to sugars is much reduced after the maxillae and labrum are removed together but is still statistically significant. Sugar receptors thus might exist on other parts of the buccal region as well. The overall reduction in intake rate after the combined removal of the maxillae and labrum was found to be due to an adverse effect of these operations on the larval feeding ability.

DISCUSSION AND CONCLUSIONS

The failure of inositol to enhance feeding activity in the armyworm larva places a question mark behind the functional significance of the inositol-sensitive chemoreceptors in the maxillary styloconic sensilla. The possibility that these receptors may mediate rejectance does not seem very likely. If this would be the case they would be remarkably ineffective and would be of no significance under natural conditions in view of the low concentrations of inositol in green plants.

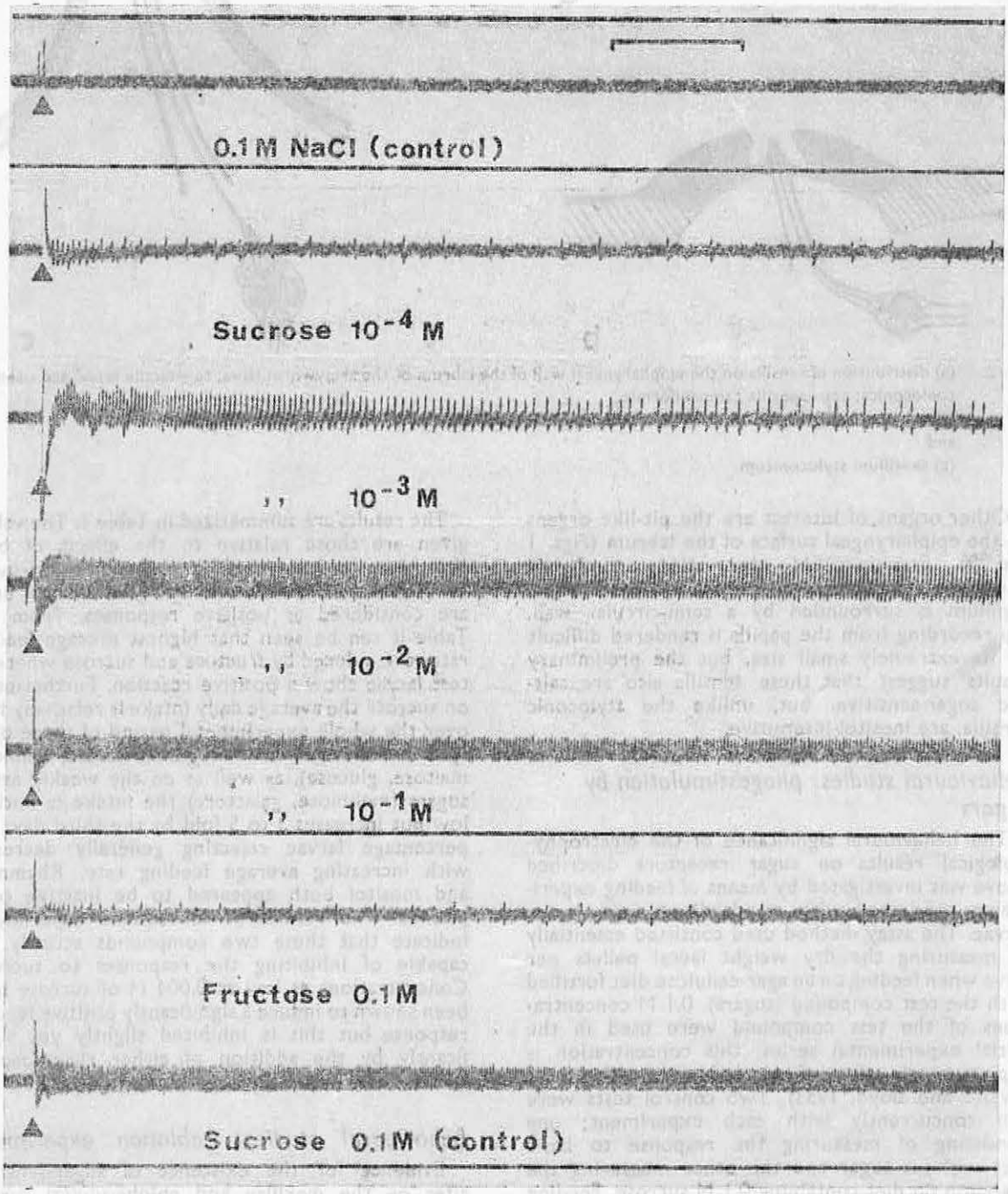

Fig. 3. Representative oscillographs recorded from a lateral sensillum styloconicum of *Spodoptera exempta* upon stimulation with sodium chloride, sucrose and fructose.

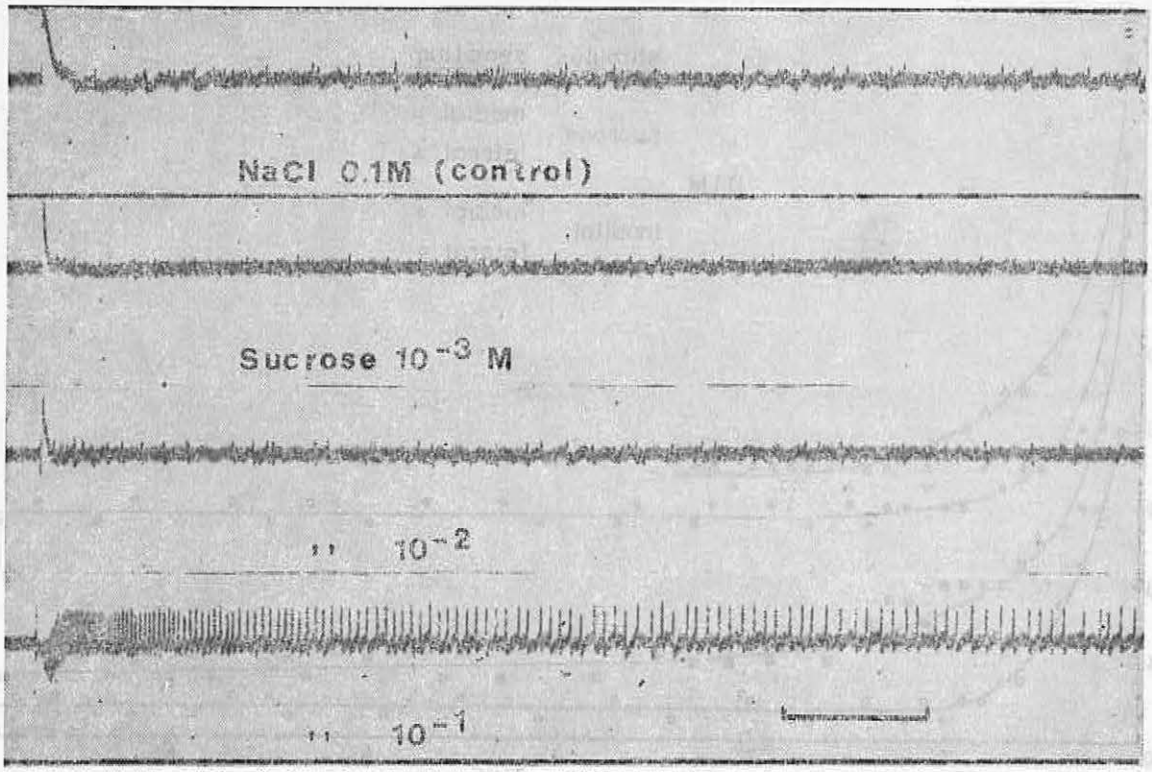

Fig. 4. Oscillograph of responses from the medial sensilla styloconica to stimulation with sodium chloride and sucrose at different concentrations, Calibration mark 200 msec.

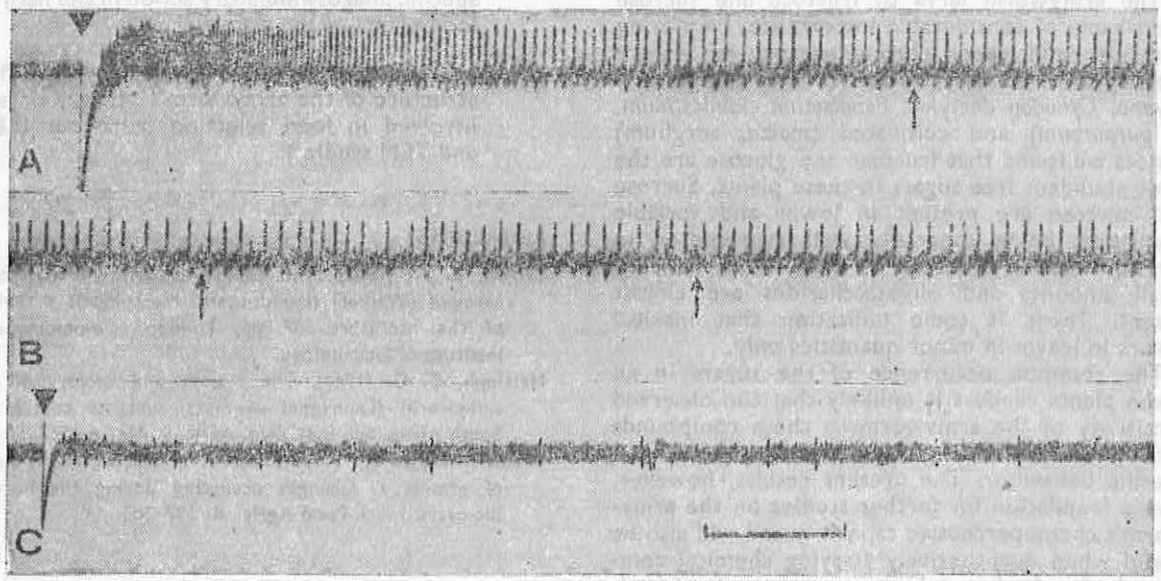

Fig. 5. Oscillograph of responses from the medial and lateral sensilla styloconica to stimulation with 50 mM concentration of meso-inositol: (A) medial sensillum, first second of stimulation; (B) 10th second of stimulation; (C) lateral sensillum.

Phagostimulatory active sugars as determined by faecal dry weight, can be arranged in the following order of decreasing effectiveness: sucrose, fructose, raffinose, maltose, glucose, melibiose and galactose. Rhamnose is inactive, behaviourally as well as electrophysiologically on the armyworm's sugar receptors. Apart from being the most active phagostimulants fructose and sucrose were also the most effective stimuli for the maxillary and epipharyngeal sugar receptors. Thus, in so far as the effect of sugars is concerned, there is a good correspondence between behavioural and electrophysiological data.

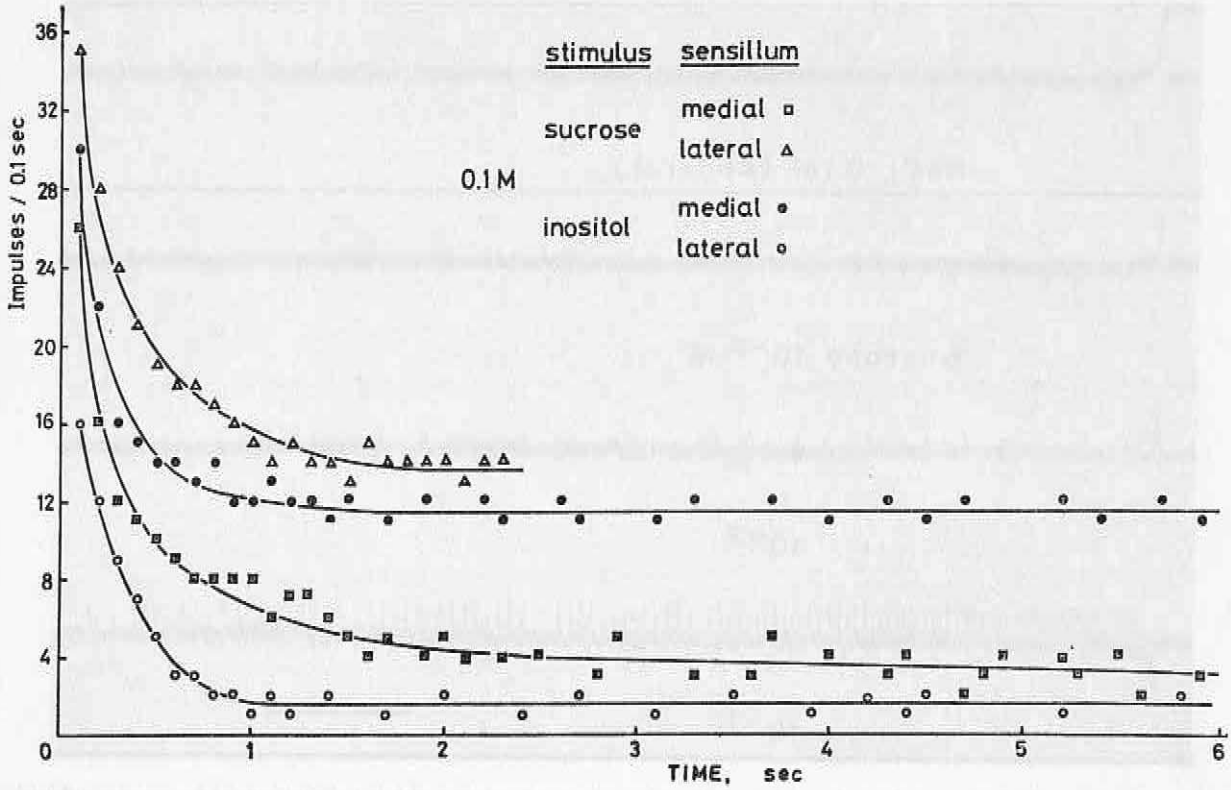

Fig. 6. Adaptation rates of action potentials from sugar-and inositol-sensitive receptor cells in the medial and lateral mixillary sensilla styloconica.

Also under natural conditions the taste sensitivity of the armyworm larva to fructose and sucrose is probably of considerable importance. Employing thin-layer and paper chromatography of watery extracts of dried leaf powders of wild (e.g. *Chloris gayana*, *Cynodon dactylon*, *Pennisetum clandestinum*, *P. purpureum*) and cultivated (maize, sorghum) grasses we found that fructose and glucose are the most abundant free sugars in these plants. Sucrose and maltose are present in lower and variable quantities, being especially well represented in sorghum leaves. Pentose is only found in very small amounts and oligosaccharides are almost absent. There is some indication that inositol occurs in leaves in minor quantities only.

The common occurrence of the sugars in all green plants renders it unlikely that the observed sensitivity of the armyworm to these compounds can by itself provide a significant basis for a food specific behaviour. The present results, however, give a foundation for further studies on the armyworm's chemoperceptive capability and will also be useful when quantitatively assaying chemical compounds for feeding inhibitory activity (anti-feedants).

Objectives of present research

Our research objectives may be summarized as follows:

1. to work out the electrophysiological results in more detail;
2. to focus attention on chemical compounds with anti-feedant effect in electrophysiological and behavioural studies;
3. to continue searching for compounds with specific phagostimulatory action in the natural food plants of the armyworm;
4. to further study the morphology and fine structure of the armyworm's sensory organs involved in food selection behaviour (SEM and TEM studies).

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GROWTH AND DEVELOPMENT IN RELATION TO TEMPERATURE AND FOOD

INTRODUCTION

When studying the sensory physiology and behaviour of the African armyworm it is often necessary to work with standardized material. It is for this reason that observations have been carried out concerning growth and development

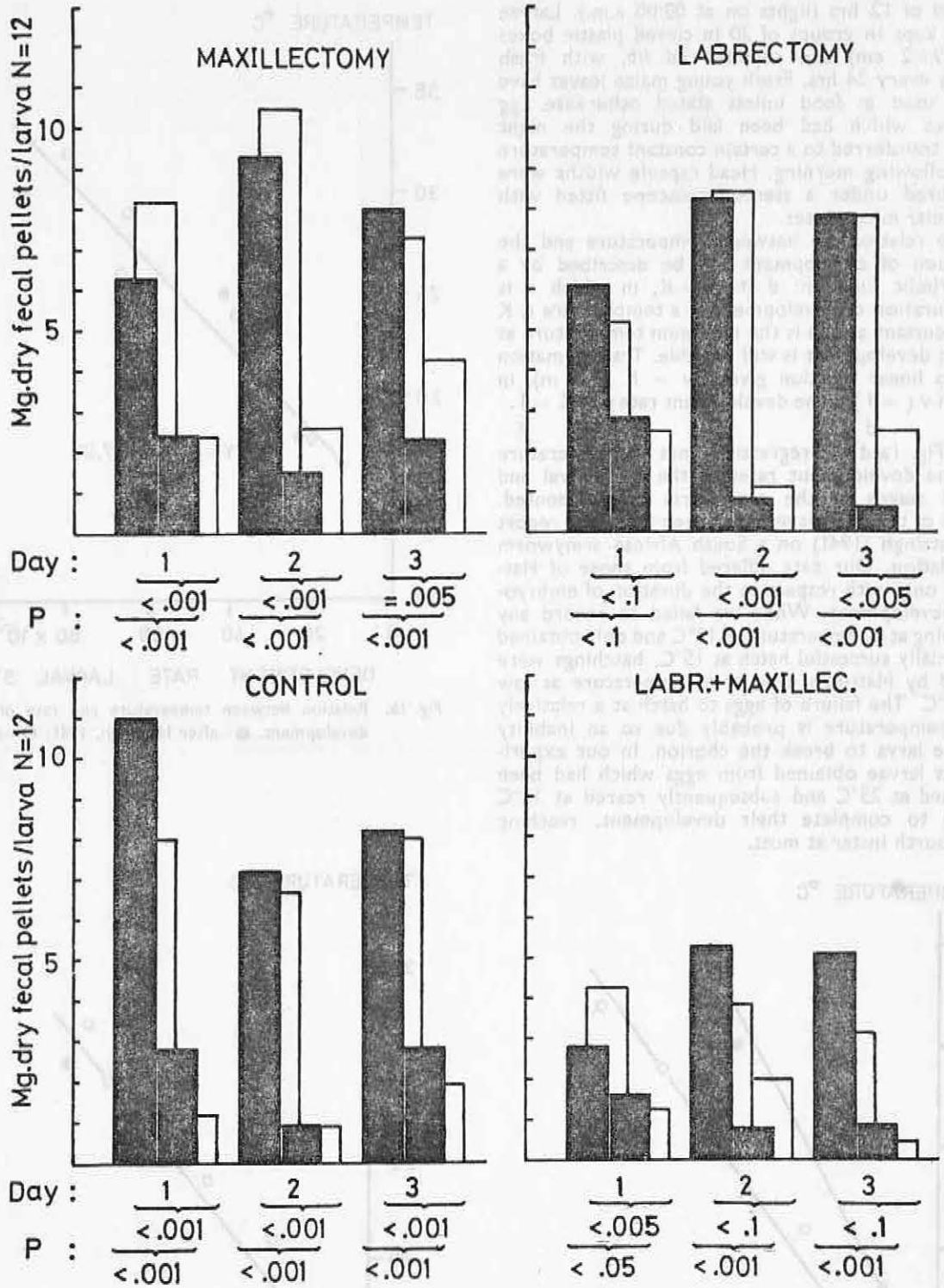

Fig. 7. Effects of various treatments on the chemotactic responses of *S. exempta* to sucrose (left column of each pair of black columns) and to fructose (left column of each pair of white columns). The right column of each column gives the average response to diet without any sugar added. P means the probability that there is no difference between responses on diets with and without sugar (t-test).

under certain laboratory conditions. Since during the past years we have been suffering from very high mortality rates in the ICIPE armyworm breeding colony some data on mortality are also presented.

RESULTS

Influence of temperature on development rates

Rates of development were followed in Hotpack controlled temperature chambers set at a photo-

period of 12 hrs (lights on at 08:00 a.m.). Larvae were kept in groups of 20 in closed plastic boxes (14×9×2 cm) and supplied *ad lib*, with fresh leaves every 24 hrs. Fresh young maize leaves have been used as food unless stated otherwise. Egg batches which had been laid during the night were transferred to a certain constant temperature the following morning. Head capsule widths were measured under a stereomicroscope fitted with an ocular micrometer.

The relationship between temperature and the duration of development can be described by a hyperbolic function: $d\ (t-m)=K$, in which d is the duration of development at a temperature t, K is a constant and m is the minimum temperature at which development is still possible. Transformation into a linear function gives : $v = K\ (t - m)$, in which $v\ (=\frac{1}{d})$ is the development rate and $K = \frac{1}{K}$.

In Fig. 1a-d the regression lines of temperature on the development rates of the egg, larval and pupal stages of the armyworm are presented. Some of the data have been taken from the report of Hattingh (1941) on a South African armyworm population. Our data differed from those of Hattingh only with respect to the duration of embryonic development. While we failed to record any hatching at a temperature of 12°C and only obtained a partially successful hatch at 15°C, hatchings were found by Hattingh even at a temperature as low as 10°C The failure of eggs to hatch at a relatively low temperature is probably due to an inability of the larva to break the chorion. In our experiments larvae obtained from eggs which had been hatched at 25°C and subsequently reared at 15°C failed to complete their development, reaching the fourth instar at most.

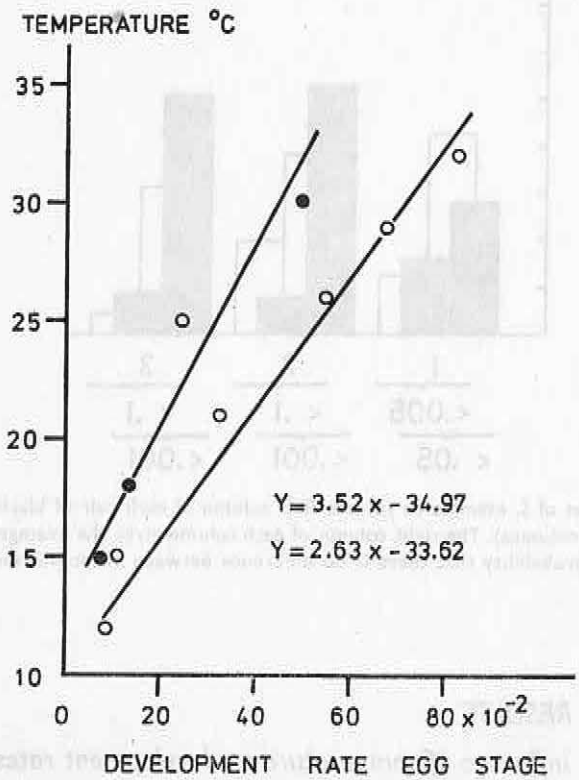

Fig. 1a. Relation between temperature and rate of egg development. ●=after Hattingh, 1941; O=author.

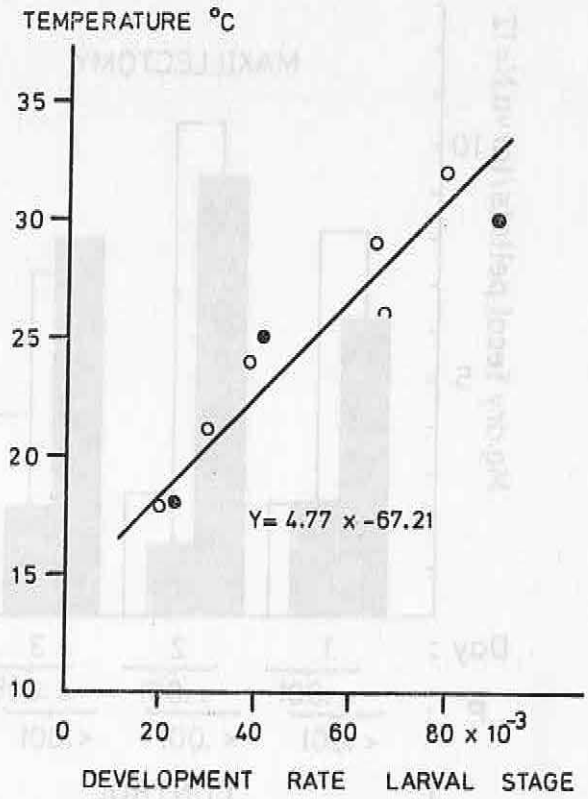

Fig. 1b. Relation between temperature and rate of larval development. ●=after Hattingh, 1941; O=author.

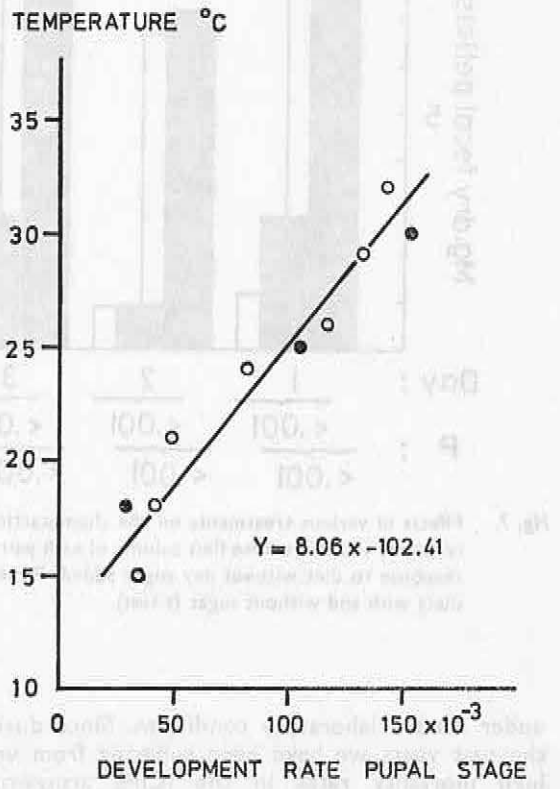

Fig. 1c. Relation between temperature and rate of pupal development. ●=after Hattingh, 1941; O=author.

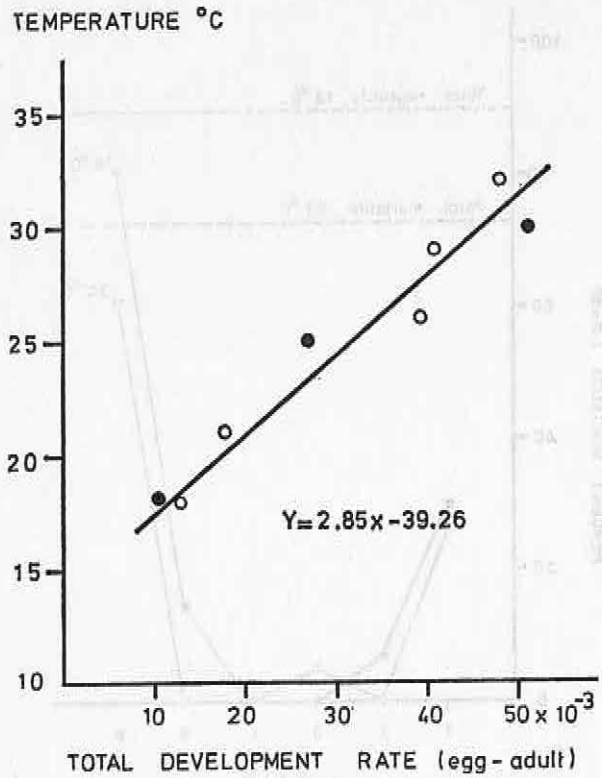

Fig. 1d. Relation between temperature and rate of developm ent of *S. exempta* from the moment of deposition of the egg until adult eclosion. ●=after Hattingh, 1941; O=author.

Influence of temperature and food on growth rate and mortality

Larval growth as measured from increments in width of the head capsule in the consecutive instars is shown in Fig 2. The geometric progression has a growth ratio of 1.55, which is neither influenced by the specific temperature condition nor by the type of larval food supplied. In Fig. 3 the logarithm of the mean head width has been plotted against the number of days at which 50% of the population concerned had moulted into the next instar. Only the first instar shows a disproportionally prolonged duration at a temperature of 18°C. Not shown by Fig. 3 is the pronounced influence which temperature has on the length of the last larval instar. Under temperature conditions of 30, 25 and 18°C the mean duration of the last instar increases linearly to 3, 8 and 20 days respectively. The main responsible factor was the high temperature sensitivity of the prepupal period.

Almost all grasses appear as approximately equally suitable to support growth and development of the armyworm. Notable exceptions are: (a) red oat grasses (*Themeda triandra*) which proved to be nutritionally inadequate, and (b) wheat seedlings, which were consistently found to cause a virtually 100% mortality in the post-feeding last instar larvae.

In Fig. 4 the pooled data are shown for growth rates of larvae reared at 25°C on the following grasses: sorghum, maize, napier (*Pennisetum purpureum*), couch (*Digitaria scalarum*), star (*Cynodon dactylon*), Kikuyu (*Pennisetum clandestinum*), nut (*Cyperus rigidifolius*) and rhodes grass (*Chloris gayana*). The largest variation occurs in the first and sixth instar.

A high total (cumulative) larval mortality was normally recorded during rearing of the armyworm, which ranged from 50 to more than 70 per cent. As shown in Fig. 5 the mortality distribution among the different instars shows a U-shape with highest death rate occurring during the first and last instar. The latter is mainly caused by death in the prepupal phase. A similar U-shaped mortality distribution is obtained when rearing either at a higher (30°C) or at a lower (18°C) temperature (Fig. 6).

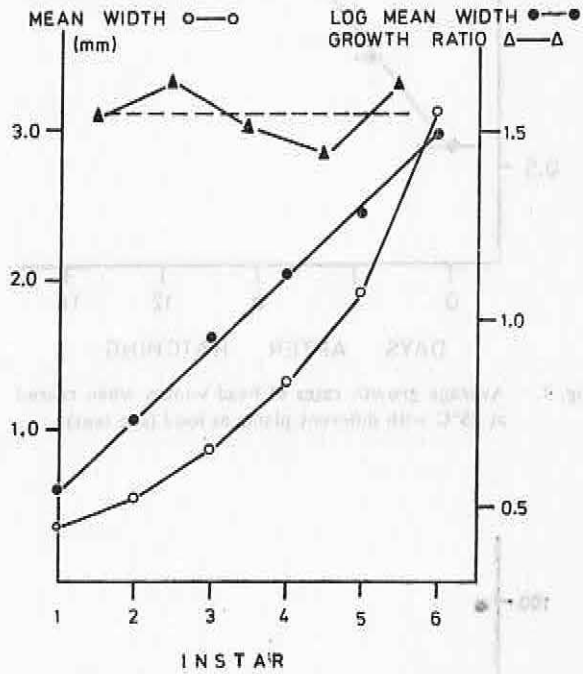

Fig. 2. Growth in head width and growth ratio of *S. exempta*.

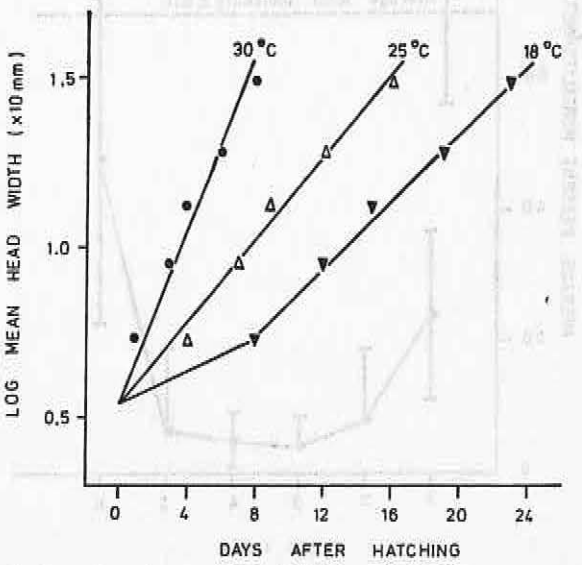

Fig. 3. Growth rate of head width at three different temperatures.

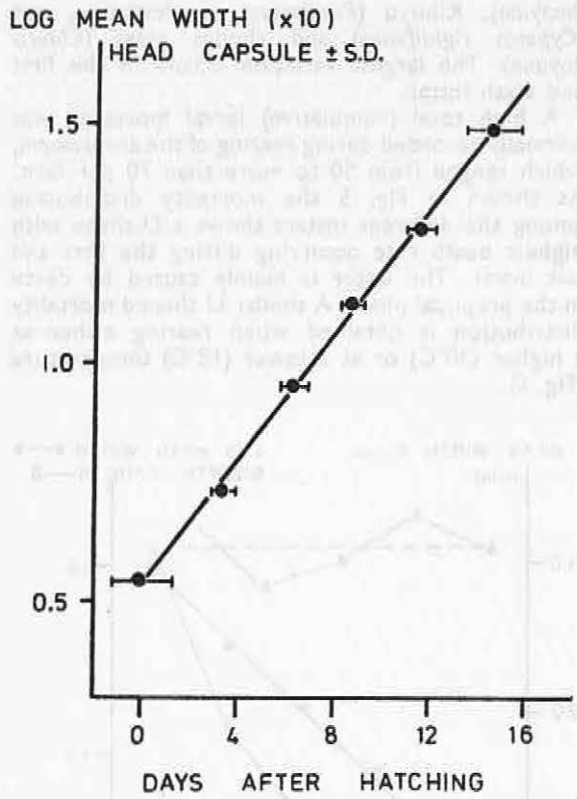

Fig. 4. Average growth rates of head widths when reared at 25°C with different plants as food (see text).

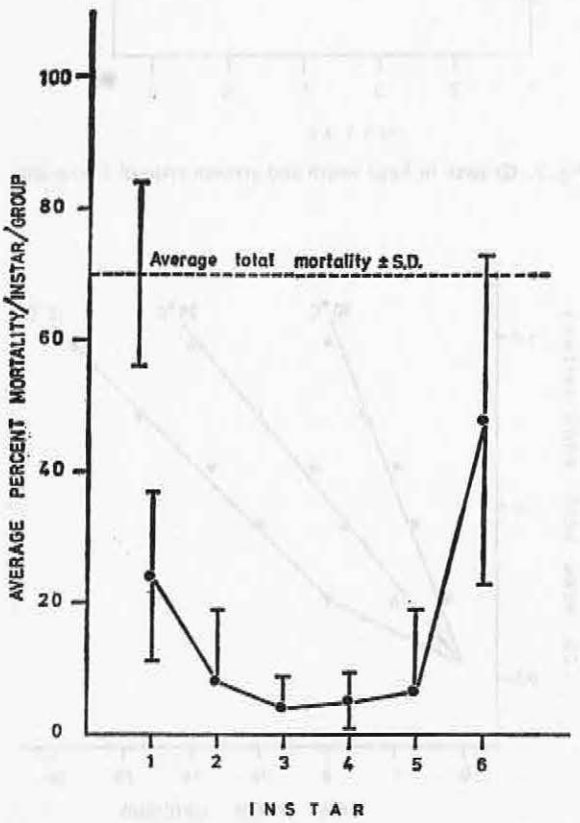

Fig. 5. Average per cent mortality distribution among 8 groups of 30 larvae each (± s.d.) at 25°C.

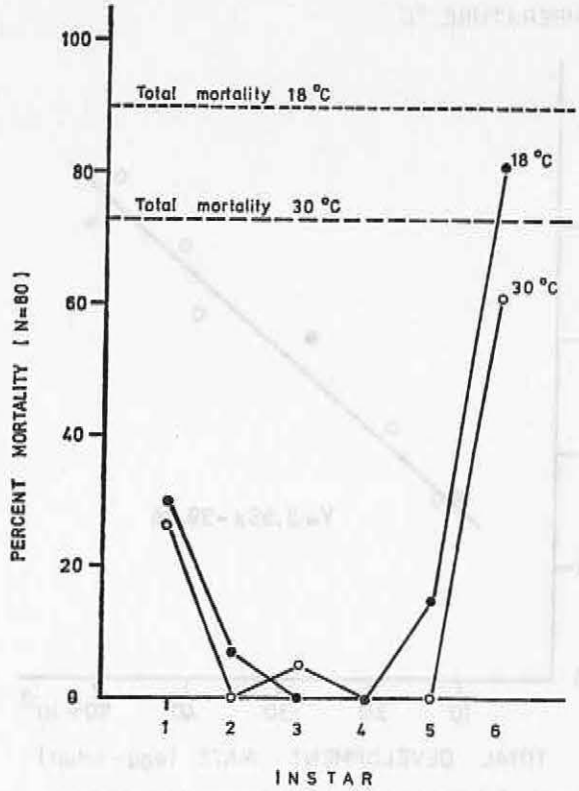

Fig. 6. As in Fig. 5 for temperatures of 18 and 30°C.

DISCUSSION

The present observations will enable us to recognize the larval instar by measuring the head width. Also the duration needed to reach a certain instar at a given temperature can now be estimated. This sort of information will be necessary in some of our experiments concerning the sensory physiology and behaviour of the armyworm.

Our observations indicate that the African armyworm has a high temperature requirement as compared to armyworm species originating from more temperate regions. For instance, in *Leucania separata* temperature higher than 25°C results in retardation of larval growth (Sinchaisri and Sogawa, 1969), whereas in the African armyworm fastest development is reached at the highest temperature studied (32°C). Mortality rates are not greater at such high temperatures than at lower temperatures. When assuming a pre-oviposition period of 2 days (determined from personal observations) and an unlimited food supply our data indicate that the armyworm could reach a maximum of 11 consecutive generations per year.

During the larval stage mortality was rather high in our experiments, ranging from 50 to 70 per cent. This more or less corresponds with the average mortality found in the insectary. The cause of mortality is not known, but apparently it is not related to inadequate food. Since our observations have been made at constant temperatures it remains to be investigated how mortality can be influenced by fluctuating temperature regimes.

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ANALYSIS OF THE SEX PHEROMONE

Gas-liquid chromatography (GLC) with electron capture (EC) Detector

Ten abdominal tips of two-to-four-day-old females were extracted with benzene, filtered, and concentrated. The concentrated solution was divided into two equal portions (each portion equivalent to 5 female tips). One portion was directly trichloroacetylated and the other was hydrolized and then trichloroacetylated. The trichloroacetylated products were dissolved in 20 µl of solvent and analyzed by a GLC with an EC detector (3mm by 75 cm column, 5% OU-17, 190°, N_2 carrier gas at 25 ml/min).

The GLC retention times of the above trichloroacetylated products were compared with standard trichloroacetates of unsaturated alcohols. The existence of the following compounds is suggested from this experiment.

C_{14} (conj. F2) alcohol (10 ratio)
C_{14} (non-conj. F2) alcohol (1 ratio)
C_{14} (conj. F2) acetate (20 ratio)
C_{14} (non-conj. F2) acetate (2 ratio)

This analysis was carried out by T. Yonehara and Y. Kuwuhara of Kyoto University.

Gas-liquid chromatography (GLC) with a flame ionization detector (FID)

About 50 female abdomen tips were extracted with ether and filtered through a silica gel bed (lg). The solution was then analysed by GLC (FID, column, 5% carbowax 20M, 3mm × lm, 150°). Two components were observed that had a retention time corresponding to that of *cis*-9, *trans*-11-tetradecadien-1-yl acetate and *cis*-9, *trans*-12-tetradecadien-1-yl acetate (*cis*-9, *trans*-11-tetradecadien-1-yl acetate supplied by Y. Tamaki, T. Yushima, and T. Kono).

Meanwhile it has been shown that *Spodoptera littoralis* has a group of compounds (tetradecen-1-yl acetate, *cis*-9-tetradecen-1-yl acetate, *trans*-11-tetradecen-1-yl acetate, *cis*-9 *trans*-11-tetradecadien-1-yl acetate) as a sex pheromone (Nature, 244, 208 1973). Also, *cis*-9, *trans*-11-tetradecadien-1-yl acetate and *cis*-9, *trans*-12-tetradecadien-1-yl acetate were found as a sex pheromone of *Spodoptera litura* (Y. Tamaki, T. Kono, private communication, in press). (*Cis*-9, *trans*-11-tetradecadien-1-yl acetate was supplied to me by Tamaki *et al.* above).

It seems possible then that the sex pheromone of the African Armyworm (*Spodoptera exempta*) may contain *cis*-9, *trans*-11-tetradecadien-1-yl acetate and *cis*-9, *trans*-12-tetradecadien-1-yl acetate as the sex pheromone. As the two methods in the GLC indicated, this is quite likely.

ADULT BEHAVIOUR AND SEX PHEROMONE BIOASSAY

INTRODUCTION

Studies on mating behaviour and bioassays for female sex pheromones were initiated on laboratory-reared *Spodoptera exempta* during January 1974. Female sex pheromones have been reported and identified for other species of *Spodoptera*. Brady and Gaynard (1972) have identified the female sex pheromone of *S. exigua* (Beet Armyworm), while Sekul and Sparks (1967) and Jacobson *et al.* (1970) have isolated, identified, and synthesized sex pheromones of the Fall armyworm and Southern armyworm, respectively. The response of male Southern armyworm to a synthetic pheromone "Prodenialure" in field cages has been reported by Redfern *et al.* (1971).

In order to utilize the behavioural sequence of a male moth responding to pheromone-releasing females for bioassays, the phenomenon must be studied closely. The investigations reported here include four main sections: (1) study of the reproductive behaviour of both sexes; (2) bioassays; (3) field investigations on adult behaviour; and (4) data collection towards construction of life tables.

These studies are intended to provide information regarding the behaviour of both sexes of moths that leads to successful copulation. It is hoped that such information will allow us to get an insight into some of the aspects of the population dynamics of the African armyworm, which in turn might enable us to manipulate conditions or factors so as to disrupt their normal behaviour and thus control this pest.

Reproduction behaviour

Reproductive behaviour was studied in respect to the pre-mating (courtship) and post-mating behaviour (behaviour during the time the two sexes are coupled is a passive phase). For reasons of convenience pre-mating and post-mating behaviour are dealt with separately.

The object of studying the pre-mating behaviour was to get an insight into:

(i) the responses of sexually mature males to females of different ages;

(ii) the age of females at which pheromone production and/or eliciting behaviour response in males begins;

(iii) the age at which males can perceive and/or respond to females; and

(iv) the time of day or night most preferred for mating.

It is essential in such studies to use males that have not been exposed to the pheromone odour. This was accomplished by keeping the male and female pupae in different rooms, fairly well isolated from each other. The emerging adults were collected into Kilner jars provided with 10% sugar solution marked for the day of emergence, and maintained in these rooms until put with the opposite sex.

Observations were made on 18 single pairs as well as 2 groups of five pairs of adults obtained from the laboratory culture. The two sexes were put together in both cases between 9.00 a.m. and 11.00 a.m., when individuals were 24 hours old. Observations were made round the clock at hourly intervals. 50 individual pairs of adults obtained from pupae collected in the field (outbreaks) were also studied similarly.

Results from these observations are presented in Table 1. Observations indicated that males are not sexually active (behaviourally) until 2 days after emergence, in that no males up to this age showed any response to females. On the other hand, 12 of the 41 responding wild males did so only 24 hours after emergence. This difference may be due to the varying physiologies of the wild and laboratory populations, but is not being elaborated at present. Females take at least 2½ days (60 hours) in the case of laboratory reared moths and 2 days (48 hours) in the case of wild before they start attracting males and eliciting pre-mating behavioural response in males. Of the 28 laboratory males observed, only 22 showed response in this way while 41 of the 50 wild ones also responded. The others did not respond throughout their lives. Of the responding males of either kind, there was considerable variation in the behaviour of one individual from another; however, the following sequence of behavioural responses was observed and can be considered as being a general behaviour pattern.

Table 1. The extent of sexual responsiveness and mating in males of *S. exempta*

Source of adults	*No. of Pairs studied*	*Responding* ♂♂		*Males mated*	
		No.	*%*	*No.*	*%*
Laboratory bred	28	22	78.57	18	64.28
Wild collected	50	41	82.00	39	78.00

Sequences of behavioural responses of males

General alerting by raising antennae from resting position.
Vibrating wings uniformly.
Vibrating wings as well as antennae.
Periodic jerking or fluttering of wings and antennae.
Raising the body on legs to assume a standing position.
Movement—starting to walk.
Bending the tip of abdomen down to give the body a curved appearance.
Walking rapidly and haphazardly and/or starting to fly.
Making copulatory attempts—opening up the scent brush plume and swaying of the rear abdominal portion on either side.

It should be pointed out here that the first three steps of behaviour seem to be rather general, in that any stimulation such as a change in light intensity or blowing of air would also produce these responses. More observations (possibly electrophysiological) are needed to understand the significance of these three steps of behaviour.

Other behavioural traits that were noted were as follows:

1. Of the 28 laboratory pairs, there were 18 pairs that mated; and all the matings occurred between 12.30 a.m. and 3.00 a.m. over a period of observation of about 10 days;
2. 39 of the 50 wild pairs mated and all of these also mated between 12.30 a.m. and 3.00 a.m.
3. Some of the unmated females laid eggs that did not hatch (unfertilized).
4. Data from table 1 indicate that males of this species exhibit a rather erratic mating behaviour. Not all males in a given population may respond to pheromone eliciting females, and an even lower proportion of males really can mate and inseminate females (64.28% and 78.00% only). This could be even more so in nature where females are not necessarily confined with males in a limited space, as under the experimental conditions. This is at present under investigation in the laboratory under a series of experiments on the reiterative mating ability of males of this species.

Bioassays

Bioassays were conducted to note the response of males to (i) live females, (ii) crude extract of female abdomen tips; and (iii) suspected female sex pheromone compound (*cis*-9, *trans*-11-tetradecadien-1-yl acetate). The tests were conducted in two major types of set-up: (a) air flow system and (b) direct exposure by means of air puffs with a dropper.

(*a*) The air flow system consisted of an air pump leading to a humidifying chamber, to a stabilizer, to an air flow meter, to the test chambers. The flow of air was adjusted to ca. 8.0 cu. ft. per hour and let into the first chamber with live females inside, which was in turn connected to a second chamber with males.

(*b*) Under this set-up, males in a Kilner jar were provided with pheromone-loaded air puffs from a laboratory dropper, the pheromone solution being first taken in the dropper and the solvent allowed to evaporate. Results from the system are presented in Table 2. All bioassaying was done between midnight and 1.30 a.m.

Table 2. Response of males to live females, crude extract of female abdomen tips, and suspected synthetic pheromone chemical

Sources of stimulus	*No. of ♂♂ used and age*	*♂♂ responded* No.	%	*Degree of * response*
Live females				
3 ♀♀, 3½ days old	2½ days old	0	0.0	0
5 ♀♀, 3½ days old	5 3½ days old	1	20.0	moderate
5 ♀♀, 4½ days old	5 2½ days old	3	60.0	complete
Crude extract				
0.1, equivalent ♀♀	22 1½ days old	1	50.0	moderate
0.1, equivalent ♀♀	5 2½ days old	2	40.0	good
0.1 ♀♀ equivalent	3 2½ days old	3	100.0	complete
0.1 ♀♀ equivalent	5 2½ days old	1	20.0	slight
0.1 ♀♀ equivalent	5 2½ days old	3	60.0	complete
0.1 ♀♀ equivalent	5 2½ days old	5	100.0	complete
Synthetic pheromone				
trans—11—trans 12 mixt.	3 2½ days old	2	60.6	
0.002 µg **	3 3½ days old	3	100.0	Slight
	10 1½ days old	0	0.0	—
0.02 µg in 10 ml	3 3½ days old		50.0	moderate
	10 1½ days old	0	100.0	—
0.2 µg in 10 ml	3 3½ days old	3	100.0	complete
	10 1½ days old	0	0.0	—
2.0 µg in 10 ml	3 3½ days old	100.0		complete
	10 1½ days old	0	0.0	—

*At present the degree of response is being considered as slight, moderate, good, and complete until more is learned about the sequence of behaviour responses.

**The same males were used after a 20 min gap.

The exposure time was 10 min. It must be pointed out here that, several test attempts to run the bioassays before midnight, did not produce any response, while the same sets of individuals tested right after midnight (a gap of 30 min to 60 min.) produced remarkable response. This further confirms the studies on mating behaviour and goes even further to show that the sexes do not respond to each other, apart from the set period of time preferred for mating.

Results from Table 2 suggest that females were not able to produce as good a response as the crude extract or the synthetic pheromone. Males less than 2 days old do not seem to respond as strongly as older males, suggesting that they are not fully mature sexually by this time.

The difference in response to live female and crude extract needs attention. The crude extract was prepared from laboratory-reared females about 6–7 months ago, and has provided good response. The lack of response to live females suggests that either these females have lost production of the pheromone, or their release mechanisms have been affected in some way due to continuous laboratory rearing. This, however, needs to be investigated in more detail.

One female of a related species, *S. triturata*, was caught at light and used to study the response of males of *S. exempta* under the air-flow system. Complete response was shown by all the 5 males tested, all males responding within 5 seconds of being exposed to the air current. However limited, this small experiment on the one hand tells us that there may be cross attraction; similar chemicals acting as pheromones; and on the other hand provides additional evidence to the hypotheses that laboratory-reared females may be affected in their pheromone production and/or release.

Bioassays were also conducted on adults reared out of pupae collected in the wild when outbreaks of armyworm occurred in Kenya—April to June 1974. As before male responses were studied in relation to live females and the suspected synthetic pheromone mixture. The procedure followed was identical to the one described above. Results from these bioassays are presented in table 3.

From the sequence of behaviour of males responding to pheromone producing/releasing females, responses of individual males could be quantified by assigning points in ascending order corresponding to the successive steps in the sequence. The overall response of a group of males (say 10) in a bioassay is then computed by a cumulative average of individual responses.

Results from table 3 indicate several trends. The very marked difference in responses to 3 day and 2 day old females (76.0% vs 3.0%) confirm the previous bioassays on laboratory reared females. However, it is also worth noting that while no response was noticed to 2 day old females of the lab-reared population, some response (3% and 9%) was noted in wild females. This would mean that in the wild, females can start producing/releasing pheromone after only 48 hours of emergence while the lab-reared females require at least 60 hours to do so. Also, the difference in males is noteworthy. A certain proportion of 1 day old males, although a small proportion (30%, 30% and 40%), did respond to live females and the synthetic pheromone. As against this the lab-reared males were not able to perceive/respond to

pheromone releasing/producing females or the chemical unless they were at least 36 hours old. This same trend is also evident from their responses to the pheromone (30% and 40% of 24 hour old males) responding to at least some degree to increasing concentrations respectively. A gradual increase in the response of fully mature males to increasing concentrations of the pheromone is also evident. The only sharp difference in this sort of response is noted between concentrations of 0.002 µg per 10 ml and 0.02 µg per 10 ml whereas the increase in response to concentrations above 0.02 µg per 10 ml is at best gradual. It therefore seems likely that 0.02 µg per 10 ml is a threshold concentration for fully mature and perceiving males.

Table 3. Response of males to live females and the suspected pheromone (Wild population)

Source of stimulus	*No. of ♂♂ used and age*	*♂♂ responding No.*	*%*	*Overall * response*
Live females				
5 ♀♀, 3 days old	10, 3 days old	9	90.0	76.6%
5 ♀♀, 2 days old	10, 2 days old	1	10.0	3.0%
5 ♀♀, 1 days old	10, 2 days old	0	0.0	0.0%
5 ♀♀, 3 days old	10, 3 days old	8	80.0	80.0%
5 ♀♀, 2 days old	10, 1 days old	3	30.0	9.0%
Synthetic pheromone				
0.002 µg per 10 m.l.	10, 3 days old	7	70.0	42.0%
0.002 µg per 10 m.l.	10, 2 days old	2	20.0	6.0%
0.02 µg per 10 m.l.	10, 3 days old	9	90.0	72.0%
0.02 µg per 10 m.l.	10, 2 days old	4	40.0	24.0%
0.02 µg per 10 m.l.	10, 1 day old	3	30.0	0.9%
0.2 µg per 10 m.l.	10, 3 days old	8	80.0	80.0%
0.2 µg per 10 m.l.	10, 2 days old	3	30.0	12.0%
2.0 µg per 10 m.l.	10, 3 days old	10	100.0	86.0%
2.0 µg per 10 m.l.	10, 2 days old	6	60.0	60.0%

*Refer text for detail about computation of overall response

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FIELD RESEARCH

INTRODUCTION

Armyworm outbreaks in Kenya commenced in early April 1974 and continued into early June by which time they covered most of the country (see map). Since *Spodoptera* outbreaks tend to be sporadic—indeed this was the first such occurrence in at least 2½ years—a study of the insects which was to embrace:

1. pupation sites
2. emergence behaviour
3. post-emergence behaviour
4. reproductive behaviour
5. release of sex pheromones

was initiated immediately. It should be emphasised that—due to the broad spectrum of studies undertaken and also, due to the relatively large number of areas on which these investigations were replicated—the information gathered is more qualitative than quantitative. Conclusions drawn are therefore to be treated as only tentative.

The time factor together with other limitations permitted a study of only 4 of the approximately 30 outbreaks (April to June 1974). The first site was located in the Lambwe Valley game reserve near Lake Victoria, the second at the Taita Hills in the south west, the third near Kampi-ya-Moto in the Central Province and the fourth in Nairobi. The observations recorded in the following pages depict a general pattern for all the outbreaks studied, unless specified otherwise.

RESULTS AND DISCUSSION

Pupation sites

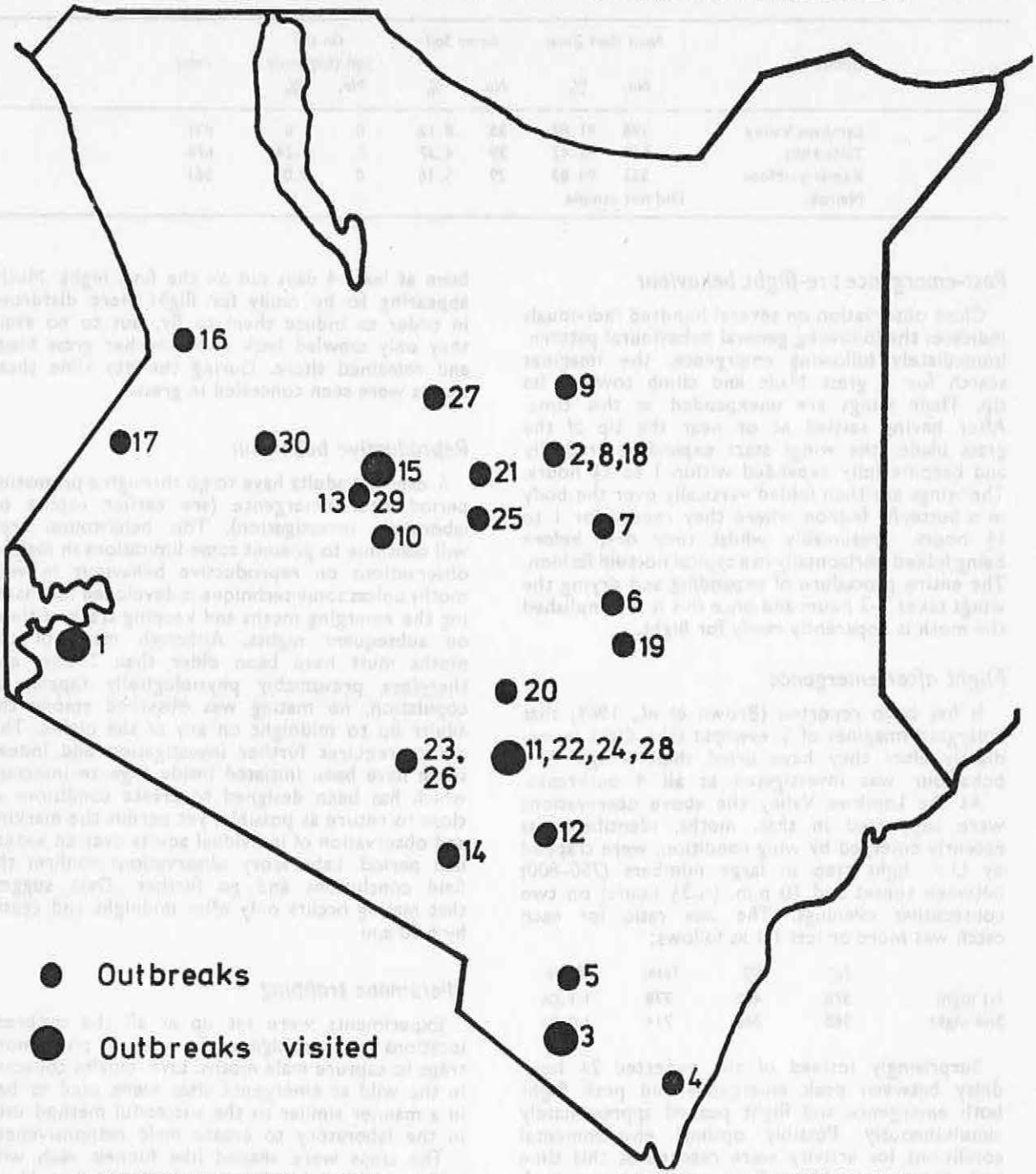
At all the locations pupation sites were studied by the examination of topsoil samples measuring approximately 1 sq metre in area by 10 cm in depth. Four to five such samples were distributed for approximately every acre of land sampled.

It was found at each location that pupation takes place 2–10 cms below the soil surface and that the majority of pupae were recovered from around the root zone of clumps of grasses (Table a).

It would appear therefore that pre-pupal larvae burrow into the soil in the immediate vicinity of grass clumps. This behaviour becomes very relevant when their post emergence behaviour is looked at in the following pages.

Adult emergence takes place during the period between sunset (approx. 6.45 p.m.) and midnight. Emergence commences immediately after sunset, reaches a peak 1½ to 2½ hours later and then falls off to a minimum by approximately 3½ to 4 hours after sunset. However, infrequent emergences continue until about midnight after which the phenomenon is rare. At Lambwe Valley a definite pattern of emergence was observed in that for the first 1 to 1½ hours males predominated but thereafter the sex ratio swung increasingly in favour of females. However, at the other outbreaks the sex ratio remained approximately at a constant unity.

MAP OF KENYA SHOWING THE ARMYWORM OUTBREAKS DURING April-June 1974



OUTBREAKS IN RELATION TO TIME

Outbreak No.	*Period*
1	April 1st. Week
2	—do—
3	April 4th Week
4	—do—
5	May 2nd. Week
6	May 3rd. Week
7	—do—
8	—do—
9	—do—
10	—do—
11	—do—
12	—do—
13	May 4th. Week
14	—do—
15	June 1st. Week

OUTBREAKS IN RELATION TO TIME

Outbreak No.	*Period*
16	—do—
17	—do—
18	—do—
19	—do—
20	—do—
21	—do—
22	—do—
23	—do—
24	June 2nd. Week
25	—do—
26	—do—
27	—do—
28	June 3rd. Week
29	—do—
30	June 4th. Week

Table a. Distribution of pupae in the soil

Outbreak	Near Root Zone		Baren Soil		On the Soil (Exposed)		Total
	No.	%	No.	%	No.	%	
Lambwe Valley	396	91.87	35	8.12	0	0	431
Taita Hills	647	95.42	29	4.27	2	0.24	678
Kambi-ya-Moto	532	94.83	29	5.16	0	0.0	561
Nairobi	Did not sample						

Post-emergence pre-flight behaviour

Close observation on several hundred individuals indicates the following general behavioural pattern. Immediately following emergence, the imagines search for a grass blade and climb towards its tip. Their wings are unexpanded at this time. After having settled at or near the tip of the grass blade, the wings start expanding gradually and become fully expanded within 1 to 1½ hours. The wings are then folded vertically over the body in a butterfly fashion where they remain for 1 to 1½ hours, presumably whilst they dry, before being folded horizontally in a typical noctuid fashion. The entire procedure of expanding and drying the wings takes 2–3 hours and once this is accomplished the moth is apparently ready for flight.

Flight after emergence

It has been reported (Brown *et al.*, 1969) that emerging imagines of *S. exempta* take flight immediately after they have dried their wings. This behaviour was investigated at all 4 outbreaks.

At the Lambwe Valley the above observations were supported in that, moths, identifiable as recently emerged by wing-condition, were trapped by U.V. light trap in large numbers (750–800) between sunset and 10 p.m. (~3½ hours) on two consecutive evenings. The sex ratio for each catch was more or less 1:1 as follows:

	♂♂	♀♀	*Total*	*Ratio*
1st night	376	402	778	1:1.06
2nd night	368	346	714	1:0.94

Surprisingly instead of the expected 2½ hour delay between peak emergence and peak flight both emergence and flight peaked approximately simultaneously. Possibly optimal environmental conditions for activity were reached at this time and these probably influence or trigger both activities simultaneously.

At the other outbreaks the behaviour of the emerging adults was very different. The light trap was operated at all the locations on each of 4, 5 and 8 nights respectively from just before sunset until approximately midnight. Emergence was studied within a radius of about 90 metres from the trap for about 4–5 hours each night. These observations were made by using head torches whose beams were reflected by the eyes of the moths in a manner similar to cats' eyes.

On each night at each of the locations 300–400 freshly emerged and emerging moths were observed. However, not a single moth flew for 4–5 consecutive nights even though many may have been at least 4 days old on the final night. Moths appearing to be ready for flight were disturbed in order to induce them to fly, but to no avail, they only crawled back onto another grass blade and remained there. During the day time these moths were seen concealed in grass.

Reproductive behaviour

S. exempta adults have to go through a premating period after emergence (see earlier report on laboratory investigation). This behavioural trait will continue to present some limitations in making observations on reproductive behaviour in wild moths unless some technique is developed for marking the emerging moths and keeping track of these on subsequent nights. Although many of the moths must have been older than 2 days and therefore presumably physiologically capable of copulation, no mating was observed among the adults up to midnight on any of the nights. This aspect requires further investigation and indeed these have been initiated inside a gauze insectary which has been designed to create conditions as close to nature as possible, yet permit the marking and observation of individual adults over an extended period. Laboratory observations confirm the field conclusions and go further. Data suggest that mating occurs only *after* midnight and ceases by 6.00 a.m.

Pheromone trapping

Experiments were set up at all the outbreak locations to investigate the use of pheromone traps to capture male moths. Live females collected in the wild at emergence sites were used as bait in a manner similar to the successful method used in the laboratory to create male responsiveness.

The traps were shaped like funnels each with a 45 cm diameter opening at the top and an 11 cm opening at the narrower bottom. The narrow portion was fitted with screw tops of kilner jars so that the latter could be screwed on or off. The jars were partially filled with water and the inner surfaces of the funnels were smeared with a glue substance; these together were to serve as a trapping device. Clear petroleum jelly was used as the glue; since a non-drying insect trapping glue such as "Tanglefoot" was not available.

The females used as bait were held in wire cages hung in the centre of the funnel. The traps contained either 5 or 10 females representing two treatment levels. Each treatment was replicated four times and individual trap locations were randomized within treatments.

No male moths were trapped in any of the treatments. This was not unexpected during the first two days since females do not release the pheromone until they are at least 2 days old. The reason for the absence of captures on subsequent nights is not known, but there are abundant possible explanations only some of which are listed below:

1. trap design, size and shape;
2. height of traps above ground level;
3. age of males;
4. quality of the sticky substance—petroleum jelly may have been responsible directly as a deterrent and indirectly by modifying the pheromone odour from the releasing females;
5. absence of pheromone release for unknown reasons.

Giving consideration to the points listed above, these experiments will be repeated at the next armyworm outbreak.

General observations

When larval outbreaks in the Lambwe Valley were visited during mid-April, the local farmers stated that during mid-March a less severe larval outbreak occurred which was preceded by another even smaller one during mid-February. It seems therefore, that this outbreak was endemic and that at least three generations of the armyworm occurred before the population reached outbreak proportions in mid-April.

On the three other outbreak sites visited, all stages of the insect were observed—eggs, 1st, 2nd, 3rd, 4th and 5th instar larvae, pupae of different ages, and adults of different ages. This absence of synchrony, such obvious staggering of life history stages, together with the obvious lack of flight behaviour mentioned earlier are all markedly atypical of a migrant species. Indeed these observations strongly support the hypothesis mentioned above that these outbreaks arose from endemic populations that reach outbreak numbers under favourable ecological conditions.

In conclusion therefore, it is tentatively suggested that Armyworm outbreaks in Kenya are not necessarily a consequence of migrations as has been suggested in the past (Brown *et al.*, 1969) Brown and Swaine 1966) but may on the contrary be due to rapid increases in the endemic populations as a result of optimal environmental conditions. Whether these large populations subsequently give rise to migration has yet to be determined.

Data collection for lifetable analyses

The African Armyworm *Spodoptera exempta* Wlk. is todate considered as an outbreak pest, outbreaks being restricted to only certain periods in any given year. The population dynamics of this pest during the rest of the year is not fully known, nor has it been investigated in the past; although it is not uncommon to collect stray adults in light traps during such periods. It becomes practically impossible to study the population dynamics in the field due to the specialized mode of life and reproduction of this pest. In view of this difficulty a gauze insectary has been erected to provide an enclosed stand of the food plant (maize) on which can be reared self-perpetuating populations of the armyworm.

It was planned to start a population inside the gauze insectary with known numbers of individuals of the same age to provide a uniform cohort on which observations could be recorded periodically to obtain data towards compiling a life-table for the species. Also intended were studies on the adult behaviour including emergence, reproductive and flight behaviour, which, as mentioned in an earlier section, are extremely difficult to conduct on a wild population.

The gauze insectary is a platform of earth measuring 10 meters by 5 meters with cement walls holding the earth from all four sides. A wooden frame-work supporting plastic gauze stands $\sim 2\frac{1}{2}$ meters above the surface. The roof is also constructed like the walls. The entire set-up is insect proof and is provided with a double door entrance which serves the purpose of an anteroom. Under the set-up the physical environmental factors (temperatures, humidity, rainfall and sunshine) are the same as in the open.

The available land space was divided into 2 halves by means of gauze partition and only one half was utilized for the studies in question. The half of the land was further divided approximately into 3 sections of $\sim$ 5 sq. meters each. Maize was planted in these three plots at 15 day intervals so as to provide the larvae with maize of different ages.

A week after the last plot was planted, 300 newly hatched larvae from the same egg batch were introduced in the three plots. This cohort of larvae was monitored throughout the developmental stages. Observations were intended to be made twice a week on the number of larvae and their growth stage; initiation of pupation, termination of pupation and emergence of adults. Records were made of mortality factors associated with different growth stages of the larvae and the pupae. The temperature and humidity were also monitored by operating a thermophygrograph set inside the insectary and the ranges of the maximum and minimum are presented in Appendix I.

Counts on the first and second instar larvae could not be made because of their extremely small size, and therefore, they were counted from the 3rd instar onwards. It was not intended to make absolute counts, but only so these would serve as samples from each plot. Data are presented in table b. It is indicated by this sampling data that plots II and III (later planted) harboured greater numbers in comparison to plot I. This suggests that there probably is a preference for a particular age of maize plants exhibited by the later instar larvae, which were also observed to migrate out of plot I into the plots with younger maize.

The data at present are very limited and do not permit any interpretations.

Table b. Observations on the larvae recorded from three adjacent maize plots in the enclosed insectary

Date	No. and stage of larvae						
	Plot I		Plot II		Plot III		Total
May 13, 1974	7	3rd instar	35	3rd instar	9	3rd instar	
	15	4th instar	41	4th instar	35	4th instar	
	22		76		44		142
May 20, 1974	30	5th instar	49	5th instar	83	5th instar	
	9	6th instar	8	6th instar	13	6th instar	
	39		57		96		192
May 24, 1974	44	6th instar	62	6th instar	40	6th instar	146
May 28, 1974	21	6th instar	36	6th instar	35	6th instar	92

APPENDIX I

Date	HUMIDITY		TEMPERATURE	
	Maximum (Range)	*Minimum (Range)*	*Maximum (Range)*	*Minimum (Range)*
May 16–2	94–96	40–43	29°C–31°C	14°C–15°C
May 20–27 * *	96–97	34–52	22°C–38°C	18°C–16°C
May 27–June 3 * *	96–97	36–51	27°C–34°C	13°C–15°C
June 3–June 9	93–96	32–51	28°C–34°C	9°C–12°C
June 9–16	94–96	31–52	28°C–34°C	9°C–12°C
June 17–24	95–96	41–58	21°C–27°C	9°C–13°C
June 24–30	92–95	38–76	19°C–27°C	11°C–13°C
July 1–7	94–97	48–62	15°C–26°C	11°C–13°C
July 8–15 *	94–96	41–58	20°C–23°C	10°C–13°C
July 15–21	94–96	52–58	18°C–22°C	10°C–13°C
July 21–28	95–97	35–65	21°C–26°C	11°C–13°C
July 28–August 5	96–97	32–70	16°C–31°C	8°C–12°C
Aug. 5–12	96–99	36–64	20°C–28°C	11°C–13°C
Aug. 12–19	95–99	47–64	18°C–27°C	9°C–13°C
Aug. 19–26	97–99	27–57	23°C–28°C	7°C–14°C
Aug. 26–Sept. 2	97–99	39–59	21°C–27°C	9°C–14°C
Sept. 2–9	97–99	37–65	20°C–26°C	9°C–13°C
Sept. 9–15	98–99	37–53	21°C–24°C	9°C–13°C
Septe. 15–23	97–99	48–70	16°C–23°C	8°C–12°C
Sept. 23–30	97–98	30–46	23°C–28°C	7°C–13°C
Oct. 1–7	96–98	34–45	29°C–31°C	7°C–14°C
Oct. 7–14	96–98	22–29	31°C–33°C	7°C–13°C
Oct. 14–22	93–98	26–36	31°C–34°C	9°C–12°C
Oct. 22–28	96–96	32–56	33.5°C–23°C	11°C–13°C

*Adult emergence.
* *Pupation

Pupation was observed to have started on May 24 and by June 1, (approx. 1 week) all larvae had gone under the soil. Maximum and minimum temperatures during this period ranged 22°C–38°C and 13°C–16°C respectively with the humidities fluctuating between a low range of 34% R.H.—52% R.H. and high range of 96% R.H.—97% R.H. (Appendix I).

The first moths to emerge did so on July 9. If these were first ones pupated (May 24th) then they had spent at least 6 weeks as pupae. This extended pupal duration suggests, among other possibilities physiological adaptability (diapause?) and is planned to be investigated in detail in future. Any comments on this behaviour are therefore reserved until the phenomenon is investigated in some detail. However, it is worth mentioning here that pupae dug out during mid-June and exposed to optimum conditions of temperature and humidity (ca. 25°C and ca. 70% respectively) in the laboratory, emerged within 2 days. Total emergence in the enclosed insectary till the end of July amounted to 9 ♀♀ and 8 ♂♂, a total of 17.

With an initial cohort of 300 first instar larvae only 17 (5.6%) adults emerged. This fact seems to be of significance from the point of view of constructing a life-table, especially when viewed in the light of mortalities during the growth stages discussed below:

Mortality factors

No apparent mortality was detected as long as the larvae were in the 1st and 2nd instars. 52 (17.3%) 5th instar dead larvae were counted and the symptoms shown indicated a virus infection.

Pupae examined by digging revealed mortality due to ants and physical damage. Some pupae were found dead without any apparent cause and were thus thought to have died due to physiological reasons. In all a total of 65 (21.6%) dead pupae were observed.

It seems that both the larval stages and the pupal stages of this species are subject to considerable mortality, an appreciable proportion of any given population being regulated by various regulatory factors. However, information from several such generations needs to be accumulated before we can even begin to understand the dynamics of armyworm populations and indeed, this is one of the aims of initiating the studies reported above.

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Brown, E. S., Elizabeth Betts and R. C. Riney, (1969). Seasonal changes in distribution of the African Armyworm, *Spodoptera exempta* (Wlk). (Lepidoptera: Noctuidae), with special reference to Eastern Africa. *Bull. Ent. Res. 58*, Part 4: 661-728.

6

MOSQUITO RESEARCH

Director of Research
Professor G. B. Craig, Jr. (1970)

Scientists:

Dr. D. Fanara	1971–1973)
Dr. W. Hausermann	(1971–1974)
Dr. L. P. Lounibos	(1974)
Dr. N. Lorimer	(1974)
Dr. P. McDonald	(1971–1974)
Dr. F. Ogah	(1973)
Mr. J. Petersen	(1974)

Abstract

Ecological studies were minimized and emphasis was on a village-scale field release. We released males carrying chromosome translocation heterozygotes conferring about 67% sterility. The release extended over 10 weeks, with about 1000 males released each day. The released males were fully competitive and inseminated field females at the predicted rate. Egg hatchability in the village dropped from 90%+ to 30–40%; when the release terminated, it slowly rose but had reached only 67% 10 weeks afterwards. When compared to a control village where no treatment was conducted, the release village showed a marked drop in adults collected while landing and biting. However, there was no reduction in rate of oviposition, perhaps because the experiment was too short. In a third village, treated by twice-weekly removal of larvae, the adult population declined virtually to zero. This initial experiment shows that translocations can be introduced into field populations and that they can survive long after introduction. This accomplishment is a first for any disease vector. These data further indicate that our original idea of population replacement with a translocation homozygote might be feasible. Therefore, release experiments in year 4 will attempt replacement rather than suppression of populations.

INTRODUCTION

Aedes aegypti is well-known as a major vector of arbovirus disease over most of the tropical world. In East Africa, the species is widespread and abundant. Even minimal control is not economically feasible in most areas. To compound the problem, there are numerous behaviourally distinct populations within the species. In East Africa, one population is domestic, breeding inside houses and feeding on man, while another is sylvan, breeding in treeholes and feeding on other animals. The relative importance of these forms to disease transmission needs clarification.

In recent years, entomologists have been searching for biological methods to regulate vector species, in the hopes that such methods would avoid both insecticide resistance and environmental pollution. One biological approach is "genetic control", the use of genetic manipulation to turn a species against itself. While the use of radiation-sterilized males has attracted wide attention, this method has had little use for mosquitoes; reduced fitness of irradiated males makes the method impractical. However, there are many other ways of inducing sterility; sophisticated methods of genetic breeding allow production of males that confer sterility on their mating partners and yet are fit and competitive. Chromosome translocations conferring semi-sterility are particularly promising, both for *Aedes aegypti* and for many other pest and vector insects. This method requires field trials; the present project is so directed.

PROJECT OBJECTIVES

Stated objectives of this contract include:

1. Develop a study area for intensive research on a population of *A. aegypti*.
   a. Determine absolute number and population fluctuation.
   b. Develop predictive life tables.
2. Establish 10–20 replicate sites for later use in release experiments. Monitor each population for at least one year.
3. Determine relationships between sylvan and domestic populations.
4. Develop chromosome translocations for use in genetic control.
5. Develop facilities for mass production of mosquitoes.
6. Make trial releases of lab-reared mosquitoes for methods development.
7. Conduct subsidiary research on bionomics of other *Stegomyia* mosquitoes.
8. Develop a major research component, the Mosquito Biology Unit (MBU) of the International Centre for Insect Physiology and Ecology (ICIPE).
9. Provide training opportunities for African scientists and technicians.
10. Serve as a mechanism to assist in strengthening the East African Community by affiliation with research institutes and universities.

METHODS

Preparation for release experiments began in January of 1974. The production of release males started with the build-up in numbers of a marker strain and two strains heterozygous for single translocations. The production of the release males necessitated four subsidiary crosses between these three strains. An account of the translocation homozygotes and the genetical methods for preparing material for release is given in Annexes two and three.

Releases began in mid-March and soon reached a peak of about 1200 males per day. A further increase was not possible, partly because of space and working hour limitations and partly because of a chronic water shortage and repeated breakdown of the water supply due to the severe dry season.

Originally the release study was organized on the basis of two triplets of experimental villages. Each triplet was to contain one release village. The first triplet consisted of large villages of near or above 100 inhabitants, the second of small villages of around 50 inhabitants. Due to rearing difficulties our male production did not reach the envisaged 2000 males per day and we decided to concentrate our study on the triplet of large villages alone (Fig. 1, Table 1). Chibarani village was selected for the releases, Kwa Dzivo was used as village for source reduction and Majengo was designated as reference village receiving no treatment. All three villages were monitored the same way for population density, sterility, and survival of translocations and genetic markers (Table 2).

Table 1. Description of release villages prior to release study

Name of village	*No. Inhabitants*	*No. Houses*	*No. Containers*	*No. Containers with larvae or pupae*	*L-B catch/man-hour*	
					Total	♀♀
CHIBARANI (= CHI)	161	31	48	34	3.7	2.6
KWA DZIVO (= KDZ)	108	31	36	26	6.4	5.3
MAJENGO (= MAJ)	82	28	43	32	3.6	1.4
Average 15 other Rabai villages	87.9	21.9	35.2	19.7	3.4	2.6

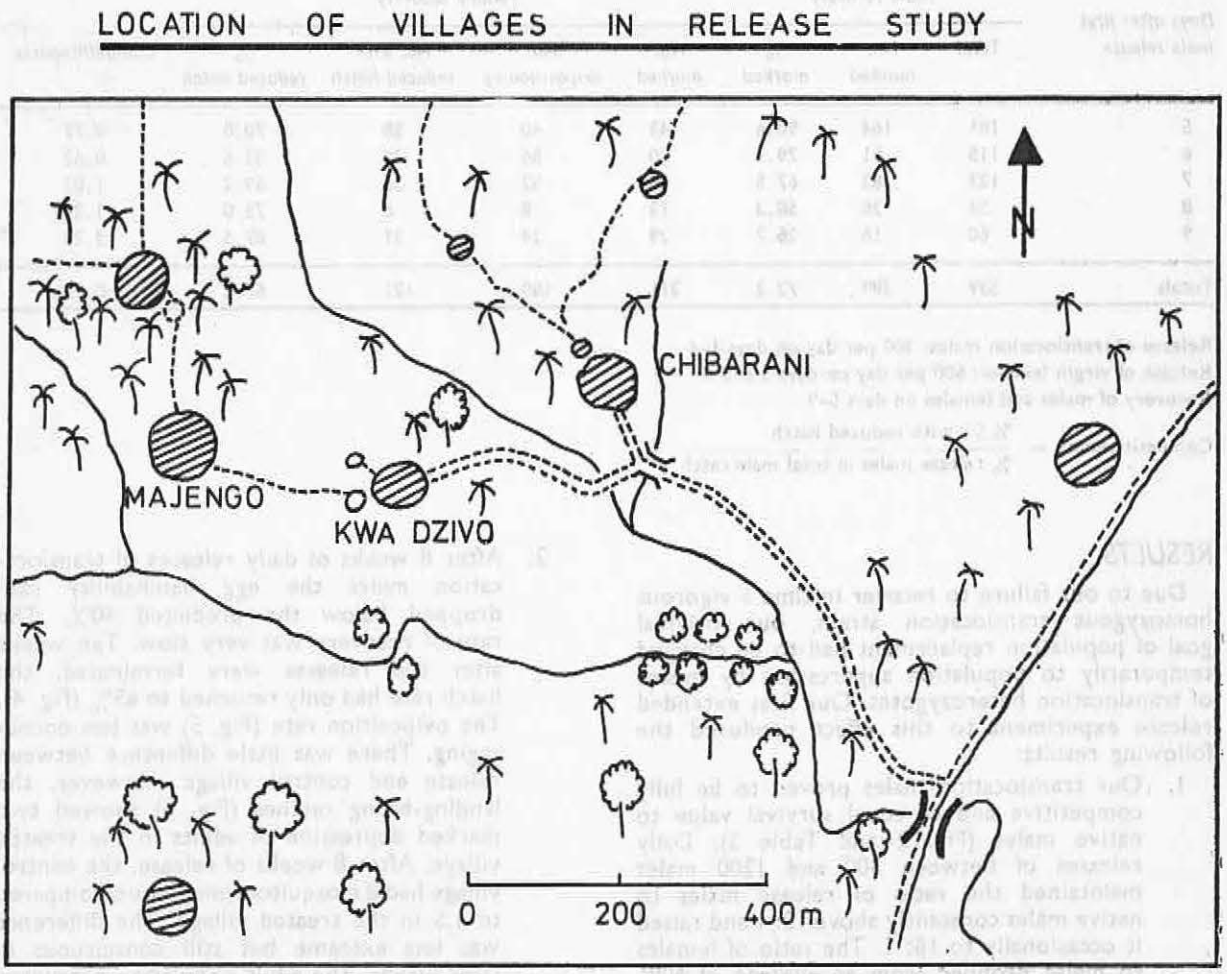


Fig. 1.

Table 2. Treatments and population assessments of the three villages in release experiment

Village	*Treatment*	*Weekly population assessments for all three villages*		*Additional assessments*
		Population size	*Sterility*	
CHI	Daily release of 300–1200 double heterozygote males from March 29 to June 9	1. Oviposition rates with 1 oviposition trap per house for 3 days per week	1. % hatchability of eggs collected in oviposition traps	1. Estimates for total adult populations at beginning and half time of release experiment
KDZ	Cleaning of domestic water pots every Wednesday and Saturday from March 30 till to date	2. Landing-biting rates with 3 men for 10 minutes per house once a week	2. % hatchability in egg batches of single females collected in L-B catch	2. Estimate for survival of release males and ratios of release males to native males for first half of release period
MAJ	No special treatment	3. Container index once a week		3. Assessment of competitiveness of release males in additional village
		4. Total pupal counts once a week		4. Assessment for survival of genetic marker and translocations from May 20 till to date.

Table 3. Competitiveness of double heterozygote release males

Days after first male release	*Male recovery*			*Female recovery*				*Competitiveness*
	Total	*No. marked*	*% marked*	*No. marked*	*No. ovipositioning*	*No. with reduced hatch*	*% reduced hatch*	
5	181	164	90.6	43	40	28	70.0	0.77
6	115	91	79.1	70	56	30	53.6	0.62
7	123	83	67.5	56	52	36	69.2	1.02
8	53	28	58.3	13	8	6	75.0	1.29
9	60	16	26.7	29	24	21	87.5	3.28
Totals	539	389	72.2	211	180	121	67.2	0.93

Release of translocation males: 500 per day on days 1–4
Release of virgin females: 600 per day on days 5 and 6
Recovery of males and females on days 5–9

$$\text{Competitiveness} = \frac{\%\ ♀♀ \text{ with reduced hatch}}{\%\ \text{release males in total male catch}}$$

RESULTS

Due to our failure to recover in time a vigorous homozygous translocation strain, our original goal of population replacement had to be changed temporarily to population suppression by means of translocation heterozygotes. Our first extended release experiment to this effect produced the following results:

1. Our translocation males proved to be fully competitive and of equal survival value to native males (Fig. 2 and Table 3). Daily releases of between 300 and 1200 males maintained the ratio of release males to native males constantly above 10: 1 and raised it occasionally to 16: 1. The ratio of females to males dropped from an average of 60% to below 20% (Fig. 3).
2. After 8 weeks of daily releases of translocation males the egg hatchability rate dropped below the predicted 40%. The rate of recovery was very slow. Ten weeks after the releases were terminated, the hatch rate had only returned to 65% (Fig. 4). The oviposition rate (Fig. 5) was less encouraging. There was little difference between release and control village. However, the landing-biting catches (Fig. 6) showed two marked depression of adults in the treated village. After 8 weeks of release, the control village had 8 mosquitoes/man/hour, compared to 1.5 in the treated village. The difference was less extreme but still conspicuous in later weeks. The adult population recovered quickly when releases were stopped.

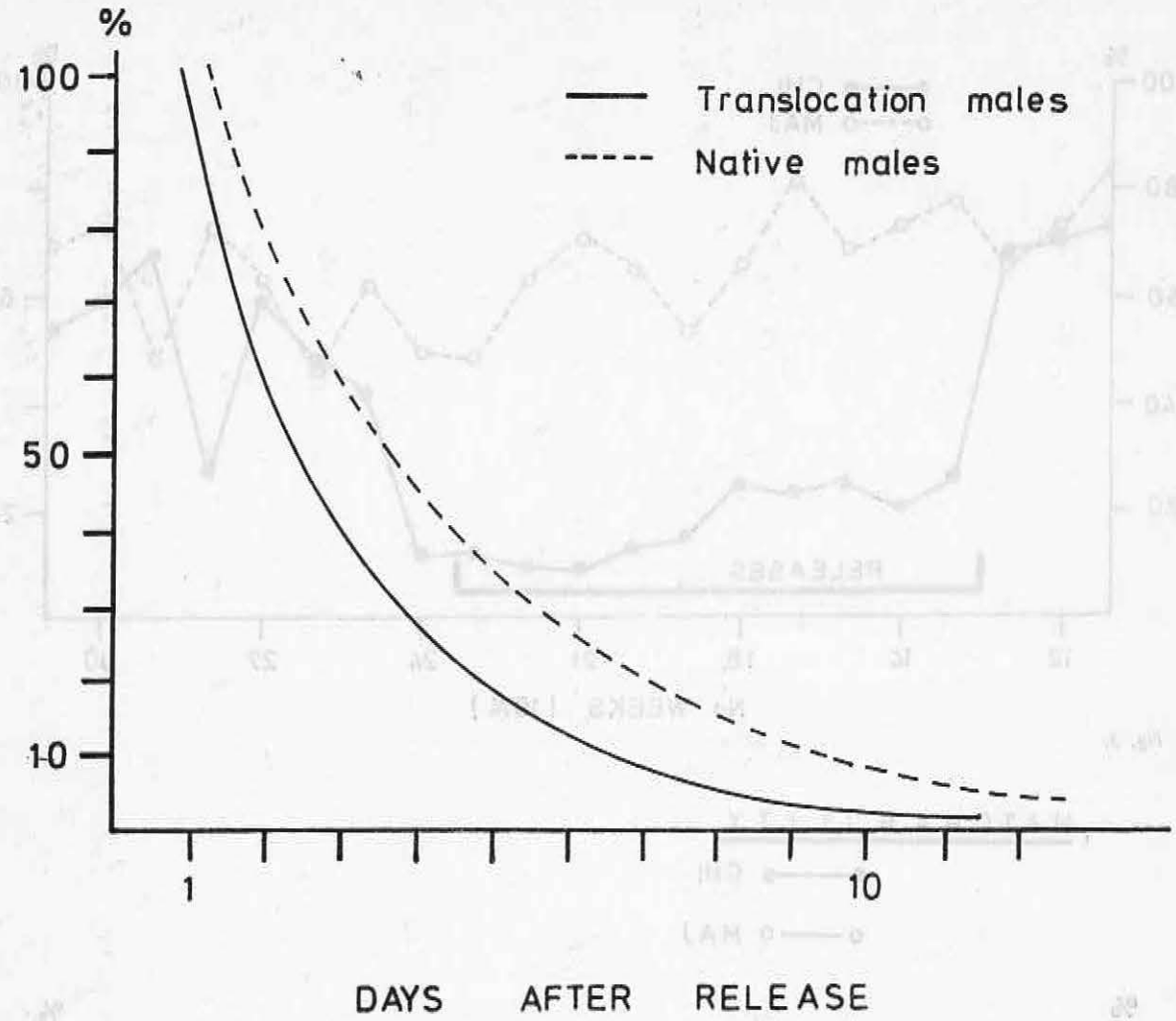


Fig. 2.

3. The killing of all larvae and pupae twice a week in Kwa Dzivo reduced the adult mosquito population rapidly and virtual eradication of the native population of domestic *A. aegypti* type form was achieved after 9 weeks. Immigration of domestic *A. aegypti* from neighbouring Chibarani and Majengo was minimal. A small amount of domestic oviposition by peridomestic *A. aegypti* ssp. *formosus* was observed, however, through the whole period. This intrusion of ssp. *formosus* into the domestic habitat is still taking place today after source reduction has been continued for 10 weeks beyond the originally determined date.
4. Migration between the release villages was during the whole release period negligibly small. Fluorescent dust marked males from Chibarani were recovered only once in Majengo and twice in Kwa Dzivo. A significant depression in fertility was observed only twice in Majengo, but regularly in Kwa Dzivo before the native population was removed. This low migration was not unexpected since we had dry season conditions for the whole duration of the releases, the rains being two months later than usual. See Annex 4.
5. Genetic analysis of egg collection and pupal collections demonstrated an unexpectedly slow disappearance of the genetic markers introduced with the translocations and indicated so far a stabilisation of the M-linked translocation at around 50% (Fig. 7).

DISCUSSION

We have demonstrated the ability to introduce a translocation into a population and to induce sterility long after the releases are over. We believe this is a major accomplishment.

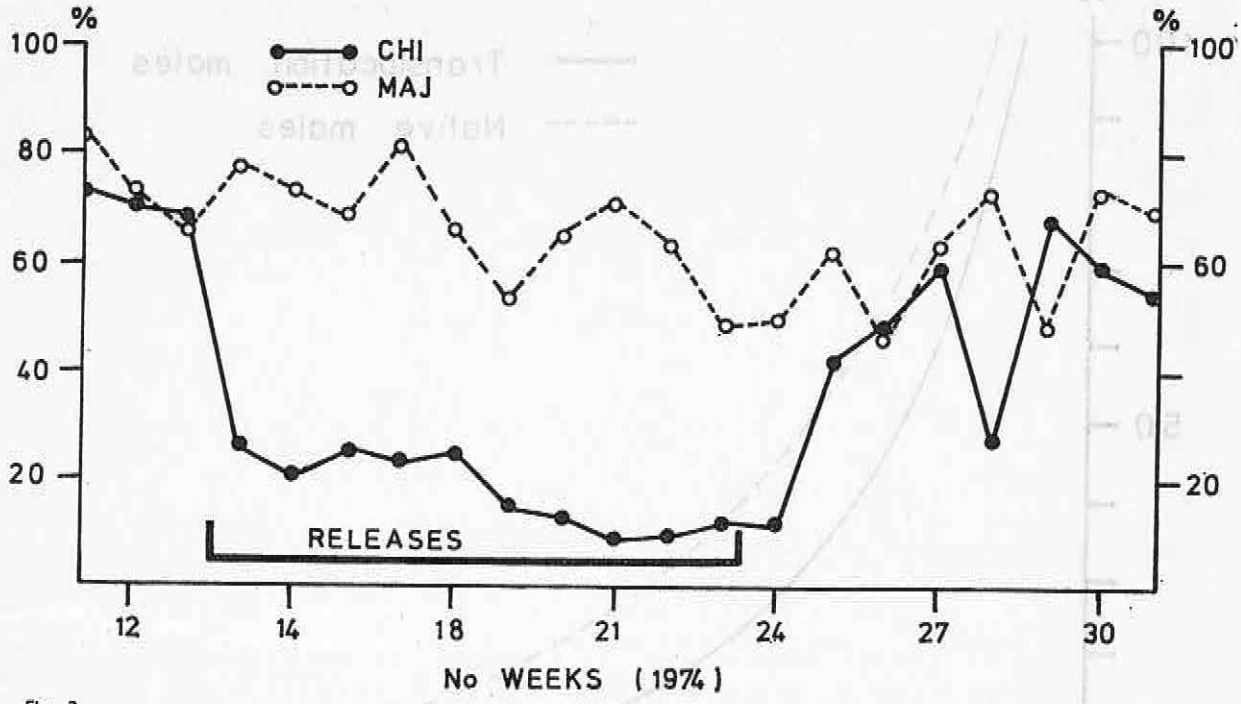

FEMALE RATIOS IN RELEASE● AND REFERENCE○ VILLAGE
%
100
80
60
40
20
CHI
MAJ
RELEASES
12
14
18
21
24
27
30
No WEEKS (1974)

Fig. 3.

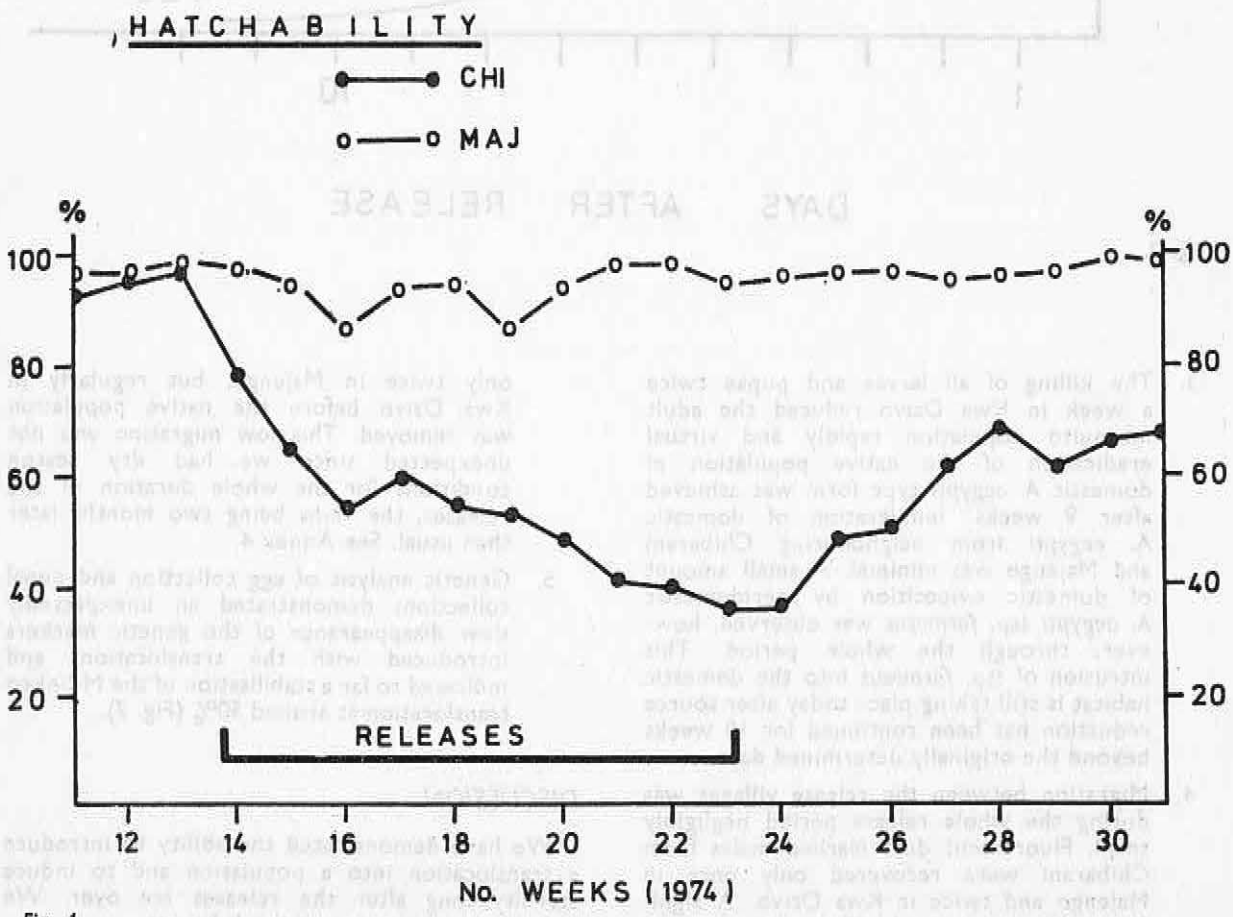

HATCHABILITY
CHI
MAJ
%
100
80
60
40
20
RELEASES
12
14
16
18
20
22
24
26
28
30
No. WEEKS (1974)

Fig. 4.

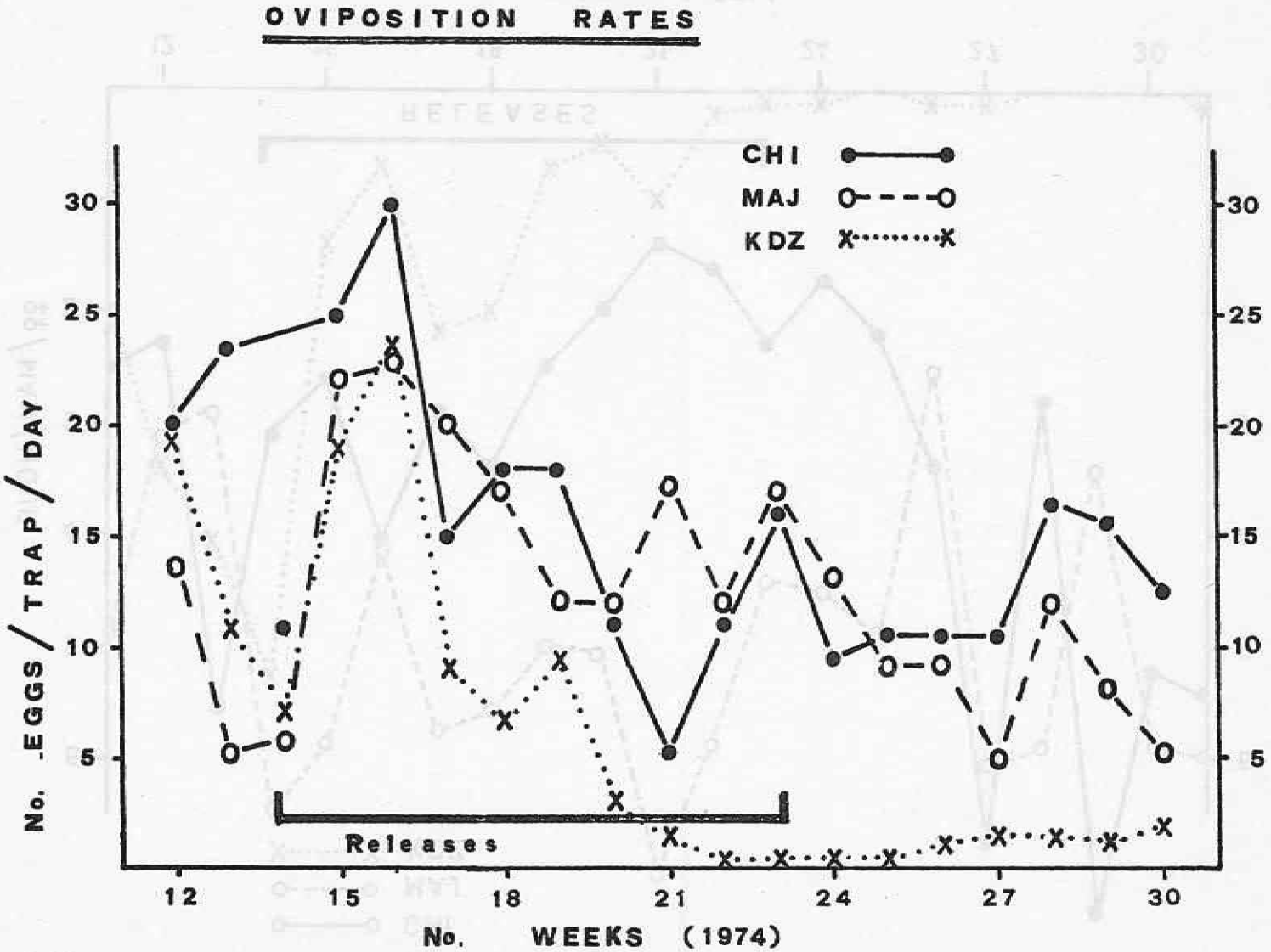

OVIPOSITION RATES
CHI
MAJ
KDZ
No. EGGS / TRAP / DAY
30
25
20
15
10
5
Releases
12
15
18
21
24
27
30
No. WEEKS (1974)

Fig. 5.

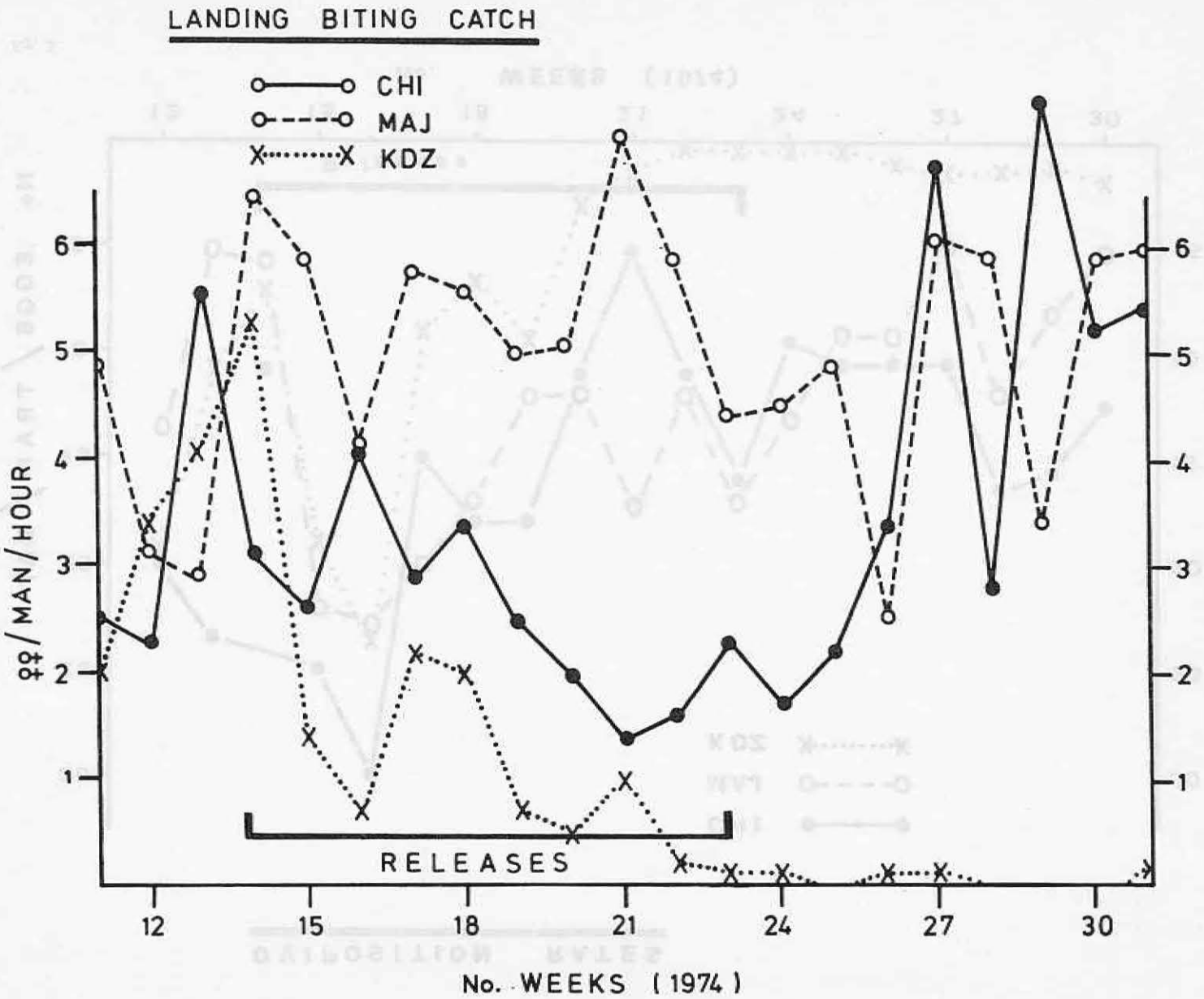

LANDING BITING CATCH
CHI
MAJ
KDZ
♀♀/ MAN / HOUR
RELEASES
No. WEEKS (1974)
12
15
18
21
24
27
30
1
2
3
4
5
6

Fig. 6.

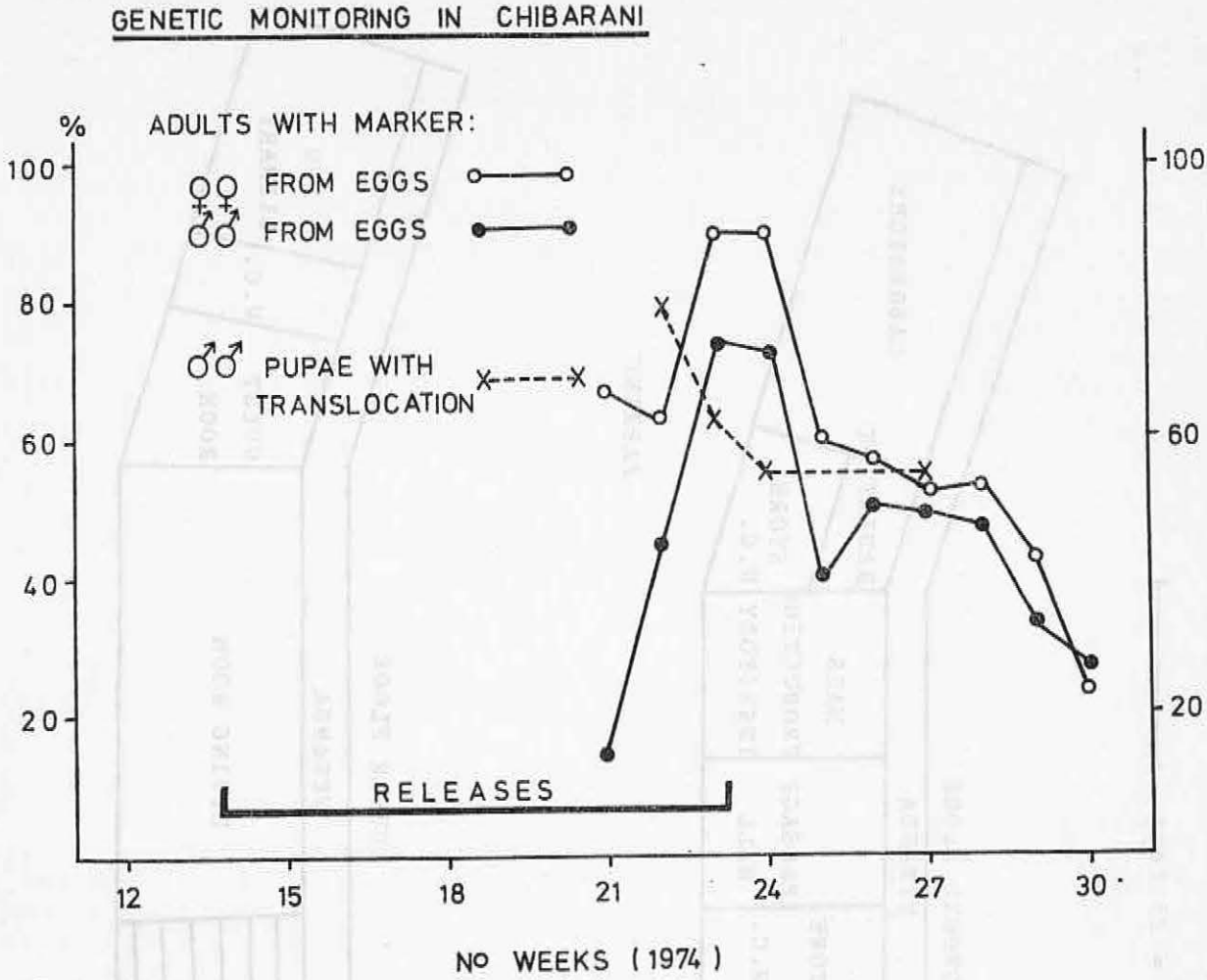


Fig. 7.

The failure to maintain a significant reduction in adult population size does not prove a failure of genetic control by means of translocations. The double heterozygote release males introduced into the population the maximum level of sterility which could be expected. That the population size was not reduced as much as the sterility would lead one to expect confirms our earlier findings, that most of the mortality occurs in the larval stage and is density dependent. In domestic habitats egg mortality just reduces density dependent larval mortality.

The persistence of the male-linked translocation in the population for more than ten weeks after releases stopped indicates that selected translocations can be established in field populations for extended periods and suggests strongly that the original idea of population replacement is feasible once an appropriate homozygous translocation is available. This might very well be the case with the now available strain.

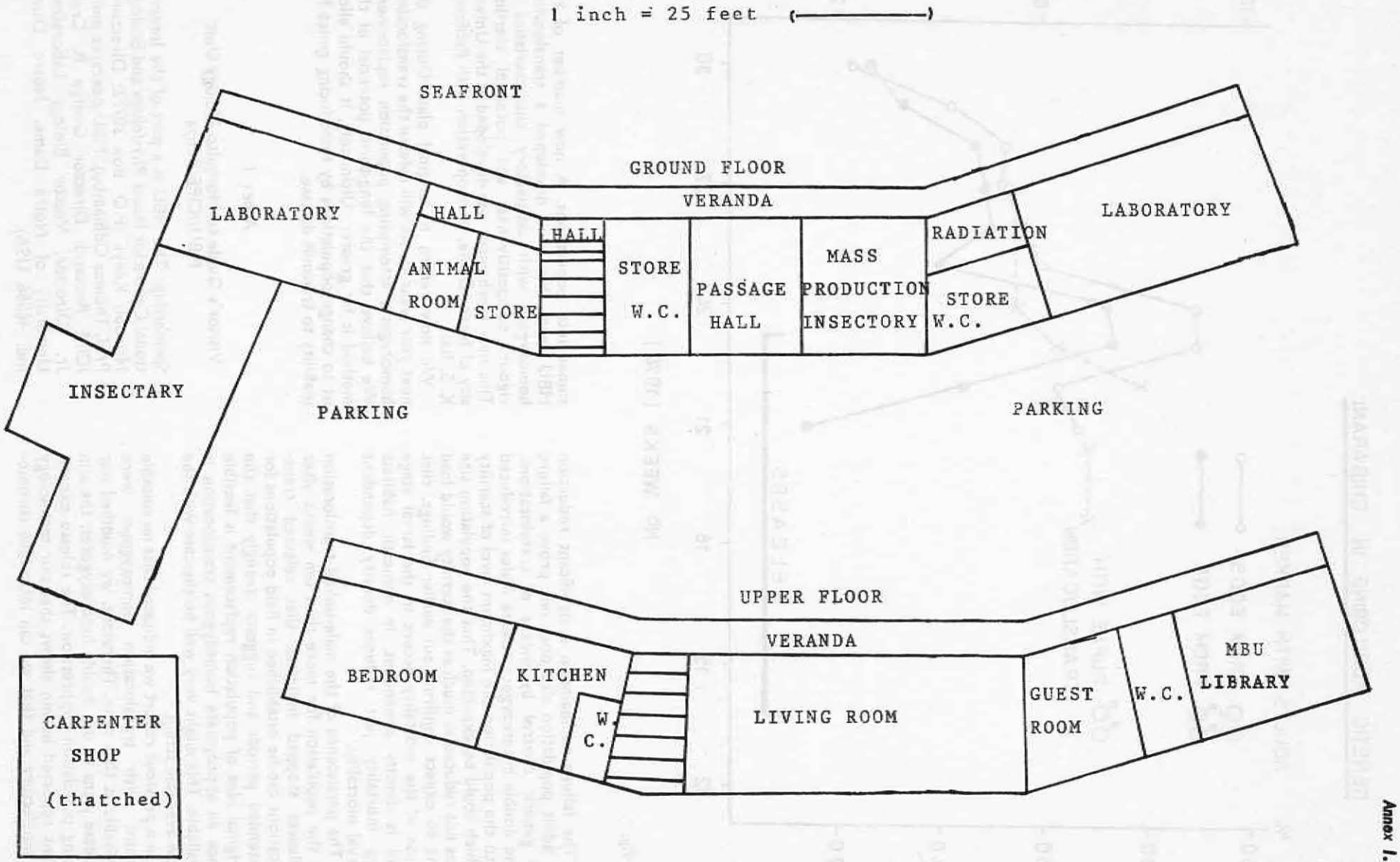
In a previous report we indicated that no suitable strains with translocation homozygotes were available at that time. Therefore, we modified our release plans to use double heterozygotes, the aim being population suppression. The release experiment reported herein shows that our technology is satisfactory and that we can introduce chromosomes into populations. A new member of the MBU team, has now developed a translocation homozygote with satisfactory characteristics of vigour, competitiveness and potential sterility. This new mechanism was developed at the University of Notre Dame, in cooperation with Professor K. S. Rai.

We now return to original plan. During the next year (year 4), we will release the translocation homozygote, attempting population replacement. We believe that the long-term potential of this method is far greater. Ultimately, it should allow us to change populations by introducing genes for inability to transmit disease.

Annex 1.

Visitor's Guide to Mosquito Biology Unit
MBU/ICIPE/Kenya

1. *Sponsorship:* The MBU is a part of the International Centre for Insect Physiology and Ecology, Nairobi, Kenya (P.O. Box 30772; Director—Prof. Thomas Odhiambo). MBU operates under ICIPE Research Director George B. Craig, Jr. (Director, Vector Biology Laboratory, University of Notre Dame, Notre Dame, Ind. 46556, USA).


1 inch = 25 feet
SEAFRONT
GROUND FLOOR
VERANDA
LABORATORY
HALL
ANIMAL ROOM
STORE
HALL
STORE
W.C.
PASSAGE HALL
MASS PRODUCTION INSECTORY
RADIATION
STORE
W.C.
LABORATORY
INSECTARY
PARKING
PARKING
UPPER FLOOR
VERANDA
BEDROOM
KITCHEN
W. C.
LIVING ROOM
GUEST ROOM
W.C.
MBU
LIBRARY
CARPENTER SHOP
(thatched)

2. *Funding:* MBU operates on a 4-year research contract to the University of Notre Dame from the U.S. Agency for International Development. This contract terminates on 1 July 1975. Funds are also provided by the UNDP, via ICIPE, and by the U.S. Public Health Service.

3. *Location:* The laboratory is located in a 15 room home at Nyali Beach, north of Mombasa. P.O. Box 80804; Phone Mombasa 71653. A diagram is attached. Field studies are conducted in rural villages in Rabai District, west of Mombasa, a 30 minute drive from the laboratory.

4. *Personnel:*

Scientists—1st 3 years—Walter Hausermann, Project Leader (now Ciba-Geigy, Basel) Paul MacDonald, Geneticist (now U. Calif,. Berkeley) Dean Fanara, Ecologist (now WHO, Indonesia)
Year 4—L.Philip Lounibos, Project Leader (NIH PD Fellow)
Nancy Lorimer, Cytogeneticist
John Petersen, Ecologist
Fedelis Ogah, Population Geneticist (ICIPE Fellow)

Technicians: Rosbella Chesang—Laboratory management, microscopic work
David Maina—Special genetic strains, translocations
Samuel Mburu—Mass production
George Tsuma—Rearing of laboratory strains
Nicoloas Juma—Electrophoresis,

Field Assistants: Julius Mwandadu, Livingstone Ndenge

Workshop Assistants: James Rimba, Steve Wanjohi

Temporary Assistants., Daily Basis:
Karim Iddi, Peter Kimani, Kenneth Saha, Malaki Ralik, Eduard Mkuzi

5. *Project Objectives:*

(*a*) To study the ecology of the yellow fever mosquito, *Aedes aegypti*, in detail, to recognize the key environmental factors and to predict its responses to seasonal change and control operations.

(*b*) To attempt population suppression by genetic manipulation of populations, the so-called "genetic control".

6. *Research Progress:*

The research was organized in two phases. After a preliminary period devoted to site selection (Kenya Coast has heavy year-round populations of *A. aegypti*), two years (June 71 to June 73) were devoted to studies of selected sylvan and domestic populations of *A. aegypti*. Simultaneously, strains with chromosome translocations were selected and developed in the laboratory. From July of 1973 onwards, primary emphasis shifted toward small-scale field trials of released males bearing translocations for genetic control. Results to date have been exciting. When males were released daily at a 10:1 ratio, a high level of sterility was established in a village. More important, sterility continued long after the releases were halted. By June of 1975, we expect to be able to make a well-documented recommendation on the feasibility of genetic control.

7. *Summary—Ecological Research:*

(*a*) Population composition—On the Kenya coast, *A. aegypti* occurs in two distinct populations, domestic and sylvan. These populations have very little genetic exchange, although their habitats overlap. The populations differ in their dependence on rainfall and in their preference for oviposition sites, hunting sites and host species. They can also be distinguished on the basis of coloration of both larvae and adults. The domestic form, subspecies *aegypti*, (a) has adults that are brownish and creamy white, with some pale mutants of the *queenslandensis* variety, (b) has a high frequency of yellow larva, (c) lives inside houses, with larvae breeding in clay pots and other water containers, (d) prefers man as host. The sylvan form, subspecies *formosus*, (a.) has adults that are black and silvery white, (b) has no yellow larva, (c) lives in forests, with larvae breeding in treeholes, (d) prefers other animals, not man, as host. The numbers of the domestic form are reasonably constant throughout the year, whereas the sylvan form is abundant only during the rainy season. Also during the rainy season a third form, peridomestic, appear, breeding in outdoor habitats such as old tyres, discarded containers, etc. The peridomestic form results from hybridization of the other two forms.

(*b*) Many experiments have been conducted on marking and releasing mosquitoes for later recapture, in studies of population size and dispersal. Mosquitoes are either dusted with fluorescent dyes or individually painted with dots in a distinctive pattern. Recovery rates on these experiments have been in the vicinity of 30–50%, exceptionally high for this sort of work. We find that in a typical village of the Rabai District, with 15–20 houses, there is an ongoing population of about 1000 mosquitoes, with 50–100 new adults emerging each day. Both sexes move at random through the village, from house to house Relatively few adults leave the village, the number depending on the distance to the adjacent village. Less than 1% will move as far as 800 meters away from the village of origin. These characteristics make the Rabai area an excellent study arena for

experiments on genetic control; each village may be considered as an ecological island.

8. *Summary—Genetic Research*

(*a*) In the laboratory, chromosome translocations have been induced by radiation and bred and analysed with genetic marker stocks. In two years, 78 translocation heterozygotes have been isolated. Initially, we had hoped to obtain translocation homozygotes and to use them in experiments on genetic manipulation by replacement of populations. However, we were unable to obtain homozygotes in the time available. Therefore, we have developed a system of double heterozygotes which confer high sterility. Field females mated by males carrying this system have 20–30% egg hatch; moreover, almost all resulting progeny also carry the system. The population will continue to be affected long after the initial release.

(*b*) A field experiment on genetic control was initiated in April, 1974. Three villages were used; one was for the release of translocated males, the second was for control by removal of larvae and the third was untreated. All villages were monitored weekly for landing-biting adults, pupal output, oviposition rate and sterility. Males were released at the rate of 1000 per day, every day for 60 days. This gave a ratio of better than 10:1 of release males over native males.

(*c*) Results of the first field experiment are still coming in at the time this is written. However, there is no doubt that the high sterility was established in the experimental population. The villages all started with 94–98% egg hatch. In the translocation release village, the egg hatch rate declined steadily to 40% hatch after six weeks and remained at this level for 10 additional weeks (to the present). Note that releases were halted after the first 8 weeks.

9. *Visit to the Field Site:*

(*a*) All of MBU's experimental field sites are situated in the area of the Rabai people in Rabai and Ruruma of Kilifi District, just west of Mombasa. The reasons for selecting sites away from the immediate coast and outside the municipal boundaries of Mombasa are twofold:

(*a*) Mombasa exercises within its boundaries a vigorous mosquito control programme which prohibits long range field observations. No mosquito control (or other insect control) is conducted in Rabai.

(*b*) The people in the mosquito-producing areas of Mombasa and the coastal tourist belt are heterogenous, mobile and highly individualistic. Full cooperation of all people, which is essential for our kind of work, is very difficult to obtain on the basis of friendly persuasion. In Rabai, the population is homogeneous and extensive cooperation with the villagers can be obtained almost everywhere after proper introduction and acquintance with the village elders. In practice, the people have given us a warm and friendly welcome. We are deeply grateful to them.

(*b*) The field sites are reached after a 30–40 minute drive from the MBU laboratory. The approach leads through Mombasa Island and then follows the road to Nairobi for about 10 miles. After climbing the coastal foothills in Mazeras, the Rabai area lies to the Northeast of the Nairobi road, in the coconut belt. Please see the attached sketch map. In the Chief's Camp, we have located an ICIPE Mobile Laboratory; testing for egg hatch and microscopic examination for translocations are done here. The three experimental villages are typical of hundreds of others in the coastal belt. They were treated as follows:

(*a*) Chibarani—Translocated males released, 1000/day for 8 weeks

(*b*) Kwa Dzivo—Larvae in domestic water containers (clay pots) removed twice weekly by cleaning pots

(*c*) Majengo—No control.

The distances between these villages are 240 m and 205, m, respectively. Biting and domestic breeding in all villages monitored weekly.

(*c*) Subsequent experiments will be done in villages that are further apart. More effort to prevent immigration will be made. Two more releases are planned, one in October of 1974 (short rains) and one in January of 1975 (long rains).

10. *Outreach: MBU has working contacts with the following agencies:*

(*a*) University College, Nairobi—Entomol. Dept.—Prof. Odhiambo, Botany Dept.—Prof. Olembo, ICIPE home laboratory.

(*b*) Division of Vector-Borne Disease, Kenya Medical Dept., Nairobi Director—Dr. J. D. Roberts; Entomologist—V. J. Prashar.

(*c*) East African Institute of Malaria & Vector-Borne Disease, Amani, Tanzania Director—Philip Wegesa; Entomologist—John Raybold.

(*d*) Dept. Preventive Medicine, School of Medicine, Univ. Dar-es-Salaam, Tanz. Senior Lecturer—Wenceslaus Kilama, Filariasis.

(*e*) East African Inst. of Virus Research, Entebbe, Uganda Entomologists—Louis Mukwaya, S. Sempala.

MBU FIELD STUDY Rabai, Kilifi District W. of Mombasa

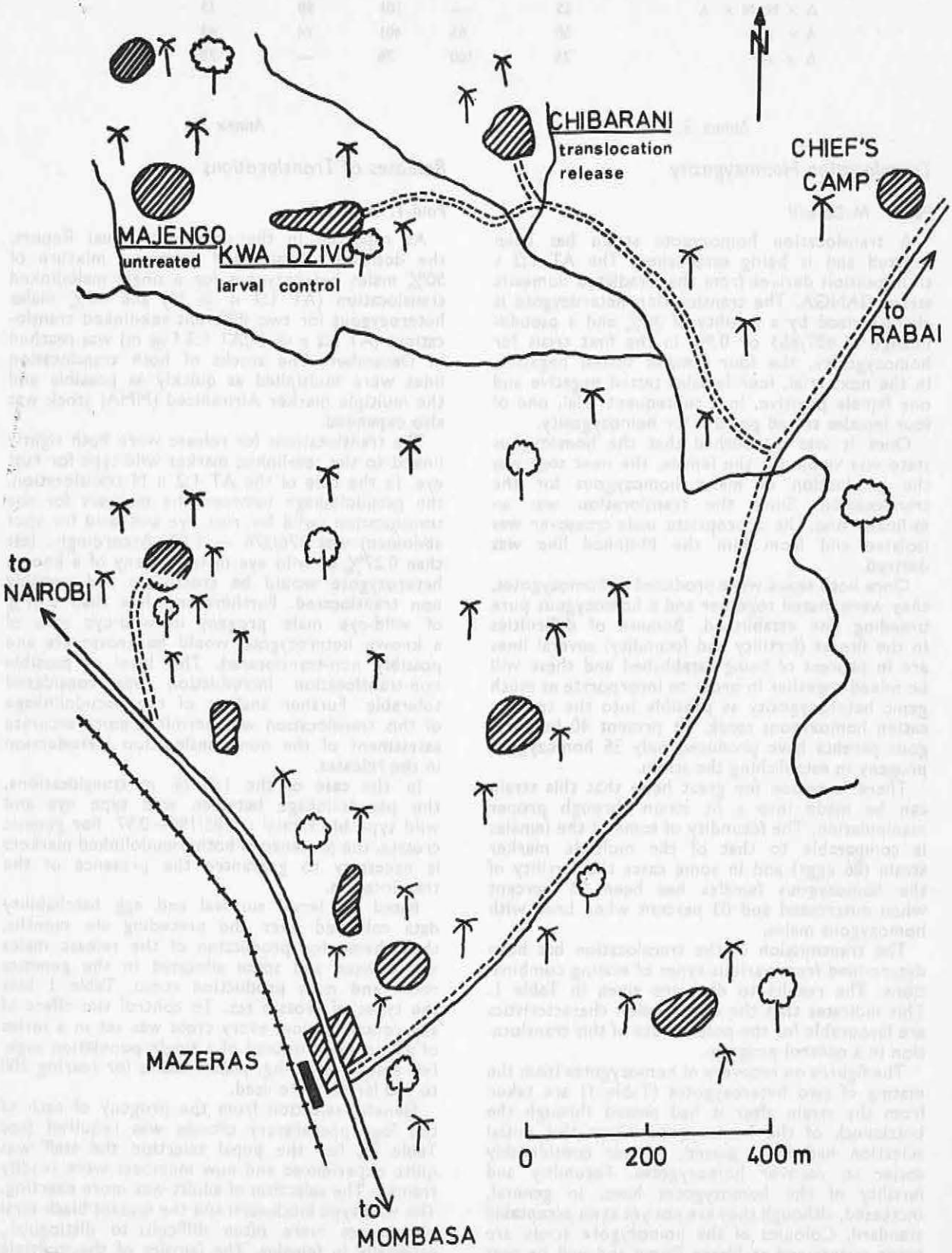


Table 1. Transmission of Translocation at 1:2 t

Parental Cross	Percent Translocation in Parent	Progeny Δ	Progeny Δ	Progeny N	Percent Translocation in Progeny
Δ × N; N × Δ	25	—	101	98	25
Δ × Δ	50	65	401	74	49
Δ × Δ	75	100	76	—	78

Annex 2.

Translocation Homozygosity

Paul T. McDonald

A translocation homozygote strain has been isolated and is being established. The AT 1:2 t translocation derives from the irradiated domestic strain GANGA. The translocation heterozygote is characterized by a fertility of 30% and a pseudolinkage of 637/653 or 0.98. In the first trials for homozygosity, the four females tested negative. In the next trial, four females tested negative and one female positive. In a subsequent trial, one of four females tested positive for homozygosity.

Once it was established that the homozygous state was viable for the female, the next step was the production of males homozygous for the translocation. Since the translocation was an m-linked one, the appropriate male crossover was isolated and from him the M-linked line was derived.

Once both sexes were produced as homozygotes, they were mated together and a homozygous pure breeding line established. Because of difficulties in the fitness (fertility and fecundity) several lines are in process of being established and these will be mixed together in order to incorporate as much genic heterozygosity as possible into the translocation homozygous stock. At present 40 homozygous parents have produced only 35 homozygous progeny in establishing the strain.

There is reason for great hope that this strain can be made into a fit strain through proper manipulation. The fecundity of some of the females is comparable to that of the multiple marker strain (86 eggs) and in some cases the fertility of the homozygous females has been 95 percent when outcrossed and 83 percent when bred with homozygous males.

The transmission of the translocation has been determined from various types of mating combinations. The results to date are given in Table 1. This indicates that the transmission characteristics are favourable for the possible use of this translocation in a control program.

The figures on recovery of homozygotes from the mating of two heterozygotes (Table 1) are taken from the strain after it had passed through the bottleneck of the homozygote. Once this initial selection had been passed, it was considerably easier to recover homozygotes. Fecundity and fertility of the homozygotes have, in general, increased, although they are not yet at an acceptable standard. Colonies of the homozygote strain are being maintained at Notre Dame and will be sent to Mombasa once they are well established.

Annex 3.

Releases of Translocations

Paul T. McDonald

As reported in the previous Annual Report, the decision to raise and release a mixture of 50% males heterozygous for a single male-linked translocation (AT 1:2 n @ M) and 50% males heterozygous for two different sex-linked translocations (AT 1:2 n @ M/AT 1:3 f @ m) was reached in December. The stocks of both translocation lines were multiplied as quickly as possible and the multiple marker Africanized (MMA) stock was also expanded.

The translocations for release were both tightly linked to the sex-linked marker wild type for rust eye. In the case of the AT 1:2 n M translocation, the pseudolinkage between the markers for the translocation (wild for rust eye and wild for spot abdomen) was 376/376 = 1.00. Accordingly, less than 0.27% of wild eye male progeny of a known heterozygote would be crossovers and possibly non translocated. Furthermore, less than 0.81% of wild-eye male progeny of wild-eye sons of a known heterozygote would be crossovers and possibly non-translocated. This level of possible non-translocation introduction was considered tolerable. Further analysis of the pseudolinkage of this translocation will permit a more accurate assessment of the non-translocation introduction in the releases.

In the case of the 1:3f @ m translocations, the pseudolinkage between wild type eye and wild type black-tarsi is 185/190=0.97. For genetic crosses, the presence of both pseudolinked markers is necessary to guarantee the presence of the translocation.

Based on larval survival and egg hatchability data collected over the preceding six months, the scheme for production of the release males was devised and space allocated in the genetics room and mass production room. Table 1 lists the types of crosses set. To control the effect of any contamination, every cross was set in a series of gallon cages instead of a single population cage. For ease of handling, plastic basins for rearing 200 to 300 larvae were used.

Genetic selection from the progeny of each of the four preparatory crosses was required (see Table 2). For the pupal selection the staff was quite experienced and new members were readily trained. The selection of adults was more exacting. The wild type black-tarsi and the mutant black-tarsi phenotypes were often difficult to distinguish, especially in females. The females of the multiple marker stock were all black-tarsi mutants, yet

the expression had come to resemble the wild type, illustrating the probable occurrence of an evolution of dominance.

The genetic crosses were either set by the geneticist or under his direct supervision. The adult progeny of each day's crosses 1, 2, and 3 were examined and checked for possible contamination. Two instances of contaminated groups were found, and in both cases the contamination was eliminated before they could be used in other crosses. The checking of adult phenotypes daily provided the critical control point in the entire production operation, in the effort to avoid and contain any contamination in the series of genetic crosses necessary for the releases.

Pupal selection alone was applied to progeny from crosses 4 and 5, and this was done in the interests of managing all the other work carefully with the staff available. (Table 2). By avoiding the adult selections, only a slight amount (less than 0.81%) of non translocation males would be included among the release males.

As a final check on the quality of individuals taken for release in the field, a sample was taken at approximately ten-day intervals, and these males were mated with wild type virgin females of the strain collected in the release village before any releases. The results are entered in Table 3.

On two occasions the phenotypes of the release males were checked, and on both occasions, the mixture was approximately 50% of each type of male.

	Single Heterozygote	*Double Heterozygote*
19 March	87	93
— June	29	24

Furthermore, a sample containing 13 single and double heterozygotes was mated with 40 virgin multiple marker females. The results indicate transmission from both types of males in equal frequency with 95% of males carrying the M-linked translocation and 33% of females carrying the m-linked translocation, based on 101 progeny.

During the course of the release, a problem developed with the feeding of the larvae, and, until new food was available, a considerable loss of the stocks occurred. In order to augment the release males of the mixture, a second line was initiated for release, and this line was the expanded stock of the single heterozygote AT 1:2 n @ M. This stock was released only when the number of males of the mixture was below 1000.

Dusted males were released during the first six weeks in order that they could be identified in collections in the field. Each day the group was dusted with a different colour or combination of colours, for a period of ten days in a colour cycle.

The objective of the rearing scheme was the provision of approximately 1000 males daily on a long term basis. The actual numbers of males released and their colours are given in Table 4 for the period 29 March-14 May.

Table 1. Scheme of crosses necessary for continuous daily production of 1,000 males heterozygous for two translocations ΔM/ Δm
1,000 males heterozygous for one translocation ΔM

Cross	*Size* ♀♀ × ♂♂	*No. cages per day* (60♀♀ × 20♂♂)	*Expected yield of eggs*	*Adults required*	*Expected yield of adults*	*No. adults needed*	*No. 1st instars set*	*No. pans per day*
Preparatory crosses in genetics room:								
1. MMA × MMA	120 × 40	2	7,200	MMA ♀	1,940	540	2,000	8
2. MMA × M Δ	120 × 40	2	7,200	ΔM ♂	580	440	1,750	7
3. Δ m × MMA	60 × 20	1	3,600	Δm ♂	140	100 ♂	750	3
				Δm ♀	140	100 ♀		
4. MMA × Δm	300 × 100	5	18,000	Δm ♀	1,440	1,200	4,800	16
Production cross in mass production room:								
5. Δm × ΔM	1200 × 400	20	72,000	ΔM/ Δm♂ ΔM	2,160	2,000	8,100	27

Table 2. Genetic Marker Selection for Crosses

Cross	*Translocation*	*Pupal Selection* *eye*	*Adult Selection* *abdomen*	*hind tarsi*
1.	Marker Stock (♂♂+♀♀)	rust	spot	black-tarsi
2.	1:2n @ M(♂♂)*	wild	wild	black-tarsi
	1:2n @ M(♂♂)**	wild	——	
3.	1:3f @ m(♂♂)	wild	spot	black-tarsi
	1:3f @ m(♀♀)	wild	spot	black-tarsi
4.	1:3f @ m(♀♀)**	wild	——	——————
5.	1:2n @ M(♂♂)	wild	——	——————
	1:2n @ M/1:3f m(♂♂)	wild	——	——————

*To renew cross 2.
**For use in cross 5; excess for release if necessary.

Table 3. Quality Control of Release Males

Date	Cross ♀♀ × ♂♂	Hatchability
11 April	Chibarini (60) × Chibarini (25)	969/1046=93%
11 April	Chibarini (60) × Mixture (25)	322/1021=32%
22 April	Chibarini (34) × Chibarini (17)	608/631=96%
22 April	Chibarini (37) × Mixture (19)	53/158=34%
1 May	Chibarini (70) × Chibarini (25)	756/829=91%
1 May	Chibarini (50) × Mixture (25)	72/113=64%
16 May	Chibarini (70) × Chibarini (25)	302/305=99%
16 May	Chibarini (50) × Mixture (25)	162/456=36%
16 May	Chibarini (50) × Single Heterozygote	180/362=50%

Table 4. Release Males at Chibarani

Date	Colour	Number *	Death	Released
29 March	HR	729	7	
30 March	HY	900	2	
31 March	DR	621	5	
1 April	DG	1086	12	
2	DO	1275	13	
3	DG, DR	777	7	SH only
4	DG, HR	420	4	
5	HG, HR	228	0	
6	HG	708	4	
7	DY	795	35	
8 April	HR	903	15	
9	HY	1000	5	(1/2 D.H. mix)
10	DR	1000	3	(1/2 D.H. mix)
11	DG	297	25	
12	DO	444	5	Mix
13	DGDR	288	1	
14	DGHR	417	5	
15	HGHR	1278	0	
16	HG	1047	1	
17	DY	963	5	
18 April	HR	633	3	
19	HY	0	0	
20	DR	420	5	
21	DG	624	4	
22	DO	291	3	
23	DGDR	402	4	
24	DGHR	657	8	
25	HGHR	1326	92	
26	HG	1005	47	
27	DY	384	2	
28 April	HR	858	16	
29	HY	1005	4	
30	DR	1005	27	
1 May	DG	834	6	
2	DO	804	29	
3	DGDR	1000	147	
4	DGHR	1059	29	
5	HGHR	1140	9	
6	HG	1200	3	
7	DY	1002	8	
8	HR	1005	9	
9	HY	1590	9	
10	DR	1200	4	
11	DG	1020	25	
12	DO & DY	450	4	
13	———	1050	0	
14	———	1325	3	

*29 March–8 April Single M-linked heterozygotes only.
9, 10 April Mixture 75%–25% Single and Double.
11 April–14 May Mixture of 50–50% Single and Double.

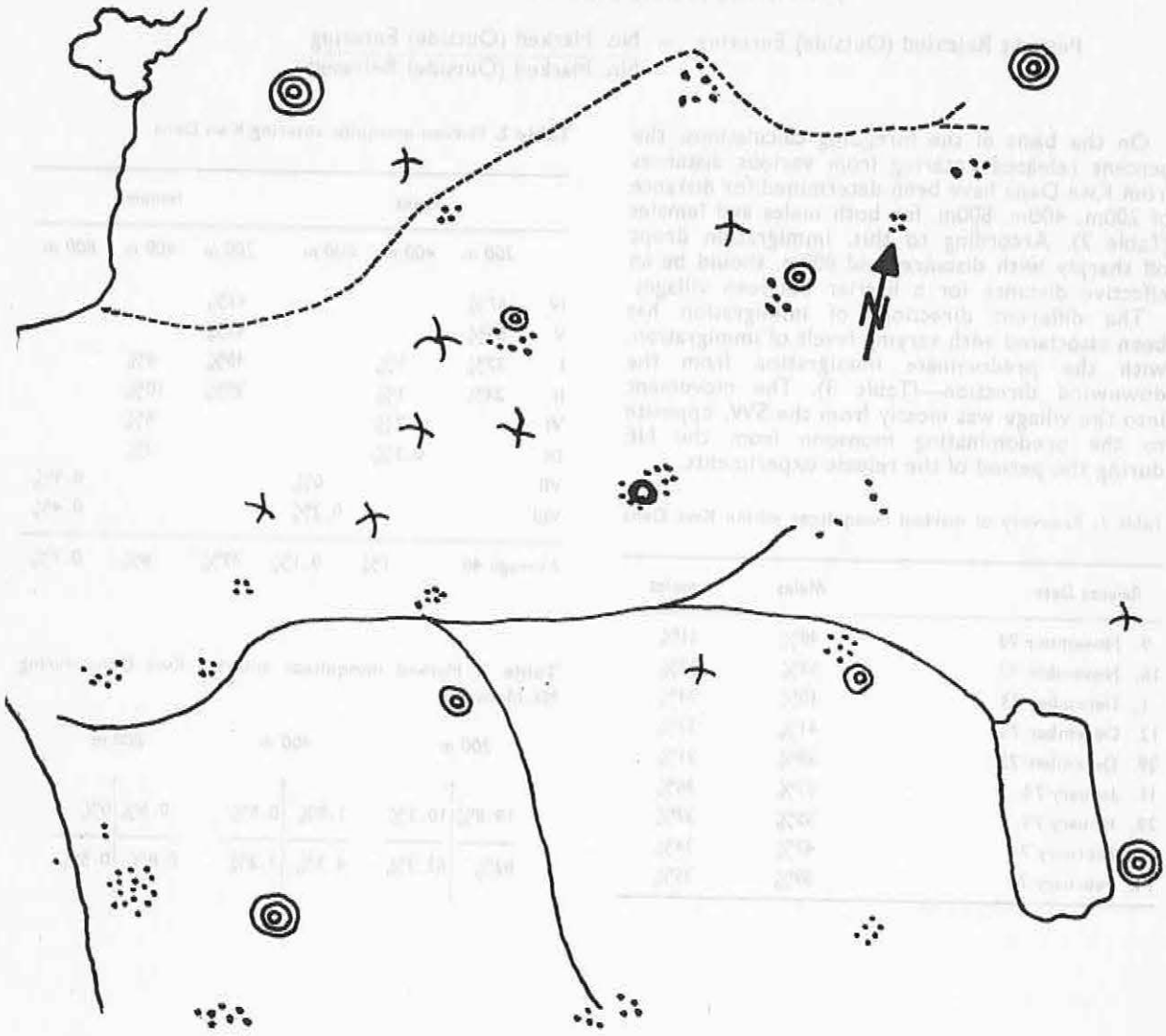
Annex 4.

Movement of Mosquitoes Between Vilages

Paul T. McDonald

Studies on movement of domestic *Aedes aegypti* between villages have been completed since the last Annual Report. A total of nine releases were made. Newly-emerged adult males and females of the RABAI DOMESTIC strain were released in various numbers within and at measured distances from the center of Kwa Dena village. A sketch map of the area including the village is included as Figure 1. The release points located at 400 meters and 800 meters from the center of the village are also indicated on the map. The daily handing-biting catches were done by two men, each spending thirty minutes in each of the 14 houses of the village.

The recoveries of the mosquitoes released within the village in the L-B catches of the nine days subsequent to release are given in Table 1. Generally the recovery was quite good except for 1 December's release. This release has been excluded in analysis of movements into the village.



LEGEND

- Kwa Dena
- 400 m
- 800 m
- Houses
- Trees
- Road, Quarry

Fig. 1.

The estimate of immigration was done as follows:

$$\text{Number Entering} = \frac{\text{No. Marked (Inside) Released}}{\text{No. Marked (Inside) Captured}} \times \text{No. Marked (Outside) Captured}$$

$$\text{Percent Released (Outside) Entering} = \frac{\text{No. Marked (Outside) Entering}}{\text{No. Marked (Outside) Released}}$$

On the basis of the foregoing calculations, the percent released entering from various distances from Kwa Dena have been determined for distance of 200m. 400m. 800m. for both males and females (Table 2). According to this, immigration drops off sharply with distance, and 800m. should be an effective distance for a barrier between villages.

The different directions of immigration has been associated with varying levels of immigration, with the predominate immigration from the downwind direction—(Table 3). The movement into the village was mostly from the SW, opposite to the predominating monsoon from the NE during the period of the release experiments.

Table 1. Recovery of marked mosquitoes within Kwa Dena

Release Date	*Males*	*Females*
9. November 73	49%	21%
16. November 73	52%	32%
1. December 73	10%	24%
12. December 73	41%	32%
29. December 73	28%	21%
11. January 74	53%	36%
28. January 74	55%	37%
5. February 74	43%	34%
14. February 74	59%	35%

Table 2. Marked mosquito entering Kwa Dena

	Males			*Females*		
	200 m	*400 m*	*800 m*	*200 m*	*400 m*	*800 m*
IV	47%			42%		
V	55%			43%		
I	32%	1%		45%	6%	
II	24%	1%		39%	10%	
VI		2%			6%	
IX		0.3%			2%	
VII			0%			0.9%
VIII			0.2%			0.4%
Average 40		1%	0.1%	42%	6%	0.7%

Table 3. Marked mosquitoes entering Kwa Dena during NE-Monsoon

200 m	*400 m*	*800 m*
19.8% | 10.3%	2.8% | 0.5%	0.5% | 0%
82% | 62.3%	4.3% | 3.3%	0.8% | 0.5%

7

TERMITE RESEARCH

Directors of Research:
Professor M. Lüscher (1970)
Dr. W. A. Sands (1973)

Scientists:
Dr. R. H. Leuthold (1971–1974)
Dr. F. C. Mathez (1973)
Dr. G. W. Oloo (1974)
Mr. K. M. Wanyonyi (1971)
Mr. O. H. Bruinsma (1974)

Collaborators:
Dr. C. J. Heather (1974) Fine Structure
Dr. M. Kaib (1973) Electrophysiology
Dr. G. D. Prestwich (1974) Chemistry
Dr. S. Takahashi (1973–1974) Chemistry

BEHAVIOUR

INTRODUCTION

The survival of termites, all of which live in complex societies, must require an efficient communication system. Recent studies show that the mechanisms underlying such communication are primarily tactile and olfactory. The use of pheromones by termites and other social insects is very extensive (Lüscher, 1970) and, if clearly understood, might be taken advantage of in controlling harmful species. Thus, termite behaviour research at the ICIPE has concentrated on the orientation and communication mechanisms of foraging termites.

Orientation and Communication in the Harvester Termite Hodotermes Mossambicus

Menotaxis (light compass orientation) and trail pheromones

Menotaxis has been shown to be a major factor for the above-ground orientation of outgoing and returning workers during harvesting activity if optical conditions are suitable. Optical conditions are provided by sunshine, moonlight or artificial light at night.

Pheromone trails are established by outcomers. Trail-dependent chemical orientation is essential when optical cues are lacking (e.g. overcast sky or moonless night), as proved in turntable experiments by shifting the trail through an angle of 90°. Using extracts from different tissues, it was demonstrated that the sternal gland produces the trail-active substance. This was expected from work on other termites. The trail-following behaviour, however, differs from the conventional type. Whereas in other termites that have been studied, a trail is perceived on rather close contact and followed tropotactically as a firm line, in *Hodotermes* it acts as a gradient chemical zone within the boundaries of which the termites vaguely keep on course (for details, see the comparative study below). This principle explains the more scattered distribution of foraging workers on overcast days compared with the rather bundled pattern when menotactic orientation takes over.

If the direction of the chemical trail is well separated (e.g. by an angle of 90°) from the trained azimuth, a 100% menotactic orientation is observed when there is a central light source (turntable and mirror experiments), whereas trail orientation is performed when the sky is overcast. When clouds cover the sun but blue patches of sky are visible, the outcomers follow the azimuth of the trained direction (whether the pattern of polarized sky is the cue for orientation is a matter for further investigation). If the trail is shifted only a little from the trained direction (22°), the termites leaving the nest in full sunshine, first pursue the chemical trail and then change over to sun orientation. The returning termites first keep an intermediate course and then run according to sun orientation.

To summarize, optical orientation dominates over chemical orientation in clearly segregated alternative situations. Chemical trails influence the orientation if their active area overlaps with the path determined by optical cues. With decreasing strength of the optical stimulus, the chemical means of orientation gains increasing dominance. Termites going out from the nest tend to use pheromone orientation more readily than returning termites.

Memory

Memory for the homing distance has been proved by means of displacement experiments. Returning foragers displaced from food baits far from home (100 cm) walk significantly further before they stop orientating than do those displaced from food baits near home (40 cm).

Structure and Function of Sternal and Tergal Glands

The sternal gland: polymorphism and pheromone bioassays

The two systematically unrelated termite species *Trinervitermes bettonianus* and *Hodotermes mossambicus* are both rare exceptions with regard to the caste polymorphism of their sternal glands. In *Hodotermes* an enormous gland hypertrophy is found in the swarmed male whereas in *Trinervitermes* a similar phenomenon is found in swarmed females. In contrast to *Hodotermes*, the increase of glandular size in *Trinervitermes* is accompanied by multiple increase of the gland's trail-laying activity (Leuthold and Lüscher, 1974).

A comparison of the volume of the sternal gland in the various castes of *T. bettonianus* showed the following ratios:— worker : newly dealate

male : newly dealate female = approx. 1 : 10 : 65. The corresponding trail-activity exhibits the much higher contrast of approximately 1 : 70 : 1,200 respectively.

The increase in physiological trail activity between worker and female alates is about 20 times larger than the increase in glandular volume. This indicates a relatively higher capacity of either the pheromone producing or pheromone storing organelles of the gland. Ultrastructural comparative analysis of these glands promises to reveal fundamental principles of the pathway of pheromone production. Cooperative investigation of this problem is now progressing with the collaboration of Dr. A. Quennedey in Dijon.

Behavioural Association of the Sternal and Tergal Glands

In a series of behavioural investigations it was demonstrated that caste polymorphism of the sternal gland in *T. bettonianus* is reflected in the caste's specialized behaviour. Only the workers take part actively in exploration for food and in recruitment. They are the only ones that lay foraging trails. The soldiers with their almost vestigial glands do not lay discernible trails. The large sternal glands of the imagines reveal their function during courtship behaviour. The female's sternal gland, together with the tergal gland, is exposed during "calling" for the attraction of the male. After meeting, male and female dealates perform their so-called "tandem-run" (the behaviour of close following of the female by the male). During the tandem-run the female's sternal gland increases the affinity of the male to the female. The very strong pheromone trails established by both sexes when walking enable the couple, if disconnected, to join again.

A further series of experiments demonstrates the relative significance of both the tergal and sternal glands in the female. From isolated glands as well as glandular extracts it is shown that the tergal gland dominates male attraction at distances from 10 to 2 cm, whereas attraction at less than 2 cm is dominated by the sternal gland. From a cross experiment comparing both workers' and imagines' sternal extracts in both competitive trail bioassay with workers and sex-attraction bioassay with males, evidence points to the trail pheromones of workers and imagines possessing identical properties.

Chemical Analysis of Hodotermes Trail Pheromone

A quantitative laboratory bioassay for trail pheromone extracts has been designed with consideration of the particular mechanism of trail-following response as mentioned above. After we had established this routine test, our chemist succeeded in the stepwise chemical purification of the pheromone to the extent of obtaining a five-component mixture that contains the trail-active component.

Preliminary work on purification. A portion of abdominal sternite IV and V, including the trail pheromone gland, was excised under the microscope and extracted with methylene chloride. A solution of 100 glands in 100 µl methylene chloride was bioassayed and shown to be active: 10 µl solution containing 0.01 glands spread over 20 cm trail, was followed 100% by workers.

The gland extract was chromatographed on TLC. The TLC was developed with 1% ether in n-hexane and divided into 5 portions. RF 0.25 ~ 0.45 showed activity.

The whole body extract of 800 workers was collected and concentrated. This extract had an activity of 0.01 ~ 0.1 worker/10 µl (15 + out of 20), and gave 150 mg of crude oil. The oil was hydrolised with KOH in methanol at room temperature. The non-saponifiable layer showed activity at 0.04 worker/10 µl. (16 + out of 20, yield 34 mg).

The active crude solution was lowered in activity by the addition of a drop of Br_2 in CCl_4. The activity was tested after purification.

The activity was retained after collecting through *GLC* at 150° for 20 minutes using either 10% S.E.–30 or 5% Carbowax 20M (Column 3 mm × 1m).

Bioassay. A group of large and small workers was collected from the field in Kajiado, Kenya, and kept in a container with moist soil.

The bioassay was carried out on a blank paper with double Y-shaped lines. The room was kept under red light, constant temperature, and a high relative humidity. On the lines marked with + on a drawing, 5 µl of the sample solution was spread evenly with a capillary. A certain number of workers in a small box was placed at Point 0 at the bottom of the Y. One worker in each test was allowed to enter the testing maze and was twice given a choice between following lines+ and lines−.

Collection. At night, when workers came out of the nest to forage, they were collected. Extraction was done with methylene chloride (7,755 workers). The crude oil was then hydrolised with KOH in methanol to remove the lipid. The non-saponifiable part was then chromatographed on silica gel (100 g, 60 ~ 80 mesh). The portion eluted with 5% ether in n-pentane was collected; and crude oil obtained from the eluate was chromatographed on TLC (silica gel 0.25mm, precoated by Merck). First, Rf 0.05 ~ 0.6 was collected as the active portion by development with IZ ether in n-pentane. This was repeated, and Rf 0.05 ~ 0.45 collected by a second TLC. The solution of mg/ml gave 100% response (20 + out of 20 tested).

This active fraction was subjected to preparative GLC, using a 3mm × 1m glass column packed with 5% Carbowax 20M on Chromasorb W, AW-DMCS 60–80 mesh at 150%. The fraction collected between 0 and 20 was active in the bioassay. Since this active fraction contains several components, further purification by preparative GLC is necessary.

The pheromone is suspected to be an unsaturated alcohol of the molecular weight of 250–300. Unfortunately, our chemist left the ICIPE before accomplishing the chemical identification of the pheromone; however, he hopes to finalize this work in his laboratory in Japan using extracts prepared here.

The relative trail activity of the extracted sternal glands of various castes was determined by means of the bioassay. No significant difference was found between major workers, minor workers, soldiers, and swarmed females. Extracts of swarmed males,

however, revealed no trail activity in the range of concentrations used for the test. This is in striking contrast to the size of the sternal gland: the volume of a male gland is approx. 30 times that of a female and 700 times that of a major worker. The glands differ from each other in shape and structure and histological sections of the glands prepared in Bern reveal such exceptional features that experts in termite ultrastructure in Prof. Ch. Noirot's laboratory in Dijon immediately become interested in collaboration on this problem. The ongoing cooperative work with Dr. A. Quennedey will contribute an interesting chapter to his comparative survey of the ultrastructure of termite sternal glands.

A Comparative Study of Trail Pherome Mechanisms

Hodotermes mossambicus workers follow artificial pheromone trails in a manner which differs from that of *Schedorhinotermes lamanianus* and other termite species. They do not stay in close contact with the trail as do the other species but walk sinuously, recrossing the trail regularly. This observation indicates an osmoklinotactical orientation, i.e. the termite orientates up an odour gradient towards the higher concentration.

Experiments have been carried out in an attempt to prove the osmoklinotaxis. The antennae of *Hodotermes* workers are moved horizontally. The orientation of termites with one immobilised antenna does not differ significantly from those with one amputated antenna. In both instances the horizontal movement of the remaining or intact antenna increases and overall progress is slower due to increased deviations. After immobilising or amputating both antennae, the termites are no longer able to follow a trail.

As with other termite species, *Schedorhinotermes* workers follow trails in very close contact, trying to keep the trail between the antennae. This indicates an osmotropotactical orientation in which the termite compares the sensory input from both antennae and moves forward keeping both inputs equal. Theoretically, one would expect that the amputation of one antenna would prevent trail following. This is not the case however, directly after amputation the termite turns away from the trail towards the side having the intact antenna and follows the trail sinuously for a certain distance. After a short period however, it reverts to following the trail in a straight line, but to one side of it. The antenna under these circumstances now moves horizontally. Immobilising one or both antennae has no effect. These results indicate that *Schedorhinotermes* workers use an osmotropotactical orientation, but are able to switch to osmoklinotaxis after the loss of one antenna. This switch-over however, appears to be incomplete.

CASTE FORMATION

INTRODUCTION

We have noted earlier that communication within the termite society is of fundamental importance for survival. There also exists, however, a second high priority requirement, the balanced production of individuals of different castes which evidently constitutes the basis for the division of labour within the colony. Although studies on lower termites indicate caste development is under hormonal regulation (Lüscher, 1960; Wanyonyi, 1974), corresponding information on higher termites is fragmentary or even lacking. In addition to observations on colony composition in the field, attempts have been made to rear lower and higher termites under laboratory conditions and to study factors which control their caste development.

Net Structure and Mature Colony Composition of Odontotermes

A study of 3 nests permits the following description Fig. 1. The external evidence of a nest is a number of chimneys, which measure about 4–10 cm in diameter and which may be up to 64 cm deep. They do not open directly into the nest.

The nest consists of many chambers (11 to 68) of very variable sizes. Most of the chambers contain fungus gardens in which the termites live. A few chambers have been found empty. In two nests with 11–15 chambers each, there was one central chamber larger than all the others at the bottom of which the royal cell was located. In the third nest, with 68 chambers, there was no evidence of a central chamber.

In all three cases studied, a single royal cell with one physogastric queen and one king was found. Larvae were the most numerous, followed by the workers.

Although the chambers closest to the royal cell harboured most of the eggs, there was no indication that the composition of individuals varied from chamber to chamber. The function of the empty chambers is unknown.

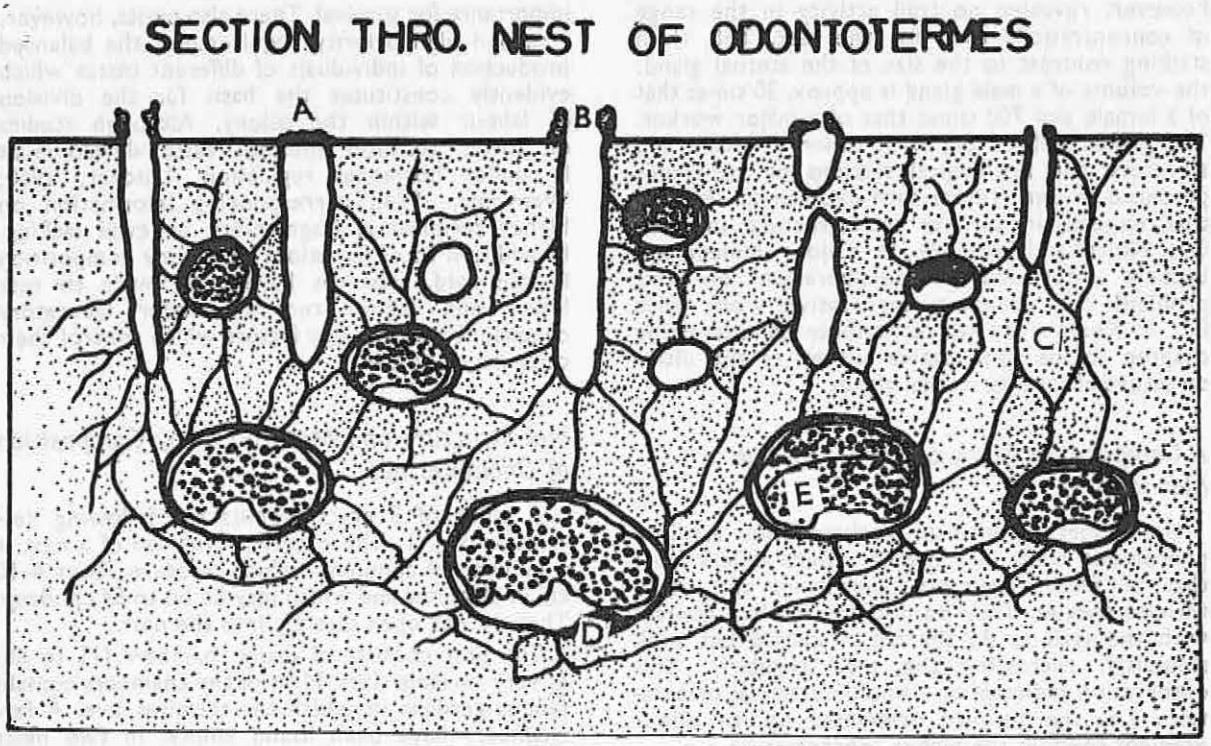
These studies will be extended to cover a 12-month period so as to obtain detailed information of a complete seasonal cycle.

Rearing Under Laboratory Conditions

Incipient colonies of *Odontotermes* were established in sterilized soil in aluminium pots and kept at 29°C±1°C, ca. 80–90% r.h. The colonies were watered from time to time, and when workers were observed to be foraging on the surface for food they were provided with suitable food materials.

Of about 500 colonies so established between the 6th and 8th November 1973, 324 are still being cared for in the insectary. About one third of these colonies have been observed foraging. Examination of 11 of the foraging colonies three months after their establishment gave the following compositions:— Table 1.

Similarly a large number of *Hodotermes* colonies have now been successfully established. We also have a few colonies of *Microtermes* which are doing very well and have recently started to breed *Macrotermes subhyalinus*.



A Closed Chimney
B Open Chimney
C Interconecting Channels
D Royal Cell
E Fungus Garden

Table I. Composition of 3-month-old incipient colonies of *Odontotermes* (18.2.1974)

	Original Colony Number	*No. of larvae*	*No. of workers*	*No. of presoldiers*	*No. of Soldiers*	*Total*
1.	F106	17	25	1	3	45
2.	F120	23	20	–	3	46
3.	F212	24	25	–	3	51
4.	F246	19	14	–	2	35
5.	F247	22	27	–	3	52
6.	F255	27	26	–	2	55
7.	F280	23	33	–	3	59
8.	F281	29	35	–	3	67
9.	F282	22	30	–	3	55
10.	F283A	24	32	–	3	59
11.	F283B	20	16	–	4	40

Odontotermes Endocrine Glands

The endocrine glands of an *Odontotermes* larvae have been described in the first report. They include the *corpora cardiaca*, *corpora allata* and the moult or prothoracic glands. We have now attempted a comparative study of the size of these glands in various castes of the termite.

Materials and Methods

The materials and the histological methods remain the same as already described in the first report.

Serial sections of the required termites were studied using Wild M20 stereomicroscope and camera lucida drawings of the glands were made at a fixed magnification.

The magnification was accurately determined with the help of Thoma counting chamber whose small square is equivalent to 1/400mm^2. The area of the drawing was determined with the use of Coradi Cora-Senior Planimeter. Since the magnification factor is known, the actual cross-sectional area of the gland can be calculated. The mean value in a series of serial, sagittal sections was taken as the size of the gland.

Results and Discussion

The sizes of the *corpora cardiaca* in the castes investigated are shown in Table 2.

Table 2. *Corpus Cardiacum* size (Cross-sectional area in μ^2)

	Larvae	*Presoldiers*	*Soldiers*	*Workers*
1.	781.25	923.61	1562.50	906.25
2.	958.30	1161.75		937.50
3.	1156.25	1453.13		1072.92
4.	1500.00	1619.10		1171.86
5.	1604.00	1625.00		1171.86
6.	1999.50			1171.86
Mean	1333.22	1356.66	1562.50	1072.04

Results show no significant size variations in the *corpus cardiacum* from caste to caste. It may be recalled that the function of the *corpus cardiacum* is to store neurosecretory material from the *pars intercerebalis* of the brain. Our histological technique has so far not been aimed at demonstrating the presence of neurosecretory material. The function of the *corpus cardiacum* as an endocrine gland in its own right has not been investigated in termites. Our present studies show that the *corpus cardiacum* probably does not play a significant direct role in termite caste development.

The *corpus allatum*, however, shows interesting size variations as summarized in Table 3.

Table 3. *Corpus Allatum* size (Cross-sectional area in μ^2)

	Larvae	*Presoldiers*	*Soldiers*	*Workers*
1.	539.06	3500.00	906.25	484.38
2.	567.71	4572.92		500.00
3.	713.54	4832.15		510.42
4.	828.13	5765.63		520.92
5.	976.56	5833.33		687.25
6.	1046.88			796.88
Mean	778.65	4900.81	906.25	583.31

The *corpus allatum* is largest in presoldiers, smallest in workers and intermediate in size in larvae. Since the presoldiers stage is short and transitional (presoldiers are destined to develop into soldiers within about 14 days), it can be inferred that the formation of the soldier in *Odontotermes* requires a high level of juvenile hormone. Although we cannot indicate when the *corpora allata* become adequately large to produce the juvenile hormone required for soldier differentiation, our results support existing evidence that, in lower termites, a high juvenile hormone titer is necessary for soldier differentiation.

It is not possible to make a similar conclusion with regard to worker differentiation. But since half of the workers fixed were only lightly pigmented and probably still young as workers, the possibility of worker differentiation occurring under low juvenile hormone titer must be considered.

According to Table 4, the moult glands of *Odontotermes* are largest in soldiers but smallest in workers. The sizes of larval and presoldier prothoracic glands lie between the two extremes.

Table 4. Moult or Prothoracic Gland size (Cross-sectional area μ^2).

	Larvae	*Presoldiers*	*Soldiers*	*Workers*
1.	1612.31	1083.25	3487.50	522.32
2.	1060.13	1125.00	8750.00	714.88
3.		1515.63		790.65
4.		1703.13		820.31
5.				1195.31
6.				1206.25
Mean	1336.22	1356.75	6118.75	874.95

Since only sagittal sections were made for these measurements, presoldiers and soldiers which possess elongate heads probably show higher values of prothoracic glands size than they should. Examination of the cellular structure, however, clearly indicates that the prothoracic glands of presoldiers possess larger and more granular cells than those of larvae and soldiers, while the prothoracic glands of workers have small and degenerate cells. It can be concluded that the prothoracic glands of presoldiers probably actively secrete moulting hormone necessary for the soldier moult. Had more larvae been dissected, one would expect to find similarly active cells in those larvae approaching the moult stage.

Group Effect and Reaction to JH Analogues

Effect of artificially increasing a colony's population in Hodotermes

12 incipient colonies each received the larvae and eggs (but not the adults) of another colony. When the resulting populations were compared to control colonies six months later, it was evident that artificially increasing the incipient populations had induced no changes. The experimental populations and their composition were not significantly different from the control colonies.

The Influence of Soldiers upon Caste Determination in Hodotermes

A total of 84 colonies, half containing 1 soldier each and the other half being control colonies lacking soldiers, were scrutinised approximately 150, 175 and 200 days after swarming. No significant differences were noted between colonies with or without soldiers.

Effect of J. H. Analogue Applications on Hodotermes

Applications to very young colonies have not, as yet, induced the formation of soldiers. However,

depending upon the concentration of analogue application, the hormone may prove lethal to adults, larvae and eggs or may cause females to oviposit three times the normal quantity of eggs all of which are infertile.

Presoldier Formation in Hodotermes

Scrutiny of incipient colonies over an extended period indicates that the time taken to produce the first presoldiers in colonies varies considerably. The first presoldier appeared 100 days after flight whilst some colonies still lacked presoldiers after 200 days. Furthermore, the number of presoldiers in each colony showed considerable variation.

Effects of Altering Presoldier Content of a Colony in Macrotermes

Pilot experiments indicate that the presoldier population is maintained as a definite proportion of the population as a whole. The removal or addition of presoldiers results in the formation of replacements or the eventual elimination of supernumaries respectively.

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8

TICK RESEARCH

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INTRODUCTION

Ticks are among the most important vectors of disease of man and his livestock, and in East Africa the disease caused by *Theileria parva*, known as East Coast Fever (E.C.F.), is the most serious disease of cattle, causing high mortality in unprotected cattle. E.C.F. is transmitted by the brown ear tick *Rhipicephalus appendiculatus* and very expensive measures, including the frequent dipping of cattle in acaricides, must be taken to protect stock from the disease. Little is known of the physiology, and less of the ecology, of *R. appendiculatus* and for this reason a study, jointly undertaken by workers at I.C.I.P.E. and at the East African Veterinary Research Organization, at Muguga, has begun.

The work falls under two heads; physiology and ecology. In both cases we are trying to study those aspects of the biology of *R. appendiculatus* that seem to us most likely to yield results that may be of importance in helping to devise non-acaricidal methods of control of the tick.

Soft ticks (Argasidae) are also important in the transmission of disease and part of our work concerns especially *Ornithodoros moubata*, the vector of human relapsing fever, a disease of considerable importance in Africa, and also of swine fever. All this work so far has been physiological.

PHYSIOLOGY

RHIPICEPHALUS APPENDICULATUS

Sexual attraction

Experiments were carried out on the movement of partially fed *R. appendiculatus* ticks toward other ticks of the same species. The general procedure of the experiments was to enclose 80 ticks in a calico bag on each ear of a rabbit. Unattached ticks were removed the following day. After a certain number of feeding days the single ear bags were removed and the number of ticks reduced to 50. A large ear bag was then placed over both ears of the rabbit, thus permitting tick migration.

In the first experiment male ticks were placed on the left ear of 7 rabbits, and groups of males, females, or nymphs on the right ear. Ticks were allowed to feed 5 days before the ears were put together. The results of this experiment (Fig. 1) showed 60% (30/50) migration of male to female ticks within 2 days. There was no migration between male ticks and nymphs: migration of females to males was negligible. Sites where other ticks had fed had no attraction. Ticks of each sex clustered at the various feeding sites rather than attaching at random.

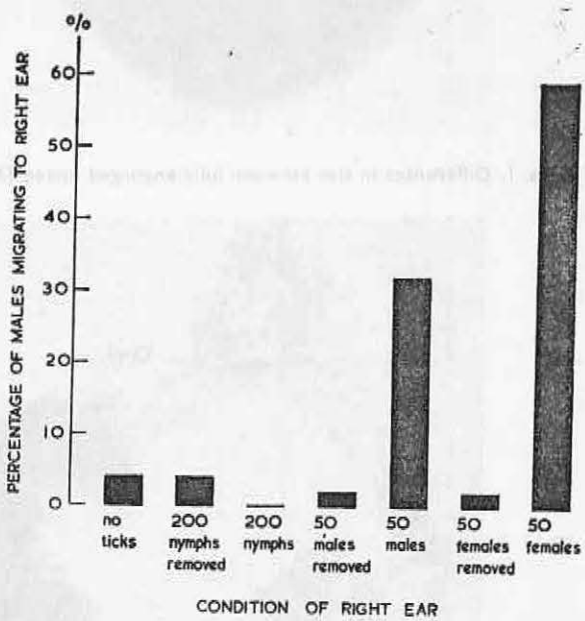

Fig. 1. Percentage of males *R. appendiculatus* migrating to right ear of rabbit within 2 days.

The production of attractant by female ticks and the response of male ticks to it was investigated with relation to feeding periods of both sexes ranging from 0 to 11 days. It appeared that male ticks that had fed for more than 3 days were more readily attracted to females than those fed for fewer days (Fig. 2). Similarly, female ticks fed 3 days or more exhibited a greater attraction for males (Fig. 3). It is suggested that the male ticks are attracted by a sex pheromone, as has been shown for other ixodid tick species (Berger *et al.*, 1971). The apparent attraction of males to males shown in the first experiment is the first suggestion of a male-produced pheromone inducing aggregation in male ixodid ticks. Additional experiments indicate that females also produce a pheromone that induces assembly of females

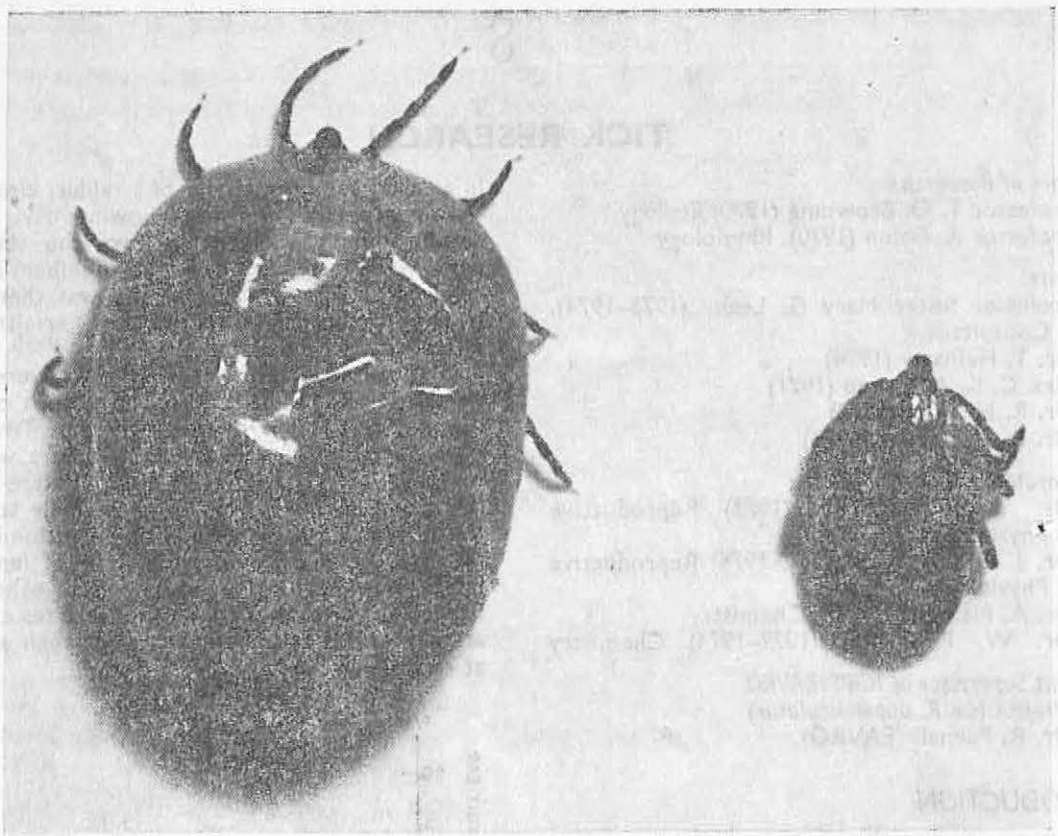

Photo. 1. Differences in size between fully engorged mated (A) and partially engorged virgin (B) *R. appendiculatus* females.

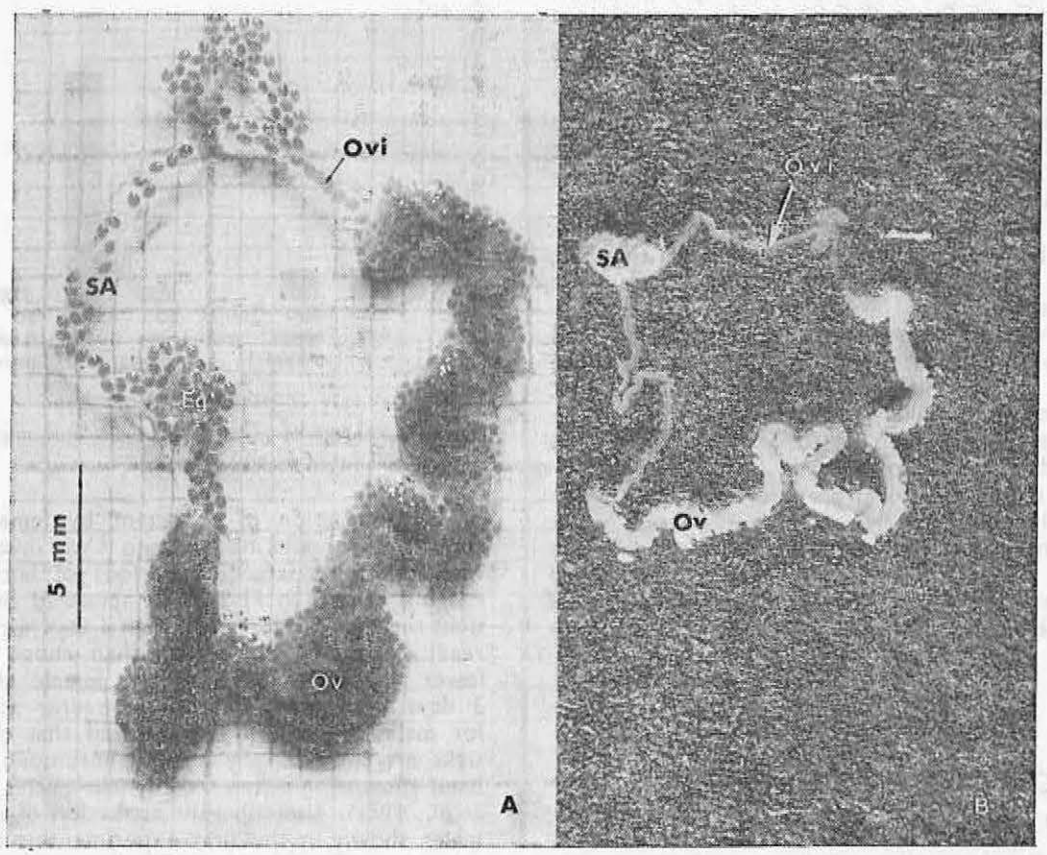

Photo. 2. Developmental differences between reproductive systems of fully engorged mated (A) and partially engorged virgin (B) *R. appendiculatus* females four days after dropping off the host. Eg = Eggs; SA = Seminal receptacle and accessory gland; OV = Ovary; Ovi = Oviduct. Same scale for both A & B.

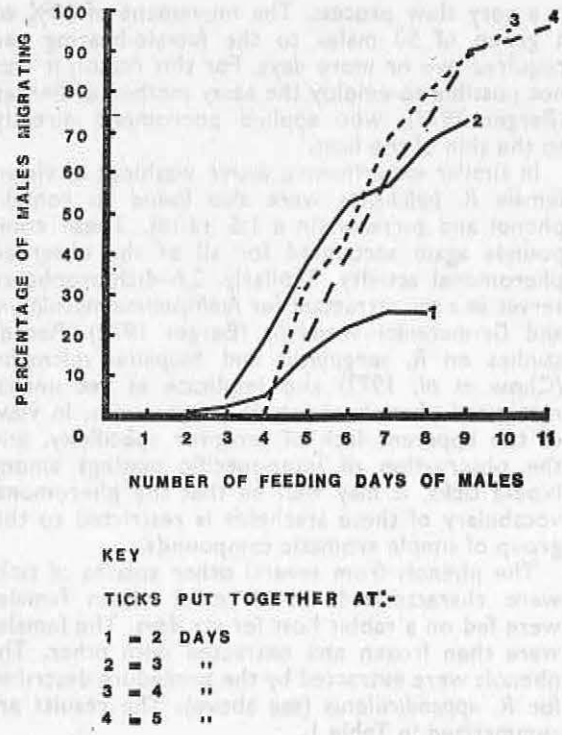

Fig. 2. Effect of length of feeding period of males on the percentage migrating to females. Females had fed 4 days when first exposed to males.

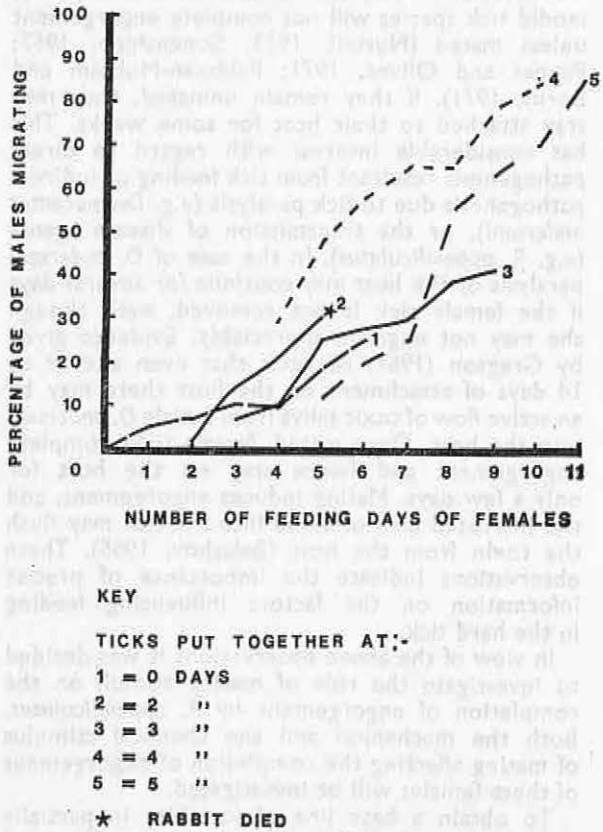

Fig. 3. Effect of length of feeding period of females on percentage of migration of males that had fed 4days.

An in vitro method of bioassay of the sex attractant was designed and standardized. In developing this method the pheromone material was obtained by placing filter paper in a container of virgin females overnight. The general method of assay for the presence of pheromone was to use a glass T-tube, which stood upright on the table. The disc of filter paper (from the container of females) was inserted into one arm of the T, and clean paper was placed in the other. An unmated male (of appropriate age and feeding condition) was allowed to climb up the tube. When he reached the top of the tube, observations were made on (a) his first choice of arm, and (b) the length of of time spent (within 2 minutes) in each arm. This gave information on attractant and arrestant activity of the test paper.

Chemical studies (on *R. Appendiculatus* and other ticks)

The chemical isolation and identification of the sex pheromone of *R. appendiculatus* was carried out as follows. Virgin females (ca. 7000) were fed on rabbit ears for six days and then removed and frozen. Simple washing of the intact, frozen bodies with ether gave an extract which was active in the T-tube bioassay.

Extraction of the initial ethereal female washing with 1 N sodium hydroxide left an inactive organic phase; acidification and ether extraction of the aqueous phase yielded an active, acidic fraction (Fig. 4). Further washing with aqueous sodium bicarbonate, to remove carboxylic acids, left a biologically active ether solution which should contain weak, organic acids. Gas chromatographic examination of this material showed the presence of only two major, volatile components (1 m × 3mm glass column, 5% Carbowax 20M, 150°). These had retention times indistinguishable from those of phenol and p-cresol and direct gas chromatographic and mass spectral comparisons with authentic samples of phenol and p-cresol provided unambiguous identification of both compounds. In

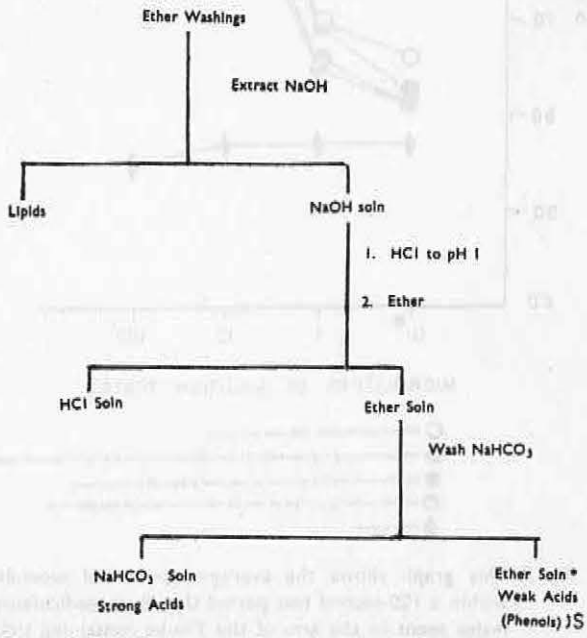

Figure 4. Tick Pheromone Extraction Procedure.
*All activity of the original ether washings are found in this fraction. (*R. appendiculatus* and *R. pulchellus*).

two such isolation experiments, the p-cresol recovered amounted to ca. 3.5 ng per female while the phenol content ranged from 4.5 to 13 ng.

Fig. 5 shows comparative T-tube bioassay results for the original ether washings, a mixture of phenol and p-cresol (i.e., "synthetic pheromone") and solutions of phenol and p-cresol alone. From the results summarized in the figure, we conclude that the mixture of phenol and p-cresol can account for the attractivity of the natural extract. Furthermore, in contrast to observations on some insect species whose pheromones are mixtures, the individual phenolic components also show activity, although there is some suggestion that it may be less than that of the mixture.

Washings from unfed females did not contain significant amounts of pheromone, implying either that they do not yet contain these compounds, or that they do not release them until they are ready to mate. Male washings show no comparable activity.

Migration of male ticks to females, when each is placed on a different ear of the same rabbit,

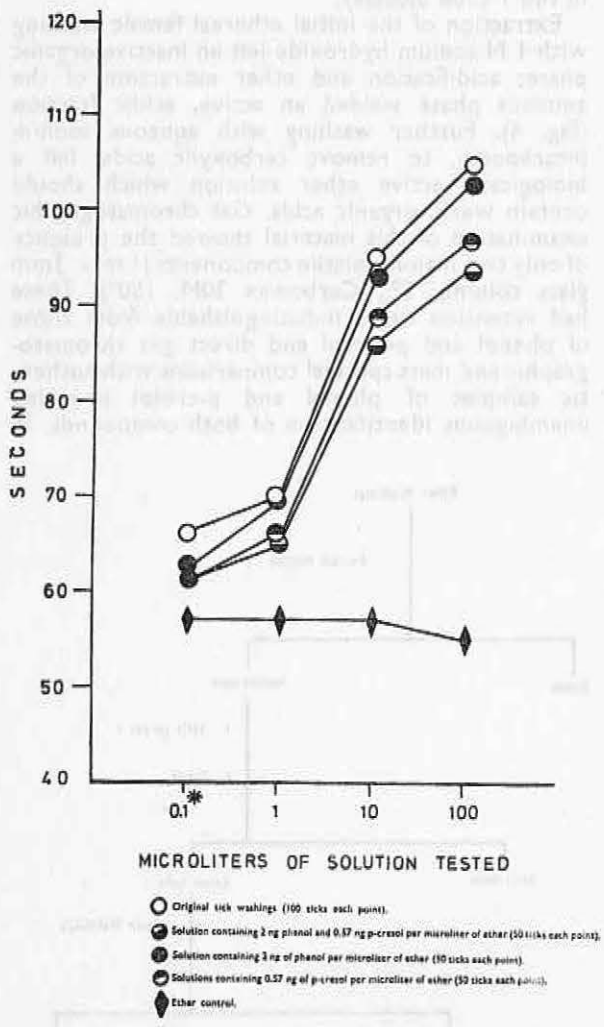

Fig. 5. This graph shows the average number of seconds within a 120-second test period that *R. appendiculatus* males spent in the arm of the T-tube containing tick extracts or synthetic pheromone solutions plotted against the volume of solution tested. *One microliter of ether in a 1:10 dilution of stock solutions.

is a very slow process. The movement of 60% of a group of 50 males to the female-bearing ear requires two or more days. For this reason it was not possible to employ the assay method of Berger (Berger 1972), who applied pheromone directly to the skin of the host.

In similar experiments, active washings of virgin female *R. pulchellus* were also found to contain phenol and p-cresol (in a 1:5 ratio). These compounds again accounted for all of the observed pheromonal activity. Similarly, 2,6–dichlorophenol serves as a sex attractant for *Amblyomma maculatum* and *Dermacentor variabilis* (Berger 1972). Recent studies on *R. sanguineus* and *Boophilus microplus* (Chow *et al.* 1972) also implicate as yet uncharacterized phenols as female pheromones. In view of the apparent lack of receptor specificity, and the observation of inter-specific matings among ixodid ticks, it may well be that the pheromonal vocabulary of these arachnids is restricted to this group of simple aromatic compounds.

The phenols from several other species of ticks were characterized. In all cases virgin females were fed on a rabbit host for six days. The females were then frozen and extracted with ether. The phenols were extracted by the procedure described for *R. appendiculatus* (see above). The results are summarized in Table I.

Effect of mating on engorgement in females

It is known that the females of a number of ixodid tick species will not complete engorgement unless mated (Nuttall, 1913; Sonenshine, 1967; Pappas and Oliver, 1971; Feldman-Muhsam and Borut, 1971). If they remain unmated, they may stay attached to their host for some weeks. This has considerable interest with regard to direct pathogenesis resultant from tick feeding or indirect pathogenesis due to tick paralysis (e.g. *Dermacentor andersoni*), or the transmission of disease agents (e.g. *R. appendiculatus*). In the case of *D. andersoni* paralysis of the host may continue for several days if the female tick is not removed, even though she may not engorge appreciably. Evidence given by Gregson (1967) suggests that even after 9 to 14 days of attachment on the host there may be an active flow of toxic saliva from female *D. andersoni* into the host. Once mated, female ticks complete engorgement and hence stay on the host for only a few days. Mating induces engorgement, and the increased flow of blood into the tick may flush the toxin from the host (Balashov, 1965). These observations indicate the importance of precise information on the factors influencing feeding in the hard tick.

In view of the above observations it was decided to investigate the role of mating stimuli on the completion of engorgement by *R. appendiculatus*. Both the mechanical and the chemical stimulus of mating affecting the completion of engorgement of these females will be investigated.

To obtain a base line of variables in partially engorged virgin (PEV) and fully engorged mated (FEM) females, some female ticks were placed in an ear bag on one ear of a rabbit whilst others were placed together on the other ear. The ticks were allowed to feed for eight days. During this period, the ticks were examined daily, and those that had either engorged and dropped, or had not

attached, were removed. On the eighth day all the female ticks were collected and observations were made on their weight, colour, egg development (by measuring the diameter of the 5 largest eggs), size of seminal receptacle, and distribution of spermiophores in the seminal receptacle, oviducts and ovary.

Observations were also made of changes in weight, egg development in the oviduct and the ovary, and sperm distribution on day 0 to day 4 after the FEM female had dropped from the host.

Table 2 demonstrates the striking differences between virgin and mated females after 8 days of feeding. Whereas the FEM females are tanned grey, the PEV females are yellowish brown in colour. The average weight of an FEM tick (347.0 ± 2.36mg) is twenty-fold over the average weight of a PEV female (16.9±2.67 mg). There is a three-fold increase in size of an FEM female over a PEV female (Photograph 1). Because of the presence of spermatophore(s) in a FEM female, its seminal receptacle is three times larger than that of a PEV female.

A gradual development of the eggs is observed (Table 3) in the FEM females that have dropped from the host. The average egg size increases from 106±18 µm on the day of dropping to 427±60 µm on the fourth day of dropping. However, on the fourth day eggs 498±37 µm in diameter were observed in the oviduct (Photograph 2). In a few observations the oviposited eggs measured about 600 µm. The colour of the eggs from day 0 to day 4 ranged from white to brown. In the PEV females egg development was almost nil, even 4 days after dropping (Photograph 2). The eggs were white and the average egg size was 95±4 µm.

The colour of the FEM female ticks did not change over the four-day period after dropping. A decrease in average weight was observed from day 0 to day 4 since dropping. This is probably due to water loss and excretion.

Table 1. Phenolic Compounds in hard ticks *in nanograms per Tick*

Species of Tick	*Number Washed*	*Salicylaldehyde*	*Phenol*	*p-Cresol*	*2,6-Dichlorophenol*
1. *Rhipicephalus appendiculatus*	7,000	—	4.5–13	3.5	—
2. *R. pulchellus*	2,700	—	1.8	10	—
3. *R. compositus*	1,750	0.3 *	3.2	1.1	—
4. *Amblyomma americanum*	1,900	0.3 *	4.6	2.9	2.2
5. *A. variegatum*	523	3.6	52	47	16
6. *Hyalomma truncatum*	459	7.5	67	4.4	2.2

*Identified by glc retention time only.

Table 2. Comparison of virgin and mated females after 8 days feeding

Females			*Egg* *		*Seminal Receptacle*		
Condition	*No*	*Colour*	*Average Weight (mg)*	*Size (µm)±S.E.*	*Sperm*	*Appearance*	*Diameter (mm)±S.E.*
Virgin	17	Yellow Brown	16.9±2.67	95+4	0	Collapsed	0.47±0.03
Mated	11	Tan Grey	347.0±2.36	106±18	+++	Inflated	1.38±0.13

*5 largest eggs per female.
±S.E. of the mean.

Table 3. Changes occurring in the mated females after dropping off the host

Females			*Egg Size (µm) **		*Seminal Receptacle (S.R) Diameter*	*Sperm*		
Days after Detachment	*Number*	*Average Weight (mg)*	*in Oviduct ± S.E.*	*in Ovary ± S.E.*	*(mm)± S.E.*	*S.R.*	*Oviduct*	*Ovary*
0	11	347.0±2.36	None	106±18	1.38±0.13	++++	0	0
1	25	304.7±18.61	None	212±84	1.5±0.06	++++	+	0
2	11	341.0±37.20	None	289±114	1.41±0.08	+++	+	0
3	14	290±30.01	None	382±91	1.28±0.06	++	++	0
4	20	242.3±16.30	498±37	427±60	1.32±0.05	+	+	0

*5 largest eggs per female.

Sperm from the seminal receptacle of a FEM female were first observed migrating near the end of day 1. About 50% of the spermiophores had migrated into the oviduct by day 3 since dropping from the host. Only on day 1, when the eggs in the ovary were still whitish, were spermiophores occasionaly observed in the ovary. Spermiophores in the oviduct were slightly active. An ampulla-like structure is located about one-third of the length of the oviduct from the ovary. This ampulla is normally filled with motile sperm by the third day after the tick drops from the host. By the fourth day very few sperm were observed in the oviduct or spermatophore(s) in the seminal receptacle. On this day the walls of the spermatophore were globular giving the appearance of degeneration. Also at this time a granular homogeneous secretion was observed in the seminal receptacle. Up to 4 spermatophores in such cases were of decreasing size suggestive of mating with a single male tick.

During the past few months efforts have been made to investigate the effect of mechanical stimulus of copulating males on the parameters of weight and colour of females. Genitalia of fed males and PEV females were blocked with balsam:paraffin mixture (1:1 ratio) (Pappas and Oliver, 1972) and then put on rabbit ears for reattachment. Preliminary results indicate that with just the mechanical stimulus of copulating males, PEV females will engorge about seven to eight fold their previous weight. Under normal conditions the average weight of a FEM female tick (347.0±2.36 mg) is twenty fold over the average weight of a PEV female (16.9±2.67 mg).

Experiments are now underway to investigate the effect of chemical stimuli from copulating males upon the feeding of females.

ORNITHODOROS MOUBATA AND OTHER ARGASID TICKS

Assembly in O. moubata

The presence of pheromones in soft ticks was first reported in the fowl tick *Argas persicus* (Leahy, Vandehey, and Galun, 1973). The present study investigates the possibility of such a pheromone in *O. moubata*, the vector of human relapsing fever and swine fever. Additional experiments consider the relation of possible receptors of the ticks to its perception of the pheromone.

The first method for obtaining assembly material was to place filter paper in the container of recently fed ticks. After 2 days or longer the papers were removed and then bioassayed for the ticks' response to them. As an alternative method, ticks were washed in saline. These washings were then transferred to clean discs of filter paper, air-dried, and bioassayed.

Since aggregation in *O. moubata* could not be detected by the method for *A. persicus*, we introduced the following adaptations. Petri dishes (9 cm) were divided into 4 sectors, each sector with discs of filter paper, 1.5 cm. Three paper discs on top of each other provided sufficient material for definitive bioassay.

At assay of the assembly material, discs that had previously been exposed to ticks were placed in one sector (No. 4). The remaining 3 sectors had clean papers. At the beginning of the experiment 10 ticks were placed in the centre of the dish. When the test discs contained saline extract of the assembly material, the control discs were clean or contained 0.5 ml of 1–20% NaCl and were also air-dried. Since the normal tendency of ticks is to go to the edge of the dish, the discs were placed about 1 cm from the periphery.

The tests were carried out in a dark-room at 28±2°C. Variable humidity was provided by damp cloths placed around the tray containing the open test dishes. Observations under a red light were made at half-hour intervals.

Our experiments indicate that female *O. moubata* produce a material that induces assembly of males (Fig. 6). Male *O. moubata* also produce an assembly substance to which males respond (Fig. 7).

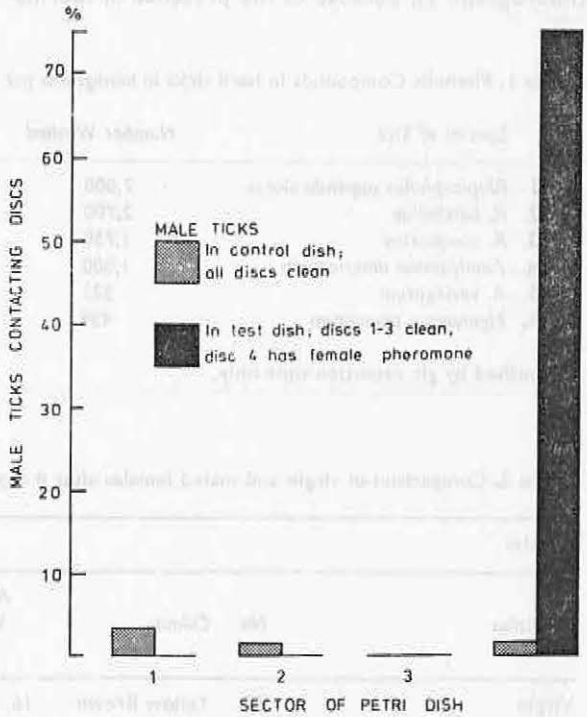

Fig. 6. Summary of 6 experiments showing aggregation of males *O. moubata* to discs with female pheromone.

Various other materials were also tested: water, saline, and saline washings from flies (*Sarcophaga*), termites (*Hodotermes*), and ants (*Pheidole*). None of these induced assembly of males.

Papers previously exposed to about 200 virgin females were washed in 4 ml pentane. One ml of the washings was transferred to clean discs, air-dried, and tested against males. The original papers were then washed in ether and the same procedure followed. A third washing was made in acetone, a fourth in methanol, a fifth in water, and the last in saline.

The results of the extraction studies are shown in Fig. 8. When tested for male assembly, there was no activity from washings of pentane, methanol, acetone, ether, or water; however, when the original papers were finally washed in saline, the latter showed high activity.

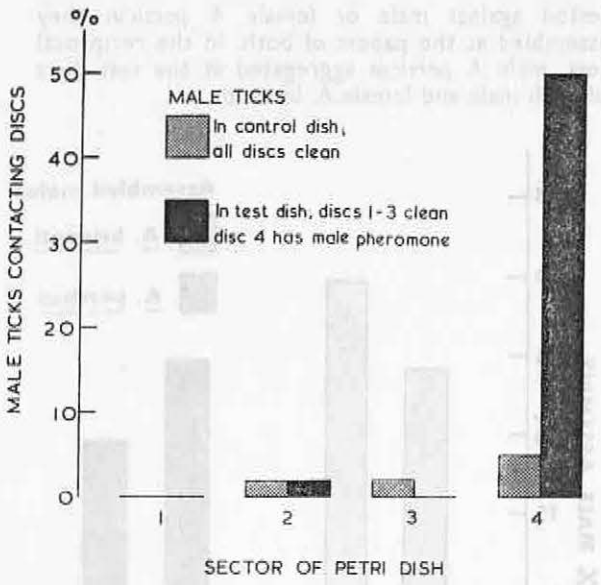

Fig. 7. Summary of 6 experiments showing aggregation within 1 hr of male *O. moubata* (n=60) on discs previously contacted by unmated males.

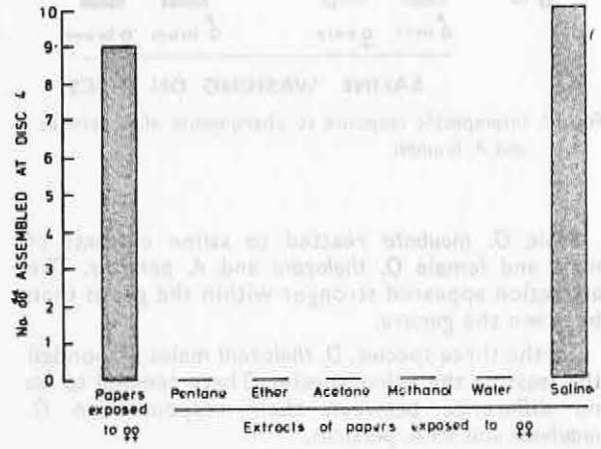

Fig. 8. Assembly of male *O. moubata* at papers in Sector 4.

The effect of temperature change on saline extracts of assembly material was considered. Papers with extracts that were held at 4°C for 48 h or 100°C for 5 min retained their activity.

An experiment was designed to determine whether the ticks detect the pheromone when they were unable to make physical contact with the pheromone-impregnated discs. A cylindrical dish (9 cm) was fitted with a screen (0.3 mm thick, 23 mesh/cm) 0.5 cm above the floor of the dish. This separated the ticks below from the discs on the surface. After taking readings, the screen and dish were washed and the position of the ticks and papers was reversed. As a control the test was carried out without the screen.

This experiment demonstrated that the ticks did indeed assemble under circumstances in which they had no direct contact with the pheromone papers: therefore, it would seem that the pheromone involves an olfactory component.

Assembly in O. tholozani

The major vectors of tick-borne human relapsing fever are in the genus *Ornithodoros*. The report below demonstrates the presence of an assembly pheromone in the carrier of Asiatic relapsing fever, *O. tholozani*.

O. tholozani is considerably smaller than *O. moubata* and it does not aggregate unless the discs are situated about $\frac{1}{4}$ in. from the periphery of the dish. The test discs contained saline washings from about 15 ticks.

Male *O. tholozani* tend to assemble at discs with saline extracts from either females or males (Fig. 9), but not at discs with saline only. Females also respond to assembly material produced by either males or females (Fig. 10).

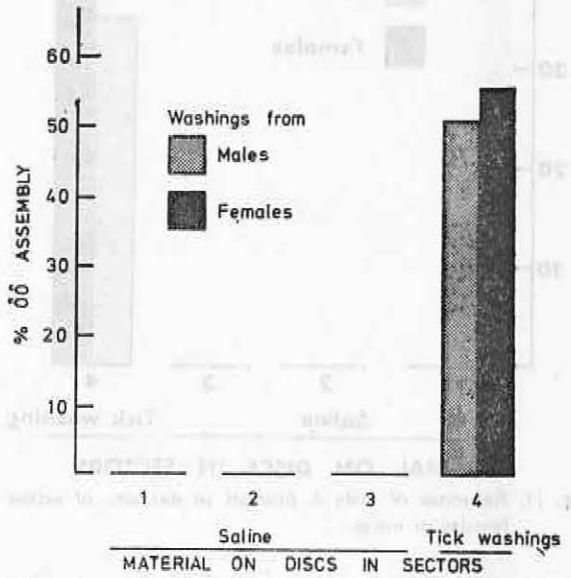

Fig. 9. Assembly of male *O. tholozani* at discs with saline extracts of either females or males.

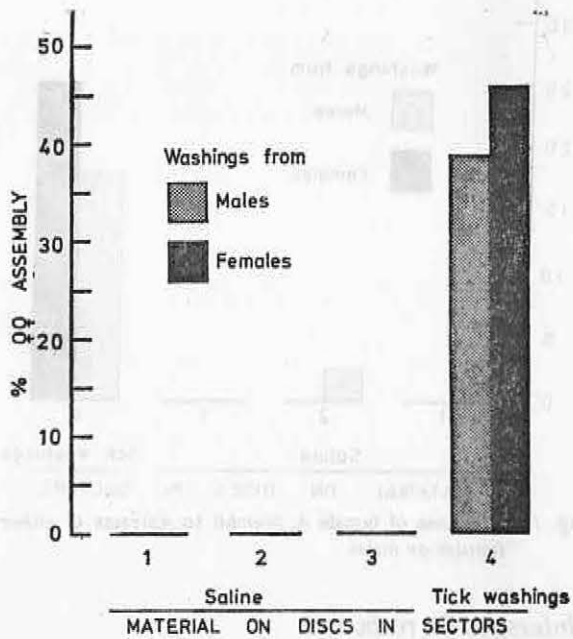

Fig. 10. Assembly of female *O. tholozani* at discs with saline extracts of either females or males.

Assembly in Argas brumpti

Kenyans claim that the bite of this tick causes pain and sickness (Hoogstraal, 1956). Because of the large size of the tick, petri dishes 6 inches in diameter were employed and discs were placed ½ in. from the edge. Extract from 15 ticks was used in each test.

Figures 11 and 12 show that *A. brumpti* aggregates both inter-and intra-sexually.

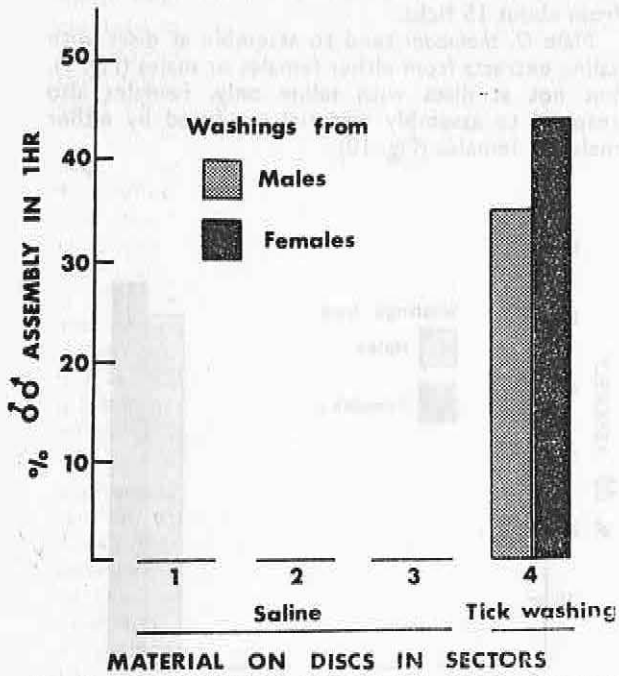

Fig. 11. Response of male *A. brumpti* to extracts of either females or males.

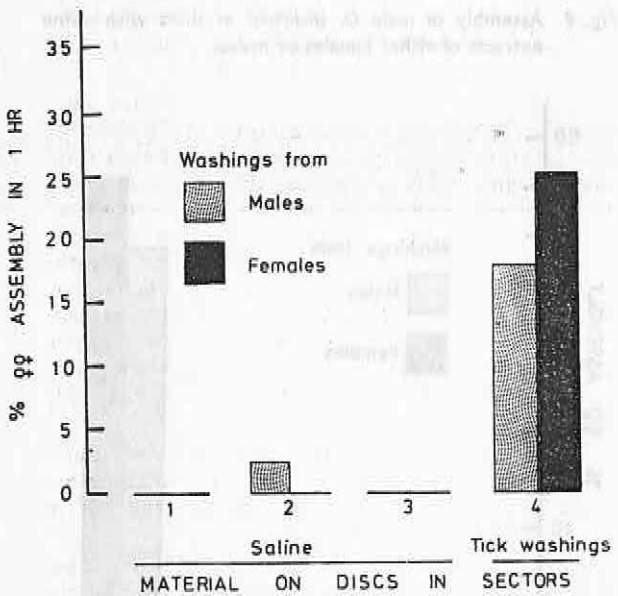

Fig. 12. Response of female *A. brumpti* to extracts of either females or males.

Interspecific responses

The results of heterologous experiments are shown in Fig. 13. When *A. brumpti* males were tested against male or female *A. persicus* they assembled at the papers of both. In the reciprocal test, male *A. persicus* aggregated at the test discs of both male and female *A. brumpti*.

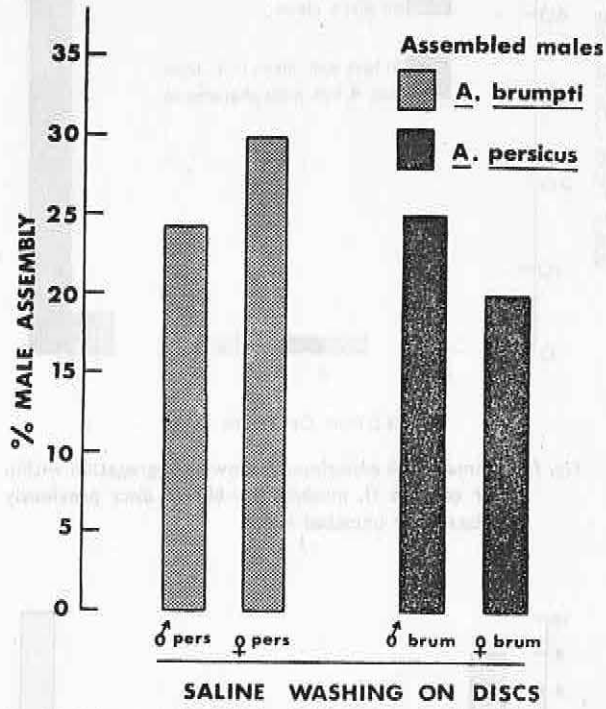

Fig. 13. Interspecific response to pheromones of *A. persicus* and *A. brumpti*.

Male *O. moubata* reacted to saline extracts of male and female *O. tholozani* and *A. persicus*. The attraction appeared stronger within the genus than between the genera.

Of the three species, *O. tholozani* males responded the least to the other species. There seemed to be no difference between their responses to *O. moubata* and to *A. persicus*.

Male *A. persicus* appeared more attracted to extracts of *O. tholozani* than to those of *O. moubata*. *A. persicus* had the highest rate of assembly in the interspecific crosses. This may be a reflection of its own high assembly rate (Leahy *et al.*, 1973). When one or more ticks find the pheromone discs, their presence may act as an additional factor in the resultant aggregation.

In general, males of the three species responded more readily to extracts of heterologous females than to those of heterologous males.

The effects of ecdysone, juvenile hormone and their analogues on O. moubata

Kitaoka (1972) reported oviposition—inhibitory, supermoulting and killing effects on engorged adults of *O. moubata* by Ponasterone A, β-ecdysone and Inokosterone at doses ranging from 0.5–10 μg per ml of blood ingested through an artificial membrane. He also observed moulting in non-moulting nymphs fed on an insufficient amount of blood or non-blood meal. The aim of this study therefore, is to investigate the effect of ecdysone,

juvenile hormone and their analogues on *O. moubata* and *R. appendiculatus*.

Adult *O. moubata* were fed artificially through bat wing membranes on pig's blood in which β-ecdysone or Ponasterone A had been incorporated at doses ranging from 1µl to 5µl/ml of blood. The ticks were kept individually and observed for supermoulting, mortality or egg laying.

Supermoulting was observed with both β-ecdysone and Ponasterone A. With Ponasterone A, at a dose of 5µl/ml of blood, almost 100% mortality was observed. Experiments are underway for more data.

Sperm capacitation in O. moubata

Tick sperm undergo a complicated morphogenesis in the male until they reach an "invaginated" state at which point morphogenesis ceases. After the sperm have been transferred to the female, they evaginate, becoming twice as long as they are in the male, and acquire the capacity for motility. These processes take 2 to 3 days inside the spermatophore within the uterus of the female (Samson, 1909; Robinson and Davidson, 1914); Wagner-Jevseenko, 1958). Wagner-Jevseenko suggested that the development of the sperm of *Ornithodoros moubata* in the uterus might be stimulated by secretions of the male's accessory glands, which are combined with the sperm during ejaculation. The work described below sets out to test this suggestion.

The factor(s) responsible for development of the sperm in the uterus does indeed come from the male's accessory glands. Sperm taken from the vasa deferentia of the male will mature *in vitro* without any contribution from the female if the secretions of the male's accessory glands are added to the sperm. The active principle is present in no more than 3 of the 8 different lobes of the accessory glands, and appears to be most concentrated in one of these lobes. The active principle is heat-labile and trypsin-sensitive.

Materials and methods. Ticks used were virigin male *Ornithodoros moubata* reared on rabbits in the ICIPE insectary.

Male ticks were routinely dissected in "arachnid Ringer" (Rothschild, 1961) or Calliphora saline (Barridge, 1966). Vasa deferentia were excised as much intact as possible and placed on "Parafilm" in a moist chamber, removing as much Ringer as possible. 5 to 15 µl of the sperm medium (see below) was then added, in which the vasa deferentia were broken open. After the sperm were forced out, the walls of the vasa deferentia were removed.

Various artificial media were assessed as diluents for the sperm, but rarely survived more than 48 hours in these media and often died within 24 hours. Unheated, sterilized calf serum (Oxoid Tissue Culture Serum NO. SR 41) routinely allowed sperm to survive more than 72 hours, thus permitting full elongation and the development of motility.

Accessory glands were removed whole and excess Ringer removed. 4 to 6 glands were homogenized in 10 to 15 µl of a saline solution or in 0.1 M HEPES-KOH, pH 7.6, with 10 mM $CaCl_2$ added. The homogenates were centrifuged to remove most of the insoluble material and were used immediately. Fresh preparations were made for each experiment.

When preparations were made of each lobe of the accessory gland, each lobe was removed with clean forceps from 3 accessory gland complexes and placed in 10 µl of saline solution. (The areas where the lobes join the base of the glands were discarded). Each of the preparations was then homogenized and centrifuged.

Routinely about 2 to 3 µl of sperm suspension was mixed with ½ to µl of accessory gland homogenate (or saline or buffer in the case of controls) on a glass cover slip. The cover slip was then inverted over a depression slide and sealed with hot wax. Elongation was observed in the phase microscope at 12-hour intervals thereafter.

0.5 µl of 1 mg/ml bovine pancreatic trypsin (crystalline) in 0.1 *M* HEPES-KOH buffer, pH 7.6 with 10 m*M* $CaCl_2$ added was mixed with 2 µl of an accessory gland extract and maintained at room temperature (24 to 27°C). At the end of one hour, this solution was mixed with 0.5 µl of 10 mg/ml soybean trypsin inhibitor in the same buffer and then assayed for capacity to induce elongation. As a control, 0.5 µl of inhibitor solution was mixed with 0.5 µl of trypsin solution and 2 µl of accessory gland extract, and incubated for the same period as the trypsin solution.

Results and discussion. Whole spermatophores were stolen from copulating ticks before the male placed them in the female's genital opening. At least one of these was not contaminated with coxal fluid. These spermatophores were then everted (see Feldman-Muhsam *et al.* 1973) and were maintained on Parafilm in a moist chamber. In all cases, sperm elongated as they would normally in the spermatophore in the uterus of the female tick.

Spermatophores collected in a similar fashion were broken open in a few µl of saline solution and maintained as a drop on Parafilm in a moist chamber. These sperm also elongated normally.

Sperm were then dissected from the vasa deferentia of the male and combined with extracts of male accessory secretions as described above. These sperm also elongated normally and often developed motility, especially if they had been suspended in calf serum. Dilutions of the accessory gland extracts were also assayed. Since greater than 3-fold dilutions were usually ineffective, the amount of elongation-promoting material in the extracts was evidently not large. Control preparations, i.e., sperm mixed with homogenizing solution only, never showed more than 5% elongation.

These results imply that the initiation of sperm maturation in the female is due solely to contributions from the male's accessory glands and not to any influence from the female.

Assays of extracts of each of the different lobes of the accessory glands revealed that 3 out of the 8 lobes, those designated A, B. and C by Wagner-Jevseenko (1958) (Fig. 14) and most likely homologous with the anterior dorsal, posterior dorsal, and third lateral granular lobes of *Argas persicus* (Tatchell, 1963), are the only lobes capable of inducing elongation. Extracts of lobe B evinced the most marked elongation.

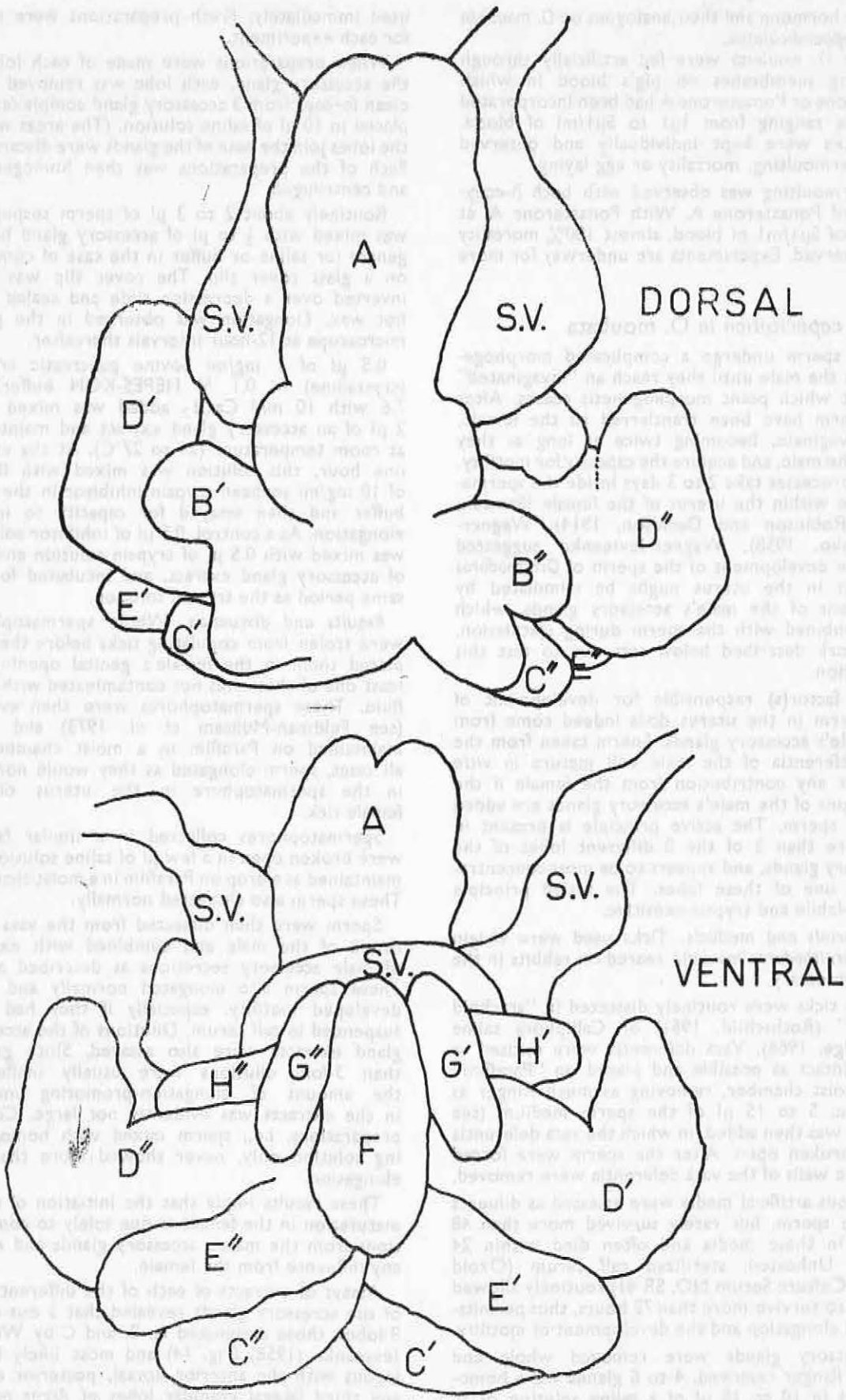

Fig. 14. Drawings of the dorsal and ventral aspects of the accessory glands of virgin male *O. moubata*. Letters indicate the different lobes as designated by Wagner-Jevseenko (1958). ′ = left member of a pair of lobes, ″ = right member, s.v. = seminal vesicles.

Accessory gland extracts were heated in sealed capillaries for 30 to 40 min. respectively at 95°C (temperature of boiling water in Nairobi). Elongation occurred even after this treatment.

An extract of accessory glands incubated with 1 mg/ml trypsin for one hour induced much less elongation than the native extract, although slightly more than the control assay. Probably the factor promoting elongation is destroyed by trypsin.

ECOLOGY

A long-term study of the population of *R. appendiculatus* in an experimental plot.

The aim of the work is to study the components of the environment of *R. appendiculatus* that may influence its abundance and distribution.

METHODS

Two paddocks, each of about 2 hectares were made available at E.A.V.R.O. Muguga. A thorough check, by dragging a cloth over the vegetation and by exposing cattle in the paddocks, showed that they contained only very few *R. appendiculatus*. One of the paddocks served as a control and cattle are not grazed in it regularly. The other was the experimental paddock.

In October 1973 five cattle, artificially infected with E.C.F. were introduced into the experimental paddock. On that day each animal was infested with approximately 11,000 nymphs and 20,000 larvae of *R. appendiculatus*. One week later, a further 20,000 larvae and 2,000 nymphs were applied to each of the surviving animals. All the animals were examined each day. For one animal in turn the whole body was examined and all the ticks were counted *in situ* and recorded separately for the various parts of the body. On the other cattle only the ticks on the ears were counted. By the thirtieth day four of the cattle had died and the survivor was removed.

After 37 days, when it was estimated that nymphs and adults would be starting to moult from the larvae and nymphs that had fed on the infected cattle, five more cattle, susceptible to E.C.F., were introduced into the paddock. They were examined as described above until they too died of E.C.F., when they were replaced by successive groups of susceptible cattle. So far 13 such groups have been exposed.

During the year two groups of cattle immunized against E.C.F. by different methods have also been exposed in the paddock for field challenge, using some of the susceptible groups mentioned above as controls. All the immunes survived the challenge during exposure for 60 days in the paddock for each group. All the ECF susceptibles 12/12 in the first group and 8/8 in the second group, died of ECF.

In order to assess the distribution of ticks both paddocks were marked out in equal-sized plots (100 in the experimental and 125 in the control). Sampling the vegetation for active ticks was done by dragging a blanket over the plot and by sweeping with a net (Milne, 1943). Three plots are randomly chosen from each paddock (once a week for the control and three times a week for the experimental). Sampling is done at 08.30, 12.00 and 15.30 hr. Larvae nymphs and adults caught are counted and a sub-sample preserved to check for the presence of other species.

Various live-traps for mammals are set in both fields in alternate months. Captured animals are identified, anaesthetised, searched for ticks, marked and released. The ticks are preserved and identified.

The development and survival of the three instars under nearly natural conditions are being followed. Samples of engorged larvae, nymphs and adults from the experimental cattle, together with laboratory-reared specimen fed on rabbits, are put out at regular intervals in gauze-plugged tubes in the grass at the edge of the paddock in both open and shaded sites.

In June-July 1974, three E.C.F. susceptible cattle were exposed in the control paddock for 40 days to see if there were any E.C.F.—infected ticks.

RESULTS AND DISCUSSION

The tick population in the infested paddock. Adult ticks were rarely collected from the vegetation, and their numbers were able to be assessed only on the cattle (Table 4). Larvae, on the other hand, are readily sampled from the grass and accurately counted on the net or blanket (Fig. 15). but are difficult to find on the host when not engorged, and impossible to count accurately. Nymphs can be sampled both from the grass and on the host but can be counted accurately on the host only on the ears, which are not their main site of attachment (Fig. 15, Table 4).

Adults were detected in the first sampling (December 1973) and their numbers reached a maximum in April 1974, at the beginning of the long rains. Nymphs showed a small peak in numbers in January 1974 and much larger numbers in October. Larvae appeared briefly in March 1974 and again in late May, reaching peak numbers in August. All larvae seen in 1974 must have been bred in the paddock. The first nymphal peak derived from the larvae introduced originally, the second one from the indigenous larvae. Similarly the first adults must have come from the original introduction of nymphs, with subsequent recruitment from the first nymphal cohort of nymphs (Fig. 15).

Survival in the relatively cool and well-vegetated conditions at Muguga is certainly high, and the adults, in particular, have tended to maintain a relatively uniform level of activity. Larval and nymphal activity can therefore be expected to level off if the supply of hosts continues as at present.

Sites of attachment of R. appendiculatus *on cattle.* The counts of the numbers of larvae, nymphs and adults on different parts of the body in relation to the season (before, during, or after the long rains) have been analysed and show significant differences. The immature ticks seem to attach close to the place on the body where they brushed against the cattle, and this is higher on the body when the grass on which they were waiting is longest. The general pattern of distribution agrees with the findings of Yeoman and Walker (1967) and Baker and Ducasse (1967).

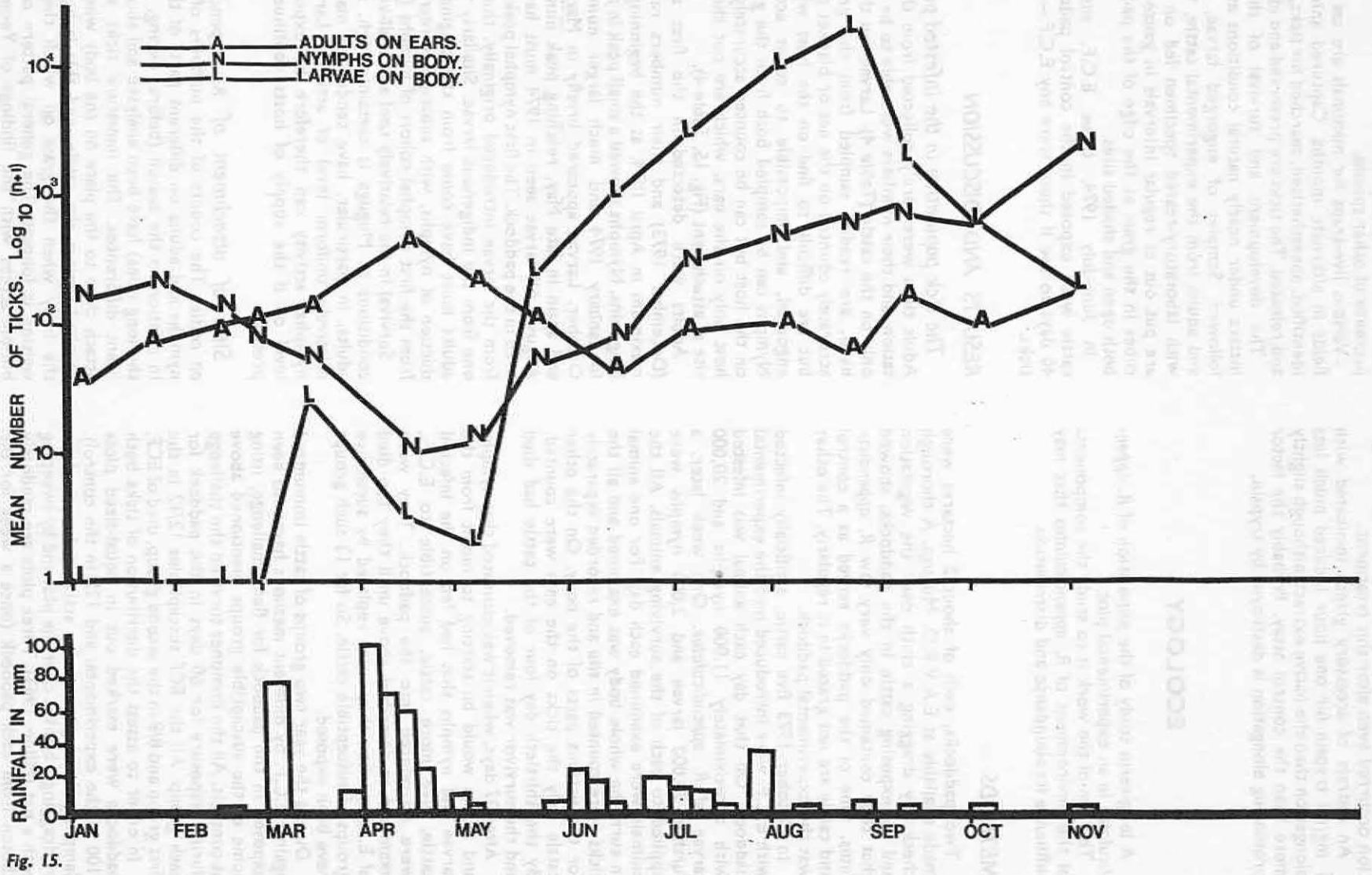
MEAN NUMBER OF RHIPICEPHALUS APPENDICULATUS ON CATTLE COUNTED ON DAYS 11—15 OF EXPOSURE IN THE PADDOCK, CORRELATED WITH RAINFALL.
A ADULTS ON EARS.
N NYMPHS ON BODY.
L LARVAE ON BODY.
MEAN NUMBER OF TICKS. Log 10 (n+1)
10⁴
10³
10²
10
1
RAINFALL IN mm
100
80
60
40
20
0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV

Fig. 15.

Table 4. Mean number of *Rhipicephalus appendiculatus*. Ticks per animal per day recorded on days 11–15 of exposure. (Susceptible cattle only)

Group Number	Number of Animals per Group	Period	Adults on Ears	Adults on Body	Nymphs on Ears	Nymphs on Body	Larvae on Ears	Larvae on Body
I	5	1.1.74–5.1.74	35.9	9.0	2.5	29.2	0.0	0.0
II	2	23.1.74–27.1.74	71.8	37.6	1.3	100.4	0.0	0.0
III	3	13.2.74–17.2.74	91.1	48.1	0.7	40.1	0.0	0.0
IV	2	23.2.74–27.2.74	107.8	25.4	0.1	35.8	0.0	0.0
V	5	13.3.74–17.3.74	134.8	11.2	0.1	10.8	0.0	0.0
VI	4	12.4.74–15.4.74	311.3	82.8	0.0	3.1	0.0	1.6
VII	4	3.5.74–7.5.74	207.9	80.7	0.0	2.8	0.0	0.5
VIII	4	21.5.74–23.5.74	131.9	46.9	13.1	11.7	90.5	64.2
IX	2	15.6.74–19.6.74	54.5	50.4	13.8	40.4	268.0	455.5
X	2	8.7.74–12.7.74	91.0	62.4	77.2	153.9	1129.6	1660.7
XI	2	4.8.74–8.8.74	92.8	64.6	75.7	223.9	2224.2	4966.6
XII	2	28.8.74–7.9.74	66.7	58.4	119.5	279.1	4565.2	9445.4
XIII	4	4.10.74–8.10.74	104.4	313.0	615.8	1847.4	664.5	2658.2
XIV	4	5.11.74–9.11.74	185.4	741.5	2479.8	9918.3	208.8	835.3

Table 5. Ticks on wild mammals captured on the study area—November 1973—October 1974 in 1578 trapnights (Large traps) and 1929 trapnights (Small traps)

Host	No. of different individuals captured	Total captures	No. of occasions animals infested with ticks	*Rhipicephalus appendiculatus*			Other *Rhipicephalus* ticks			*Haemaphysalis* spp.			*Ixodes* spp.		
				A	N	L	A	N	L	A	N	L	A	N	L
White-tailed Mongoose (*Ichneumia albicauda*)	13	20	11	—	20	8	—	—	—	8	1	1	10	4	3
Silver-backed Jackal (*Canis mesomelas*) *	1	1	1	—	—	—	26	—	—	349	3	1	1	—	—
Zorilla (*Ictonyx striatus*)	4	5	4	—	—	1	—	—	—	4	4	1	—	—	—
Striped ground squirrel (*Xerus erythropus*)	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Domestic cat (*Felis cattus*)	5	5	5	1	—	9	—	—	—	71	2	—	4	—	—
Large spotted genet (*Genetta tigrina*)	5	6	5	—	1	16	—	—	—	6	11	6	—	91	56
Shrew (*Crocidura* sp.)	1	1	1	—	—	—	—	—	—	—	—	—	—	4	2
Groove-toothed rat (*Otomys* sp.)	2	2	2	—	—	—	—	—	—	—	2	2	—	2	—
Unstriped grass mouse (*Arvicanthis abyssinicus*)	19	22	2	—	—	—	—	1	—	—	1	—	—	—	1
Domestic dog (*Canis familiaris*)	1	1	1	—	—	—	1	—	—	4	—	—	—	—	—
Striped grass mouse (*Lemniscomys* sp.)	12	13	4	—	—	—	—	—	—	—	7	2	—	—	3

*—The animal was run over by a vehicle 200 metres from the paddock.

Collection of ticks from the vegetation. Samples of larvae and nymphs from the vegetation showed similar trends to the approximate data from counts made on the cattle. There were no significant differences between the numbers caught by sweeping or dragging and there were no significant differences in the catches of ticks when sampled in the morning, at mid-day, or in the afternoon. No immature ticks were ever caught in the control paddock.

Ticks on wild animals. The results of collecting ticks from wild hosts caught in the study area are shown in Table 5. Most harboured ticks, but only the white-tailed mongoose, the large-spotted genet and the domestic cat were found to be carrying *R. appendiculatus* apart from a single larva on a zorilla.

Exposure of E.C.F.-susceptible cattle in the control paddock. Three cattle were exposed for a period of 43 days, by which time 2 had died of E.C.F. and the third had also become infected, but recovered. The following ticks were counted on the cattle:— *R. appendiculatus*, *R. hurti*, *R. simus*, *Boophilus decoloratus*. The *R. appendiculatus* increased in numbers in the last 18 days, and it is suspected that many of them were brought into the paddock on the clothes of the assistants examining the cattle.

CONCLUSION

There is no doubt that a flourishing and still-increasing population of *R. appendiculatus* has become established in the paddock from the initial seeding, and that nymphs and adults are readily capable of infecting susceptible cattle with E.C.F. whenever they are put into the pasture.

Further studies should throw light on the development and survival of all stages under the conditions prevailing in the paddock.

Evidence so far is that wild animals play a small part in maintaining the population, and their importance may lie in their carrying unfed, infective ticks passively into or out of the paddock, and that these may infect a susceptible bovine host.

So far the density of cattle in the paddock has been maintained at a very high level. Future studies will aim at an assessment of the influence of the density of hosts on the survival and multiplication of a tick population.

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9

TSETSE RESEARCH

REPRODUCTIVE PHYSIOLOGY
(Glossina morsitans)

Directors of Research:
Professor Thomas R. Odhiambo (1970)
Professor Jan de Wilde (1970)

Scientists:
Dr. F. B. Chaudhury (1974)
Dr. D. L. Denlinger (1972–1974)
Mr. T. S. Dhadialla (1973)
Mr. J. Kawooya (1973)

Collaborators:
Dr. W. C. Ma (1972)

SALIVARY GLAND PHYSIOLOGY (G. morsitans, G. pallidipes and G. austeni)

Director of Research:
Professor Thomas R. Odhiambo (1970)

Scientists:
Dr. L. H. Otieno (1973)
Dr. A. Youdeowei, Research Associate (1973)

ISOLATION MECHANISMS (Glossina pallidipes)

Director of Research:
Dr. Wim Helle (1971)

Scientist:
Dr. van Etten (1974)

Collaborator:
Mr. J. Kawooya (1974)

SOUND PRODUCTION

Director of Research:
Professor A. R. Møller (1973)

Research Assistant:
Miss I. E. C. Erickson (April to June 1974)

OVULATION
(Glossina morsitans)

INTRODUCTION

Tsetse flies exhibit adenotrophic viviparity with such peculiarities as reduction of ovaries to two ovarioles each, maturation of one egg at a time in alternate ovaries, successive ovulation of single mature egg into the uterus, subsequent *in utero* development of the embryo and the deposition of full grown larva. As each larva is deposited, another mature egg is ovulated, fertilized, hatched and the cycle is repeated.

The fact that the egg is not released from the ovary into the uterus in virgin females whereas the mated females are normally ovulated (Odhiambo 1971, Saunders and Dodd 1972), indicates that mating is necessary to initiate the process. Saunders and Dodd (1974) additionally report that transfer of sperm is not necessary to initiate ovulation but the duration of mating experienced by the female is an important factor in the control of ovulation. Foster (1974) reports that ablations of median neurosecretory cells inhibit ovulation in *Glossina austeni*. Whether the ablation removes the source of a stimulatory hormone or interferes with nervous pathways necessary for hormone release at some other site is not known.

Thus, the physiological events leading from copulation to ovulation in tsetse flies are not known clearly. Because the ovulation, in a real sense, is the initiation of the tsetse reproductive cycle, a thorough knowledge of this event is of importance not only because of the uniqueness of the method of reproduction but also because the low biotic potential of the tsetse reproductive process represents a potential weakness that might be exploitable in terms of control.

The objectives of this research project, are to examine in detail the physiological events related to ovulation and to determine the factors responsible for the initiation and regulation of ovulation in the tsetse fly. Attempts will be made to determine the relationship of ovarian development and mating to ovulation and analyse elements in the mating process that are responsible for the initiation of ovulation. The exact role of the median neurosecretory cells and corpora allata in the control of ovulation will be investigated; the cyclical activity of the neuroendocrine system during the first pregnancy cycle will be studied and any correlation of such event with ovulation will be investigated.

This report describes the phase of research which is examining the relationship of ovarian development and mating to the ovulation in the tsetse fly, *Glossina morsitans morsitans*.

MATERIALS AND METHODS

Flies used for the present study were obtained from the ICIPE laboratory colony and were reared and maintained under conditions described by Denlinger and Ma (1974).

To study the relationship of ovarian development to ovulation, female flies were either kept virgin or were allowed to mate with 7 to 15 day-old males 2 days after emergence. Flies were kept in small polystyrene cages and offered a blood meal daily (except Sundays) from rabbit ears (Nash *et. al.*, 1966) and were dissected at various ages in *Calliphora* saline to determine the stages of ovarian development, the presence or absence of sperm in spermathecae and whether ovulation had occured. The ovarian development was scored by measuring the length of the first developing follicle and the length of the yolk material therein and expressing the measurements in ratio. A chorionated egg was considered to have a ratio of one.

In order to study the effect of delayed mating on ovulation, female flies were allowed to mate 12 days after emergence and were dissected either immediately after the completion of copulation or at various time-intervals thereafter to check insemination and ovulation.

To study the effect on ovulation of mating with spermic males, the males were first made aspermic by allowing them to mate with different females repeatedly for 10 to 14 times. The last mated female was then examined to determine whether insemination or ovulation had occured.

The effect of multiple but brief matings (preventing any sperm transfer as well as spermatophore formation but allowing prolonged mating) on ovulation was studied by allowing 12-day-old females to mate with a series of fresh unmated males for consecutive specific periods not exceeding 15, 20 or 30 min. *in copula* for each male and an accumulative period of up to 90 min. *in copula* for a female. Females were dissected after 24 hours to determine whether ovulation had occured. Control females in each case were dissected immediately to check any spermatophore formation.

Unless otherwise mentioned, calculation of the percentages in the above experiments are based on the observation made on 20 insects in each treatment.

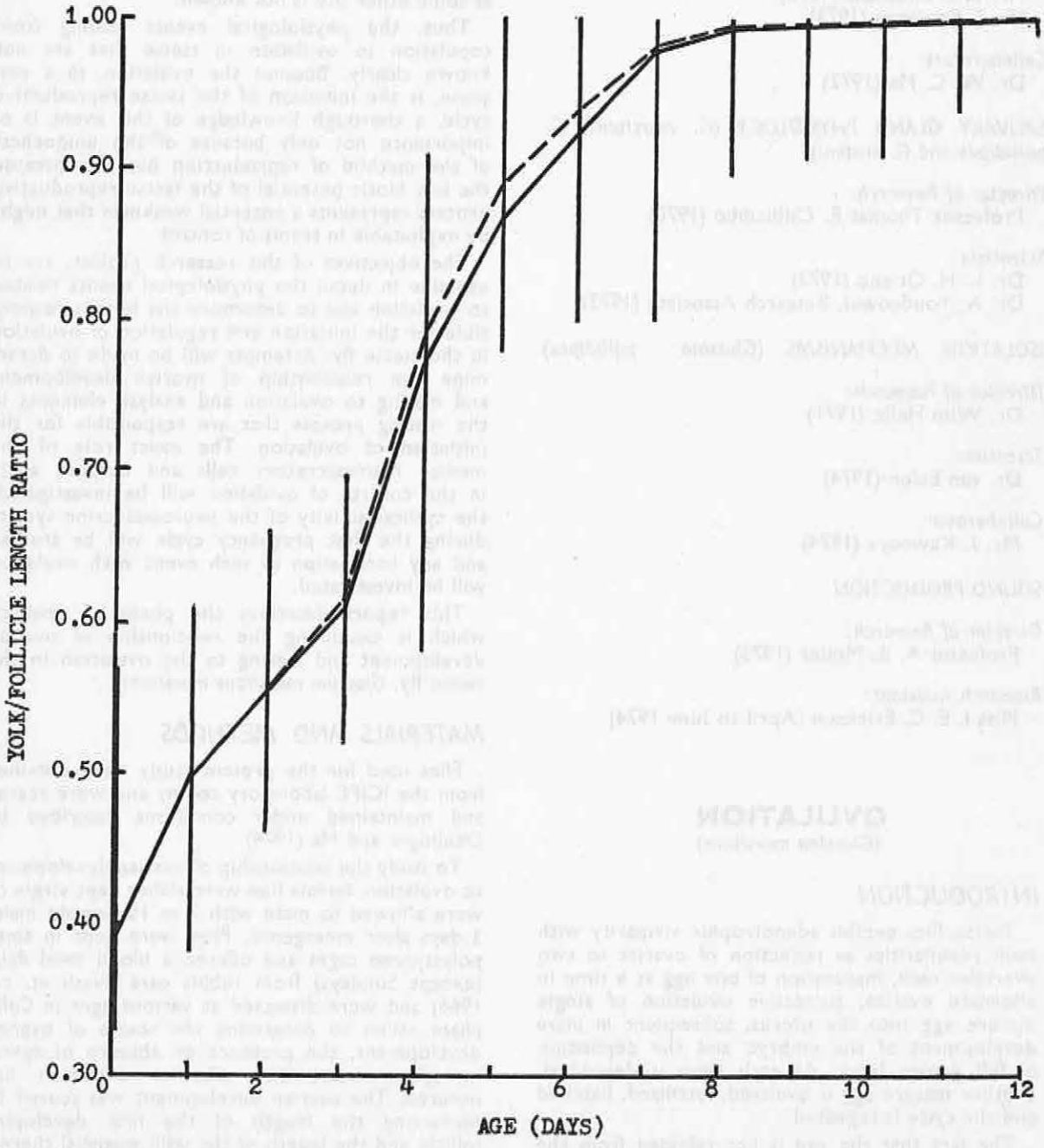

Fig. 1. Mean yolk/follicle length ratio in mated (———) and virgin (— — —) *Glossina morsitans morsitans*. Vertical line indicates the range of the ratio in the females of indicated age and 1.00 on the ordinate indicates the presence of a chorionated egg.

RESULTS

Observations on the ovarian development in virgin and mated females are presented in Fig. 1, which shows the ratio of the length of yolk material to the length of the entire follicle of the first developing egg to reach maturity in the ovary of 0 to 12-day-old virgin females and 3 to 12-day-old mated females. At emergence the yolk material occupied about one third of the length of the follicle. The incorporation of yolk material steadily increased as the fly grew older. The first chorionated egg was observed in 5 day-old females (both virgin and mated); however the number of females possessing chorionated eggs at this age did not exceed 20%. The number of females with first chorionated eggs increased steadily with age until at 9 days the percentage was above 90. On rare occasions abnormal follicular growth was observed when not enough yolk deposition and no chorionation took place even although the age of the insects ranged from 9 to 12 days.

Percentages of the 5 to 12 days mated females having a chorionated and ovulated egg are presented in Fig. 2. No ovulation was observed in 5 day-old females even though 20% of them had a chorionated egg. The youngest flies showing ovulation were 15% of 6 day-old females, even though more than 40% of them possessed chorionated eggs. Eighty percent or more of the 7 and 8-day-old females had chorionated eggs but all these did not ovulate. All of the 9 to 12-day-old females which possessed chorionated eggs had normally ovulated.

Fig. 3 shows the ovulation pattern in females mated when they were 12 days old. None of the females ovulated during the 4-hour period following mating although they all possessed chorionated eggs. Only 15% females had ovulated 8 hrs after the completion of mating. Thereafter however percent ovulation increased until at 20 hrs after mating all had ovulated.

Results of the mating with aspermic males are summarized in Table 1. All of the females copulated with aspermic males ovulated normally. Although sperm were absent in the spermathecae and uterus, spermatophore formation might have had occured in the uterus as evidenced by observations on the control insects. No spermatophores were present in the uteri of the ovulated females.

Table 2 shows that 12-day-old females, which were allowed to mate repeatedly with fresh unmated males for short durations and accumulate mating experience, were able to ovulate successfully. Observations on the control insects indicated that such repeated matings with fresh males for short durations prevents sperm transfer as well the spermatophore formation.

DISCUSSION

The results of the investigation show that ovulation in the tsetse fly is dependent on the availability of a mature chorionated egg in her ovary as well as copulation. Although the female normally copulates 2 or 3 days after emrgence, a "mature" chorionated egg is not available for ovulation until she is about 8 or 9 days old. It is probable that newly chorionated eggs observed in some younger females are not ready to be released until further maturation.

Observations on the delayed-mated females indicate that a female with a mature egg in the ovary is capable of ovulating within a few hours following mating. In fact, 65% ovulate within 12 hrs and 100% within 20 hrs.

Results also indicate that there is no significant difference in the development of the first follicle in the mated and virgin females.

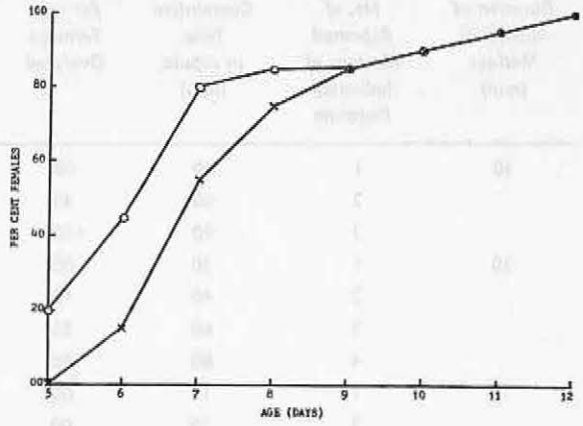

Fig. 2. Per cent mated females of *Glossina morsitans morsitans* with chorionated (O–O–O) and ovulated (X–X–X) eggs at indicated age. Females were allowed to mate two days after emergence.

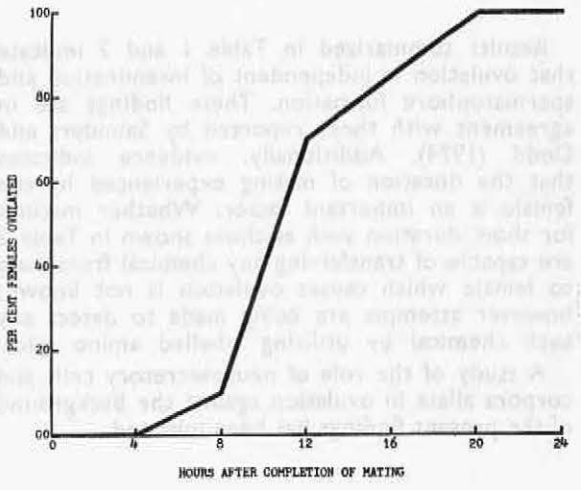

Fig. 3. Per cent mated females of *Glossina morsitans morsitans* ovulated after 0 to 24 hrs post copulation. Females were allowed to mate 12 days after emergence.

Table 1. Ovulation in 12-day-old female *Glossina morsitans morsitans* when mated with aspermic males. The spermathecae and uteri of all test and control insects were devoid of sperm.

Mating No. for Male Used	*No. of Females Mated*	*No. of Females Ovulated*
10 th	11	11
11 th	8	8
12 th	5	5
13 th	5	5
14 th	4	4

Table 2. The effect of mating 12-day-old female *Glossina morsitans morsitans* repeatedly with fresh unmated males, for various short durations, on ovulation. Virgin controls showed no ovulation. Control females experiencing uninterrupted mating with single male ovulated. Dissection of two mated females from each of the above test groups immediately after the mating was interrupted revealed no spermatophore in the uteri.

Duration of Individual Matings (min)	*No. of Repeated Matings of Indicated Duration*	*Cumulative Time in copula (min)*	*Per cent Females Ovulated*
30	1	30	00
	2	60	45
	3	90	100
20	1	20	00
	2	40	10
	3	60	55
	4	80	85
15	1	15	00
	2	30	00
	3	45	25
	4	60	65
	5	75	85
	6	90	95

Results summarized in Table 1 and 2 indicate that ovulation is independent of insemination and spermatophore formation. These findings are in agreement with those reported by Saunders and Dodd (1974). Additionally, evidence indicates that the duration of mating experienced by the female is an important factor. Whether matings for short duration such as those shown in Table 2 are capable of transferring any chemical from male to female which causes ovulation is not known; however attempts are being made to detect any such chemical by utilizing labelled amino acids.

A study of the role of neurosecretory cells and corpora allata in ovulation against the background of the present findings has been initiated.

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MOTHER-LARVA INTERACTION

(Glossina morsitans)

INTRODUCTION

A successful pregnancy in tsetse requires the integration of several interdependent development processes. During the 9-day period required for the *in utero* development of the progeny, the "milk" gland of the female undergoes a cycle of activity that is coordinated with the larva's requirements for nutriment. In turn, the maternal and larval energy requirements and the space available within the females abdomen dictate a cycle of adult feeding activity during pregnancy. Oocyte development is also coordinated within the 9-day cycle; at the time of larviposition, the next oocyte is completely developed and ovulation occurs within 1–2 hours after the previous larva has been expelled from the uterus. In the first phase of this work on the mother-larva interaction in tsetse the normal sequence of events during pregnancy has been defined as a prelude to research on disruption of the pregnancy cycle for purposes of possible control.

RESULTS AND DISCUSSION

The dynamics of the pregnancy cycle is summarized in Figure 1. The data are discussed in further detail by Denlinger and Ma (1974). The investigation on the milk gland has also provided information on the mechanism of secretory discharge. Earlier work suggested that milk is released directly into the lumen of the gland by apocrine secretion. Present observations on the structure of the gland do not support such a mechanism, but rather a novel type of exocrine discharge in which secretion is stored in an extracellular reservoir and released into the lumen through a dense cuticular network. A schematic representation of the structural organization of the milk gland tubules is seen in Figure 2. At the points of milk release the lumen is frequently inhabited by Gram-negative bacteria, which have not previously been described in milk glands. The secretory discharge and microflora of the milk gland are discussed in further detail by Ma and Denlinger (1974).

Reproductive abnormalities such as abortion and *in utero* pupariation occur occasionally in wild and laboratory populations of tsetse. Research is presently focussed on mechanisms for inducing such abnormalities in the laboratory. If parturition is prevented by physical blockage, the larva has the potential to delay pupariation for 1–2 days; but after a maximum of 2 days pupariation will proceed even if the larva is still *in utero*. If the uterus is plugged with a puparium, the reproductive future of the female is obviously terminated: the female is unable to take a blood meal due to the lack of space in the abdomen and consequently she dies within 4–5 days. Future work will explore potential chemical mechanisms for causing *In utero* pupariation.

Abortion has been experimentally induced in pregnant females with injections of ecdysoids and topical applications of juvenoids. Abortion has been induced in all stages of progeny development: egg, 1st, 2nd, and 3rd instars. Even an aborted

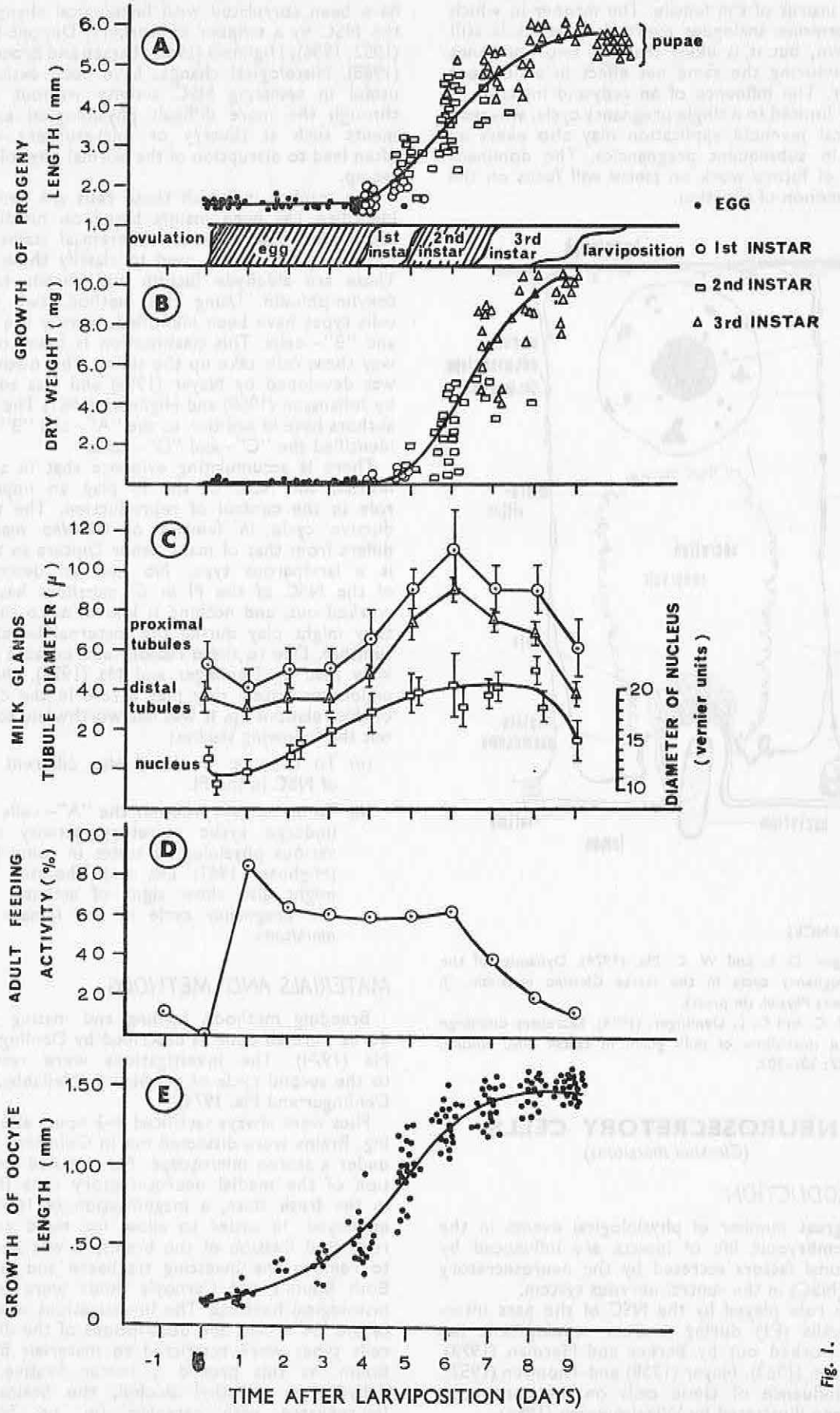
A
GROWTH OF PROGENY
LENGTH (mm)
6.0
5.0
4.0
3.0
2.0
1.0
pupae
ovulation
egg
1st instar
2nd instar
3rd instar
larviposition
• EGG
o 1st INSTAR
□ 2nd INSTAR
△ 3rd INSTAR
B
DRY WEIGHT (mg)
10.0
8.0
6.0
4.0
2.0
C
MILK GLANDS
TUBULE DIAMETER (μ)
120
100
80
60
40
20
0
proximal tubules
distal tubules
nucleus
DIAMETER OF NUCLEUS (vernier units)
20
15
10
D
ADULT FEEDING ACTIVITY (%)
100
80
60
40
20
E
GROWTH OF OOCYTE
LENGTH (mm)
1.50
1.00
.50
0
-1
1
2
3
4
5
6
7
8
9
TIME AFTER LARVIPOSITION (DAYS)

Fig. 1.

3rd instar larva is not capable of surviving outside of the uterus of the female. The manner in which the hormone analogues exert their effect is still unknown, but it is likely that the two hormones are producing the same net effect in a different manner. The influence of an ecdysoid injection is usually limited to a single pregnancy cycle, whereas, a topical juvenoid application may also exert an effect in subsequent pregnancies. The dominant thrust of future work on tsetse will focus on the phenomenon of abortion.

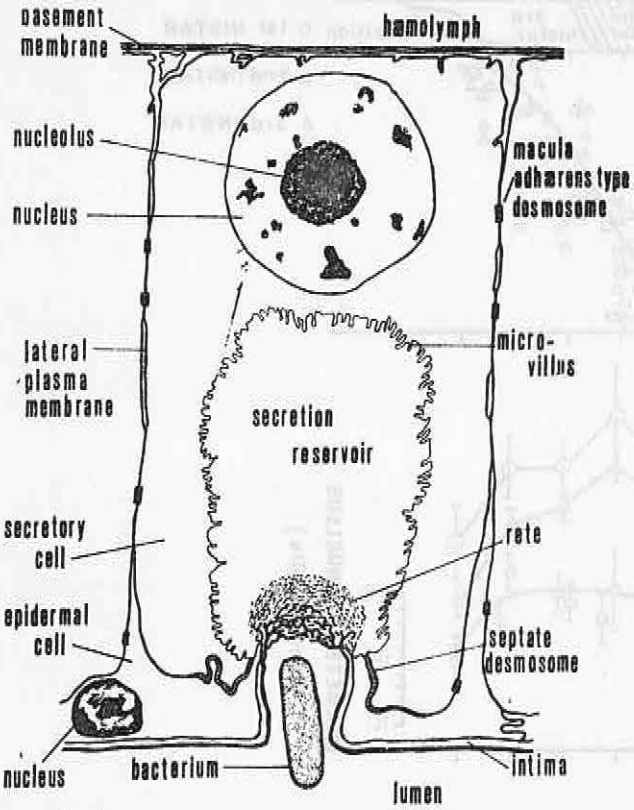

Fig. 2.

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NEUROSECRETORY CELLS

(*Glossina morsitans*)

INTRODUCTION

A great number of physiological events in the post-embryonic life of insects are influenced by hormonal factors secreted by the neurosecretory cells (NSC) in the central nervous system.

The role played by the NSC of the pars intercerebralis (PI) during ovarian development has been worked out by Barker and Herman (1973), Highnam (1962), Nayer (1958) and Thomsen (1952). The influence of these cells on metamorphosis has been illustrated by Wigglesworth (1954).

Various physiological phenomena in insects have been correlated with histological changes in the NSC by a number of workers: Dupont-Raabe (1952; 1956); Highnam (1962); Larsen and Broadbent (1968). Histological changes have been extremely useful in assessing NSC activity without going through the more difficult physiological experiments such as cautery or microsurgery which often lead to disruption of the normal physiological set-up.

The manner in which these cells are generally identified has been mainly based on histological techniques. Two types of differential stains have for a long time been used to classify these cells. These are aldehyde fuchsin and chrome-haematoxylin-phloxin. Using this method, two major cells types have been identified, namely the "A"– and "B"– cells. This classification is based on the way these cells take up the stains. This distinction was developed by Nayar (1955) and was adopted by Johansson (1958) and Highnam (1961) The latter authors have in addition to the "A"– and "B"– cells identified the "C"– and "D"– cells.

There is accumulating evidence that in several insects, the NSC of the PI play an important role in the control of reproduction. The reproductive cycle in females of *Glossina morsitans* differs from that of many other Diptera in that it is a larviparous type. No detailed description of the NSC of the PI in *G. morsitans* has been worked out, and nothing is known as to the role they might play during the maternal larval relationship. Due to these reasons and because of the view held by Denlinger and Ma (1974), that the endocrine system may play a role in the control of this relationship, it was felt worthwhile to carry out the following studies:

(*a*) To describe in detail the different types of NSC in the PI.

(*b*) To investigate whether the "A"– cells which undergo cyclic secretory activity during various physiological states in some insects (Highnam 1961; Lea and Thomsen 1962), might also show signs of activity during the pregnancy cycle in the female of *G. morsitans*.

MATERIALS AND METHODS

Breeding methods, feeding and mating procedures were all done as described by Denlinger and Ma (1974). The investigations were restricted to the second cycle of pregnancy (Mellanby, 1937; Denlinger and Ma, 1974).

Flies were always sacrificed 2–3 hours after feeding. Brains were dissected out in *Calliphora* Ringer under a stereo microscope. For detailed examination of the medial neurosecretory cells (MNSC) in the fresh state, a magnification of 16×4 was employed. In order to allow for rapid and unrestricted fixation of the brains, it was necessary to remove the investing trachaeae and air sacs. Both Bouin's and Carnoy's fluids were used as histological fixatives. The investigations of activity of the "A"– cells and descriptions of the different cells types were restricted to materials fixed in Bouin, as this proved a better fixative. After dehydration in ethyl alcohol, the brains were impregnated with paraplast (m. pt. 56–57°C.

Sherwood Medical Industrial Inc.) for 1-hr. (changing the paraplast after every 20 min.). For general histological studies, both thin (5μ), and thick (8–10μ), sections were cut, whereas for specific studies on synthetic activity only one thickness (7μ) were cut. Four staining procedures were employed to identify the different cell types. These were azan (Pantin, 1959); chrome-haematoxylin-phloxin (Gomori, 1941); paraldehyde fuchsin (Ewen, 1962); and victoria blue (Dogra and Tandan, 1964). In the latter case either wholly processed brains were mounted on the slide, or were embedded in paraplast and then cut at 7μ. In all cases Canada balsam in xylene was used as the mounting medium. The diameters of the cells and nuclei were measured by means of an eyepiece micrometer under oil immersion. In order to reduce errors in cell counting (Thomsen, 1965), nucleoli were used as land marks for identification of individual cells. The average of least and greatest diameters of the cells were determined, and the ratio of the nucleus to the cytoplasm was considered as a measure of synthetic activity for the "A"-cells. N refers to the number of flies under investigation. Attempts were also made to estimate the sizes of the nucleoli of the "A"–cells by division into 3 categories. Small (less than 2μ); medium (2–3μ), and large (more than 3μ). The description and classification of the different cells types were confined to flies that had just larviposited the 1st larva, but before the 2nd ovulation occurred.

RESULTS

Anatomy and histology of the neurosecretary cells:

The gross anatomy of the brain of *G. morsitans* (Fig. 1) is very similar to that of *Calliphora erythrocephala* as described by Thomsen (1965). Four main groups of NSC are present in the brain of *G. morsitans* (Fig. 1). These are (a) one paired group of lateral cells, and (b) one paired group of medial cells.

(a) *One paired group of lateral cells:*

Both lateral groups of NSC are composed of 3 similar cells. The cells are located on the dorsal aspect of the protocerebral hemispheres, and are close to each other. Each cell has an average nuclear and cellular diameter of 11.6μ and 20.5μ respectively. (S.D. = ±3; N= 15). They are rounded in shape with large centrally located rounded nuclei. The cytoplasmic inclusions of these cells stain green or greenish brown in paraldehyde fuchsin, sky blue or light blue in azan, and bright red or various shades of red in chrome-haematoxylin (i.e. phloxinophilic). All these cells are termed the "B"–cells of the lateral group. The variations in the staining intensity are due to the various degrees of differentiation employed. The cells do not show up in the victoria blue stain. Neurosecretory material (NSM) can be traced along the fibres of these cells.

(b) *The paired group of medial cells:*

The two medial NSC groups are located in the dorsal region of the PI protocerebrum, and actually merge in the median furrow of this region to form a more or less single group. Four types of cells are present in this group, namely "A"–, "B"–, "C"– and "D"–. This classification is based on the morphology, size and staining characteristics of the cells (Figs. 1 and 2).

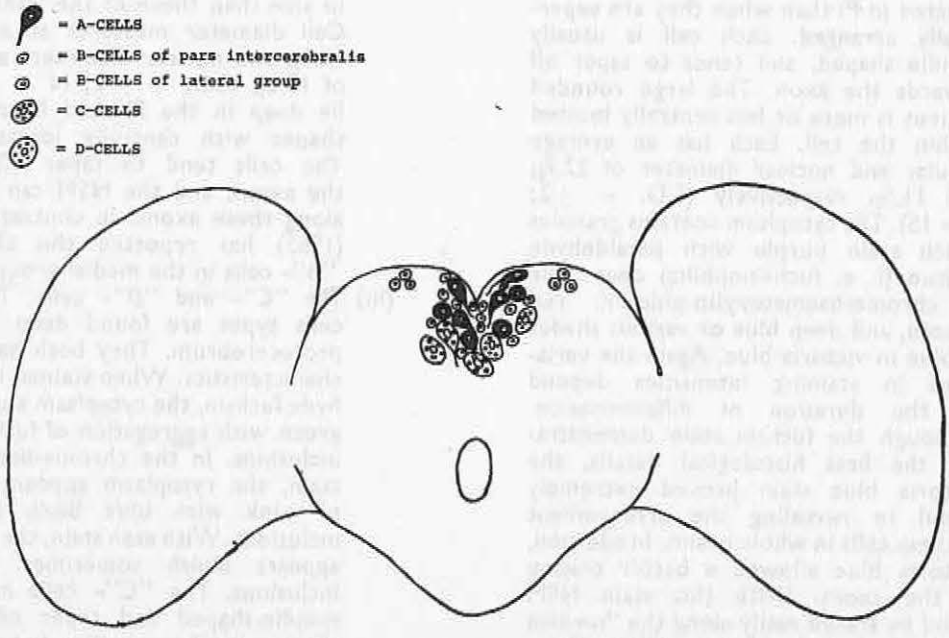

Fig. 1. Brain of *Glossina morsitans* showing the distribution of different types of neurosecretory cells.

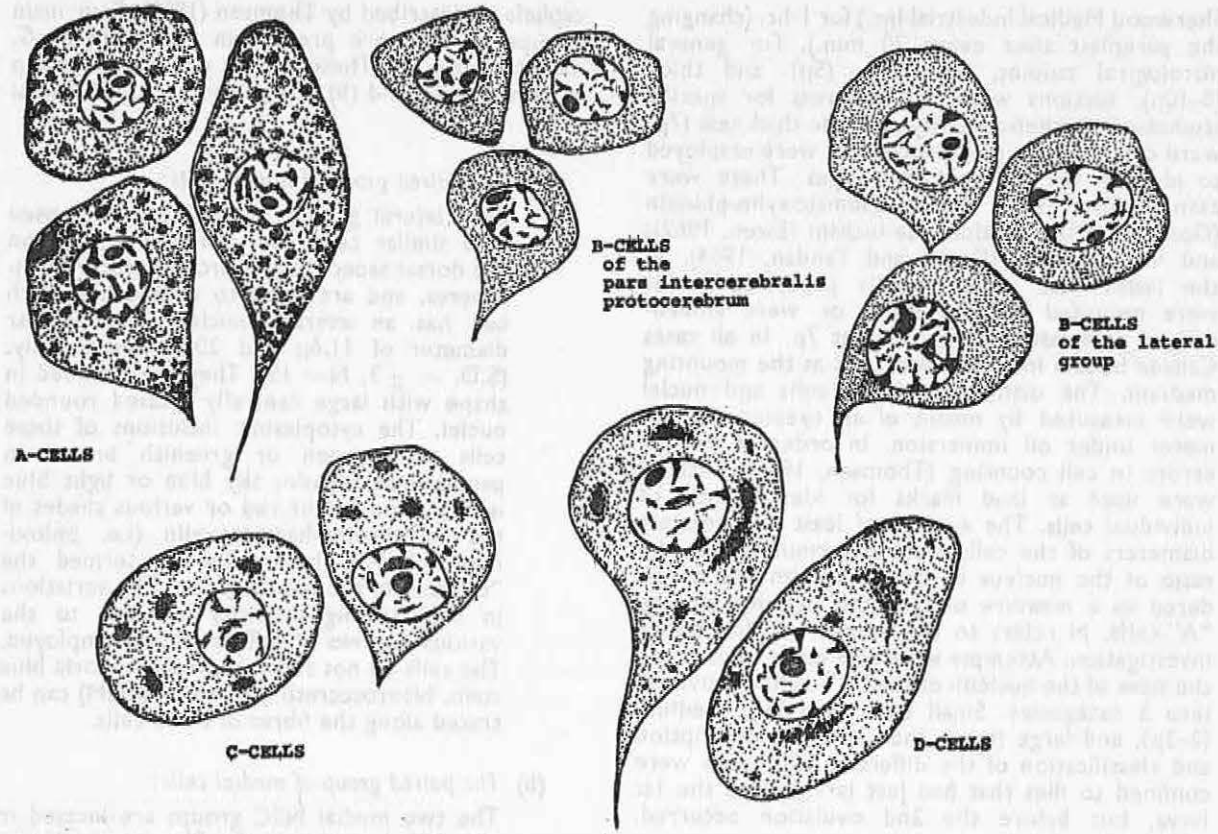

Fig. 2. Different types of neuresecretory cells in the protocerebrum of *G. morsitans*.

(i) *The "A"– cells.* Both medial NSC groups include 4 "A"– cells. In most cases, the cells lie superficially on both sides of the median furrow. In certain instances, however, they occur in a form of a cluster within this furrow. In the latter case, some of these cells are more distally situated in PI than when they are superficially arranged. Each cell is usually spindle shaped, and tends to taper off towards the axon. The large rounded nucleus is more or less centrally located within the cell. Each has an average cellular and nuclear diameter of 22.7μ and 11.5μ respectively (S.D. = ±2; N= 15). The cytoplasm contains granules which stain purple with paraldehyde fuchsin (i. e. fuchsinophilic) deep blue in chrome-haematoxylin-phloxin, red in azan, and deep blue or various shades of blue in victoria blue. Again the variations in staining intensities depend on the duration of differentiation. Although the fuchsin stain demonstrated the best histological details, the victoria blue stain proved extremely useful in revealing the arrangement of these cells in whole brains. In addition, victoria blue allowed a better tracing of the axons. With this stain NSM could be traced easily along the "median bundle" of Power (Power, 1943). These cells are always packed with NSM.

(ii) *The "B"– Cells.* The two medial groups of NSC include approximately 23–32 cells which show staining characteristics that are very similar to those of the "B"– cells of the lateral groups. These are referred to as the "B"– cells of the PI protocerebrum. They are smaller in size than those of the lateral group. Cell diameter measures an average of 18.5μ and nuclear diameters an average of 10.2μ (S.D. = ±2; N = 15). They lie deep in the PI, and have rounded shapes with centrally located nuclei. The cells tend to taper off towards the axons, and the NSM can be traced along these axons. In contrast, Langley (1965) has reported the absence of "B"– cells in the medial groups.

(iii) *The "C"– and "D"– cells.* These two cells types are found deep in the PI protocerebrum. They both have similar characteristics. When stained in paraldehyde fuchsin, the cytoplasm appears light green with aggregation of fuchsinophilic inclusions. In the chrome-haematoxylin stain, the cytoplasm appears light red or pink with blue black or mauve inclusions. With azan stain, the cytoplasm appears bluish sometimes with red inclusions. The "C"– cells are usually spindle-shaped and taper off towards the axons; they number between 6–10, and have average cell and nuclear

diameters of 24.5μ and 13.4μ (S.D. = ±2; N = 15) respectively. The large centrally located nucleus is rounded. NSM is more abundant than in "D"– cells and it can easily be traced along the axons. The "D"– cells which vary in number between 4–6 are largest of all the NSC. They are usually rounded with large more or less oval centrally located nuclei. The cells taper off towards the axons. The "D"– cells measure an average of 28.2μ and nuclear diameters average 14.5 = 14.5μ (S.D. = ±3; N = 15). They differ from the "C"– cells in that they are large and the NSM is less in quantity and not easy to trace along the axons.

Cyclic activity of the "A" -cells

There are no visible changes with regard to the density of the fuchsinophilic material in the cytoplasm of the "A" cells during the course of the pregnancy cycle. There are, however, notable synthetic changes in these cells as measured in terms of nucleus/cytoplasm ratio (Fig. 3). At the time of the first larviposition but before the next ovulation (30–60 mins after larviposition), there is a fairly low activity ratio. There is a continuous decrease reaching a minimal level at Day 1, that is 24 hours after ovulation. At this time there is already an egg in the uterus. On these two days the nucleoli appear small in size (Fig. 4). On Day 2, there is a sharp rise in activity and the nucleoli enlarge to a medium size. By Day 3 the nucleus/cytoplasm ratio reaches a maximum and the nucleoli acquire a maximum size. There is a slight but gradual decrease in the activity at Day 4, which thereafter falls to a constant level at Day 5, through Days 6, 7, and 8. On all these days the nucleoli appear large in size. There is a significant fall in cellular activity from day 8, which also corresponds with a decrease to medium size of the nucleoli. Thirty to sixty minutes after parturition, there is a further decrease in cellular activity, and the nucleoli appear small.

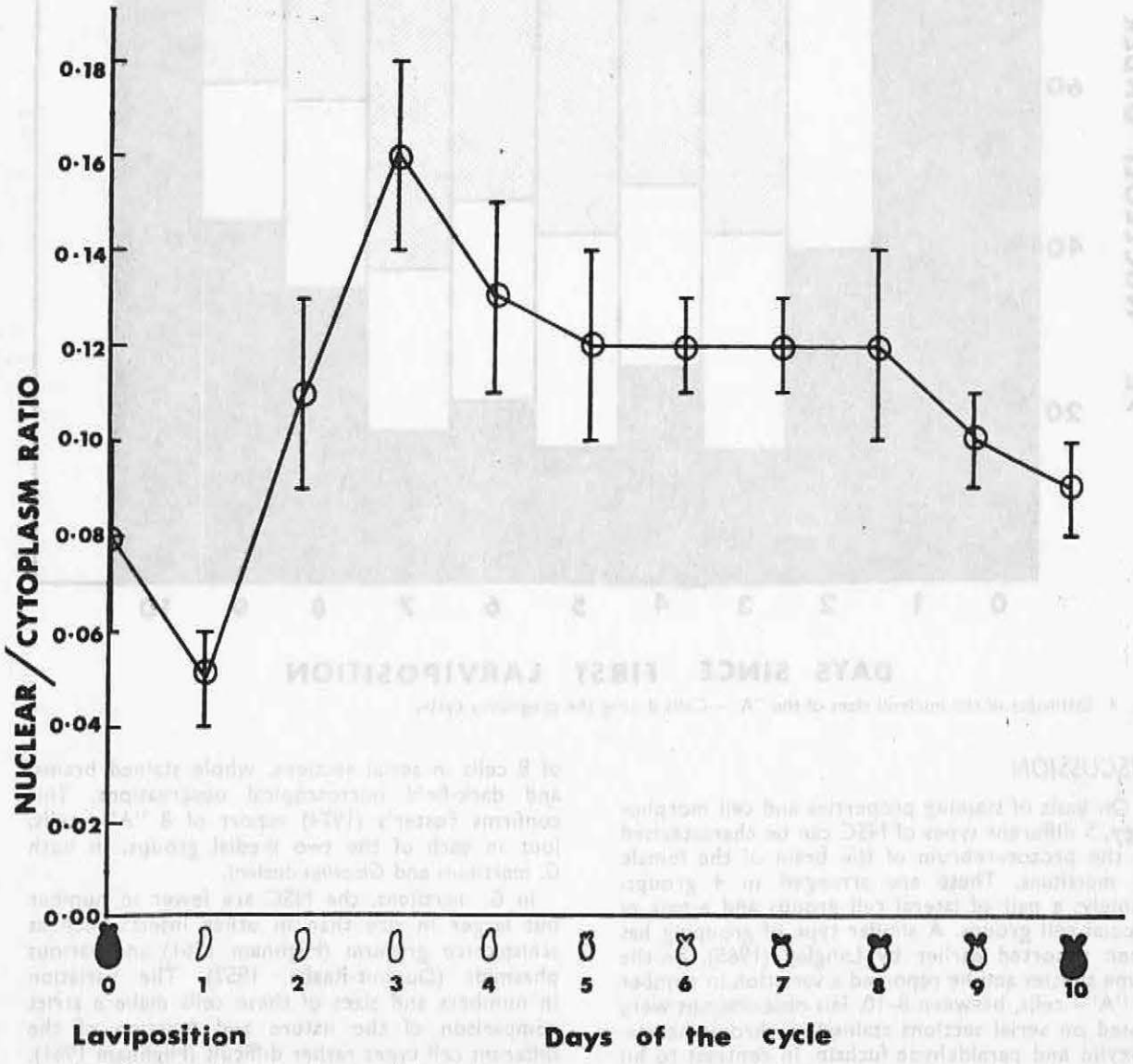

Fig. 3. Cellular activity of the "A—Cells" during the pregnancy cycle.

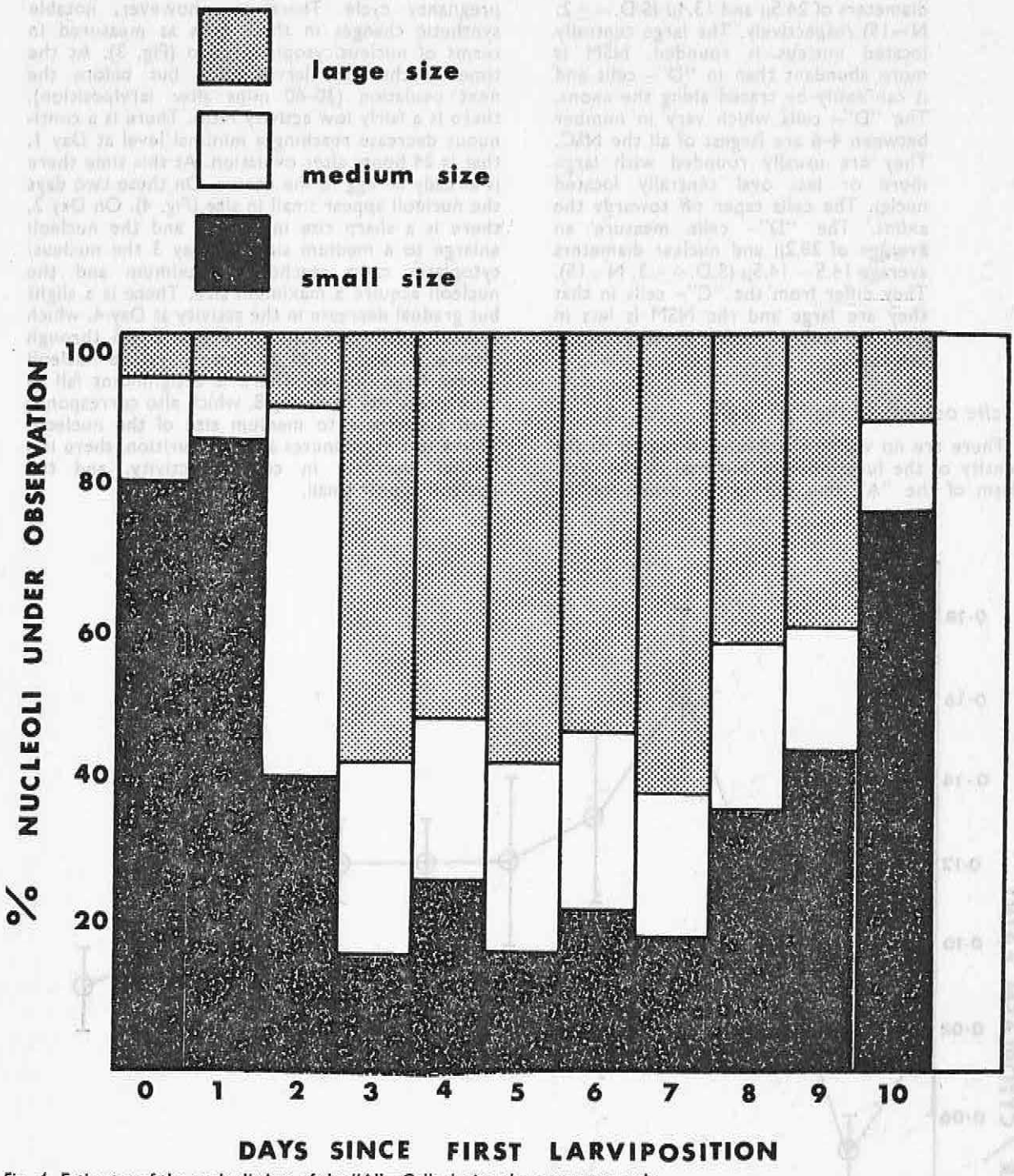

Fig. 4. Estimates of the nucleoli sizes of the "A"—Cells during the pregnancy cycle.

DISCUSSION

On basis of staining properties and cell morphology, 5 different types of NSC can be characterised in the protocerebrum of the brain of the female *G. morsitans*. These are arranged in 4 groups, namely; a pair of lateral cell groups and a pair of medial cell groups. A similar type of grouping has been reported earlier by Langley (1965), on the same species and he reported a variation in number of "A"– cells, between 8–10. His observations were based on serial sections stained in chrome-haematoxylin and paraldehyde fuchsin. In contrast to his findings, this investigation found a constant number of 8 cells in serial sections, whole stained brains, and dark-field microscopical observations. This confirms Foster's (1974) report of 8 "A"– cells, four in each of the two medial groups, in both *G. morsitans* and *Glossina austeni*.

In *G. morsitans*, the NSC are fewer in number but larger in size than in other insects such as *schistocerca gregaria* (Highnam 1961) and various phasmids (Dupont-Raabe, 1952). The variation in numbers and sizes of these cells make a strict comparison of the nature and function of the different cell types rather difficult (Highnam 1961).

The cytoplasmic inclusions of the "A"–cells are

fuchsinophilic, while those of the "B"– cells are phloxinophilic. Both these materials are traceable along the "median bundle" of Power (1943). At no time do the two types of material appear in the same cell. The two cells types are of a different shape and size. These observations are consistent throughout the cycle of pregnancy. There is therefore no doubt that the "A"– and "B"– cells are two different cell types in *G. morsitans*, and not phases of secretory activity as has been reported by Nayar (1955) in *Iphita limbata*. Highnam (1961) has indicated that the "A"– cells are different from the "B"– cells in *S. gregaria*. Thomsen (1965) has reported in *C. erythrocephala*, "giant neurons', which when over-stained in paraldehyde fuchsin reveal granules in the neuroplasm. The same author has observed in overstained sections numerous thread-like and branched indentations of the cell membrane on the cell surface. No such features could be observed in the "C"– and "D"– cells of *G. morsitans* after the same treatment. Both cells in *G. morsitans* show granules in their neuroplasm even without overstaining. It is likely that these cells are different from the "giant neurons" described by Thomsen. The two cell types differ from the "A"– cells in that they are negative to victoria blue stain. Both cells have different morphology and sizes, and occupy positions different from those of the "A"– cells. For example the "A"– cells are always packed with NSM unlike the "C"– and "D"– cells. Unlike the "D"– cells, the "C"– cells are smaller in size, and have more NSM in the neuroplasm. This material is always traceable along the axons. In contrast, the "D"– cells are very large, with usually less NSM which is rarely traceable along the axons.

Different cyclic phases of emptying and filling of the NSC's with NSM have been reported by a number of workers (Rehm 1951, Arvy and Gabe 1954, Larsen and Broadbent 1968 Highnam 1961), during various different physiological states of various insects. On the other hand Clarke and Langley (1962) have reported an absence of such changes during the 3rd, 4th and 5th stage nymphs of *Locusta migratoria*. Langley (1966) has also stated that in *G. morsitans*, unlike most other insects, there is no cycle of activity alternating with inactivity in the neuroendocrine system. Foster (1973) has indicated that in *G. austeni* no cycle of secretion has been detected in the median NSC's. The present observations on the "A"– cells during the 2nd cycle of pregnancy have revealed that, although there are no detectable cyclic phases of filling and emptying with NSM, there is a strong indication that these cells undergo cyclic phases of synthesis (nucleus/cytoplasm ratio and nucleoli sizes) that could be correlated with the different days of the pregnancy cycle. It has already been established (Hisao and Frankel 1966, Highnam 1962) that an actively synthesizing or secreting NSC has a small quantity of stainable material, while a cell in an inactive or storage state has its cytoplasm packed with this material. Lea and Thomsen (1962), have reported that in *C. erythrocephala* there is a correlation between the size of the nucleus and the quantity of NSC in the cytoplasm. In the latter fly, following a protein meal and during this time, there is an increase in size of the nuclei and nuceoli. There is a strong indication from the present studies that in *G. morsitans*, rates of synthesis of NSM are either equal to or lower than the rates of release at all stages of the pregnancy cycle; for phases of filling and emptying were not observed.

The present work shows that there may be a close relationship between the activity of and synthesis in the "A"– cells and structural changes in the milk glands during the cycle of pregnancy as described by Denlinger and Ma (1974). The latter workers reported a regression of glandular activity from day 8 to day 1. This regression correlates with a low level of synthesis in the "A"– cells. The onset of the milk gland activity on day 2 correlates with a sharp rise in the "A"– cells activity, which reaches a peak on day 3. On this latter day Denlinger and Ma (1974) reported an apparent increase in the size of the milk gland cells, and the beginning of the accumulation of the milk. After the peak on day 3, there is a slight but gradual fall in the "A"– cells' activity on days 4 and 5, which levels off from day 5 to day 8. According to changes in a nuclear diameter, the secretory activity of milk gland cells reach a maximum value on days 5 to 8.

Since Denlinger and Ma (1974) reported the parallel development of the milk gland activity and oocyte growth, the activity of the "A"– cells could possibly be involved in the regulation of both processes. These correlations further support the view held by Denlinger and Ma (1974) that there may be a regulation of the pregnancy cycle by the tsetse endocrine system. This view is in addition supported by Foster (1973) who has shown that the MNC or nervous tissue closely associated with them are to some extent necessary for larval development.

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SALIVARY GLAND PHYSIOLOGY

SALIVATION

INTRODUCTION

When a tsetse fly feeds on a mammalian host infected with trypanosome parasites, it takes in the parasites with the blood meal and thus becomes infected. Trypanosomes of the *brucei* subgroup develop in the alimentary system of the fly and later invade the salivary glands and saliva. If such an infected fly feeds on a healthy mammalian host, it transfers the parasites when it salivates into the tissues of the host. The conditions in the fly that stimulate salivation are little understood; and it is hoped that information on this aspect of tsetse fly behaviour will throw some light on the factors that favour the transmission of the trypanosomes from the fly to the mammalian host.

METHODS AND RESULTS

Most of the work was confined to *Glossina morsitans*, which is the species being bred in the largest numbers at the ICIPE Research Centre. Some observations were also made on the saliva of *G. austeni*.

Collection of and observations on saliva

A quick and simple method for collecting and observing the saliva of the tsetse fly was developed. A hole 3.2 cm in diameter was cut in the centre of a rectangular perspex plate 14 cm × 4.5 cm and 3 mm thick, and a piece of wing membrane taken from the bat *Otomops martiensseni* was stretched across this hole and held down with sellotape. Individual flies were confined in 2″ × 1″ plastic tubes with nylon mesh at both ends. One end of each tube was attached to the hole in the perspex plate so that the fly could make tarsal contact with the bat-wing membrane. The assembled apparatus was observed under the microscope, with light from a microscope directed into the membrane at an angle of about 30° (Figs. 1 and 2).

As soon as the tsetse fly made tarsal contact with the bat-wing membrane, it probed and salivated. Prior to probing, the labium was released from its normal resting position and the labellum was used to cut the membrane by rasping action. During this time drops of saliva just sufficient to moisten the labium were produced. After a few seconds of rasping the membrane was pierced and the labium protruded through it.

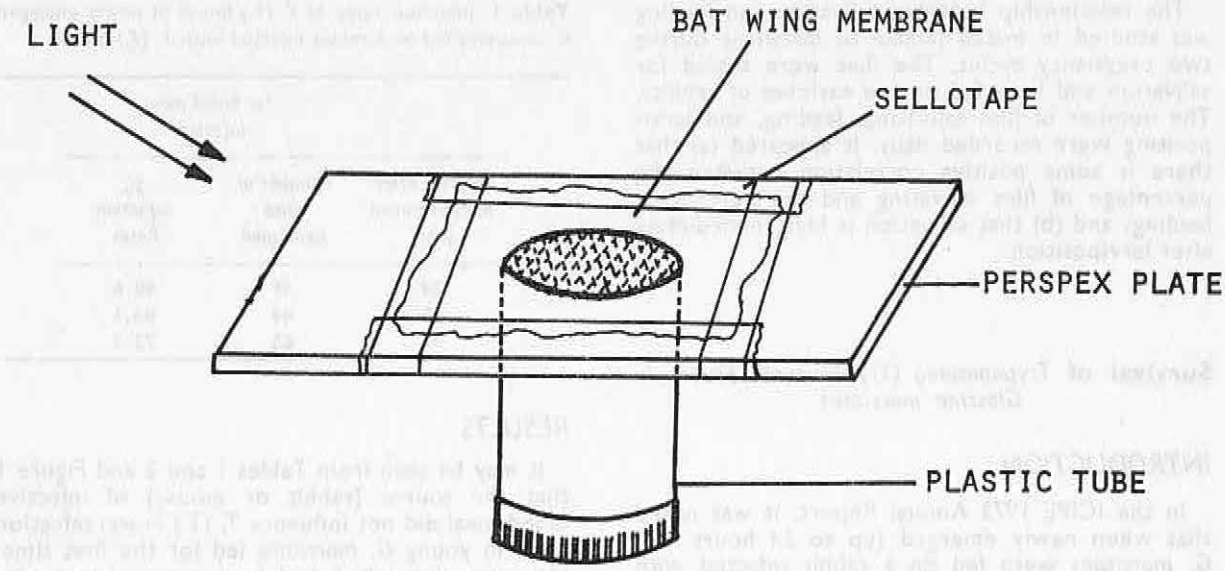
As soon as the labium protruded there was a copious flow of saliva to the tip of the labium. A drop of saliva was left on the surface of the membrane when the labium was withdrawn. Flies probed and salivated within 20 seconds of making tarsal contact with the bat-wing membrane.

The saliva was collected on clean glass slides as it was produced by the fly. It was fixed in methanol, dried, and stained with Giemsa for 40 minutes. In this way it was possible to identify the flies whose salivary glands were infected with trypanosomes. Out of a total of 55 tsetse flies examined over a period of 2 months, salivary infection was detected in 3 flies on the 42nd, 56th and 56th days respectively after the infected feed. This gave a salivary gland infection rate of 5.46%, which is considered high when compared with natural salivary infection rates for *brucei* subgroup trypanosomes obtained by other workers for field-collected flies.

Direct observation of the salivation process made by means of the bat-wing membrane technique described here led to the following conclusions:

(*a*) Tsetse flies produce saliva on the surface of the host while probing.

(*b*) There is copious salivation only when the tissues of the host have actually been pierced: this ensures that the trypanosomes are deposited within the host tissues.



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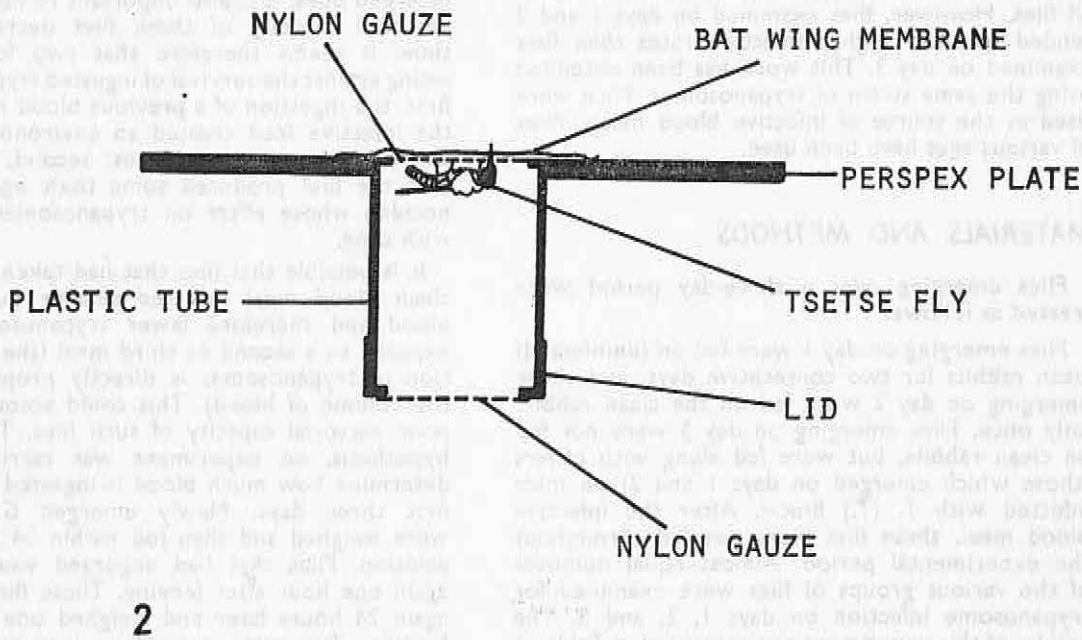

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Fig. 1. The apparatus for collecting tsetse fly saliva.
Fig. 2. Sectional view of the apparatus for collecting tsetse fly saliva.

(*c*) Tsetse flies salivate intermittently and do not exhaust their saliva in a single probe. It seems that there is a mechanism in the fly that causes rapid secretion of saliva.

(*d*) The saliva of *G. morsitans* and *G. austeni* is a clear, colourless, and slightly viscous liquid that dries quickly.

There was no apparent difference between the appearance of the saliva of flies infected with trypanosomes and that of infection-free flies.

Salivation in starved flies

Salivation in relation to the hunger cycle of newly emerged male *G. morsitans* was studied. The results (Table 1) show that the proportion of flies salivating increased with the intensity of starvation. When flies were starved for 3 days, over 70% of them salivated. The mortality observed when flies were starved more than 3 days was very high, and the few flies that survived were too weak to probe and salivate.

Table 1. The effect of starvation on salivation and mortality in male *Glossina morsitans*

No. of days starved	*Mean % flies salivating*	*No. of flies tested*	*Mortality %*
Less than 1	13.7	68	2.9
1	27.9	80	20.0
2	56.1	76	23.2
3	72.1	39	43.5

The relationship between salivation and feeding was studied in mated female *G. morsitans* during two pregnancy cycles. The flies were tested for salivation and later fed on the earlobes of rabbits. The number of flies salivating, feeding, and larvipositing were recorded daily. It appeared (a) that there is some positive correlation between the percentage of flies salivating and the percentage feeding; and (b) that salivation is high immediately after larviposition.

Survival of *Trypanosoma (Trypanozoon) brucei* in *Glossina morsitans*

INTRODUCTION

In the ICIPE 1973 Annual Report, it was noted that when newly emerged (up to 24 hours old) *G. morsitans* were fed on a rabbit infected with *T. (T.) brucei* and examined for the presence of trypanosome infection, one, two, or three days later, infection could be demonstrated in nearly all flies. However, flies examined on days 1 and 2 tended to show higher infection rates than flies examined on day 3. This work has been extended using the same strain of trypanosomes. Mice were used as the source of infective blood meals. Flies of various ages have been used.

MATERIALS AND METHODS

Flies emerging over a three-day period were treated as follows:

Flies emerging on day 1 were fed on (uninfected) clean rabbits for two consecutive days, and those emerging on day 2 were fed on the clean rabbits only once. Flies emerging on day 3 were not fed on clean rabbits, but were fed along with others (those which emerged on days 1 and 2) on mice infected with *T. (T.) brucei*. After the infective blood meal, these flies were not fed throughout the experimental period. Almost equal numbers of the various groups of flies were examined for trypanosome infection on days 1, 2, and 3. The results of this experiment are expressed in Tables 1 and 2 and Figure 1.

Table 1. Infection rates of *T. (T.) brucei* in newly emerged *G. morsitans* fed on a rabbit infected with *T. (T.) brucei*

	1st Blood meal Infective	
Interval after Infective meal (hrs)	*Number of Flies Examined*	*% Infection Rates*
24	35	88.6
48	44	84.1
72	65	72.3

RESULTS

It may be seen from Tables 1 and 2 and Figure 1 that the source (rabbit or mouse) of infective blood meal did not influence *T. (T.) brucei* infection rates in young *G. morsitans* fed for the first time. However, flies which had been exposed to a previous blood meal before the infective feed were not as good hosts of *T. (T.) brucei* as the newly emerged ones. It is also important to note that the vectorial capacity of these flies decreased with time. It seems therefore that two forces were acting against the survival of ingested trypanosomes; first, the ingestion of a previous blood meal before the infective feed created an environment which was hostile to trypanosomes; second, it appears that the flies produced some toxic agent (trypanocidal) whose effect on trypanosomes increased with time.

It is possible that flies that had taken a previous clean blood meal ingested smaller quantities of blood and therefore fewer trypanosomes when exposed to a second or third meal (the concentration of trypanosomes is directly proportional to the volume of blood). This could account for the poor vectorial capacity of such flies. To test this hypothesis, an experiment was carried out to determine how much blood is ingested during the first three days. Newly emerged *G. morsitans* were weighed and then fed within 24 hours after eclosion. Flies that had engorged were weighed again one hour after feeding. These flies were fed again 24 hours later and weighed one hour after feeding. The same procedure was repeated the following day.

Table 2. Infection rates of *T. (T.) brucei* in young *G. morsitans*. The flies were fed on heavily parasitaemic mice either at their first, second or third meal

	1st Blood Meal Infective		*2nd Blood Meal Infective*		*3rd Blood Meal Infective*	
Interval after Infective Meal (hours)	*Flies Examined*	*% Infection Rates*	*Flies Examined*	*% Infection Rates*	*Flies Examined*	*% Infection Rates*
24	28	96.4	31	93.5	32	59.3
48	42	88.1	45	71.1	44	56.8
72	45	71.1	48	37.5	44	18.1

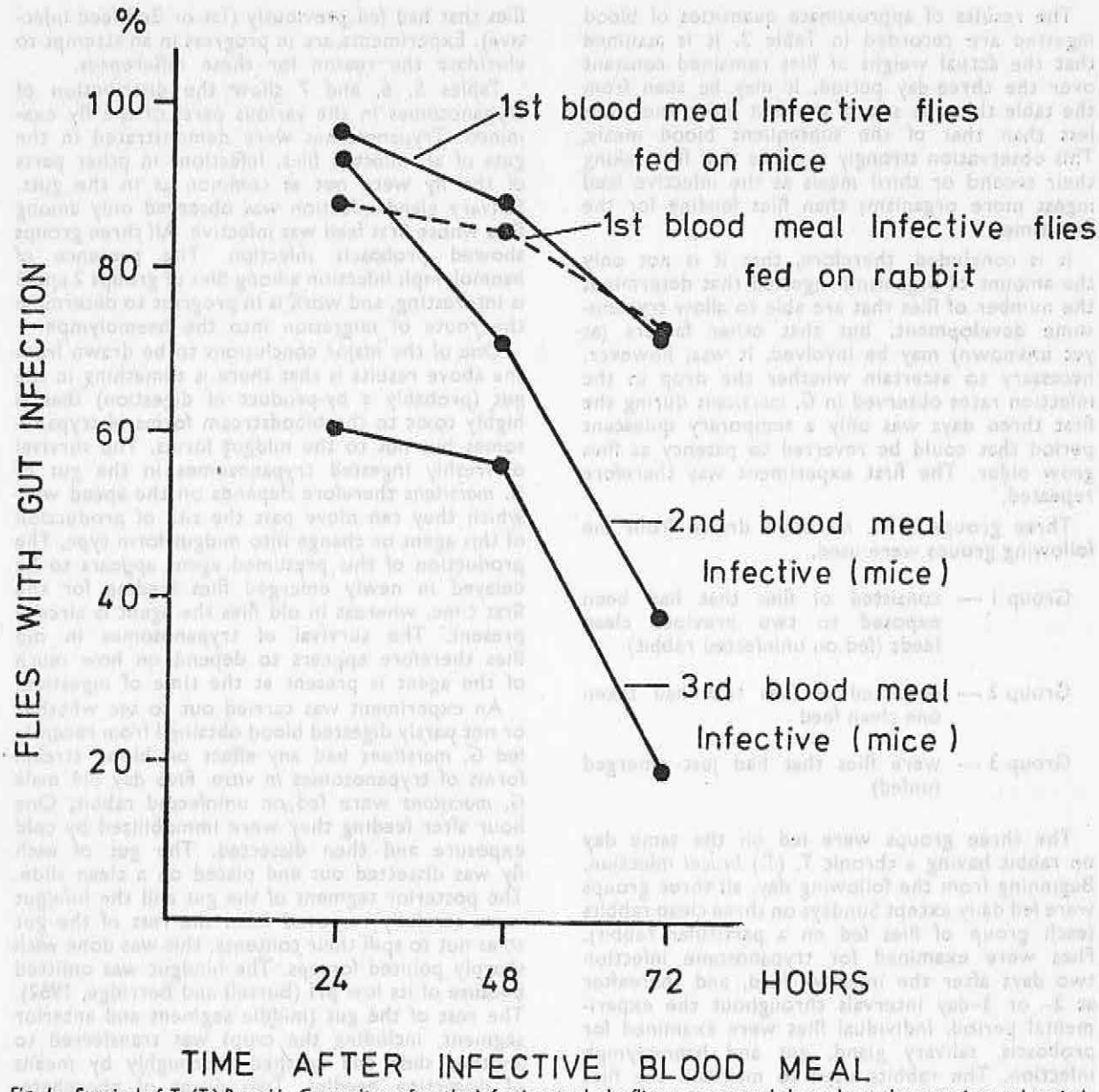

Fig. 1. Survival of *T. (T.) Brucei* in *G. morsitans* after the infective meal, the flies were starved throughout the experimental period.

Table 3. The size of blood meals taken by *G. morsitans* over a three-day period

	MALES				*FEMALES*			
	Number Weighed	*Mean Wt. (mg)*	*Weight range (mg)*	*Approx. ingested blood (mg)*	*Number Weighed*	*Mean Wt. (gm)*	*Weight range (mg)*	*Approx. ingested blood (mg)*
Unfed, newly emerged (up to 24 hours old)	52	18.4	10.3–22.9	—	41	18.1	11.3–26.6	—
1st blood meal (weighed one hour after feeding)	46	23.6	14.6–33.1	5.2	33	32.2	19.8–48.6	14.1
2nd blood meal (one hour after feeding)	34	31.1	21.2–45.8	12.7	19*	35.3	24.2–52.7	17.2
3rd blood meal (one hour after feeding)	34	38.8	24.5–58.3	20.4	29	37.1	28.6–52.7	19.0

*Power failure before all flies were weighed.

The results of approximate quantities of blood ingested are recorded in Table 3. It is assumed that the actual weight of flies remained constant over the three-day period. It may be seen from the table that the size of the first blood meal was less than that of the subsequent blood meals. This observation strongly suggests that flies taking their second or third meals as the infective feed ingest more organisms than flies feeding for the first time.

It is concluded, therefore, that it is not only the amount of organisms ingested that determines the number of flies that are able to allow trypanosome development, but that other factors (as yet unknown) may be involved. It was, however, necessary to ascertain whether the drop in the infection rates observed in *G. morsitans* during the first three days was only a temporary quiescent period that could be reverted to patency as flies grow older. The first experiment was therefore repeated.

Three groups of *G. morsitans* drawn from the following groups were used.

Group 1 — consisted of flies that had been exposed to two previous clean feeds (fed on uninfected rabbit)

Group 2 — consisted of flies that had taken one clean feed

Group 3 — were flies that had just emerged (unfed)

The three groups were fed on the same day on rabbit having a chronic *T.* (*T.*) *brucei* infection. Beginning from the following day, all three groups were fed daily except Sundays on three clean rabbits (each group of flies fed on a particular rabbit). Flies were examined for trypanosome infection two days after the infective feed, and thereafter at 2– or 3–day intervals throughout the experimental period. Individual flies were examined for proboscis, salivary gland, gut and haemolymph infection. The rabbits used to maintain the flies were examined every other day for trypanosome infection. As soon as one of these rabbits became infected it was destroyed and replaced with a clean rabbit. The rabbit used to maintain group 3 flies (flies whose first feed was infected) was found infected after 14 days. The other two rabbits were not parasitaemic when the experiment ended.

It may be seen from Table 4 that infection rates in flies examined two days after infected feed were high for all groups of flies. However, the incidence of infection dropped sharply during the next three to four days. From then onwards, infection rates did not rise very much beyond 20%. This observation is in line with the earlier findings that *T.* (*T.*) *brucei* infection rates in young flies decreased rapidly during the first three days.

No infected flies were observed after 12 days among flies that had been exposed to a previous blood meal before the infecting feed. But of the flies whose first feed was infective, infection was evident even at the completion of this experiment. In general, infection rates in these flies (first feed infective) were higher than in the flies that had fed previously (1st or 2nd feed infective). Experiments are in progress in an attempt to elucidate the reason for these differences.

Tables 5, 6, and 7 show the distribution of trypanosomes in the various parts of the fly examined. Trypanosomes were demonstrated in the guts of all infected flies. Infections in other parts of the fly were not as common as in the guts. Salivary gland infection was observed only among flies whose first feed was infective. All three groups showed proboscis infection. The presence of haemolymph infection among flies of groups 2 and 3 is interesting, and work is in progress to determine the route of migration into the haemolymph.

One of the major conclusions to be drawn from the above results is that there is something in the gut (probably a by-product of digestion) that is highly toxic to the bloodstream forms of trypanosomes but not to the midgut forms. The survival of freshly ingested trypanosomes in the gut of *G. morsitans* therefore depends on the speed with which they can move past the site of production of this agent or change into midgut-form type. The production of this presumed agent appears to be delayed in newly emerged flies feeding for the first time, whereas in old flies the agent is already present. The survival of trypanosomes in old flies therefore appears to depend on how much of the agent is present at the time of ingestion.

An experiment was carried out to see whether or not partly digested blood obtained from recently fed *G. morsitans* had any effect on blood stream forms of trypanosomes *in vitro*. Five day old male *G. morsitans* were fed on uninfected rabbit. One hour after feeding they were immobilized by cold exposure and then dissected. The gut of each fly was dissected out and placed on a clean slide. The posterior segment of the gut and the hindgut were carefully removed from the rest of the gut so as not to spill their contents: this was done with sharply pointed forceps. The hindgut was omitted because of its low pH (Bursell and Berridge, 1962). The rest of the gut (middle segment and anterior segment, including the crop) was transferred to another slide and crushed thoroughly by means of dissecting needles. Two drops of phosphate-buffered saline (PBS) pH 7.2 were added to the contents and mixed well. Four drops of blood freshly drawn from a tail of a mouse heavily infected with *T.* (*T.*) *brucei* were added to the mixture of the gut contents and PBS. The mixture was then incubated in a humid chamber at room temperature for two hours. For control, PBS was used instead of the gut contents. After incubation thin blood smears of the mixture were prepared, dried, and stained with 10% Giemsa's stain for 40 minutes.

Slides prepared from control samples showed normal trypanosomes, whereas slides prepared from trypanosomes incubated with gut contents showed many variations in trypanosomes behaviour. Many organisms divided into multinucleate forms; other dividing forms failed to separate. In general, many organisms tended to stick together. In extreme cases they stuck together in a rosette form. The significance of this rosette formation is not clear, though it is tempting to associate this crowding phenomenon with the effect of interactions between trypanosomes and the tsetse fly gut contents.

Table 4. *T.* (*T.*) *brucei* infections in the gut of *G. morsitans* of various age groups. The flies were exposed to one infective meal and maintained on clean rabbits

	1st Meal infective		*2nd Meal infective*			*3rd Meal infective*		
Days after infective meal	*Flies examined*	*% infection rates*	*Days after infective meal*	*Flies examined*	*Days after infection rates*	*Flies infective meal*	*% examined*	*infection rates*
2	13	100	2	17	64.7	2	17	88.2
5	10	20	6	21	9.5	5	17	11.7
8	13	15.4	8	19	5.3	9	18	0.0
11	17	6.0	11	15	0.0	12	19	15.7
14	13	0.0	14	15	0.0	16	18	0.0
16	14	21.5	16	21	0.0	17	20	0.0
19	11	18.1	18	16	0.0	20	19	0.0
21	10	0.0	20	20	0.0	22	16	0.0
23	12	0.0	—	—	—	—	—	—
26	9	22.2	—	—	—	—	—	—
Total	112	22.3	—	144	9.7	—	144	13.9

Table 5. *T.* (*T.*) *brucei* infections in *G. morsitans*. Newly emerged flies were first fed on infected rabbit and then maintained on clean rabbit.

Days after infected feed	*Number of flies examined*	*% infected*	*Prevalence of trypanosome infection in these organs*			
			Proboscis	*Salivary glands*	*Entire gut*	*Haemolymph*
2	13	100	—	—	13	—
5	10	20	—	—	2	—
8	13	15.4	—	—	2	—
11	17	6.0	—	—	1	—
14	13	—	—	—	—	—
16	14	21.5	1	—	3	1
19	11	18.1	2	2	2	—
21	10	—	—	—	—	—
23	12	—	—	—	—	—
26	9	22.2	—	—	2	—
Total	112	22.3	3	2	25	1

Table 6. *T.* (*T.*) *brucei* infection in *G. morsitans*. The flies were first fed on clean rabbit. Their second feed was on infected rabbit, after which they were maintained on clean rabbit

Days after infected feed	*Number of flies examined*	*% infected*	*Prevalence of trypanosome infection in these organs*			
			Proboscis	*Salivary glands*	*Entire gut*	*Haemolymph*
2	17	64.7	—	—	11	1
6	21	9.5	—	—	2	—
8	19	5.3	1	—	1	—
11	15	—	—	—	—	—
14	15	—	—	—	—	—
16	21	—	—	—	—	—
18	16	—	—	—	—	—
20	20	—	—	—	—	—
Total	144	9.7	1	—	14	1

Table 7. *T. (T.) brucei* infections in *G. morsitans*. The flies were exposed to two previous clean feeds before they fed an infected rabbit

Days after infected feed	Number of flies examined	% infected	Prevalence of trypanosome infection in these organs			
			Proboscis	Salivary glands	Entire gut	Haemolymph
2	17	88.2	—	—	15	—
5	17	11.7	—	—	2	—
9	18	—	—	—	—	—
12	19	15.7	1	—	3	—
16	18	—	—	—	—	—
17	20	—	—	—	—	—
20	19	—	—	—	—	—
22	16	—	—	—	—	—
Total	144	13.9	1	—	20	—

Location of site of development of T. (T.) brucei *in the gut of* G. morsitans

INTRODUCTION

It was observed in the ICIPE Annual Report for 1973 that there was a progressive migration of trypanosomes from the crop, anterior, middle, and posterior segments of the midgut and the hindgut. By the time the trypanosomes had reached the hindgut, they had all changed morphologically into typical midgut forms, and this happened within two days. The midgut forms could be seen all along the different segments of the midgut and hindgut. Using labelled trypanosomes, attempts have been made to see whether or not a clearer picture of trypanosome movement within *Glossina* could be obtained.

MATERIALS AND METHODS

The source of trypanosomes used to inoculate mice was stabilate materials of *T. (T.) brucei* EATRO 1587, whose history has been outlined in the 1973 ICIPE Annual Report.

The mice used were Swiss White TO strain, bred at the ICIPE animal house. Both sexes were used at the age 4–8 weeks.

500 µl of (6 3H) thymidine (Radiochemical Centre, Amersham, England) sp. act. 20,000–30,000 (Ci/mmol) was added to 10 ml of heavily infected defibrinated mouse blood obtained from mice inoculated with *T. (T.) brucei* seven days earlier. The infected blood-thymidine mixture was incubated at 37°C for one hour after which the blood cells were separated from trypanosomes by centrifugation. The plasma obtained was further centrifuged for 5 minutes at 1000 r.p.m. (International Clinical Centrifuge) to separate trypanosomes from plasma. The plasma was discarded. The sediment (trypanosomes) was washed six times in Tris-Buffer, balanced salts solution, pH, 7.4. After the last washing trypanosomes were resuspended in defibrinated, uninfected mouse blood. Using an artificial membrane (parafilm) feeding method, newly emerged *G. morsitans* were allowed to feed on the trypanosome suspension.

Flies that fed were separated into two groups. One group was killed 3 hours and the other killed 24 hours after feeding. Their guts were isolated and solubilized in soluene T.M. 100 (quarternary ammonium hydroxide) overnight. The rest of the carcasses was also solubilized in soluene. Scintillation fluid (ppo– 7 gms, m_2POPOP—0.6 gm, Naphthalene 150 gm, isopropyl alcohol, 300 ml, Toluene to make 100 ml) was then added to the digested tissues. Radio-activity in these samples was checked, using a liquid scintillation counter.

RESULTS

There was almost no radioactivity detected in the guts of flies dissected 24 hours after feeding, whereas high activity was detected in their carcasses. On the other hand, flies examined 3 hours after feeding showed activity both in the gut and in the carcass. There was, however, more activity in the guts than in the carcasses.

Table 8. Detection of radioactivity in *G. morsitans* fed on labelled trypanosomes

Interval After Feeding	Counts/Minute	
	Gut	Carcass
3 hrs	675	259
	572	249
	824	332
24 hrs	139	4944
	129	3765
	145	6178
	144	3120

These results indicate that either the labelled organisms were being disintegrated as a result of digestion and therefore being absorbed through the haemolymph, or the organisms were not being washed thoroughly. The two possibilities are being investigated.

ISOLATION MECHANISMS

(Glossina pallidipes)

INTRODUCTION

Tsetse flies occur in widely diverging habitats. The assessment of the degree of isolation between discrete populations in varying habitats, together with analysis of the relevant isolation mechanisms, are the general objectives of this project.

The practical value of a basic knowledge of the mechanisms which maintain the isolation of different populations within a species cannot be overstressed—particularly in this age of attempted control by sterile-male release. Where a species is distributed over vast areas of Africa with no regard for international boundaries, the possibility of simultaneouse control throughout is most unlikely. A more promising approach appears to be to seek out the isolated populations, define their boundaries, determine and understand the mechanisms that control this isolation and then apply control measures—isolated population by isolated population—in the knowledge that the potential for success would be much enhanced with such reduced areas, and that the likelihood of repopulation from adjacent populations would be greatly reduced to the now-defined isolation barriers.

Elucidation of reproductive compatibilities both at the species as well as at the population level is also worthwhile, since genetic incompatibilities may be useful for tsetse control. Releases of alien types to control tsetse populations were seriously considered by Vanderplank (1947), when studying the closely related species *G. morsitans* and *G. swynnertoni*. After the regrettable cessation of this research by Vanderplank it was Curtis who reintroduced explorations in this field by studying hybrid sterility between subspecies of *G. morsitans*. His recent work (Curtis, 1972) stresses the potential advantages of this approach as compared with other genetic techniques.

Glossina pallidipes has been selected as the first tsetse to be studied in this project, primarily because of its economic importance, its wide distribution throughout East Africa and the remarkably varied habitats in which it survives.

It cannot be concealed that with this choice the performance of the programme will encounter very serious difficulties. Background knowledge on this species is limited. The fact, for instance, that the preferred breeding sites of *G. pallidipes* are not known, places an immediate restriction on advanced field studies. Laboratory studies on genetic affinities between populations (which implies a quantification of eventual hybrid sterility and/or hybrid breakdown) cannot be initiated because it is not yet possible to maintain *G. pallidipes* colonies in insectaries. In spite of Rogers' (1971) work on *pallidipes*-rearing the requirements for rearing this species are still problematic. As compared to *G. morsitans* and *G. austeni*, the fecundity of *G. pallidipes* is discouragingly low. It is emphasised however that should the ICIPE succeed in culturing *G. pallidipes* successfully, the benefits will not be limited to the Isolation Mechanism Project alone, since the availability of these flies to other scientists will permit in-depth studies currently oriented solely on *G. morsitans*.

The principal objective in the first phase of the Project is to study in the field a number of characteristics of *G. pallidipes*, such as resting places, breeding sites and activity periods. These field studies will be done in two selected areas. It is hoped that these investigations will provide essential information with respect to the requirements for rearing *G. pallidipes* in the laboratory.

The second objective is to maintain *G. pallidipes* populations of the selected habitats as self-supporting strains in the laboratory, both at the ICIPE as well as in the laboratory for Experimental Entomology in Amsterdam. Interstrain fertility will be studied in Nairobi, while other characteristics, such as the usefulness of iso-enzyme patterns (zymograms) as markers for the population under study will be investigated at Amsterdam. As soon as the electrophoretic techniques have been perfected, this work can be transferred as a routine to the ICIPE in Nairobi.

The third phase in the project will deal with releases of flies originating from alien habitats into the study areas. Subsequently examination of zymograms of recaptured flies will provide information on the extent and efficacy of the possible isolation barriers between the various populations.

Discussion of field studies

In February 1974 the field studies commenced with surveys of potential target areas of *G. pallidipes*. Of these, two areas have been selected: Nkruman, the area between the Ewaso-Ngyro-river (west of Magadi) and the Loita Hills, and the Coastal area. In the coastal area several places have been surveyed very thoroughly in a search for a suitable area. During the last three months studies have been concentrated mainly in two places: the Gogoni Forest, about 50 km. south of Mombasa and Mwalewa Forest, near Lunga Lunga at the Tanzanian border. Future work at the coast will be carried out only in the latter area.

Present research is focusing on the following aspects: trapcatching; catching in and on the car (a one-ton pick-up Landrover with green canvas covering)—using the car as standing object; determining activity times; and searching for breeding sites and resting places. Two types of traps are used: the Langridge Box Screen (LBS) and the Awning Screen-skirt (AS-S) Moloo, in press). Initial results from the three studied areas are summarized in table 1. Though total trapping days are relatively few, two points might be worth mentioning. Firstly, the ratio of males to females caught differs in the two study areas. The traps placed in Nkruman catch a much lower proportion (21.4%) of females than do the same traps in the coastal area (approximately 56%).

Table 1. Comparative catches of *G. pallidipes* in two types of traps Langridge Box Screen (LBS) and Awning Screen Skirt (AS-S) in Nkruman and two coastal areas

	Nkruman					*Malewa-Forest*					*Gogoni-Forest*				
	♀	♂	*Tot*	%♀	*Trapping days*	♀	♂	*Tot*	%♀	*Trapping days*	♀	♂	*Tot*	%♀	*Trapping days*
LBS	45	158	203	22.1	4	333	230	563	59.1	9	155	106	261	59.3	6
ASS	66	259	325	20.3	4	300	248	548	54.7	9	119	117	236	50.4	6
Totals	111	417	528	21.4	8	633	478	1111	56.9	18	274	223	497	55.1	12

No other worker has reported catches containing less than 70% females. So whilst the proportion of females caught in traps in the coastal areas are rather low, the results in Nkruman are remarkable and might well represent behavioural (genetic) divergence. Secondly, there are no striking differences between the total catches of the two types of traps as was reported by Moloo (in press). He caught approximately three times more flies in the ASS than in the LBS.

The results of catching by hand inside and outside the car or just around the car are summarized in table 2. The catches from Gogoni Forest are too low (less than 100) to be included. In contrast to the trap catches, these results show little difference in the proportion of females in the two areas. This makes differences in the proportion of females in the trapcatches even more striking.

Table 2. Catches of *G. pallidipes* by hand inside, outside and around the car in Nkruman and Mwalewa-Forest (coastal area).

	♀	♂	*Tot*	%♀
Nkruman	334	389	723	46.2
Mwalewa-Forest	158	210	368	42.9

The study of activity times, resting sites and breeding places are still in the initial phase. Thus far 37 living pupae have been found in Nkruman in a typical "floorsites" breeding place. The apparently small collection is nevertheless encouraging when compared to the finds of other workers (Le Roux, pers. comm. and others). Of the 37 pupae, 30 have emerged in the laboratory to give 13 males and 17 females. These have now been mated and the subsequent reproductive cycles will be followed closely.

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ISOENZYME RESEARCH

INTRODUCTION

The increasing application of isoenzymes as tracers in population studies of insects has prompted us to establish an investigation on various isoenzymes of tsetse flies. This work was initiated by J. van Etten and executed in collaboration with Dr. L. P. S. van der Geest at the Laboratory of Experimental Entomology in Amsterdam. The research is directed to isoenzyme-polymorphism. Five different isoenzymes are so far being looked into namely Leucine Amino Peptidase (LAP); Lactate Dehydrogenase (LAD); Malate Dehydrogenase (MDH) and the Esterases. The effects of age, sex, feeding and freezing are also being checked in order to obtain base line data for future applications. Three different strains of *Glossina morsitans* are used for comparative studies of their isoenzyme patterns. The strains originating from three different laboratories, namely Tanga (Tanzania); Itard (Paris) and Langford (Bristol).

MATERIALS AND METHODS

Isoenzyme patterns were determined by means of the horizontal starch gel electrophoresis method, using a discontinuous tris-borate buffer system (Poulik, 1957). Electrophoresis was carried out at 0°–2°C with a current of 1–1,5 mA/am. Using the Brown Mini-Potter, individual flies were homogenized in 0.2 ml of the gel buffer. The homogenates were absorbed in small (6×5 cm) pieces of Whatmann 3 MM chromatography papers, and applied into a slit cut across the gel.

After electrophoresis (4–4½ hrs), the gels were sliced lengthwise in 3-parts and incubated at 37°C in the appropriate staining solutions till the bands appeared. After staining, the gels were thoroughly rinsed in water and fixed in methanol.

Photos of the gels were taken using a Polaroid camera.

RESULTS

1. Leucine Amino Peptidase

The zymograms obtained from teneral flies of both sexes shortly after ecdysis show three distinct zones of (LAP)-activity. These are indicated as fast (F); intermediate (M) and slow (S) (Fig. 1): In most cases the three mobility zones (F), (M) and (S), each show only a single band in these flies. In the (M) and (S) zones, variants are found in older flies, as indicated by double bands. However, the frequency of these bands is very low. The (F) zone consists usually of a single band, which in most cases is very faint.

There are no differences in the zymogram patterns with respect to sexes. Flies which have not had a blood meal 24 hours prior to the experiments also give similar bands to those that have not fed for 2 days. Flies starved beyond this period produce weak bands in the three zones. Three mobility zones similar to those of teneral flies are present in old flies (10–30 days) (Fig. 1). The activity of (S) zone is lower in old flies compared with that observed in young flies. In contrast to flies, an extremely high activity in the (S) zone is detected in pupae.

2. Lactate Dehydrogenase (LDH)

Lactate Dehydrogenase activity is not detected in pupae, and teneral flies. Flies analysed 24 to 36 hours after a blood meal show a single zone of activity (Fig. 2). This zone consisting of single band, is similar to the one obtained from the host blood. Adult flies starved beyond 2 days do not show LDH-activity. There are no sex differences with regard to the bands obtained, and no variations have so far been detected.

3. Malate Dehydrogenase (MDH)

Two mobility zones (A)+(B) are obtained for the MDH-activity in pupae, teneral and old flies (Fig. 3). The (A) zone consisting of 2 bands close together show a very high MDH-activity in young pupae (1–6 days old), become weak in older pupae (28 days), and very weak in teneral flies. Zone (B) consists of 2 bands which are weak in young pupae but become intense in older pupae, teneral and adult flies. There is a remarkable increase in the rate of mobility of zones (A)+(B) with increase in age. Flies analysed 24 hours after a blood meal give a strong reaction in the (A)+(B) and an extra weak band in the (C) zone. Host blood gives bands in the (C) and (D) zones but none in the (A) and (B) zones. There are no differences in the electrophoretic patterns with regard to sex, and no variations have been detected yet.

4. Esterases

Three zones of mobility (W), (X) and (Y), are recorded in flies analysed 24 hours after a blood meal (Fig. 4). The band in zone (Y) is missing in pupae and in flies that have not fed for more than 2 days. The bands in (W) and (X) zones are weak in teneral flies. In all flies analysed, zone (W) consists of a single band, while the (X)-zone consists of a double band. No variations have been detected yet in the two zones (X) and (W). Both sexes give similar patterns. Fresh and frozen materials produced similar results in all isoenzymes under investigation.

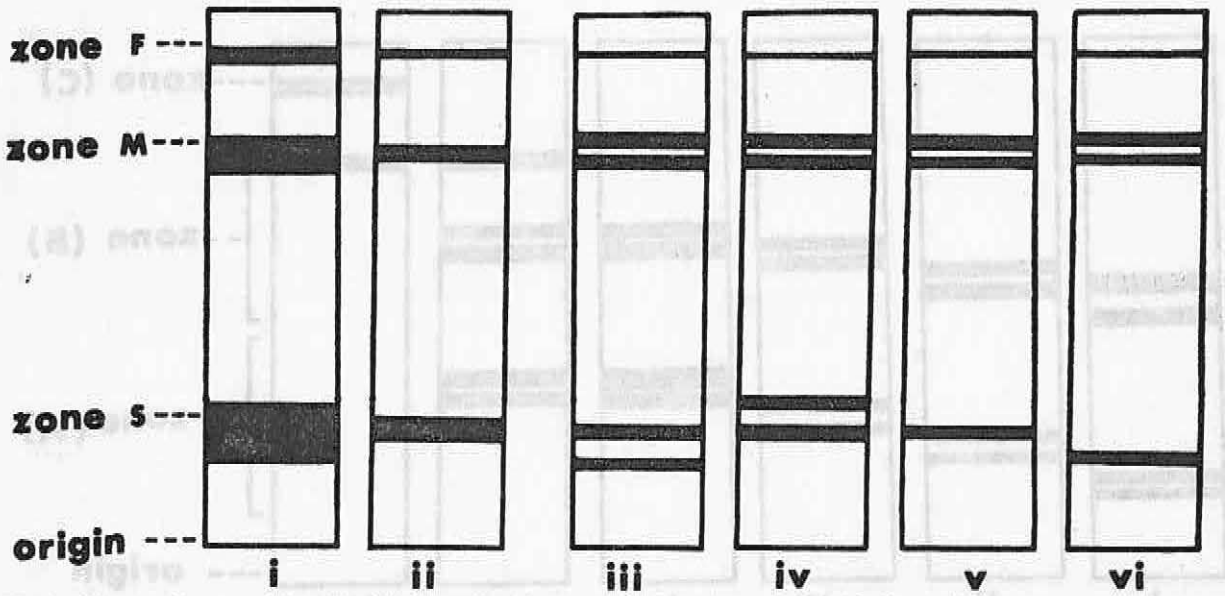

Fig. 1. Diagramatic presentation of (LAP) electrophoretic patterns in pupae, teneral and adult *G. morsitans.*

(i) pupae
(ii) teneral flies
(iii) — (vi variants of (LAP) in adult flies
a= faster
b= fast
c= intermediate
d= slow

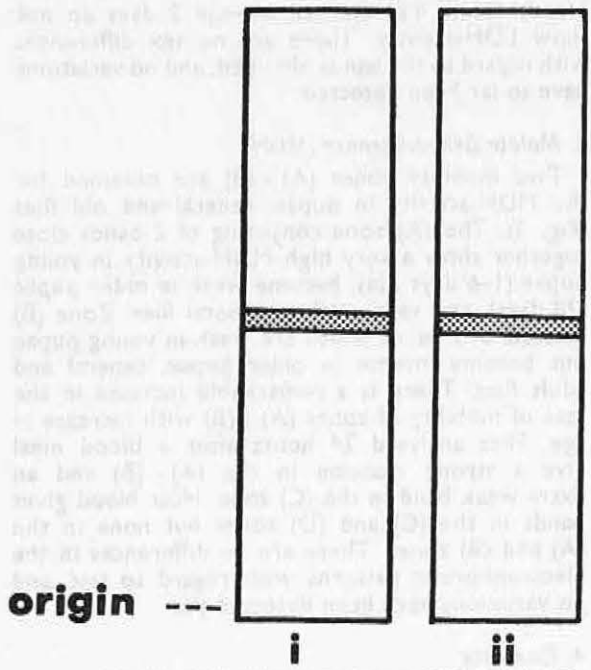

Fig. 2. Electrophoretic patterns of (LDH) in adult *G. morsitans* and rabbit's blood.
(i) adult *G. morsitans*
(ii) blood from lop-eared rabbit

DISCUSSION

In (LAP), the doubling of the (M)-band is apparently age dependent. Flies at 3–4 days after emergence show double bands in this zone while in teneral flies, only single bands are detected (Fig. 1). In zone (S), five different variants have been detected (Fig. 1). There is an indication that the proportion in which these variants occur may differ among different populations. The band in the (F) zone is usually not clear and in most cases it is missing. Due to a limited supply of flies, it has not yet been possible to interpret the data with respect to the question whether (F) and (S) are under control of distinct gene loci.

The absence of (LDH) in pupae and teneral flies cannot be interpreted as an absolute lack of this enzyme, but may indicate levels below the limit of detectability of the test conditions used. The presence of bands in fed flies, but lacking in flies that have not been fed has made the study of this isoenzyme difficult. The situation has been further complicated by the fact that flies fed 24 hours prior to analysis give bands that are similar to those of the host blood.

In (MDH) the (C)-zone-band which is detected in flies fed 24 hours prior to analysis is apparently dependent on a blood meal. Since a similar band is detected in the host blood, it is very likely that the band is a result of undigested enzymes of host blood. A similar account can be given for the (Y) band detected in the Esterases.

The high activity of (LAP), (MDH) and Esterases detected in pupae can be interpreted in terms of the rapid developmental processes that are going on during this stage With regard to the effects of feeding, age and sex the results indicate that the most suitable animals to work with are adult flies fed 48 hours prior to analysis. Presently, it is too early to draw any conclusions with regard to variants of isoenzyme polymorphisms in the 3-strains. The present results show that both fresh and frozen materials give equally good results. This factor will enable in future the analysis of frozen material collected from different populations in the field.

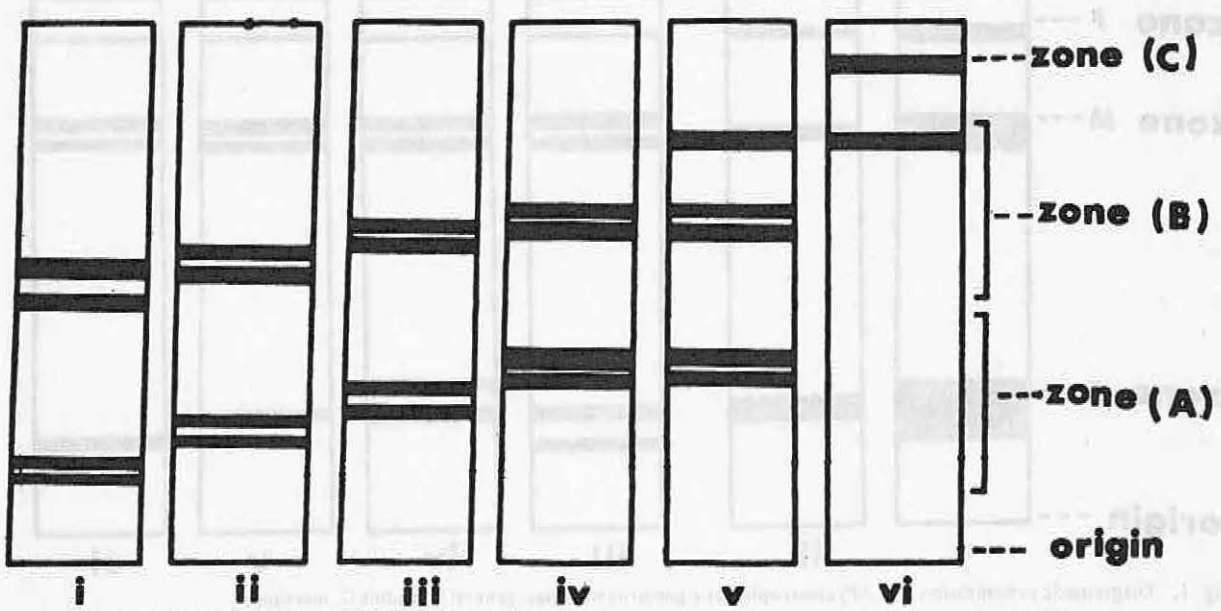

Fig. 3. Electrophoretic patterns of (MDH) in *G. morsitans*
(i) young pupae 1—5 days old
(ii) pupae 10—15 days old
(iii) pupae 26—28 days old and newly emerged flies
(iv) adult flies 48 hours after a blood meal
(v) adult flies 24 hours after a blood meal
(vi) rabbit's blood

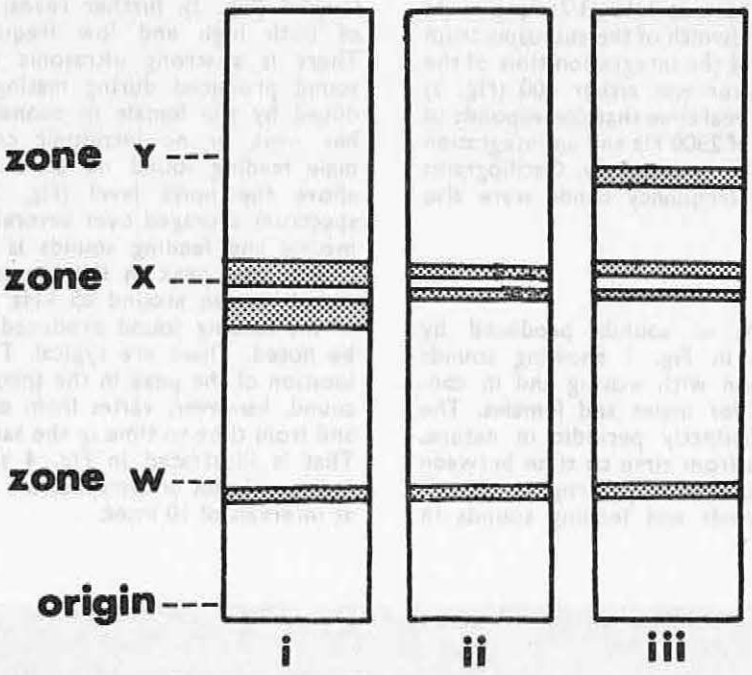

Fig. 4. Electrophoretic patterns of Esterases in *G. morsitans*
(i) pupae
(ii) adult flies 48 hours after a blood meal
(iii) adult flies 24 hours after a blood meal

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SOUND PRODUCTION
(*Glossina morsitans morsitans*)

INTRODUCTION

It is known that many insects produce various types of sounds. It is also known that several insects have auditory receptors. Sound may thus play an important role for the behaviour of insects. That such is the case has been verified experimentally in a few insects. Sound production and reception are, however, far less studied than is the role of chemical agents in communication, the effect and production of pheromones having drawn considerable attention during the past few years.

As regards the tsetse fly, the results of several studies indicate that it does not possess pheromones (Dean *et al.*, 1969; Turner, 1971). Yet it remians difficult to explain the behaviour of the tsetse fly without assuming some form of communication among individual flies. This assumption is based on, for example, the fact that individual tsetse flies appear at certain times in widely dispersed populations in the bush, and later appear as a swarm (Glasgow, 1963). It is also known that tsetse flies, despite their being very particular about selection of a feeding host, can survive a low host density. They therefore must have means on an individual basis to find a suitable host and, having found one, to communicate this information to other flies. Communication among individual tsetse flies is furthermore assumed to be necessary in order to make it possible for flies of opposite sex to find each other for mating.

It has been known for a long time that various species of tsetse flies produce sound and sound has consequently been suggested as a means of communication (Carpenter, 1924; Glover, 1967; Kartman, 1946; Kolbe, 1973; Vanderplank, 1948). So far no experimental proof has been put forth showing that sound is used by any species of the tsetse fly for communication. Previous studies have, however, only been concerned with the frequency range that is audible to man. In the present study we show that the sound produced by the tsetse fly *Glossina morsitans morsitans* has ultrasonic components. The character of the ultrasound connected with feeding is different to that connected with mating. There is moreover a consistent difference between the sounds produced by the male and the female.

METHODS

Sounds were recorded from tsetse flies under laboratory conditions at a distance of 2–3 cm using a Brüel and Kjaer ¼″ microphone (type 4135) and a microphone amplifier (type 2618). The flies were kept in small boxes covered with mosquito net. They were fed by placing the box on one of the ears of a rabbit. Magnetic tape recordings were made by a Precision Instrument tape recorder (type PI 6200) used in its direct mode at 37.5 ips. A highpass filter (cut-off 470 Hz) was inserted between the microphone amplifier and the tape recorder. The over-all frequency response of the system was 470–100.000 Hz (±3 dB). The recorded sounds were analyzed using a "Voice print" sonagraph (Fig. 2) and hybrid audiospectrum analyzer

with the tape played back at 1/10 (3.75 ips) of its original speed. The bandwidth of the audiospectrum analyzer was 250 Hz and the integration time of the subsequent lowpass filter was either 400 (Fig. 3) or 100 msec (Fig. 4). In real time that corresponds to an analysis bandwidth of 2500 Hz and an integration time of 40 and 10 msec respectively. Oscillograms of sound in different frequency bands were also made.

RESULTS

Typical oscillograms of sounds produced by tsetse flies are seen in Fig. 1 showing sounds produced in connection with mating and in connection with feeding for males and females. The sounds are always distinctly periodic in nature. The periodicity varies from time to time between about 300 and 2000 Hz. Sound spectrograms (sonograms) of mating sounds and feeding sounds in females (Fig. 2), further reveal a periodic nature of both high and low frequency components. There is a strong ultrasonic component in the sound produced during mating. The sound produced by the female in connection with feeding has weak or no intrasonic components. In the male feeding sound no ultrasound is discernible above the noise level (Fig. 2). The frequency spectrum averaged over several periods of typical mating and feeding sounds is seen in Fig. 3. A pronounced peak in the spectrum of the mating sound is seen around 65 kHz and a double peak in the feeding sound produced by the female can be noted. These are typical. The exact frequency location of the peak in the spectrum of the mating sound, however, varies from occasion to occasion and from time to time in the same sound sequence. That is illustrated in Fig. 4 showing a three-dimensional plot of the spectrum taken consecutively at intervals of 10 msec.

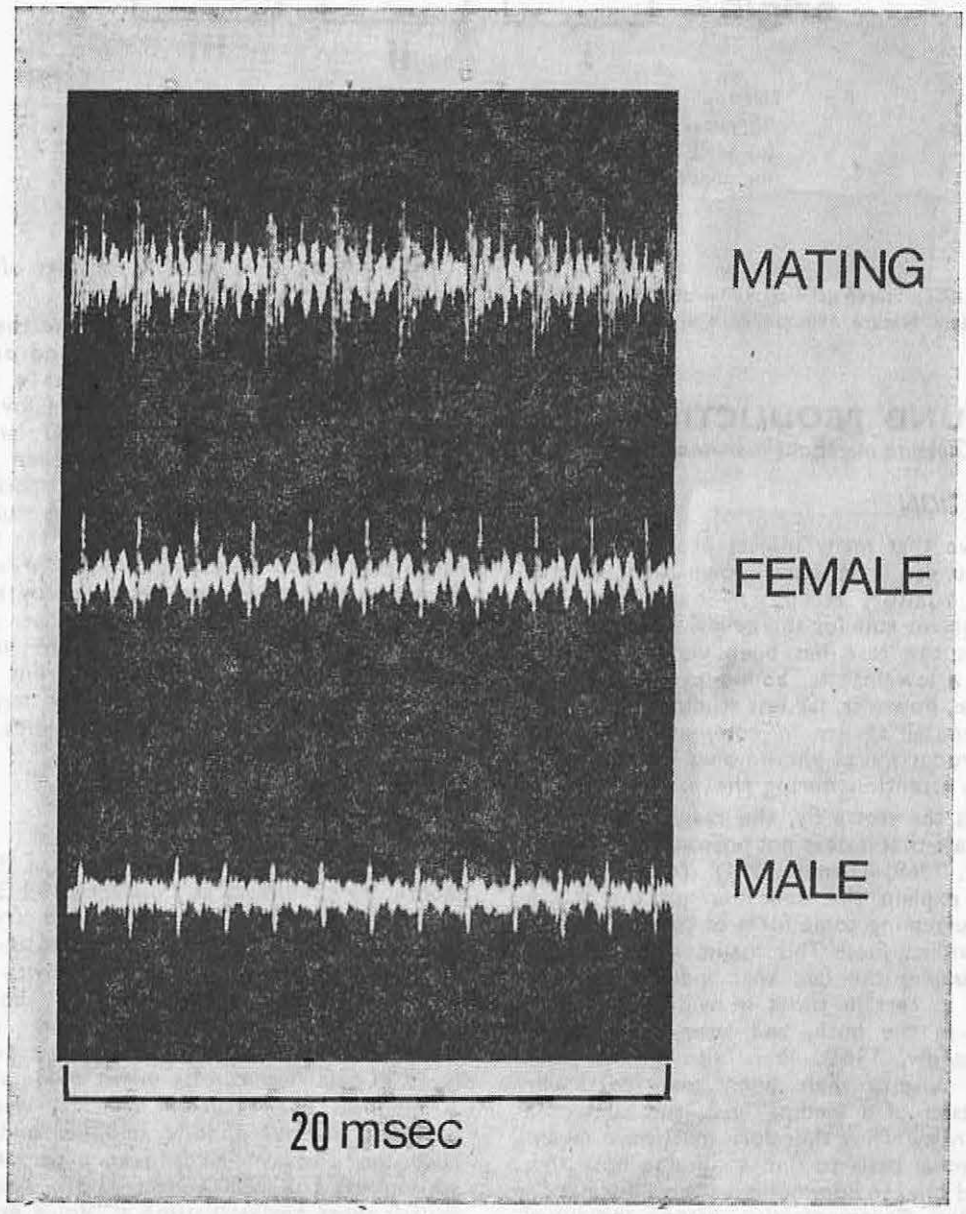

Fig. 1. **Oscillograms of typical sounds produced by Glossina morsitnas.**

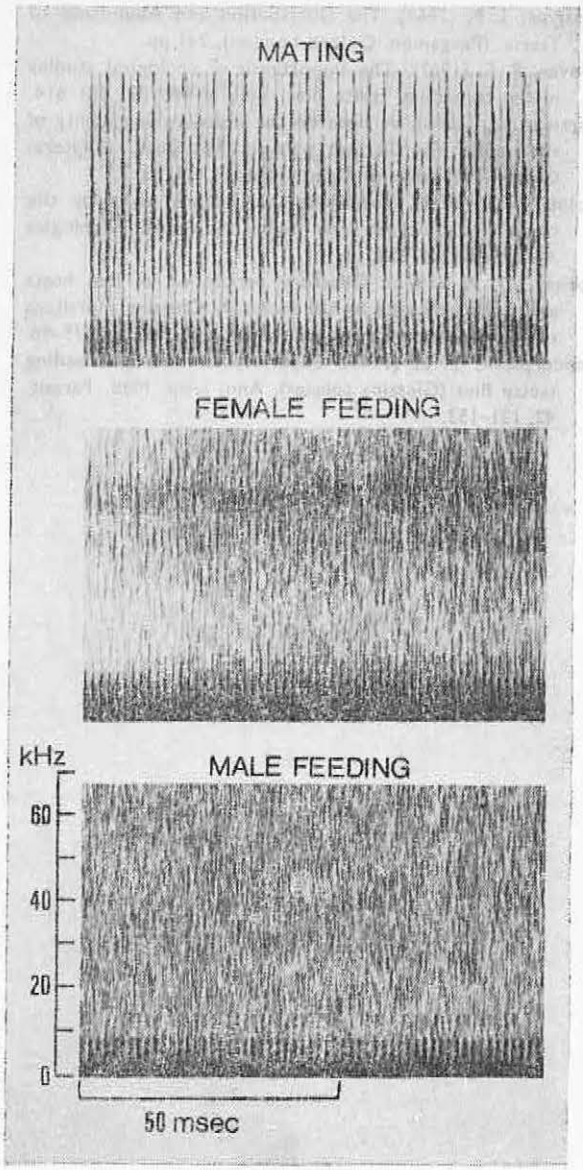

Fig. 2. Sonograms of typical sounds.

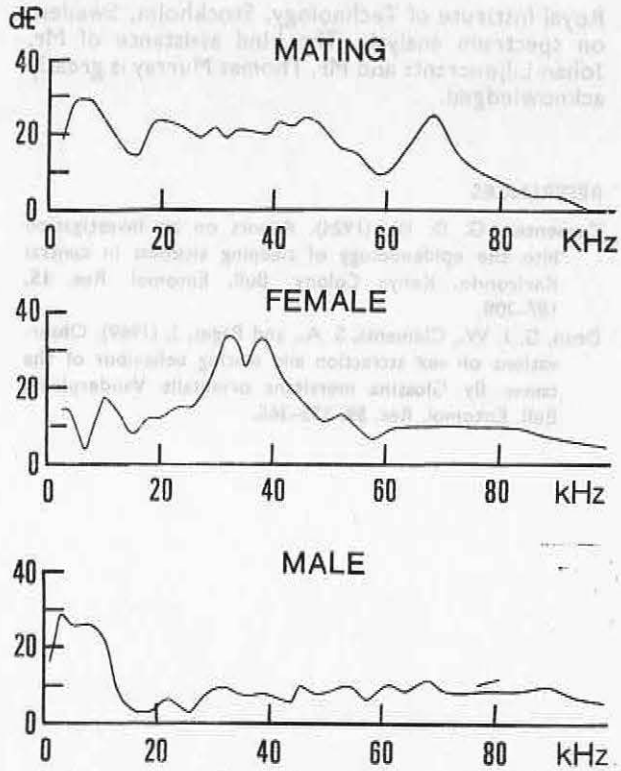

Fig. 3. Spectrograms of typical sounds. Analysis bandwidth was 250 Hz, corresponding to 2500 Hz in real time. The integration time was 40 msec real time. Zero dB corresponds approximately to 50 dB SPL.

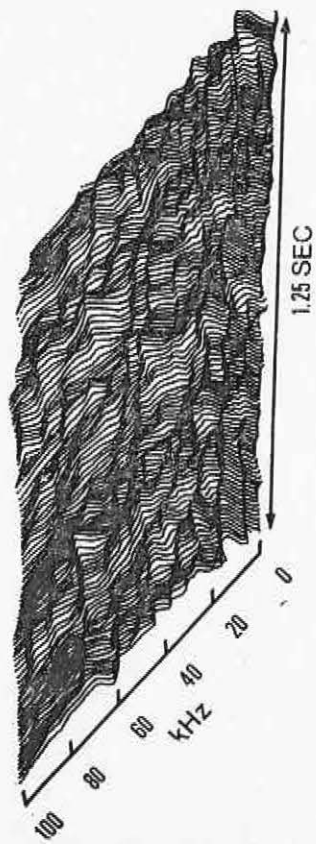

Fig. 4. Three-dimensional plot of consecutive spectra of mating sounds taken every 10 msec. Integration time was 10 msec (real time).

DISCUSSION

The results of the present study show a consistent difference in amplitude of the ultrasonic component of the sounds produced by the tsetse fly during feeding and during mating. Furthermore, the sound produced by the female differs from that produced by the male with regard to presence of ultrasonic components. The low frequency components also show some differences but these are far less prominent than the ones displayed by the high frequency components.

We hypothesize on basis hereof that sound may constitute the prerequisite for communication between tsetse flies and that the ultrasonic components of the sounds most likely carry the most important part of the information.

ACKNOWLEDGEMENTS

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10

AESTIVATION DIAPAUSE IN SOME EQUATORIAL INSECT PESTS

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FLESH FLIES
(*Sarcophagidae*)

INTRODUCTION

In the temperate regions insects commonly circumvent adverse environmental conditions by entering diapause. In this environment seasonal change in day-length plays a dominant role as an environmental token informing the insect of the advent of the adverse period. In the tropics insects are not confronted with the same seasonal environmental changes, and on the equator the potential for utilizing changes in photoperiod as a seasonal cue is minimal. This project was undertaken to investigate whether the potential for diapause exists in equatorial insects and if so, what the environmental factors causing its induction are and how mediated. Flesh flies (Sarcophagidae) have been used as the experimental animals. Weekly trap samples of insects have also been analyzed since August 1972 in order to get an over-view of insect seasonality in the East African environment.

RESULTS AND DISCUSSION

Nine different species or strains of flesh flies representing populations from Nairobi (1°S, highland), Mombasa (4°S, lowland), Addis Ababa, Ethiopia (9°N, highland) Mbeya, Tanzania (9°S, highland) have been reared in the laboratory for investigations on environmental regulation of diapause. A pupal diapause has been found in flies from Nairobi, Addis, and Mbeya; definitive experiments have not yet been completed for the Mombasa flies. Photoperiod is ineffective for diapause induction. Temperature during larval development is the important factor responsible for diapause induction. As is seen in Figure 1, at 18°C a few individuals enter diapause and increasingly more enter this state with lower temperatures. Moisture content of the larval medium also influences the induction of diapause. A 10% increase in moisture content increases the incidence of diapause by about 10%; conversely a decrease in moisture content decreases the incidence of diapause. The diapause is terminated by high temperature; diapausing pupae at 12°C will begin developing after 3 consecutive days at 25°C.

The ecological importance of diapause is still vague. Mean temperatures during August, the coldest month in Nairobi, border the threshold for diapause induction, but apparently many years can elapse without the fly actually entering diapause. Two species have been reared outside throughout the year in Nairobi and only occasional pupae were seen to enter diapause in August; none entered diapause during other times of the year. Thus it appears that whilst the potential for diapause is widerspread in the gene pool of the equatorial flies, it is infrequently used.

Neuroendocrine aspects of the equatorial diapause have been examined most extensively in *Sarcophaga inzi*. Injection of ecdysterone can terminate the diapause—thus supporting the concept that pupal diapause in *Sarcophaga* is an ecdysone-deficiency syndrome. Several small doses of ecdysterone are more effective than a single dose of the same total amount. This observation suggests that pupae are caused to enter diapause by the reduction of ecdysone production. The role of juvenile hormone in diapause termination is obscure; by itself it is ineffective, but when applied simultaneously with ecdysterone the speed at which diapause is terminated is much greater than when ecdysterone alone is applied. Topical application of a wide variety of chemicals (e.g. acetone, hexane, etc.) can also trigger the termination of diapause, but they are effective only if the pupal brain is intact. The normal mechanism for diapause termination therefore appears to require the initial activation of the brain.

Data collected from Malaise traps in the Nairobi National Park and the Olorgesaille Prehistoric Site presents an approximate picture of insect seasonality that can be correlated with environmental variables such as rainfall and temperature. This trapping was established to roughly identify seasonal periods characterized by low numbers of insects which in turn might serve to identify diapause seasons. It is appreciated that several years of data would be required to adequately assess seasonal changes in insect density and diversity. From the 20 months of data already collected it is apparent that there is a great deal of variability from year to year; the extensive drought preceding the long rains in 1974 reduced the insect density to an unprecedented low, and the peak in activity following the short rains in 1972 was not repeated in 1973. Trapping will be terminated at the low rainfall period of Olorgesaille after completion of the 2-year period, but records will continue to be kept from the trap in the Nairobi National Park.

The analysis of data collected from insect traps and laboratory experiments on environmental and endocrine regulation of diapause in equatorial flesh flies will, it is hoped, provide basic information on the mechanism employed by equatorial insects for survival during adverse periods in the tropical environment.

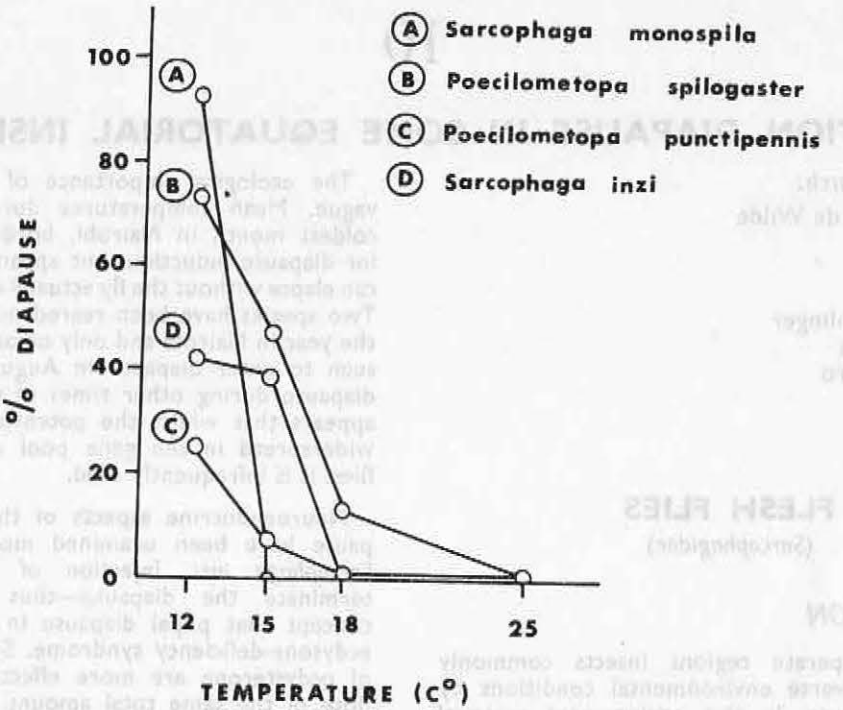

Fig. 1. The influence of temperature upon diapause in flesh flies.

MAIZE STEM-BORERS

(*Chilo argyrolepia* Hmps. and *C. zonellus* Swinhoe)

INTRODUCTION

Lepidopterous stem-borers have long been considered major international pests. Whilst the damage to maize has not been assessed, it is known that infestation of both maize and sorghum may vary between 10% and 40%. Reduction in rice harvests of 1%–10% and sugar cane sucrose content of 2–3% (below the normal 15–18% sucrose content) are not uncommon (Jepson, 1954).

Whilst considerable research has been orientated towards the active phases of stem-borers in the tropics, little attention has been paid to the aestivating stage. Considering that survival through the dry seasons between crops is probably dependant upon diapause, this research omission is remarkable. An understanding of the factors which induce and terminate aestivation might well provide the means for control without the pollution that is so prevalent in this age.

MATERIALS AND METHODS

All field material and field data are obtained from the National Agricultural Research Station in Kikambala (Coast Province). Of the 3 naturally occurring maize stem-borers, only *Chilo zonellus* Swinhoe and *C. argyrolepia* Hmps. are being studied and of these *C. zonellus* alone is reared in the Nairobi laboratories on wheat germ diet (Chatterji *et al.*, 1968).

A maize stem is recorded as infested if it is found to contain one or more living larvae.

The following environmental criteria:— temperature, relative humidity, rain, hours of bright sunshine, evaporation, growing stage and water content of the plant—are correlated with percentage diapausing larvae.

Diapause in *Chilo* occurs during the final larval instar. This condition permits survival as a larva for several months. Relatively rapid pupation or death (computed statistically) is therefore a reliable criterium for determining the absence of diapause. This study utilises two environmental conditions with this assessment:

Method 1, where the larvae are provided with wheat germ diet or pieces of fresh maize stem (Katiyar and Long, 1961; Usua, 1973) and Method 2, where the larvae are permitted to crawl into holes bored in old, completely dry maize stems without other food. Since results indicate that the environment in Method 1 is effective in breaking diapause, Method 2 in which non-aestivating larvae either die from lack of food or pupate (Fig. 1) is preferred.

Oxygen consumption is also used as an indication of diapause and is measured with a Scholander micro volumetric respirometer Model VR–300.

The juvenile hormone mimic is ZR 619 (ethyl 11-methoxy-3, 7, 11-trimethyldodeca-2, 4 diene-thiolate, Zoëcon).

RESULTS AND DISCUSSION

Ecological studies

The level of *Chilo* infestation of maize stems tends to be relatively low (15% with occasional peaks of up to 30%) in maize planted early (April/May) in the rainy season. Understandably, infestation is greater (up to 40–50%) in maize planted later (June/July) in the rains or under irrigation in the dry season (Jan/Feb).

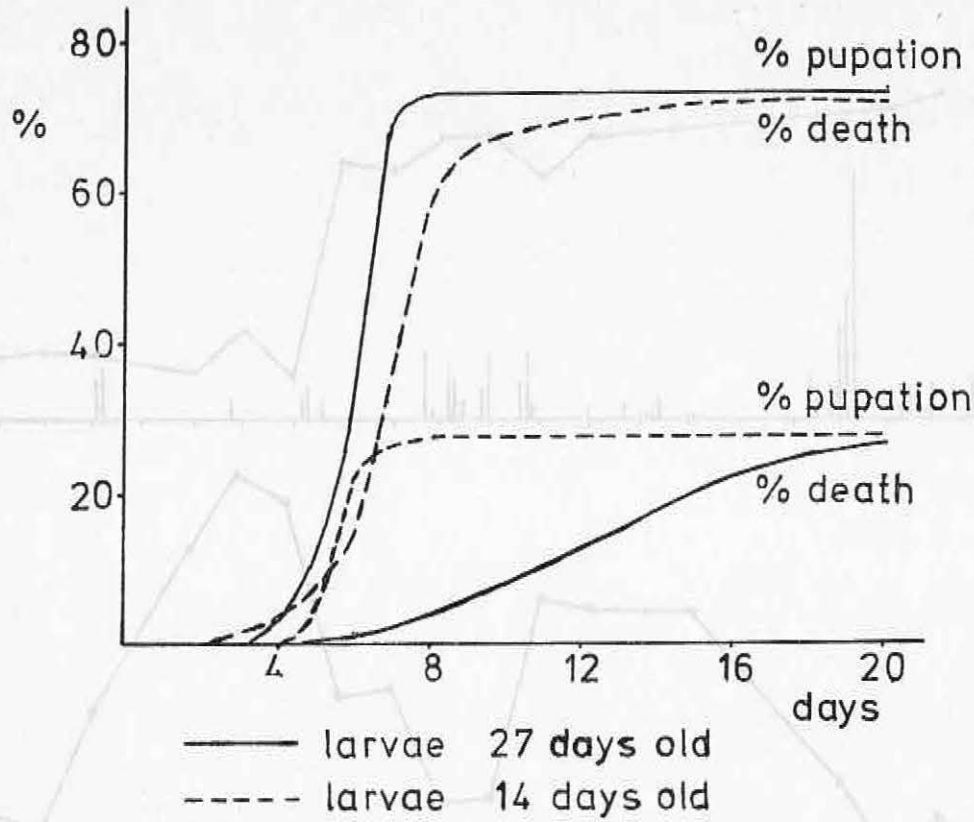

Fig. 1. Pupation and death rate of non-diapausing larvae of different age placed in pieces of dry maize stem.

One and sometimes two larval peaks may occur in a single crop. Normally young stems are more heavily infested than older plants. Infestations generally fluctuate from 0.3–0.5 larvae/stem, but an average of 1 larva/stem is not unusual. Whilst pupae may be common in young stems, they become increasingly rare as the plants dry out, and are absent in dry stems where final instar larvae arrest development by aestivation. Only on the re-occurrence of rains are pupae again found.

Earlier observations summarised in the 1973 report lead to the tentative suggestion that conditions within the host plant were the major factors influencing diapause. These investigations have been continued and the environmental factors prevailing during two peak diapause periods are noted in Fig. 2 and Table I. As can be seen, these comparative data do little to clarify the issue—many factors, both climatical and the physiological conditions of the plant, vary widely—yet in both instances aestivation peaked. The only obviously common environmental feature was lack of rain. Of interest in these data in the implication that neither the water content of the stem nor its age seem to be deciding factors in the induction of aestivation—for in the earlier peak, the stems were not yet mature and their water content was relatively high ($\sim$ 80%).

The influences of the water content of diet (reduced from 86% to 75%), glucose and casein content of diet and (Table II) temperature upon the induction of diapause were investigated in the laboratory. Although in some instances (low glucose and/or casein contents, high temperature) the larval lifespan could be prolonged significantly, it has yet to be proven whether or not these factors influence diapause itself.

Since repeatedly disturbed (for purposes of examination) diapausing larvae deposit considerable quantities of faecal pellets, the possibility that diapausing larvae require food was investigated. Results (Table III) clearly indicate that food is unnecessary since 8-month aestivating undisturbed larvae produce only half the faeces deposited in 1 week by disturbed larvae. It is therefore suggested that these faeces merely represent the amount of stem ingested for purposes of excavating suitable pupation chambers. The weights of aestivating larvae never increase as do those of non-aestivating larvae, in fact they usually decline slightly whether or not diet is provided. This supports the suggestion that aestivating larvae do not normally feed.

Morphological studies

Field studies indicate that non-aestivating larvae gradually lose their pinacular spots as they increasingly enter the diapause condition. In totally dry maize, all larvae are immaculate (without spots).

Physiological studies

Oxygen consumption and water content were studied in relation to non-diapausing and diapausing *Chilo* larvae. There is a marked drop in water content and decrease in oxygen consumption with diapause. These findings are comparable with those of other authors on different insect species (Table IV).

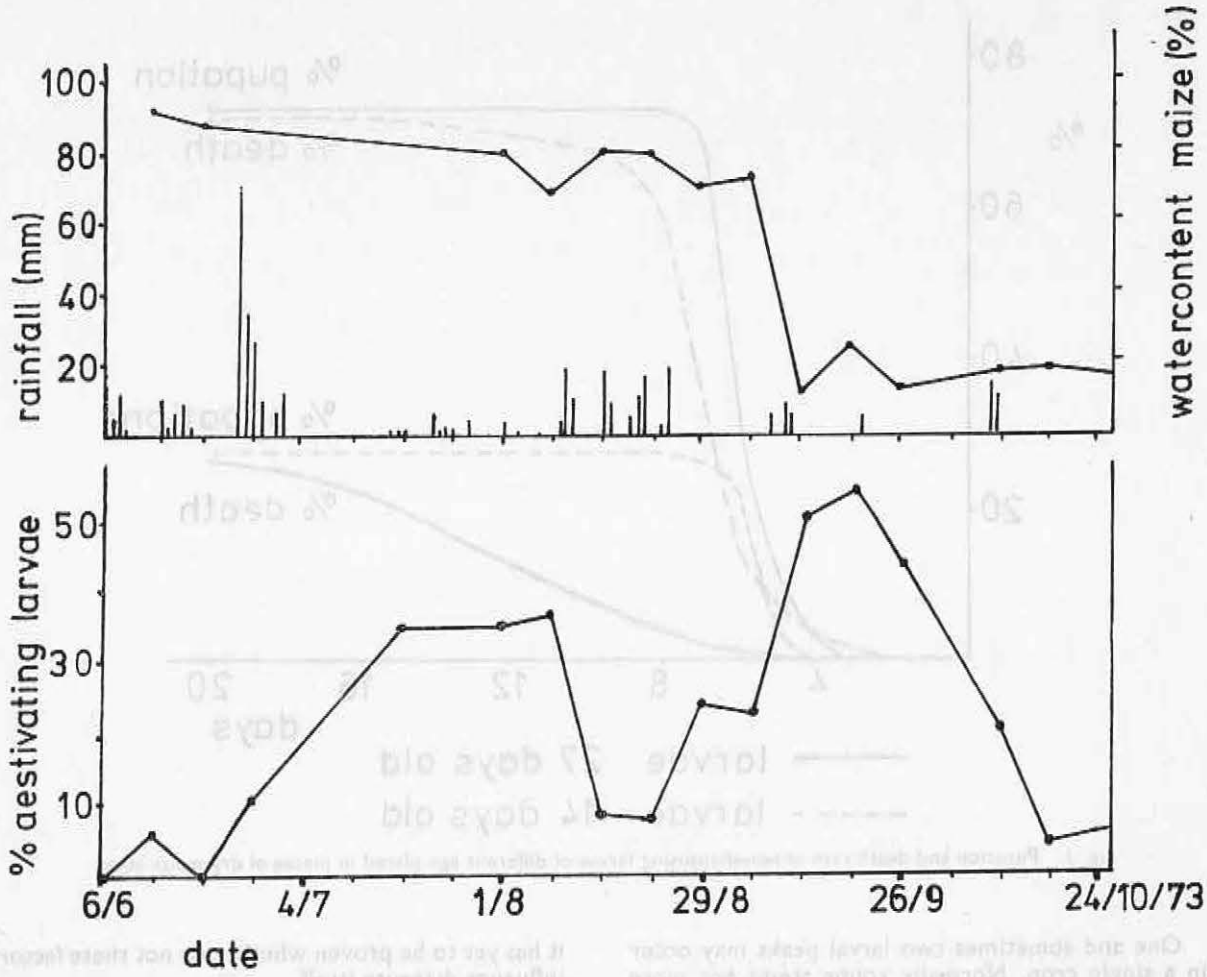

Fig. 2. Percentage of aestivating larvae of *Chilo zonellus* and *Chilo argyrolenia* in relation to rainfall and watercontent of the maize-plant in an experimental field at Kikambala, Coast Province, Kenya.

Table 1. Environmental, numerical, morphological and physiological differences of two groups of aestivating Chilo larvae, obtained from maize stems at different times of the year in one experimental field at Kikambala, Coast Province, Kenya

	Period during which aestivating larvae were obtained	
	18.7–8.8.73	12.9–26.9.73
Climatic conditions two weeks before and during the period that aestivating larvae were obtained:		
minimum temp.	~ 20°C	~ 22°C
maximum temp.	~ 28°C	~ 30°C
RH (average 9.00 a.m. and 3.00 p.m.)	~ 80%	~ 75%
rain (mm.)	18.0	25.3
hrs. of bright sunshine		
per day: ≥ 8 hrs %	58	90
≥ 10 hrs %	19	43
evaporation ≥ 5 mm. %	42	93
Plant physiological conditions:		
stage	shortly after tasselling	one month after maturity
watercontent stem (%)	70–80	10–20
Morphological differences of larvae:		
colouration	spotted	immaculate
headcapsule width (mm)	1.4–1.5	1.6–1.7
Physiological differences of larvae:		
life span of larvae (days)	17.6–22.0	20.6–24.3
maximum % aestivation	35	54

Table II: Influence of temperature on the time required for pupation of 1st, 3rd and 5th instar larvae of *Chilo zonellus*

Temp. regime *	Time required for pupation (days ± S.D.) for 1st instar larvae		3rd instar larvae		5th instar larvae	
	♀	♂	♀	♂	♀	♂
25/25°C	35.8±6.8	29.1±4.8	28.8±6.6	21.8±6.9	21.0±6.3	14.4±4.4
35/25°C	29.8±2.7	26.9±7.3	23.3±3.4	20.0±4.7	15.7±4.7	10.5±6.7
37/25°C	35.0±2.7	35.1±11.4	24.7±6.2	21.6±2.4	16.4±6.2	9.2±3.9
41/25°C	37.0±5.9	42.3±11.6	44.2±15.3	37.1±11.2	27.4±5.0	14.2±5.1

*6 hrs "high temperature"
18 hrs "low temperature"

Table III. Excrement production (mg dry weight of dry maize/100 mg larva ± S.D.) of aestivating larvae in dry maize stems when disturbed twice a week or when left undisturbed

larvae disturbed 2× week				larvae undisturbed
total excrement production after a period of				
1 week	2 weeks	3 weeks	4 weeks	8 months
6.28±5.12	9.76±4.17	13.33±5.21	14.08±5.51	3.01±7.34

Table IV. Comparison of oxygenconsumption and watercontent of some fully grown diapausing and non-diapausing stemborer larvae

Species	oxygenconsumption (µl/mg dry/hr)		water (%)		Author (s)
	diapause	non-diap.	diapause	non-diap.	
Pectinophora gossypiella	0.61	3.63			Adkisson, Bell and Wellso (1963)
Pyrausta nubilalis	1.47±0.41 (early diap) 0.59±0.20 (late diap)	2.20±0.38	66.5 (early diap) 52.2 (late diap)	76.6	Beck and Hanec (1960)
Diatraea grandiosella	0.85±0.04 (early diap) 0.45±0.00 (late diap)	2.32±0.11			Yin and Chippendale (1974)
Diatraea grandiosella			65.0		Chippendale and Reddy (1972)
Chilo zonellus	2.61±0.77 (early diap) 1.06±0.25 (late diap)	4.26±1.20	68.5	80.8	

Preliminary investigations concerning the influence of juvenile hormone and ecdysterone upon diapause tentatively indicate that the induction and termination of aestivation are largely determined by varying the balance of these two hormones.

Twice weekly applications (of 2µg.) of juvenile hormone in acetone for a 4-week period onto the thorax of newly moulted L5 larvae induced 1–3 stationary ecdyses. These giant larvae were immaculate which in the field is an indication of aestivation. The larvae were divided into two groups, one of which was fed on wheat germ diet and the other placed into dry maize stems. Whilst all surviving larvae placed in the artificial diet pupated, none of those in the dry maize pupated within the following month. Reference to Fig. 1 suggests that the latter larvae had entered diapause. This interpretation is supported by the oxygen consumption figures which were recorded 3 days after the larvae were divided into 2 groups (Table V).

These data, it is suggested, indicate that a high titre of juvenile hormone is a requirement for the induction of the aestivation-diapause condition in *C. zonellus*.

The influences of hormones upon the termination of diapause were studied using field-collected aestivating larvae (Table VI).

Results indicate that neither a reduced titre of juvenile hormone alone (ligated controls) nor high titres of both ecdysterone and juvenile hormone (non-ligated ecdysterone-inoculated larvae) are effective in terminating diapause. It would appear that aestivation is terminated by the combined influences of an increase in ecdysterone together with a reduction in juvenile hormone titres (ligated, ecdysterone-inoculated larvae). It is understood of course that it is still necessary to prove that the "head factor" (prevented from entering the body by ligation) is indeed juvenile hormone.

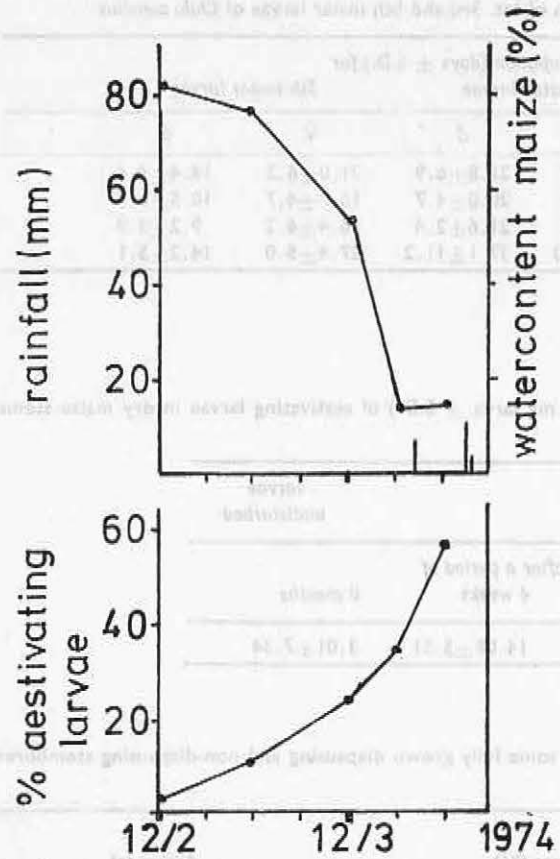

Fig. 3. Percentage aestivating larvae of *Chilo zonellus* and *Chilo argyrolepia* in relation to rainfall and watercontent of the maizeplant in an experimental field at Kikambala, Coast Province, Kenya.

Table V. Oxygen consumption of experimental and control larvae (See text)

Oxygen consumption (µl O_2/mg wet weight/hr)

Experimental larvae		*Control larvae*	
in wheat germ diet	*in dry maize stems*	*Non-diapause (laboratory)*	*Deep diapause (field)*
0.52 (±0.13)	0.38 (±0.09)	0.81 (±0,26)	0.25 (±0.09)

Table VI. Influence of ecdysterone and juvenile hormone (ligation) upon termination of diapause

Aestivating Larvae				
		Controls		
5µ Ecdysterone inoculated		*5µ saline inoculated*		*No. treatment*
Not ligated	*Head ligated*	*Not ligated*	*Head ligated*	
Stationary ecdysis (ie. remained in diapause)	Pupated (i.e. Diapause terminated)	Nil	Nil	

CONCLUSION

In the field, it would appear that lack of rain results in, and the advent of rain terminates, diapause. The manner in which information concerning rainfall is conveyed to the larvae has still to be determined.

Evidence tentatively indicates that aestivation is induced by a high titre of juvenile hormone and is terminated by a combination of high ecdysterone and low juvenile hormone titres. Similar results have been obtained for *C. suppressalis* (Fukaya and Mitsuhashi 1958) and *Diatrea grandiosella* (Yin and Chippendale 1973, 1974).

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SORGHUM SHOOT FLY

(*Atherigona varia* ssp *soccata*)

INTRODUCTION

Sorghum (*Sorghum bicolor*) has a complex of shoot flies now generally known by the name of *Atherigona varia soccata* (Rond). This name was first used by Hennig (1961) to include all African, Indian and Mediterranean populations from sorghum. This inevitably led to taxonomic confusion (Pont 1971) which continues today.

Initial investigations (carried out at the Kibos

field station) therefore are orientated to the determination of the taxonomic position of the local shoot flies, taxonomic characters of the larvae and the life histories of the flies. Diapause studies will follow.

RESULTS AND DISCUSSION

Preliminary life history data are recorded in Table I and Fig. I and are self-explanatory. Oviposition occurs on the host plant and continues with increasing irregularity throughout the female's life. The maximum number of eggs recorded for one female is 75. Although several eggs may be oviposited on individual plants, only a single larva may parasitise the central shoot. Pupation occurs at the base of the plant. The full life cycle is concluded in 27–30 days.

Taxonomic studies of the larval instars await the development of an artificial diet.

Table I. Life-Cycle (days) of *Atherigona varia soccata* (Rondani) on *Sorghum bicolor* at Kibos

	Eggs	*larva*	*Pupa*	*Average total life-cycle*	*Adult Longevity*	
					Females	*Males*
Range	2–5	11–15	10–14		10–46	7–39
Average	3 approx.	13.14	11.78	27.92	26.4	19.7
Number of observations	726	24	74		60	30

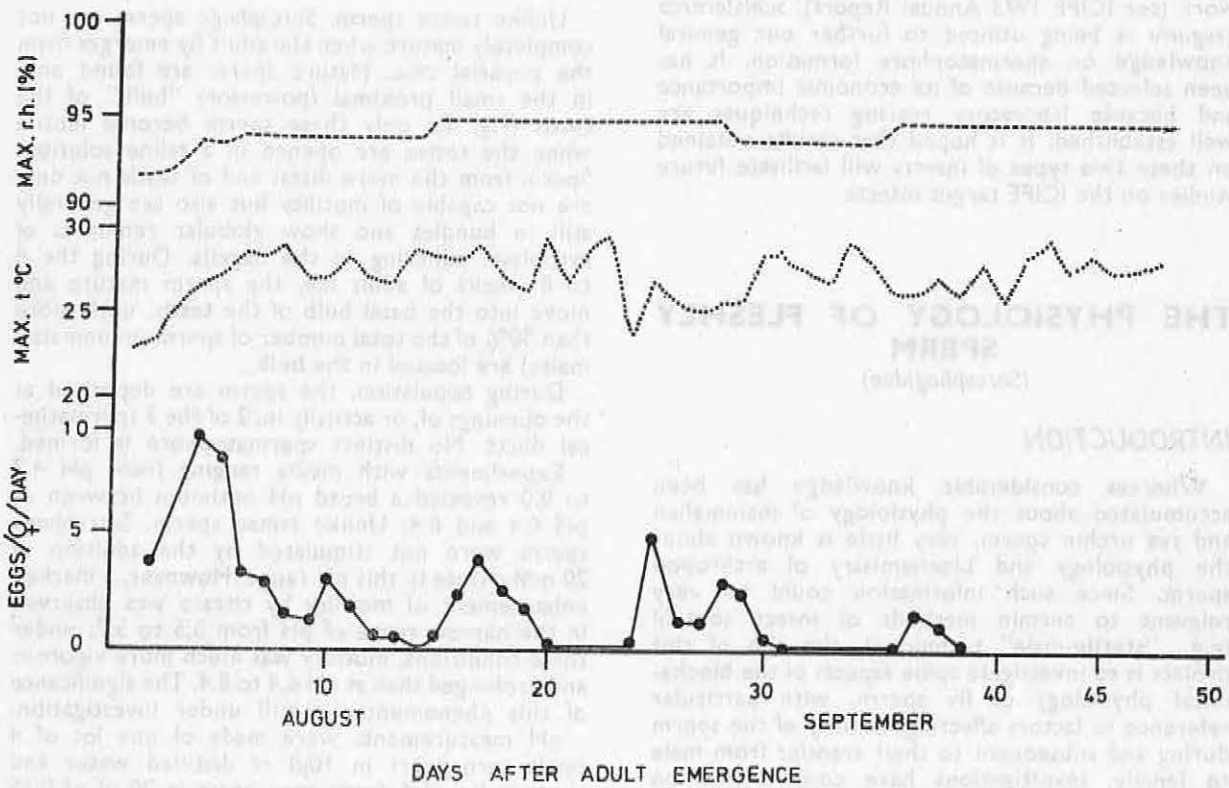

Fig. I.

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11

REPRODUCTIVE PHYSIOLOGY OF THE MALE INSECT

Directors of Research:

Professor Thomas Odhiambo (1970)
Professor Jan de Wilde (1970)

Scientists:

Dr. J. G. Shepherd (1972–1974)
Mr. T. S. Dhadialla (1973)

Neither the fleshfly nor the locust, which are discussed below, are target insects. Research on the reproductive physiology of target insects has been included under the title of the insect concerned. Investigations were initiated on fleshflies as a substitute for tsetse flies whose sperm were found to be unsuitable for certain physiological work (see ICIPE 1973 Annual Report). *Schistocerca gregaria* is being utilised to further our general knowledge on spermatophore formation. It has been selected because of its economic importance and because laboratory rearing techniques are well established. It is hoped that results obtained on these two types of insects will facilitate future studies on the ICIPE target insects.

THE PHYSIOLOGY OF FLESHLY SPERM

(*Sarcophagidae*)

INTRODUCTION

Whereas considerable knowledge has been accumulated about the physiology of mammalian and sea urchin sperm, very little is known about the physiology and biochemistry of arthropod sperm. Since such information could be very relevant to certain methods of insect control (e.g., "sterile-male" technique), the aim of this project is to investigate some aspects of the biochemical physiology of fly sperm, with particular reference to factors affecting motility of the sperm during and subsequent to their transfer from male to female. Investigations have concentrated on sperm activation during copulation.

Work on sperm activation in fleshflies stems from the observation that sperm are generally immotile in male flies until they are ejaculated, when they become vigorously active. Results reported in the first ICIPE Annual Report indicated that the sperm of tsetse flies could be activated simply by releasing the sperm from the tsetse into a buffered saline solution. However, motility was found to be short-lived under such conditions unless certain substrates, e.g., citrate, were added to the saline.

The work reported below includes a preliminary analysis of conditions suitable for motility of *Sarcophaga* (flesh fly) sperm and measurements of their oxygen consumption.

MATERIALS AND METHODS

The methods of dissection of sperm, media used, and the nature of the respirometer were described in the 1st ICIPE Annual Report. Sperm were always taken from the "bulbs" (see results) of the testise of 3 to 5 week old virgin males. A medium containing 0.12 *M* NaCl, 0.03 *M* KCl, 2 m*M* $CaCl_2$, 1m*M* $MgCl_2$, 50 m*M* HEPES (or Tris), 10 mg/ml bovine serum albumin (crystalline), 50 µg/l penicillin, and 300 µg/l streptomycin was used for measurements of pH optima. Respiration of sperm from the testis of virgin males was measured in 5 to 6µl of 0.02 *M* HEPES–KOH, pH 6.9 or 7.2, containing 10 mg/ml bovine serum albumin.

RESULTS AND DISCUSSION

Unlike tsetse sperm, *Sarcophaga* sperm are not completely mature when the adult fly emerges from the puparial case. Mature sperm are found only in the small proximal (posterior) "bulb" of the testis (Fig. 1); only these sperm become motile when the testes are opened in a saline solution. Sperm from the more distal end of testis not only are not capable of motility but also are generally still in bundles and show globular remnants of cytoplasm adhering to the flagella. During the 4 to 8 weeks of adult life, the sperm mature and move into the basal bulb of the testis, until more than 50% of the total number of sperm (in unmated males) are located in the bulb.

During copulation, the sperm are deposited at the openings of, or actually in, 2 of the 3 spermathecal ducts. No distinct spermatophore is formed.

Experiments with media ranging from pH 4.7 to 9.0 revealed a broad pH optimum between ca pH 6.4 and 8.4. Unlike tsetse sperm, *Sarcophaga* sperm were not stimulated by the addition of 20 m*M* citrate in this pH range. However, a marked enhancement of motility by citrate was observed in the narrow range of pH from 5.5 to 5.7: under these conditions, motility was much more vigorous and prolonged than at pH 6.4 to 8.4. The significance of this phenomenon is still under investigation.

pH measurements were made of one lot of 4 testis torn apart in 10µl of distilled water and another lot of 6 testis torn apart in 20 µl of 0.15 *M* NaCl. These showed pH values of 6.8 and 6.5, respectively. The pH of ejaculated sperm was not measured due to its very small volume.

In several experiments, the oxygen-consumption of *Sarcophaga* sperm was initially (i.e., 30 min. after the start of incubation to allow for equilibration of the respirometer) about 40 to 50 µl/10^8 sperm/hour at 25°C. In one experiment, the oxygen consumption after 3 hrs was about ½ of the original value; microscopic observation of the sperm indicated motility was still quite vigorous. In a second experiment, the oxygen consumption after 3 hrs was about 1/10 of the original value; in this case, motility had ceased altogether. Whether this reflects death of some of the sperm as well as the cessation of motility is not yet clear.

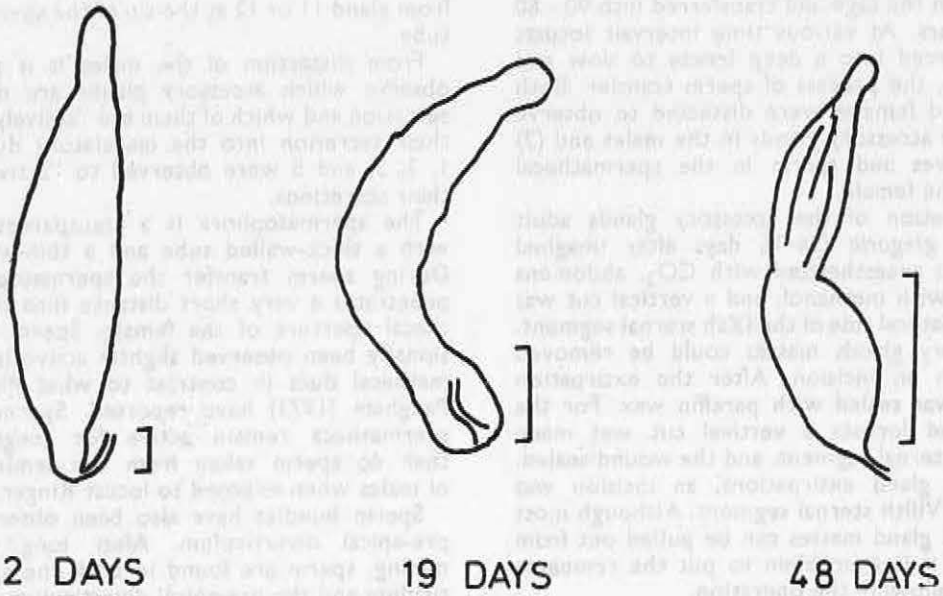

Fig. 1. Tests of virgin male *Sarcephaga* at various ages after adult eclosion. The bars indicate the increasing relative size of the "bulbs".

Since these respiratory rates were surprisingly high, the oxygen consumption of fresh bull sperm (obtained through the courtesy of the Veterinary Laboratory Kabete) was measured in the micro-respirometer. The oxygen consumption of both undiluted semen and semen diluted 1:3 with egg york-phosphate buffer (Mann, 1964) were comparable with literature values (Bishop *et al.*, 1954; Bishop and Salisbury, 1955), indicating that the apparatus was functioning correctly.

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SPERMATOPHORE FORMATION IN LOCUST

(*Schistocerca gregaria*)

INTRODUCTION

In insects that make use of a spermatophore for the transfer of seminal fluid from the male to the female, the spermatophore is usually formed from secretions of the male accessory reproductive glands. Spermatophores have been reported in several orders of insects (Davey, 1960) and occur particularly widely in the Orthoptera. In spite of the wide occurrence of spermatophores for sperm transfer, little is known about their chemical composition and formation.

Detailed investigations have been reported on the formation and fate of the spermatophore in *Locusta migratoria migratorioides* (Gregory, 1955). In this species the fully formed spermatophore consists of a thin-walled, dilated reservoir that remains within the male copulatory organ during copulation, and a long, blind-ended tube that passes into the spermatheca of the female. *Schistocerca* differs from *Locusta* in that *Schistocerca gregaria* produces more than one spermatophore during each mating (Pickford and Padgham, 1973). As many as 14 small (1.5 mm) spermatophores may be produced during a 4-hour mating period. The 16 pairs of accessory glands in *S. gregaria* have been examined histologically and histochemically and classified into 9 types (Odhiambo, 1969). Analysis of the secretions with disc electrophoresis has shown that each of the 16 glands have different staining patterns for protein and carbohydrate.

The purpose of the present study is to investigate the contribution from different accessory reproductive glands for sperm transfer and spermatophore formation in *S. gregaria*. This objective will be achieved by: (i) investigating the events leading to spermatophore formation (much of this has been done by Pickford and Padgham, 1973); (ii) extirpating individual pairs of male accessory glands and allowing these operated males to mate with virgin females; and (iii) investigating changes occurring in the protein, carbohydrate, and lipid patterns in the functional accessory glands at different times during spermatophore formation. Histochemical and chemical analysis of the spermatophore itself at different stages of formation will also be done.

During the last months the first objective has been achieved, and attempts have been made on the second objective.

MATERIALS AND METHODS

To initiate mating, virgin females were introduced into cages containing males only. After they had mated for 5–7 minutes, pairs were gently

removed from the cage and transferred into 90×80 mm kilner jars. At various time intervals locusts were transferred into a deep freeze to slow and possibly stop, the process of sperm transfer. Both the males and females were dissected to observe (1) the active accessory glands in the males and (2) spermatophores and sperm in the spermathecal complex of the female.

For extirpation of the accessory glands adult males of *S. gregaria* (14–16 days after imaginal ecdysis) were anaesthetized with CO_2, abdomens were wiped with methanol, and a vertical cut was made on the lateral side of the IXth sternal segment. Both accessory glands masses could be removed through such an incision. After the extirpation the wound was sealed with paraffin wax. For the sham-operated locusts a vertical cut was made on the IXth sternal segment, and the wound sealed.

For single gland extirpations, an incision was made on the VIIIth sternal segment. Although most of the paired gland masses can be pulled out from the incision, it is a problem to put the remnants back intact following the operation.

RESULTS

In these experiments observations were made by interrupting copulating pairs at different time intervals from 10 minutes up to 120 minutes after the onset of copulation (mating can last as long as 11 hours). The observations have been grouped as follows: 0–30 minutes; 30–60 minutes; and 60–120 minutes.

0–30 Minutes

The earliest that a spermatophore has been observed was 10 minutes after copulation, at which time sperm were also present in the spermathecal duct. During the 30-minutes period the spermatophore was observed in between the female ovipositors. Inactive sperm were collected from in between the ovipositors and/or around the aedegus. After 30 minutes sperm were in the spermathecal duct or the preapical diverticulum of the spermatheca.

30–60 Minutes

At least one and a maximum of two spermatophores were observed in between the ovipositors. An additional spermatophore was normally seen being extruded out of the aedegus.

60–120 Minutes

Normally 2 spermatophores were observed in between the ovipositors with a third extruding or already extruded out of the male aedegus. Spermatophores that were being extruded out of the male aedegus had an oil secretion inside the spermatophore and a secretion resembling that from gland 11 or 12 at the tip of the spermatophore tube.

From dissection of the males it is possible to observe which accessory glands are depleted of secretion and which of them are "actively pumping" their secretion into the ejaculatory duct. Glands 1, 2, 3, and 5 were observed to "actively pump" their secretions.

The spermatophore is a transparent structure with a thick-walled tube and a thin-walled bulb. During sperm transfer the spermatophore tube penetrates a very short distance into the spermathecal aperture of the female. Sperm have occasionally been observed slightly active in the spermathecal duct in contrast to what Pickford and Padgham (1973) have reported. Sperm from the spermatheca remain active for longer periods than do sperm taken from the seminal vesicles of males when exposed to locust Ringer.

Sperm bundles have also been observed in the pre-apical diverticulum. After long periods of mating, sperm are found in both the apical diverticulum and the pre-apical diverticulum.

The operated males were used for mating with virgin females a week after the operation. About 90% survival of the operated males was obtained. Results indicate that extirpation of male accessory glands prevents sperm transfer and spermatophore formation.

Sham-operated males copulate with and inseminate virgin females successfully. Spermatophore production is normal, i.e. about 6 spermatophores were formed when some of these males were allowed to mate for 4 hours or longer. Loose sperm and sperm bundles were also present in the spermatheca of the mated females.

Although quite a good survival rate has been obtained amongst males with a single pair of accessory glands extirpated, none of these males have successfully inseminated virgin females. The difficulty arises when, after selective extirpation of individual pairs of accessory glands, the rest have to be put back intact. It is suspected that the remaining glands are severely damaged during the operation.

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HORMONES, FLIGHT AND MATING BEHAVIOUR

Scientist:
Professor M. P. Pener, Research Associate

NEUROSECRETION AND FLIGHT ACTIVITY

(*Locusta migratoria migratorioides*)

INTRODUCTION

Several endocrine glands, namely the corpus allatum (CA) and the corpus cardiacum (CC) seem to affect flight activity in locusts and other insects (Wajc and Pener, 1971; Goldsworthy *et al.*, 1972; Caldwell and Rankin, personal communication). Nevertheless, the possible effect of the median neurosecretory cells (MNSC) of the pars intercerebralis (PI) upon flight performance was not investigated in insects. After initiation of the present study, Michel and Bernard (1973) reported that destruction of the PI drastically reduces sustained flight in the desert locust (*Schistocerca gregaria*), but Goldsworthy (personal communication) concluded that cauterization of the MNSC does not affect flight in the African migratory locust. The possible role of the MNSC in control of flight activity of the African migratory locust constituted the subject of the present study. Michel and Bernard's (1973) and Goldsworthy's (personal communication) investigations did not invalidate this subject, since the conclusions drawn by these workers are controversial.

MATERIALS AND METHODS

A culture of *Locusta migratoria migratorioides* originating from the Anti-Locust Research Centre (now Centre for Overseas Pest Research), London, had been maintained from 1953 till 1972 in the Department of Entomology, The Hebrew University, Jerusalem. In 1972 eggs were sent to the International Centre of Insect Physiology and Ecology, Nairobi, and the breeding developed from these eggs constituted the stock material. Locusts were kept under conditions of crowding.

Only male locusts were employed for the present investigations.

Electrocoagulation of the PI was performed by Girardie's (1966) method (see also Pener *et al.*, 1972). Allatectomy was carried out according to Joly's (1960) technique. Sham-operations included all the surgical interference, except actual coagulation of the PI, or actual removal of the CA. All these treatments were performed 2 ± 1 days following moult to adult stage. Unoperated controls were simply removed from the stock at the same age. All operated and control locusts were individually marked.

Investigations on flight performance were initiated 8 days (Series I), 6 days (Series II), or 9 days (Series III) following surgical or control treatments. The locusts were flown individually on a roundabout without any specific stimulation and every consecutive week were tested twice for 30 minutes (Series I), or 15 minutes (Series II and III) each time. Each roundabout was connected to an electric counter and the number of revolutions made by a locust within such a test was noted. The results were averaged within each series for a similarly treated group of locusts flown on the same day. These averages constituted the parameter for measuring flight activity and are shown in the figures.

Experimental locusts were kept under 12 hours light and 12 hours darkness in a temperature-conditioned room. The electric bulbs providing the illumination heated the locust cages from the outside and so raised the temperature within the cages during the daytime over that of the room. Nightly minimum temperatures of 24–26°C were recorded in the room (and in the cages), while maximum day-temperatures in the cages fluctuated between 33–40°C. Flight performance was tested at 28 ± 1°C.

RESULTS AND CONCLUSIONS

Series I

Initially 12 PI-coagulated males, 12 sham-operated controls and 12 unoperated controls were used. If a male died or its wings were damaged (unfortunately, locusts kept together under crowding eat and damage each other's wings) it was replaced by a similarly treated one, insofar as these were available. The experiment was concluded at the end of the 5th week following treatment; at this time 9 males remained and were tested in each group.

The average numbers of revolutions per 30 minutes, as performed by these males in each test, are shown in Fig. 1, which demonstrates that there is no marked difference in the flight performance of sham-operated and unoperated controls. On the other hand, the figure indicates less intense flight in PI-coagulated males. Individual variations were, however, extremely high and preliminary statistical analyses (*t*-tests) revealed that concerning single tests of flight, in most cases the difference between PI-coagulated males and either group of controls was statistically not significant.

It was necessary, therefore, to employ a greater number of locusts for subsequent investigations.

Series II

Since the available number of roundabouts was limited to two, only PI-coagulated males and sham-operated controls were used in this series, and flight duration was reduced to 15 minutes in each test (twice per week). Initially 46 males were employed for each group, but in contrast to Series I, if a male died or its wings were damaged it was not replaced. Thus, the number of locusts decreased from test to test, but even at the beginning of the 6th week, when the experiment was concluded, 29 sham-operated and 31 PI-coagulated males were flown (Fig. 2).

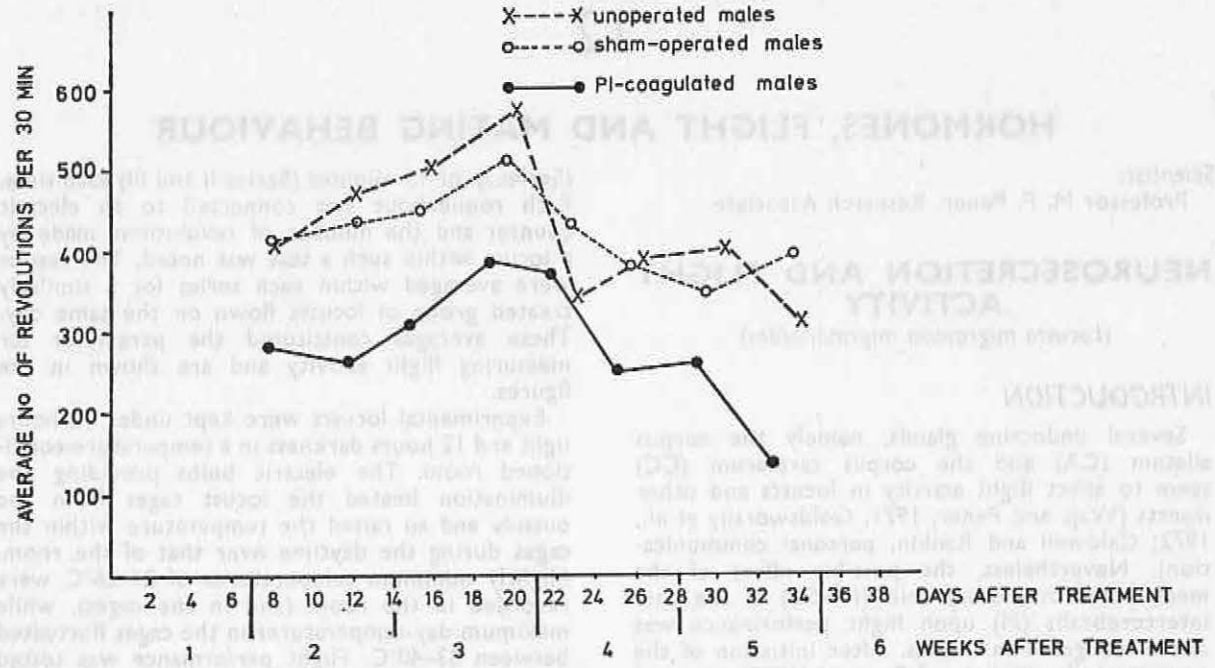

Fig. 1. Flight performance of locusts in Series I of the experiments X---X = unoperated males; O----O = sham-operated males ●——● = PI-coagulated males.

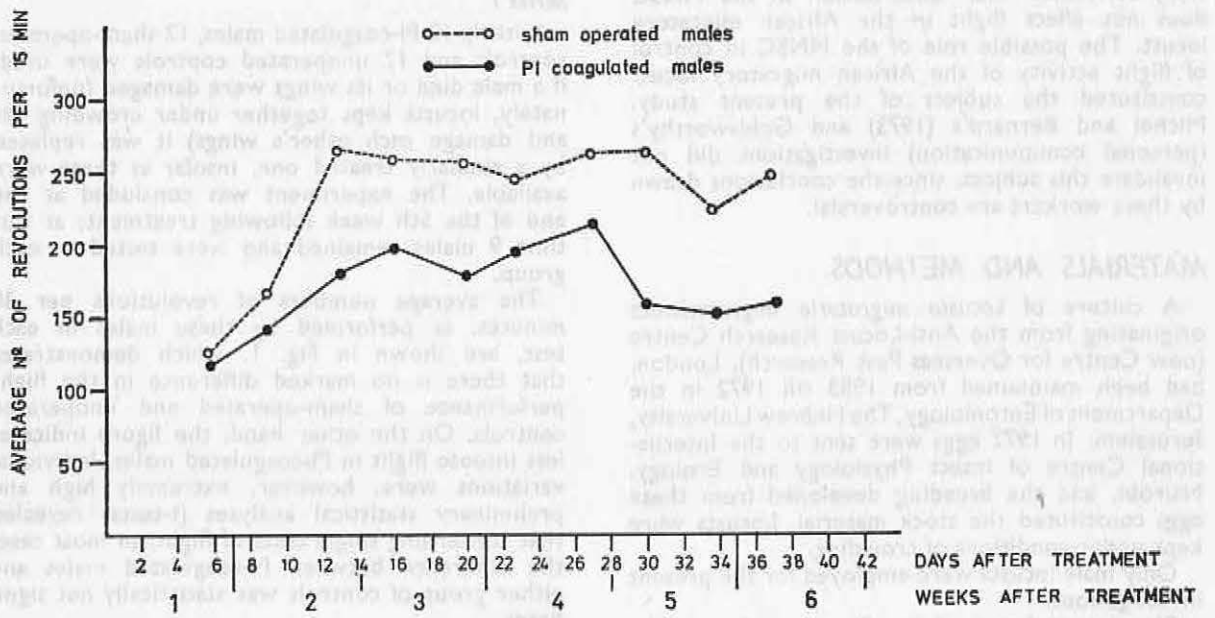

Fig. 2. Flight performance of locusts in Series II of the experiments O----O = sham-operated males; ●——● = PI-coagulated males.

Preliminary statistical analyses (t-tests) revealed that the differences between PI-coagulated males and sham-operated controls were statistically not significant in the sole test of the first week ($p > 0.5$) and in the first test of the 2nd week ($0.4 > p > 0.2$). However, in the second test of the 2nd week, the difference was highly significant ($0.005 > p > 0.001$). In the majority of the later tests the differences were significant.

The t-test must be regarded as a preliminary statistical analysis. The usage of this test is, in fact, incorrect, since the same, or almost the same locusts were employed in consecutive flight tests. It is intended to use *analysis of variance* for those males that were flown during the whole duration of this experimental series.

However, summarizing the *cumulative* flight performance of all the males flown from the second test of the 2nd week (first case when the difference was found to be significant by t-test), till the first (and only) test of the 6th week, allows for a justified usage of the t-test. This summary shows a cumulative flight performance of 2136.86 ± 833.94 (average $\pm$ s.d) revolutions for sham-operated controls

(n=29), and of 1500.16±999.65 revolutions for PI-coagulated males. Despite the huge variations, the difference is statistically significant ($p=0.01$). Taking the flight performance of sham-operated controls as 100%, that of the PI-coagulated males is about 70%.

Electrocoagulation of the PI, therefore, seems to reduce flight activity in male African migratory locusts. The effect, however, is slight during the first 6–9 days after operation (8–11 days after moult to adult stage) and only becomes marked later.

Series III.

The effect of the PI- on flight performance of the African migratory locust may be direct, indirect, or both. Since in this insect the C-cells of the MNSC activate the CA (Girardie, 1966; Pener *et al.*, 1972), and the CA itself affects flight performance (Wajc and Pener, 1971; Goldsworthy *et al.*, 1972), lower flight activity of the P-coagulated males may well be a result of the inactivity of the CA. This possibility was investigated in Series III of the experiments.

Initially, 16 PI-coagulated males, 26 allatectomized males and 28 sham-operated controls (14 sham-operated locusts for PI-coagulation and the other 14 for allatectomy) were used. Males that died, or those in which the wings became damaged, were not replaced by others. Due to unknown reason(s) mortality was high, especially in the PI-coagulated and the sham-operated groups. In the first half of the 5th week, when the experiment was completed, only 6 PI-coagulated males, 23 allatectomized males and 15 sham-operated controls (8 for PI-coagulation and 7 for allatectomy) were tested. Results of the flight performance are summed up in Fig. 3.

Preliminary statistical analyses (t-tests) revealed that PI-coagulated males exhibited less intense flight than the controls (in four tests, $p \leq 0.05$; in three tests, $0.1 > p > 0.05$). No significant difference was found in either the first or the second test of the 2nd week between allatectomized and sham-operated males ($p > 0.5$); in the first test of the 3rd week the difference became more marked ($0.1 > p > 0.05$), and in all later tests it was significant ($p < 0.025$). Finally, allatectomized males flew significantly ($p < 0.025$) better than PI-coagulated males in the first test of the 2nd week, but less so in the second test of the same week ($0.1 > p > 0.05$); from the 3rd week onwards no significant differences were found between these two groups (in all five tests $p > 0.5$).

Fig. 3 and these statistical analyses show that flight performance of the PI-coagulated males was less intense than that of the controls. Flight performance of the allatectomized males was similar to that of the controls in both tests of the 2nd week, it decreased in the first test of the 3rd week; and in subsequent tests, it became roughly the same as that of the PI-coagulated males.

Inactivity of the CA seems to be, therefore, the main causative factor leading to less intense flight of the PI-coagulated males. This activity, however, cannot constitute the only factor involved, since even complete lack of the CA (allatectomy) resulted in a decrease of the flight only from the 3rd week onwards, while flight performance of the PI-coagulated males was already reduced in the 2nd week.

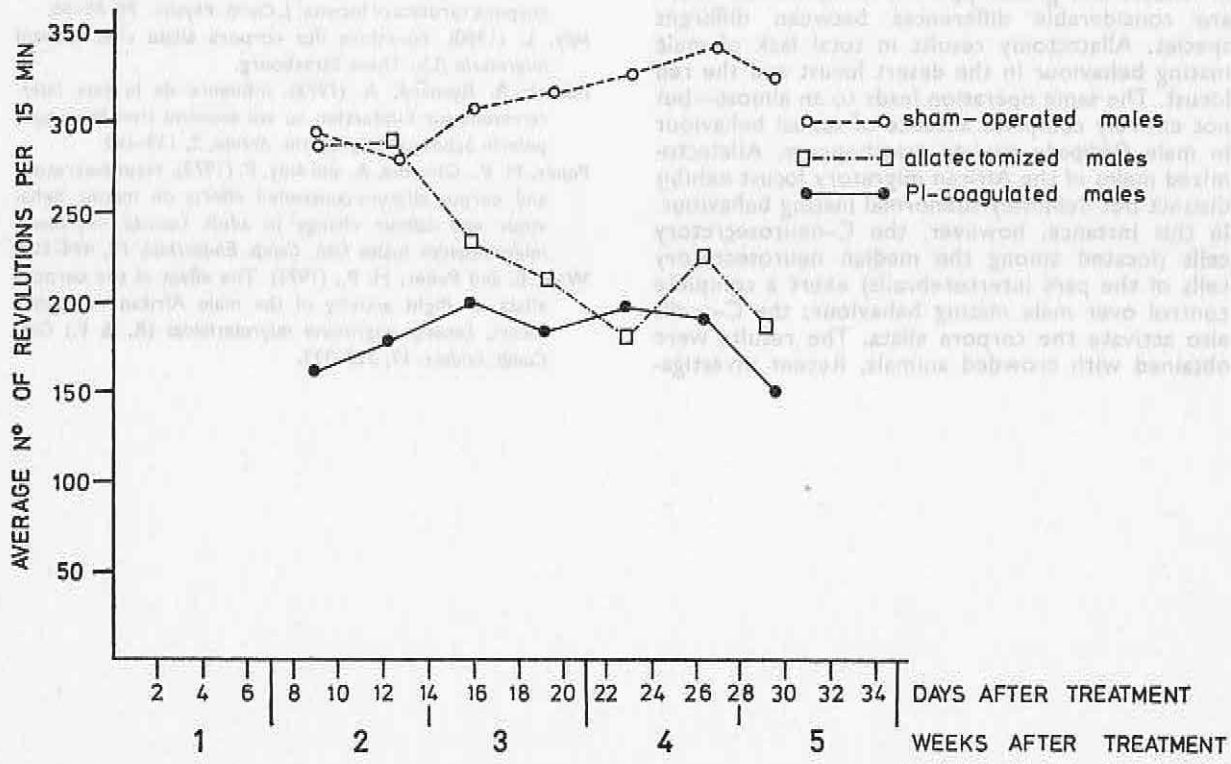

Fig. 3. Flight performance of locusts in Series III of the experiments O- - - -O = sham-operated males; ●——● = PI-coagulated males □——□ = allatectomized males.

The latter finding does not necessarily contradict the results of Series II in which no significant differences were found during the first 6–9 days between PI-coagulated males and controls. In the present series the tests started only 9 days after operations, while in Series II they started on the 6th day. Furthermore, day- (but not night-) temperatures in the locust cages were higher by about 2°C in Series III, and thus locusts having the same chronological age (9 days) were physiologically older in the present series than in the previous one. The fact that Fig. 3 does not show increasing flight performance with increasing age in the first tests for the controls (such an increase is very clear in Fig. 2 and detectable in Fig. 1) also suggests that the tests of Series III were initiated with physiologically older locusts.

The individual variations in flight performance were very considerable in all experiments, but always the highest in the PI-coagulated males. Perhaps this finding is a result of imperfect coagulation of the MNSC in some males, who may be "better flyers" than those in which the coagulation was complete. Since these brains were dissected out, fixed and embeded, it is intended to scrutinise stained sections of each in order to determine the effectiveness of individual operations and to compare these observations with individual performances.

HORMONES AND MATING BEHAVIOUR

DISCUSSION

Endocrine control of male sexual behaviour in locusts and grasshoppers is complex and there are considerable differences between different species. Allatectomy results in total lack of male mating behaviour in the desert locust and the red locust. The same operation leads to an almost—but not entirely complete absence of sexual behaviour in male *Oedipoda miniata* grasshoppers. Allatectomized males of the African migratory locust exhibit distinct but definitely subnormal mating behaviour. In this instance, however, the C–neurosecretory cells (located among the median neurosecretory cells of the pars intercerebralis) exert a complete control over male mating behaviour; the C– cells also activate the corpora allata. The results were obtained with crowded animals. Recent investigations demonstrated, however, that endocrine effects on male sexual behaviour may be different in crowded (gregarious) and isolated (solitary) locusts of the same species.

Allatectomy leads to a decrease of flight performance in *Locusta migratoria migratorioides* males. Coagulation of the pars intercerebralis also reduces flight performance, though the results differ in certain details from those obtained after allatectomy. In this species, therefore, the pars intercerebralis seems to affect flight mainly, but not solely, through the activation of the corpora allata. Data in the literature indicate that endocrine effects on flight activity (like on sexual activity) differ in different species.

In influencing behaviour hormones may exert general effects, or may serve as releasers (or inhibitors) of preprogrammed behavioural patterns. These two conceptions are not mutually exclusive, but neither one seems to be in full accord with all the experimental results. Involvement of further endocrine organs in controlling behaviour cannot be excluded, but this assumption needs experimental proof. The extraordinary flexibility of hormonal effects on behaviour in different species may constitute a partial answer as to why and how insects adapt themselves through relatively rapid evolutional processes to different or changing environments.

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CHEMISTRY

Directors of Research:

Professor M. Barbier (1971)
Professor Jerrold Meinwald (1970)
Professor Koji Nakanishi (1970)

Scientists:

Dr. Asafu Maradufu (1974)
Dr. Isao Kubo (1974)
Dr. Shozo Takahasi (1973–74)
Dr. W. F. Wood (1972–74)

Since this annual report is intended to review the year's research progress on target insects rather than the individual scientist's findings, results obtained by the Chemistry Unit are included under the relevant target insect headings outlined below:

Ticks

(a) Sex pheromones of the hard tick *Rhipicephalus appendiculatus*
(Dr W. F. Wood)

(b) Pheromones of the soft tick *Ornithodorus moubata*
(Dr Asafu Maradufu)

Termites

Trail pheromone of *Hodotermes mossambicus*
(Dr Shozo Takahashi)

African Armyworm

Sex pheromone of *Spodoptera exempta* (Dr Shozo Takahashi in collaboration with Dr T. Yonehara and Dr Y. Kuwuhara of Kyoto University)

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ELECTROPHYSIOLOGY

Directors of Research:

Professor D. Schneider (1970)
Professor F. Huber (1970)

Scientists:

Dr. M. Kaib (1973)
Dr. G. R. Karuhize (1972)
Dr. Ma Wei-Chun (1972)

INTRODUCTION

The general outline of research problems, approach and workplan are summarized in the 1973 Annual Report.

Since these reports are intended to review the year's research progress on target insects rather than the individual scientist's findings, results obtained by the ICIPE electrophysiologists are included under the relevant target insect headings as outlined below.

ELECTROPHYSIOLOGICAL STUDIES

Since the end of 1973 three electrophysiological recording units have been operational:

(1) Taste cell recordings (Dr. Ma Wei-Chun)

(2) Microrecording from single olfactory cells (Dr. M. Kaib)

(3) Electroantennogram bioassay (n.n.)

Experimental animals:

(a) Armyworm larva (food taste cells, see Armyworm Research Report: Dr. Ma Wei-Chun)

(b) Tsetse fly (antennal odour receptors: Dr. G. R. Karuhize and Dr. M. Kaib)

(c) Termites (antennal pheromone receptors: Dr. M. Kaib)

(d) Tineid caterpillar (antennal rectors: Dr. M. Kaib)

BEHAVIOUR STUDIES

(a) Armyworm food plant selection: (Dr. Ma Wei-Chun)

(b) Termite trail-following behaviour. This work (done by Dr. R. Leuthold and Dr. Kaib) is the basis for a forthcoming electrophysiological study of the pheromone receptors on the termite antenna (Dr. M. Kaib).

(c) Pheromone-and humidity-controlled behaviour of soft ticks (Dr. G. R. Karuhize).

MORPHOLOGICAL STUDIES

Since any research on sensory receptors needs detailed knowledge of the distribution and the structure of sensilla, histological work on the following systems has been started:

(a) Tsetse fly antenna (funiculus):

Scanning electron microscopical survey of the patterning of sensilla, and transmission electron microscopical study of the fine structure of the sensilla of the antennal pit organs (Dr. George Karuhize).

(b) Termite antenna:

Distribution of different types of sensilla on the antennal flagellum in different species and castes (Dr. Manfred Kaib).

(c) Sensilla on the palps in soft ticks (Dr. George Karuhize).

Studies on sensory function as outlined above are undertaken in close collaboration with several projects, i.e. armyworm, tick and termite research, as well as the fine structure and chemistry units.

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FINE STRUCTURE UNIT

Directors of Research:

Professor T. R. Odhiambo (1970)
Professor D. S. Smith (1970)

Scientists:

Dr. C. J. Heather (1974)
Mr. J. Owor (1973)
Miss L. Sequeira (1972)

GENERAL OBJECTIVES AND METHODOLOGY

Work completed so far has been mainly concerned with establishing, organising and determining the terms of reference for the Fine Structure Research Unit (FSRU); collaborative work together with the specific projects of the FSRU staff has now begun.

The Unit conducts research into basic insect structure and has its own trained personnel who offer a range of collaborative services to other research workers, including both scanning (SEM) and transmission (TEM) electron microscope facilities and various aspects of light microscopy (LM) and photomicrography. In this way it provides an effective additional instrumentation to the basic research programmes of ICIPE.

The technical skills that are made available are of necessity very specialised and it is anticipated that the major part of any work will be carried out by the trained FSRU staff only.

Once the Unit is fully operative, these services will include:

	LM	TEM	SEM
1. Tissue processing to resin embedding	+	+	ø
2. Thin section cutting	+	+	ø
3. Gross preparations/metal coating	ø	ø	+
4. Examination and photography	++	+	+
5. Fluorescence microscopy	++	ø	ø
6. Histochemistry	++	+	ø
8. Advisory services	+	+	+
9. Occasional training/courses for non-FSRU staff	+	+	+

++ facilities and/or services
\+ services only
ø Not applicable

PROPOSED PROGRAMMES

The FSRU will concentrate on various aspects of 3 body systems in the principal insect under investigation, the tsetse fly *Glossina morsitans*, with parallel studies on termites, locusts and ticks. The systems for specific study will be (1) the reproductive system; (2) the digestive system; and (3) the central nervous system and endocrine glands.

The techniques will include those used in basic anatomical studies, histochemistry, autoradiography, direct (surface) morphological studies, and the examination of material following replication and surface stripping.

REPRODUCTIVE SYSTEM

Spermathecae (tsetse)

Tissue organisation in spermatheca bulbs and ducts has already been examined in sections prepared from resin-embedded material prepared for light microscopy. Electron microscope observations are expected to be carried out in the next few months. It is hoped that this study will elucidate the phenomena of long-term sperm viability (observed up to 6 months in tsetse spermathecae) and sperm migration through the female reproductive tract. Any observed changes in sperm structure during this period will also be fully investigated.

Egg development (tsetse)

Studies on egg development including vitellogenesis in the adult tsetse as well as a general survey of gross morphological changes in newly merged tsetse have commenced. A study of the structural changes related to ovulation and pregnancy will be initiated in the near future.

Accessory glands (male tsetse)

The roles of these glands and the ejaculatory duct in spermatophore formation will be investigated.

DIGESTIVE SYSTEM

Salivary glands (tsetse)

The distribution and condition of trypanosomes carried in the salivary glands of infected *G. morsitans* is already being studied with the collaboration of Dr. L. H. Otieno from the ICIPE Salivary Gland Physiology Programme. These studies are at the stage of LM observation, following resin embedding. The relationship between *Glossina* and the trypanosomes it carries will be investigated anatomically, histochemically and if possible, immunologically.

Gut anatomy

A survey of the organisation of the gut is being undertaken. Preliminary micrographs of stages in the secretion of the peritrophic membrane have been obtained and this work will be continued and extended.

CENTRAL NERVOUS SYSTEM AND ENDOCRINE GLANDS

These projects are still at a very early stage. TEM photographs of corpora allata of *G. morsitans* have been taken and are being studied in order to establish an accurate reference collection of photographs of this tissue.

The project emphasis will be on the basic morphology and changes associated with hormone production.

CURRENT WORK

The electron microscopes have only recently become functional. Light microscope techniques have been used to prepare material from *G. morsitans* with a view to establishing reference collection of low-and medium-power photographs of normal tissues. Thin sections have been cut from resin-embedded gut, spermathecae, ovaries, thoracic muscles, brain and thoracic ganglia, and halteres. After staining in toluidine blue in borax, some have been photographed, and other material is now being prepared.

When a reasonable initial collection has been made, other staining techniques will be employed to establish a variable light microscope staining schedule for thin sections prepared in this way.

COLLABORATIVE WORK

Discussions have been held with various members of the ICIPE research staff who have specifically asked for guidance and/or assistance with various aspects of their own projects. Several projects have been suggested.
These include:

a. TEM/SEM studies on armyworm (*Spodoptera exempta*) scent brushes (male), pheromone-producing glands (female), and flight muscles.
b. LM and possibly TEM/SEM studies on termite heads and brains.
c. TEM of sensory structures in *Spodoptera* buccal cavity.
d. SEM/TEM studies on caterpillar antennae.

COMPLETED STUDIES

Tsetse antennae

Studies on the occurrence and distribution of sensory (? olfactory) cells, associated with host-seeking, have been conducted by Dr. G. R. Karuhize.

Tsetse uterine gland

Dr. W. C. Ma and Dr. D. L. Denlinger, in collaboration with Prof. D. S. Smith and Miss U. Järlfors have investigated changes in the uterine ("milk") glands throughout the gestatory cycle. These studies have included a description of the relationship between the secretory epithelium and adjacent epidermal layer, including the specialised cuticular plugs through which secreted material passes from the extracellular reservoir invaginated from the apical cell surface of the secretory component to the uterine cavity. These studies were carried out jointly between the ICIPE laboratory and the University of Miami Laboratory.

Trypanosomes

The fine structure of bloodstream forms of *T. brucei* has been studied by Dr. A. Njogu (EATRO), Prof. D. S. Smith, and Miss U. Järlfors, in materials prepared during Dr. Njogu's visit to the University of Miami. This work has included preparation of pure trypanosome fractions, free from blood cells and plasma, and their examination by scanning microscopy and transmission electron microscopy of thin sections and carbon-platinum replicas of freeze-fractured material. Part of this work, primarily concerning the fine structure of the plasma membrane, and intra-membrane specialisation associated with flagellar adhesion was reported by Dr. Njogu at the 1973 Bellagio Tsetse Conference and is currently in press: a further account of the techniques for preparation of pure isolates, and their processing for SEM studies (by centrifugation onto Thermonox slides and critical-point drying) and for TEM works is in preparation.

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