R. P. ORTEGA

ICIPE

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

THIRD ANNUAL REPORT

1975

Nairobi, March 1976

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Ibadan, Nigeria, May 1974: The then Chairman of the ICIPE Committee Dr. M.E.A. Materu replying to the opening address at the ICIPE second meeting by the Permanent Secretary, Ministry of Agriculture Mr. Adigun (on extreme right). Seated in the Centre is the Committee Secretary Mr. J.M. Ojal who is also Deputy Director of the ICIPE.

RESEARCH STAFF

RESEARCH SCIENTISTS	NATIONALITY	DATE OF APPOINTMENT	PROJECT/UNIT
Dr Nabil Abo-Khatwa	Egyptian	15.11.73	Termite
Dr Oebele H. Bruinsma	Dutch	1.11.74	Termite
Dr Muhammed Chaudhury	Awaiting U.S. Citizenship	1. 3.74	Insect Development and Reproductive Physiology
Dr Marius den Boer	Netherlands	1.10.74	Armyworm
Dr George Buhlmann	Swiss	1. 4.75	Termite
Dr John R. Clearwater	New Zealand	11. 7.75	Insect Dev. Reproductive
Dr Johana P.E.C. Darlington	British	1.10.74	Termite
Dr Christopher Heather	British	9. 2.74 – Feb. 1976	Fine Structure
Dr Manfred Kaib	German	1. 9.73	Electrophysiology
Dr George R. Karuhize	Ugandan	16. 6.72 – 1975	Electrophysiology
Dr Syed Khasimuddin	Indian	1.12.73	Armyworm
Dr Michel G. Lepage	French	24. 7.75	Termite
Dr Asafu Maradufu	Tanzanian	1. 3.74	Chemistry
Dr Robin Newson	British	1. 9.74	Tick
Dr Leonard H. Otieno	Kenyan	1. 2.73	Salivary Gland Physiology
Dr G.W. Oloo	Kenyan	1. 5.74	Termite
Dr Glenn Prestwich	U.S.A.	1. 7.74	Chemistry
Dr. Paul Scheltes	Dutch	1.10.71	Reproductive Physiology
Dr. R.A. Steinbrecht	German	9. 9.75	Electrophysiology
Rr. Jaap van Etten	Dutch	31. 1.74	Isolation Mechanisms
Dr R. Subra	French	1975	*Aedes egypti* mosquito
Dr Philip Lounibos	U.S.A.	15. 7.74	*Aedes egypti* mosquito
Dr Fidelis Ogah	Nigerian	1.10.73	Genertic Variability
Mr John L. Petersen	U.S.A.	1. 6.74 – Jan. 1975	*Aedes egypti* mosquito

EXPERIMENTAL OFFICERS	NATIONALITY	DATE OF APPOINTMENT	PROJECT/UNIT
Mr Tarlochan S. Dhadialla	Kenyan	1.10.73	Insect Development and Reproductive Physiology
Mr John Kawooya	Ugandan	1. 9.73	Insect Development and Reproductive Physiology
Mrs. Christine K.A. Mango	Kenyan	1. 7.71	Tick
Mr Kenuel Ogwaro	Ugandan	1. 9.73	Insect Development and Productive Physiology
Mr Bernard B. Otindo	Kenyan	2. 1.75	Armyworm
Mr Joseph Owor	Ugandan	1.12.73	Fine Structure
Mrs Nirmala Y. Patel	Kenyan	1. 3.75	Salivary Gland Physiology
Mr Daniel Punyua	Kenyan	1. 9.73	Tick
Mrs Elizabeth D. Kokwaro	Kenyan	1.12.75	Fine Structure
Mr B.M. Okot-kotber	Ugandan	13. 2.76	Termite Programme

RESEARCH ASSISTANTS

Miss Nafisha F. Darji	Kenyan	1.10.74	Salivary Gland Physiology
Mrs Jedida Kongoro	Kenyan	16. 4.74	Salivary Gland Physiology

TECHNICAL STAFF

	NATIONALITY	DATE OF APPOINTMENT	PROJECT/UNIT
Mr Julius O. Apale	Kenyan	1. 5.74	Isolation Mechanisms
Mr Mathayo Chimtawi	Tanzanian	15. 1.74	Fine Structure
Mr Andrew Chapya	Kenyan	16. 4.74	Chemistry
Mr Joshua Kilori	Kenyan	1.11.72	Armyworm
Mr Hilary M. Kahoro	Kenyan	1. 5.75	Electrophysiology
Mr James Kagoiya	Kenyan	1.10.73	Insectary
Mr Peter Lisamulla	Kenyan	1. 2.73	Fine Structure
Mr Morton Lubega	Ugandan	1. 3.74	Armyworm
Mr Atashili Mando	Cameroonian	1. 3.73	Controller Technical Services
Mr James Maina	Kenyan	1. 3.75	Workshops
Mr Dominic Mathenge	Kenyan	1. 6.73	Armyworm
Mr Frederick Mukunza	Kenyan	14.11.73	Insect Development and Reproductive Physiology
Mr Yohana Musili	Kenyan	1. 5.74	Workshops
Mr Patroba O. Nyachieo	Kenyan	1.12.73	Workshops
Mr Raphael S. Ochieng'	Kenyan	1. 5.73	Insectary
Mr James Ongudha	Kenyan	1.10.73	Insectary
Mr Philip Onyango	Kenyan	1. 1.74	Salivary Gland Physiology
Mr Stephen Othieno	Kenyan	1. 4.73	Insect Development and Reproductive Physiology
Mr John Wanyonje	Kenyan	1. 6.70	Insectary
Mr James Atema	Kenyan	1.10.75	Insectary

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PREFACE

The year under review saw notable advances being made in all the research activities in which the ICIPE was engaged. Some of the highlights are:

- The discovery of resident populations of African armyworm moths in parts of East Africa that may well modify the current migratory theory of armyworm outbreaks
- The discovery that moulting hormone analogues applied to soft ticks lead to the production of 'super ticks', which are larger, feed better, and are more fertile
- The continuing work on the elucidation of the chemical communication process in termite mound societies
- The demonstration that tsetse flies can indeed communicate effectively by means of sounds, most of which is at ultrasonic level
- The discovery that infective trypanosomes can and do live in the haemocoele of tsetse flies, which is now leading to a complete re-examination of the developmental history of *Trypanosome brucei* organisms within tsetse flies.

There are equally impressive developments elsewhere: the full establishment and high usage of the Fine Structure Unit, which is giving support to much of the research going in the ICIPE, as well as in collaborating institutes within East Africa; the highly supportive Chemistry Research Unit, which has completed several analytical projects of pheromones, active natural products, and similar compounds important for insect life (or its disruption); and the intensification of ICIPE's training activities.

These developments have been made attainable by a combination of vital factors: the internationality of the scientific community in the ICIPE, the international support that the ICIPE obtains and the realisation that it is serving the developing countries in a critical area of their developmental concerns, and the adherence to high quality as a criterion for evaluating ICIPE's research.

In this respect, the results and recommendations of the programme review by an independent international panel a year ago — to which I referred in the Second Annual Report (1974) — is germane. We are encouraged that the panel gave approval to the level of excellence and the orientation to relevance that we had established during the first three years of our functional existence.

The task for the future, as we enter our fifth year of research, is a challenging one: to continue to serve the developing world in their critical pest problem areas by carrying on high-quality fundamental research on those vital questions that need to be answered in order that these pests might be controlled on a long-term basis, with the least ecological and environmental pertubations. Our task is therefore goal-oriented, tuned to practical application.

Thomas R. Odhiambo

ICIPE Research Centre
Nairobi
10th March 1976

ADMINISTRATION

A number of landmarks were recorded at the ICIPE in the past year.

"The Northern Star" Building, ICIPE's first permanent building was formally inaugurated by Professor C.G. Bernhard on 9th June 1975. The building now houses the Fine Structure and Chemistry Research Units, the Library and the Administration. The Riverside Drive house was purchased but will not be used by the Centre until later.

Further afield, a laboratory, with living accommodation attached, was completed at the ICIPE Kajiado Field Station for use in research on Termite ecology. The Permanent Secretary, Ministry of Agriculture, Mr. S.D. Gathiuni was due to officially open the Mchwa Building of the station in January 1976. The Field Station was constructed with funds donated by the Swiss Agency for Technical Co-operation.

The Mombasa Biology Unit has now been renamed the ICIPE Coastal Research Station and a gift from the Netherlands will enable the Centre to maintain it until December 1976. At Mbita Point, near Homa Boy in South Nyanza District, negotiations are fairly advanced for acquiring land for an ICIPE field station for tsetse fly, armyworm, sorghum shootfly and stem-borer research.

A visiting Group headed by Professor Harvey Brooks evaluated the programmes of the ICIPE and the quality of its scientific research in January 1975. Its report was discussed in the Annual Research Conference at the ICIPE in June 1975.

Following the proposal made on behalf of the ICIPE by the United Nations Development Programme during August 1974 to the Technical Advisory Committee of the Consultative Group on International Agricultural Centres that operates under the CGIAR, the ICIPE Governing Board made a detailed application for acceptance and funding of its core programmes and activities from January 1977. ICIPE's report to the Centres, bearing detailed proposals on the planned research programmes and the justification for proposed capital development plans, was discussed by the Joint TAC/CGIAR in a meeting held at Chiromo on 22nd - 25th September 1975.

In order to strengthen the ICIPE management, the Governing Board decided on a number of changes. These included having a full-time Director from January 1976 or thereafter; the appointment of Senior Research Scientists as on-the-spot programme leaders and that of Consultant Directors of Research; and the appointment of a Director of Research for Pest Management. Long-term capital development planning was approved and the possibilities of acquiring land at Langata and Kabete were investigated. The decision was put to wind up the ICIPE Company, which will be replaced by a Board of Trustees composed of a consortium of donors and Academies of Sciences.

During the period, all the annual meetings of the ICIPE were, for the first time, held at Chiromo. The important meetings held during the period were:

(a) **The Annual Meeting of the Governing Board**, from 9th to 14th June 1975. Earlier, on 24th and 25th March 1975, the Board had held a meeting to prepare the ground for detailed discussions on the CGIAR Application by several ICIPE bodies in June 1975. The Executive Committee of the Governing Board met at Chiromo on 25th September 1975.

(b) **The Policy Advisory Committee** held its third meeting on 7th June 1975. The Committee reviewed Tsetse and Termite Research Programmes on 5th and 6th June 1975, respectively.

(c) **The African Committee** held its 6th Meeting on 11th and 12th June 1975, part of the session held on 11th being a joint meeting with the International Committee.

(d) **The International Committee** held its 6th Meeting on 10th and 11th June 1975, part of the session held on 11th June being a joint meeting with the African Committee.

(e) **The Directors of Research** held their 5th Annual Meeting on 2nd and 3rd June 1975 and reviewed Armyworm, Mosquito and Tick Research Programmes on 4th June 1975.

(f) **The ICIPE Company** held its Annual General Meeting on 10th June 1975 to consider and accept Estimates, the Annual Budget and other matters.

(g) **The Scientific Staff Advisory Committee** was instituted at the ICIPE, the Resident Science Council having been abolished.

The ICIPE was also the venue for a number of international conferences.

(i) **The Armyworm Study Workshops**
The ICIPE was host for the ICIPE/COPR/EAAFRO Armyworm Study Workshop, held at Chiromo from 6th-9th January 1975. The Study Workshop was very successful and suggestions have been made for similar seminars to be held from time to time.

(ii) **The International Atomic Energy Agency Conference** held on 24th-28th March 1975 and hosted by the Kenya Government.

During the period, six Research Scientists, two Experimental Officers and one Research Assistant joined the ICIPE, while four scientists and one Experimental Officer left after the completion of their assignments. The ICIPE Administration was strengthened with the recruitment of an Administrative Manager and a Communications and Training Officer, an Accountant and a Librarian.

The ICIPE said farewell to Mr. Brian Berry (Clerk of Works) and suffered the grevious loss of Dr. T. Hefnawy and Mr. K. Wanyonyi through fatal motor accidents.

The period may be described as transitional between the activities of the ICIPE since its inception and its future role as a fully established institution. And altogether more than 350 people visited the Centre.

TRAINING

One of the major objectives of the ICIPE is the training of young African post-doctoral scientists and senior technical staff through participation in its programmes. Its potential for specialised training is considerable, and post-doctoral training is by itself an important ingredient for a vigorous scientific community, particularly on the African scene.

The initial training programme was a modest one mainly because of the demands of administering a training project and its possible impediment to the growth of a young enterprise like the ICIPE. The ICIPE started in a small way by granting post-doctoral fellowships for two young African scientists who were enabled to spend considerable time at the Centre working as "Research Associates" on ICIPE core programmes. Their contribution was significant while their experience at the ICIPE stimulated them to mount similar research projects at their home institutions on their return.

The programme of Research Associateship is to be intensified and provision is being made for upto 6 fellowships a year from 1977. The Fellows will be able to spend six months at the Centre in the first year, and three months each for the following two years.

The Fellows will thus maintain organic collaborative contacts with the ICIPE for 3 years and in time there should be an alumni of a development – oriented invisible college of great benefit to pest research in Africa.

RESEARCH ASSOCIATESHIP PROGRAMME – SCHEDULE

	NEW	CONTINUING
1976	2 x 6	—
1977	2 x 6	2 x 3
1978	2 x 6	2 x 3 + 2 x 3
1979	2 x 6	2 x 3 + 2 x 3 + 2 x 3
1980	2 x 6	2 x 3 + 2 x 3 + 2 x 3

The first Fellow under the Scheme (from Ghana) for 1976 is already at the Centre and is training on Reproductive Physiology and Aestivation in Maize Stem-borers. But the programme of top priority for the ICIPE is the Research Training Fellowships in which it is hoped to take on 6 African fellows a year to undertake postgraduate work in Insect Science partly in their "home Universities" and partly at the ICIPE for both masters and doctoral degrees. Under the scheme, the fellows are to spend 1-2 years at the ICIPE, having done the course work at their home Universities. The ICIPE research would lead to a University of Nairobi degree and would be financed by the Centre. The aim is for the students to engage in areas the ICIPE is concentrating in and thereby add to the sum-total of ICIPE knowledge as well as enabling the ICIPE scientists to supervise their work. The first such student, sponsored by the East African Trypanosomiasis Research Organisation, Tororo, joined the ICIPE in 1975. And 3 ICIPE staff have registered (1976) with the University of Nairobi for the Masters degree.

SCHEDULE – RESEARCH TRAINING FELLOWSHIPS

	NEW	CONTINUING	TOTAL
1976	3	—	3
1977	6	3	9
1978	6	6	12
1979	6	6	12
1980	6	6	12

In the field of Professional training, high premium is placed on the training of technical staff, especially those involved in research programmes, workshops, and the insect and animal breeding unit. The Professional technical manpower training programme is another area of top priority and the first full programmes (to start in 1977) will involve short-term (4-6 months) courses for senior technicians from African countries. The training will be in specialised areas of the ICIPE's technical expertise. Five trainees will be taken on in the first half of each year and another 5 in the second half so that 40 trainees are covered in 4 years.

At the moment, technical training is partly at the Centre as well as at the Kenya Polytechnic. Where necessary, this training has been topped up by training in institutions abroad. In this latter connection, ICIPE technical staff have had advanced training at the U.S. Department of Agriculture research institutions (insectary training), International Atomic Energy Agency (IAEA) in Vienna (tsetse breeding), Cambridge and Manchester Universities (radioisotope techniques), Miami and Cambridge Universities (Electron Microscopy) University of Berne (termite research techniques) Karolinska Institute in Stockholm (acoustic research techniques) and the International Institute of Tropical Agriculture in Ibadan (for greenhouse techniques and the breeding of phytophagous insects). This training is continuing as new projects come into being, new staff are engaged and as it becomes necessary to update the skills of experienced staff.

The ICIPE has also from time to time mounted specialised courses for training senior technicians from other African countries. Tsetse breeding at the ICIPE is a noted success and already two technicians from Zambia and Nigeria have undergone training at the ICIPE in this field. A second Zambian trainee from the Zambia Museum is at the Centre for the training in Insect Breeding and Care and will soon be joined by a third technician (from the Zambian National Council for Scientific Research) to train in tick breeding. From Nigeria, a senior technician has been taking specialist training in Electron microscopy at the ICIPE. Proposed similar specialised training will be in scientific photography, electrophysiological studies, Fine Structure techniques, and the maintenance of electronic equipment.

In addition to all this, the ICIPE has organised in-house training courses for its own technical staff to increase their knowledge in biological laboratory techniques.

A syllabus was arranged, the first term of which covers fairly general topics in Insect Biology, and the second term

concentrate on Laboratory techniques related to the requirements of the various ICIPE projects. The first full course (involving 12 students) was successfully completed in May 1975, and the first term of the second course was completed in January, 1976. The syllabus for the course consisted of the following:

Insect Biology, Animal and Insect Care at ICIPE, Insect Ecology and Behaviour, Insect Collection and Preservation in the field, Laboratory equipment, Basic microscopy, Basic histology, Chemical separation and analysis, Bioassay techniques, Statistics and Photography. There was also relevant practical work in each of these fields, where possible. At the completion of the course, eight students sat for two three hour examinations (theory and practical) and six of them reached a satisfactory standard.

LIBRARY AND DOCUMENTATION

1975 was a year of improvement in the Library Services. The Library moved into its present accommodation towards the end of 1974. Library furnishing and equipment donated by Britain through the British Council were received and installed in the year.

The Library added more than 300 books and added over 30 current periodical titles and purchased over 200 volumes of periodical back numbers covering 1965 to 1974. This was also made possible by a grant from the British Government. About 1000 reprints were received and work is progressing to have these reprints indexed.

The Library is endeavouring to collect as many reprints as possible on insect sciences especially those that are related to the type of research being carried out at the ICIPE.

ICIPE PUBLICATIONS

ICIPE Research Staff published over 40 papers in various journals and reprints of these papers are available from the Library on request. (See list of publication).

AFRICAN ARMYWORM RESEARCH

Director or Research:
Professor J.W.S. Pringle

Scientists:

1. Dr. D.J. Aidley 1973-74	Research Associate
2. Dr. M.H. den Boer 1974	Research Scientist
3. Dr. S. Khasimuddin (1973)	Research Scientist
4. Dr. Wei-Chun Ma (1972-75)	Research Scientist
5. Dr. B.L. Otindo (1975)	Experimental officer

PHYSIOLOGY

INTRODUCTION

The migratory behaviour in the armyworm moth, *S. exempta* is thought to redistribute the moth to regions where there are freshly sprouted food plants for the larval stage, thereby ensuring better survival.

A proportion of pupae reared from wild larvae have been found to have an extended pupal duration lasting approximately six months instead of the normal 7-10 days (Khasimuddin, this report).

This is a preliminary report of the distribution in field collected pupae of pupal duration and the rate of oxygen consumption.

Reproductive behaviour

Extensive armyworm outbreaks in Kenya during 1975 made it possible to collect larvae from various areas and observe the behaviour of the adult moths emerging from these larvae in the laboratory.

In all samples, 95-100% of the adults to emerge did so between 18.00 and 24.00 hrs and there were some emergences between midnight and 12.00 hours. As reported in the 1974 report, both male and female moths attain sexual maturity (ability to attract or be attracted by the opposite sex) only after 2½-3 days.

In order to estimate the probability of multiple matings, the attractiveness of females was determined (by the bioassay method previously described) at various periods after mating. In separate experiments, it was shown that a single mating is adequate to enable a female to lay fertile eggs throughout her life.

The ability of males to mate with and inseminate more than one female could have a bearing on the population dynamics. By determining the ability of females subsequently to lay fertile eggs, it was shown that a male could inseminate only one female each night, but could inseminate fresh virgin females successfully for 5 successive nights.

It has been suggested that the fertility of females depends on their ability to feed on nectar. Preliminary results reported in 1974 suggested that access to water and sugar increased longevity. Further experiments were therefore performed in which females were presented on emergence with either distilled water, 10% or 20% sugar solutions and allowed to mate for 24 hours at 3 days. The average number of eggs laid was significantly greater in the females given 10% sugar solution from those given distilled water or 20% sugar solution, and there were indications that the period of oviposition was slightly extended.

The relative importance of migration and residual populations

It is of major importance in predicting the likelihood of outbreaks to know whether all infestations in Kenya are the result of migration from other areas or are derived from insects which have survived an unfavourable dry period. Early in 1975, it was discovered that 4-5% of the pupae resulting from larvae collected in the field in June emerged only after a period of 6 months, suggesting the occurrence of facultative diapause in a part of the population.

During successive visits to this area (Lambwe Valley Game Reserve) in the early months of 1975, three successive generations of larvae and moths were observed under circumstances that made it highly probable that they were derived from a residual population.

Larvae collected from various outbreaks and reared in the laboratory provided further evidence of delayed emergence from the pupa. One pupa, which did not emerge for 58 days compared with the normal 14-21 days, had an exceptionally low respiratory rate for 48 days of its pupal life and was probably in a diapausing condition.

In a separate series of investigations, a different method has been used to estimate the relative importance of migration. If there is extensive migration throughout the geographical range of the armyworm, populations are likely to be genetically uniform, whereas, if local races exist, some variation might be expected due to differing selection in different areas. The extent of genetic diversity is being determined by measuring the isoenzyme ratios in samples from different outbreaks in Tanzania, Kenya, Ethiopia and the Arab Republic of the Yemen. Only one isoenzyme system, for an esterase, has so far been investigated and no significant diversity has been found in the ratios of three Mendelian alleles.

Evidence of differences among fractions of the armyworm population has been found by analysis of measurements of wing lenghts in 6000 moths caught by light traps at various sites in East Africa between December 1973 and July 1974. Pooling the data by station-months it

is clear that although there is considerable variation within each sample, there are significant differences between samples which suggest mass movement of moths from one place to another.

Extended Pupal Duration in *Spodoptera exempta* *Lep Noctuidae)*

INTRODUCTION

Brown *et al.* (1969) considered the importance of migration in the armyworm moth, *S. exempta.* The migratory behaviour is thought to redistribute the armyworm moth to regions where there are freshly sprouted food plants for the larval stage, thereby ensuring better survival.

A proportion of pupae reared from wild larvae have been found to have an extended pupal duration lasting approximately six months instead of the normal 7-10 days (Khasimuddin, this report). Reporting on armyworm outbreaks in South Africa, Carnegie (1975) states that pupal duration in the species lasts from one to five weeks. Oliver (1969) recognizes as diapause a failure by individuals to eclose within the time taken by the majority. Mansingh & Smallman (1967) further characterized diapause by determining rates of oxygen consumption and development.

This is a preliminary report of the distribution in field collected pupae of pupal duration and the rate of oxygen consumption.

Table 1: Distribution of field collected pupae.

RANGE OF GROUP	NO. OF INDIVIDUALS	% INDIVIDUALS PER GROUP
a) Respiratory rates (μL 0_2/100 mg/h)		
1.1-20.0	8	7.4
20.1-40.0	50	46.3
40.1-60.0	30	29.6
60.1-80.0	14	13.0
80.1-100.0	4	3.7
b) Adult emergence (days)		
4-7	67	6.1
8-11	238	21.8
12-15	472	43.3
16-19	233	21.4
20-23	80	7.3

a) Respiratory rates (μL 0_2/mg/h)

MATERIALS AND METHODS

Pupae obtained mainly from the armyworm infestation on a wheat farm, Ruplex Estates, Nakuru, were reared in groups of 50 to 100 in Kilner jars under controlled laboratory conditions.

Oxygen consumption was measured using a Scholander microvolumetric respirometer at 25° C water bath temperature. Readings were normally taken at hourly or half-hourly intervals but in the case of a single low respiring pupa the duration was fifteen hours. Standardized insectary pupae were used as control. Emergence was recorded daily. Sex was determined by pulling the tuft of hairs at the tip of the abdomen with fine forceps (Brown & Dewhurst, 1975).

RESULTS

The mean live weight of field collected pupae of unknown age was 173.3 + 34.5 (S.D.) mg, n = 27. Table 1 and Fig. 1 show oxygen uptake and pupal duration of these pupae. Respiratory rates ranged between 30.1 and 40.0 ul/100 ng/h Of the eight (out of 106) pupae shown in Table 1 to have a rate below 20.0 ul/100 mg/h, one had a

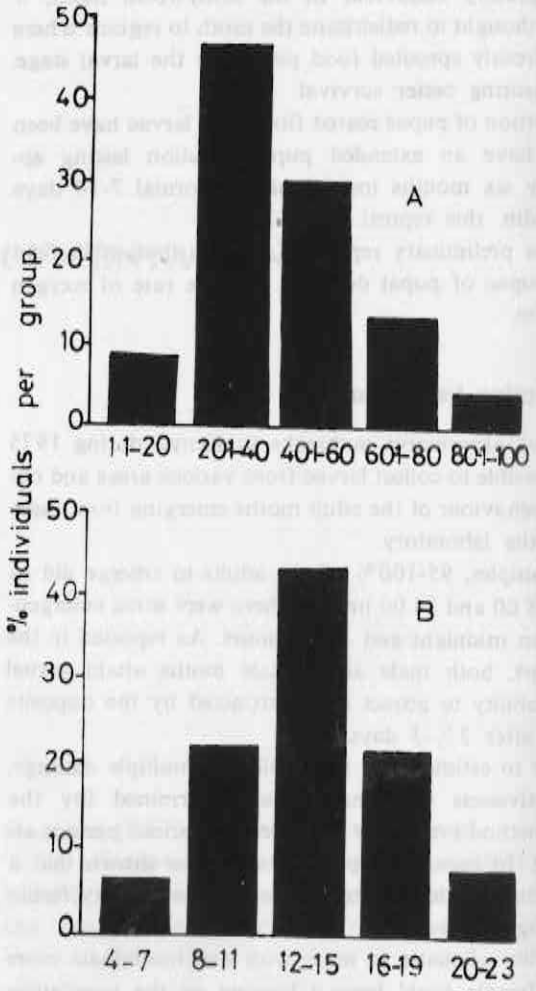


Figure 1 Percentage distribution of field collected pupae:a) rate of respiration, 1 0_2/100 mg/h; b) rate of emergence (days); n = 221 (16.2%) individuals failed to eclose.

low value of 1.3 ul/100 mg/h and the other seven had values about 13.0 ul/100 mg/h. Table 2 and Fig. 2 give the results for the low respiring pupa.

Table 3 shows live weight changes and respiratory rate for a sample of standardized insectary pupae.

Sex ratio was unity in all samples.

Table 2: Respiratory rate and weight changes of a single pupa with longer duration.

AGE (days)	LIVE WT mg	RESPIRATORY RATE $\mu L\ 0_2$/100 mg/h	EYE & BODY COLOUR
2	203.3	1.3	uniformly reddish-brown.
30	198.8	1.8	—do—
42	196.6	3.1	eyes & antennae darkening.
48	193.2	9.5	dark brown eyes & leg bristles.
55	185.4	20.4	increased abdomenal mobility; dark brown.
58	179.6	28.9	—do—

Table 3: Oxygen consumption rates and weight changes of standardised insectary pupae.

AGE	LIVE WT mg	$\mu L\ 0_2$/100 mg/h
days	Mean ± S.D.	Mean ± S.D.
−1 (prepupa)	142.4 ± 30.6	45.9 ± 7.0
0	120.8 ± 29.8	60.0 ± 17.0
1	118.9 ± 35.9	25.6 ± 9.7
2	115.5 ± 35.0	27.4 ± 7.0
3	111.4 ± 28.5	28.6 ± 8.3
4	106.0 ± 36.0	32.0 ± 12.5
5	101.6 ± 36.5	39.3 ± 13.7
6	97.9 ± 30.8	46.7 ± 18.5
7	96.1 ± 28.7	51.3 ± 13.4
8	92.1 ± 31.2	59.5 ± 11.8
9	89.5 ± 29.0	71.0 ± 12.9

(S.D = standard deviation; n = 18)

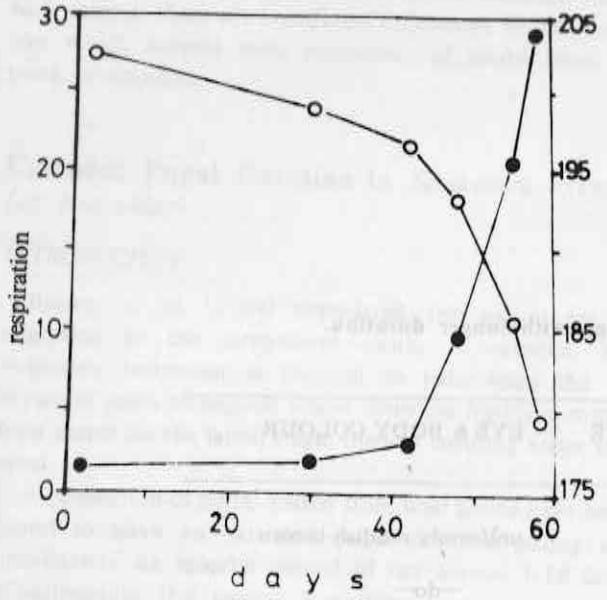


Figure 2. Rate of respiration and weight changes of a single pupa with longer duration (— —0_2 uptake, l/100mg/h; —0— weight, mg)

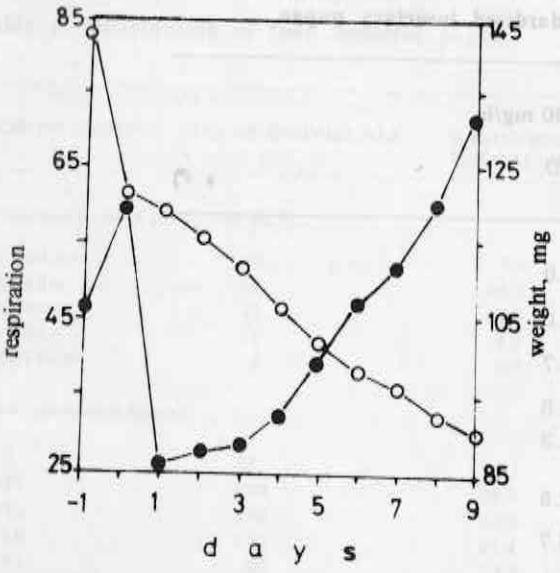


Fig. 3: Rate of respiration and weight changes of standardized insectary pupae (– – 02 uptake, 1/100gm/h; –0– weight,

DISCUSSION

Aidley (1974) found oxygen uptake values of 13.4 and 70.4 ul/100 mg/h for early and late insectary pupae respectively. These figures are consistent with those shown in Table 3. The pupa with an exceptionally low initial respiratory rate of 1.3 ul/100 mg/h had a pupal duration of 58 days and is probably in diapause. The condition of pupae with respiratory rates below 20 ul/100 mg/h is uncertain and more observations are needed before reliable conclusions can be drawn. Nevertheless a comparison of Tables 2 and 3 shows that at least one pupa exhibited a respiratory rate near maintenance level for a period longer than the normal total period of pupal development.

REPRODUCTIVE BEHAVIOUR

Studies on reproductive behaviour reported here are a continuation of the studies reported earlier (ICIPE Annual Report 1974). In the last report we discussed aspects pertaining to courtship behaviour in males, and the time required for sexual maturity in both sexes, etc. The present report discusses aspects of reproductive behaviour pertaining to the time of day adult emergence takes place, the reiterative mating ability of the males, attractiveness of mated females of varying ages to sexually mature males and oviposition by mated females under different sources of nutrition.

Adult Emergence

MATERIALS AND METHODS

Pupae and late larval instars were collected from various outbreaks and brought back to the laboratory. The pupae were transferred into kilner jars with sterilized soil, not more than 50 pupae per jar to avoid crowding of the emerging adults. For observational convenience the 24 hour cycle was divided into 4 quarters of six hours each starting at 00.00 hours. Observations on emergence were made at the end of each quarter and the number and sex of adults for the quarter recorded.

RESULTS

As the data on emergence presented in Table 4 shows, it is clear that adult emergence is predominantly during the period 18.00 - 24 hours i.e. from dusk to mid-night. This confirms earlier observations on the emergence and eclosion behaviour of adults at outbreaks in the field (ICIPE Annual Report, 1974). Some emergence does occur in the 12.00 - 18.00 hours period (see Table 4.) – a phenomenon also observed in the field (see Field Studies).

Table 4: Emergence pattern of adults, from different sources

	Sample 1 (from KASARANI) Total Pupae – 980 EMERGENCE				Sample 2 (from NAIROBI) Total Pupae – 230 EMERGENCE				Sample 3 (from KAMPI YA MOTO) Total Pupae – 125 EMERGENCE				Sample 4 (from TAITA HILLS) Total Pupae – 120 EMERGENCE				Control (Pupae from lab. culture) Total Pupae – 130 EMERGENCE			
	♂♂	♀♀	TOTAL	%	♀♀	♂♂	TOTAL	%	♀♀	♂♂	TOTAL	%	♀♀	♂♂	TOTAL	%	♀♀	♂♂	TOTAL	%
0.00-06.00 hrs.	4	4	8	1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
06.00-12.00 hrs.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.00-18.00 hrs.	16	7	23	3.83	3	3	6	4.40	2	2	3.07		0	0	0		0	0	0	0
18.00-24.00 hrs.	302	266	568	94.82	65	65	130	95.58	29	34	63	96.92	35	34	69	100.00	33	72		100.00
Sex ratio (♀♀:♂♂)	1.0:0.86				1.0:1.0				1.0:1.24				1.0:0.98				1.0:0.84			
Percentage total emergence	61.12				59.13				52.0				57.50				55.38			

Overall sex ratio 1.0:0.98

Overall percentage emergence = 57.026

Regardless of the place of collection of the pupae and larvae, the sex ration of adults that emerged successfully was fairly uniform (1:1). Even more interesting and significant is the data on overall emergence. Among the various samples, the percentage of pupae yielding adults ranged from 52.0% - 61.12% and the overall figure was computed as 57.06%. About 40% of original pupae died due to no apparent cause and this high mortality was consistent with our earlier studies (ICIPE Annual Report 1974) and emphasises the point even more.

Laboratory Evidence On the Occurrence of An Extended Pupal Period

In the course of studies on reproductive and post-eclosion behaviour pupae from field outbreaks were utilised in addition to pupae from the laboratory colony. Field collections were made mostly in June 1974 and pupations among the field collected larvae occured before the end of June 1974.

Most of the emergence occured between a week and 15 days of pupation and the resulting adults were utilized for behaviour studies. However, a considerable proportion of the pupae did not emerge at all, although these looked healthy from external appearance. Weekly observations were therefore made starting July for any late emergence. But till end of December 1974 there were no results, and during the first half of January 1975, no observations could be made. However, when the jars were opened in the last week of January 1975, adults which had emerged and died were found.

This emergence was definitely after December 1974 and the death of the moths was in all probability due to the non-availability of any food. It was unfortunate that no observations could be made during the first 3 weeks of January because the exact date of the late emergence cannot now be determined. However, it is quite clear that 4% - 5% of the pupae took as long as 6 months in the pupal stage as against the normal period of 7 - 10 days. Whether this is due to a diapause or aestivation and if so, what factors are responsible and how such pupae are different physiologically from normal pupae, are some of the questions posed at present and are to be investigated in the future.

Until these questions are answered the occurrence of such a phenomenon cannot be ignored for it is consistent with what happens in East Africa, where after a gap of 6 months, fresh outbreaks of a season start again in January-February in Southern Tanzania. For fresh outbreaks to start after a virtual absence of the insect there has to be a nucleus somewhere; and such a mechanism of extended pupal life may be the nucleus.

Attractiveness of Mated Females to Sexually Mature Males

Virgin females after attaining sexual maturiy are attractive to sexually mature males in that they can elicit courtship behaviour in such males (ICIPE Annual Report 1974). The attraction of males to mated females is of considerable importance to the understanding of population dynamics in this species, especially as adults have been reported to migrate during their pre-mating period (Brown, *et. al.* 1969).

MATERIALS AND METHODS

Test females were obtained by setting up single pair matings of sexually mature females (2 ½-3 days) and males (2-2 ½ days). After a mating period of 24 hours the females were separated and marked for the day of mating. They were maintained for use in the experiments and were tested at age intervals of 24 hours starting from zero hours after mating.

Five females of each class were tested against varying numbers of males, under the bioassy set-up described earler (ICIPE Annual Report 1973). The response of each individual male in any given group was observed and the overall group response computed. The response produced by virgin females (sexually mature) was also computed and treated as the control.

RESULTS

Results from these tests are presented in Table 5 and the data are graphed in Figure 1. It is clear that the overall response produced by females keeps decreasing progressively after mating. As will be seen in a later section, one mating is sufficient for a female to be able to produce fertile eggs throughout her ovipositing life. This suggests that the production and/or release of the pheromone by females is not necessary after they have mated successfully.

Table 5: Response of sexually mature males to mated females of different ages

Age (hrs) of ♂♂ after mating ♀♀	No. of ♂♂ tested	Overall response (%)
0 hours	25	76.0
24 "	25	32.0
48 "	31	18.38
72 hours	38	6.15
96 hours	27	2.1
120 hours	21	0.0
144 hours	16	0.0
Virgin (3 day old)	25	80.0

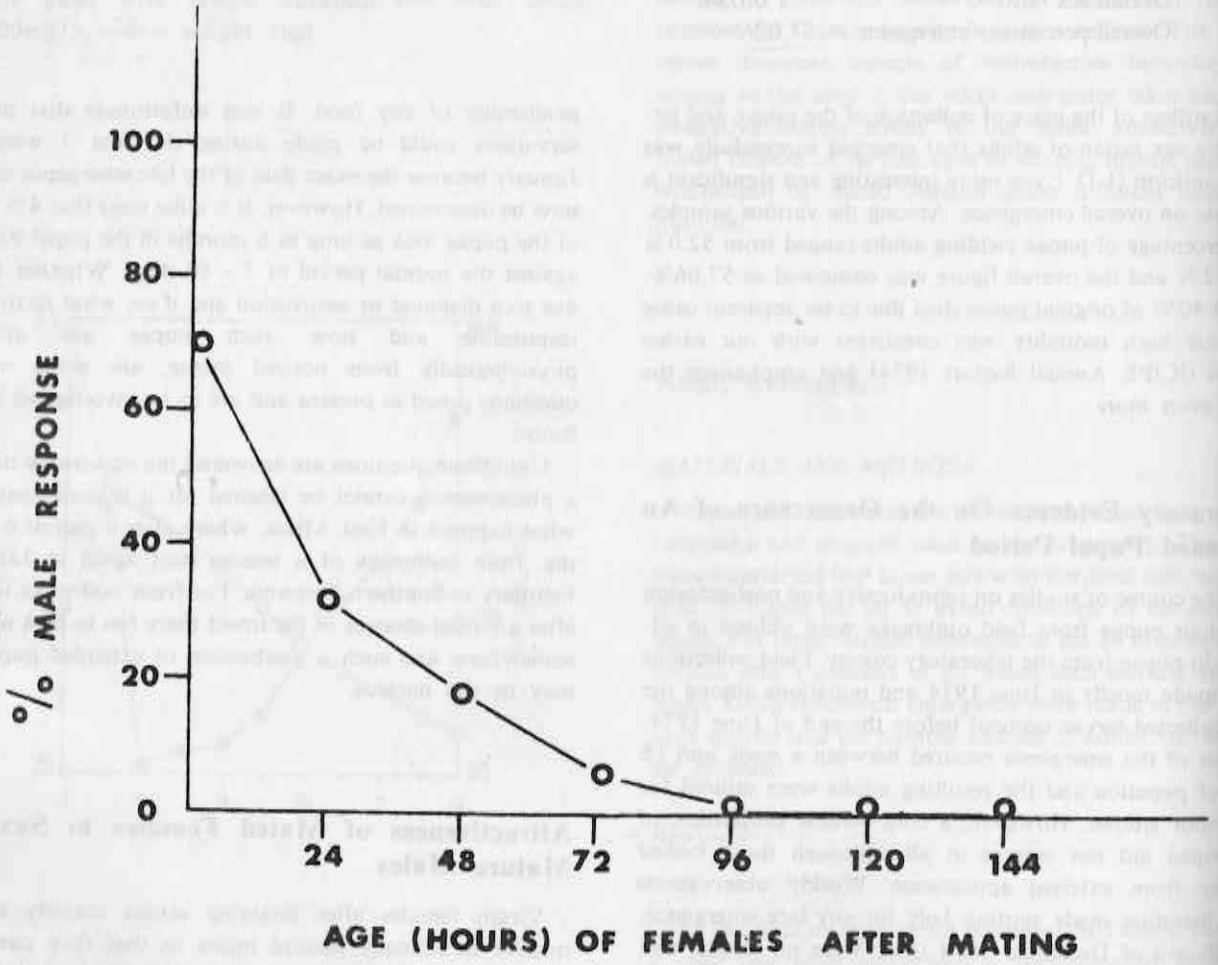


Figure 1: Courtship response of Males to Females of different post-mating ages, (Response by virgin Females 80%)

The Reiterative Mating Ability of a Single Male: The frequency distribution of males based on the number of matings performed by individual males is represented in Figure 2. The highest frequency was that of males performing 6 matings in a life-time followed by those performing 3 and zero matings. The high frequency of males failing to mate and inseminate is consistent with results reported earlier (ICIPE Annual Report 1974) and agrees with data concerning a related species, *S. litura* F, reported by Fugiie and Miyashita (1973). These authors speculated that the reason for such behaviour in males is continuous inbreeding (3 generations in their case). In *S. exempta* however, such erratic behaviour is also exhibited by males during courtship (ICIPE Annual Report 1974). It is difficult, therefore to assume any such reasons for this erratic behaviour though it seems to be a characteristic of this species.

The number of matings performed varied considerably between individuals, but when these were plotted against the longevity of respective individual males, the result was a linear correlation (Fig. 3). The number of females inseminated by a given male increased with the longevity of that male. The same trend has been reported for the related species *S. litura* by Fugiie & Miyashita (1973).

The data on oviposition by the inseminated females indicated that all females inseminated by single male would produce fertile eggs to the same extent regardless of whether a female was mated on the first night of the male's life. However in *S. litura* the fertilization rate of eggs differed from one experiment to another (Fugiie and Miyashita, 1973). But this difference could have been due to the fact that these authors let the eggs remain under uncontrolled conditions. It has been proved that even fertilised eggs dry up and shrivel under low humidity conditions.

As noted earlier and as indicated in a further trend of the data on the fertility of eggs one mating enables a female to lay fertilized eggs throughout her ovipositing life.

SUMMARY

Based on the data the following conclusions can be drawn:

1. Males exhibit very erratic behaviour in mating and inseminating females.
2. Any given male can only mate with and inseminate one female per night.
3. Males are capable of performing more than one mating during their lifetime, with equal efficiency.
4. The number of matings performed by a single male seems to be correlated to its longevity.
5. The fertility of eggs laid by mated females is independent of whether the effective copulation was the first or last mating of a given male.
6. One mating enables any female to lay fertilized eggs throughout her ovipositing life.

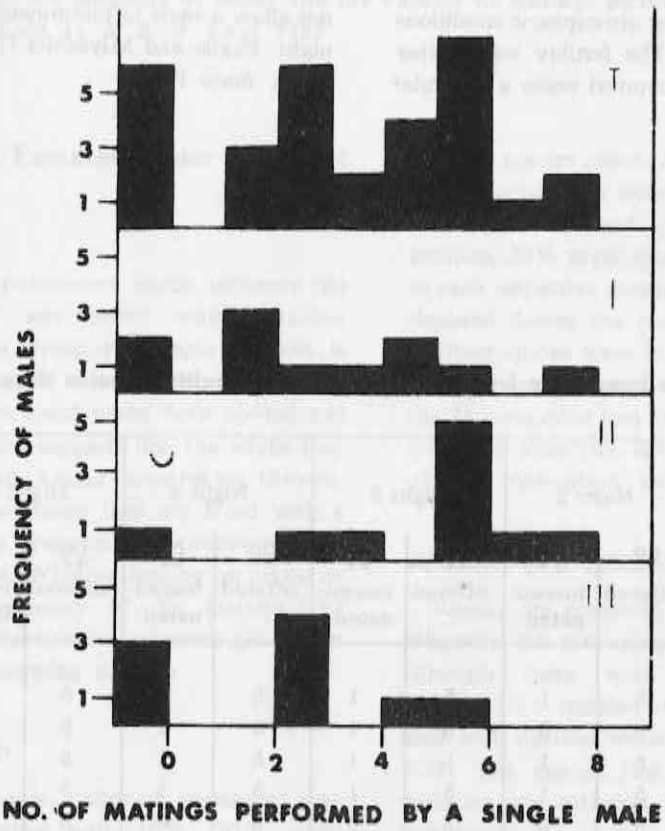


Figure 2: Frequency distribution of Males classified by number of matings during life (T is the total of 3 replicates).

Reiterative Mating Ability of Males

The ability of the male to mate and inseminate more than one female is of considerable importance in the entire study of the mating behaviour of this species.

MATERIALS AND METHODS

All the sexually mature females and males utilized in the experiments were derived from the laboratory colony, bred on maize leaves. Experiments were conducted under uncontrolled conditions of temperature, humidity and light.

Each male was kept in a 1 pint kilner jar provided with a cotton swab dipped in a 10% sugar solution for food. A 2" wide filter paper strip was introduced into the jar to serve as a resting place for the test insects.

After each female was introduced to a male, a mating period of 24 hours was allowed before the females were removed and set up for oviposition in kilner jars prepared in a similar manner as described above except that in the latter the inner walls of the jars were also covered by a hollow cylinder of filter paper corresponding to the diameter of the jar. This was done to avoid oviposition on the inner glass walls of the jars. Each male until its death was provided every 24 hours with a fresh sexually mature female.

The success or failure of mating was determined by the fertility of the eggs laid by the mated females — virgin females only lay unfertile eggs — (ICIPE Annual Report 1974). The eggs were then kept under controlled temperature and humidity room with c.a. 25 + 2° C and c.a. 75 + 5% R.H. Eggs kept under drier atmospheric conditions usually dry up before hatching. The fertility was further determined by (i) hatching larvae counted under a binocular microscope as well as (ii) eggs that changed colour; darke shades indicating development of the embryo (fertility) a against eggs not changing in colour and shrivelling (infertil — no embryonic development). Observations were also made (after mating) on the first egg batch laid, longevity o each female and the total number of eggs laid by each female. The experiment was repeated three times with a different set of males each time. The ability of the males to mate and inseminate more than one female during the same night was also tested by providing individual males with 5 females each night and testing the fertility of eggs laid subsequently by such females.

RESULTS AND DISCUSSION:

Under the given condition of multiple choice not a single male, of the 8 tested, could inseminate more than one female each night for five consequetive nights. One of the eight males (12.5%) was not able to inseminate even a single female of the 25 offered (Table 6).

The data therefore suggest that a sexually mature male cannot mate and inseminate more than one receptive female per night. And it would appear that the preferred time of mating in this species which only lasts between 00.00-03.30 hours (ICIPE Annual Report 1974) is responsible for this. Under natural conditions, a male locates a calling female but before mating can occur successfully the two sexes both have to be physiologically ready. Such behavioural traits coupled with the relatively limited daily mating period may not allow a male to inseminate more than one female each night. Fugiie and Miyashita (1973) report similar findings for *S. litura* Fujiie.

Table 6: The ability of males to inseminate females offered under a multiple choise situation.

Male No.	Night 1		Nighr 2		Night 3		Night 4		Night 5		TOTAL	
	♀♀ offered	♀♀ insemi-nated	♀♀ offered	♀♀ insemi-nated	♀♀ offered	♀♀ insemi-nated	♀♀ offered	♀♀ insemi-nated	♀♀ offered	♀♀ insemi-nated	♀♀ offered	♀♀ insemi-nated
1	5		5	1	5	1	5	1	5	1	25	5
2	5	1	5	0	5	1	5	1	5	1	25	4
3	5	1	5	1	5	1	5	1	5	1	25	5
4	5	1	5	1	5	1	5	1	5	1	25	5
5	5	0	5	0	5	0	5	0	5	0	25	0
6	5	0	5	1	5	1	5	0	5	1	25	3
7	5	1	5	1	5	1	5	1	5	1	25	5
8	5	1	5	1	5	1	5	1	5	1	25	5

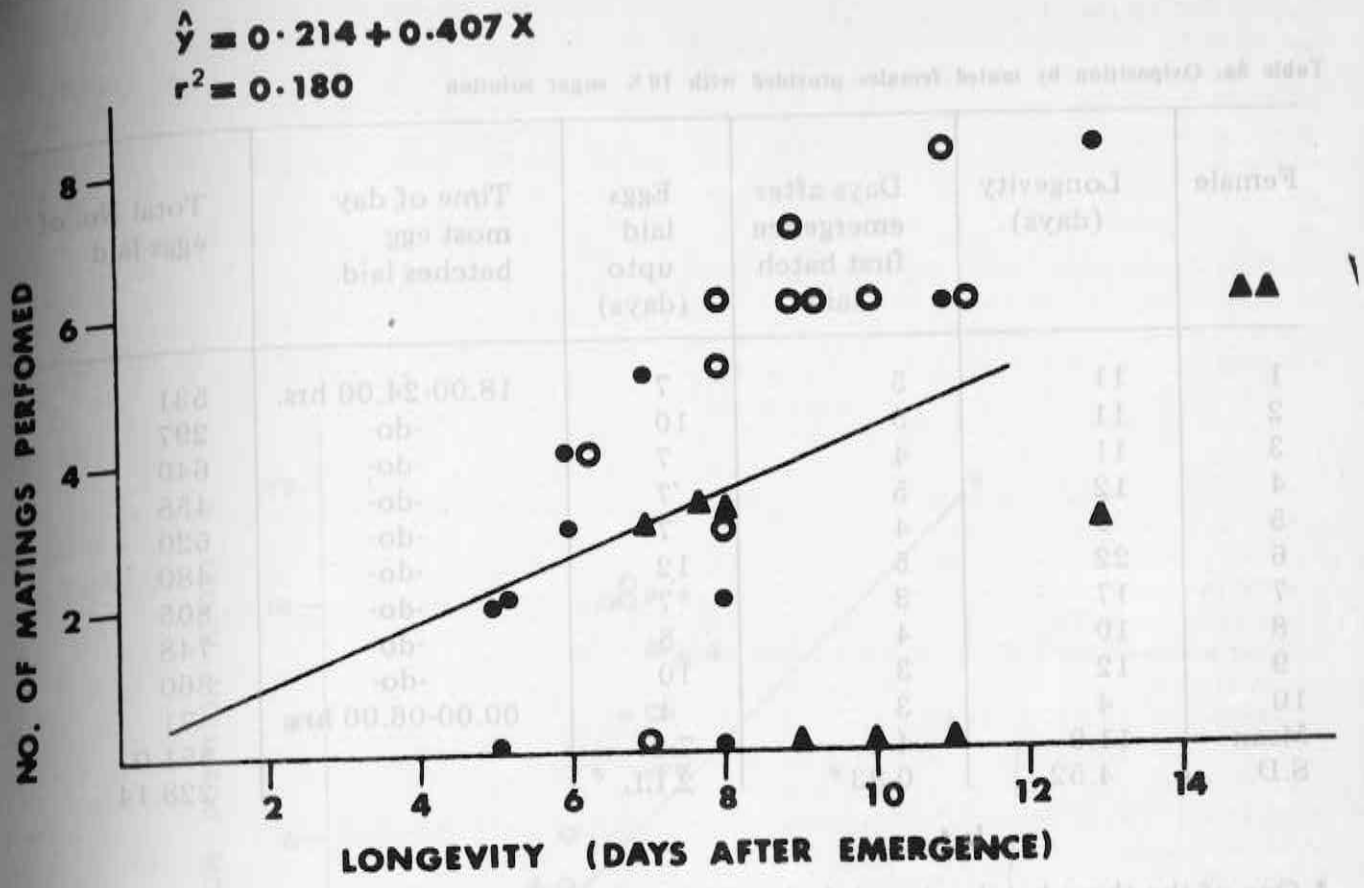


Figure 3: Relationship between longevity of males and the number of matings performed (● = Expt 1; ● = Expt 11 & ▲ = Expt 111)

Oviposition by Mated Females Under Different Sources of Nutrition

An investigation of the parameters which influence the reproductive potential of any insect which exhibits sporadicity to the extent that *Spodoptera exempta Wlk* does, is of prime importance with regard to an understanding of the population dynamics of this insect under both normal and outbreak situations. Literature suggests that the adults feed on nectar from sorghum head, Acacia blossoms etc. (Brown, *et. al.* 1969) and if for any reason they are faced with a situation where nectar is not available, they probably utilize dew drops as a water source. Whether feeding on nectar or dew drops affects the fecundity of the females and ultimately the size of population in the following generation was the purpose of the following studies.

MATERIALS AND METHOD

Freshly emerged females were collected, segregated into three kilner jars and supplied with 10%, 20% sugar solution or distilled water respectively until sexual maturity. These three sets of females were isolated from males for 3 days, and then single pair matings were arranged with sexually mature males. After a 24 hours mating period each mated female was isolated and set up for oviposition in a kilner jar. For food cotton wool dipped in 10% sugar solution, 20% sugar solution or distilled water was provided to each respective group. This was replenished as and when depleted during the course of the experiment.

Observations were made and oviposition times recorded for each female four times every 24 hours, having divided the 24 hour cycle into four quarters. Data was also recorded on female longevity, age of first oviposition, duration (days) of total oviposition, and fecundity.

RESULTS AND DISCUSSION

Results are presented in Tables 5a, 5b and 5c. Female longevity did not appear to be unduly influenced by diet although there were indications that longevity at 10% > 20% > distilled water. However the variation was least with distilled water (S.D = 1.48 as against 4.52 and 5.36). The age of first oviposition showed little if any variation with different diets—(4.10 days to 4.59 days after emergence).

Observations on the time of day most preferred for oviposition (tables 5a, 5b & 5c) showed that the preferred time of egg laying is between 18.00 - 24.00 hours.

Table 8a. Oviposition by mated females provided with 10% sugar solution

Female	Longevity (days)	Days after emergence first batch laid	Eggs laid upto (days)	Time of day most egg batches laid	Total No. of eggs laid
1	11	5	7	18.00-24.00 hrs.	531
2	11	5	10	-do-	297
3	11	4	7	-do-	640
4	12	5	7	-do-	458
5	9	4	7	-do-	620
6	22	5	12	-do-	480
7	17	3	7	-do-	805
8	10	4	8	-do-	748
9	12	3	10	-do-	860
10	4	3	4	00.00-06.00 hrs.	71
Mean	11.9	4.1	7.9		551.0
S.D.	4.52	0.83	2.LL		228.14

* One of the three batches layed during 00.00-06.00 hrs.
Total egg batches laid by females was 43, of which 3 (6.9%) were laid during 00.00-06.00 hrs.

Table 8b. Oviposition by mated females provided with 20% sugar solution

Females	Longevity (days)	Days after emergence first batch laid	Eggs laid upto (days)	Time of day most egg batches laid	Total No. of egg laid
1	13	4	9	18.00-24.00 hrs.	728
2	11	4	9	-do-	410
3	7	3	5	-do-	261
4	13	5	9	-do-	142
5	11	4	9	-do-	192
6	7	2	3	-do-	386
7	7	4	4	00.00-06.00 hrs	210
8	7	NO EGGS LAID			ZERO
9	12	9	9	18.00-24.00 hrs	7
10	14	NO EGGS LAID			ZERO
Mean	10.2	3.5 or 4.37	5.70 or 7.12		233.6 or 292.0 per laying female
S.D.	5.36		3.60		

* Only one batch laid
Total egg batches laid by 10 females was 20, of which only 1 (5.0%) was laid during 00.00-06.00 hrs.

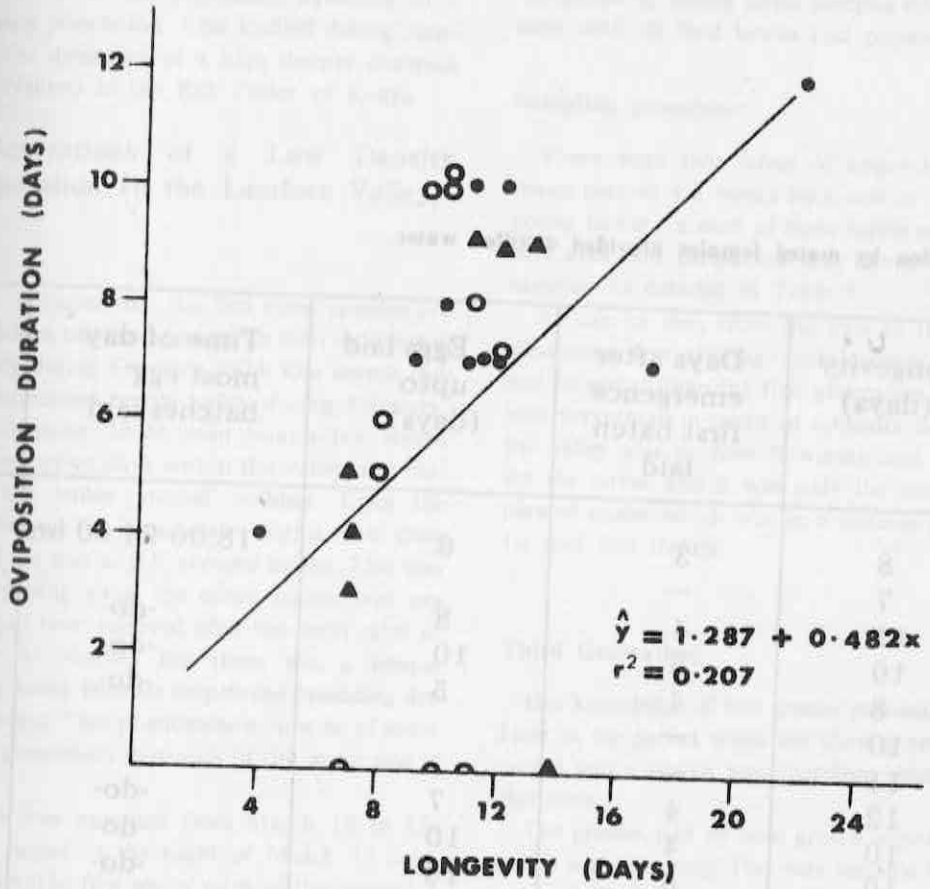


Figure 4: Relationship between age of female and duration of ovipositing period in mated females of *S. Exempta*. (● = 10% Sugar Solution; ▲ 20% Sugar Solution & ● = Distilled water

The average number of eggs laid by females fed 10% sugar solution was significantly higher than by those fed the other two diets (551 as against 233 and 192.1). This is understandable since the concentration of sugar in the nectar of wild flowers is closest to 10% than to the other two concentrations. The lower fecundity resulting from 20% sugar solution is consistent with what was reported earlier (ICIPE Annual Report 1974). However, what is of particular significance is that nectar is not an essential prerequisite for oviposition. Although the average fecundity is lower when sugar (nectar) is not available, the availability of water (dew) nevertheless permits satisfactory fecundity. Indeed imbibition of dew has been noted in the field regularly.

When the ovipositing life of a female is plotted against her longevity (Fig. 4) a positive correlation is evident. In effect, these data indicate that oviposition is a continuous process and is not limited to a particular period of a female's life.

Table 8c. Oviposition by mated females provided distilled water.

Female No.	Longevity (days)	Days after emergence first batch laid	Eggs laid upto (days)	Time of day most egg batches laid	Total No. of eggs laid
1	8	3	6	18.00-24.00 hrs	437
2	7	—			
3	11	4	8	-do-	247
4	10	5	10	-do-	310
5	8	4	5	-do-	99
6	10	—			
7	11	—			
8	12	4	7	-do-	344
9	10	4	10	-do-	251
10	10	8	10	-do-	233
Mean	9.70	3.2 or 4.57	5.6 or 8.0		192.1 OR 274.42 per laying female
S.D.	1.48	2.44	4.00		149.82

FIELD RESEARCH

The 1974 ICIPE Annual Report included discussion on the aspects of field research pertaining to pupation sites, post-eclosion behaviour and flight after emergence. It was also recorded that there existed local oral evidence for the build-up of populations in successive generations at the Lambwe Valley. To investigate this aspect, a sustained study was carried out at the Valley from February 1975 to the end of May 1975 on the population dynamics of a resident low density population. Also studied during June-July 1975 were the dynamics of a high density outbreak population near Nakuru in the Rift Valley of Kenya.

Successive Generations of a Low Density (Resident) Population In the Lambwe Valley

INTRODUCTION

Our 1974 data indicated that the first three consecutive armyworm generations became apparent to local residents in the Lambwe Valley during February 1974. Our search this year therefore commenced in the valley during February.

However, an intensive 20-24 man hour-a-day search mounted for 2 consecutive days within the valley and outside (South) of the valley yielded nothing. Even the operation of a light-trap for 3 successive nights from dusk until 2.30 a.m. did not trap any *S. exempta* moths. This was not altogether surprising since the entire region was dry because no rains had been received after the short rains in August 1974 (for 6 months). But there was a unique phenomenon in the valley because despite the preceding dry spell grass had sprouted. This phenomenon must be of some significance to the population dynamics of the insect and is discussed later.

A second search was mounted from March 10 to 13, 1975. But after it rained in the night of March 10 continuously for 4 hours (the first major rains of the season) 2 males were caught in a light trap on the night of March 11 and appeared to have freshly emerged from their wing condition. This indicated the presence of the first adults trapped during that season in the valley and it was presumed that if breeding took place in the area, the first larval population would be detectable in 2½ to 3 weeks time. Field inspections phased to coincide with successive generations were therefore organised.

First Generation:

The third search was therefore made from April 2 - 4, 1975.

The fresh grass was now fairly tough and about to flower. But there was still no signs of armyworm larvae on the grasses. However, a search of a small maize field (1½-2 acres) with young maize (2-3 weeks after germination) yielded 4 green larvae, 3 in the 5th and 1 in the 3rd instars which when taken to the laboratory and maintained under ca. 25° C and 75 + 5% R.H. constants, turned black within two days. These larvae presumably resulted from the same generation of moths that were trapped during the second week of March. But no attempt was made to collect all the larvae in the valley to check whether another generation would occur in the same area.

Second Generation:

Between May 6th and 16th 1975 larvae in their 4th, 5th and 6th instars were discovered on maize planted during the 2nd and 3rd weeks of April. And this was the 2nd consecutive generation in the same place. This generation was monitored by taking larval samples every day for about 10 days until all field larvae had pupated.

Sampling procedure:

There were two farms of approximately 2-3 acres of maize planted 4-5 weeks back and in the suitable stage for young larvae. In each of these farms an approximately half acre area was earmarked and larvae in these areas were sampled as detailed in Table 9.

As can be seen from the data in the table this second generation was although considerably greater in numbers and in spread than the first generation (April, 1st week), it was very small in terms of outbreak density. The grass in the valley was by now flowering and therefore unsuitable for the larvae and it was only the small area of recently planted maize which was in a suitable state to support the 1st and 2nd instars.

Third Generation:

Our knowledge of this species pointed to the first week of June as the period when the third generation could be expected and a search was therefore timed to coincide with this date.

The grasses had by now grown almost chest-tall and the maize was maturing. The only suitable food source for the young larvae seemed to be a small patch of maize (3-4 weeks old) of about 1 acre.

A careful search of the older maize and grasses did not reveal any larvae, as had been expected. However, the 1 acre of young maize harboured five, green 3rd instar and one black 5th instar larvae. The drastic reduction in the population size from that of the preceding generation was very obvious. The duration between successive larval populations coincides with the normal development time (egg-adults) of the species (Hattingh, 1941).

It was therefore clear that between April and June 1975 at least three successive generations had occurred in the valley. Although the second generation increased in size, an outbreak was prevented probably due to lack of synchrony in the rains, the sprouting of fresh grass, the planting of maize, and the occurrence of the first adults of the season. This information points to the continuous presence (an endemic population) of armyworm in this area. Since environmental conditions were unfavourable at this time, no effort was made to check for a fourth generation.

Table 9: Sampling of larvae on maize on two farms in the Lambwe Valley.

Date	Farm I				Farm II			Total
	Larval Instar	COLOUR		Total	Larval Instar	COLOUR		
		Black I	Non-black			Black	Non-black	
1975								
May 8th	4th, 5th & 6th	—	6	6	4th, 5th 6th	1	32	33
9th	4th, 5th & 6th	—	5	5		3	22	23
10th	4th, 5th & 6th	—	5	5	3rd, 4th & 5th, 6th	3	26*	29
11th	5th & 6th	—	3	3	4th, 5th, 6th	1	12	13
12th	6th	—	2	2	5th & 6th	—	8	8
13th	6th	—	1	1	5th & 6th	—	5	5
14th	—	—	—	0	6th	1	2	2
15th	—	—	—	0	6th	—	1	1
16th	—	—	—	0	—	—	—	—

* include 3 larvae in their 3rd instar

Towards Understanding The Dynamics Of a High Density Population

Armyworm larvae were detected during the first week of June 1975 on wheat about 4-5 weeks old in a private farm at "Bahati," 12 miles east of Nakuru. These larvae were fairly uniformly spread over approximately 500 acres of wheat. A detailed study aimed at understanding the population dynamics of this species was therefore initiated on June 9th. The investigation lasted for an entire month and was based on daily population sampling of all different life-history stages.

Larval and pre-pupal sampling:

A study area of about 60 acres was selected, which was in two blocks of ~ 25 and ~ 35 acres divided by a farm road. Sampling of larvae was carried out on 25 fixed units of one square meter each distributed in a stratified random manner over the 60 acres which were marked and labelled. Each day the number of larvae, instars, and pre-pupae were recorded for each sample unit. Movement of larvae (5th and 6th instar) was observed particularly during the latter part of the study, when the food source was becoming depleted. This sampling no doubt introduces a variable into the data but is not discussed at the moment.

Table 9 gives the mean number of larvae of each instar and mean number of pre-pupae from the 25 samples for 17 consecutive days. Since detection of the first two instars is extremely difficult, these are not recorded. However, it is worth mentioning that these earlier instars (1st and 2nd) were also occasionally observed during the latter part of the study when the population was taking off, suggesting a new generation and overlapping broods or indeed an arrival of new migrants.

Table 10: Larval and pre-pupal densities per square meter in the study area. (Values are averaged from 25 samples each day)

Date	3rd instar	4th instar	5th instar	6th instar	Pre-pupae	Total
June 9th	45.0	187.88	176.96	96.72		496.56
10th	5.0	173.48	100.76	79.44	—	358.68
11th	16.5	142.72	191.92	169.56	—	520.70
12th	1.24	69.52	152.32	349.52	—	572.60
13th	6.36	45.88	111.92	377.36	—	541.52
14th	0.88	2.76	58.28	351.52	—	413.42
15th	—	—	13.4	685.52	5.80	704.72
16th	0.40	0.24	7.28	212.40	14.40	234.74
17th	2.64	9.20	16.04	74.04	25.88	127.80
18th	3.96	8.68	8.76	28.60	22.20	72.20
19th	1.68	3.32	3.04	13.44	7.60	31.16
20th	1.68	3.32	3.04	5.24	9.76	23.04
21st	1.44	2.96	2.64	7.08	16.04	30.16
22nd	1.44	0.92	2.36	5.64	10.88	19.60
23rd	—	1.12	1.04	4.16	5.48	11.80
24th	0.24	0.24	0.44	3.28	2.16	6.36
25th	0.16	—	0.32	2.64	0.08	3.20

Pupal sampling:

Pupae were sampled by digging up 1 square meter soil samples to a depth of 5 cm. Varying numbers of samples were dug up each day and the data is presented in Table 10 As can be seen the outbreak was particularly severe, with an average count of 466.33 pupae per sample and a highest count of 697.

In the later pupal samples in July, a considerably number of moths were found to have died during eclosion. In order to assess the mortality due to this cause two 1 sq. meter samples were assessed, the data from which are tabulated below.

It can be seen that this single factor alone accounted for between 20-30% mortality.

Predation on the pupae by an unidentified Staphilinid beetle larvae was also observed.

	Sample 1 (8.7.75)	Sample 2 (9.7.75)
Pupal	163	76
Unemerged pupae	79	20
Dead moths	103	27
% mortality during eclosion	29.85	21.95

Sampling for adults:

In order to assess the adult eclosion per unit area 3 emergence traps, 1 x 1 x 1 metres were set up at random and emerging adults were counted and removed each morning and recorded. Data is given in Table 11.

Emergence of adults was observed to follow the same pattern as reported earlier (ICIPE Annual Report 1974). However, it was observed that a good proportion of adults did not fly away after emergence since many could be seen each day in the vegetation, on the bare ground and under mud clods during the day time.

Samples of the fresh emergence were taken each evening and each day of the adults found during day time. Each sampling included the counting of all adults in approximately a half acre area chosen at random each time, care being taken to avoid the same area or parts of the same area more than once. Data from these samples is presented below:-

Table 11. Mean number of pupae per sample of 1sq. meter

Date	No. of Pupae	Remarks
18.6.75	75.00	Mean of 8 samples
19.6.75	172.00	Mean of 3 samples
21.6.75	244.00	Mean of 4 samples
24.6.75	231.50	Mean of 4 samples
25.6.75	412.50	Mean of 2 samples
29.6.75	466.33	Mean of 3 samples
3.7.75	236.00	Mean of 2 samples
8.7.75	53.0	One Sample only
9.7.75	123.00	One Sample only

Total pupal samples made = 28

As the data shows the major emergence took place for 4-5 consecutive nights. The moths seen during day time were disturbed in order to observe their behaviour under these circumstances. Most flew for a few yards only before resettling whilst others merely crawled along the ground for a few yards. Such adults were noticeable all through the day.

Flight Behaviour:

Moths freshly emerging during the evening were close observed for their flight behaviour. No activity was o served until mid-night when the moths which had be resting on the ground or grass-blades would start flyi around. On five consecutive nights (30th June to 4th Ju there was an extremely strong South to North wind th prevailed almost continuously. On each of these 5 nights t moths became wind born after mid-night and hundreds thousands were carried away in this manner.

A torch beam and the full beam of the head-lamps of car parked on a slope were used to make observations flight.

Adults appeared to be divisible into the two majo categories of "fliers" and "non-fliers" (observed during day-time). Close observations of the fliers indicated that they could be grouped into 3 sub-categories as follows:-

(i) Those that would fly 10 feet or higher above ground level and be carried away by the wind for unknown distances – these would be in the hundreds of thousands and by far the majority.

(ii) those that would fly at between 5-10 feet above the ground and would only actively fly for 50-100 yds before settling.

(ii) those that flew below a height of 5 feet and would fly distances not more than 5 yds before settling. It is likely that these were the adults observed during the day-time.

It would have been interesting to investigate physiological differences, if any, among these three categories, but the present studies did not permit such investigations. It is planned to undertake such investigations in the future if such outbreaks are observed again.

The most fascinating factor in all these flight studies was the coincidence of peak emergence and heavy winds. The question that arises is whether the moths carried away by these winds would have left the area at all in the absence of such wind. Answers to this can only be found by more detailed investigations. Further more, such flight was observed only during the nights of heavy wind and peak emergence. Although emergence continued afterwards, no mass "flight" was again observed.

Mass flights of this nature are traditionally regarded as migratory flights – (Brown, *et. al* 1969 and Brown, & Swain, 1966). But it is essential to differentiate the phenomena of "active migration" from that of "dispersal" of an insect species. The latter can and will occur for any given species of insects under given conditions such as extremely high densities and depletion of food sources in a given area. The flight observed during the present study in conjunction with the winds, cannot therefore be easily classified as *active migration* or simple *dispersal* and needs detailed investigation in order to understand the field-biology of the species.

remained in ... detected and observations on these are recorded below:

dicating the occurrence of overlapping broods. ... heavy larval density reached earlier, completely deple food supply and the larvae of the later broods were s on extremely rare occasional grass-blades.

During the later portion of the field study thousa moths were found to have died "natural" deaths. A generation from the hatching egg batches observe prevented probably due to the total depletion of the food source.

Date	No. of Batches	Hatched on	Remarks
15.6.75	2	19.6.65	In all cases hatching first instar larvae were observed to hang and drop on to vegetation by means of fine silken threads
16.6.75	2	19.6.75	
17.6.	1	21.6.65	
18.6.	5	23.6.75	
19.6.	2	23.6.65	
20.6.	4	24.6.65	
21.6.	3	23.6.75	
10.7.75	3		

Table 12: Adult emergence pattern as studied by using emergence traps

Date	NUMBER EMERGED			REMARKS
	Trap 1	Trap 2	Trap 3	
3.7.75	8	—	21	
4.7.75	44	—	21	
5.7.75	32	15	24	
6.7.75	28	28	25	
7.7.75	20	3	4	
8.7.75	15	3	1	
9.7.75	20	47	6	
10.7.75	9	31	2	
11.7.75	32	117	14	Heavy rain previous night
Unemerged live	34	268	6	
Dead	67	75	12	
TOTAL	309	587	136	

SENSORY PHYSIOLOGY AND FOOD SELECTION BEHAVIOUR

Involvement of Styloconic Pegs in Food Recognition

ICIPE laboratory tests have proved, contrary to earlier claims, that *S. exempta* larvae do not feed on non-graminaceous plants. The oligophagy of the species is clearly a strict one and cannot normally be broken even under condition of severe starvation stress. However, we have now found one particular non-graminaceous plant, cassava *(Manihot esculenta)*, on which at least part of a diet-reared population of larvae is able to survive. Within a population, the decline in resistance to feeding on cassava in the course of starvation is presented graphically in Fig. 5. It is shown that the rate of decline is much faster in larvae which had been reared entirely on artificial diet than in those which had been allowed to feed on maize. The two categories did

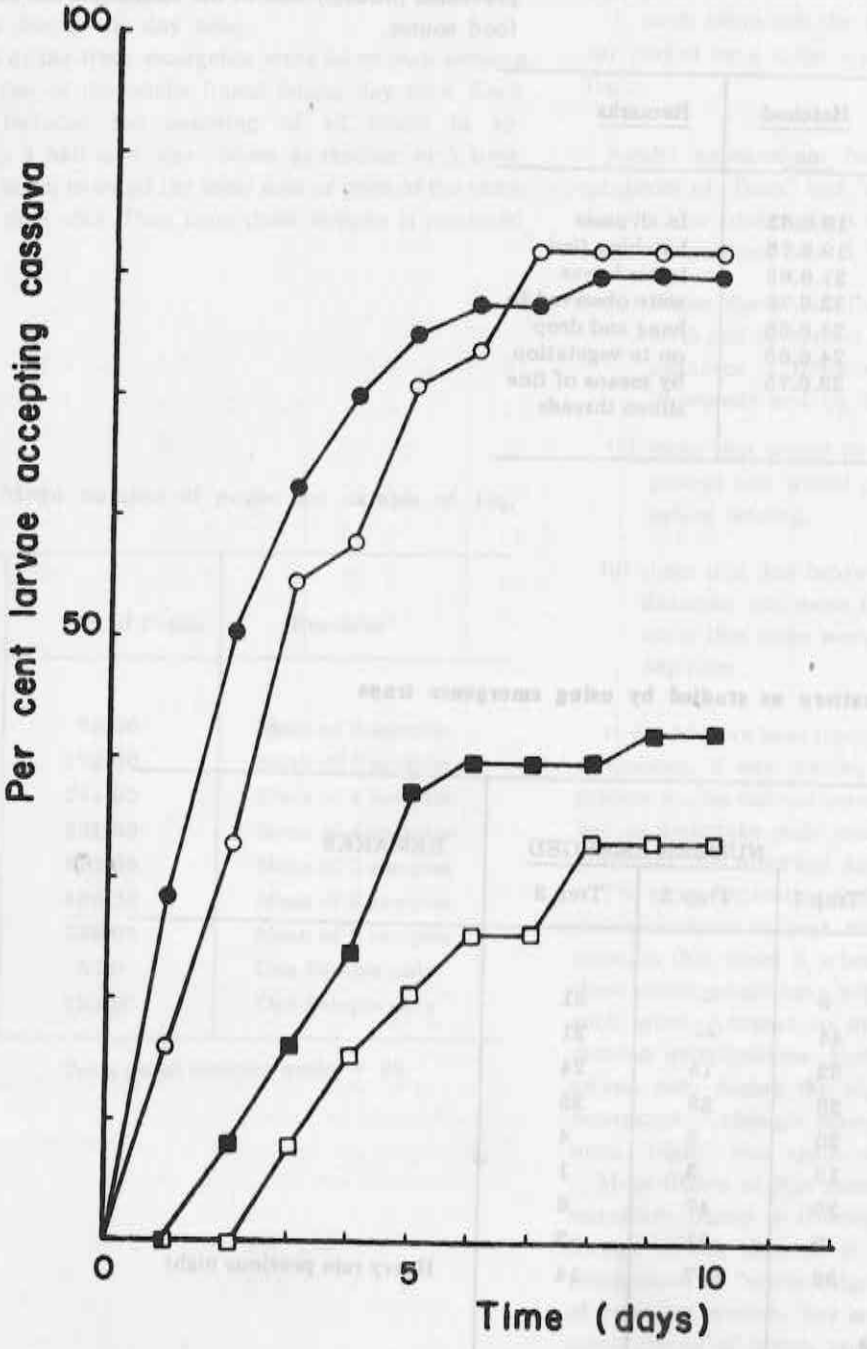


Fig. 5: Average cumulative rates of acceptance of cassava by diet-reared larvae after feeding for 18 hr on maize. Larvae were treated as follows:
bilateral maxillectomy (●); labrectomy (■); amputation of one abdominal leg (□). Another control group consisted of larvae bred entirely on artificial diet (O). Plots obtained from larvae with one abdominal leg amputated do not differ from the untreated control.

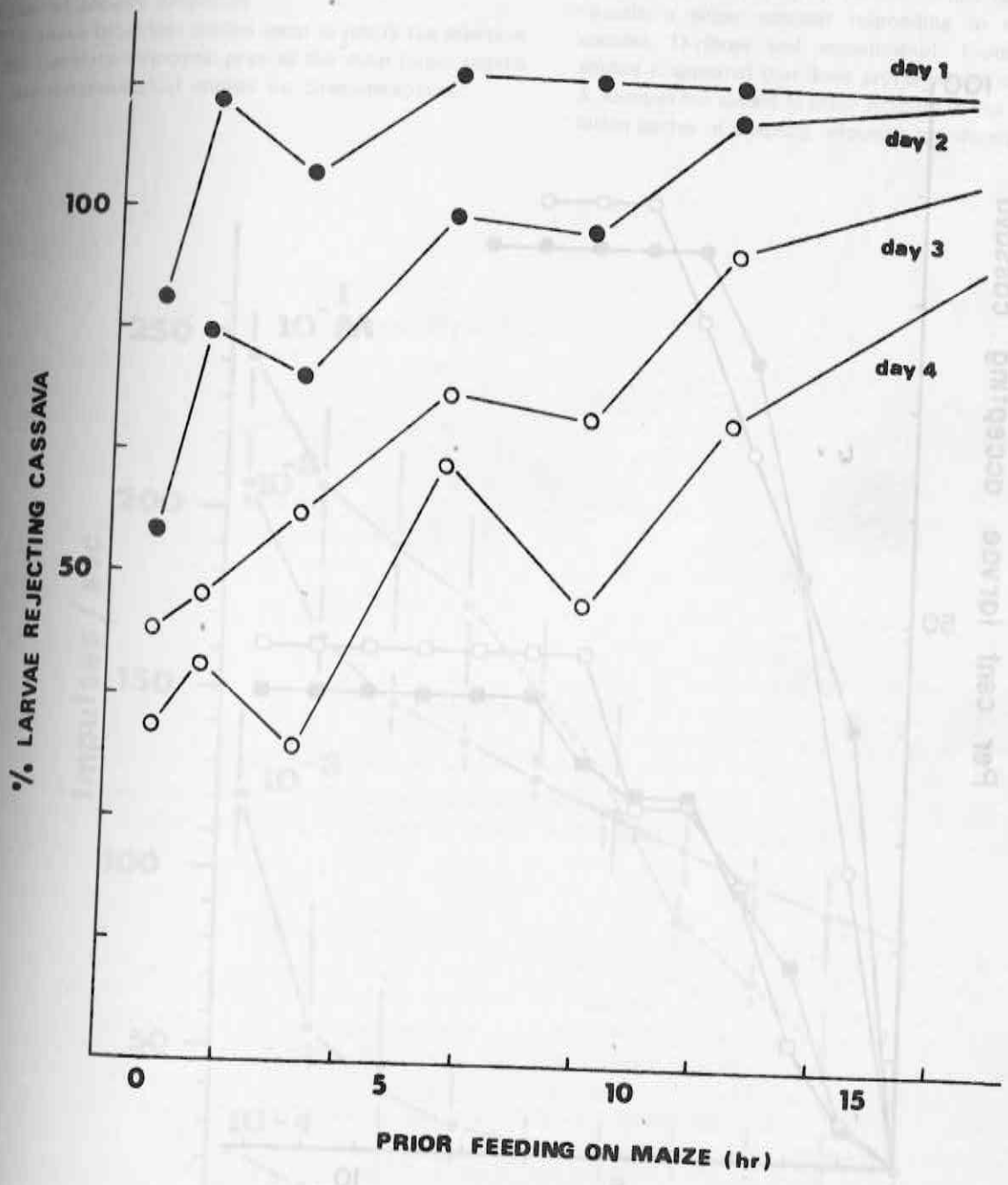


Fig. 6: The relationship between time spent by diet-bred larvae in feeding on maize and subsequent rejection of cassava.

not differ in longevity in the absence of food. If diet-reared larvae were allowed to feed on fresh maize during increasing periods of time the subsequent rejection of cassava increased accordingly (Fig. 6). Fig. 5 further shows that the food-influenced change in cassava-aversive behaviour could still be induced if the larvae were deprived of their labral sensilla, but this was no longer the case if the maxillary se silla were removed. Since the maxillae bear styloconic pe as well as palps the test was further refined by removi either the galeae (bearing the styloconic pegs) or the palp The results shown in Fig. 7 indicate that for induction of t behaviour change, only the styloconic pegs seem to

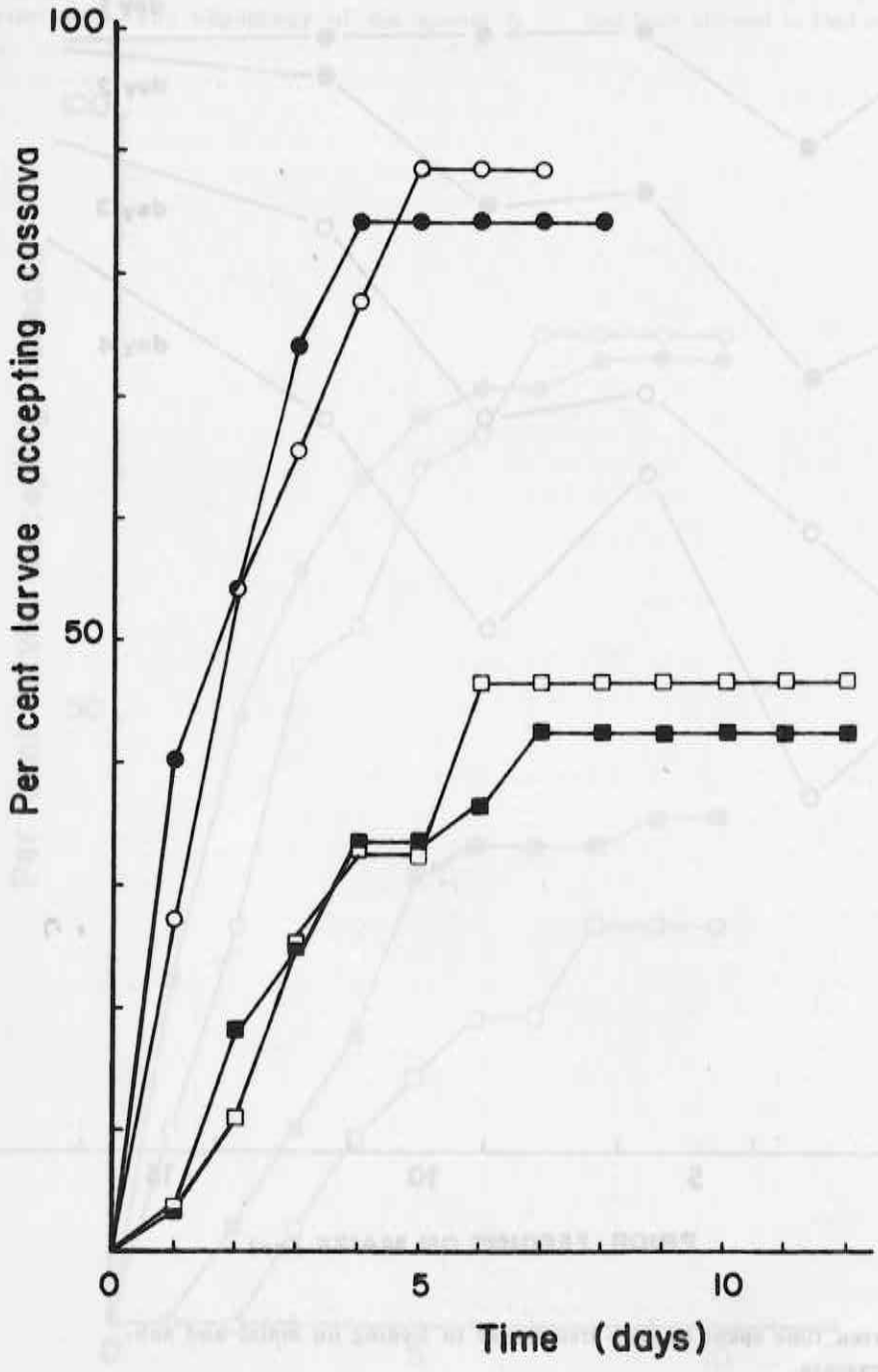


Fig. 7 Average cumulative rates of acceptance of cassava by diet-reared larvae after feeding for 18 hr on maize. Larvae were treated as follows: bilateral maxillectomy (●); bilateral palpectomy (□); bilateral galeactomy (○) amputation of one abdominal leg (■)

relevant. The effect apparently is associated with sensory information received by the larva via its styloconic pegs when it feeds on fresh maize. This particular information thus is absent in the artificial diet and presumably is related to certain foodplant-specific factors. It is of interest to note that an alien plant such as a cassava is nutritionally an adequate food for the armyworm but in the field is not attacked because of sensory properties.

The above behaviour studies seem to justify the selection of the maxillary styloconic pegs as the main target organs for electrophysiological studies on chemoreception.

Sugar Receptors

We briefly dealt with the importance of sugars in eliciting feeding behaviour in the larva (ICIPE Annual Report, 1974). Detailed investigations now afford a more exact picture of the two different sugar receptors possessed by the larva. The sugar receptor in the lateral pegs has a predominant sensitivity to sucrose, while the medial pegs contain a sugar receptor responding to stimulation by sucrose, D-ribose and mesoinositol. From comparative studies it appeared that these properties are not confined to *S. exempta* but extend to other Noctuid larvae. Typical adaptation curves of adapting responses are shown in Fig. 8 for

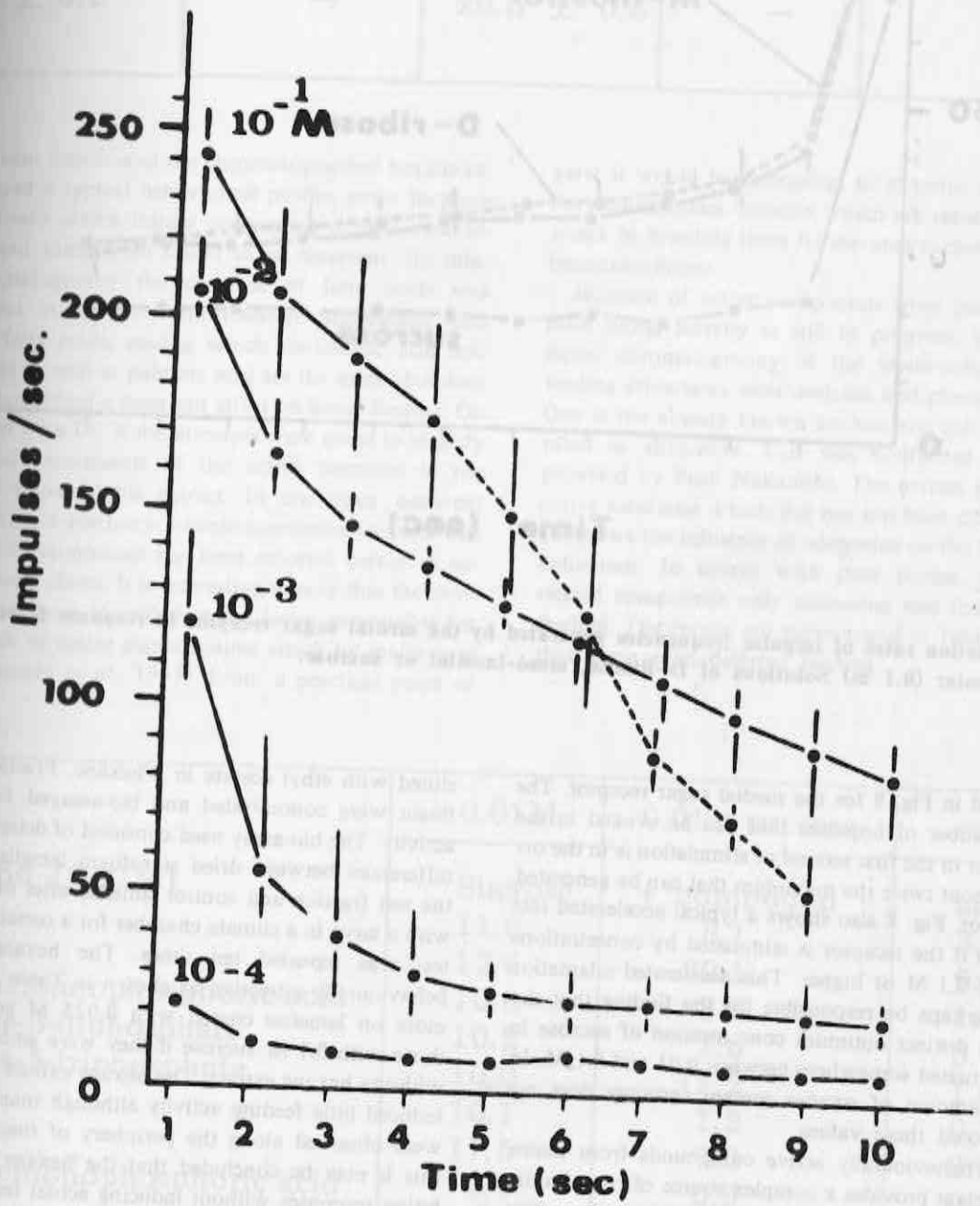


Fig. 8: Adaptation rates of impulse frequencies generated by the lateral sugar receptor in response to various concentrations of sucrose. Average values from 3 series of stimulations with extreme values (vertical bars) are shown.

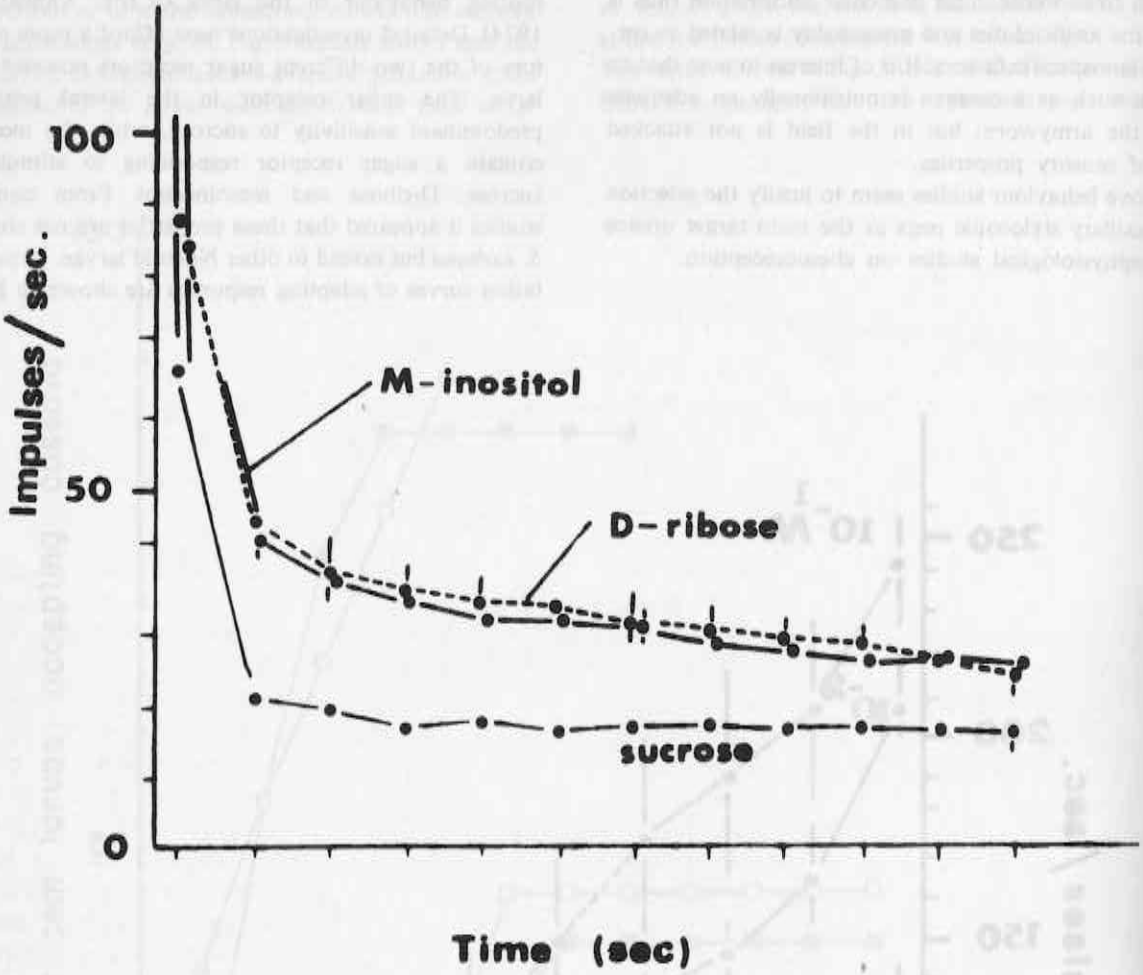


Fig. 9: Adaptation rates of impulse frequencies generated by the medial sugar receptor in response to stimulation by equimolar (0.1 m) Solutions of D–Ribose, meso-inositol or sucrose.

the lateral and in Fig. 9 for the medial sugar receptor. The maximum number of impulses that can be evoked in the lateral receptor in the first second of stimulation is in the order of 200, about twice the maximum that can be generated by the receptor. Fig. 8 also shows a typical accelerated rate of adaptation if the receptor is stimulated by concetrations of sucrose of 0.1 M or higher. This accelerated adaptation rate could perhaps be responsible for the finding that the larva have a distinct optimum concentration of sucrose in their food, situated somewhere between 0.01 and 0.1 M. In grasses the amount of sucrose present certainly does not normally exceed these values.

Isolation of behaviourally active compounds from maize

The foodplant provides a complex source of chemical information for the larva. In order to determine the active chemical contituents fresh maize was subjected to chemical fractionation. Young leaves were macerated in a blender with 80 per cent aqueous ethanol and the resulting extract was concentrated to water and re-extracted with n-hexane. The hexane extract was applied to a silica gel column and eluted with ethyl acetate in n-hexane. Fractions of the effluent were concentrated and bio-assayed for behavioural activity. The bio-assay used consisted of determining weight differences between dried styrofoam lamellae coated with the test fraction and control lamellae after incubating them with a larva in a climate chamber for a certain period. Each test was repeated ten times. The hexane extract was behaviourally attractive as shown in Table 13. Larvae fed more on lamellae coated with 0.025 M sucrose than on those with 0.1 M sucrose if they were additionally treated with the hexane extract. The hexane extract itself, however, induced little feeding activity although many feeding scars were observed along the periphery of the lamellae. From this it may be concluded that the hexane extract induces biting responses without inducing actual feeding (ingestion) responses. The water-soluble extract, on the contrary, induces distinct feeding responses. The concerted action of both types of stimuli would result in optimal intake as occurs when the larva feeds on its foodplant.

Table 13:

Assay	Blank Control	0.1 M Sucrose	n-hexane extract	0.025 M Sucrose	0.025 M sucrose + hexane extract
1		22.2 ± 1.4	0.8 ± 0.3	—	—
2		16.6 ± 3.0		3.1 ± 0.5	9.39 ± 1.4
3	0.6 ± 0.2	—	20.5 ± 0.6	—	—

The effluent fractions of the chromatographed hexane extract rendered a typical behavioural profile: some fractions were positively active (biting responses), others were indifferent, and some were found to be deterrent. By thin-layer chromatography the presence of fatty acids was demonstrated in one deterrent fraction. Subsequent tests with pure fatty acids, among which cis-linoleic acid and linolenic acid as well as palmitic acid are the most abundant in grasses, confirmed a deterrent effect on larval feeding. On collaboration with Dr. Kubo attempts were made to identify the chemical constituents of the active fractions in the hexane-and water-soluble extract. In one other deterrent hexane fraction 6-methoxy-2-benzoxazolinone (coixol) was identified. This compound has been reported before as occurring in maize plants. It is interesting to note that the same compound has been identified as the factor responsible for that resistance of maize plants against attack by maize corn borers (Smissman *et al.*, 1957). From a practical point of view it would be interesting to examine the possibility of developing maize varieties which are resistant to *Spodoptera* attack by breeding them for elevated contents of 6-methoxy-benzoxazolinone.

Isolation of active compounds from fractions which induce biting activity is still in progress. By high-pressure liquid chromatography of the water-soluble extract two feeding stimulants were isolated and chemically identified. One is the already known sucrose and the other was identified as adenosine. This was confirmed by NMR data provided by Prof. Nakanishi. The extract also contains an active substance which still has not been characterized. Fig. 10 shows the influence of adenosine on the larval preference behaviour. In assays with pure purine, pyrimidine and related compounds only adenosine was found to stimulate feeding. The results are summarized in Table 14, and show that some even deterred feeding.

Table 14:

Compound (0.01M)	0.01M Sucrose	0.01M sucrose + compound	compound alone
Adenine	11.6	8.1	3.0
Adenosine	12.6	20.1	9.2
Adenosinemonophosphoric acid	10.8	8.8	1.0
Adenosine-5-diphosphate	10.2	7.5	0.3
Adenosine-5-triphosphate	10,0	11.2	1.0
Caffeine	10.1	1.2	0.1
Guanosine	17.5	7.0	2.0
Guanosinemonophosphoric acid	9.9	0.3	0.1
Cytidine	14.8	14.6	1.8
Uridine-5-diphosphate	13.3	7.9	1.1
Uridine	11.3	6.9	0.7

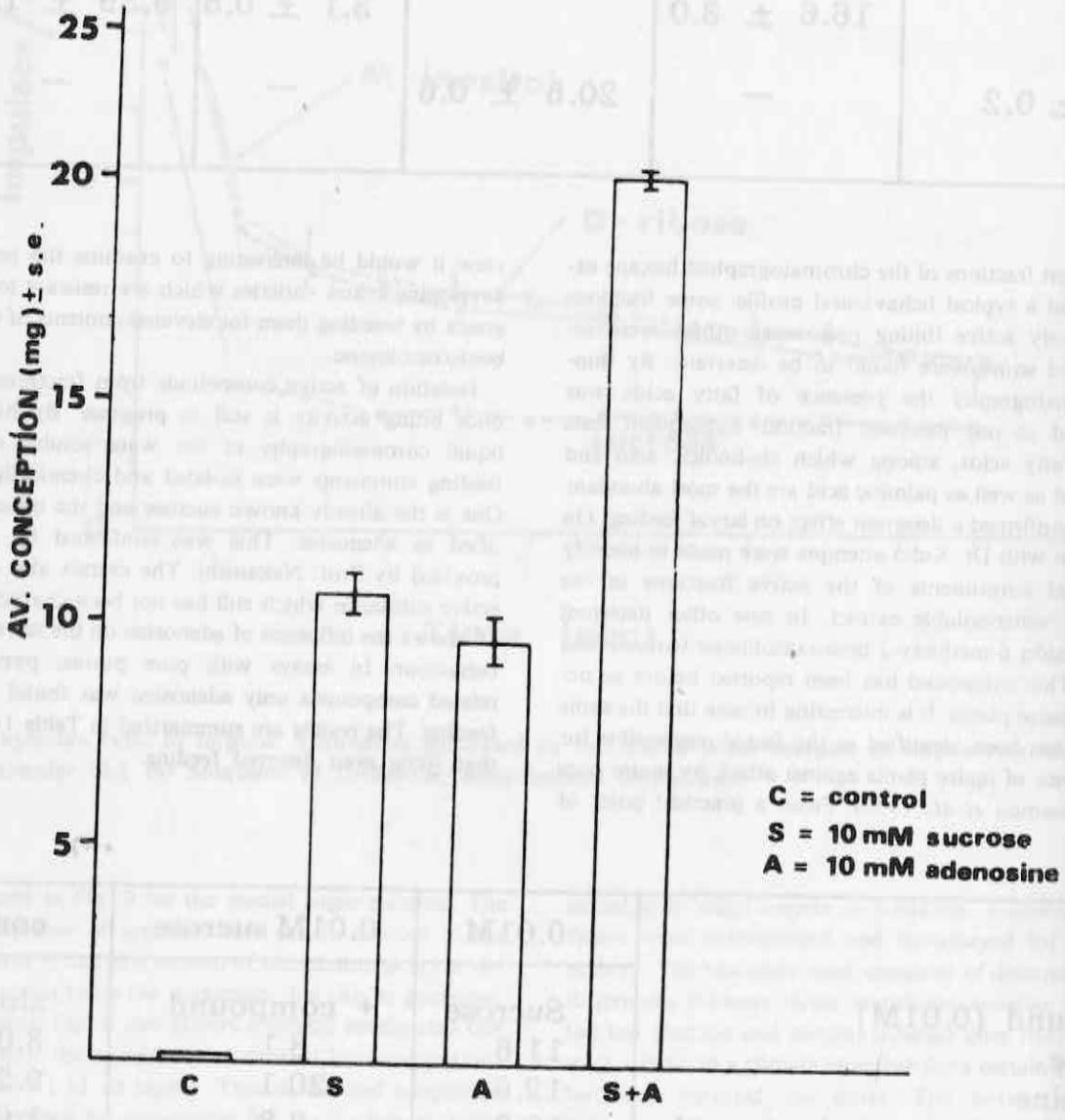


Fig. 10: Larval preference in a four-choice test between lamellae treated with sucrose, adenosine, mixture of sucrose and a denosine, or with solvent only (Control)

Adenosine receptors

In electrophysiological examination a receptor was identified in the lateral maxillary pegs of *S. exempta* which responded to stimulation by adenosine. The concentration-response dependence of this adenosine receptor is shown in Fig. 11. The arrow in Fig. 11 indicates that the amount of adenosine which we have isolated from fresh maize is within the range of the physiological sensitivity of the adenosine receptor which has a threshold near 0.01mM adenosine. Fig. 12 shows the adaptive responses upon continuous stimulation. In comparative studies with other *Spodoptera* species, including *S. littoralis* and *S. frugiperda*, we found an identical receptor in all species. As to how widespread the adenosine sensitivity is among plant-feeding insects is currently being investigated. In all species grasses form part of the range of food plants. In any case the finding of sucrose and adenosine sensitive receptors in the armyworm provides clear evidence for the importance of these chemicals in regulating feeding activity.

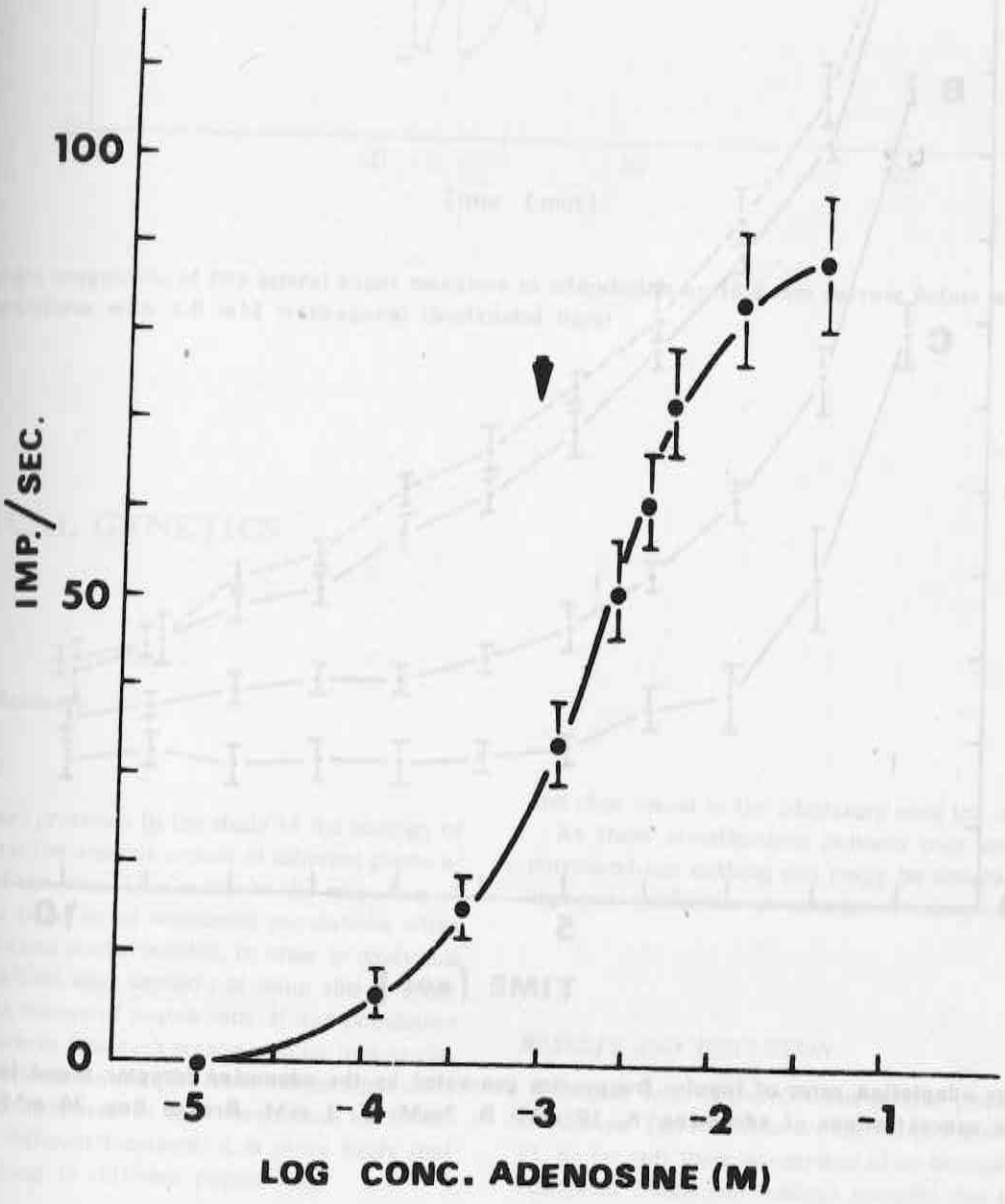


Fig. 11: Concentration–response dependence of the adenosine receptor present in the lateral maxillary styloconic pegs of *S. exempta*. The arrow indicates the approximate concentration of adenosine isolated from free maize leaves.

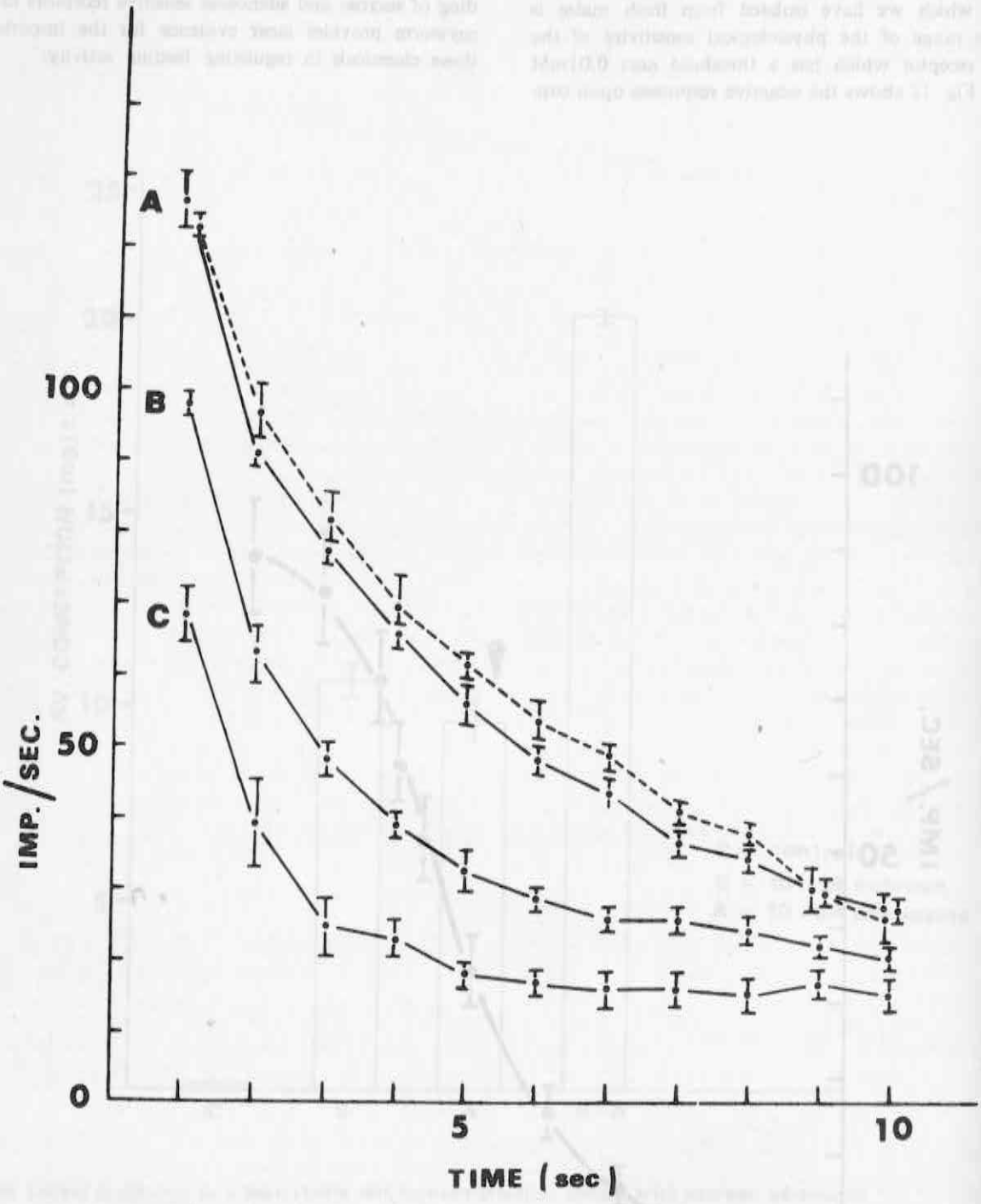


Fig. 12: Average adaptation rates of inpulse frequencies generated by the adenosine receptor found in *S. exempta* to various concentrations of adenosine. A, 10 mM; B, 3mM; C, 1 mM. Broken line, 30 mM.

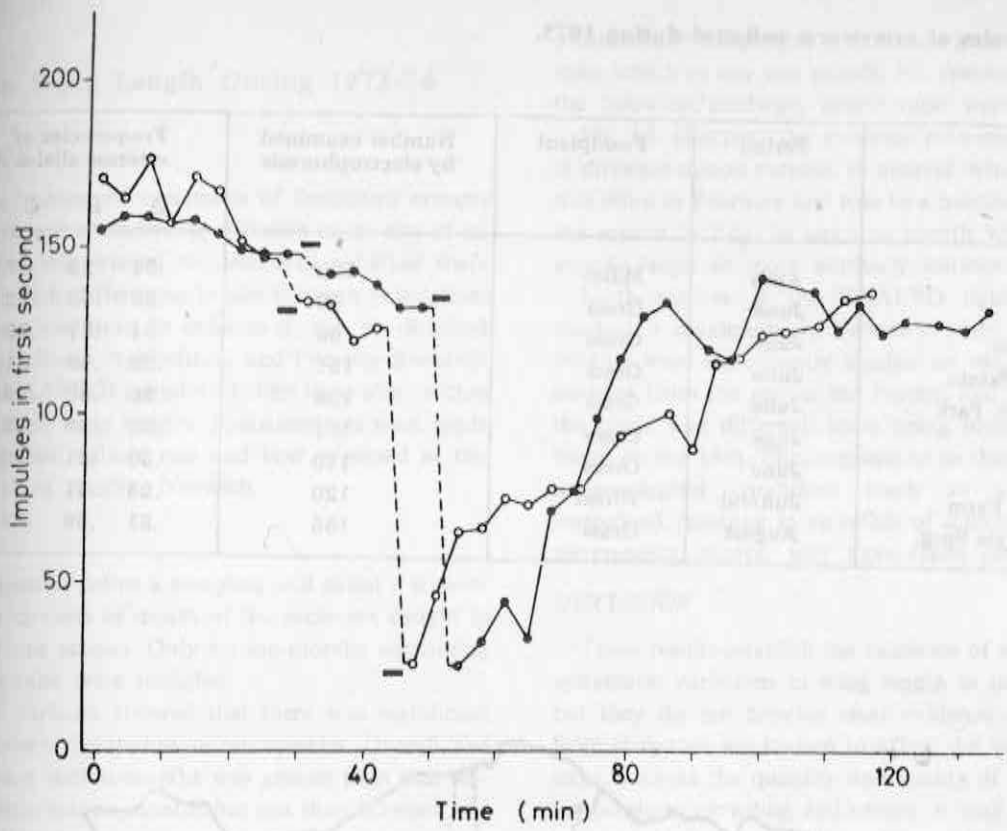


Fig. 13: Response magnitude of two lateral sugar receptors to stimulation by 10.0 mm sucrose before and after treating the sensillum with 1.0 mM warbuganal (horizontal bars)

ECOLOGICAL GENETICS

Alleloenzyme Research

INTRODUCTION

One of the great problems in the study of the ecology of armyworms is that the animals appear in different places at different times of the year. This is due to the migration of the moths or the build up of residential populations when the climatic conditions are favourable. In order to study this problem, investigations were carried out using alleloenzyme polymorphisms as tracers of populations. If one population spreads over the whole area each season, similar frequencies of alleles can be expected in samples from different places. If on the other hand differences in allele frequencies are found in samples from different locations, it is more likely that these samples belong to different populations.

MATERIALS AND METHODS

Samples of armyworms were taken from as many different places as possible. Normally, larvae were collected and then reared in the laboratory until the moths emerged.

As these investigations concern only one alleloenzyme polymorphism nothing can really be concluded from these apparent similarities in esterase frequencies.

RESULTS AND DISCUSSION

Samples were collected from several places in Tanzania and Kenya. The locations are shown in Figure 14 and Table 15. So far only three isoenzymes of an esterase are being investigated. Single pair matings revealed that they really are *alleloenzymes*. The frequencies of the three alleles are given in Table 15. From these data it can be shown that there are no significant differences between the frequencies of the three alleles in the samples from the different places.

Table 15: Samples of armywarm collected during 1975.

Location	Period	Foodplant	Number examined by electrophoresis	Frequencies of esterase alleles		
				A	B	C
Moshi	April	Millet	102	.34	.29	.37
Kajiado	June	Grass	60	.36	.43	.22
Olorgesaillie	June	Grass	66	.34	.37	.29
Drumvale Estate	June	Grass	151	.29	.39	.31
Nairobi Nat. Park	June	Grass	124	.26	.46	.29
Dagoretti	June	Grass	111	.32	.36	.33
Nakinya	June	Grass	120	.30	.41	.30
Nakuru B. Farm	Jun/Jul	Wheat	120	.25	.41	.34
Lodwar-Elyie Sprg.	August	Grass	155	.31	.36	.33

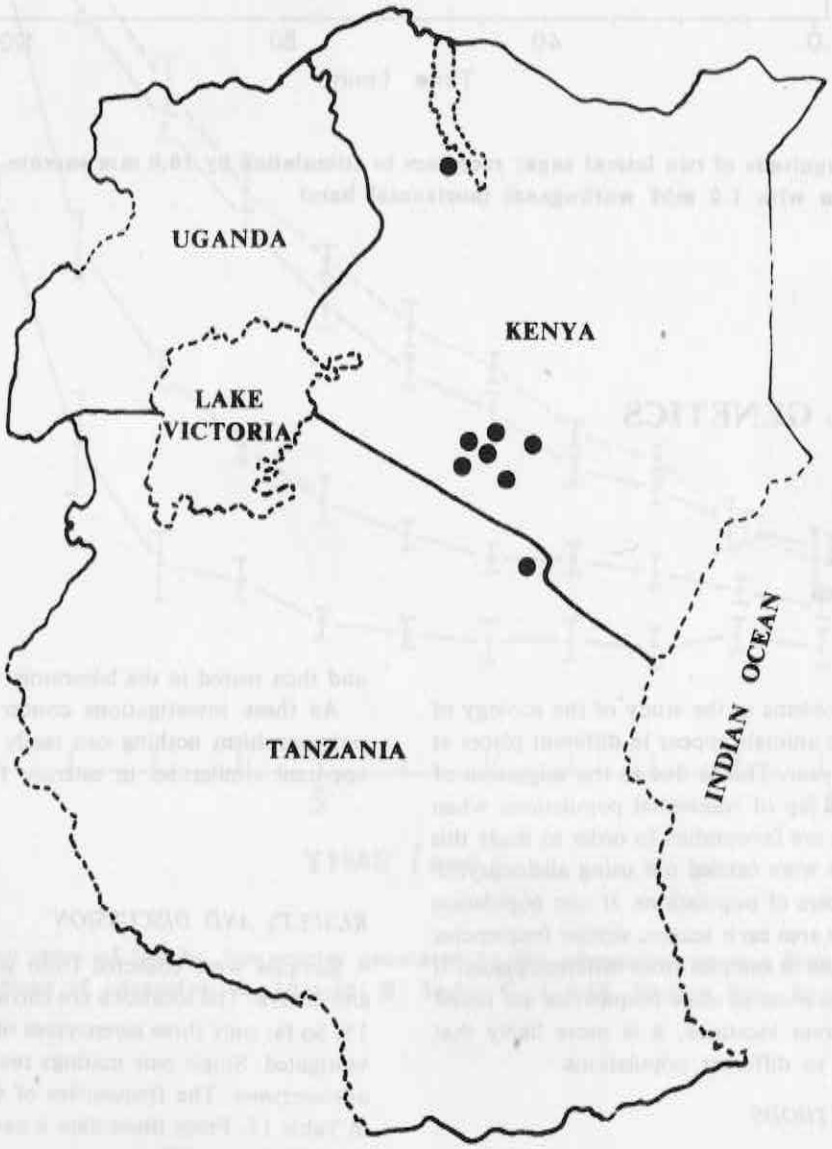


Fig. 14: Distribution of Location in East Africa where Armywarm are collected

Variation in Wing Length During 1973-74

INTRODUCTION

Inspection of preserved specimens of *Spodoptera exempta* showed that there is considerable variation in the size of individual moths. We wished to determine whether there were any systematic differences in size between populations separate in space and time. In order to do this we obtained from the East African Agricultural and Forestry Research Organisation (EAAFRO) samples of their light trap catches and measured their wing lengths. Measurements were made with a vernier steel caliper rule and later analysed at the University of East Anglia, Norwich.

RESULTS

It is convenient to define a sampling unit called a station-month, which consists of moths of the same sex caught in one month at one station. Only station-months containing 20 or more moths were included.

Analysis of variance showed that there was significant variability between the station-month samples. Overall, the variance between station-months was greater than that between days within station-months but less than between observations within days.

There was a negative correlation between the mean wing length in a station-month and its standard deviation. For male forewings, for example, $r = -0.72$ ($P > .001$). The mean female winglength was always greater than the mean male length in any one month. No systematic variations in the forewing/hindwing length ratio were detected.

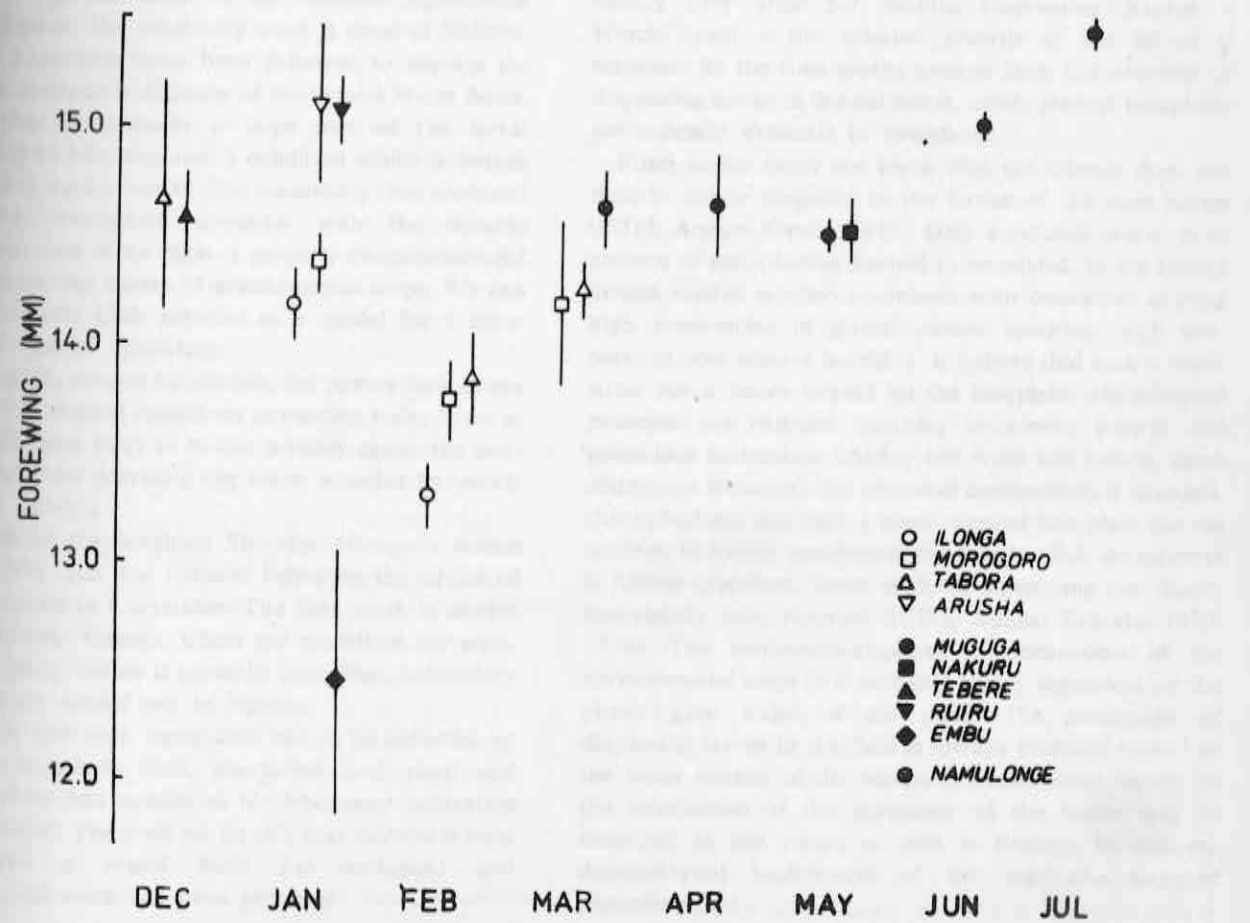
Fig. 15, illustrates the variation between the mean values in different station months. In general, wing length was at a minimum in February and rose to a maximum at the end of the season in July. In any one month wing lengths were usually larger at more northerly stations.

Moth catches at the EAAFRO light trap at Ilonga reached a maximum on February 16th. The mean wing lengths were significantly smaller on this day than in the samples from the rest of the month, and the sex ration of the catch was different, there being proportionately more males on the 16th. This suggests to us that some change in meteorological conditions (such as an incursion of westerlies), resutling in an influx of moths from a different geographical source, may have taken place at that time.

DISCUSSION

These results establish the existence of a phenomenon — systematic variations in wing length in time and place — but they do not provide clear evidence as to its origins. Several factors are known to affect the size of various insects, such as the quantity and quality of the food supply, temperature, crowding and others. It may be that large insects arise in relatively favourable conditions; if this is so it might be possible to use wing-length measurements in the prediction of future population changes. Further work is required to establish this possibility.

Fig. 15: Variation between mean values in different station months



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MAIZE-BORER AND SORGHUM SHOOTFLY RESEARCH

Director of Research:
Professor Jan de Wilde

Scientists:
Dr. John Clearwater (Sorghum Shootfly) — Research Scientist (July 1975)
Mr. Kenuel Ogwaro (Sorghum Shootfly) — Experimental Officer(1974)
Ir. Paul Scheltes (Maize-borer) — Research Scientist(1972)

INTRODUCTION

The Maize Borer and Sorghum Shoot fly *Atherigona soccata* project emerged from our former project on Seasonality and Aestivation diapause.

The Maize Borer programme was started in 1971. The field experiments are carried out in the Coastal area near Kikambala, with assistance of the National Agricultural Research Station. The laboratory work is done in Nairobi.

Several hypothesis have been followed to explain the remarkable aestivation diapause of the pyralid Maize Borer, *Chilo partellus*. Periodically a large part of the larval population goes into diapause, a condition which is broken by moistening during rainfall. The seasonality thus produced assures that oviposition coincides with the suitable physiological state of the plant, a property characteristic for many lepidopterous borers of graminaceous crops. We can therefore consider *Chilo partellus* as a model for a more generalised type of infestation.

After rejecting several hypothesis, the picture now seems clearer. Meteorological conditions promoting water stress in the plant are most likely to be the primary cause, the condition of the plant providing the token stimulus by which diapause is induced.

The study of the Sorghum Shootfly *Atherigona soccata* started in 1974, and was initiated following the advice of the Policy Advisory Committee. The field work is carried out in Kibos near Kisumu, where the conditions are semi-arid and sorghum culture is generally prevailing. Laboratory experiments are carried out in Nairobi.

During the first year, many data had to be collected on bionomics, taxonomic state, alternative host plant and seasonal activity and conditions for laboratory cultivation had to be defined. The goals set for this year have now been reached, and a sound basis for ecological and ecophysiological work has been provided.

Aestivation-diapause in *Chilo partellus (Swinhoe)* and *Chilo orichalcociliella* (Strand)

INTRODUCTION

The life-cycle of the two stem-borers which are currently studied – the Spotted Stalk-borer, *Chilo partellus* (Swinhoe) = *C. zonellus* (Swinhoe) and the Coastal Stalk-borer, *Chilo orichalcociliella* (Strand) = *C. argyrolepia* Hmps. show an almost perfect synchronization with both the climate and the growth-cycle of the hostplant (maize, sorghum, millet). The last instar larvae of the two stem-borers stop their development and enter a stage of dormancy when the weather conditions are unfavourable for further development of the plant. Only when a more favourable situation (rains) returns – under field condition in the Coast Province usually only after 5-7 months (September/October – March/April) – the arrested growth of the larvae is resumed. By the time moths emerge from the reservoir of diapausing larvae in the old stems, newly planted hostplants are normally available in abundance.

From earlier work we know that the climate does not directly induce diapause in the larvae of the stem-borers (ICIPE Annual Report, 1974). Only a reduced rate or even absence of precipitation seemed to be related. In the tropics limited rainfall is often associated with conditions causing high evaporation in plants: intense sunshine, high temperature, low relative humidity. It follows that such a water stress has a heavy impact on the hostplant: physiological processes are retarded, resulting in stunted growth and precocious maturation (thicker cell walls and cuticle, more compound structure); the chemical composition is changed. Our hypothesis that such a water stressed host plant has the qualities to induce aestivation-diapause in *Chilo* stem-borers is further examined. Some evidence supporting this theory has already been reported (ICIPE Annual Reports, 1973, 1974). The aestivation-diapause is independent of the developmental stage (but probably highly dependent on the physiological stage) of the plant. The percentage of diapausing larvae in the field is always inversely related to the water content of the hostplant stems. Some results on the termination of the dormancy of the larvae will be described in this report as well as findings on the endocrinological background of the aestivation-diapause phenomenon.

MATERIALS AND METHODS

Larvae were obtained from the experimental maize and sorghum fields of the National Agricultural Research Station in Kikambala (Coast Province) and the data on the induction and termination of diapause in *Chilo partellus* and *Chilo orichalcociliella* was obtained from this larval material. Experiments were also carried out with *C. partellus* larvae bred on a wheat-germ diet at the ICIPE insectary. A larva is considered to be in diapause when it survives in a piece of old, dry maize stem for a period longer than it takes 97.5% of non-diapausing larvae before they die or pupate under the same conditions. Figures on the incidence of diapause in the field, which are obtained by making use of this criterium, are conservative because the death rate of a larva in diapause is certainly accellerated by the biweekly disturbance of the larvae to check their survival.

Water contents of larvae and plant stems were determined by drying the material at 90° C till constant weight. The water content is expressed as percentage of the fresh weight.

Oxygen consumption of the larvae was measured with a Scholander Micro Volumetric Respirometer Model VR-300.

For the bio-assay of the juvenile hormone titer in the haemolymph, extracts were applied to *Galleria* pupae as described by de Wilde *et al.* (1968).

RESULTS AND DISCUSSIONS

I. Induction of Aestivation-Diapause

1. Field Results

Fig. 1 (A) shows the incidence of diapause as a percentage of the total larval population in an experimental field planted on June 16, 1974. Also plotted in this figure are data on the pigmentation of the larvae (1.A) (larvae entering diapause gradually loose their black pinacular spots and turn immaculate), the water content of the maize stems from which the larvae were collected (1B), the water content (1C) and the oxygen consumption (1D) of the larvae and the rainfall during the observation period (1E). The observations started at the time that the maize plants were still green and about to tassel, but – as it turned out – only shortly before the first immaculate larvae were observed.

As usual a decrease in water content of the maize stems corresponded well with an increase in the percentage of diapausing and immaculate larvae. The water content of the larvae which fluctuated around a 70% level at the start of the sampling, was found to be only 60% at the end of the experiment. There was a distinct relation with the rainfall indicating that diapausing larvae were capable of quickly restoring their water balance when water is available. Oxygen consumption of larvae in a sample averaged 2.6 μl O_2/mg dry weight/hr at the beginning of the observations and then gradually decreased to only 0.75 μl O_2/mg dry weight/hr at the end. Note also the decreasing standard deviation in the course of the experiment. A very remarkable observation is that both the water content and the oxygen consumption of the larvae were at the start of the observations – well below the figures obtained for non-diapausing larvae from the laboratory stock which had a water content of 80.0± 2.55% and oxygen consumption of 4.2 ± 1.2 $\mu l O_2$/mg dry weight/hr. It is tempting to speculate that our observations started during the prediapause period when the induction of diapause took place. During this period larvae are already physiologically preparing for the adverse environment which soon becomes very severe when the plant starts drying up completely. At that time larvae may still be in the process of turning immaculate and diapause may then not be detectable (fig. 1A) by our method. This is possibly because of a rapid change in available water when the larve are changed from the still fairly wet maize stem in the field to a piece of dry maize stem at the laboratory (material and methods) to which they are not yet fully adapted. It is of interest to see that in this experiment the maize plants were under considerable water stress when the first sample was taken following a period of two months of very light precipitation (Fig. 1E).

Conclusion: the initial response of the host plant to water stress seems to act as a token simulus for the larvae inside the plant, which anticipates unfavourable conditions for feeding and growth, constituted by the rapid lowering of the water content of plant tissues from 70-80% to 10-20%. They react to this warning signal by starting to prepare for diapause.

2. Laboratory Results

If the above statement is true, it should be possible to induce diapause "artificially" by transferring non-diapausing larvae into stems of maize for which these conditions are fulfilled. This has been tried in the following experiment with maize plants which had for six weeks been under dry (field) conditions after maturity. The leaves and leafsheaths of the plant were all completely dry, but the underlying stems were still green. Before the start of the experiment the plants were transplanted into plastic pots and watered for three days. Then all the leaves and leafsheaths were removed to facilitate the introduction of newly moulted 5th instar larvae into pre-drilled holes which were then plugged with plasticine. When a stem was later dissected, the number of larvae and pupae found inside and the colour of the larvae was noted. Likewise the water content of the different internodes were determined from time to time. Results are given in Table 1.

The normal period required for pupation of 5th instar larvae (on diet at 25° C) is 20.7± 6.25 days for females and 14.4 = ± 4.4 days for males. This period was well exceeded by many larvae. After 23 days no pupae were found, (only exuviae). Further evidence that diapause induction had indeed occurred was the colour of the larvae; they were still spotted after 6 days, but had almost all turned immaculate within 17 days. Unfortunately the deathrate of the larvae was rather high due to some technical problems during the introduction into the stems. When the experiment is repeated, a larger number of plants and larvae will be used and the observations will be made more regularly. It is however felt that the results are sufficiently convincing to permit the claim that we managed to induce diapause.

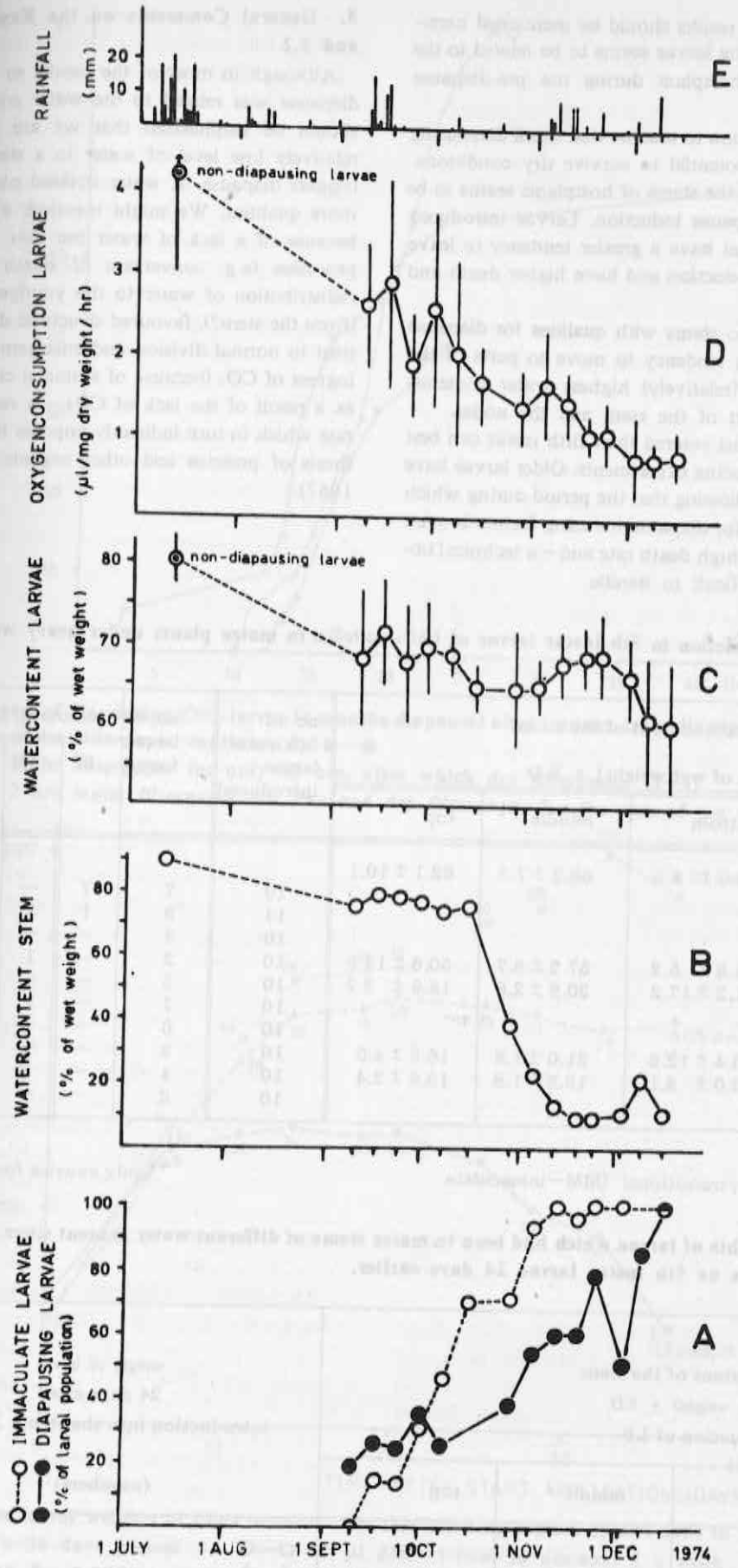

Fig. 1: Some aspects of diapause in relation to the water content of the hostplant and the rainfall in an experimental maizefield in Kikambala, Coast Province.

A few more tentative results should be mentioned here:-
–The weight of diapausing larvae seems to be related to the water content of the hostplant during the pre-diapause period (Table II).
It requires little imagination to assume that small diapausing larvae have a limited potential to survive dry conditions.
– A 65% water level in the stems of hostplants seems to be about the limit for diapause induction. Larvae introduced into stems drier than that have a greater tendency to leave the stems after the introduction and have higher death and pupation rates.
– Larvae introduced into stems with qualities for diapause induction have a strong tendency to move to parts of the stem which have the (relatively) highest water contents: namely the bottom part of the stem and the nodes.
– Larvae which have just entered their fifth instar can best be used in diapause inducing experiments. Older larvae have a high pupation rate indicating that the period during which the larvae are sensitive for diapause inducing factors is over. Younger larvae show a high death rate and – a technical objection – are more difficult to handle.

3. General Comments on the Experiments under 1.1 and 1.2

Although in most of the results so far the incidence of diapause was related to the water content of the stem it should be emphasized that we are not yet sure that a relatively low level of water in a stem is *the* factor that triggers diapause. A water stressed plant changes in many more qualities. We might mention: a low water potential because of a lack of water but also because of chemical processes (e.g. conversion of starch to sugars), internal redistribution of water to the youngest parts of the plant (from the stem?), favoured structural differentiation (in contrast to normal division and enlargement of cells), retarded ingress of CO_2 (because of stomatal closure) and – partially as a result of the lack of CO_2 – a reduced photosynthetic rate which in turn indirectly imposes limitations on the synthesis of proteins and other organic compounds (Kramer 1967).

Table 1: Diapause induction in 5th instar larvae of *Chilo partellus* in maize plants under heavy water stress (see text).

Days after introduction of the larvae	Water content of the stoms (% of wet weight) + S.D.			no. of 5th instar larvae introduced	no. of larvae found	colour of larvae			no. of pupae found
	bottom	middle	top			SP	TR	IMM	
0	73.0 ± 8.5	68.2 ± 7.3	62.1 ± 10.1						
6				10	7	7	—	—	—
13				14	9	1	3	5	1
17				10	4	—	—	4	2
18	65.8 ± 5.2	57.2 ± 8.7	50.6 ± 14.9	10	2	—	1	1	2
23	51.2 ± 17.2	20.9 ± 2.8	18.6 ± 3.7	10	3	—	—	3	—
31				10	7	—	1	6	1++
32				10	6	—	—	6	1++
42	23.4 ± 12.6	21.0 ± 1.8	16.5 ± 4.0	10	2	—	—	2	2++
88	13.0 ± 3.1	18.8 ± 1.8	15.6 ± 2.4	10	4	—	—	4	2++
88				10	2	—	—	2	1++

+SP—spotted TR—transitional IMM—inmaculate

++only exuviae found

Table 11. Fresh weights of larvae which had been in maize stems of different water content since their introduction into these stems as 5th instar larvae 24 days earlier.

Water content of the stem (% of wet weight) ± S.D. at introduction of L5			weight of larvae 24 days after introduction into the stems ± S.D.
bottom	middle	top	(numbers)
73.0 ± 8.5	68.2 ± 7.3	62.1 ± 10.1	108.2 ± 56.5 (21)
65.8 ± 5.2	57.2 ± 8.7	50.6 ± 14.9	35.6 ± 13.3 (11)

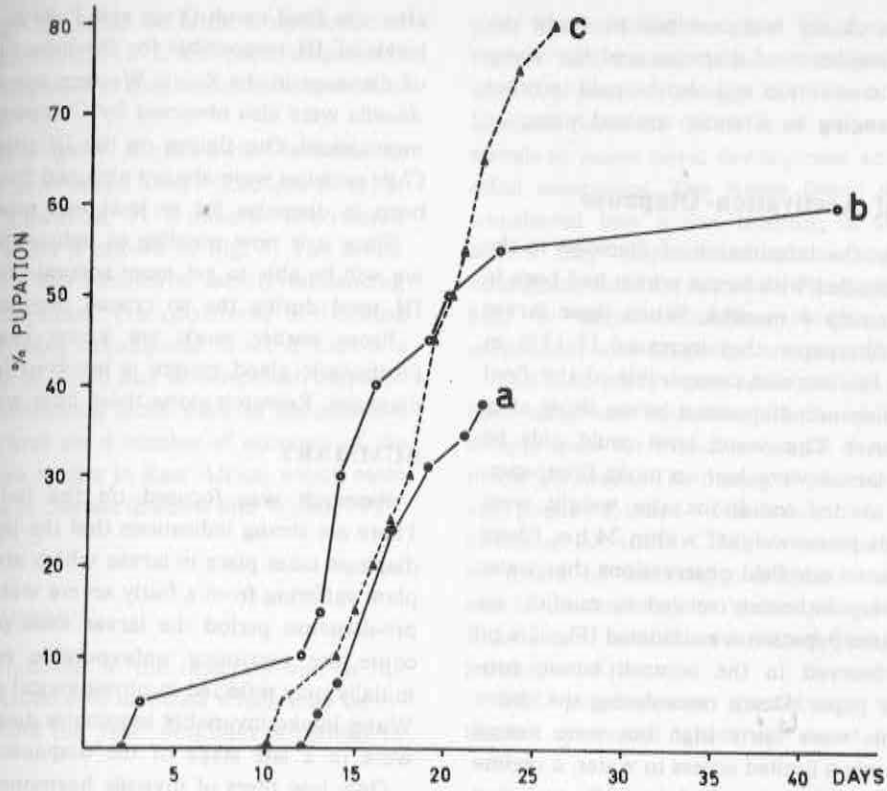

Fig. 2: Pupation rates of diapausing *Chilo* larvae (4 months diapause) after transfer from diapause maintaining conditions to:

a. moist filterpaper continuously ●—●

b. moist filterpaper for only 24 hrs. after which dry filterpaper O—O

c. 2 hrs moist filterpaper par day and dry filterpaper for the rest of the day ▲---▲

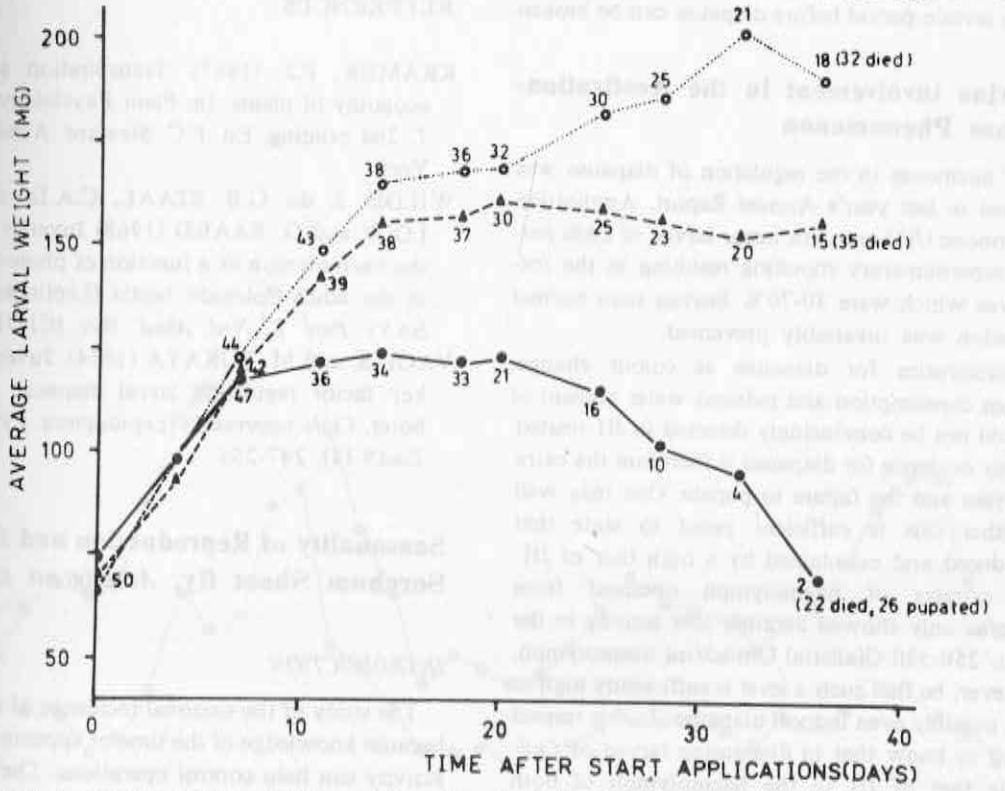

Fig. 3: Changes in the larval weights of *Chilo partellus* after the following topical applications to 5th instar larvae were made during a 36 days period:

O—O 2 µl JH* (1:1000 in acetone) 2 x week

▲---▲ 2 µl JH (1:1000 in acetone) 1 x week

●—● 2 µl acetone 2 x week (control)

***JH used is a mimic from Zoecon (ZR 619)**

In next year's research we will continue to study the relation between the incidence of diapause and the water content of the stem, but attention will also be paid to other factors which are changing in a water stressed plant.

II. Termination of Aestivation-Diapause

The role of water in the termination of diapause in the *Chilo* larvae was only studied with larvae which had been in diapause for approximately 4 months. When these larvae were placed on moist filterpaper, they increased 15-18% in weight within 24 hrs (an increase comparable to the final decrease in weight which non-diapausing larvae show after having entered diapause). This water level could only be maintained when the larvae were *kept* on moist filterpaper. When re-transferred to dry coniditons, the weight went back to the original "diapause weight" within 24 hrs. (these results are very similar to our field observations that water levels in larvae are very intimately related to rainfall, see Fig. 1.) But in both cases pupation was initiated (Fig. 2 a,b.) No pupation was observed in the control: larvae continuously on dry filter paper. Death rates during the above mentioned experiments were fairly high but were lowest when the larvae had only a limited access to water: a regime of 2 hrs moist filterpaper/day resulted in 80% pupation (and thus only 20% death) (Fig. 2c).

Experiments are now carried out to see whether diapause can be terminated by water at any time during the diapause period. It may be that a diapausing larva needs to be in diapause for a certain period before diapause can be broken.

III. Endocrine involvement in the Aestivation-Diapause Phenomenon

The role of hormones in the regulation of diapause was briefly discussed in last year's Annual Report. Application of juvenile hormone (JH) onto 5th instar larvae of *Chilo partellus* caused supernumerary moulting resulting in the formation of larvae which were 30-70% heavier than normal (Fig. 3). Pupation was invariably prevented.

Other characteristics for diapause as colour change, reduced oxygen consumption and reduced water content of the larvae, could not be convincingly detected in JH-treated larvae. The only evidence for diapause is therefore the extra stationary ecdyses and the failure to pupate. One may well question whether this is sufficient proof to state that diapause is induced and maintained by a high titer of JH.

Thus far extracts of haemolymph obtained from diapausing larvae only showed a rather low activity in the *Galleria*-biotest: 250-580 G(alleria) U(nits)/ml haemolymph. It might, however, be that such a level is sufficiently high to maintain (and possibly even induce) diapause. In this respect it is interesting to know that in diapausing larvae of *Chilo suppressalis* the titer of JH in the haemolymph of both diapausing and non-diapausing larvae is high immediately after the final moult (3000-4000 G.U./ml haemolymph) and then a) in non-diapausing larvae decreases very rapidly to a level lower than 30 G.U./ml.h. and b) in diapausing larvae also decreases but only slowly and to a level of 400-500 G.U./ml.h. Such a level had already been reached 15 days after the final moult (Yagi and Fukaya 1974). Intermediate levels of JH responsible for the induction and maintenance of diapause in the South Western cornborer *Diatraea grandiosella* were also observed by Chippendale (Personal Communication). Our figures on the JH titer in haemolymph of *Chilo partellus* were always obtained from larvae which had been in diapause for at least one month.

Since it is now possible to induce diapause "artificially" we will be able to get more accurate figures on changes in JH level during the so crucial pre-diapause period.

From earlier work we know that an active brain-prothoracic gland system is involved in the termination of diapause. Research along these lines will also be continued.

SUMMARY

Research was focused on the induction of diapause. There are strong indications that the inductive phase of the diapause takes place in larvae which are tunneling in a host-plant suffering from a fairly severe water stress. During this pre-diapause period the larvae start preparations to overcome the oncoming unfavourable environment. This is initially only reflected in physiological changes in the larvae. Water intake invariably terminates diapause in larvae which were in a late stage of the diapause.

Only low titers of juvenile hormone can be found in the haemolymph of diapausing larvae. The possible impact of such a low level for the aestivation-diapause phenomenon is discussed.

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Seasonality of Reproduction and Development in Sorghum Shoot fly, *Atherigona soccata (ROND*

INTRODUCTION

The study of the seasonal incidence of a pest is important because knowledge of the time of appearance and maximum activity can help control operations. The period of activity of the sorghum shoot fly, *Atherigona soccata* is not the same in different geographical regonsh Kundu *et al* (1971) observed only one peak period of infestation in a year in Udaipurt, India. Granados (1972) observed two peak periods in Thailand. *Atherigona* is known to be adversely affected mainly by extremes of temperatures (Kundu *et al*

1971), possibly through the effect on adult reproduction or diapause in certain areas (Barry, 1972) and pupal diapause in others. However, development is continuous as long as conditions are favourable.

This study of *Antherigona soccata* was initiated in August 1974 at Kibos in Western Kenya (Latitude 0° .03's; Longitude, 34 38'E; Elevation, 1173 meters). The rainfall at Kibos for over 15 years is shown in Fig. 1. The initial study is focused on the environmental factors influencing reproduction and development. The occurrence of diapause within the life-cycle is being investigated to see if there is a corelation between reproduction and development/diapause and any obvious environmental factor such as temperature, humidity or rainfall. There are a number of accounts of the life-history of *Atherigona soccata* in East Africa, which seem to vary with variations in climate (Swaine and Wyatt, 1954; Nye, 1960; Barry, 1972).

MATERIALS AND METHODS

Experiments are conducted in the insectary and in the field to examine the incidence of diapause which may be induced at any time during the year. Sorghum is planted in pots and enclosed in small glass cages where pairs of females and males are released. Oviposition is checked daily at 17.00 hours. The eggs are counted and infested plants labelled. Some of the infested plants are dissected at intervals to assess larval development and others are left until adult emergence. The larvae found during dissection are transferred into a diet medium in short specimen tubes where they pupate and adults emerge. Pupation and emergence dates are recorded. The newly emerged adults are kept in cages containing seedlings until death. The oviposition and longevity are recorded.

The field study is on adult activity during the year and both adult and immature stages are being sampled. The sample units for immature stages consist of 100 damaged plants per week. The damaged plants are dissected and larvae/pupae counted. The adults are sampled daily by sweeping 100 times. Climate records are taken continuously by means of thermohygrographs placed both in the field and in the insectary to record temperature and humidity. The data for rainfall are collected from the Cotton Research Station, at Kibos.

Wild graminaceous grasses are searched for "dead hearts" symptoms which are caged until adults emerge.

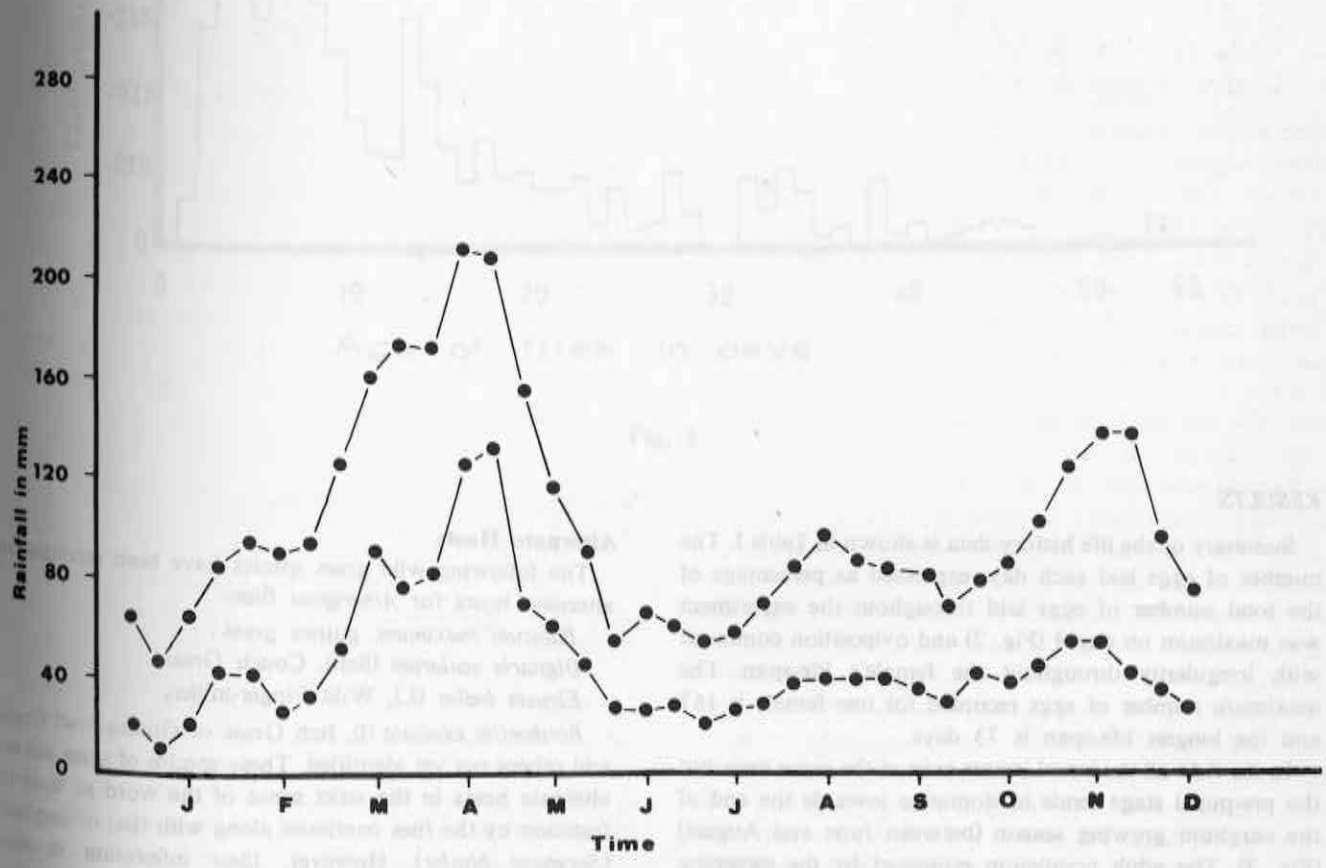

Fig. 1: Maximum and Minimum Rainfall recorded over 15 years.

Table 1: Summary of Life Cycle of *Atherigona Soccata Rond.* (indays)

	Eggs	Larvae	Pupae	Complete life cycle	Adult longevity	
					Females	Males
Average	2.5	13.2	11.3	27.9	33.2	21.1
Range	2-5	11-15	7-15	24-31	9-73	9-51
Mean daily temp. in degree oC	23.75	21.5	22	20.95	21.9	23.25
Mean daily r.h.%	85	87	90.1	72.3	70.5	77.5

RESULTS

Summary of the life history data is shown in Table I. The number of eggs laid each day, expressed as percentage of the total number of eggs laid throughout the experiment was maximum on day 4 (Fig. 2) and oviposition continued with irregularity throughout the female's life-span. The maximum number of eggs recorded for one female is 167 and the longest life-span is 73 days.

In the field all the larval instars exist at the same time, but the pre-pupal stage tends to dominate towards the end of the sorghum growing season (between June and August) (Fig. 3). The adult population estimated by the sweeping method (Fig. 4) shows the major peak in October and minor peaks in May, August and November.

Alternate Hosts

The following wild grass species have been recorded as alternate hosts for *Atherigona* flies:-

Panicum maximum, guinea grass ;

Digitaria scalarum (Sch), Couch Grass;

Eleusin indica (L), Wild Finger-miller;

Rottboellia exaltata (l), Itch Grass or Guinea-fowl Grass, and others not yet identified. These species of grass are not alternate hosts in the strict sense of the word as their infestation by the flies continues along with that of sorghum (*Sorghum bicolor*). However, their infestation is more pronounced during the time of continuous rains and is less during the dry spells.

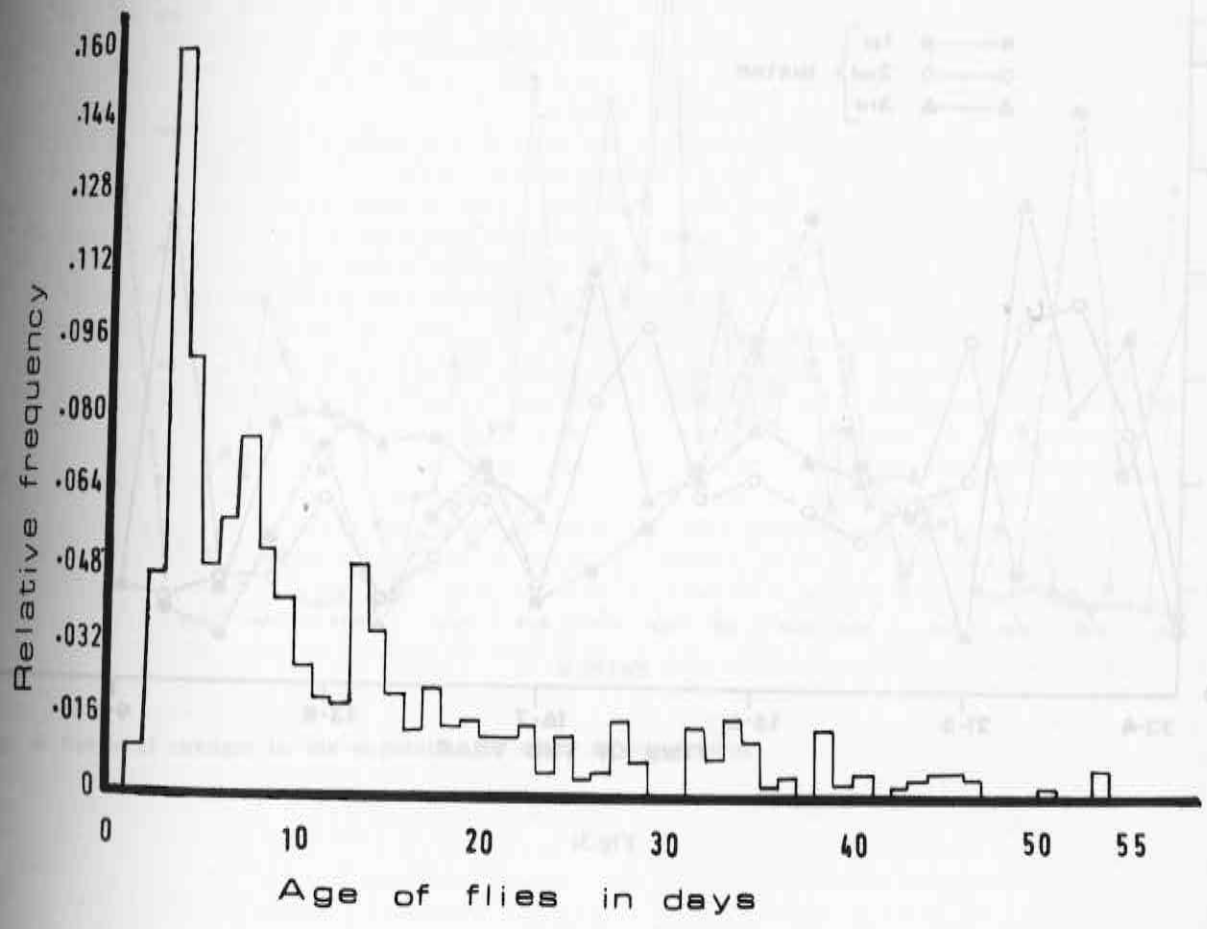
.160
.144
.128
.112
.096
.080
.064
.048
.032
.016
0
Relative frequency
0
10
20
30
40
50
55
Age of flies in days

Fig. 2

DISCUSSION

We have recorded life-cycle extention up to 43 days, the average being 27 days. This could be the crucial period for aestivation-diapause incidence. Throughout our observation so far we have recorded only one larva developing each time within a single shoot, although up to 30 eggs have been recorded in one plant. We have not observed cannibalism in our case (though this has been observed among larvae feeding on artificial diet).

SUMMARY

The life-cycle has been studied. Egg to adult development takes an average of 27 days. The adults live up to 73 days under green-house conditions, laying up to 167 eggs. Oviposition occurs throughout the female's life. All the three larval instars exist in the field at the same time showing an overlap in generations. Four wild grass species have been recorded as alternate hosts.

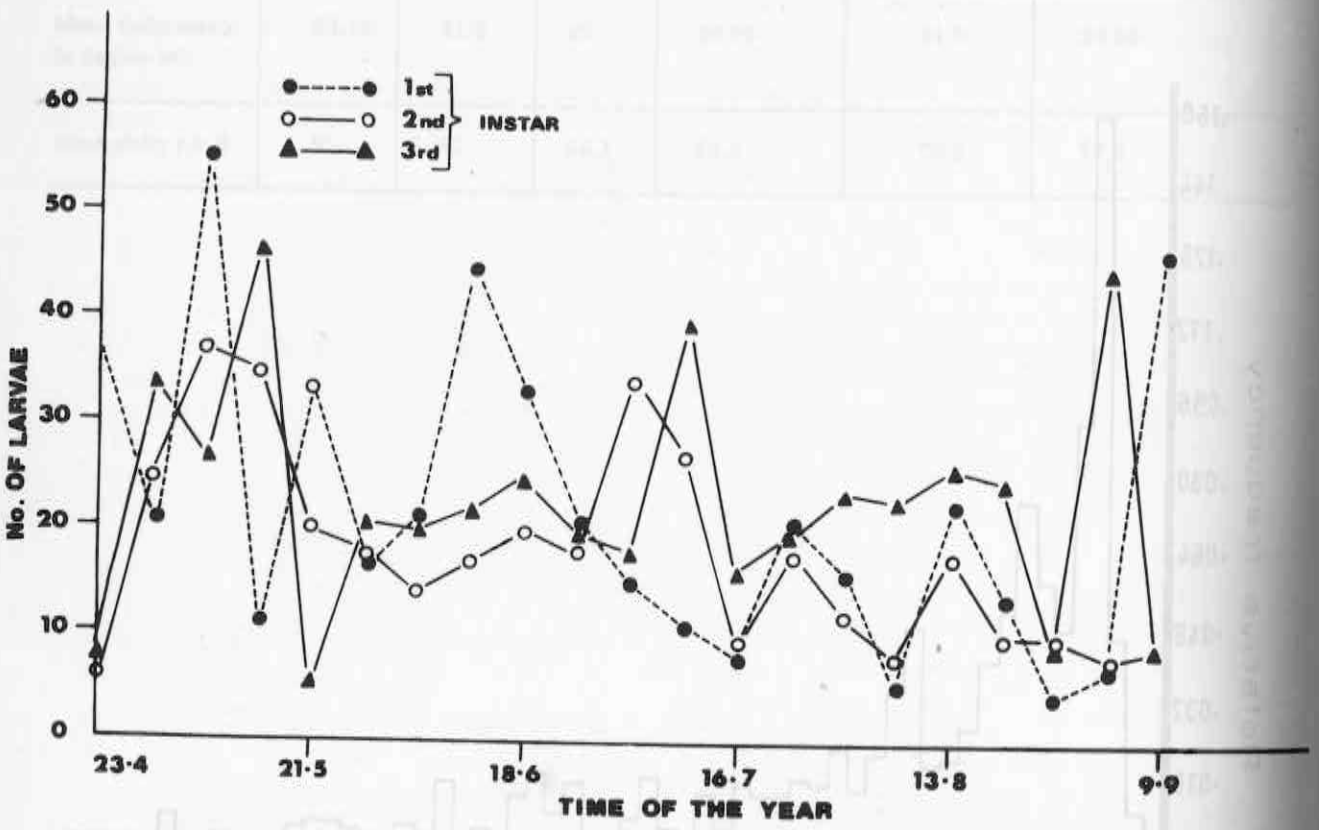

Fig.3:

Rearing of Sorghum Shoot Fly *Atherigona spp.* on Artificial Diets

The pyralid stalk borer, *Chilo zonellus* is the second most important pest of sorghum and its food requirements may be similar to those of the Sorghum Shoot Fly, *Atherigona* spp. The *Chilo* borer is being reared on Keaster and Harrendorf (1965) diet at ICIPE. The *Chilo* diet, the modification of it by adding ground sorghum seedlings and diets used in the Armyworm project were tried out for rearing *Atherigona* flies.

MATERIALS AND METHODS

The samples used in assessing larval population in the field were introduced to the three diets contained in 3x1 specimen tubes and were reared to adulthood. The number of emerging adults was recorded. The survival of larvae was calculated from the number of pupating larvae as percentage of the total number introduced; and that of pupae from emerging adults as percentage of the total pupae.

RESULTS

The results are summarised (Table 1).

The Development of Atherigona Larvae on Artificial Diets

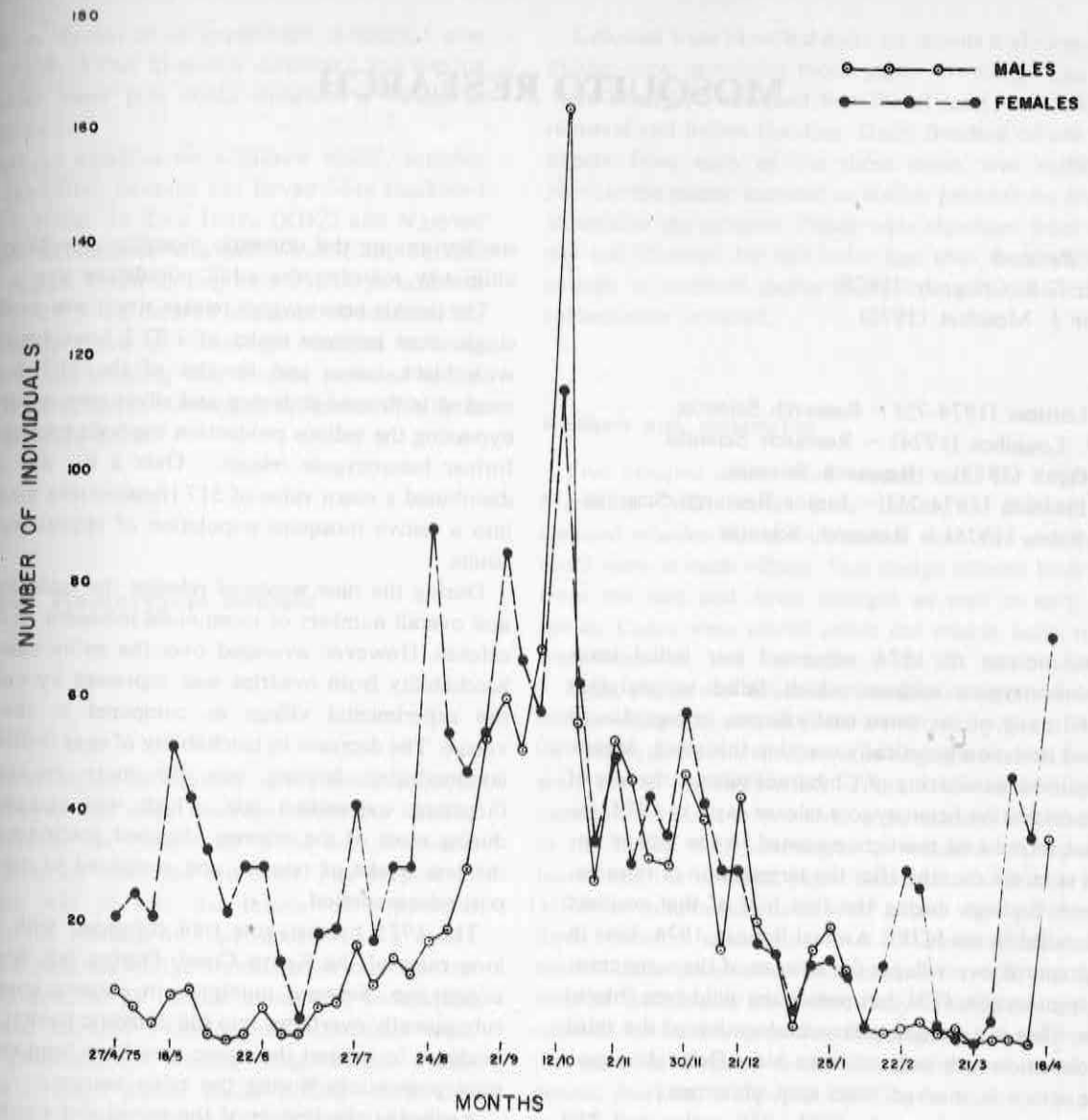

Fig. 4: Seasonal changes in the population of adult *Atherigona* flies

DISCUSSION AND CONCLUSION

The Keaster and Harrendorf (1965) diet *(Chilo* diet) gave the best results. When the culture was started with early first instar larvae, 33.3% developed to the pupal stage. The diet will probably prove more successful by varying some ingredients quantitatively or by addition of new ingredients. Many Diptera have been successfully reared on diets containing some lipids which the *Chilo* diet does not have. It is evident from the successful development of introduced very young first instar larvae, that the culture could also very well be started from eggs.

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MOSQUITO RESEARCH

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RESUME

The last months of 1974 witnessed our initial translocation homozygote releases which failed to maintain themselves in any of the three trial villages, in part due to the red-eyed mutation genetically marking this stock. Meanwhile, continued monitoring of Chibarani village, the site of the first translocation heterozygote release early in 1974, indicated that introduced markers persisted in the village environment even six months after the termination of releases. Our research findings during the first half of that contract year are detailed in the ICIPE Annual Report, 1974. Late in 1974 we prepared two villages for releases of the same translocation homozygote (T3), but possessing wild-type (black) eye colour. This is a homozygous translocation of the third and first chromosomes isolated from New Delhi *A. aegypti* stock; the strain is marked with spot abdomen.

Beginning in early January, 1975, 250 males and 250 females were released as adults daily for ten consecutive weeks into both Kwa Bendegwa (KBW) and Mgandini (MGN) villages. The success of this attempt at population replacement was measured by the increase in frequency in our weekly monitoring of the spot mutant in the two release villages. This genetic marker, which occurs at a low natural frequency among Rabai *A. aegypti*, was monitored in a nearby reference village, Majengo (MAJ).

Field and laboratory tests compared the T3 homozygote to native domestic mosquitoes and pinpointed certain liabilities of the translocation strain which likely impeded population replacement. The principal breeding sites of domestic *A. aegypti* in Rabai are earthenware jars. The release mosquito possessed reduced adult survivorship and mating competitiveness and incomplete fertility, each a handicap toward the introduction and colonization of the homozygote stock in the presence of the adaptively fit, native *A. aegypti*.

In April of 1975 we initiated daily releases of a double heterozygote translocation in a single Rabai village (Mngamboni-MBI), designed to complement the success of the 1974 heterozygote releases which induced a level of 65% sterility among native female mosquitoes (ICIPE Annual Report 1974). Our goal in 1975 was to implement a yet greater sterility among the domestic mosquito population thereby ultimately reducing the adult population size.

The double heterozygote release strain was produced by a single cross between males of a TI:2 homozygote marked with black tarsus and females of the TI:3 homozygote marked with spot abdomen and silver mesonotum, thereby bypassing the tedious production methods employed for the former heterozygote release. Over a 61 day period we distributed a mean value of 517 translocation males per day into a native mosquito population of approximately 1200 adults.

During the nine weeks of releases the male/female ratio and overall numbers of mosquitoes increased in indoor bait catches. However, averaged over the entire release period, hatchability from ovistrips was depressed by only 16% in the experimental village as compared to the reference village. The decrease in hatchability of eggs from individual, landing-biting females was far more promising. Furthermore, oviposition rate, which was exceedingly high during most of the releases, dropped precipitously toward the last weeks of releases and continued to decline in the post-release period.

The 1975 heterozygote trail coincided with the annual long rains of the Kenya Coast. During this wet period *A. aegypti* ssp. *formosus* multiplies in outdoor containers and subsequently overflows into the domestic habitat. There was evidence to suggest that there may have been two overlapping populations during the rainy season.

Analyzing the impact of the introduced sterility presents further difficulties in data interpretation. The decrease in oviposition rate observed in MBI after releases might be attributable in part to sterility introduced by the translocation, but analysis is confounded by similar trends in the reference villages as the rains subside and ambient temperature drops. Nonetheless, the magnitude of decreases in egg, pupal, and adult numbers tends to indicate the possibility that the translocation heterozygote stimulated a decline in population size of the domestic mosquito in the release village.

During the period covered by this report we continued weekly population monitoring of some eight Rabai villages. Our most striking finding to date is the upsurge in oviposition indoors following the onset of the long rains. Although a certain proportion of the increase in numbers of eggs from ovistrips may be accounted for by the house entry of *A. aegypti formosus*, a sizeable component of the rise must in certain villages be attributed to the domestic "type form" mosquito. This observation qualifies our earlier reports which stated that indoor populations are relatively stable in numbers throughout the year; the current data suggests that oviposition frequency fluctuates seasonally, but pupal and adult numbers are less susceptible to change, perhaps as a consequence of density-dependent larval mortality.

We further confirmed in an experiment conducted over 14 weeks in 1974-75 that bi-weekly scrubbing and sieving of the domestic water pots could eliminate a village *A. aegypti* population.

In three outdoor localities we withdrew weekly samples of mosquito eggs from ovitraps and larvae from blackened tins filled with water. In Kwa Dzivo (KDZ) and Ngeyeni (NGY) the habitat sampled was peridomestic; in Birikani (BKI) region, sylvan. From scoring over 35,000 mosquitoes during Jan-July, 1975 we derived measures of seasonal intensity and composition of breeding outdoors. During February and March breeding was severely curtailed by the lack of natural precipitation but surged in April and May in response to the long rains.

Translocation Homozygote Release

INTRODUCTION

Since the *Aedes aegypti* mosquitoes in the first release in 1974 were red-eyed and were recovered from the field in very low frequency, the mosquitoes in the second release were homozygous for wild type eye color. The purpose of the experiment was to test the theory of population replacement by the fixation of a translocation in a native population. The progress of the translocation through the population was detected by routine monitoring techniques for markers and sterility. Translocated individuals were recovered in abundance in landing-biting catches inside houses and in ovitraps placed inside houses. However, fewer than expected were collected from natural breeding sites. Laboratory and field experiments revealed that the homozygotes scored lower for various fitness measurements, including oviposition preference for other than the natural sites.

MATERIALS AND METHODS

The stock used for the releases was T3, a sex-linked homozygote involving chromosomes 1 and 3, induced in mosquitoes collected from a population in Delhi, India, homozygous for the recessive mutant marker *spot abdomen* (*s*) on chromosome 2. A proportion of the released material also carried the semi-dominant marker *Silver mesonotum* (Si) on their second chromosomes. T3 was reared under ambient conditions in an insectary used for this purpose alone. Three or more colonies in wooden cages 60 x 60 x 60 cm. were maintained separately to ensure continuity of production in the event that one colony was contaminated by non-translocated mosquitoes. Occasionally progeny from the colonies were intercrossed to increase the likelihood of producing a strain with heterotic qualities. Quality control techniques also included daily checks of genetic markers and periodic single pair crosses of individuals selected from each cage.

Colonies were bloodfed daily on rabbits and oviposited in plastic cups containing moist paper towelling. Egg papers were dried in a screened box for at least three days after removal and before flooding. Daily flooding of one of four papers from each of the three cages was sufficient to provide the release material as well as progeny for the maintenance of the colonies. Pupae were separated from the larvae and checked for eye color and then were allowed to emerge in screened gallon cages from which adults were subsequently released.

Releases and monitoring

Five hundred adults were released daily for 10 weeks in Mgandini (MGN) and Kwa Bendegwa (KBW). Three elevated wooden boxes with slatted sides and over-hanging roofs were in each village. This design offered both shelter from the rain and direct sunlight as well as easy escape routes. Cages were placed inside the release units, the tops were removed, and the cages were left for 24 hours.

Eggs, pupae and adults were collected weekly from each release village as well as from a reference village, Majengo (MAJ). Eggs from ovitraps set in each house for three days were scored for hatchability. Traps consisted of large juice cans painted black with a strip of upholsterer's cloth clipped to the inside, half submerged in water. The larvae were reared and the resulting pupae and adults were checked for *(s)* Indoor human bait catches of adults were performed by three collectors spending a total of one-half man-hour per house. Both sexes were examined for *s* and the females isolated individually in oviposition vials. These eggs were counted for hatchability after flooding, and the offspring of the captured females were also reared and checked for genetic markers. All pupae breeding in water storage pots were counted once a week and the pupae from half of the houses were brought to the laboratory where emerged adults were examined for *s*. Some of the males were crossed in single pairs with virgin females from a laboratory stock to determine fertility as another means of identifying translocated individuals.

RESULTS

1. For the 19 weeks prior to releases, the average frequencies of *s* in ovitraps in the experimental villages and the reference village were 1.8% and 6.7% respectively. During releases, however, the frequency in the experimental villages increased to 45.0% while the reference village remained at 6.0%.
2. *S* rose from 5.6% and 4.9% in the adult catches to 84.7% and 11.4% from the experimental and the reference villages respectively.
3. The change in frequency of *s* individuals collected from the clay pots as pupae was very slight. During the pre-release period 1.4% of pupae sampled in experimental villages possessed the *s* phenotype and 6.2% in MAJ. During the releases the incidence of *s* increased to 3.4% and 9.9% respectively.

4. The fertility of individuals collected as pupae did not indicate the presence of heterozygotes breeding in the pots. Sixty-eight single pair crosses of adults from pupal samples in release villages gave an overall hatchability which was not significantly different from the release villages. However, the presence among the pupae of a few individuals marked with *Si* showed that some offspring of released mosquitoes did make it through the larval stages inside the pots.
5. Average female fertility was a function of genotype. T3 females were 75% fertile compared to wild type females which were 93% fertile. The fertility of T3 females was also a function of the genotype of their mates. T3 females which mated with wild type males averaged a fertility of 84% while T3 females which mated with T3 males averaged 70%.
6. Female fecundity was influenced neither by the genotype of the female nor by her mate. In all cases fecundity averaged 44 eggs per female for one blood meal.
7. Of 172 homozygote females which had been released and subsequently recaptured, 35.3% had been mated with wild type males, despite the fact that homozygote males and females were confined together for about 48 hours prior to release. Of 22 captured wild type females 31.8% had mated with homozygote males. This information confirms the impression of reduced mating competitive ability of T3 males in the field.
8. To investigate the possibility of stage-specific mortality in the local breeding containers, all larvae and pupae were collected from the pots of half of the houses in one experimental village and brought to the laboratory. The instars were separated, reared to adulthood and scored for *s*. If T3 individuals had been deposited in the pots as eggs but were unable to complete development to the pupal, and, eventually, the adult stage, then a greater percentage of *s* would have been noted among the younger instars. However, a statistical test showed that among the 1481 immatures scored as adults, phenotype frequency was independent of instar.
9. Adult survival was compared in the laboratory in two 60 x 60 x 60 cages, one of which held 100 T3 males and 100 T3 females, and the other 100 wild type of each sex. The number of males and females which died was counted daily. Both T3 males and females suffered a higher mortality rate than did the wild type. Especially noteworthy was the discrepancy in survivorship between males of the two strains; by day 24 after emergence 79% of the wild type strain and only 23% of the T3 males were still alive.
10. Time to pupation was measured by counting 100 T3 first instars into each of six small clay pots and 100 wild type first instars into each of six different pots. The larvae in four pots, two with T3 and two with wild type, were reared with 5 cc. of food (10g. liver powder per 400 cc. water), four with 10cc. and four with 15cc. The slower development rate of the homozygote was significantly different from the rate of the wild type strain at all food levels.
11. To ascertain the frequency of T3 eggs laid in pots during releases, we placed one water pot inside 1[illegible] houses in KBW. After three days the pots were removed. The accumulated eggs were flooded and the larvae reared in the laboratory. 23.2% of the adults reared from those eggs were *s*.
12. Since the progeny of released homozygotes were found in ovitraps but seldom in the natural breeding sites, oviposition preference of T3 females was examined in the laboratory. Forty T3 and forty wild type mated and bloodfed females were placed together in a large cage. They were offered the choice of a large black tin with a cloth strip clipped to the inside, or a small clay pot. The vessels were removed from the cage after five days and were flooded after four days. The larvae were reared to adulthood and the adults scored for *s*. Under these conditions homozygote females laid 62% of their eggs on the cloth strips compared to only 9.6% by the wild type females. This difference in oviposition preference did not change significantly after the second blood meal.

DISCUSSION

Since the purpose of replacement is to actually alter the genetic constitution of the population, the rate of alteration and the final composition of the population must be closely appraised. With the help of the marker *spot* the performance of the T3 strain could be evaluated. The data revealed that both males and females flew into houses where they were captured in abundance. The recovery of translocation-bearing eggs on ovistrips indicated that mated females took blood meals and oviposited indoors. However the monitoring of immature stages rarely supplied evidence that translocated individuals were able to undergo the subsequent steps from eggs to pupae in the village environment.

The T3 homozygote compared unfavourably with the wild type in several specific measures of fitness, namely fertility, mating competitiveness, oviposition preference, larval development time, and adult survival. Oviposition preference was a particularly interesting behavioural difference. The homozygote preferred to lay in ovitrap cans rather than in clay pots, the principal larval breeding sites of the local domestic population. The total effect of these T3 deficiencies in relation to the wild type strain as well as possible differences in other characteristics not measured in this study could have been responsible for the inability of the strain to achieve replacement.

Double Translocation Heterozygote Release

INTRODUCTION

For 9 weeks from 14 April, to 13 June 1975 an average of over 500 double translocation heterozygote males were released daily into a village where the estimated adult mosquito population was 1200. The purpose of this release

was twofold: (1) to suphppress the *Aedes aegypti* population by sterility induced by chromosomal translocations and (2) to assess the effect of rainy season conditions on the population dynamics of the indigenous and released mosquitoes. As a result of the releases, hatchability of eggs of females collected during landing-biting catches dropped from over 93% to 30-40%. For 8 weeks following the cessation of the releases, fertility remained below 70%. The reference village averaged between 80 and 99% hatch. The relative population density as measured by landing-biting catches, pupal counts and oviposition rate, declined in both the experimental and reference villages. This population decline cannot be attributed solely to the sterility induced by the translocations.

MATERIALS AND METHODS

Double translocation heterozygote males for release were obtained by crossing 2 different translocation homozygotes. One of these, designated T3, is the same translocation homozygote used during the population replacement experiments described above. The other translocation homozygote, designated DS-1D, involves chromosomes 1 and 2; chromosome 3 is marked with the mutant *black tarsi (blt)*. The DS-1D translocation was induced in a strain of *Aedes aegypti* collected locally in Rabai, Kenya and isolated at the ICIPE Coastal Research Station, Mombasa (ICIPE Annual Report 1974).

Production and Quality Control.

Translocation homozygotes are theoretically fully fertile when self-mated, so it is possible to maintain them by routine laboratory procedures. To guard against contamination all translocation homozygotes were examined for their respective markers before being used in any crosses: T3 should be homozygous for *s;* DS-1D should be homozygous for *blt*. The 2 homozygotes were crossed to obtain progeny that were heterozygous for each of the parental translocations. Consequently these mosquitoes were heterozygous for the markers *s* and *blt* and, therefore, were indistinguishable from the wild type.

In the early stages of the releases reciprocal matings were arranged using males from one homozygote with females from the other and vice-versa. However, it soon became apparent that the DS-1D strain was not as fecund nor as fertile as the T3. Hatchability of the DS-1D strain averaged 56.1% while the T3 averaged 70.3%. And the absolute numbers of adults in the maintenance colonies were dramatically different. In order to maintain the DS-1D colony all female progeny and half of the male progeny reared from each DS-1D egg paper had to be crossed to obtain the next generation. The remaining males were mated with T3 females for production of double translocation heterozygotes. Thus the low productivity of the DS-1D strain was the limiting factor in the mass production schedule.

Theoretically, double translocation heterozygotes are about 75% sterile. Release males were crossed with Rabai type form females and the egg hatchability scored. In the first trial the hatch was 23.2% (n = 23) range 3.2-37.9%. In a second trial the hatch was 23.1% (n = 13) range 15.8-34.5%.

Method of release

Only male mosquitoes were required for the releases, eliminating the problems of releasing biting females. Progeny of the homozygote crosses were sexed as pupae; the females were discarded. After being examined to ensure that they were all wild type males, mosquitoes for release were transported in the field in plastic cages.

An average of 517.2 males were released daily over a 61 day period (Fig. 1).

Monitoring

Three basic techniques were employed to monitor the release and the reference villages: (1) landing-biting catches (L-B catches), (2) indoor oviposition traps and (3) collection of pupae.

Females captured during L-B catches were blood fed and isolated in glass vials. Eggs oviposited were allowed to embryonate, hatch and scored for hatchability. The resulting larvae were reared to adults and scored for the makers *Sl, s* and *blt*.

Cloth strips served as oviposition traps. One of these traps was set inside each house of both the experimental and reference villages for 3 days of each week. After collection these strips were stored in a closed container for 5 days allowing the eggs to embryonate, were flooded to hatch the eggs and then scored for hatchability. The larvae were reared to adults and scored for the genetic markers previously mentioned. In addition, as soon as the dark form of *A. aegypti* was observed in ovitrap collections its presence was routinely monitored.

Once a week the earthenware jars inside houses were thoroughly examined for mosquito pupae. All pupae from alternate houses were brought to the laboratory at Mombasa and reared to adults which were then scored for the various genetic markers.

Mating Competition experiments.

Two mating competition experiments were set up as follows: 100 type form virgin females were added to a cage of 100 release males and 100 type form males. Two days later the females were bloodfed. Eggs collected from individual females were scored for hatchability: % hatch below 40% indicated a mating with a type form male.

RESULTS

1. Mating competition experiments demonstrated that, under laboratory conditions, translocation heterozygote males were competitive with wild type males. In the first trial 63% of matings (n = 46) were by release males; in the second 64% of matings (n = 22) were by release males.
2. As measured by the hatchability of eggs from females

collected during L-B catches, between 60-70% sterility was introduced into the domestic mosquito population of the release village. The average fertility remained below 70% for eight weeks following the cessation of the releases (Figure 2). With one exception, the hatchability of the reference village was between 80-99%.

3. The hatchability of eggs collected on oviposition strips did not indicate the same degree of sterility as the L-B data (Figure 3). During the course of the releases *Ae. aegypti* ssp. *formosus* made up an increasing proportion of the adults scored from the ovistrip collections. At the same time there was a dramatic decrease in the absolute number of type form mosquitoes. This pattern was common to several villages monitored in Rabai and is reported more fully below. Thus the monitoring of the translocation release by ovistrip data was confounded by the influx of ssp. *formosus* mosquitoes from the peridomestic habitat.
4. Three indices of relative population size indicated a substantial population decrease in the release village, MBI, concomitant with the period of the releases and continuing to the present: (1) adults/man-hr, (2) pupae/container and (3) eggs/trap/day. These are presented graphically and discussed in the following section. MAJ, the reference village, however, also shows a decline in these indices, but the observed decreases occur somewhat later (Fig. 4)
5. Recovery of genetic markers was surprisingly low considering the level of sterility observed. Results are presented in Table 3.

Table 1: Description of villages in study area prior to double heterozygote release.

Name of village	No. inhabitants	No. house	No. water containers	No. containers with larvae or pupae	Landing-biting catch mosquitoes/man-hr.
MAJENGO (MAJ)	82	28	43	27.7	4.4
MANG'AMBONI (MBI)	103	26	34	18.9	6.7

Table 2: Treatments and monitoring of the villages during the double translocation heterozygote release.

Village	Treatment	Weekly routine monitoring		
		Ecological parameters	Genetic parameters	Additional assessments
MAJENGO (MAJ)	no release; reference only.	1. Oviposition rates determined by: 1 ovitrap/house for 3 days/week.	1. % hatch of eggs collected in indoor ovitraps.	1. Estimate of absolute adult population size in both villages at beginning of experiments.
MNG'AMBONI (MBI)	release of double translocation heterozygote. (about 500/day)	2. Landing-biting rates determined by: 3 men for 10 min/house Once a week.	2. % hatch of eggs from individual females collected in L-B catches.	2. Assessment of competitiveness of release males under laboratory conditions.
		3. Containers index Once a week.	3. Assessment of frequency of genetic markers among adults reared from ovitrap and L-B catches.	
		4. Total pupal counts Once a week.		

Table 3: Recovery of genetic markers from mosquitoes collected in MNG'AMBONI

Type of collection	Genetic markers						
	s	*si/ +*	*blt*	*Si/ +*	*blt Si/ +*	*Si/ Si*	Total
L-B females whose progeny show markers	11	13	6	1	0	1	86
Progeny from indoor ovistrips	18	25	0	0	1	1	6053
Field collected pupae	3	1	0	0	0	0	565

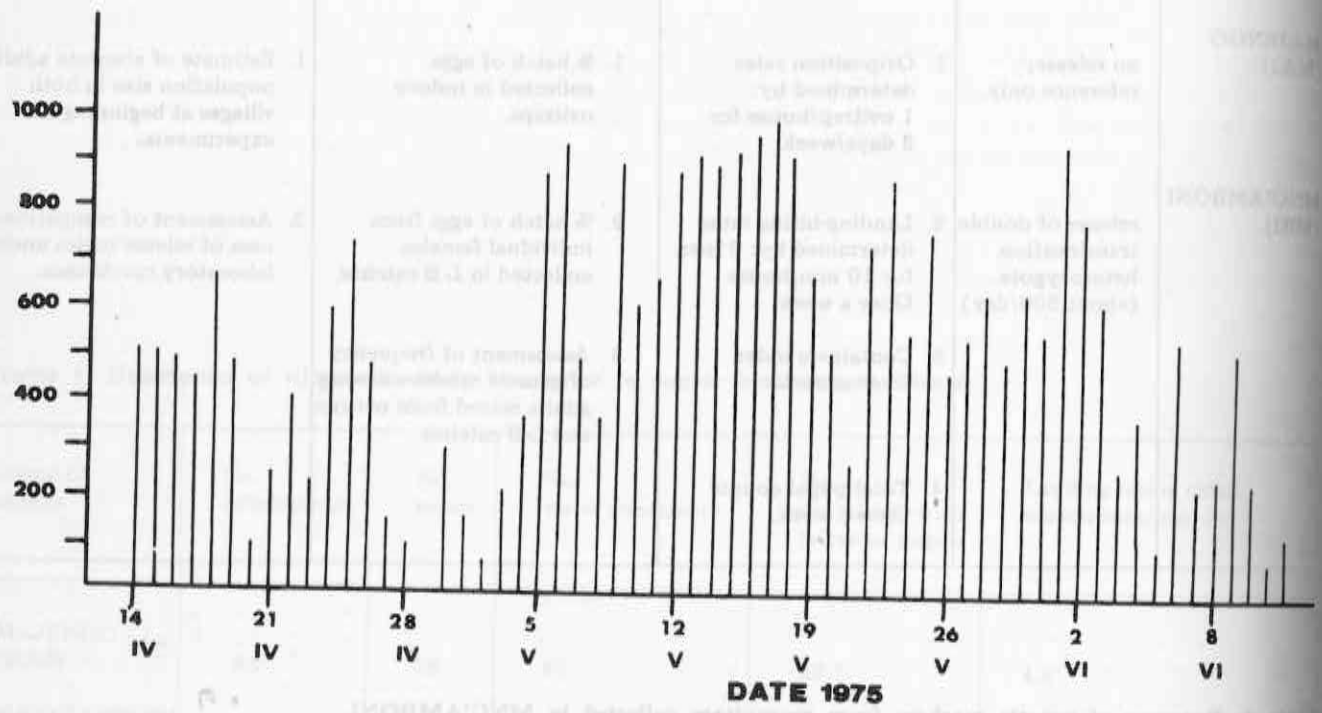


Fig. 1: Number of heterozygote males released daily in MBI

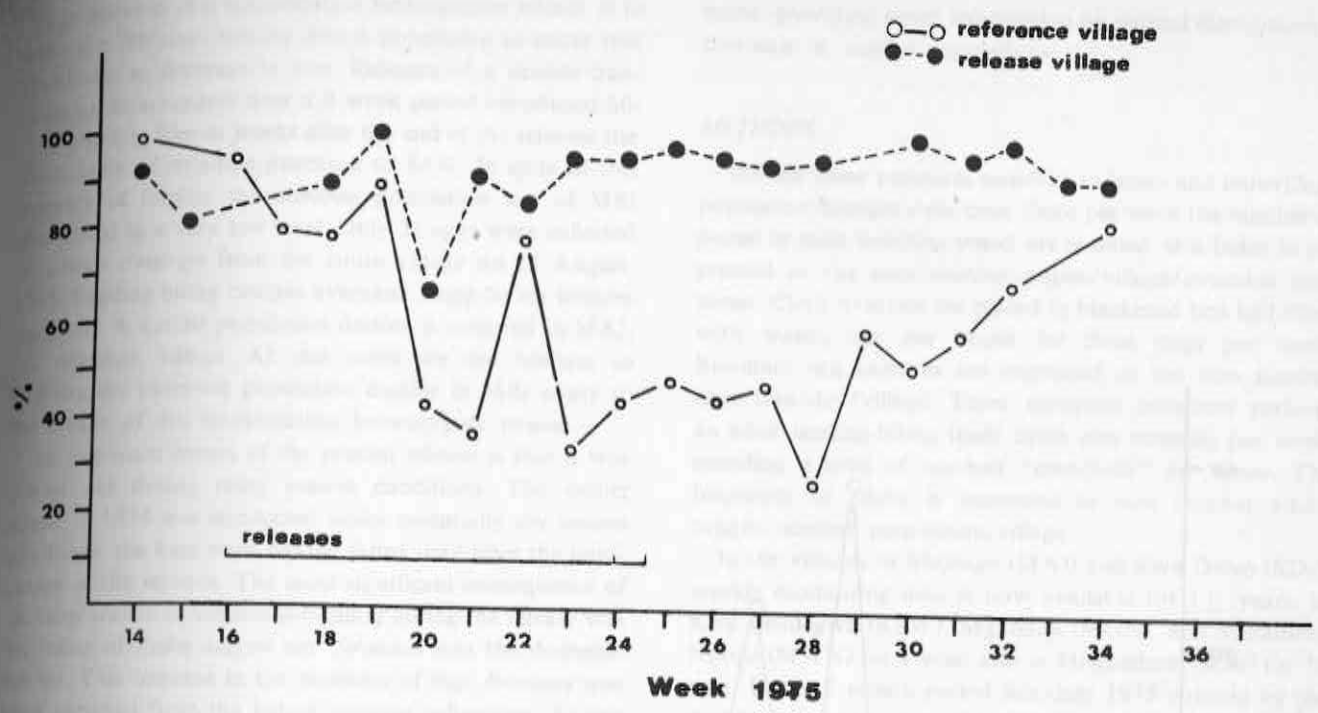


Fig. 2: Hatchability of eggs from individual L–B ♀♀

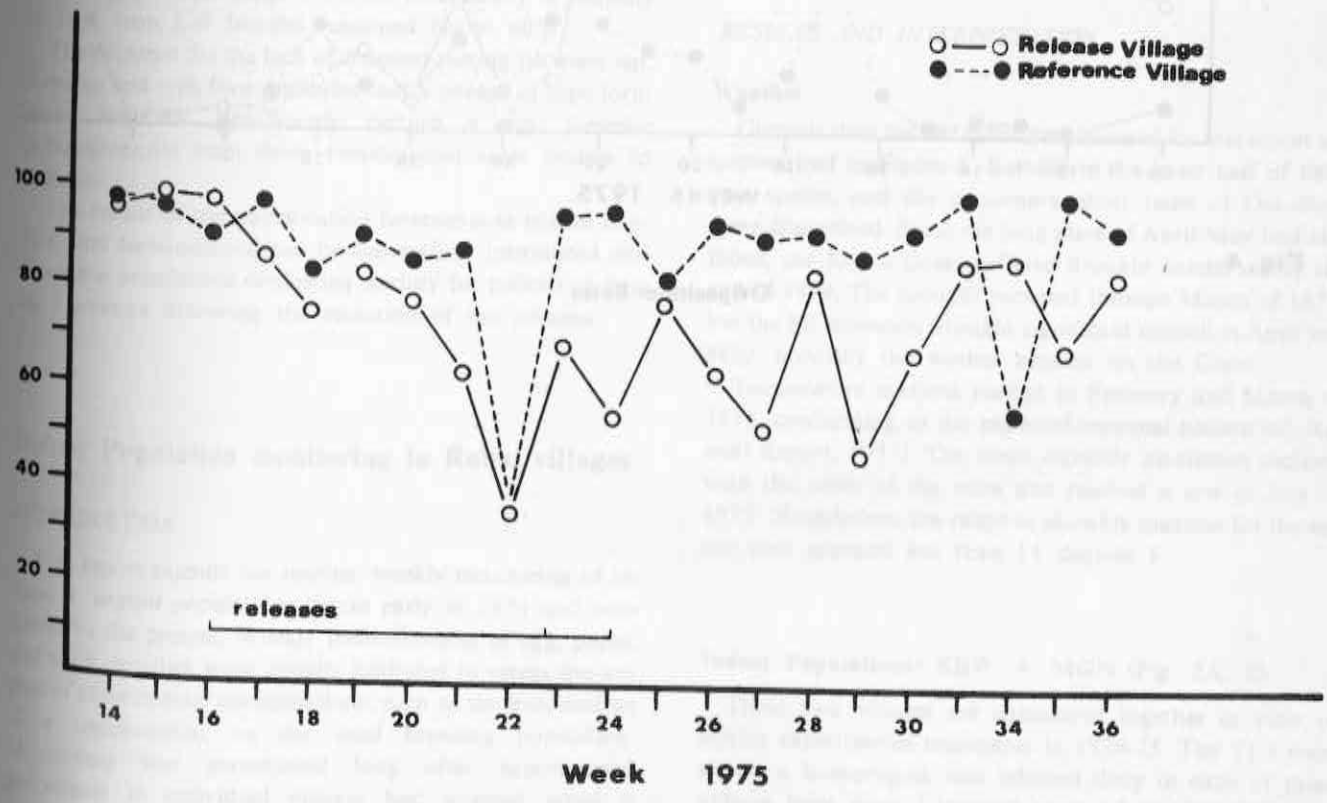


Fig. 3: Hatchability of Ovistrips

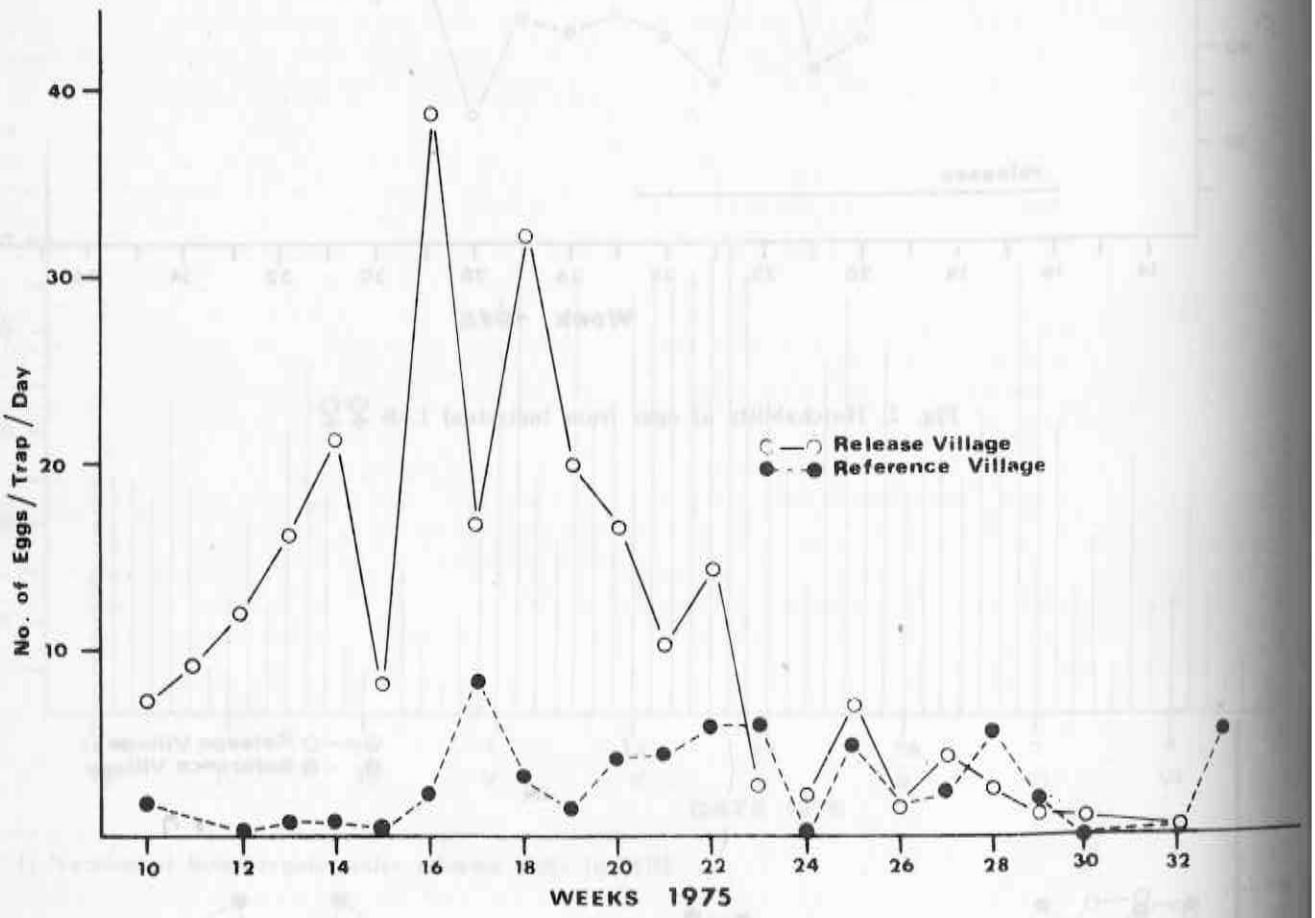


Fig. 4

Oviposition Rates

The purpose of a translocation heterozygote release is to introduce sufficient sterility into a population to cause that population to decrease in size. Releases of a double translocation heterozygote over a 9 week period introduced 60-70% sterility. Eleven weeks after the end of the releases the hatchability of ovistrips increased to 84%. In spite of this recovery of fertility the absolute population size of MBI plummeted to a very low level. Only 31 eggs were collected on indoor ovistrips from the entire village on 25 August, 1975. Landing-biting catches averaged 3 egg-laying females per catch. A similar population decline is occurred in MAJ, the reference village. At this point we are hesitant to attribute the observed population decline in MBI solely to the effects of our translocation heterozygote release.

An important aspect of the present release is that it was carried out during rainy season conditions. The earlier release in 1974 was conducted under essentially dry season conditions, the long rains having failed until after the completion of the releases. The most significant consequence of the rainy season conditions prevailing during the release was the influx of *Aedes aegypti* ssp. *formosus* into the domestic habitat. This increase in the numbers of ssp. *formosus* was most apparent from the indoor ovistrip collections. At one point 88.6% (171/193) of the adults scored from ovistrips collected in MBI were ssp. *formosus*. This abundance of ssp. *formosus* eggs on the ovistrips obscured the monitoring of the translocation heterozygote release. Since ssp. *formosus* progeny can come only from a *formosus* x *formosus* mating, it is apparent translocation heterozygote males were not readily inseminating ssp. *formosus* females. Females that oviposit inside houses may be inseminated in the peridomestic habitat before house entering. This would explain the increase in fertility observed in MBI at the time of the ssp. *formosus* surge while the hatchability of progeny of type form L-B females remained below 60%.

The evidence for the lack of frequent mating between ssp. *formosus* and type form indicates that a release of type form males will not significantly perturb a ssp. *formosus* population, not even those females that enter houses to oviposit.

The results of the translocation heterozygote release confirm that translocations can be successfully introduced into mosquito populations conferring sterility for periods as long as 2 months following the cessation of the releases.

Indoor Population monitoring in Rabai villages

INTRODUCTION

This report extends our routine, weekly monitoring of indoor *A. aegypti* populations begun early in 1974 and continued to the present. Weekly measurements of egg, pupal, and adult densities were initially instituted to assess the impact of experimental manipulations, such as the introduciton of a translocation, on the local breeding population. Monitoring was perpetuated long after experimental procedures in individual villages had stopped when it became evident that the accumulating data had instrinsic merit, providing novel information on natural fluctuations in domestic *A. aegypti* populations.

METHODS

We use three measures sensitive to inter- and intravillage population changes over time. Once per week the number of pupae in each breeding vessel are counted; this index is expressed as the sum number pupae/village/available container. Cloth ovistrips are placed in blackened tins half-filled with water, one per house for three days per week. Resultant egg captures are expressed as the sum number eggs/trap/day/village. Three mosquito collectors perform an adult landing-biting (bait) catch one morning per week, spending a total of one-half "man-hour" per house. The frequency of adults is expressed as sum number adults caught/number man-hours/village.

In the villages of Majengo (MAJ) and Kwa Dzivo (KDZ) weekly monitoring data is now available for 1 ½ years, in Kwa Bendegwa (KBW), Mgandini (MGN), and Mwamruu Ndogo (MWN) for 1 year, and in Mngamboni (MBI) for ½ year. In the 7 month period Jan.-July 1975 covered by the monitoring data presented in this section, the following experimental manipulations were performed:

translocation homozygote release – MGN, KBW
translocation heterozygote release – MBI
source reduction – MWN

The accompanying weather data was collected daily in Simakeni village, approximately 2km from our release villages MBI, KBW, and MGN, and expressed herein as monthly means of daily maximum and minimum temperatures, and total monthly accumulated rainfall.

RESULTS AND INTERPRETATION

Weather

Climatic data for the entire year covered by this report are summarized in Figure 1. Rainfall in the latter half of 1974 was sparse, and the customary short rains of Oct.-Nov. were diminished. Since the long rains of April-May had also failed, the Kenya Coast suffered drought conditions by the end of 1974. The drought persisted through March of 1975, but the SE monsoon brought significant rainfall in April and May, normally the wettest months on the Coast.

Temperature maxima peaked in February and March of 1975, conforming to the expected seasonal pattern (cf. Annual Report, 1973). The mean monthly maximum declined with the onset of the rains and reached a low in July of 1975. Nonetheless, the range in monthly maxima for the entire year spanned less than 15 degrees F.

Indoor Populations: KBW + MGN (Fig. 2A, B)

These two villages are considered together in view of similar experimental treatments in 1974-75. The T1:3 translocation homozygote was released daily in each of these villages from week 2 through week 12 of 1975 (First section). The release period co-incided with a pronounced in-

crease in frequency of adults and eggs in both villages, although the association was clearer in MGN (Fig. 2A, B). Bait and ovitrap collections produced fewer mosquitoes after the termination of releases, confirming that the introduced strain had failed to colonize the habitat. A peak in pupal production early in 1975 (KBW) did not persist during the remainder of releases. There is no evidence that this transitory peak was causally correlated with the translocation releases, which made an insignificant impact on the loc breeding population (First section).

We also observed peaks in egg production in mid-April the onset of the rainy season. Ovistrip collections continue a high yield through week 23 in MGN and at least throug week 29 in KBW. However, accompanying increases i adult or pupal collections were not apparent.

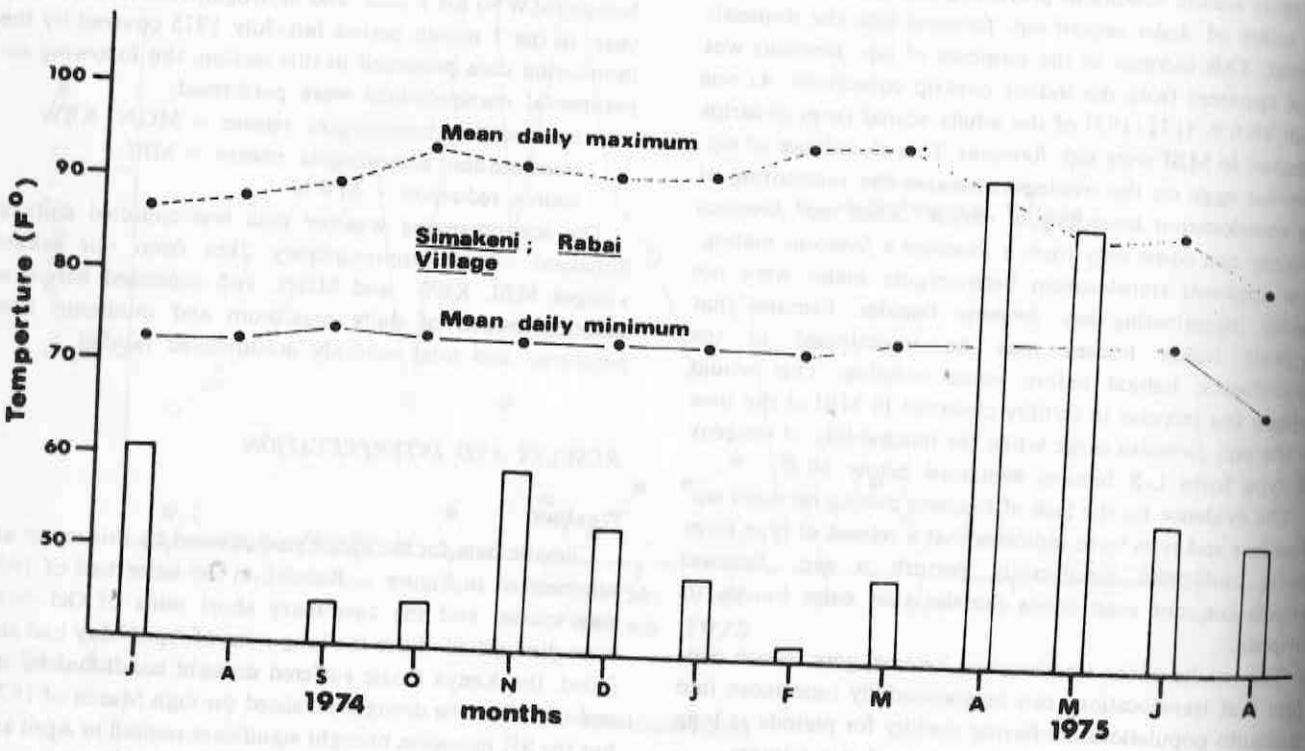


Fig. 1: Monthly temperature and rainfall data from Simakane village, Rabai Location from July 1974 through July 1975

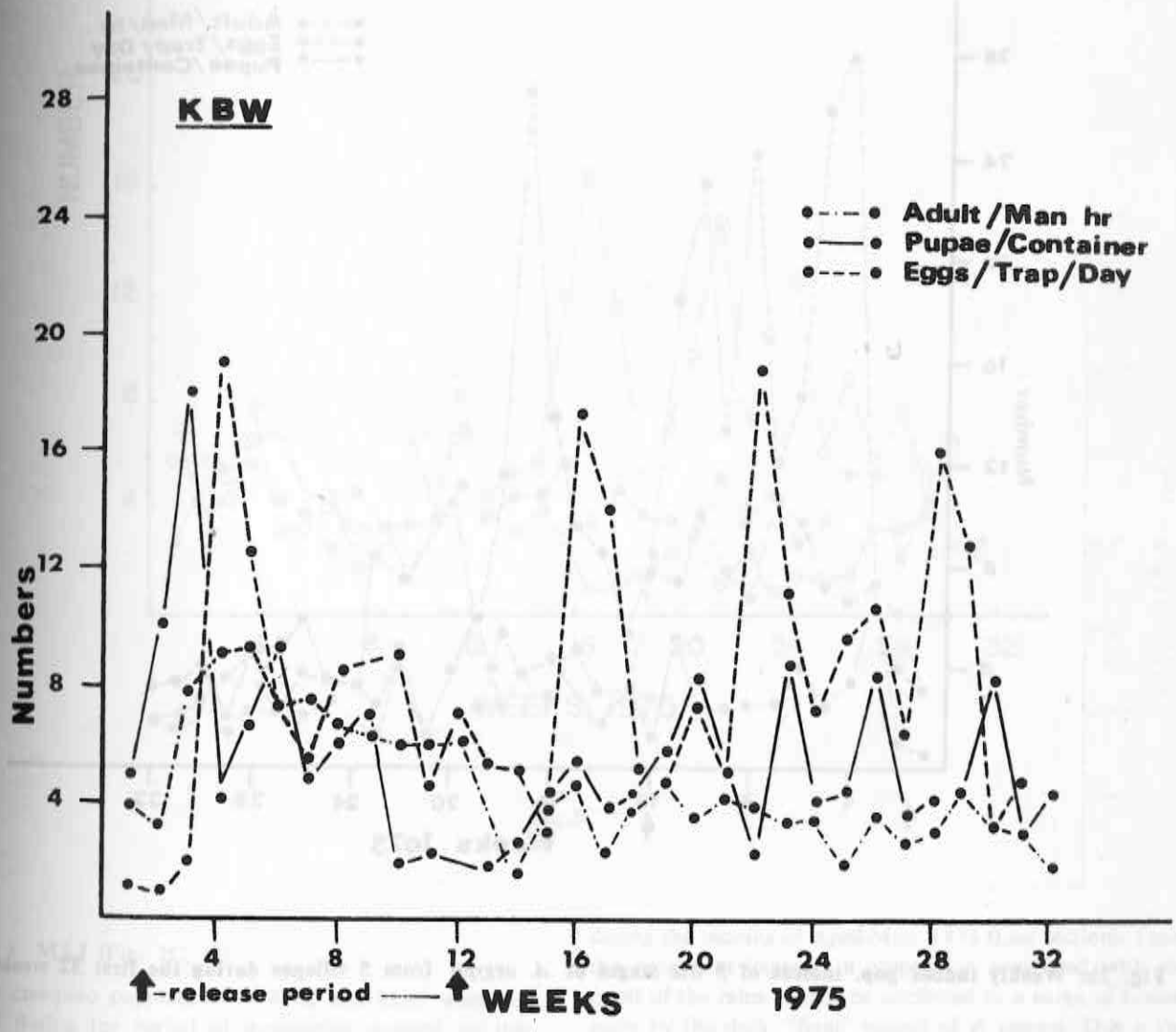


Fig. 2A-E: Weekly indoor population indices of three life stages of *A. aegypti* from five villages during the first 32 weeks of 1975. Time intervals between arrows designate release periods.

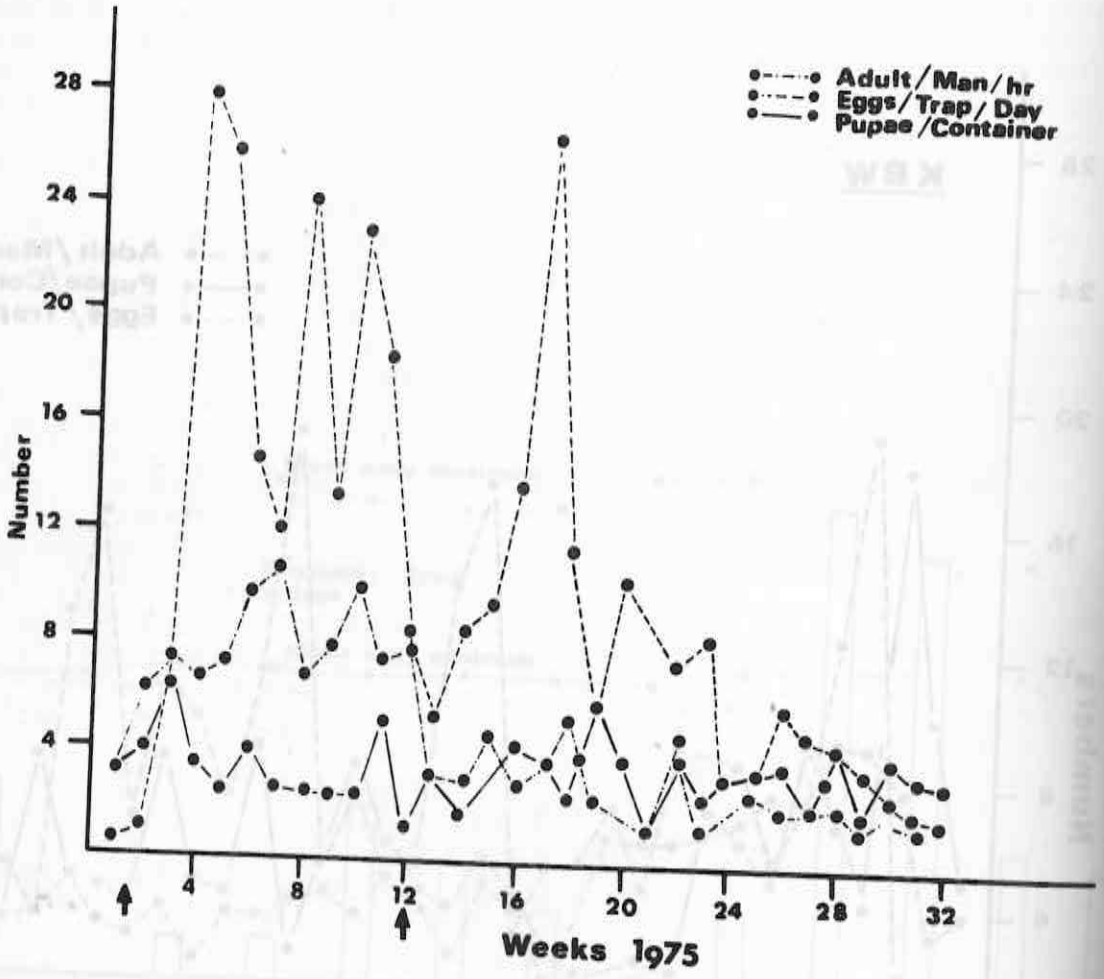


Fig. 2b: Weekly indoor pop. indices of 3 life stages of *A. aegypti* from 5 villages during the first 32 weeks, 1975

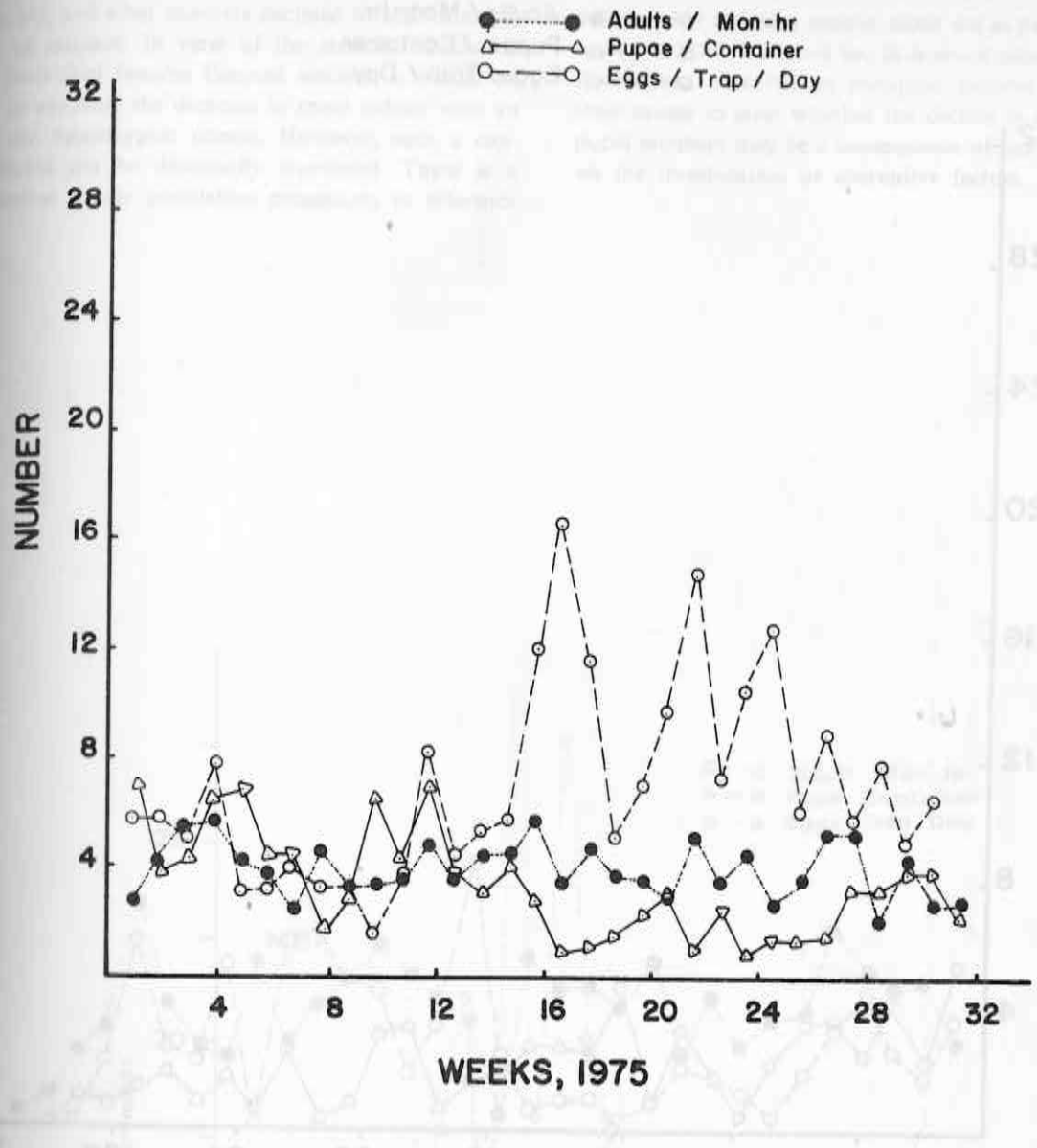


Fig.2C

KDZ + MAJ (Fig. 2C, D)

The mosquito populations of KDZ and MAJ were untreated during the period of monitoring covered by this report. Source reduction in KDZ had been terminated in week 40 of 1974 and the domestic mosquito population subsequently returned to normal. MAJ has been monitored as an undisturbed reference village since early 1974. The frequency of the dark morph, *A. aegypti formosus* in indoor collections has been noted in both KDZ and MAJ since mid-1974.

Monitored indices of population size remained relatively constant in the first 15 weeks of 1975, particularly by comparison with the release villages KBW and MGN. Egg frequency increased severalfold in KDZ beginning on week 16; the increase persisted with a gradual dampening in magnitude until week 30 of 1975. Adult mosquitoes reared from these ovitrap collections were 0 to 16% ssp. *formosus* during the months of April-May, 1975 (Last section). Thus, the prominent increase in oviposition associated with the onset of the rains cannot be attributed to a surge of house-entry by the dark, "feral" morph of *A. aegypti*. This is the first clear evidence from our study area of major changes in the frequency of any life stage of the domestic "type form" mosquito associated with the seasons. The factors linking rainfall and increased oviposition are unknown.

A single peak in egg production in MAJ on week 17 was not sustained throughout the rains, and this village did not show the overall increase in oviposition observed in KDZ.

The absence of an increase in adult and pupal production concomitant with the seasonal rise in oviposition contributes to accumulating evidence that adult numbers are regulated by density-compensating larval mortality acting within a fixed number of indoor breeding containers. Thus, the probability of an egg producing an adult during the rainy season is far less than during drier periods.

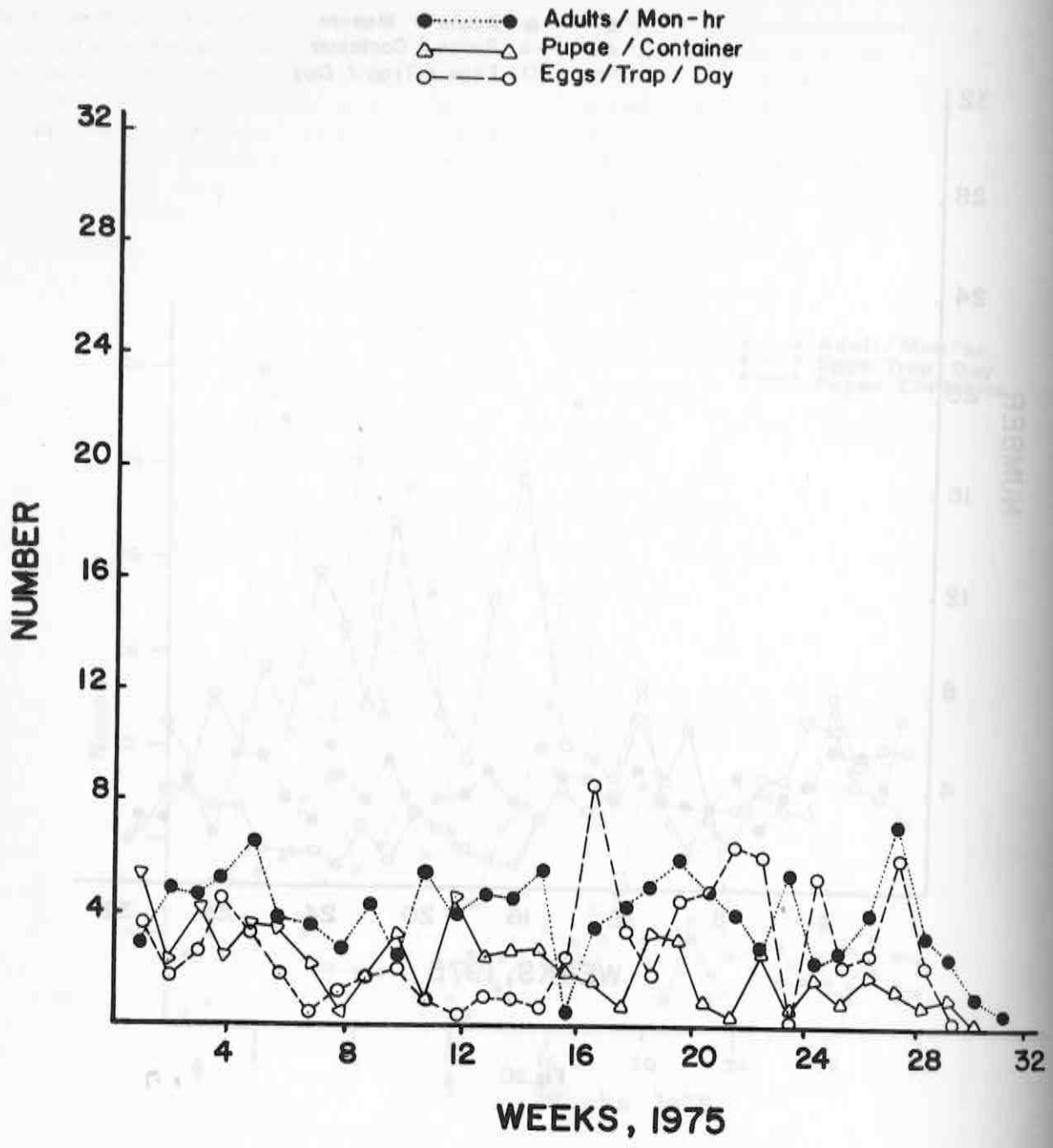


Fig.2D

MBI (Fig. 2E)

Population monitoring in this village began on week 9 of 1975, 7 weeks prior to the inception of daily double heterozygote releases in April, May, and June. Indoor oviposition increased just before the inception of releases and peaked at 38.9 eggs/trap/day on week 16, the first week of the experiment. This dramatic surge in egg frequency is not attributable to our introduced stock which consisted only of trans-location males. As in KDZ, the number of ssp. *formosus* recovered from ovistrip collections early in the rainy season is few and cannot account for the stimulation in indoor oviposition (cf. Second section).

Bait catches in MBI produced more adult *A. aegypti* during the release period (Fig. 2E). In this experimental interval the oo/oo ratio in adult catches dropped markedly in response to the daily release of translocation males (Fig. 3). A similar phenomenon was observed by Hausermann in the heterozygote release conducted in Chibarani village in 1974 (cf. Annual ICIPE Report, 1974).

Egg, pupal, and adult numbers declined in MBI after the cessation of releases. In view of the sterility induced in assayed, individual females (Second section), it would be tempting to associate the decrease in these indices with an effect of the heterozygote release. However, such a conclusion would not be necessarily warranted. There is a natural decline in the population parameters in reference villages after the rainy season, albeit not as prominent as observed in MBI. We know too little about naturally-occurring fluctuations observed in mosquito numbers indoors and their causes to state whether the decline in adult, egg, and pupal numbers may be a consequence of sterility introduced *via* the translocation or alternative factors.

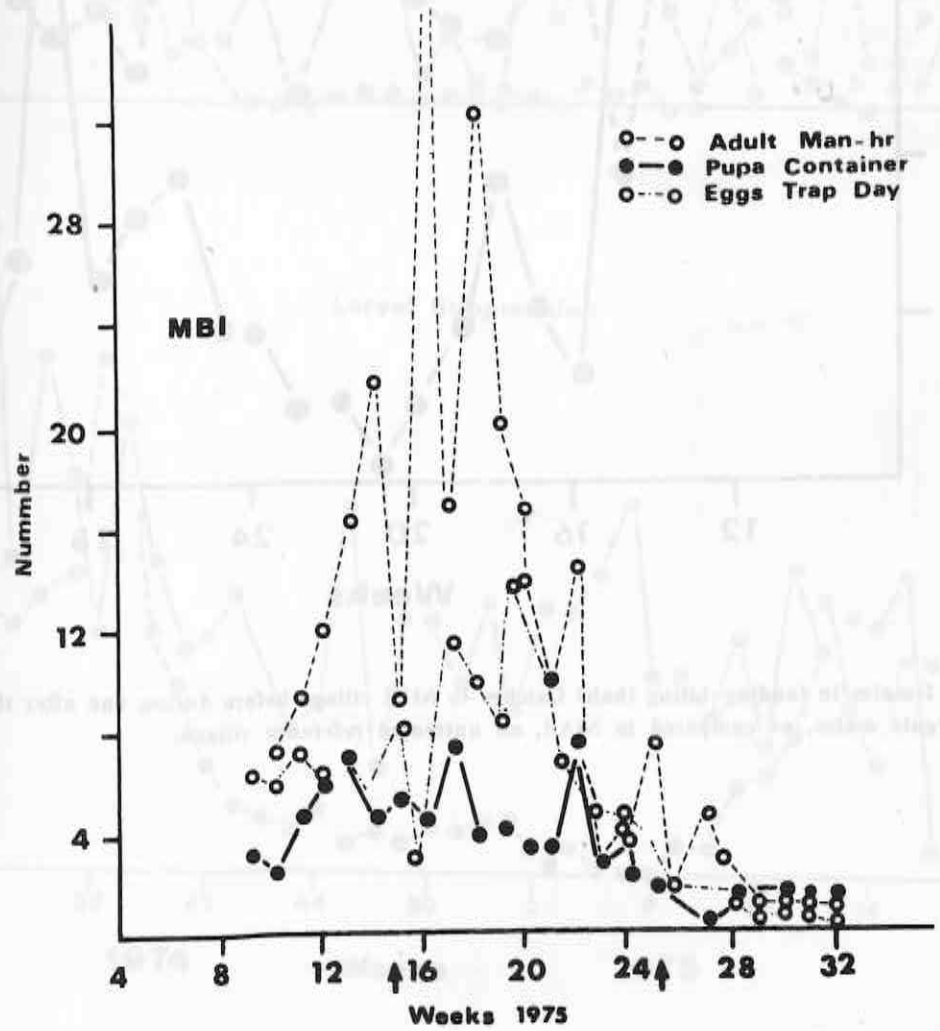


Fig. 2e: Weekly indoor pop. indices of 3 life stages of *A. aegypti* from 5 villages during first 32 weeks, 1975.

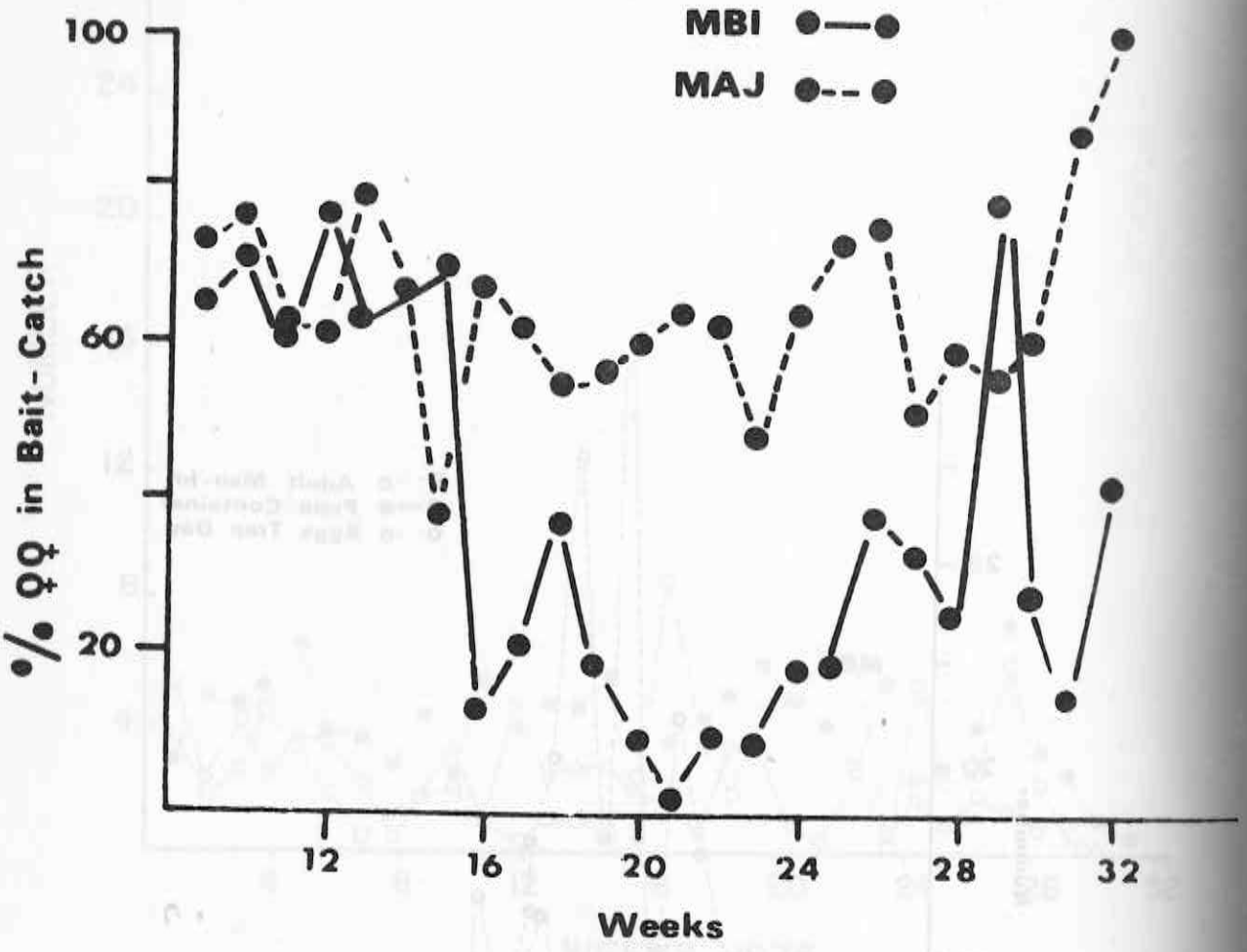


Fig. 3: Percent females in landing–biting (bait) Catches in MBI village before during and after the release of double heterozygote males, as compared to MAJ, an untreated reference village.

MWN (Fig. 4)

MWN was subjected to 16 successive weeks of "source reduction" in 1974-75 during which period the domestic water containers were scrubbed twice weekly and the contents of these vessels sieved to remove larvae. By these methods the local breeding population was reduced to zero within three weeks after the initiation of the experiment (Fig. 4). During the course of the source reduction, a few adult "type form" mosquitoes were captured in the weekly bait catches. Since breeding within the village had been eliminated, we assume these individuals represent immigrants. Recolonization and recovery of the local mosquito population required approximately 6-8 weeks following the termination of larval suppression.

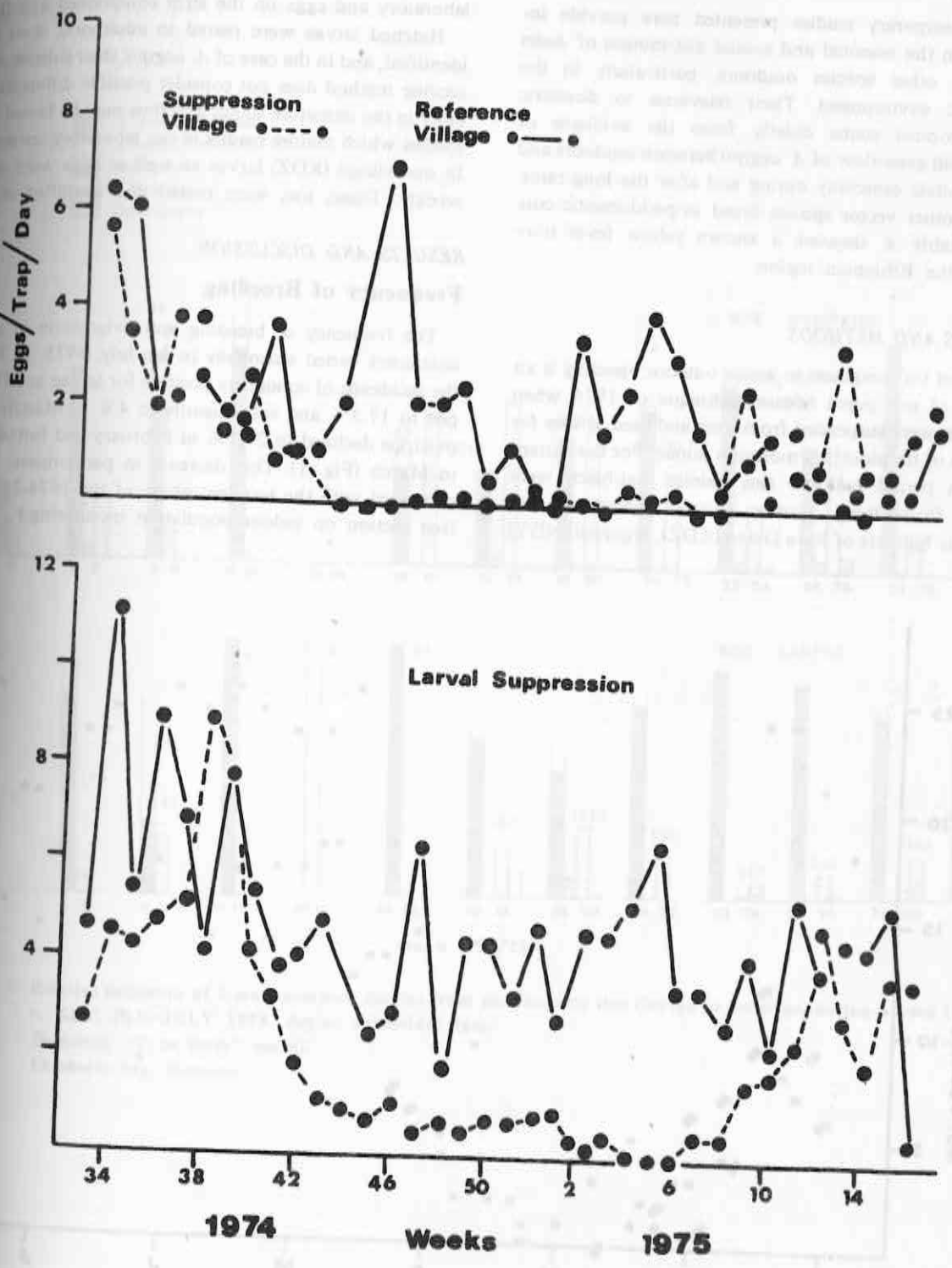


Fig. 4: Weekly egg and adult counts in MWN before, during and after source reduction (larval suppression).

Ecological Monitoring Outdoors

INTRODUCTION

The contemporary studies presented here provide information on the seasonal and spatial distribution of *Aedes aegypti* and other species outdoors, particularly in the peridomestic environment. Their relevance to domestic mosquito control stems chiefly from the evidence of migration and gene-flow of *A. aegypti* between outdoors and indoors habitats especially during and after the long rains. Moreover, other vector species breed in peridomestic containers, notably *A. simpsoni*, a known yellow fever transmitter in the Ethiopian region.

MATERIALS AND METHODS

The use of tin containers to assess outdoor breeding is an outgrowth of our pupal release technique of 1974 when these vessels were suspended from trees and used as sites for distribution of translocation mosquito pupae. For the current observation period half-litre tins painted flat-black were suspended from trees, mostly coconut palms in the peridomestic habitats of Kwa Dzivo (KDZ), Ngeyeni (NGY) and Birikani (BKI) region. Cloth ovistrips were clipped to the inside of each tin which was subsequently half-filled with water. The collection tins were cleaned weekly and replenished with fresh tap water. A strip remained in each tin for three days a week, whereafter it was brought to the laboratory and eggs on the strip conditioned and flooded.

Hatched larvae were reared to adulthood, their species identified, and in the case of *A. aegypti*, their subspecies. This scoring method does not consider possible differential mortality in the immature stages and thus may be biased toward species which mature readily in our laboratory environment. In one village (KDZ) larvae as well as eggs were collected weekly. These, too, were reared and identified as adults.

RESULTS AND DISCUSSION

Frequency of Breeding

The frequency of breeding and oviposition in outdoors containers varied seasonally in Jan-July, 1975. In February the incidence of containers positive for larvae in KDZ dropped to 17.3% and subsequently to 4% in March; positive ovistrips declined to 27.2% in February and further to 3% in March (Fig. 1). This decrease in peridomestic breeding coincided with the terminal phase of the 1974-75 drought (see section on indoor population monitoring.)

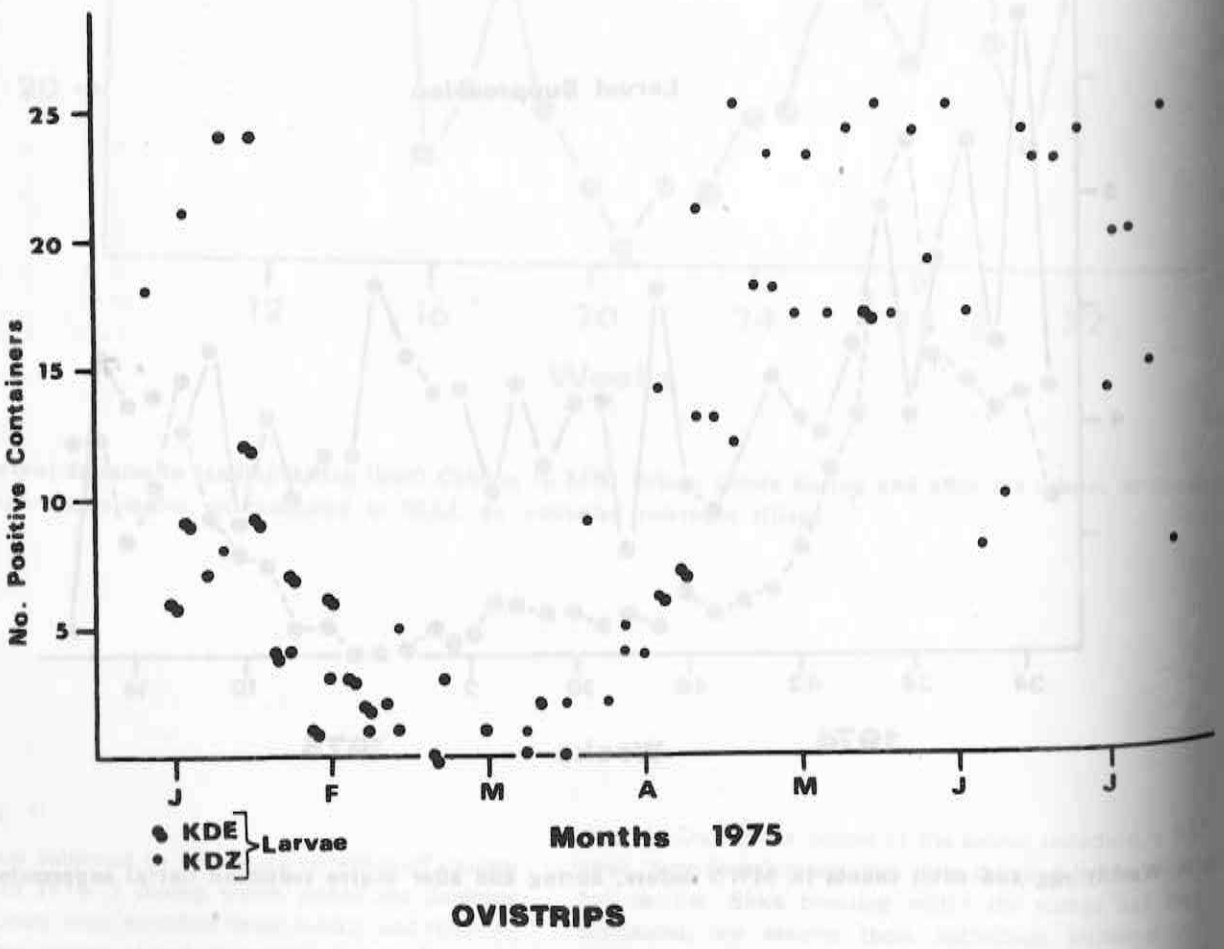


Fig. 1: Seasonal frequency of breeding in 25 tins in the peridomestic habitat of Kwa Dzivo village Jan-July, 1975.

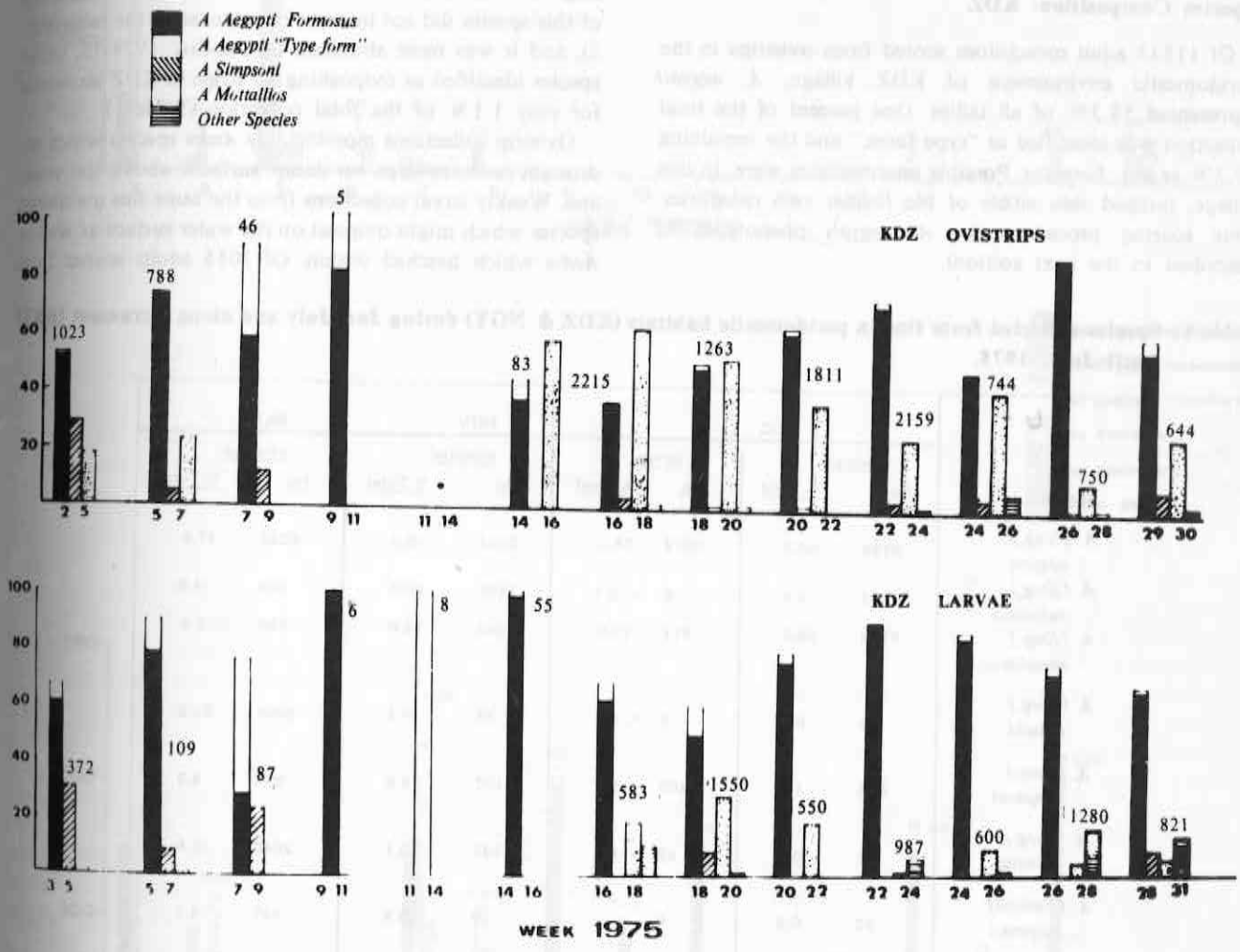


Fig. 2: Relative incidence of 3 most common species from peridomestic tins (larvae or ovistrips) during 3 week intervals in KDZ JAN–JULY 1975. *Aegypti* subdivided into:
Domestic "Type form" morph
Outdoors ssp. *Formosus*.

In April with the onset of the rains, immature mosquito stages reappeared in our sampling vessels, egg numbers increasing prior to larval forms. In May 96% of all ovistrips yielded viable eggs, and 66% of tins sampled that month contained larvae. Peridomestic container breeding remained high in June and July accompanied by a trend toward increased scatter among weekly samples (Fig. 1).

Species Composition: KDZ

Of 11533 adult mosquitoes scored from ovistrips in the peridomestic environment of KDZ village, *A. aegypti* represented 58.3% of all tallies. One percent of the total collection was identified as "type form," and the remaining 57.3% as ssp. *formosus*. Possible intermediates were, in this village, lumped into either of the former two categories. (Our scoring procedure for *A. aegypti* phenotypes is described in the next section).

A. metallicus comprised 36% of all identifications and in 3 of 12 collecting periods between Jan-July was the most common species from ovistrips (Fig. 2). These periods of *A. metallicus* abundance occurred in response to the April-May rains, and it appears from these data that *A. metallicus* may peak earlier during the wet season in the peridomestic habitat than *A. aegypti formosus*.

The third most common species was *A. simpsoni*, representing 4.6% of all individuals scored. Curiously, the frequency of this species did not increase in response to the rains (Fig. 2), and it was most abundant in Dec-Jan. 1974-75. Other species identified as ovipositing on strips in KDZ accounted for only 1.1% of the total collection (Table 1).

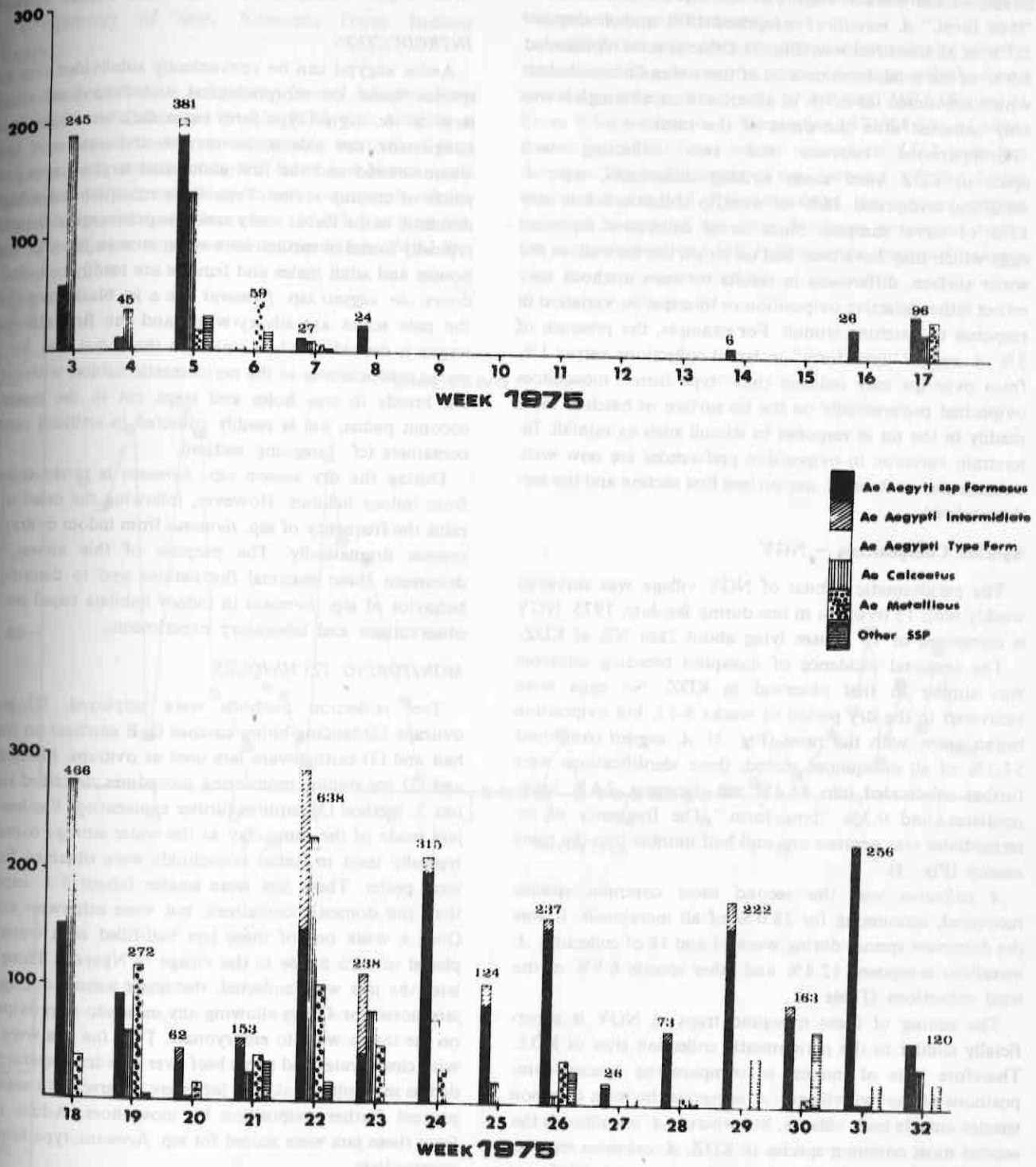
Ovistrip collections monitor only *Aedes* species which lay drought-resistant eggs on damp surfaces above the water line. Weekly larval collections from the same tins monitored species which might oviposit on the water surface as well as *Aedes* which hatched within. Of 7015 adults scored from

Table 1: Species collected from tins in peridomestic habitats (KDZ & NGY) during Jan–July and along a transect (BKI) April–July, 1975.

	KDZ				NGY		BKI	
	ovistrips		larvae		ovistrips		ovistrips	
Species	No.	% Total	No.	% Total	No.	% Total	No.	% Total
A. (Steg.) aegypti	6728	58.3	5219	74.4	2234	52.3	6233	47.5
A. (Steg.) calceatus	53	0.5	6	<0.1	1198	28.0	649	4.9
A. (Steg.) metallicus	4148	36.0	911	13.0	546	12.8	325	2.5
A. (Steg.) heischi	29	0.3	5	<0.1	15	0.4	3050	23.2
A. (Steg.) simpsoni	536	4.6	400	5.7	107	2.5	294	2.2
A. (Steg.) soleatus	15	0.1	22	0.3	133	3.1	2046	15.6
A. (Finlaya) fulgens	22	0.2	0		39	0.9	537	4.1
A. (Diceromyis) adersi	2	<0.1	0		0		0	
*A. (Diceromyia) furcifer**	0		0		2	<0.1	0	
A. (Aedimrphus) haworthi	0		0		0		7	<0.1
Culex nebulosis	0		399	5.7	0		0	
Culex horridus	0		6	<0.1	0		0	
*Culex sp.***	0		42	0.6	0		0	
Toxorhynchites brevipalpis	0		5	<0.1	0		0	
	11533		7015		4274		13141	

* Either *A. furcifer* or *A. taylori*.

** Not identified to species; may include individuals of former two species.



Mosquitoes Identified From Ngeyeme Outdoor Ovistrips in Predomestic Habitat Jan-July 1975. *A. aegypti* Subdivided as:

Dark,
Outdoors ssp. *Formosus,*
Light,
Indoors "Type Form", or
Intermediates between the 2 morphs.

larvae, 74.4% were *A. aegypti*, 69.4% ssp. *formosus* and 5% "type form." *A. metallicus* comprised 13% and *A. simpsoni* 5.7% of all identifications (Fig. 2). Other species represented 6.9% of the total; most notable of these was *Culex nebulosis* which accounted for 5.7% of all collections, although it was only collected after the onset of the rains.

Comparisons between the two collecting techniques in KDZ yield some striking differences, e.g., *A. metallicus* comprised 36% of ovistrip collections but only 13% of larval samples. Since larval collections represent eggs which may have been laid on strips, the tin wall, or the water surface, differences in results between methods may reflect either selective oviposition or interspecies variation in response to hatching stimuli. For example, the presence of 5% *A. aegypti* "type form" in larval collections versus 1% from ovistrips may indicate that "type form" mosquitoes oviposited preferentially on the tin surface or hatched more readily in the tin in response to stimuli such as rainfall. Interstrain variation in oviposition preferences are now well-documented in Rabai *A. aegypti* (see first section and the section below).

Species Composition – NGY

The peridomestic habitat of NGY village was surveyed weekly with 15 ovistrips in tins during Jan-July, 1975. NGY is composed of 13 houses lying about 2km NE of KDZ.

The seasonal incidence of mosquito breeding outdoors was similar to that observed in KDZ. No eggs were recovered in the dry period of weeks 9-13, but oviposition began anew with the rains (Fig. 3). *A. aegypti* comprised 52.3% of all mosquitoes scored; these identifications were further subdivided into 44.4% ssp. *formosus*, 7.6% intermediates, and 0.3% "type form." The frequency of intermediates was greatest one and half months into the rainy season (Fig. 3).

A calceatus was the second most common species recovered, accounting for 28.0% of all individuals. It was the dominant species during weeks 3 and 18 of collecting. *A. metallicus* composed 12.8% and other species 6.9% of the total collections (Table 1).

The setting of these mosquito traps in NGY is superficially similar to the peridomestic collection sites of KDZ. Therefore it is of interest to compare the species compositions of the two villages. *A. aegypti* is the most common species outside both villages, but whereas *A. metallicus* is the second most common species in KDZ, *A. calceatus* replaces it in NGY. By contrast, *A. calceatus* is rare outside KDZ, accounting for only 0.5% of ovistrip collections.

Species Composition: BKI

In the BKI locality 20 tins were nailed to trees along a transect in a coconut grove. The transect begins less than one kilometre away from NGY village and proceeds up a slight incline toward Benyagundo Hill in Rabai location. The collecting sites in BKI region are generally more distant from dwellings than the tins in the peridomestic habitats of KDZ or NGY.

Aedes aegypti Subspies *formo sus* from Indoor habitats

INTRODUCTION

Aedes aegypti can be conveniently subdivided into subspecies based on morphological and behavioral characteristics. *Ae. aegypti* type form has a dark brown to tannish integument, the pale scales on the abdomen tend to be cream-colored and the first abdominal tergite has a broad patch of creamy scales. Type form mosquitoes are highly domestic in the Rabai study area: the pre-imaginal forms are typically found in earthenware water storage jars kept inside houses and adult males and females are readily collected indoors. *Ae. aegypti* ssp. *formosus* has a jet black integument; the pale scales are silvery-white and the first abdominal tergite is devoid of white scales. In the Rabai area ssp. *formosus* predominates in the peridomestic habitat where it often breeds in tree holes and steps cut in the trunks of coconut palms, but is readily collected in artificial outdoor containers (cf. foregoing section).

During the dry season ssp. *formosus* is rarely collected from indoor habitats. However, following the onset of the rains the frequency of ssp. *formosus* from indoor ovitraps increases dramatically. The purpose of this annex is to document these seasonal fluctuations and to describe the behavior of ssp. *formosus* in indoor habitats based on field observations and laboratory experiments.

MONITORING TECHNIQUES

Tree collection methods were employed: (1) indoor ovitraps (2) landing-biting catches (L-B catches) on human bait and (3) earthenware jars used as ovitraps. Methods (1) and (2) are routine monitoring procedures described in Annex 3: method (3) requires further explanation. Earthenware jars made of the same clay as the water storage containers typically used in Rabai households were obtained from a local potter. These jars were smaller (about 3 l. capacity) than the domestic containers, but were otherwise similar. Once a week one of these jars half-filled with water was placed in each house in the village of Ngeyeni. Three days later the jars were collected, the water poured out and the jars stored for 4 days allowing any mosquito eggs oviposited on the inside wells to embryonate. Then the jars were filled with clean water and some beef liver powder suspension added as mosquito food. The jars were covered with netting to prevent further oviposition by mosquitoes. Adults reared from these jars were scored for ssp. *formosus*, type form and intermediate.

Classification of Phenotypes

Not all field-collected *Aedes aegypti* fit neatly into either the type form or ssp. *formosus* descriptions given above. The most common exceptions are mosquitoes with a black integument and silvery-white scales, but with a median patch of white scales on the first abdominal tergite. Such mosquitoes have been classified as intermediate, the implication being that these are F_1 hybrids between ssp. *formosus* and type form. Genetic evidence supporting this interpretation is presented below. Therefore, *A. aegypti* mosquitoes collected from indoor habitats have been classified as type form, ssp. *formosus*, or intermediate.

FIELD RESULTS

A. Frequency of ssp. *formosus* from indoor ovitraps.

Data are available for a full year from 2 villages: Kwa Dzivo (KDZ) and Majengo (MAJ). KDZ was used as a village for source reduction (twice-weekly removal of eggs and larvae from earthenware jars) from late March until early October 1974, then a translocation homozygote release was carried out until early December 1974. It has been the object of no further experimental manipulations. MAJ has served as reference village and has never been subjected to experimental perturbations.

In KDZ there was an increase in the frequency of ssp. *formosus* beginning during the period of source reduction and continuing to the first weeks of January, 1975 (Figure 1). From 9 September to 31 December 1974 58.8% of adults scored (n = 1892) from indoor ovitraps in KDZ were ssp. *formosus*. The total number of ssp. *formosus* collected during this period was 113. By comparison, the average number collected in each of the other 5 villages monitored during the same interval was 169.4. This is the greatest frequency

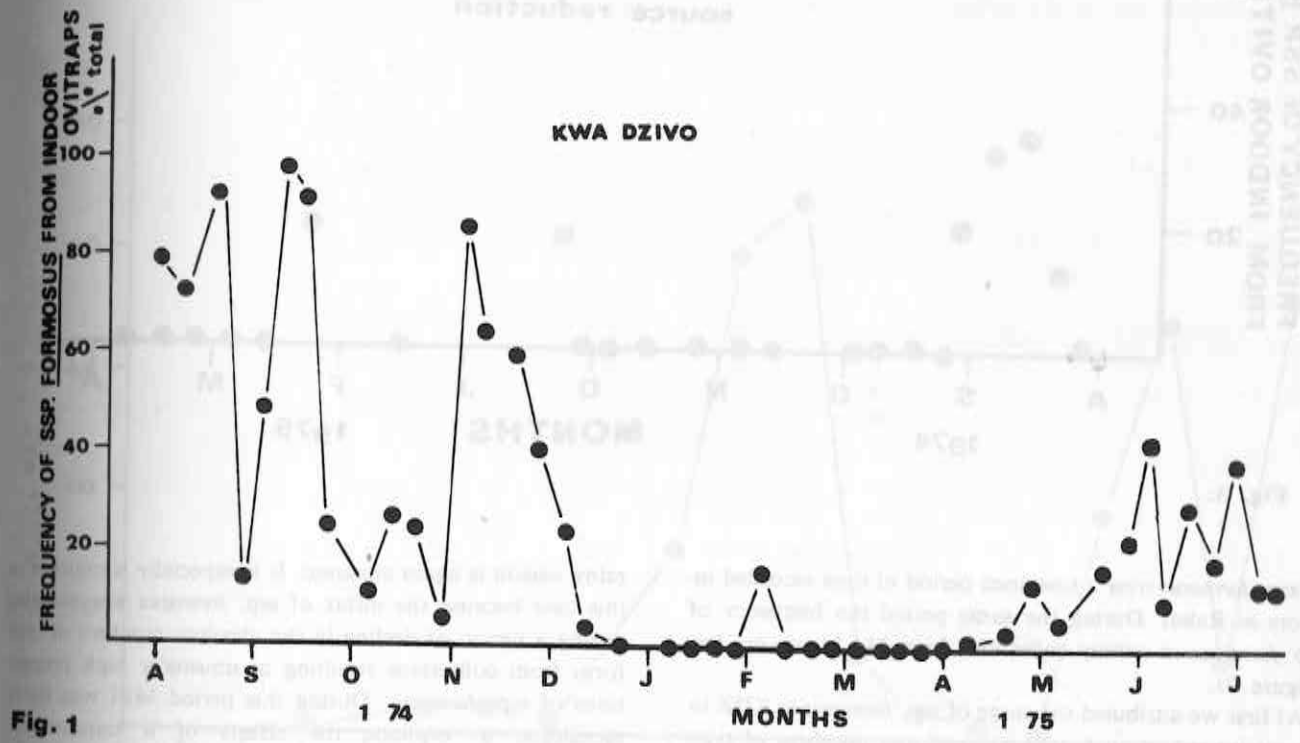


Fig. 1

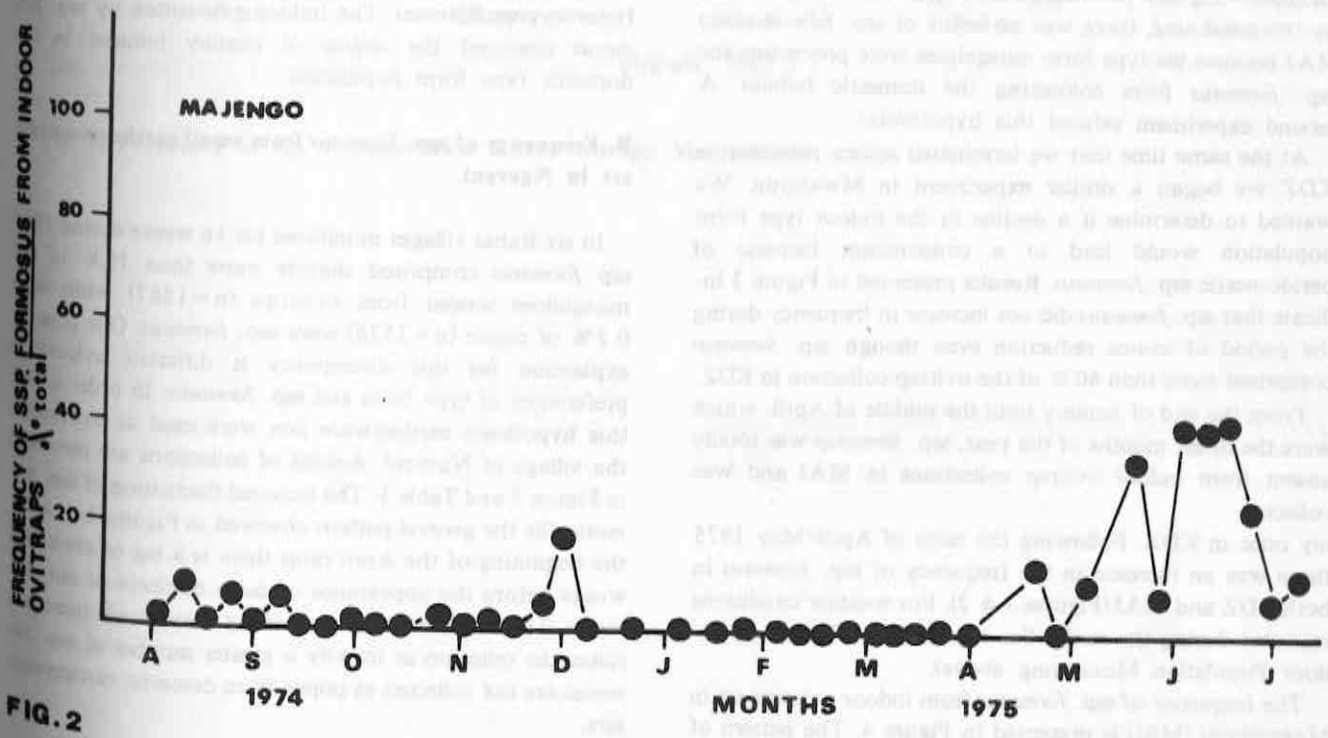


FIG. 2

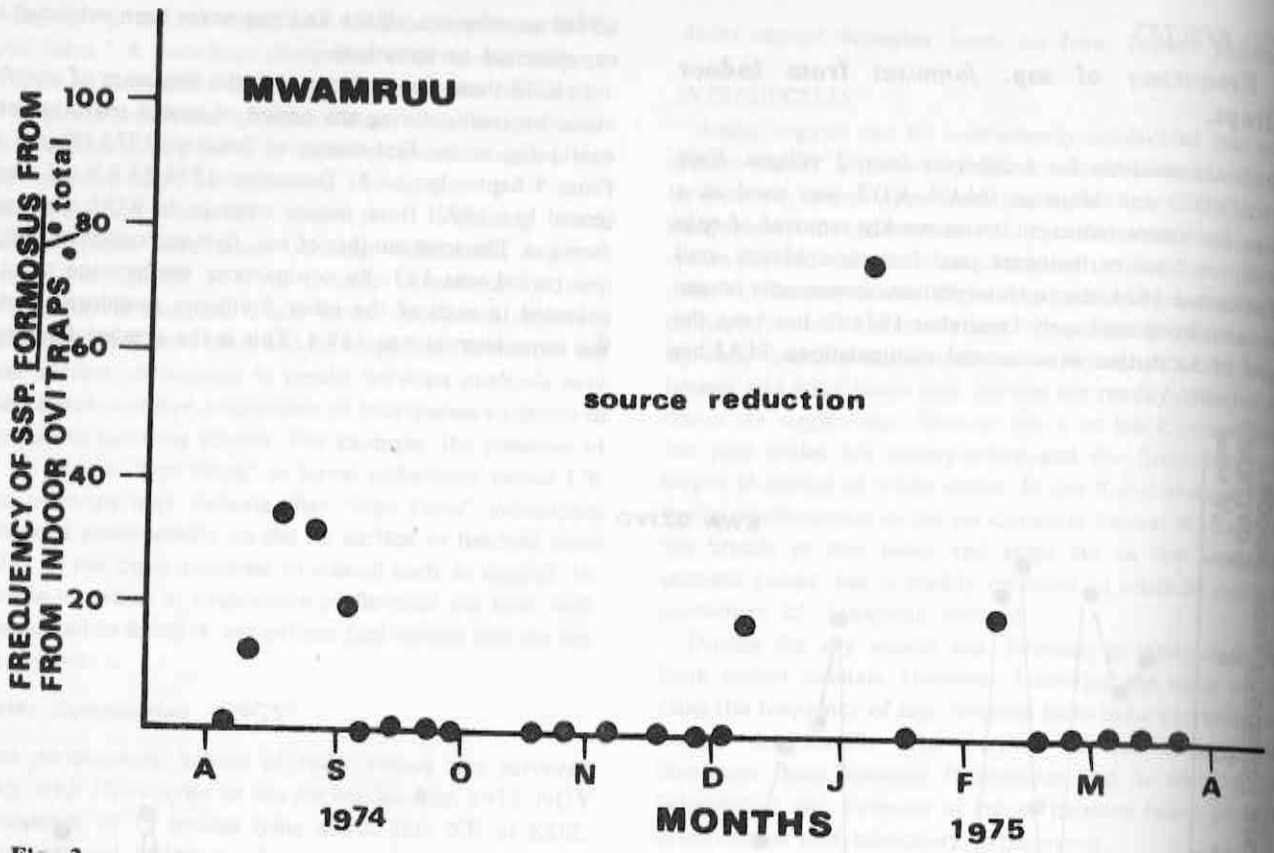


Fig. 3:

of ssp. *formosus* over a sustained period of time recorded indoors in Rabai. During the same period the frequency of ssp. *formosus* in ovitrap collections from MAJ was very low (Figure 2).

At first we attributed the surge of ssp. *formosus* in KDZ to the source reduction having reduced the numbers of type form allowing ssp. *formosus* to move into the vacated niche. By this reasoning, there was no influx of ssp. *formosus* into MAJ because the type form mosquitoes were preventing the ssp. *formosus* from colonizing the domestic habitat. A second experiment refuted this hypothesis.

At the same time that we terminated source reduction in KDZ we began a similar experiment in Mwamruu. We wanted to determine if a decline in the indoor type form population would lead to a concomitant increase of peridomestic ssp. *formosus*. Results presented in Figure 3 indicate that ssp. *formosus* did not increase in frequency during the period of source reduction even though ssp. *formosus* comprised more than 60% of the ovitrap collection in KDZ.

From the end of January until the middle of April, which were the driest months of the year, ssp. *formosus* was totally absent from indoor ovitrap collections in MAJ and was collected

nly once in KDZ. Following the rains of April/May 1975 there was an increase in the frequency of ssp. *formosus* in both KDZ and MAJ (Figures 1 & 2). For weather conditions recorded during the study, See Fig. 1 in the Section on Indoor Population Monitoring above).

The frequency of ssp. *formosus* from indoor ovitraps set in Mngamboni (MBI) is presented in Figure 4. The pattern of the rapid increase of ssp. *formosus* following the onset of the rainy season is again apparent. It is especially significant in this case because the influx of ssp. *formosus* was occuring during a period of decline in the absolute numbers of type form from collections resulting in unusually high proportions of ssp. *formosus*. During this period MBI was being monitored to evaluate the effects of a translocation heterozygote release (see section on Double Translocation Heterozygote Release). The indoor oviposition by ssp. *formosus* obscured the degree of sterility induced in the domestic type form population.

B. Frequency of ssp. *formosus* from small earthenware jars set in Ngeyeni.

In six Rabai villages monitored for 16 weeks during 1974 ssp *formosus* comprised slightly more than 10% of the mosquitoes scored from ovistrips (n = 1887) while only 0.2% of pupae (n = 2526) were ssp. *formosus*. One possible explantion for this discrepancy is different oviposition preferences of type form and ssp. *formosius*. In order to test this hypothesis earthenware jars were used as ovitraps in the village of Ngeyeni. Results of collections are presented in Figure 5 and Table 1. The seasonal fluctuation of ssp. *formosus* fits the general pattern observed in Figures 1-4. After the beginning of the April rains there is a lag of about 4-6 weeks before the appearance of large numbers of ssp. *formosus*. The observed frequency of ssp. *formosus* in these jars raises the question as to why a greater number of ssp. *formosus* are not collected as pupae from domestic earthenware jars.

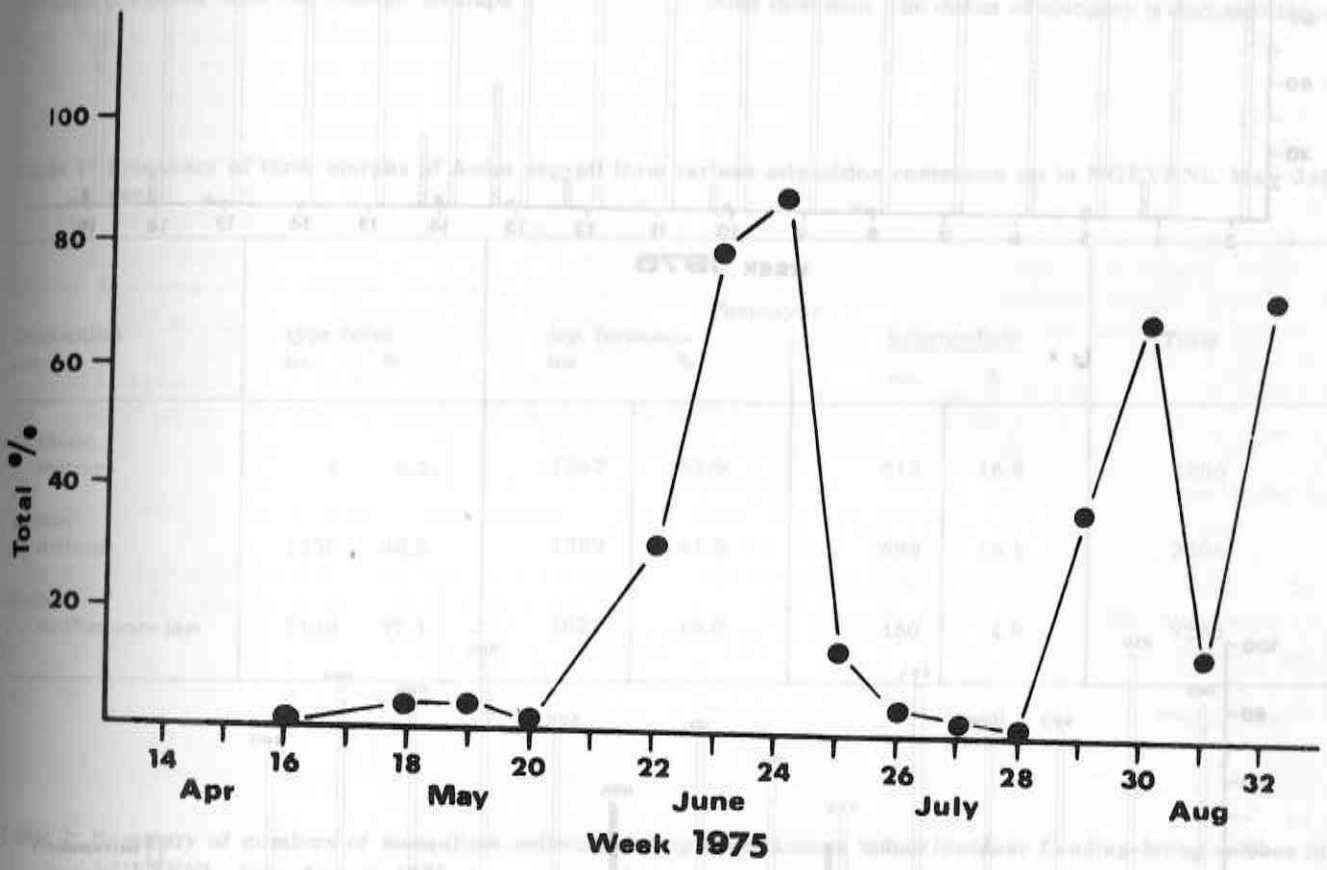


Fig. 4: Frequency of ssp. *Formosus* from indoor ovitraps: Mng'ambon.

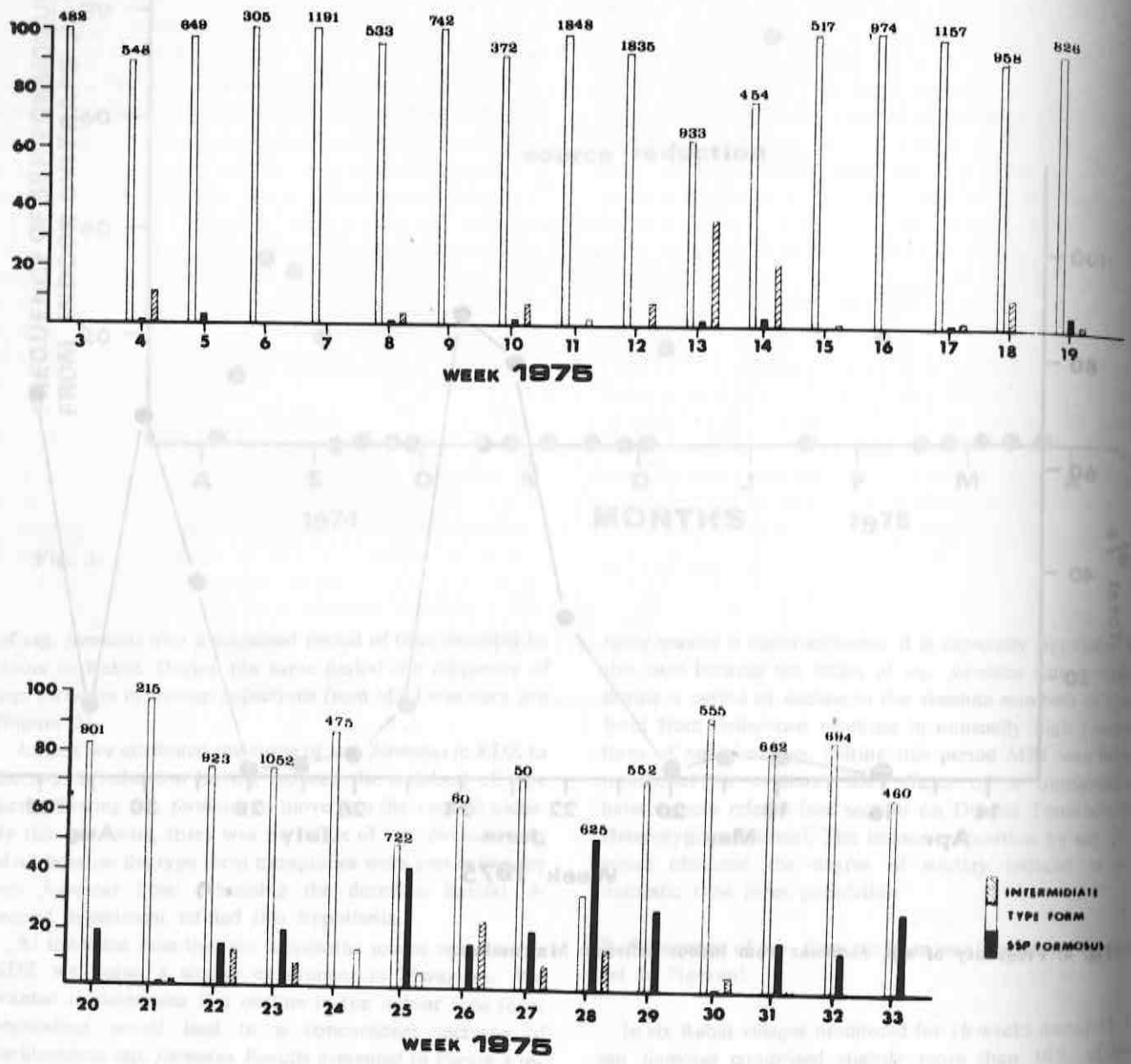


Fig. 5: Frequency of ssp. *formosus* from small earthenware jars: NGY.

C. Oviposition Preferences.

Table 1 summarizes the distribution of the 3 morphs of *A. aegypti* among 3 oviposition sites in the same village. The clearest pattern is that of type form which clearly oviposits preferentially in the indoor habitat and mostly in earthenware jars. Intermediate mosquitoes are most common in the indoor habitat where they are frequently collected in ovitraps. Based on the data given in the table it is difficult to characterize the ssp. *formosus;* while they predominate outdoors, they are more numerous in indoor collections. This may simply reflect the number and size of the indoor ovitraps compared with the outdoor ovitraps.

D. Simultaneous indoor/outdoor L-B catches. (Tables 2 & 3).

Subspecies *formosus* are rarely collected in indoor L-B catches even though they enter houses to oviposit. They do occasionally feed on man outdoors, however. It is very striking that in KDZ with its well-documented peridomestic ssp. *formosus* population, not a single ssp. *formosus* was collected during outdoor L-B catches. There are at least 2 likely explanations: (1) the mosquitoes are autogenous, i.e. they are capable of maturing their eggs without a blood meal or (2) they feed preferentially on a vertebrate host(s) other than man. The matter of autogeny is discussed below.

Table 1: Frequency of three morphs of Aedes aegypti from various oviposition containers set in NGEYENI, May–July 1975.

Oviposition site	Phenotype: type form no.	%	ssp. formosus no	%	intermediate no.	%	Total
Outdoor ovitraps	4	0.2	1543	83.0	313	16.8	1860
Indoor ovitraps	1338	40.5	1339	41.2	599	18.1	3303
Indoor earthenware jars	7149	77.1	1671	18.0	450	4.9	9270

Table 2: Summary of numbers of mosquitoes collected during simultaneous indoor/outdoor Landing-biting catches in NGEYENI, June–August 1975.

Species	Indoor	Outdoor
Ae. aegypti type form	88	13
Ae. aegypti ssp. formosus	2	25
Ae. aegypti intermediate	1	1
Ae. pembaensis	6	17
Ae. simpsoni	0	3
Ae. metallicus	0	1
Culex sitiens	0	1
Culex sp.	0	1
Total	97	62

LABORATORY EXPERIMENTS

A. Oviposition preference tests.

Forty blood fed, mated type form and fourty blood fed, mated ssp. *formosus* were placed in a large population cage provided with an earthenware jar partially filled with water and an indoor oviposition trap of the type routinely used in Rabai. After 5 days the containers were collected, the water poured off and the eggs were allowed to embryonate. The eggs from each vessel were hatched, reared to adults and scored for ssp. *formosus* or type form.

The results are presented in Table 4 Ssp. *formosus* demonstrated a preference for the ovitrap, while the type form showed a preference for the earthenware jar. These results corroborate those based on field data presented.

Table 3: Summary of numbers of mosquitoes collected during simultaneous indoor/outdoor landing-biting catches in KWA

DZIVO, July—August 1975.

Species	Indoor	Outdoor
Ae. aegypti type form	207	0
Ae. aegypti ssp. formosus	0	0
Ae. aegypti intermediate	9	0
Culex tigripes	0	—
Total	216	1

Table 4: Results of a laboratory oviposition preference test.

Vessel	Distribution of progeny in oviposition vessels				Total
	No. formosus	% formosus	No. type form	% type form	
Earthenware jar	83	6.7	1481	84.3	1564
Ovitrap	1159	93.3	275	15.7	1434
Total	1242	100.0	1756	100.0	2998

G test for heterogeneity = 2016 $p < 0.005$

B. Autogeny.

It was suggested above that the scarcity of ssp. *formosus* in L-B catches ability of females to lay eggs without a blood meal. This hypothesis has been tested as follows: strains to be examined are usually freshly field-collected eggs or larvae. These are carefully reared at optimum density (less than 100 larvae/l of water) and well fed. This care is essential since autogeny in *Aedes aegypti* is facultative, i.e. only females that were well-nourished as larvae exhibit autogeny, and then only if they are of the proper genotype. Adults reared from these larvae are provided with a carbohydrate source (honey and guava jelly mixture) and water in a small oviposition cup. They are placed in the insectary away from the cages that are blood fed to minimize mistakes. 5-10 days later the oviposition cup is checked for eggs. Resutls of autogeny tests are summarized in Table 5.

No type form strain has ever demonstrated autogeny. Most ssp. *formosus* strains are autogenous, but produce less than 1 egg/female on the average. Obviously, this is insufficient to maintain the population. Apparently, many ssp. *formosus* strains are capable of developing autogenous eggs, but require a blood meal for full-size egg batches. Autogenous eggs have been hatched and reared to adults, but a self-sustaining autogenous strain has not been established.

C. Genetic analysis of Intermediate *Aedes aegypti*.

Crosses between intermediate, type form and ssp. *formosus* in various combinations have been analyzed in an attempt to elucidate the position of the intermediates. One example will suffice here because it raises a number of interesting points.

Mosquitoes reared from indoor ovistrips are routinely sexed as pupae and scored soon after emergence. Virgin male and female intermediates were culled and crossed in single pair cages. The F_1 progeny were reared and scored as adults. The intermediates for crossing were selected for black, *formosus*-like integument, but with a small patch of white scales on the first abdominal tergite. All the progeny of these intermediate crosses showed some individuals that had no white scales on the first abdominal tergite. This indicates that all the intermediate parents were heterozygous for the gene determining scaling on the first abdominal tergite. Among the progeny of three of the crosses were some individuals that were *spot* (Table 6). This marker is recessive and in order to be expressed phenotypically it must be homozygous. Therefore, both intermediate parents must have been heterozygous for *spot*. The *spot* gene is not uncommon among type form populations in Rabai; however, it has never been observed among *A. aegypti* ssp. *formosus*. Thus the intermediate parents were carrying characteristic type form genes and characteristic ssp. *formosus* genes. These characters appeared simultaneously and in Mendelian ratios among their progeny. It is almost certain that the parental intermediates were, in fact, hybrids of ssp. *formosus* and type form. This is an important point. Carefully monitoring the frequency of intermediates may give a valuable measure of the gene flow between sylvan and domestic populations.

Table 5: FREQUENCY OF formosus FROM EGG COLLECTIONS

Outdoor Ovistrips		Indoor Ovistrips		Clay Tiles	
n	% formosus	n	% formosus	n	% formosus
!+&*	97.8	1138	4.5	616	1.1

Indoor Ovistrips In Clay Pots		Clay Pots	
n	% formosus	n	% formosus
105	9.5	7036	0.5

Table 6: SUMMARY OF AUTOGENY TESTS

Aedes aegypti type form strains		No. females tested	No. eggs laid
KDZ	ovistrips. 18 IV 75	50	0
KGY	LL ex MITUNGI. 21 IV 75	50	0
MAJ	ovistrips. 28 IV 75	47	0
NGY	LL ex MITUNGI. 28 IV 75	35	0
NGY	LL ex MITUNGI. 12 V 75	100	0
NGY	LL ex MITUNGI. 12 V 75	100	0
	Total	382	0

Aedes aegypti ssp. formosus strains	No. females tested	No. eggs laid
NGY osopts. 15 & 22 II 75	22	7
NGY LL ex TINS. 19 & 23 IV 75	30	5
BIRIKANI osopts. 24 IV 75	63	10
NGY LL ex TINS. 30 IV 75	50	1
BIRIKANI LL ex TINS. 5 V 75	24	12
BIRIKANI LL ex TINS. 5 V 75	23	0
BIRIKANI LL ex TINS. 8 V 75	43	16
BAMBO POTS—KOMBENI. 10 V 75	37	257
BAMBO POTS—KOMBENI. 19 V 75	27	0
Total	319	308

A field Trial of Supression of *Aedes aegypti* Population by Releasing Sterile Males into Ererwani domestic Population

INTRODUCTION

As a part of the project concerned with studying *Aedes aegypti* populations in Rabai, translocation heterozygote males were released into designated villages for the purpose of depressing the populations. Both males and females were also released to replace the existing populations. Both experiments (unpublished report) yielded rather discouraging results because of behaviour differences between the released mosquitoes and the wild ones. Generally, the low mating competitivenss of the sterile males also affected the results.

MATERIALS AND METHODS

Pupae under twenty-four hours old were irradiated in a nitrogen atmosphere for forty minutes and later on for thirty minutes to reduce somatic damages Hallinan and Rai (1973) and Baldwin and Chance (1970).

The radiation source used was Cesium-137 Irraditor, Model B-34 Serial No. 1144, which provides a gamma radiation intensity of about 30,00 . R/Hr in air, and for a thirty and forty minutes radiation, the gamma radiation provided 15,000 Rad and 20,000 Rad respectively. Before these doses were used a pre-release determination of percentage hatch of sterile male inseminated females was carried out (see Fig. 1).

When the pupae hatched, the adult males were put into plastic gallon cages and carried to the village, where they were released. An average of one thousand two hundred and sixty (1260) sterile males were released daily from 1st March to 30th April (sixty-one days). (see Table 1).

The release village, Ererwani, has about seventeen mud huts reinforced with wooden frames, and roofed with coconut palm leaves. It is rather isolated from other villages scattered in coconut groves in the area.

RESULTS AND DISCUSSIONS

Table 1 shows the record of daily releases. A total of over 70,000 sterile males were released. Table II shows the pre-release and post-release monitoring of egg hatchability.

Fig. II is a graphical representation of tables II and III data. As can be seen from the graph there was a progressive decrease in egg hatchability as soon as the release experiment started but this depression was not as high as expected, considering the number of males released daily. Population estimates for other larger villages put the number of mosquitoes at about two thousand. The Ererwani population should have been less than this figure and therefore the number of males released daily represented probably more than half the resident population. Table III shows the percentage hatch from eggs collected from females (broken lines in figure II are the graphical representation). The average hatchability by this method was about 69.00% compared to 92% for pre-release data from oviposition strips, 79% for post-release oviposition strip collections. The apparent lower hatchability from landing-biting catch was probably caused by sampling error-a greater probability of females inseminated by sterile males being captured by the landing-biting method than their laying eggs on oviposition strips.

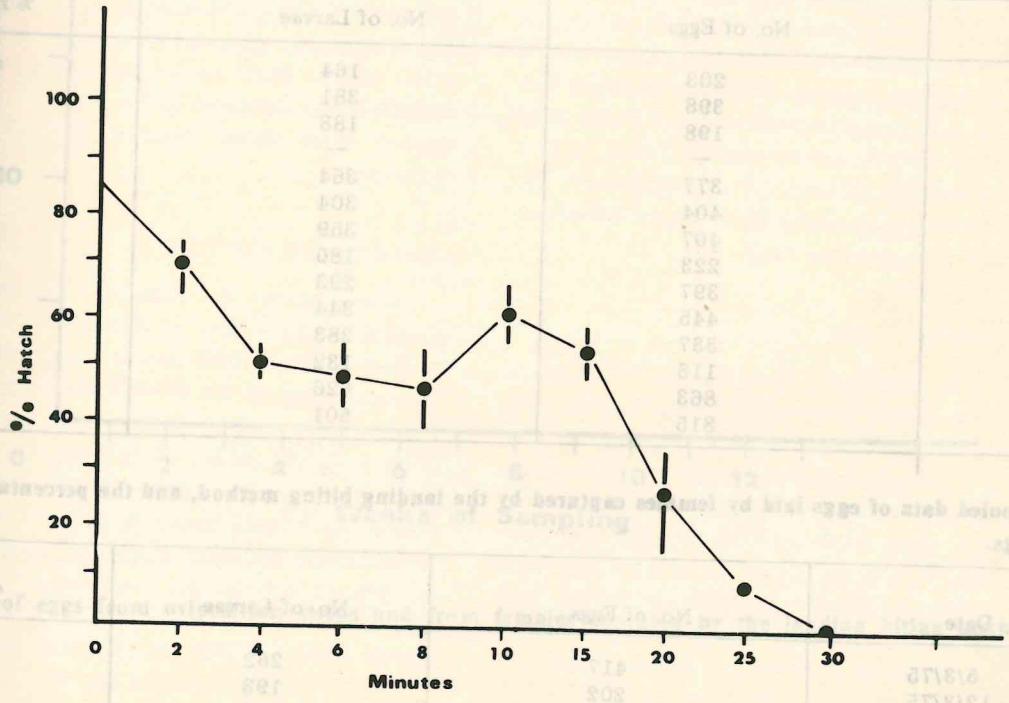


Fig. 1: % hatch of eggs of females inseminated with irradiated males.

Table 1: Record of sterile male released daily in Ererwani.

DAILY RELEASES			
Day of Release	No. of Mosquitoes	Day of Release	No. of Mosquitoes
1	500	32	1503
2	195	33	1872
3	500	34	1786
4	500	35	1000
5	500*	36	1041
6	—	37	1795
7	1583	38	1531
8	1000	39	1734
9	800	40	845
10	1300	41	1009
11	1200	42	865
12	1000	43	915
13	1155	44	795
14	1100	45	1873
15	600	46	2105
16	480	47	1696
17	760	48	1465
18	1050	49	1059
19	1650	50	1495
20	1500	51	1235
21	1150	52	1896
22	1161	53	1695
23	1100	54	2015
24	1031	55	1650
25	1095	56	1736
26	1135	57	1696
27	1353	58	1610
28	1348	59	1765
29	985	60	2156
30	1041	61	3500
31	1274		

Table 11: Record of monitoring of pre-release and post-release percentage hatchability of eggs collected from Ererwani. The asteria indicate date post-release collection started.

Date	No. of Eggs	No. of Larvae	% Hatch.
27/1/75	203	164	80.79
3/2/75	398	381	95.73
10/2/75	198	188	94.95
17/2/75	—	—	—
25/2/75	377	364	96.55
*3/3/75	404	304	75.25
10/3/75	407	359	88.21
17/3/75	223	180	80.72
24/3/75	397	293	73.43
31/3/75	445	344	77.30
7/4/75	337	283	83.98
14/4/75	116	132	79.52
21/4/75	863	626	72.54
28/4/75	815	501	61.47

Table 111: Pooled data of eggs laid by females captured by the landing biting method, and the percentage hatch of the eggs.

Date	No. of Eggs	No. of Larvae	% Hatch.
5/3/75	417	262	62.83
12/3/75	202	193	95.54
22/3/75	201	133	66.17
11/4/75	146	73	51.37

In general, the percentage hatch for both methods of monitoring was not low enough. The mating competitiveness of the sterile males is far below that of normal males as was indicated in a laboratory test. Fifty three day old males and fertile males were mixed up with a hundred-three-day old virgin females in breeding cages. The eggs laid by the females hatched separately and only eggs from fifteen out of hundred females showed complete sterility indicating that the sterile males could not effectively compete with the normal males.

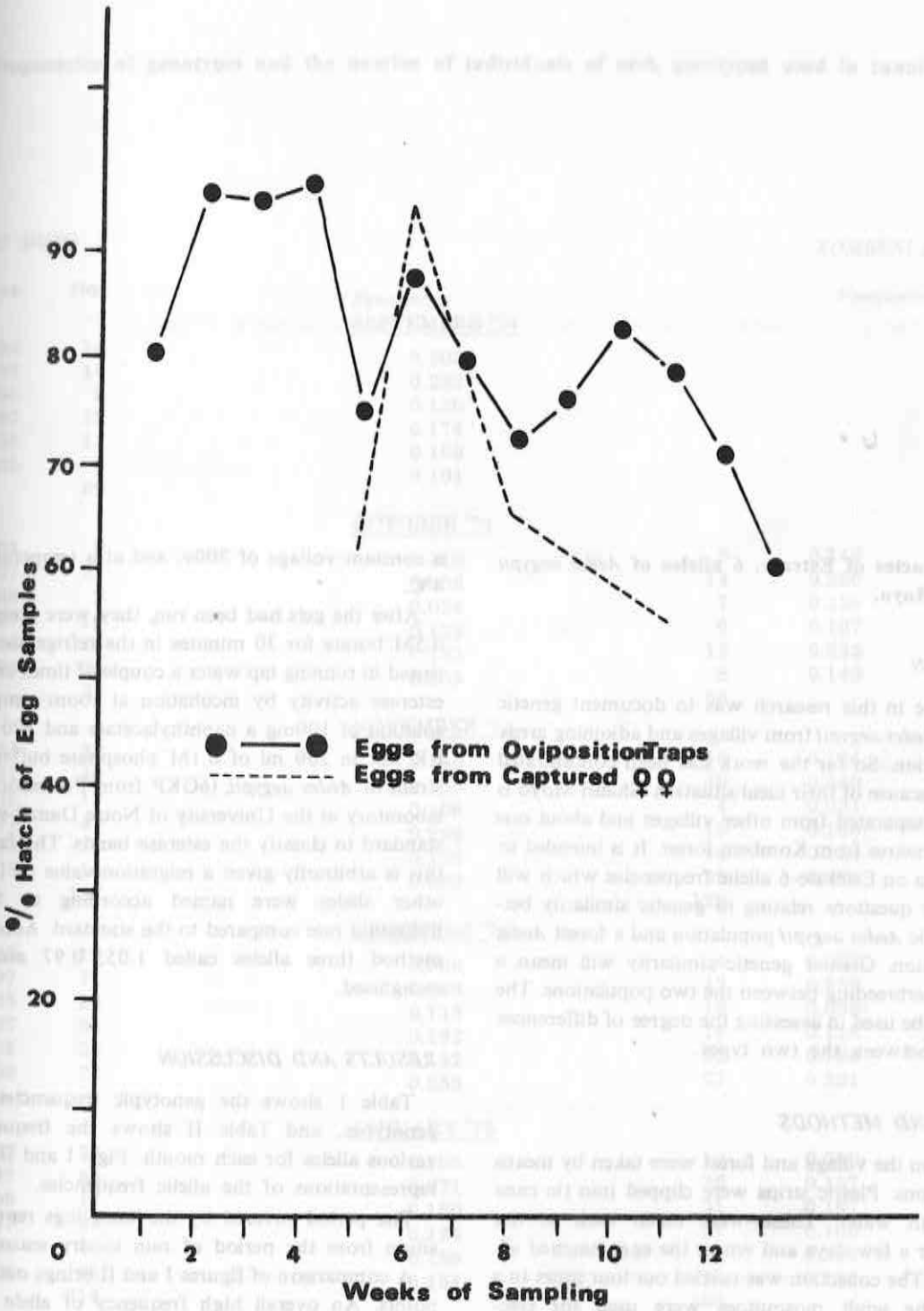


Fig. 2: % hatch of eggs from oviposition strips and from females captured by the landing biting method

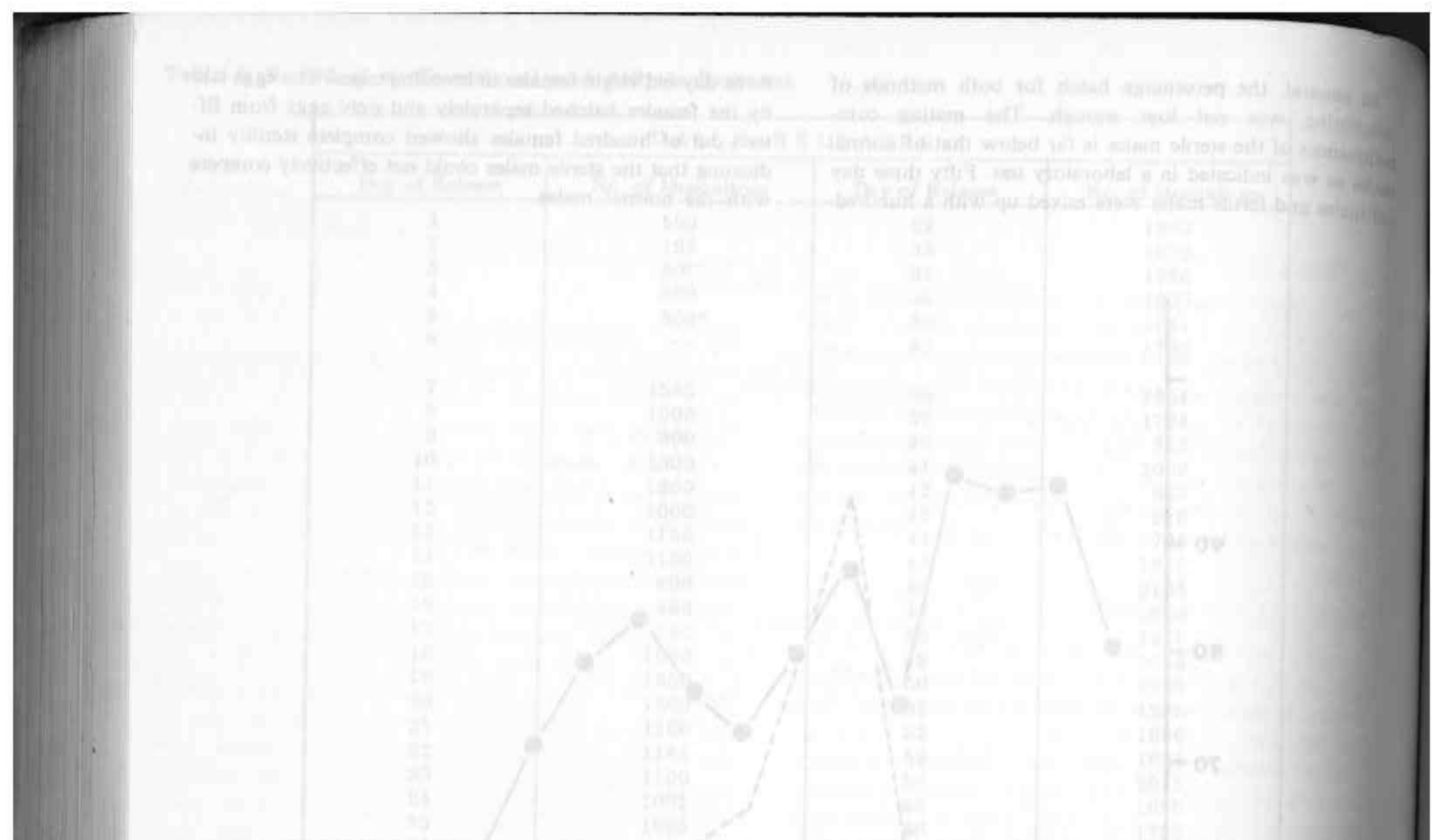


Fig. 1: Frequencies of Estrase. 6 alleles of *Aedes aegypti* from Shauri Moyo.

INTRODUCTION

The objective in this research was to document genetic differences in *Aedes aegypti* from villages and adjoining areas in Rabai Location. So far the work has been concentrated on two areas because of their ideal situation. Shauri Moyo is a village quite separated from other villages and about one and a half kilometres from Kombeni forest. It is intended to accumulate data on Esterase-6 allelic frequencies which will help to answer questions relating to genetic similarity between a domestic *Aedes aegypti* population and a forest *Aedes aegypti* population. Greater genetic similarity will mean a high rate of interbreeding between the two populations. The result will also be used in assessing the degree of differences in behaviour between the two types.

MATERIALS AND METHODS

Samples from the village and forest were taken by means of egg collections. Plastic strips were clipped into tin cans half filled with water. These were taken back to the laboratory after a few days and where the eggs hatched after three days. The collection was carried out four times in a month and the adult mosquitoes were used for electrophoresis. They were crushed in 15ul of 0.1M trisborate buffer with sucrose, and Bromophenol Blue as marker. Vertical polycylamide gel electrophoresis was carried out in 7%. Cyanogum-41 gels prepared with 0.1M tris-borate buffer, pH 8.9, containing 1.5mM EDTA and ammonium persulphate as a gelling agent. Both electrode chambers were filled with the same buffer and gels run for about 2 hours at a constant voltage of 300v, and at a temperature of about 4°C.

After the gels had been run, they were preincubated in a 0.5M borate for 30 minutes in the refrigerator. They were rinsed in running tap water a couple of times and stained for esterase activity by incubation at room temperature in a solution of 100mg a naphthylacetate and 100 mg fast blue RR salt in 200 ml of 0.1M phosphate buffer, pH 6.5. A strain of *Aedes aegypti*, (6GKP from Professor G.B. Craig's laboratory at the University of Notre Dame) was used as a standard to classify the esterase bands. The fastest allele of this is arbitrarily given a migration value of 1.00, then the other alleles were named according to their relative migration rate compared to the standard. According to this method three alleles called 1.05, 0.97 and 0.93 were recognised.

RESULTS AND DISCUSSION

Table 1 shows the genotypic frequencies for possible genotypes, and Table II shows the frequencies of the various alleles for each month. Figs. I and II are graphical representations of the allelic frequencies.

The period covered by the samplings represents a transition from the period of rain to dry season.

A comparison of figures I and II brings out the following points. An overall high frequency of allele 0.97 in both areas. In case of Shauri Moyo this remained the same up to January. In contrast the frequency of 0.97 dropped considerably from December, 1974, while allele 0.93 and 1.05 started going up. This may represent less interchange between the two populations at the height of the dry weather. In fact, at the time samples were very small in the two populations because of the dry weather.

Table 1: Frequencies of genotypes and the number of individuals of each genotyped used in running gels.

SHAURI MOYO			*KOMBENI FOREST*	
Genotype	No.	Frequency		Frequency
		SEPTEMBER '74		
0.93/0.93	14	0.203		
0.97/0.97	16	0.232		
1.05/1.05	9	0.130		
0.93/0.97	12	0.174		
0.93/1.05	11	0.159		
0.97/1.05	7	0.101		
Total	69			
		OCTOBER '74		
0.93/0.93	9	0.110	8	0.143
0.97/0.97	21	0.256	14	0.250
1.05/1.05	2	0.024	7	0.125
0.93/0.97	10	0.122	6	0.107
0.93/1.05	15	0.183	13	0.232
0.97/1.05	25	0.305	8	0.143
Total	32		56	
		NOVEMBER '74		
0.93/0.93	22	0.140	16	0.124
0.97/0.97	40	0.255	49	0.380
1.05/1.05	17	0.108	4	0.031
0.93/0.97	47	0.299	15	0.166
0.93/1.05	17	0.108	16	0.124
0.97/1.05	14	0.089	29	0.225
Total	157		129	
		DECEMBER '74		
0.93/0.93	19	0.066	4	0.033
0.97/0.97	71	0.248	43	0.352
1.05/1.05	33	0.115	12	0.098
0.93/0.97	55	0.192	14	0.115
0.93/1.05	35	0.122	22	0.180
0.97/1.05	73	0.255	27	0.221
		JANUARY '75		
0.93/0.93	33	0.105	10	0.060
0.97/0.97	85	0.271	26	0.157
1.05/1.05	47	0.150	31	0.187
0.93/0.97	39	0.124	27	0.163
0.93/1.05	49	0.156	43	0.259
0.97/1.05	61	0.194	29	0.175
Total	314		166	

Table 11: Frequencies of alleles for each month in Shauri Moyo and Kombeni Forest.

SHAURI MOYO		*KOMBENI FOREST*
	SEPTEMBER '74	
Alleles	Frequencies	Frequencies
0.93	0.370	
0.97	0.370	
1.05	0.261	
	OCTOBER '74	
0.93	0.262	0.313
0.97	0.470	0.375
1.05	0.268	0.313
	NOVEMBER '74	
0.93	0.344	0.244
0.97	0.449	0.550
1.05	0.207	0.205
	DECEMBER '74	
0.93	0.224	0.180
0.97	0.470	0.520
1.05	0.304	0.300
	JANUARY '75	
0.93	0.245	0.271
0.97	0.430	0.325
1.05	0.325	0.533

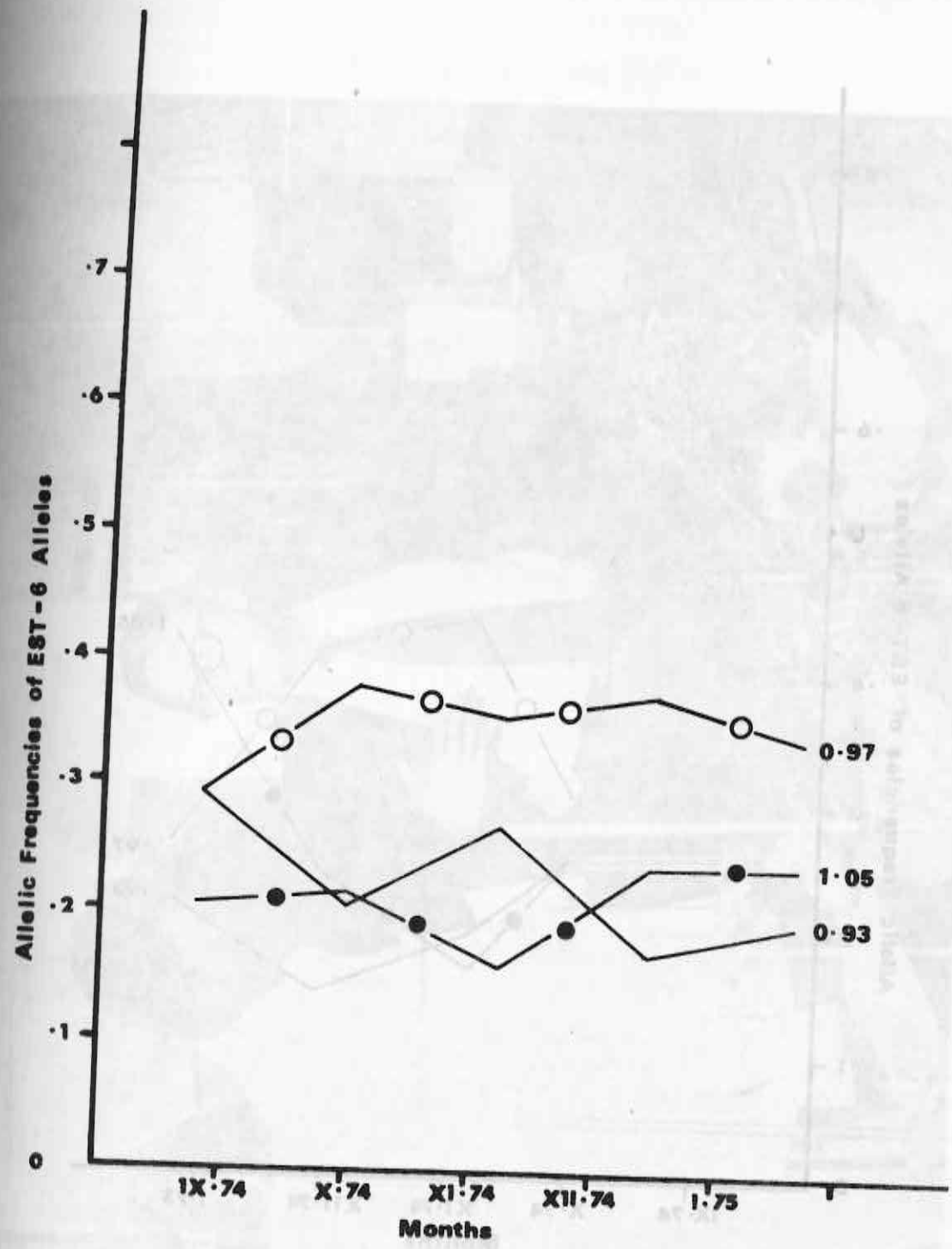


Fig. 1: Frequencies of Estrase. 6 alleles of *Aedes aegypti* from Shauri Moyo.

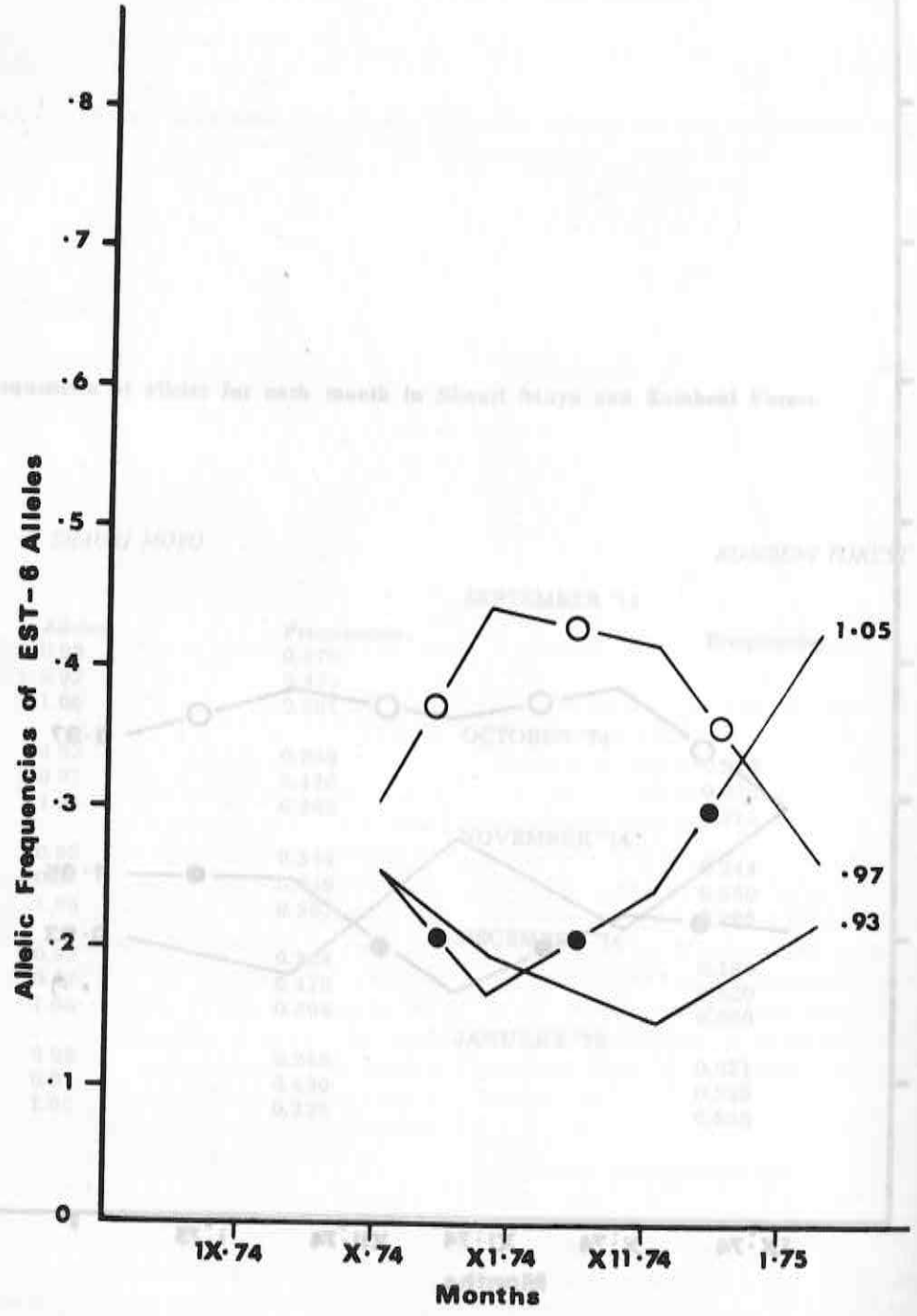


Fig. 2: Frequencies of Estrase. 6 alleles of *Aedes aegypti* from Kombeni Forest.

Dr. G.W. Oloo explaining the chemical communication process in termites to the UNDP Resident Representative, Nairobi, Dr. Karl Englund when he visited the ICIPE early 1976.

TERMITE RESEARCH

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

Scientists:

Dr. N. Abo-Khatwa (1974) – Research Scientist
Ing. O.H. Bruinsma (1974) – Research Scientist
Dr. G. Buhlmann (1975) – Research Scientist
Dr. J. Darlington (1975) – Research Scientist
Dr. M. Lepage (1975) – Research Scientist
Dr. F.C. Mathez (1973-1975) – Research Scientist
Dr. G.W. Oloo (1974) – Research Scientist
Mr. K.M. Wanyonyi (1971-1975) – Experimental Officer

Research Associate:
Dr. R. Leuthold (1974)

Collaborators:
Dr. C.J. Heather (1974) Research Scientist, Fine Structu
Unit
Dr. M. Kaib (1973-76) Research Scientist, Elec
trophysiology, Behaviour
Dr. G.D. Prestwich (1974) Research Scientist, Chemistry

Consultant
Professor C. Noirot

INTRODUCTION

felt that the significance of the physiological data can only be evaluated with a profound knowledge of the ecology of the relevant termite species. Since physiological research is more and more concentrating on *Macrotermes subhyalinus*, one of the major grass-feeding termites in semi arid regions, it was decided that the ecological research should deal mainly with this mound building species.

The behaviour studies were concentrated on the mechanisms of recruitment and on building behaviour as well as on the chemistry of the pheromones involved in these mechanisms and of defensive secretions. It was found that the trail pheromone concentration is responsible for recruitement of *Trinervitermes* workers to food sources and that at least two different pheromones are used for the coordination of building activities in *Macrotermes*. Pheromone trails of one species are followed by many but not all other species. Considerable advances were made in trail pheromone chemistry and several soldiers defensive secretions were chemically identified.

The caste determination studies were reorganized after the tragic death of K. Wanyonyi and the departure of another scientist. Emphasis was then laid on the species *Macrotermes subhyalinus* and first trials of colony foundation in the laboratory have been successful, while maintenance of small groups from large nests is still very difficult. Since it was shown earlier that juvenile hormone (JH) has a key role in caste determination in lower termites, JH concentration was determined in Macrotermes greens and eggs. The results seem to indicate a yearly cycle of activity in correlation with the appearance of wing padded nymphs and alates (Luscher and collaborators in Bern). Since JH is present in high amounts in greens of *Macrotermes*, it seemed important to study its role in egg production. Surprisingly the metabolism of homogenates of ovary or fat body of greens could not be influenced by the addition of JH but that of the ovary by ecdysone. Ultrastructure studies reveal high synthetic activity of follicular cells.

In connection with the new ecology project on food consumption it became important for the physiologists to investigate food utilization. A study of the chemistry of fungus combs of *Macrotermes* was therefore taken up and first results show that they contain besides other substances large amounts of mannitol, a sugar which cannot be metabolished by termites or by a number of fungus species but is utilized by the termite specific fungus *Termitomyces*.

The termite ecology work stated with a study of the caste and developmental stage composition of colonies of *Macrotermes* during the seasons. Study or food consumption was added later in 1975. For both studies the main task during this first period was the elaboration of the appropriate methods. Preliminary results seem to indicate that the composition of castes is nearly constant at least during one season.

ECOLOGY

ABSTRACT

The ICIPE termite ecology project on grass-feeding termites in a semi-arid grassland ecosystem began in 1975 with the arrival of the first ecologist in January and the second in June. In the first phase, attention is confined to two aspects of a single species, *Macrotermes subhyalinus*, a dominant member of the fauna and constructor of large mounds.

The internal economy of the colony as reflected by the annual cycle of changes in populations requires a destructive method of sampling for which a fumigation technique with Methyl Bromide has been developed. Mound structures have been found to vary greatly, whether for biological, or climatic/edaphic reasons is not yet known. Extremes of mound structures are described. Preliminary estimates of populations indicate that whole-mound sampling is essential to avoid bias, but that there will probably a few problems arising from individual differences between colonies. Seasonal changes should be readily detectable with a manageable sampling programme.

Foraging and feeding studies are still in an exploratory phase, but experimental areas have been selected and preliminary counts of foraging holes have been made. Distribution patterns of foraging activity are also being studied.

INTRODUCTION

Most of the ecological research on termites is being conducted at Kajiado, 100 km from Nairobi, which is the nearest place where large areas of undistributed natural vegetation with an adequate termite fauna can be found. A small field station with laboratory and living accommodation was completed in the course of the year but only became fully functional towards the end of that time.

Two main aspects of the ecology of *M. subhyalinus* are being studied. The first is the internal economy of the colony, as reflected in the annual cycle of changes in the population within the mounds. When these are properly understood, and related to the total numbers of mounds, estimates can be made of the external effects of the species on its environment. The second aspect is the foregoing cycle and feeding preferences of the species. This is the most obvious of the external effects, involving estimates of consumption of plant materials, mainly grass.

This direct approach to the ecology of the grass-feeding termites arises from our assessment of priorities for research. In the first phase of our work there are many technical problems of sampling to be overcome, without which the rest of the programme cannot be considered. When the main programme on *Macrotermes subhyalinus* is fully established, attention will have to be turned to the collection of much essential background data at present lacking. This includes environmental parameters such as plant production, soil structure and chemistry, and local variations in climate and topography. Interaction between the termites and other primary consumers, as well as turnover of biomass through predation, and many other aspects, must await the availability of additional personnel.

An Investigation of the Mounds of Macrotermes Subhyalinus

Nest Structure

During the period February to May 1975, we examined over fifty nests of *M. subhyalinus*. Most of these were in the Kajiado district and a few at Olorgesailie in the Rift Valley. For purely practical reasons, most were medium-sized or small nests. From observations on such a small and limited sample only the most tentative conclusions can be drawn.

Two extreme types of nest structure are recognised, and are named after the kind of soil in which they characteristically occur.

i) Kajiado clay type (figure 1).

This has a highly differentiated internal structure. There is a very distinct hive chamber situated below ground level, with large air passages opening from the roof and running up inside the mound. The mound itself is of coarse hard earth penetrated by a network of passages, and has no external openings. Inside this chamber is a peripheral zone of large and elaborately-shaped fungus combs in widely spaced galleries. The galleries are built of smooth laminar shelves, a few millimeters thick and supported at intervals on tapering pillars. The central part of the hive is filled with narrow, thin-walled horizontal galleries, referred to as nursery galleries because eggs and larvae of all ages are concentrated there. At the bottom of the nursery is the royal cell, surrounded by massive and elaborate defensive structures. Below this is a basement layer with low-roofed wide passages between massive walls, which presumably communicate eventually with passages leading down to the water table. The whole of the inside of the hive chamber is kept constantly moist.

ii) Olorgesailie diatomite type (figure 2).

Here the hive is mostly above ground level, and is not distinct in structure from the mound. Air passages open from the surface and extend well down inside the mound. The fungus gardens are again roughly peripheral but occur as relatively small, bun-shaped combs dispersed in separate arched cells within the fabric of the mound. The nursery area is represented by more closely spaced, smaller cells in the centre of the mound. The royal cell has no distinctive structure or surrounding defences.

These two are the extreme types of nest structure found on hard clay soils and soft friable soils respectively. On light sandy soils at Kajiado an intermediate type of structure occurs.

We can as yet offer no explanation of why some mounds have air passages opening on the surface, while others are closed. Mounds of the two types can occur side by side on the same soil (e.g. at Bissell), but one kind only tends to predominate in any one place (e.g. only closed mounds occur in the area South and West of Kajiado, while only open mounds have been seen around Voi). The local climate or microclimate may well be the determining factor.

Fig. 1: Kajiado clay type of nest.

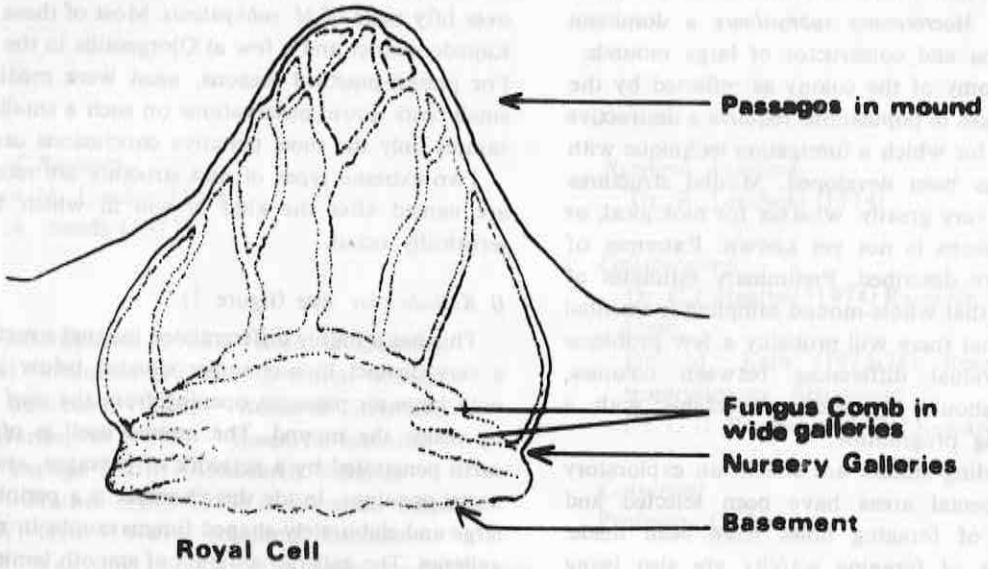
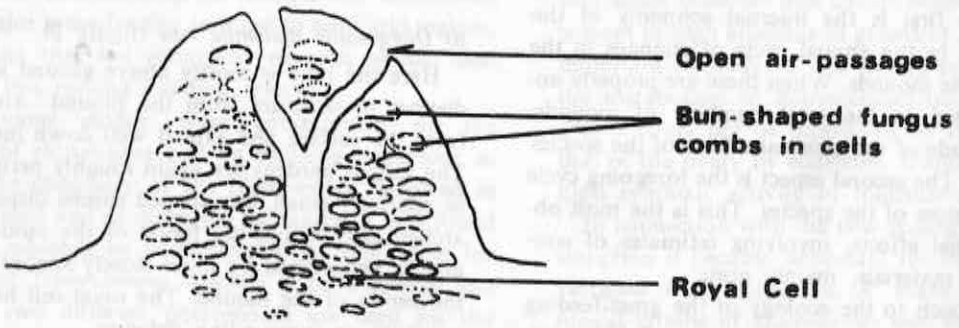


Fig. 2: Olorgesallie diatomite type of nest.



Diagrammatic vertical sections

Sampling of Nests

The aims of this project include estimating the total populations of *M. subhyalinus* nests, and quantifying seasonal changes in the caste composition. Progress has been made towards developing a suitable methodology, and this and some preliminary results are discussed below.

A method of sampling the whole population of one nest has been devised and used at Kajiado. The population is first killed in situ by administering a large dose of methyl bromide fumigant through one of the air passages in the mound. After the fumigant has dispersed the nest is opened and carefully dismantled by hand. The nest material is partly sorted on the spot and it saves quite a lot of time and trouble if most of the fungus comb can be separated out at this stage. At the laboratory the remaining nest material is separated by flotation in water into earth and wall fragments which sink, and termites and pieces of fungus comb which float to the surface. This bouyant sludge is collected with a sieve and kept cool in a refrigerator overnight. A second flotation is carried out the next day, by which time the fragments of fungus comb have become waterlogged and will mostly sink, while the termites will still float. The total dry weight of fungus comb recovered from the nest is measured. The total volume of termite sludge is determined, and ten replicate subsamples of it are taken after thorough mixing. Within these subsamples total counts are made of the four adult castes, the white soldiers, and four size-classes of larvae (and also nymphal alates if these are present), and hence the total population of the mound can be calculated.

While dismantling a nest it is simple to take samples of the population from different positions in the nest and to count these separately. The results for one nest, representing an approximate vertical sequence down the middle of the hive, are given in Figure 3. This shows that the composition of the population is quite different in parts of the nest, with hive, are given in Figure 3. This shows that the composition of the population is quite different in different parts of the nest, with larvae virtually confined to the nursery. Minor workers are the dominant caste everywhere else, while soldiers and major workers are most frequent in the air passages of the mound and the fungus comb galleries. Clearly it would be impossible to sample any one part of the nest and deduce from it the composition of the whole nest population.

So far six nests have been sampled by the methods described above (one incompletely). The results are partially summarised in Table 1, where the nests are arranged in order of increasing total population. The percentage composition of the adult castes in nearly constant in nests whose total population spans more than a full order of magnitude difference, and which were built on a variety of soil types. The percentage of adults in the total population is also very consistent. Thus it seems that there are not great individual differences in population composition between nests, and it may therefore be possible to detect seasonal changes in the composition of nest populations by sampling a fairly small number of nests at intervals through the year.

Fig. 3: Composition of the population in different parts of the nest expressed as histograms of percentage.

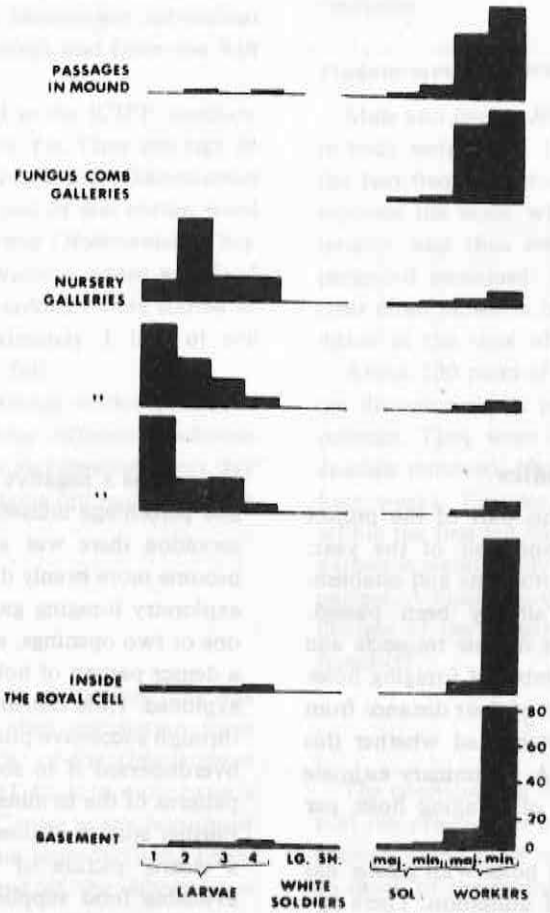


Table 1: Comparison of Six Nests

Reference number of nest	Estimated total population	Adults as % of total population	% composition of Major soldier	% composition of Minor soldier	adult Major worker	castes Minor worker	Total dry weight in g. of fungus comb in nests
24	218700	48.4	2.2	3.9	35.3	58.6	780
6	510400	43.5	1.7	2.8	35.3	60.2	2547
15	689000	47.4	2.2	2.8	29.7	65.3	3480
9 (a)	1232700	34.8	.7	2.2	23.5	73.6	5261
32	2217500	44.9	1.2	2.7	31.1	65.0	6969
48			1.1	2.6	35.0	61.3	11283

@ — not completely sampled.

B. Foraging and Feeding Studies

Only the exploratory stages of this part of the project could be accomplished in the second half of the year. However, the stage of definition of problems and establishment of experimental areas has already been passed. Preliminary counts of foraging holes on line transects and fixed plots have been made. The number of foraging holes per unit area appears to be a function of their distance from the mound, but it has yet to be established whether this relationship is linear or curvilinear. A preliminary estimate has been obtained for the number of foraging holes per mound.

Experimental masking of foraging holes with plaster has been used to estimate their levels of utilisation. There appears to be a negative correlation between number of holes and percentage utilisation. However, over the period of observation there was a tendency for the foraging holes to become more evenly distributed. This situation could arise if exploratry foraging galleries below the surface started with one or two openings, and subsequently branched to produce a denser pattern of holes nearby, until the area became fully exploited. Hole distribution would then be expected to pass through successive phases of sparse and random (or possibly overdispersed if in some way regulated by the behaviour patterns of the termites), clumped, and more densely spaced. Further studies of these patterns will be required to achieve a clearer picture of how this species makes use of its available food supplies.

Caste Formation

INTRODUCTION

There is usually only one pair of reproductives in a termite society the whole population being a large family of brothers and sisters, and forming a complex superorganism with a lot of physiological and social interactions.

The aim of our research is to clarify what factors are finally responsible for maintaining the balance among the different castes within such a society. From studies on lower termites it is evident that chemical signals (pheromones), produced by members of the society (reproductives, soldiers) regulate the direction in which the larvae will develop. In higher termites, however, distinct caste-specific features are already present in second and possibly even in first instar larvae.

To learn more about these feed-back systems it is necessary to study the social life of these animals in as many aspects as possible. Field work is necessary as well as biochemical analysis. The work presented here is confined to results obtained in the laboratory by rearing and handling termites in groups or small colonies.

MATERIALS AND METHODS

Alates of the most frequent termite species around Nairobi, *Odontotermes* sp., which fly twice a year, at the beginning of each rainy season, were collected by means of a light trap. *Hodotermes* sp., fly only once a year and our incipient colonies originate from the Kajiado area or from Olorgesailie. They are relatively easy to keep in the laboratory and constructive observations can be made.

The mound constructing termites *Macrotermes subhyalinus* were collected from the Kajiado district and from the Rift Valley.

Incipient colonies are being raised in the ICIPE insectary or in incubators at 29°C and 70-80% r.h. They are kept in glass or plastic boxes with red or black soil *(Odontotermes* and *Macrotermes)* or simply with a pad of wet cotton wool or small drinking glass filled with water *(Hodotermes)*. They are checked at least once a week, water is added and food (pieces of dry grass) replaced. Some colonies were started in covered beakers containing approximately 1 litre of soil made from house-hold aluminium foil.

Groups of freshly collected *Macrotermes* workers, soldiers, nymphs and/or larvae were held under different conditions in large and small plastic petri dishes and checked every day or every two days in order to investigate optimal conditions for breeding, feeding and survival.

RESULTS AND DISCUSSION

Aluminium Pot Colonies

The breeding of incipient colonies in aluminium pots was not successful. 14 to 18 months after installation these colonies were opened but only 2.5% of the *Odontotermes* had survived. They rarely had eggs (1.4%). In most cases a distinct royal cell was found in the Centre at the bottom of the pot containing the remains of the reproductives. There were no signs of dead larvae. None of the *Macrotermes* colonies survived. *Hodotermes* and the few *Trinervitermes bettonianus* were somewhat more successful (6.4% and 18.7% respectively survived). 5.2% of the *Hodotermes* and all of the surviving *Trinervitermes* colonies had larvae, but in both species there were no signs of eggs.

The main reason for the failure was apparently lack of proper moisture regulation. Under the insectary conditions the foil oxidised at the bottom of the pots and eventually poisoned the insects (tin impurities).

Rearing of Hodotermes

Established colonies of *Hodotermes* (five months old) were treated in different ways during a period of 10 months to find out the best rearing method. They were kept in 6 cm diameter plastic petri dishes. Some colonies got their cotton wool pad and/or the dry grass changed every week. Others just received food and/or water when necessary. Half of the colonies were kept in the insectary, half in an incubator. Both the actual numbers of eggs, larvae, pigmented larvae and soldiers and the changes since the start of the experiment, were evaluated.

The analysis shows that there is little difference between rearing in incubators and the insectary though incubators were slightly better. Weekly change of the cotton wool impaired and weekly removal of the old grass improved colony development in general.

Apparently a regular supply of fresh food is vital for the colonies welfare, whereas remaining old grass is of less nutritional value and/or contains some adverse factors e.g. mould. But changing of the cotton wool may also lead to removal of (saliva, faeces), depriving the colony of vital resources.

Hodotermes Incipient Colonies

Male and female *Hodotermes* dealates differ very distinctly in body weight (fig. 1). There is very little overlap between the two frequency distributions. This may help in future to separate the sexes without considering anatomical characteristics and thus reducing mistakes, especially for inexperienced personnel. It also shows that there is already a clear dimorphism in body size and hence in nutritional condition at the time of the flight.

About 200 pairs of these dealates were transferred into 6 cm diameter plastic petri dishes in order to form incipient colonies. They were checked every 3 or 4 days and dead animals removed. High mortality was observed in the first four weeks. Females kept singly died without exception within the first ten days. Among the pairs the death of one partner is significantly correlated with the death of the other partner. A linear regression can be calculated, indicating the lifetime of the male(y) as a function of the liftime of the female(x):

$$y = 1.49 + 0.98x$$

The coefficient of determination r^2 is 0.91. This indicates that the presence of a partner is essential for survival. The animals spend most of their time grooming each other and so death of the partner also means loss of a very efficient

cleaning device. There may also be some contagious factors affecting the surviving animal. Many of the dead insects become overgrown very soon by fungal hyphae or show signs of rapid bacterial decay.

55 females survived their males, 49 males survived their females whereas 40 pairs were recorded dead together. The distribution of the survival time, however, shows a peculiar shape (fig. 2). The statistics for skewness (g_1) and for kutosis (g_2) are both highly significant ($P < 0.1\%$) indicating that the curve is skewed to the right and heavily leptospasmic. This again reflects the contagious nature of mortality and the weaker constitution of the males. Although these conditions were very artificial, it shows how important hygiene must be for termites in nature.

Fig. 1: Frequency distribution of 140 females and 140 males of *Hodotermes* dealates 4 days after the flight.

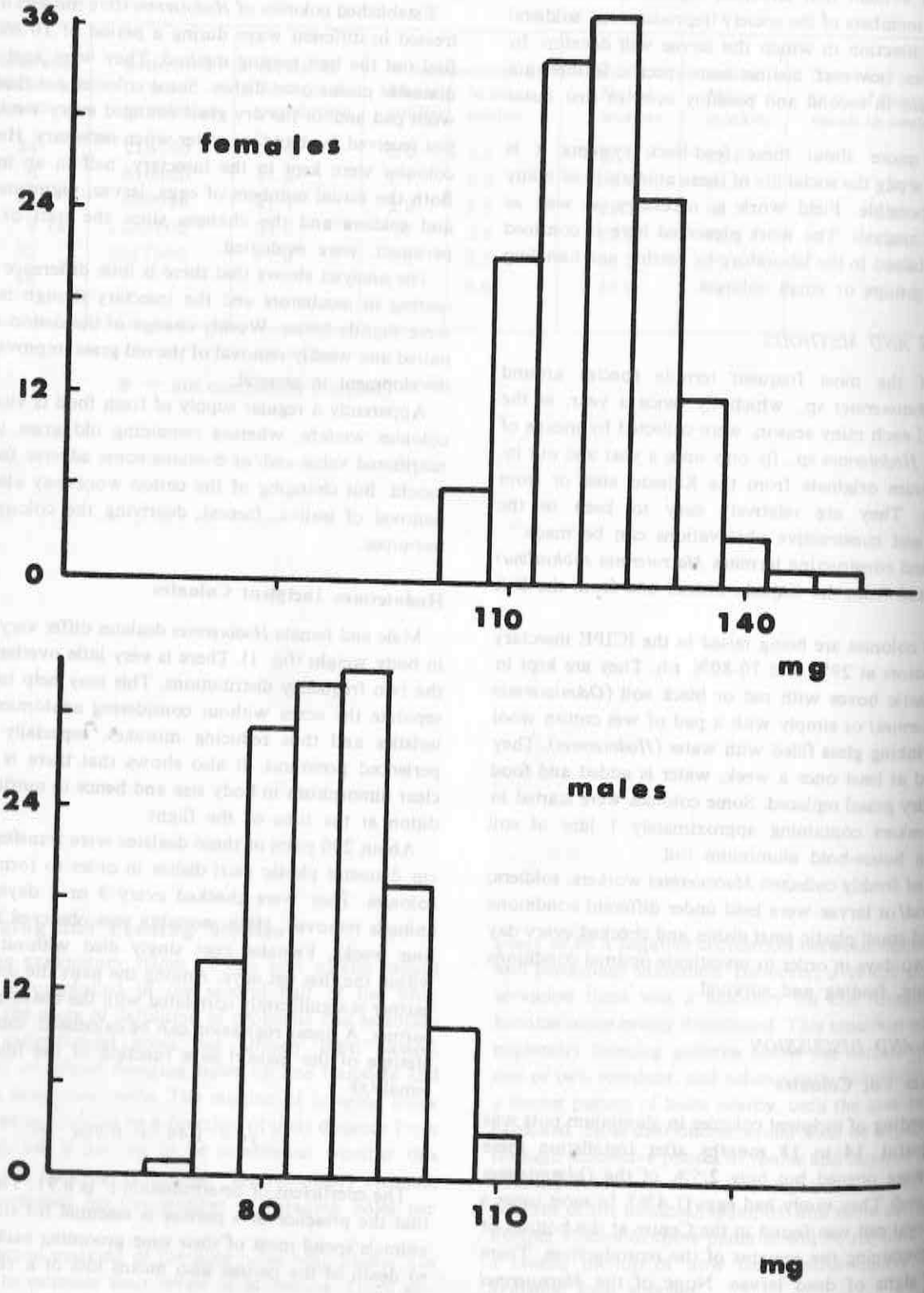


Table 1: Analysis of variance of the body weights of 50 successful *Hodotermes* dealates and 50 males and females which died within the first 10 weeks.

Source of variance	df	MS	F	
Sex	1	28981.66	387.09	***
Success	1	161.29	3.14	ns
Sex X success	1	4.75	0.06	ns
Error	96	75.3		

It is noteworthy that none of the eggs hatched when there were no parents left in the colony.

The body weights of the 50 most successful males were compared with the body weights of 50 males and females chosen at random from those that had died during the first 10 weeks. The analysis of variance indicates that it is very unlikely that the bodyweight of dealates has anything to do with the prospected success (Table 1). Most of the variation is explained by the sex dimorphism alone, whereas neither the factor "success" nor the interaction "success x sex" are significant.

Incipient Colonies of Odontotermes

As in *Hodotermes*, there is a sex dimorphism in the bodyweight of *Odontotermes* dealates. There is, however, a considerable overlap. The males' distribution is clearly bimodal which could be explained by the fact that half of them were kept in a drier container overnight before weighing. Weighing is of little help in separating sexes of *Odontotermes* dealates. (Fig. 3).

Pairs of *Odontotermes* dealates were put in small plastic petri dishes with soil. They survived better in soil that was sterilised before. Colony development, however, was in general better among the surviving animals kept in ordinary soil. This indicates that in nature reproductively weak animals may be eliminated more rapidly. As in *Hodotermes*, grooming is very important and single animals can not survive for long.

Some females were collected and kept in pairs, separated from the males straight after the flight. After about three months some of them started laying eggs. But up to now none of these eggs have hatched.

It was possible to raise successfully, incipient colonies in substrates as poor as washed diatomite earth or silica gel for column chromatography, with nothing but boiled tap water. Up to 15 larvae were fed by the reproductives until they were able to forage for food on their own.

Table 2: Median life time of different castes kept in 6 cm diameter plastic boxes

Major workers	12.33 days
Minor workers	8.10
Major soldiers	7.25
Minor soldiers	4.90
Big larvae	2.00
Medium size larvae	5.66
Small larvae	7.00
Very small larvae	6.25

Keeping Groups of Macrotermes

It is not possible to keep nonreproductives of *Macrotermes* alive for a long time under laboratory conditions. But it is possible to extend this period of time by modifying the rearing conditions. This may help to obtain some insight in their way of life. Groups of 24 animals of the different castes and four size classes of larvae were kept in small dishes with wet cotton wool and a piece of fresh fungus comb. They were checked every day and the dead were removed. Table 2 lists the median life time, i.e. the time, when only 12 animals were left.

The castes differ very much in survival capacity. Here again grooming and mutual feeding (trophallaxis) is very important. Soldiers which have mouthparts specialized for defence and not for grooming and feeding can not survive a long time. Major soldiers are much larger and stand starvation better than minor soldiers. Surprisingly the big larvae are the most fragile. Many of them were preparing to undergo a moult but deprived of any help from the other castes, most of them died.

In another experiment 12 larvae of the different size classes were held together with 12 animals of one of the four castes. Table 3 shows that more larvae were surviving day 4 when workers were present. These data were analysed as a 2 x 4 contingency table. The lifetime of the larvae is very significantly dependent on the partner caste. There was no indication that any caste would live longer if kept with a certain size class of larvae.

In similar experiments it was shown that for every caste population density is very important, animals kept in small groups dying much more rapidly. Fresh fungus material is better than old or mouldy material. The lifetime can be improved by offering a small quantity of soil in the boxes but addition of twice or three times as much does not result in any further improvement.

Table 3: Lifetime of larvae kept together with different castes

	With major soldiers	With minor soldiers	With major workers	With minor workers	Sun
Number of larvae which died before day 4	32	23	13	14	82
Number of larvae which lived longer than 4 days	16	25	35	34	110
Sums	48	48	48	48	192

$X^2 = 20.18$ $P < 0.1\%$

Fig. 2: Frequency of females surviving the male (positive values) and males surviving the female (negative values) from 144 incipient *Hodotermes* colonies. Statistical characteristics: x = -0,410; sx = 10.095; g = - 2.932, g_2 = 196.100; n = 144.

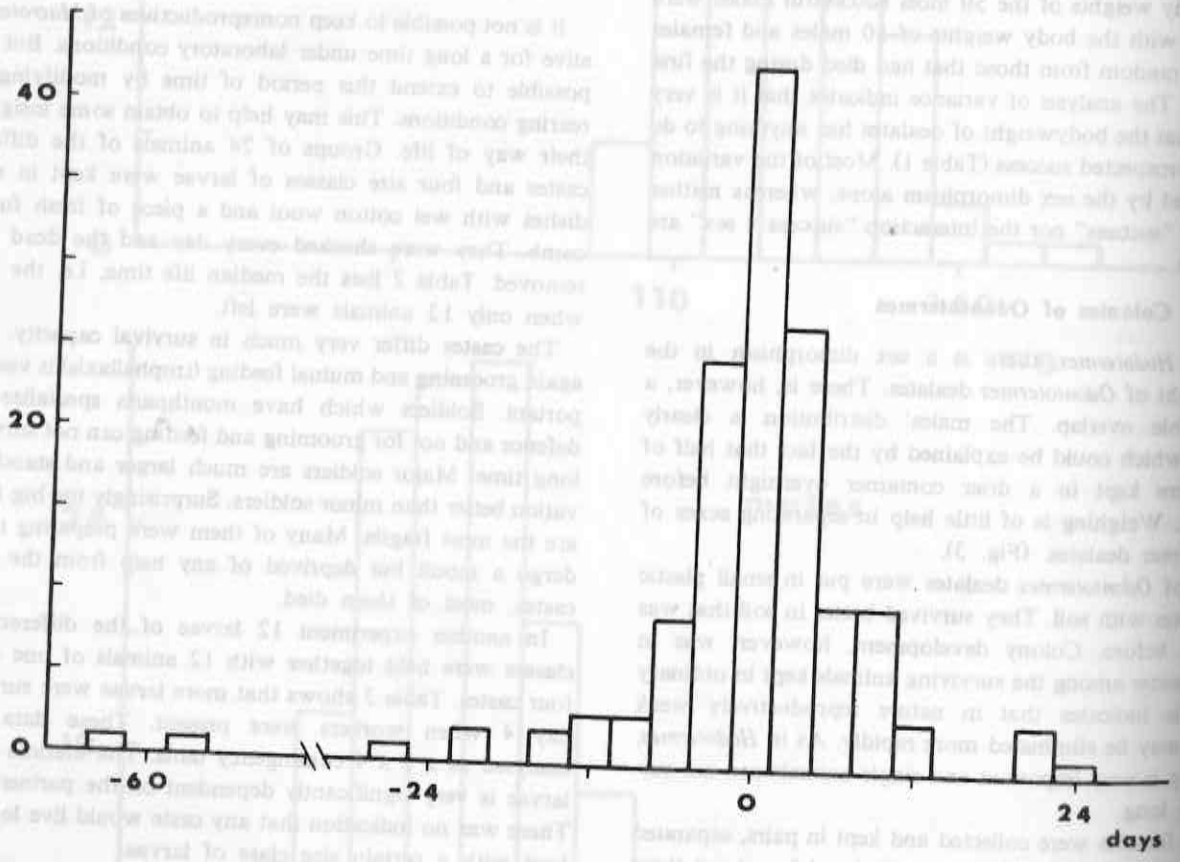


Table 4: Lifetime of soldiers kept with other soldiers or with workers

	Major or minor soldiers	Major with minor	With major workers	With minor workers	Sun
Number of deads before day 8	43	45	37	26	151
Number of soldiers surviving day 8	5	3	11	22	41
Sums	48	48	48	48	192

$X^2 = 27.14$ $P < 0.1\%$ $P < 0.1\%$

Table 5: Lifetime of workers kept with other workers or with soldiers

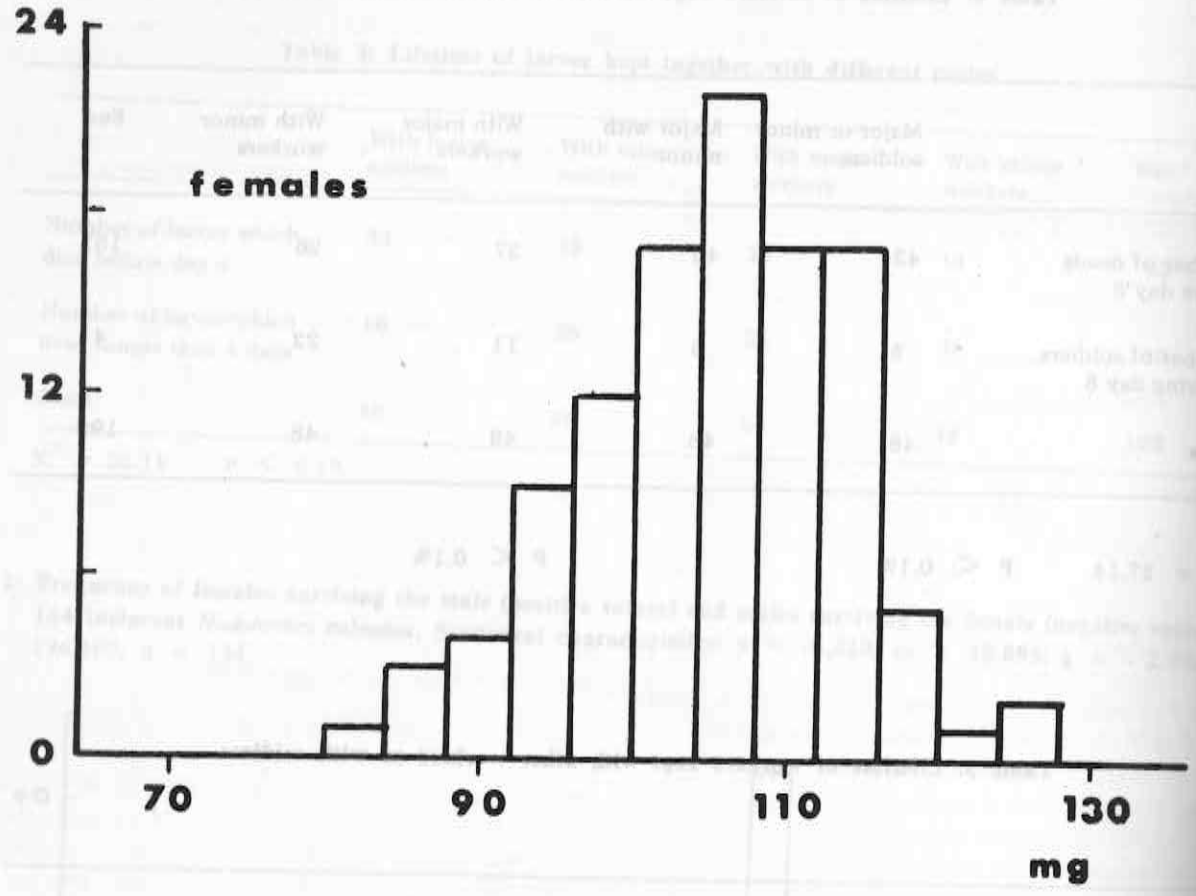
	Major or minor workers	Major with minor	With major soldiers	With minor soldiers	Sum
Number of deads before day 8	54	55	81	69	259
Number of workers surviving day 8	42	41	15	27	125
Sums	96	96	96	96	284

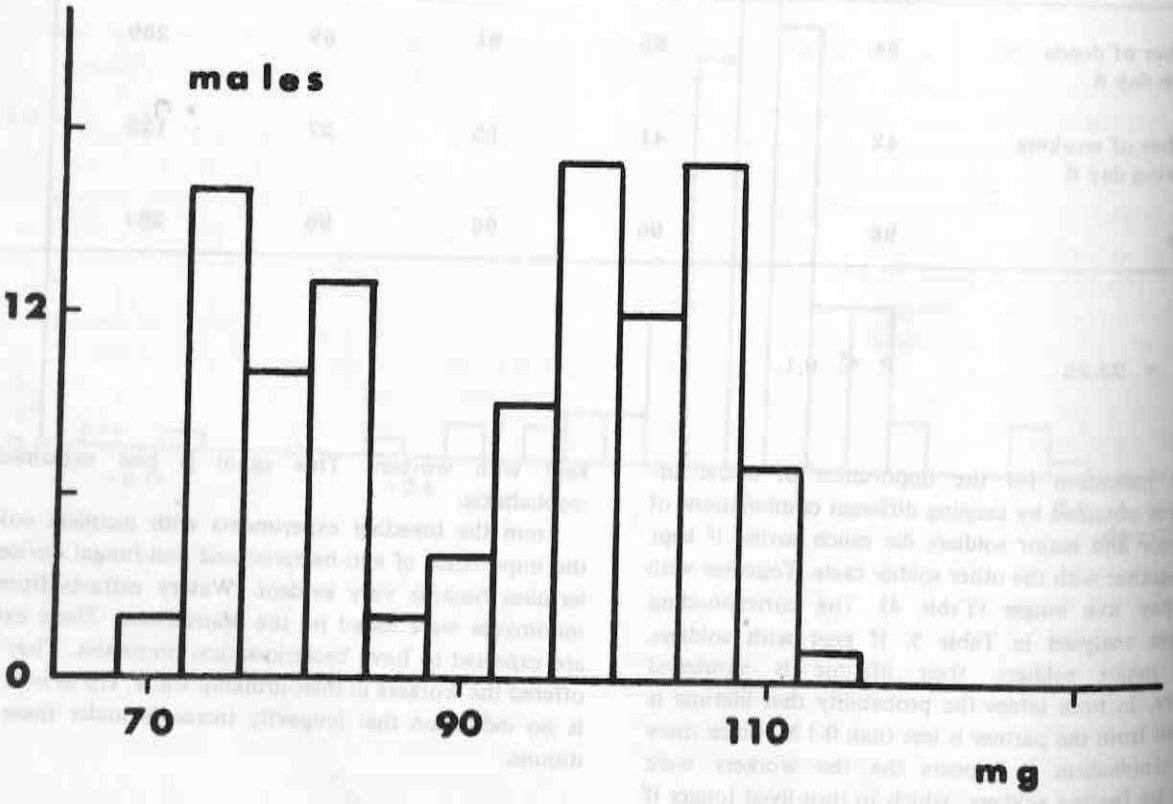
$X^2 = 23.38$ $P < 0.1$

Another indication for the importance of social interaction was obtained by keeping different combinations of castes. Minor and major soldiers die much earlier if kept alone or together with the other soldier caste. Together with workers they live longer (Table 4). The corresponding workers are analysed in Table 5. If kept with soldiers, especially major soldiers, their lifetime is shortened significantly. In both tables the probability that lifetime is independent from the partner is less than 0.1 %. Since there was no cannibalism it appears tha the workers were weakened by feeding soldiers, which in turn lived longer if kept with workers. This again is best explained by trophallaxis.

From the breeding experiments with incipient colonies the importance of anti-bacterial and anti-fungal devices for termites became very evident. Watery extracts from termitomyces were tested on the *Macrotermes*. These extracts are expected to have bacteriostatical properties. They were offered the workers in their drinking water. Up to now there is no indication that longevity increases under these conditions.

Fig. 3: Frequency distribution of 116 females and 110 males of *Odontotermes* dealates 1 day after the flight.





Recruitment Behaviour in *Trinervitermes Bettonianus*

INTRODUCTION

As was pointed out in the ICIPE Annual Report 1974, the current research on termite behaviour is focussed on the communication system. Of immediate interest is the extent to which foraging activity, building behaviour and defence (which are all vital for the survival of a colony) are dependent on chemical communication.

The present report gives preliminary results on orientation and recruitment in *Trinervitermes bettonianus*.

Orientation to Food Source

The material for all laboratory work was obtained from the field by digging up whole nests intact and maintaining them at about 24-26°C; 50-70% R.H.

The orientation experiments aimed at determining how workers and soldiers (which are blind) initially locate the food source – whether by systematic search guided by some cue or by random exploration. The observations were made on a 120cm x 120cm plywood board placed horizontally on top of a nest to provide a foraging arena. A 4mm hole in the centre served as a foraging exit. For each explorer termite, the immediate surrounding of the hole was covered with a fresh paper disc, 5.5 cm in diameter, marked with a scale of angles from 0^0 - 360^0. This was to eliminate orientation by trails laid by previous termites. The foraging arena was divided by pencil lines into guardrants. On each occasion, food (100 mg of grass material from the natural habitat) was placed 6cm from the foraging hole at random, but avoiding the previous day's location. The choice of direction made by individual explorer workers was recorded as the worker crossed a 1cm circle marked on the paper. (1cm is roughly the distance traversed by the first scouting worker before retreating back to the nest).

RESULTS AND CONCLUSIONS

From 7 tests conducted on consecutive days, there was no evidence of a preference for the food direction (Fig. 7). It was concluded that food has no positive influence on the initial choice of direction by the first explorer termites.

Development of foraging trails

As food appears to have no positive influence on orientation of pioneer exploring workers, the question arises as to how the survey for food is organized.

To study how foraging patterns develop before and after food is discovered, the board with marked quadrants was used. On each occasion, food was placed in any of the quadrants (but not that of the previous day) 20 cm from the foraging hole. Photographs were taken at 2-minute intervals from the time the first worker emerged from the nest until peak foraging activity was attained.

The experiment was repeated with a maze superimposed on the foraging board. The maze had several physically separated compartments which could be entered into by a 2-choice situation. Pictures were taken at 2-minute intervals as above. After food was discovered and peak foraging activity reached, a new food source was placed in the least-visited compartment; then further pictures taken to see whether traffic was diverted to the new food source. In either case, the board was wiped with wet cloth to remove previous day's faecal trails before the next experiment started.

RESULTS

Graphical analysis indicated no obvious initial preference for the quadrant in which food was placed. However, as soon as food was discovered, recruitment was directed to it; thereafter, the proportion of termites found in the quadrant substantially increased, while that in the rest of the foraging arena declined (Fig. 2). This randomness of exploratory trails with respect to the food source and the deviation of traffic to it after food is discovered was confirmed in the experiment with a maze (Fig. 3).

Development of Foraging Trails Described

From the foregoing, the foraging behaviour may be described as follows:

The foraging activity usually starts by a few soldiers going to the foraging hole. Then the first worker(s) may emerge and survey a short distance (about 1cm from the exit) before returning to the nest along the same path. Subsequent termites tend to follow the same path as used by the pioneer worker and, as progressively more termites come out, a column is formed. This survey column consists of workers and soldiers, with workers at the head of the column advancing short distances beyond the column before retreating along their own trials. Thus, leaders of the column are continuously replaced by individuals coming behind them, while the column is guided by the leaders' trails. If food is not discovered within a short period, several exploratory trails may form, usually with only one dominant (main) trail occurring. As the main column advances, individual workers branch off to survery peripheral areas; a few workers may also venture on their own into unexplored areas without going through established trails. It is such stray workers that often come across a food source by chance. On discovering food, a worker takes a bite and then runs back excitedly along its own trail to an established trail (and eventually to the nest). Such a worker apparently alerts nestmates by agitated physical contact; then turns and runs back towards the food source, often followed by the recruited termite. And within a few minutes, increasingly more termites are recruited to the food source. Further exploratory trails usually develop beyond the food source eventually leading to the establishment of a network of foraging trails. The foraging activity ends by (unrewarded) workers gradually withdrawing from the depleted food source and returning to the nest first, followed by soldiers. But a small number of termites will continue coming out for some time (even throughout the night) if food consumed is insufficient.

It may be noted that the orientation of the main exploratory trail is largely influenced by the direction taken by the first worker to emerge from the nest, i.e. by the first exploratory trail laid.

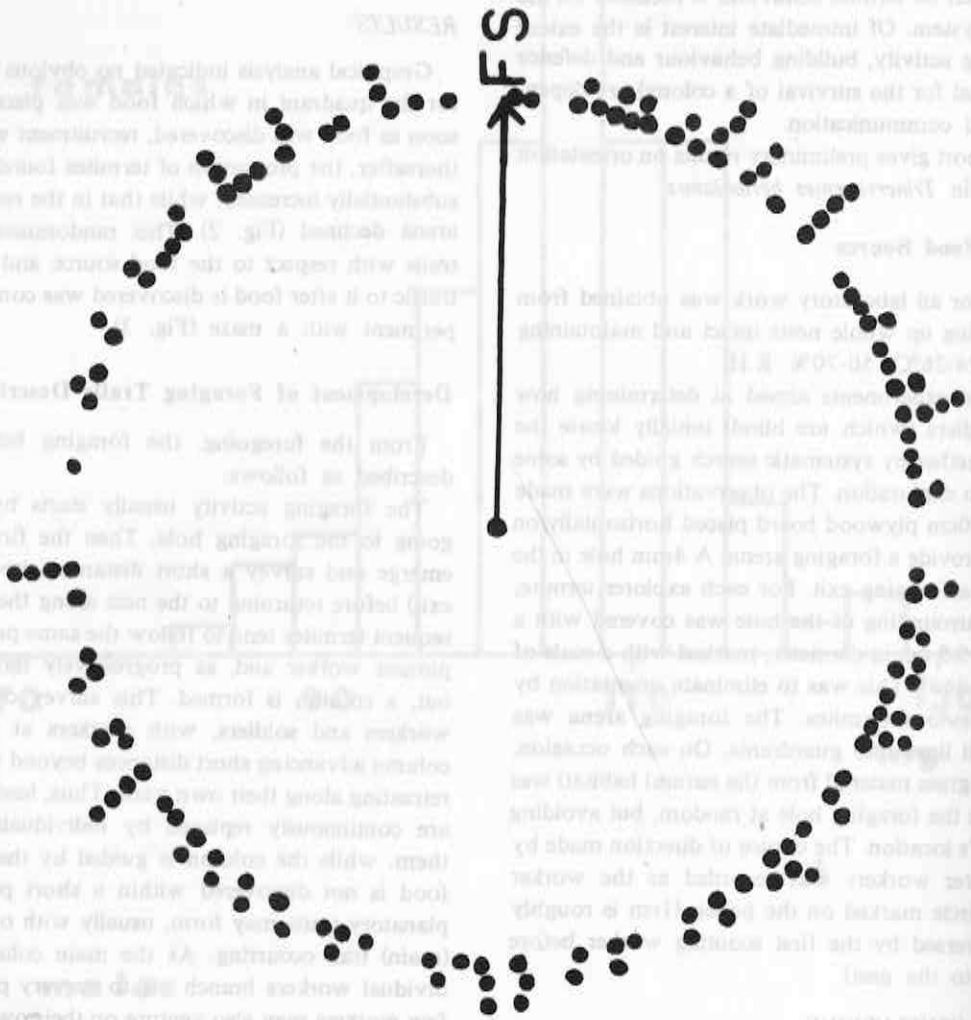


Fig. 1: Orientation to Food Source in *T. bettonianus* (A combined diagram of directions chosen by individual explorer termites from 7 experiments with a common reference of Food Direction (FS). Possible environmental factors neglected.)

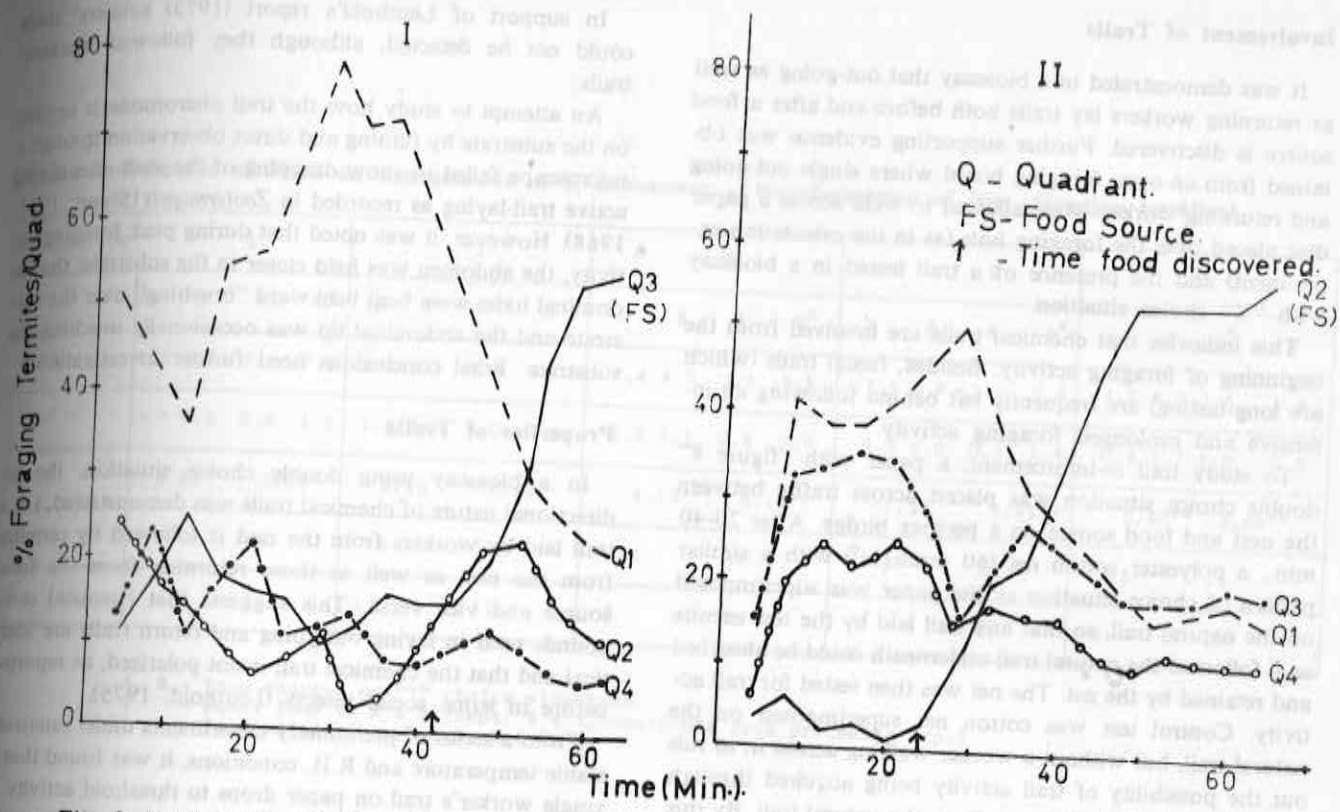
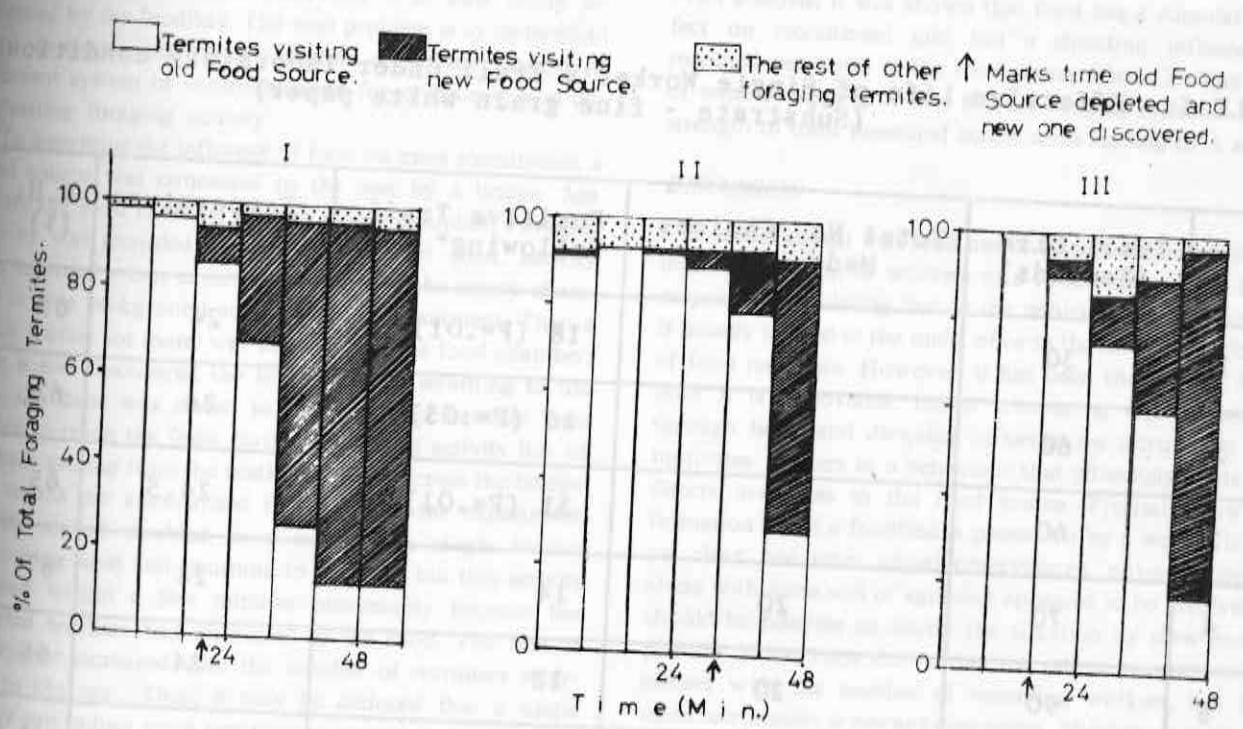


Fig. 2: Exploration and Recruitment to Food Source in *T. bettonianus.*



. 3: Diversion and Re-orientation of Recruitment to Newly-discovered Food Source after Depletion of the old one in *T. bettonianus.*

Involvement and Properties of Trails

Involvement of Trails

It was demonstrated in a bioassay that out-going as well as returning workers lay trails both before and after a food source is discovered. Further supporting evidence was obtained from an open foraging board where single out-going and returning workers were allowed to walk across a paper disc placed over the foraging hole (as in the orientation experiment) and the presence of a trail tested in a bioassay with "Y" choice situation.

This indicates that chemical trails are involved from the beginning of foraging activity. Besides, faecal trails (which are long-lasting) are frequently left behind following an intensive and prolonged foraging activity.

To study trail re-inforcement, a paper with "figure 8" double choice situation was placed across traffic between the nest and food source on a perspex birdge. After 20-40 min., a polyester cotton net (60 mesh/in^2) with a similar pattern of choice situation as the paper was superimposed on the natural trail, so that any trail laid by the test termite as it followed the natural trail underneath could be absorbed and retained by the net. The net was then tested for trail activity. Control test was cotton net superimposed on the natural trail, but without a worker walking across it, to rule out the possibility of trail activity being acquired through absorption of the pheromone from the natural trail. By this procedure, it was shown that workers re-inforce exploratory trails; well-established trails formed during peak foraging activity; and continue to re-inforce trails even 1-2 hours after food is depleted. Trail re-inforcement had earlier been reported in a related species by Tschinkel (1973).

In support of Leuthold's report (1973) soldiers' trails could not be detected, although they followed workers' trails.

An attempt to study how the trail pheromone is applied on the substrate by filming and direct observation through a microscope failed to show dragging of the abdomen during active trail-laying as recorded in *Zootermopsis* (Stuart, 1961; 1968). However, it was noted that during peak foraging activity, the abdomen was held closer to the substrate, the abdominal hairs were bent backward "brushing" over the substrate and the abdominal tip was occasionally touching the substrate. Final conclusions need further investigation.

Properties of Trails

In a bioassay using double choice situation, the bi-directional nature of chemical trails was demonstrated, i.e. a trail laid by workers from the nest is followed by termites from the nest as well as those returning from the food source and vice versa. This suggests that chemical compounds used in laying out-going and return trails are identical and that the chemical trail is not polarized, as reported before in some social insects (Leuthold, 1975).

From a series of preliminary experiments under relatively stable temperature and R.H. conditions, it was found that a single worker's trail on paper drops to threshold activity in about 60 seconds (Table 1). Further information on the properties of a trail will be obtained if chemical purification of the trail pheromone is accomplished in future.

TABLE I. Effective Life of Single Worker's Trail under laboratory conditions. (Substrate - fine grain white paper)

	Trail Life (Seconds)	Total No. Choices Made	Positive Trail Following*	Temperature (°C)	R.H. (%)
1	30	20	18 (P=.01)	24	67
2	60	20	16 (P=.05)	24	67
3	60	40	31 (P=.01)	24.5	65
4	70	20	14	24	67
5	90	20	12	24	65
6	90	20	8	24	61

*Trail laid and tested under covered channel of perspex.

Table 2: Influence of Food on Recruitment in *T. bettonianus*. √transformation of Nos. foraging termites)

REPLICATION	1				2				3				4				5			
	A	B	A	B^X	A	B	A^X	B	A	B	A	B^X	A	B	A	B^X	A	B	A^X	B
MEAN	4.3	2.7	2.8	7.5	6.2	8.3	7.4	4.0	7.4	4.0	3.4	6.1	7.5	5.9	1.9	5.8	1.2	4.5	7.6	1.8
±SD	0.7	0.5	0.8	1.2	1.8	0.9	1.0	1.1	1.0	1.1	0.8	0.9	2.2	1.0	0.5	1.4	0.3	1.7	1.9	0.5
t.05	2.22		3.08*		2.16		4.13**		4.13**		4.13**		1.57		3.41**		6.40**		3.68**	
n	6		11		6		14		14		18		5		13		13		15	
	I		II		I		II		I		II		I		II		I		II	

A, B - Food sources on "Y" choice situation.
I - Equal food in A & B (Reps. 3 & 5, continued from preceding Rep.)
II - Food added to source marked "X".

Mechanism of Recruitment

From the foregoing, it is evident that the worker's motivation to recruitment behaviour is at least partly influenced by the foodfind. The next problem is to understand how extrinsic and intrinsic factors contribute towards a coherent system of communication for organizing and coordinating foraging activity.

To determine the influence of food on mass recruitment, a food source was connected to the nest by a bridge. Just before the food chamber was an empty chamber. Foraging activity was recorded by an event recorder. First, activity was recorded when termites were visiting the empty chamber to give background activity before recruitment. Then a single worker (or more) was allowed into the food chamber; after it had discovered the food and was returning to the nest, the door was closed so that recruited termites were unable to reach the food; further records of activity (i.e. of workers coming from the nest) were taken across the bridge. The results are summarized in Fig. 4. In the experiment, activity almost doubled as a result of a single worker discovering food and returning to the nest, but this activity dropped within a few minutes presumably because the recruited workers had no access to the food. The rate of recruitment increased with the number of recruiters returning to the nest. Thus, it may be deduced that a single worker can induce mass recruitment, provided the recruited workers are rewarded.

A quantitative study of the influence of food on recruitment was carried out by a "Y" choice situation. First, equal food was provided in each arm of a "Y" terminal (A and B). The number of termites recruited to A and B were recorded until food was depleted. Then food was added to either A or B, and again numbers recorded until food was depleted. By t-test analysis, it was shown that food has a stimulating effect on recruitment and has a directing influence on recruited nestmates to the food source (Table 2). The ratios of termites visiting A and B were roughly reflected in the strength of trails measured across arms leading to A and B.

DISCUSSION

It would appear that food has no positive influence on the initial orientation of workers to a food source. This is not surprising considering that in the natural situation a colony is usually located in the midst of or in the immediate vicinity of food resources. However, it has been shown that food, once it is discovered, has a stimulating effect (possibly through taste) and directing influence on recruitment – it motivates workers to a behaviour that ultimately alerts and directs nestmates to the food source. Precisely how information about a foodfind is passed on by a worker is not yet clear, but from visual observations, physical contact along with some sort of agitation appeared to be involved. It should be possible to clarify the situation by slow-motion pictures. It has been shown that the rate of recruitment increases with the number of recruiting workers, but that mass recruitment is reward-dependent. Pheromone trails are involved from the beginning to the end of foraging activity and play a major role as a guiding cue during foraging. It is possible that the process of trail re-inforcement and trail fading gives graded information from different trails and facilities deviation from an old route to a new and more important one.

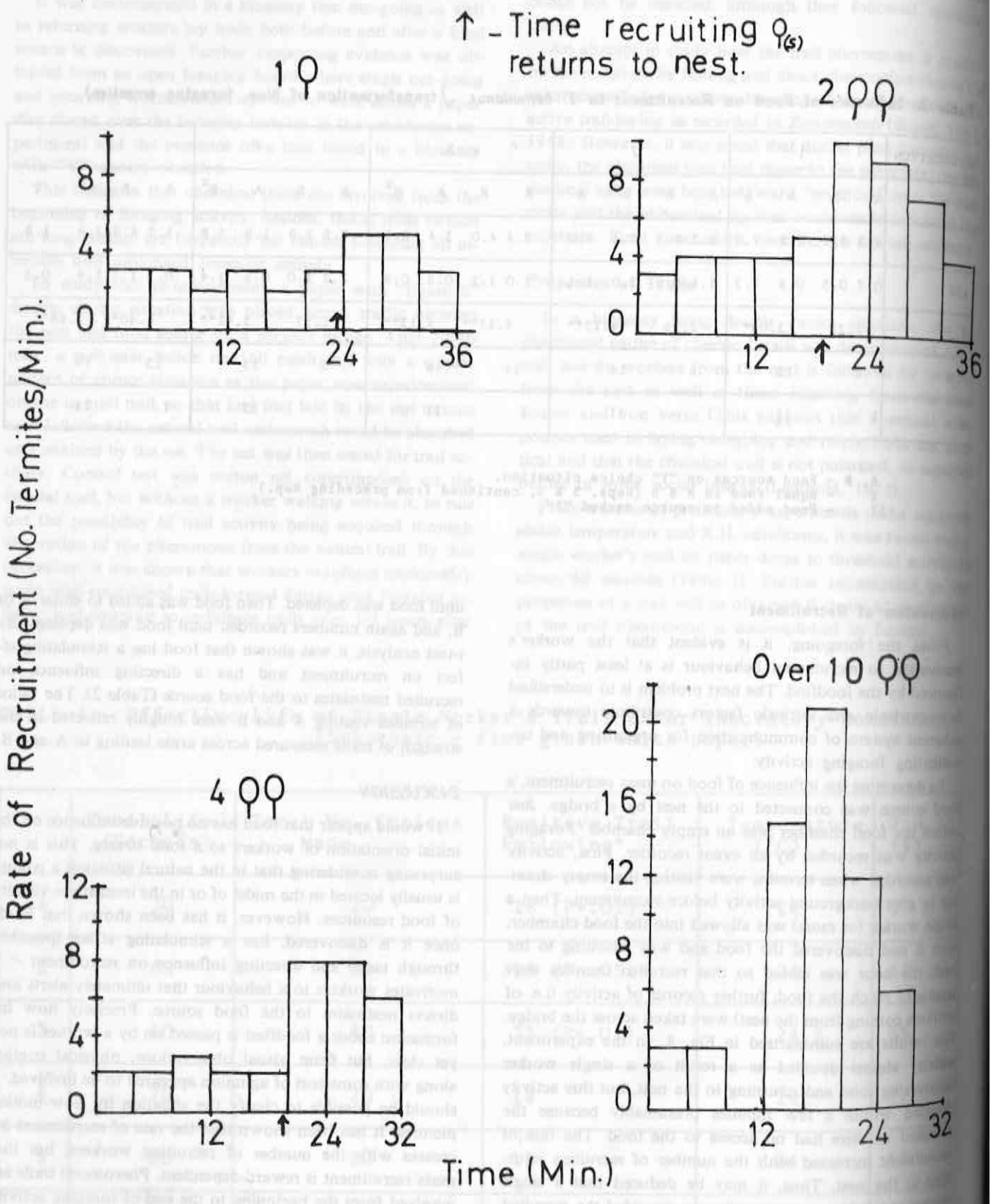


Fig. 4: Recruitment capacity of one or more workers (00) from food source in *T. bettonianus*.

Chemical communication – Behaviour

INTRODUCTION

Construction behaviour among termites is a most complex form of social interaction. The repair of damaged parts of the nest system, the construction of galleries, fungus combs and special structures like the royal cell and flying platforms during the nuptial flight, are all crucial for the survival of termites. There is some evidence that most of the building activities are defensive in nature, (Stuart, 1967). Construction activities are the result of a net-work of functionally related variables, and take place under a variety of environmental conditions. It is therefore of great importance to select a situation in which building occurs in a more or less predictable and reproduceable way.

Such a situation is available in the construction behaviour around the royal pair (the colony's reproductive female and male), and as an example, the behaviour around the physogastrict queen (female with swollen abdomen) is described. Exposing the queen creates an emergency situation for the surrounding workers who grasp soil particles, and start moving towards the queen. After a short antennal contact with her abdomen, they move back to a distance not exceeding 2.5 cm from the queen (under laboratory conditions). There they deposit the soil pellet's and cement them with saliva. A pillar-like structure is erected within a relatively short time and is then extended both in height and length, parallel to the queen's longitudinal axis. (See Fig. 1) within a couple of hours this "wall" becomes a tunnel covering the queen, whereupon construction ceases. In this study it is shown that a compound is emitted by the queen which induces building behavour and that it is effective by way of olfactory pathways. An attempt is made to clarify the chemical structure(s) of this compound – see section chemical communication chemistry. The shape and movements of the queen do not play any role in this behavour. Evidence is brought forward that a similar type of building behaviour around the king is elicited by its sternal gland secretions.

METHODS

The construction response of the workers to queens and kings or their substitutes has been assessed in two ways; first by watching construction around live, dead, treated animals and dummies. These dummies were made of a glass rod 1.4 cm 0.5 cm (covered with parts of queens or kings). Secondly by a bioassay in which construction activity was measured in extracts of the royal pair. The bioassay consisted of 3 airtight petri dishes in which soil enclosed a circular open space in the centre. Two filter papers (2 x 1cm) one treated with the extract and the other with the solvent (control) were respectively inserted vertically into two of the three petri dishes to serve as the odour carrier. Each of the three dishes had a standard number of workers, soldiers and larvae. In a period of 2 hours the number of soil pellets deposited in the open space was scored and compared after substracting from both data the number of particles placed in the centre of dish no. 3 (which had no filter paper, extract or solvent). The result was considered satisfactory if the dish with the extract scored 5 times as many pellets as the one with the solvent.

The royal pairs were collected near Kajiado township and tested within 48 hours. Workers from a nest dug not more than 3 x 24 hours before, were used. All the experiments were conducted at 25° and 55% RH.

EXPERIMENTS AND RESULTS

A series of experiments were designed to elucidate the nature and the source of the building stimuli due to the presence of the queen.

Five series of observations with live queens, 2 with the female in the natural (lengthwise) position, 2 with animals each in circular arrangements (the posterior end of the abdomen was in contact with head) and 1 female in an S-shape figure, confirmed the observation that building takes place at a certain distance – 1.5-2cm from the queen's body.

To test for a possible olfactory stimulus, a live queen was placed under a stainless wire netting which prevented the surrounding workers from making antennal contact yet allowed them to come within the possible olfactory reception distance of 1.5cm indicated above.

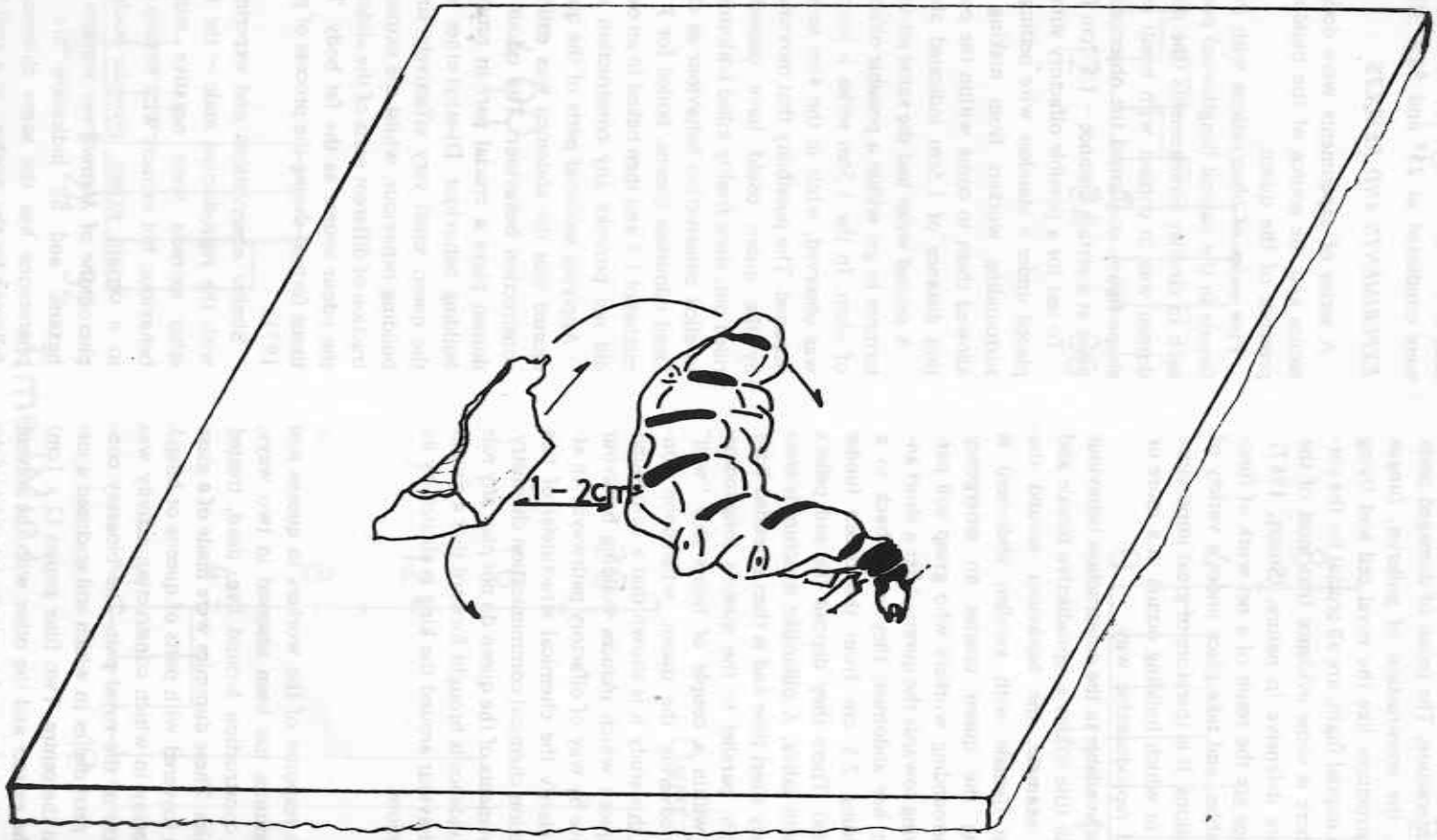
A second series had the same set-up but only allowed the termites to get within a possible olfactory reception distance of -4cm. In the 1.5cm series a normal building behaviour was observed, while in the 4cm series no building activity occurred. The possibility that movements of or sounds made by the queen could have caused the behaviour was ruled out, since freshly killed (chloroform) queen's were able to elicit construction behaviour as effectively as live ones. Dead odourless queens, boiled for 3 hours in chloroform – methanol 1:1 and then baked in an oven at 60°C and 1 hour did not provoke any construction activity.

Applying sectional parts of the queen onto a dummy indicated that the abdomen was entirely responsible for the construction behaviour. The odour emitted from the abdomen plays a crucial part in provoking and guiding the building behaviour. Di-ethyl-ether is capable of extracting the queen smell very effectively since the extract elicited building behaviour, whilst the extracted queen did not. Extraction of different parts of the abdomen led to discovery of the odour source as the fat body. This is a lipidoxidizing tissue formed during the process of physogastry (Bordereau, 1971).

Similar observations and experiments were undertaken with the reproductive male – the king. Bioassay tests of ether extracts were negative with regard to building behaviour, but extracts with hexane curiously enough could to a certain extent, provoke such behaviour. The trail pheromone of *Macrotermes* workers can be extracted with hexane, and all indication are that the king's trail pheromone has the same chemical structure since it is followed by the workers in a trail bioassay (Leuthold and Luscher, 1974).

The king's sternal gland (source of the trail pheromone) contains, compared to the workers, a relatively high amount of the pheromone (600 units versus 15). No sternal gland structure could be found in a physogastrict queen, but is

Fig. 1: Building behaviour around an exposed *Queen of macrotermes subhyalinus* **/Drawn after Photograph**



present in the female alate.

Further research is going on to elucidate the mechanisms involved in construction and the structure of the relevant chemical(s).

Attempts were made to transfer a termite colony to the laboratory, where it was rebuilt in a fibreglass container in an effort to ensure a regular supply of fresh workers and to enable a study of the construction and repair of the galleries and the nest system under controlled circumstances. Although the first efforts failed, the situation is now more promising.

DISCUSSION

It has been shown that the *Macrotermes* queen induces and controls construction activities around her by means of an odour emitted from her fat body, while the king's sternal gland elicits the same behaviour but less intensively.

The queen's odour by itself is enough to provoke building activity at a distance of 1.5-2cm. Preliminary experiments conducted to find out how the animals measure this distance, gave rise to the possibility that workers build along a threshold around the source of the smell.

In a confined area (less than 1.5cm from the walls to the dummy or filter paper) construction is hindered. Soil pellets were grasped near the source of the smell, but because space was lacking for placing them at the required distance, building hardly occurred. More experiments are planned to analyse this threshold possibility.

As indicated with the sternal gland extracts, trail pheromone is very important at least in guiding the building behaviour. This finding can be of use in explaining the mechanism involved in gallery building.

SUMMARY

Construction behaviour of workers around the royal pair has been analysed. The responsible stimuli are of chemical nature and are emitted from the queen's fat body and the king's sternal gland.

This follows from a series of experiments using live, or dead queens and kings, and parts of them either as dummies or extracts. A possible mechanism explaining the behaviour around the queen is briefly discussed.

Chemical Communication (Chemistry)

The queen of *Macrotermes subhyalinus* was found to produce an ether-extractable scent which elicits very specific building behaviour in workers of this species. The biology of this behaviour is discussed in another section. Described below are the investigations into the chemical nature of this substance.

Whole queens were extracted with solvents of various polarities and the concentrated extracts were bioassayed. Hexane extracts elicited virtually no building, and methanol extracted only part of the activity from the queen. However, ether extracts were highly active and the ether-washed queen thereafter inactive. It was then discovered that only the cuticle (with attached fat bodies) was active, so that further experiments were conducted with cuticle extracts.

Soxhelet extraction with ether was found to be of optimum efficiency. The crude extract was chromatographed on silica gel with 60:40:1 hexane: ether:acetic acid, and only the origin material showed any activity. Washing the ether extract with 1 *N* sodium hydroxide removed the activity; acidification of the base wash and re-extraction with ether provided an active extract. This active acidic organic compound was then treated with ethereal diazomethane, which destroyed the activity. Base hydrolysis of this methyl ester then restored activity, confirming the suspicion based on smell that the active component was a carboxylic acid. Finally, treatment with bromine in carbon tetrachloride destroyed the activity, suggesting the presence of unsaturation in the molecule.

Final chemical and spectral analyses are still in progress to elucidate the precise structure of this building pheromone.

Table 1: Interspecific trail following in East African termites

Workers of termite tested	extracts of workers of – 1	2	3	4	5	6	7	8	9	10	11
Amitermes unidentatus	++	+	+	nt	nt	nt	nt	nt	++	++	++
Hodotermes mossambicus	nt	nt	nt	++	0	nt	0	nt	0	nt	0
Macrotermes subhyalinus	nt	nt	nt	nt	++	nt	0	nt	+	nt	0
*Paraclystis integer**	nt	nt	nt	nt	nt	nt	nt	++	++	++	++
Schedorhinotermes lamanianus	++	+	+	—	0?	+	0	++	++	++	++
Trinervitermes bettonianus	nt	nt	nt	nt	0?	nt	nt	+	++	++	++
Trinervitermes gratiosus	nt	nt	nt	nt	nt	nt	nt	nt	++	++	++

1 = A. *unidentatus*
2 = A. *messinae*
3 = A. *lonnbergianus*
4 = H. *mossambicus*
5 = M. *subhyalinus*
6 = *Nasutitermes infuscatus*
7 = *Odontotermes badius*
8 = P. *integer* (termitophilic Teneid catterpillar w/ *S. lamanianus)*
9 = S. *lamanianus*
10 = T. *gratiosus*
11 = T. *bettonianus*

++ = following P $< .01$
+ = following P $< .05$
0 = no following
— = repellent
nt = not tested

Trail-Following Behaviour

Interspecific Trail Following

Trail following by workers of various soil and wood-dwelling termites was tested using extracts of different species. These results are summarized in Table I. Each extract was first tested with the extracted species, and then trails of 10 TU (trail units) or more were tested with termites from ICIPE colonies. No conclusions as to the meaning of this behaviour have been formulated.

Trail Activity of Chemical Analogs of Trail Pheromones

Various chemical analogs of the natural pheromone of *Reticulitermes virginicus*[1] , 3-*cis*, 6-*cis*, 8-*trans*-dodecatrien-1-01, were obtained from other workers in this field and assayed for trail activity with *S. lamanianus* and *T. bettonianus*. These data are summarized in Table II. Further information on the *S. lamanianus* pheromone will be found in the next subsection.

Fig 1: Fractination of *S. Lamanianus* worker extract

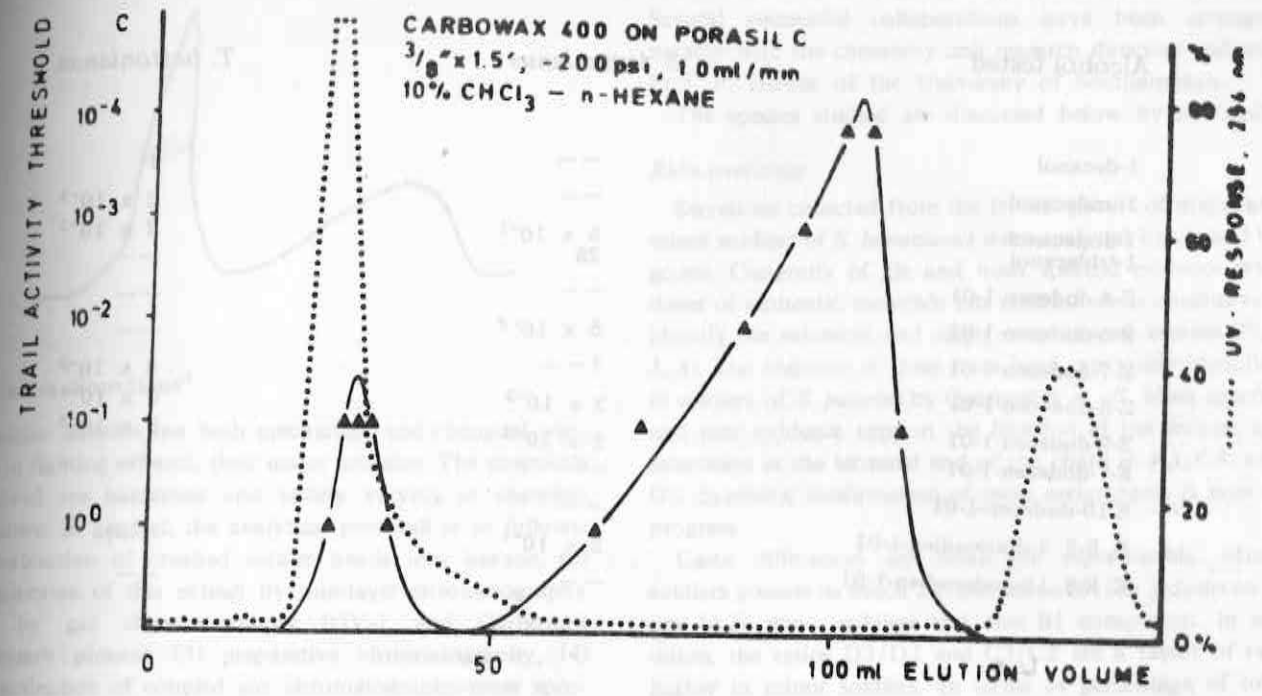
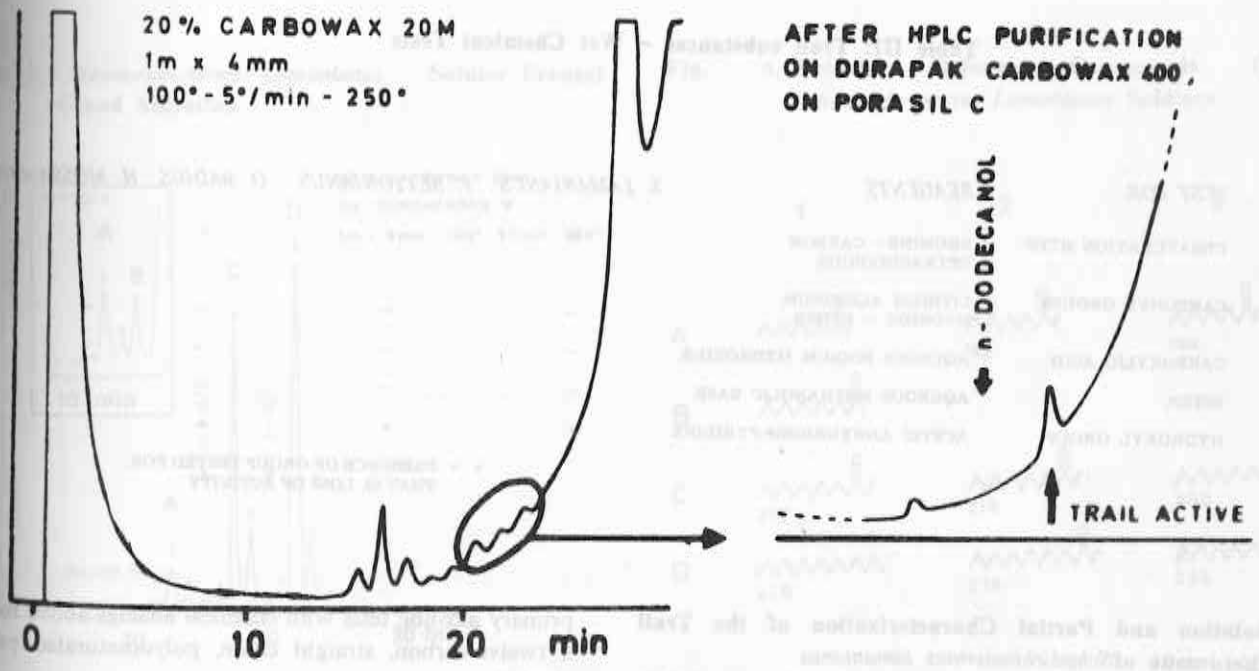


Fig. 2: *Schedorhinotermes Lamanianus*, worker extract



Examination of the pheromone by glc proved difficult, owing to miniscule amounts of pheromone amidst vast amounts of inactive lipids. It appeared from retention time comparison with 1-dodecanol that the more polar lc fraction contained an unsaturated C-12 alcohol as expected from other tests. (Fig. 2).

Assuming that 1 trail unit (TU) is approximately 50 pg, based on the maximum value for *R. virginicus*[1] and assuming 3000 TU/worker, there is in the order of 150 ng/worker. For proper purification, an overall efficiency of 1-5% could be anticipated. For 20,000 workers, then, we can expect to isolate about 100 ug of pheromone. Chemical identification is exceedingly difficult in this range with present facilities, especially considering the relative instability of this compound. Work is, nonetheless, still in progress.

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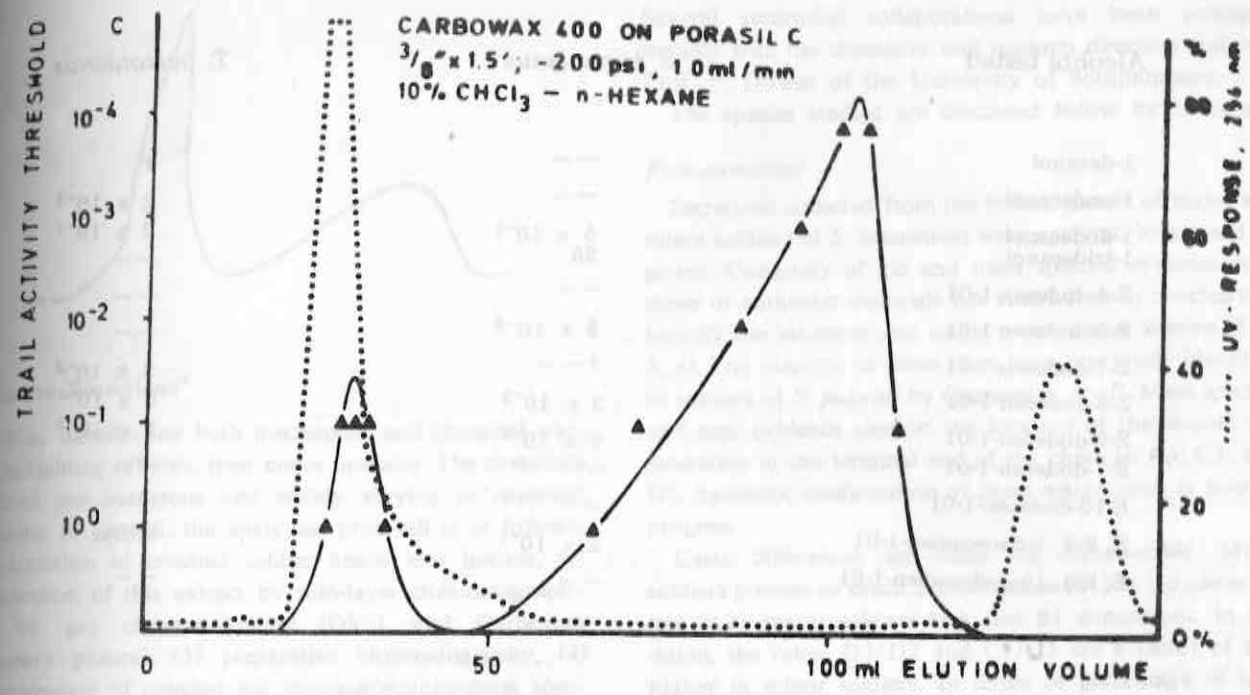

Fig. 2: *Schedorhinotermes Lamanianus*, worker extract

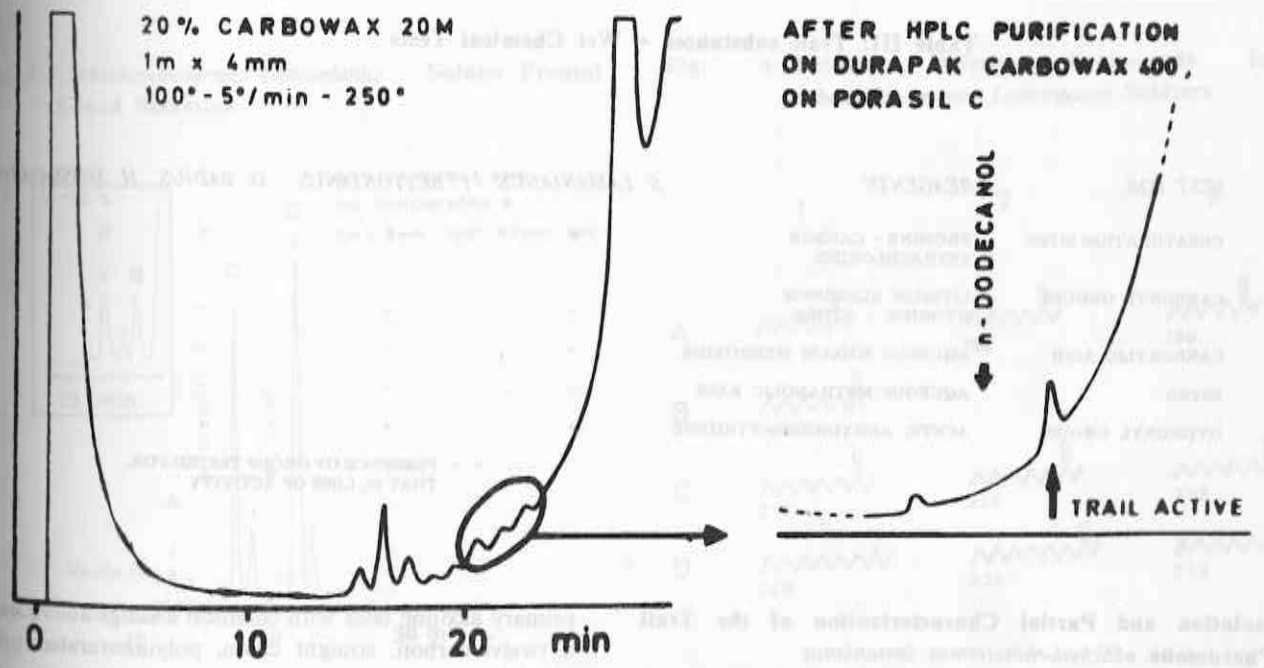

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Table 11: Trail pheromone Analgs – Threshold Values in Micrograms per 10 cm trail

Alcohol tested	*S. lamanianus*	*T. bettonianus*
1-decanol	— —	1
1-undecanol	— —	2×10^{-1}
1-dodecanol	5×10^{-1}	2×10^{-1}
1-tridecanol	25	— —
Z-4-dodecen-1-01	— —	— —
Z-6-dodecen-1-01	5×10^{-2}	— —
Z-7-dodecen-1-01	? — —	2×10^{-3}
Z-8-dodecen-1-01	2×10^{-3}	5×10^{-4}
Z-9-dodecen-1-01	2×10^{-3}	5×10^{-3}
E-7-dodecen-1-01	— —	— —
E-10-dodecen-1-01	— —	— —
E, E-2, 4-dodecadien-1-01	2×10^{-1}	— —?
E, E-8, 10-dodecadien-1-01	— —	— —

— — indicates over 10 micrograms per trail was required, or else that no discrimination at all occurred.

Table III: Trail substances – Wet Chemical Tests

TEST FOR:	*REAGENTS*	*S. LAMANIANUS*	*T. BETTONIANUS*	*O. BADIUS*	*H. MOSSAMBICUS*
UNSATURATION SITES	BROMINE – CARBON TETRACHLORIDE	+	+	+	
CARBONYL GROUPS	LITHIUM ALUMINUM HYDRIDE – ETHER	–	–	–	+
CARBOXYLIC ACID	AQUEOUS SODIUM HYDROXIDE	–	–	–	
ESTER	AQUEOUS METHANOLIC BASE	–	–	–	
HYDROXYL GROUP	ACETIC ANHYDRIDE – PYRIDINE	+	+	+	

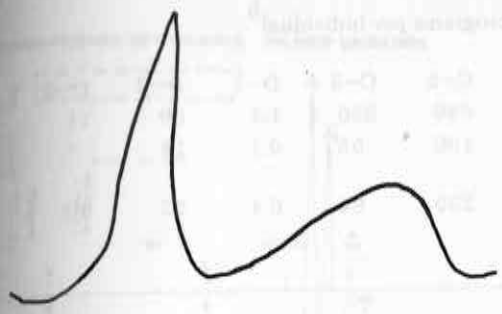
+ = PRESENCE OF GROUP TESTED FOR, THAT IS, LOSS OF ACTIVITY

Isolation and Partial Characterization of the Trail Pheromone of *Schedorhinotermes lamanianus*

Workers (20,000) of the wood-dwelling rhinotermitid *S. lamanianus* were extracted for 24-48 hr in hexane, and the extract was filtered through sodium sulphate and concentrated. An aliquot was taken and kept as a reference for trail activity. The bioassay employed is that devised by Leuthold and Luscher[2].

Initial chemical tests conducted with 1000 TU/2.5 microliter solutions of this extract are summarized in Table III. The implication is that the pheromone is an unsaturated primary alcohol; tests with chemical analogs above indicate a twelve-carbon, straight chain, polyunsaturated primary alcohol.

Purification by silica gel chromatography was of no value; however, partial purification could be achieved by liquid chromatography employing a liquid-liquid type separation (Fig. 1). Two trail active fractions were obtained, the less polar being 1000 times less active. Certain combinations of these two components resulted in a 3-10 fold synergistic effect, i.e., the mixture was more active than individual components.



Defensive Secretions[3]

Soldier termites use both mechanical and chemical warfare in fighting off ants, their major predator. The chemicals involved are numerous and widely varying in chemical structure. In general, the analytical protocall is as follows: (1) extraction of crushed soldier heads into hexane, (2) examination of this extract by thin-layer chromatography and by gas chromatography (OV-1 and Carbowax stationary phases), (3) preparative chromatography, (4) reexamination of coupled gas chromatography-mass spectrometry (Finnigan 1015D, EI mode), and (5) isolation of milligram quantities of major compounds of potential interest. Complete structure elucidation is possible in simple cases, but most compounds require more sophisticated instrumental analysis to complete the structure assignments. Several successful collaborations have been arranged, notably with the chemistry unit research directors and with Prof. P. Howse of the University of Southampton.

The species studied are discussed below by subfamily.

Rhinotermitinae

Secretions collected from the frontal glands of major and minor soldiers of *S. lamanianus* were analyzed by glc and by gc-ms. Congruity of glc and mass spectral evidence with those of authentic materials has enabled us to conclusively identify the saturated and singly unsatureated ketones (Figs 3, 4). The majority of these have been previously identified in soldiers of *S. putorius* by Quennedey *et al*[4]. Mass spectral and nmr evidence support the location of the second unsaturation at the terminal end of the chain in A3, C3, and D3. Synthetic confirmation of these assignments is now in progress.

Caste differences are small but reproducible. Minor soldiers possess as much 2-tridecanone (B1) as 1-dodecen-3-one (A2); major soldiers lack this B1 component. In addition, the ratios D3/D2 and C3/C2 are a factor of two higher in minor soldiers. In terms of percentage of total body weight, the ketonic secretion represents 13.3% in major soldiers and 10.4% in minor soldiers (Table IV).

Fig. 3: *Schedorhinotermes Lamanianus* **Soldier Frontal Gland Secretion**

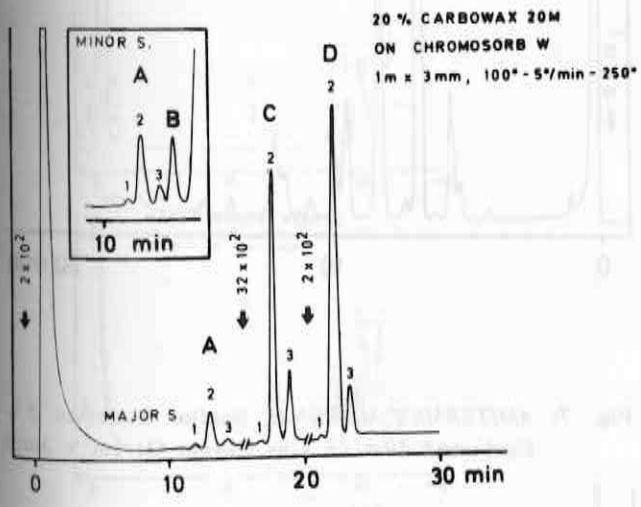


Fig. 4: Ketonic Defense Compounds from *Schedorhinotermes Lamanianus* Soldiers

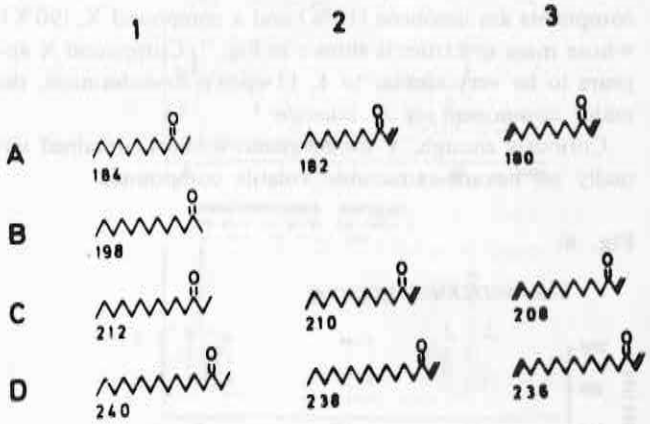


Table IV: Ketonic defensive compounds of *Schedorhinotermes lamanianus* soldiers a

	Micrograms per individual[b]									
	A–1	A–2	A–3	B	C–1	C–2	C–3	D–1	D–2	D–3
Major soldier	1.1	7.6	0.8	- -	7.8	680	230	1.1	59	11
Minor soldier	0.1	0.9	0.3	0.7	1.7	100	55	0.1	16	?
Major soldier frontal gland	[c]0.6	2.6	0.4	- -	3.4	230	64	0.4	23	4.5

√Hexane extracts of heads and abdomens were analyzed separately by glc (20% Carbowax 20M on 80/100 Gas Chrom Q, temperature programmed from 100 to 250° @ 5°/ min). Abdomens of each caste contained a factor of 3 to 5 more of each component than the heads, although the relative ratios were unaltered. This suggests that the frontal gland reservoirs extend into the abdomens. Workers lacked these ketonic compounds altogether.

+ Based on a standard solution of 3-tetradecanone (C-1), assuming equal molar detector response factors.

βCollected in mocrocapillary tubes from the frontal gland fontanelle.

Amitermitinae

Despite close ecological and morphological similarities, the single soldier castes of *Amitermes unidentatus* and *A. messinae* have vastly different defensive secretions. Hexane extracts of the heads of these soldiers have been analyzed by glc and by gc-ms. The former (Fig. 5) gives a highly complex mixture of some twenty compounds, of which 2-tridecanone and 2-pentadecanone (and a singly unsatureated pentadecenone) have been identified as three of the major components. The overall odor is a piercing ketonic smell akin to that produced by *S. lamanianus* soldiers. The *A. messinae* soldiers, on the other hand, smell pleasantly floral and have a very simple secretion (Fig. 6) whose major compnents are limonene (10%) and a compound X, (90%), whose mass spectrum is shown in Fig. 7. Compound X appears to be very similar to 4, 11-epoxy-*cis*-eudesmane, the major component of *A. evuncifer*.[5]

Curiously enough, *A. lonnbergianus* soldiers contained virtually no hexane-extractable volatile compounds.

Fig. 5: *AMITERMES UNIDENTATUS* **5% Carbowax 20m on Gas Chrom Q: 1 m x 3 mm 100° - 5° / min - 240°**

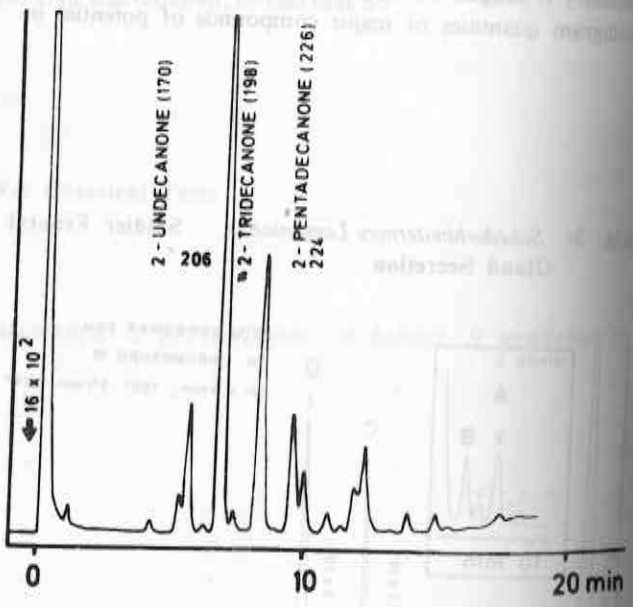


Fig. 6:

from *AMITERMES MESSINAE*

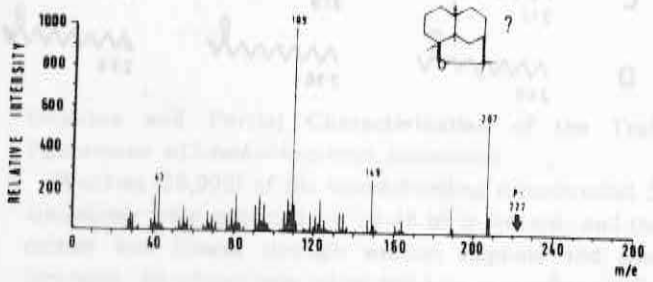


Fig. 7: *AMITERMES MESSINAE* **Soldier Secretion 5% Carbowaz 20m on Gas Chrom Q: 1m x 3mm**

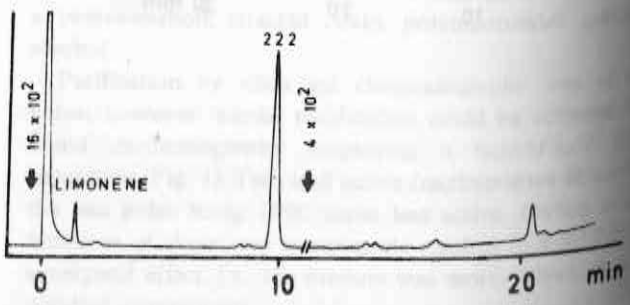
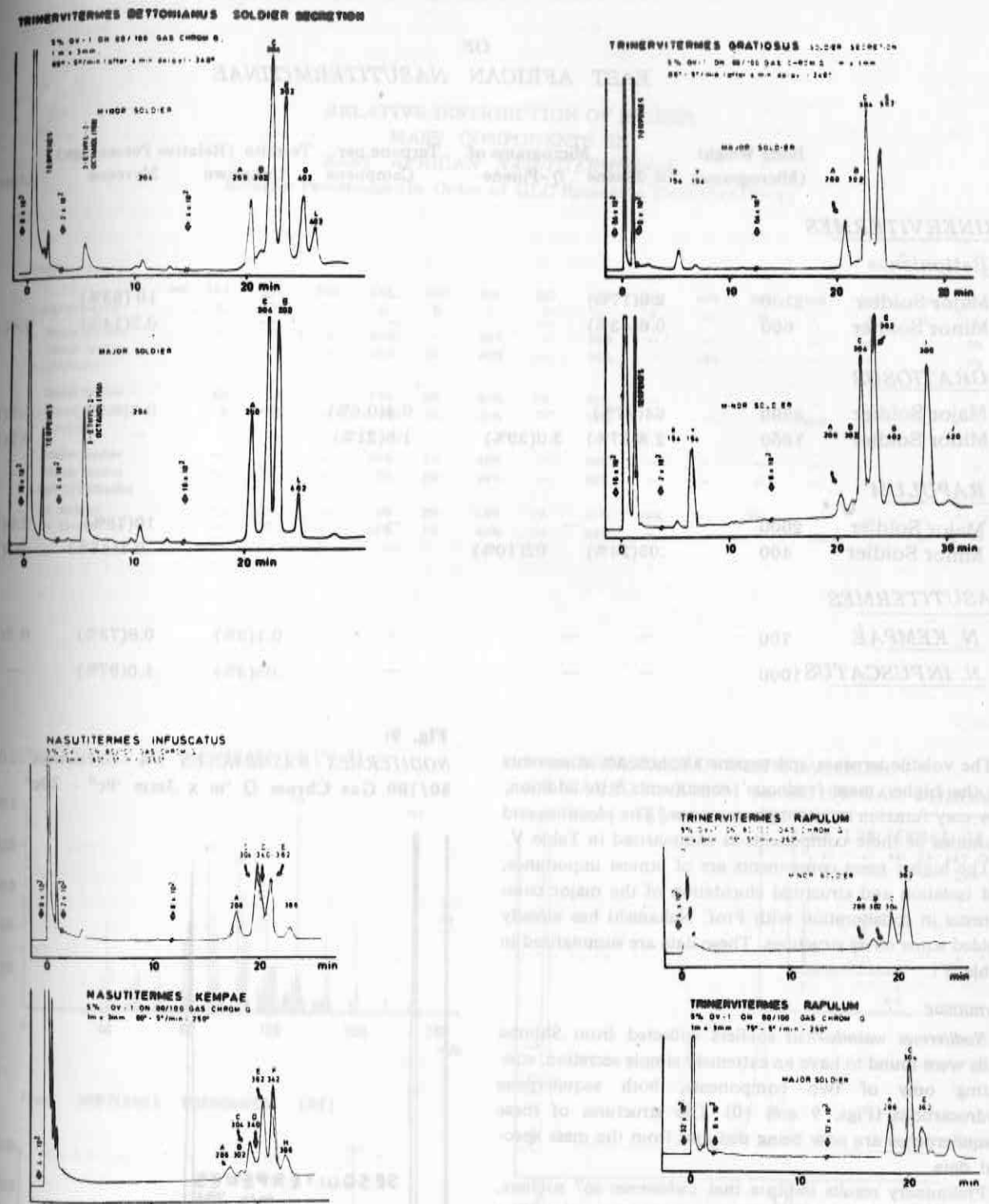


Fig. 8:



Nasutitermitinae

Work has begun at ICIPE in 1972 on *T. bettonianus*, and a few simple components were identified.[6] We have now launched a full-scale program on three *Trinervitermes* spp and two *Nasutitermes* spp. The glc traces of these species are pictured in Fig. 8.

TABLE V

TERPENE CONTENTS OF SOLDIER CEPHALIC GLANDS

OF

EAST AFRICAN *NASUTITERMITINAE*

	Body Weight (Micrograms)	Micrograms of α-Pinene	η-Pinene	Terpene per Campnene	Termite (Relative Percentage) Unknown	Myrcene	Limonene
TRINERVITERMES							
T. Bettonianus							
Major Soldier	2100	2.0(17%)	—	—	—	10 (83%)	—
Minor Soldier	600	0.6(43%)	—	—	—	0.2(14%)	0.6(43%)
T. GRATIOSUS							
Major Soldier	3900	64(97%)	—	0.4(0.6%)	—	0.6(0.9%)	1.2(2%)
Minor Soldier	1600	2.8(37%)	3.0(39%)	1.6(21%)		—	0.2(3%)
T. RAPULUM							
Major Soldier	2000	—	—	—	—	10(78%)	2.8(22%)
Minor Soldier	400	.05(24%)	.02(10%)	—	—	0.1(48%)	.04(19%)
NASUTITERMES							
N. KEMPAE	700	—	—	—	0.1(9%)	0.8(73%)	0.2(18%)
N. INFUSCATUS	1000	—	—	—	.05(3%)	1.0(97%)	—

The volatile terpenes and terpene alcohols act as solvents for the higher mass 'resinous' constituents.[7] In addition, they may function as alarm pheromones.[8] The identities and quantities of these components is summarized in Table V.

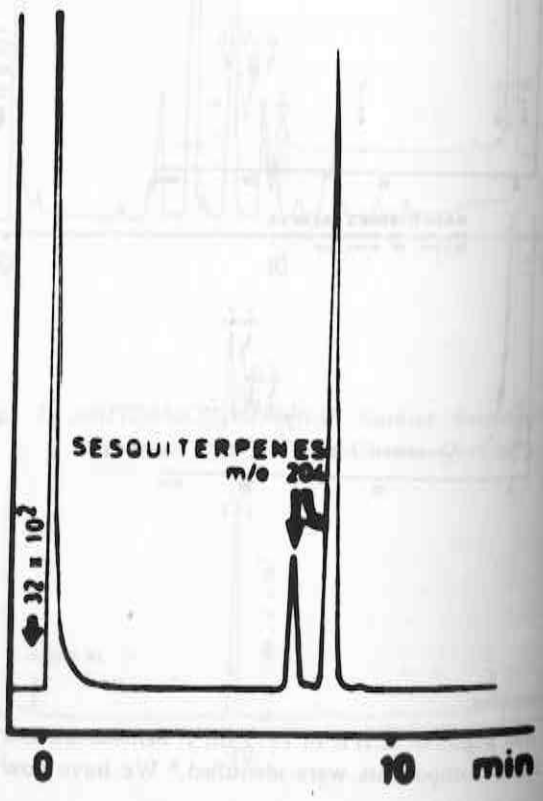
The higher mass components are of utmost importance, and isolation and structural elucidation of the major components in collaboration with Prof. Nakanishi has already yielded some novel structures. These data are summarized in Table VI.

Termitinae

Noditermes wasmbaricus soldiers collected from Shimba Hills were found to have an extremely simple secretion, consisting only of two components, both sequiterpene hydrocarbons (Figs. 9 and 10). The structures of these sesquiterpenes are now being deduced from the mass spectral data.

Preliminary results indicate that *Cubitermes* sp? soldiers, also from Shimba Hills, also posses a simple secretion (Fig. 11). Work is in progress.

Fig. 9:
***NODITERMES WASMBARICUS* 5% Carbowax 20m on 80/100 Gas Chrom Q 'm x 3mm '0c⁰ - 20c⁰**



RELATIVE DISTRIBUTION OF HIGHER MASS COMPONENTS IN EAST AFRICAN *NASUTITERMITINAE*

Relative Percentage (In Order of GLC Retention Time On OV—1)

	mt	154 X	154 Y	158	288 A	302 B	304 C	340 D	362 E	342 F	402 G	386 H	386 I	428 J	320 K	482 L
TRINERVITERMES																
T. BETTONIANUS																
Major Soldier		—	—	—	20%	—	38%	—	33%	—	—	—	—	—	—	9%
Minor Soldier		—	—	—	11%	2%	36%	—	30%	—	14%	—	—	—	—	7%
T. GRATIOSUS																
Major Soldier		85	15	—	13%	2%	48%	1%	37%	—	—	—	—	—	—	—
Minor Soldier		8	92	—	4%	1%	26%	—	33%	—	5%	—	30%	1%	—	—
T. RAPULUM																
Major Soldier		—	—	—	21%	1%	48%	—	30%	—	—	—	—	—	—	—
Minor Soldier		—	—	—	7%	2%	24%	—	65%	—	—	—	—	—	—	—
NASUTITERMES																
N. KEMPAE		—	—	—	4%	2%	15%	1%	31%	38%	—	9%	—	—	—	—
N. INFUSCATUS		—	—	—	14%	1%	40%	7%	32%	—	—	—	6%	—	—	—

from *CUBITERMES SP? WASHBARICUS* [N-2]

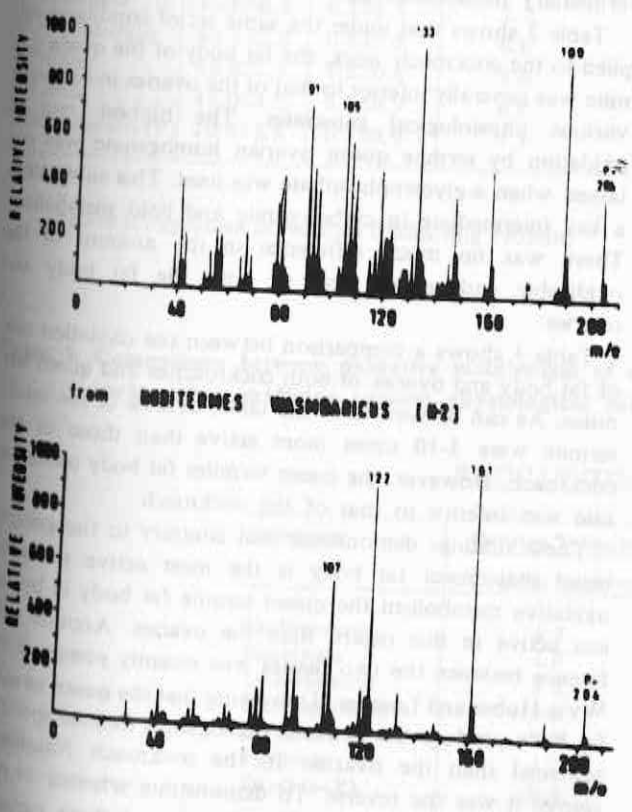


Fig. 10:

from *CUBITERMES SP?* (SHIMBA HILLS)
5% CARBOWAX 20M on 80/100 GAS CHROM Q

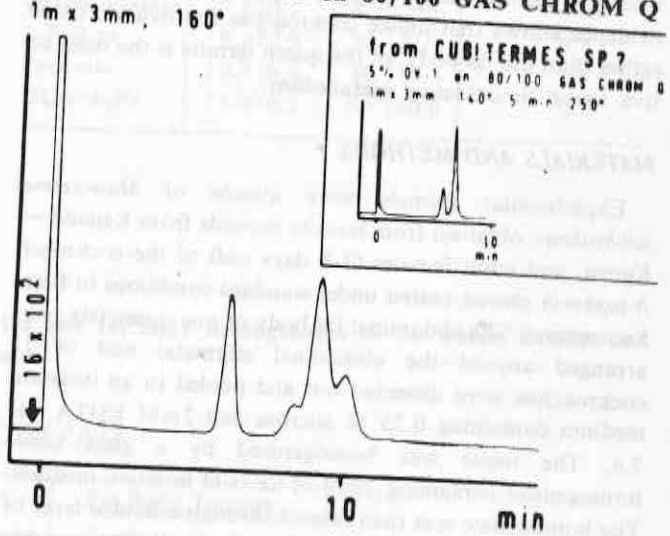


Fig. 11

Biochemistry of Termites

INTRODUCTION

As an extension of our investigation into the caste differentiation of higher termites, a biochemical approach was undertaken to study various aspects of termite biochemistry. Apparently, biochemical investigations on termites are far behind other insects. The aim of this investigation is therefore to study the termite *Macrotermes subhyalinus* focusing on:

1. The role of fat body and ovarian tissues, of the queen termite, in energy metabolism at both cellular and mitochondrial level,
2. Influence of juvenile hormones and ecdysones on oxidative metabolism of both types of tissues,
3. Biochemical characterization of flight muscle mitochondria of winged alates, and
4. The role of the fungus garden in termite colonies.

1) The Role of Fat Body and Ovaries in Energy Metabolism

The queen of *Macrotermes subhyalinus*, a common termite species in eastern Africa, has a tremendour rate of egg production. Egg production requires substantial energy input plus extremely efficient protein synthesis machinery.

It is generally agreed that protein synthesis and oxidative metabolism, two indispensable processes for egg production, occur mainly in insect fat body. Recently, however, it was shown (1) that in the termite queen vitellogenins are mainly produced in the ovary and not in the fat body as in cockroaches. We extended this study to look at the oxidative metabolism of both the ovarian and fat body tissues of termite queen in comparison to cockroaches. Our evidence shows that unlike cockroaches the ovarian tissue, rather than the fat body, of the queen termite is the most active tissue in oxidative metabolism.

MATERIALS AND METHODS:

Experimental animals were queens of *Macrotermes subhyalinus*, obtained from termite mounds from Kajiado — Kenya, and adult females (7-8 days old) of the cockroach *Nauphoeta cinerea*, reared under standard conditions in Bern-Switzerland. The abdominal fat body of one queen (six pairs arranged around the abdominal stigmata) and of 18 cockroaches were dissected out and pooled in an isolation medium containing 0.25 M sucrose and 2mM EDTA, *p*H 7.4. The tissue was homogenized by a glass tissue homogenizer containing 20ml of ice cold isolation medium. The homogenate was then filtered through a double layer of cheesecloth and the filterate was used directly for enzymic assay. The same procedure was used to obtain homogenates from ovarian tissues of both species.

For mitochondrial preparations, fat body and ovaries homogenates were subjected to differential centrifugation as described recently by Abo-Khatwa (2). The final mitochondrial pelled was washed once and suspended in cold isolation medium to give about 5mg protein/ml. Homogenate and motochondrial protein were determined colorimetrically at 546 nm using bovine serum albumin (BSA) as a standard. The results were the average from at least three independent experiments.

Measurements of the oxygen uptake of the homogenate and mitochondrial preparations were followed polargraphically, at 26°C, by using a Clark O_2 electrode coupled to a recording system. An aliquot of 0.2ml of tissue homogenate (equivalent to 1 mg protein) was added to an assay medium containing 0.25M sucrose, 30mM KH_2PO_4, 15mM KCl, 2mM EDTA, 5mM $MgCl_2$, 50mM Tris-buffer *p*H 7.4 and 20mM of a given substrate. For mitochondrial respiration, 0.3% BSA was added to the assay medium. To establish several respiration cycles of energized and non energized mitochondria, small aliquots of ADP (5ul of 200 nmoles) were added from time to time by means of a long-needle Hamilton microsyringe. The specific uncoupler 2-4 dinitrophenol (2-4DNP) and the inhibitor oligomycin were used to assess the efficiency of respiratory chain phosphorylation of mitochondrial preparations.

Isolated mitochondria from various tissues was preserved as described previously (2).

RESULTS AND DISCUSSION

Table 1 shows the ability of cockroach fat body and ovary homogenates to oxidize various physiology substrates. It is clear that the fat body was able to oxidize all the physiological substrates tested at a rate higher than that of the ovarian tissue. The oxidation rate of the fat body ranged from 6-7 times that of the ovaries. Moreover, the amount of the oxidizable endogenous pool of substrates in the fat body was about 12 times of that of the ovaries.

But these findings are not unexpected since the fat body is the tissue which is concerned with much of the intermediary metabolism and nutrient storage.

Table 2 shows that under the same set of conditions applied to the cockroach work, the fat body of the queen termite was generally inferior to that of the ovaries in oxidizing various physiological substrates. The highest rate of oxidation by termite queen ovarian homogenate was obtained when a-glycerophosphate was used. This substrate is a key intermediate in carbohydrate and lipid metabolism. There was no much difference in the amount of the oxidizable endogenous pool of both the fat body and ovaries.

Table 3 shows a comparison between the oxidation rate of fat body and ovaries of both cockroaches and queen termites. As can be seen from the table, ovaries of the queen termite were 3-10 times more active than those of the cockroach. However, the queen termites fat body oxidation rate was inferior to that of the cockroach.

These findings demonstrate that contrary to the general belief that insect fat body is the most active tissue in oxidative metabolism the queen termite fat body is by far less active in this regard than the ovaries. Another difference between the two tissues was recently presented by Wyss-Huber and Luscher (1) showing that the queen termite fat body produce less actively vitellogenins (female specific proteins) than the ovaries. In the cockroach *Nauphoeta cinerea* it was the reverse. To demonstrate whether or not these differences in the oxidative ability of these various

tissues can be traced at the mitochondrial level, our logical choice was mitochondria since cellular respiration is attributed exclusively to these organelles.

Table 4 shows that isolated mitochondria from both fat body and ovaries of the cockroach exhibited a tightly coupled respiratory-chain phosphorylation. The average ADP/O ratios (i.e. moles of ADP phosphorylated into ATP per atom of oxygen taken up; this ratio is a measure for the efficiency of the phosphorylating system) obtained from both types of mitochondira were 1.57-1.96 when a flavoprotein linked substrates such as succinate and α-glycerophosphate were oxidized. While the average ADP/O ratios were 1.82-2.83 when NAD-linked substrates such as oxoglutarate and glutamate were oxidized. These values correspond with the theoretical values expected when such substrates are oxidized by mitochondria isolated from mammalian systems such as rat liver.

When the phosphate acceptor (i.e. ADP) was added, both types of mitochondria exhibited a typical state 4/3/4 cycle of nonenergized/energized/nonenergized states. The energized state (i.e. state 3) is characterized by a tremendous increase of O_2 uptake. This increase in O_2 uptake was greater in the case of mitochondria obtained from the cockroach fat body than that of the ovaries with a ratio ranging from 1-9 times increase. This difference among both mitochondrial populations of the fat body and the ovaries of the cockroach must be attributed to differences in the make up of these organelles in terms of the concentration of the enzymic system, phosphorlating assemblies and cytochromal system. Such differences could be manifested structurally and this question is currently under investigation using electron microscopy

Table 1: Oxidation Rate*

Comparison Between Fat Body and Ovaries Homegenates of the Termite Queens of *Macrotermes subhyalinus* Oxidizing Physiological Substrates.

Substrate	Fat Body	Ovaries	Fat Body/Ovaries
Endogenous	28.1±1.8	2.4±0.9	11.7
Succinate	60.3±4.0	8.4±1.5	7.2
L-Glutamate	43.6±0.8	6.1±1.1	7.2
α-Oxoglutarate	43.1±2.8	6.5±0.8	6.6
L-Proline	42.8±1.5	7.2±0.4	6.0
Pyruvate	43.0±4.2	6.5±0.9	6.6
DL-α Glycero-(P)	40.6±2.5	5.7±0.9	7.1

*Rate is expressed as Natoms 0/Min/mig Protein.

Table 2: Comparison Between Fat Body and Ovaries Homegenates of the Cockroach *Nauphoeta cinerea* (7-8. D.O. + [9]) Oxidizing various Physiological Substrates.

Substrate	Fat Body	Ovaries	Fat body/Ovaries
Endogenous	12.0±0.3	10.0±0.2	1.2
Succinate	49.9±0.4	40.2±0.4	1.2
L-Glutamate	17.9±0.4	20.2±1.1	0.9
α-Oxoglutarate	17.6±0.2	31.0±0.9	0.6
L-Proline	9.9±0.9	20.1±1.5	0.5
Pyruvate	13.8±0.3	30.5±5.3	0.6
DL-α G-(P)	15.2±2.1	53.7±0.8	0.3

Table 3: Comparison between oxidative metabolism of ovaries and fat body homogenates of the queen termite and cockroaches oxidizing various physiological substrates

RATIO OF OXIDATION RATE

Substrate	Ovaries Termite / Ovaries Cockroaches	Fat Body Termite / Fat Body Cockroaches
Endogenous	4.2	0.66
Succinate	4.8	0.83
L-Glutamate	3.3	0.41
α-Oxoglutamate	4.8	0.41
L-Proline	2.8	0.23
Pyruvate	4.7	0.32
DL-α G-(P)	9.4	0.37

Table 4: Comparison between Fat Bosy and Ovary Mitochondria of Cockroaches (7-8 D.O. + 0) Oxidizing Various Physiological Substrates.

	FAT BODY				OVARY				
		Rate of Oxidation[a]				Rate of Oxidation[a]			St. 3 P.R.
Substrate	ADP/O	St. 3[b]	St. 4[c]	RCI[d]	ADP/O	St. 3[b]	St. 4[c]	RCI	St. 30V.
Endogenous	—	6.20	5.20	1.20	—	1.35	1.35	1.00	4.6
Succinate		89.40	25.11	3.56	1.57	17.61	6.17	2.85	5.1
α-Oxoglut	2.88	47.71	12.52	3.81	1.82	7.25	3.30	2.20	6.6
L-Glutatmate	2.16	28.43	6.90	4.12	2.22	6.00	2.00	3.00	4.7
Pyruvate	1.77	26.10	10.73	2.43	—	2.87	2.87	1.00	9.1
Pruv. + L-Prol.	1.81	25.80	8.13	3.17	2.23	7.37	1.77	4.16	3.5
L-Proline	—	11.00	7.42	1.48	2.18	10.93	2.73	4.00	1.0
DL-α G-(P)	—	16.71	11.96	1.40	1.96	5.92	2.83	2.10	2.8
NADH	—	13.70	13.70	1.00	—	2.80	2.80	1.00	4.9
NADH[e]	—	14.10	14.10	1.00	—	3.31	3.31	1.00	4.3

[a] Rate is expressed as natoms 0/min/mg Protein.
[b] In the presence of phosphate acceptor (ADP).
[c] In the absence of phosphate acceptor (ADP).
[d] Respiratory control index; a measure of the tightness of mitochondrial coupling between oxidation and phosphorlation.
[e] Aged mitochondria for 1 hour at 30°C.

Table 5: Comparison between Fat Body and Ovary Mitochondria of The Termite Queen *Macrotermes Subhyalinus* Oxidizing Various Physiological Substrates.

	FAT BODY				OVARY				
		Rate of Oxidation				Rate of Oxidation			St. 3P. V
Substrate	ADP/O	St. 3	St. 4	RCI	ADP/O	St. 3	St. 4	RCI	St. 3 OV
Endogenous	—	4.85	4.85	1.00	—	11.85	11.85	1.00	0.4
Succinate	1.49	59.14	23.63	2.50	1.60	105.00	3.97	3.15	0.6
α-Oxoglut	2.28	26.50	10.50	2.08	2.08	61.88	16.40	3.80	0.4
L-Glutamate	2.28	25.75	8.63	2.86	2.68	45.53	9.40	4.91	0.6
Pyruvate	—	28.90	17.60	1.64	—	30.23	25.00	1.21	0.9
Pyruv. †L-Orol.	2.08	25.00	9.30	2.70	2.30	68.90	14.70	4.70	0.4
L-Proline	2.08	25.00	17.60	1.43	1.30	101.70	24.17	4.24	0.3
DL-α G-(P)	1.80	26.45	15.15	1.77	1.59	59.67	18.23	3.27	0.4
NADH	—	11.13	11.13	1.00	—	14.30	14.30	1.00	0.8
NADH*	—	12.17	12.17	1.00	—	13.70	13.70	1.00	0.9
Palmitate	—	18.50	18.50	1.00	—	9.00	9.00	1.00	2.1
Palmitoyl-Carnitine ester	—	44.00	44.00	1.00	—	26.00	26.00	1.00	1.7

*Aged mitochondria for 1 hour at 30°C.

Table 5 shows the respiratory activity, respiratory-chain phosphorylation (ADP/O) and respiratory control index (RCI) of mitochondria isolated from fat body and ovaries of the queen termite *Macrotermes subhyalinus*. For all substrates tested, ovarian mitochondria showed higher oxidative rates than those of the fat body. Exceptions were, however, when plamitic acid ($CH_3(CH_2)_{14}COOH$) and its carnitine ester were utilized. In this case fat body mitochondria had superior oxidative rates (almost doubled) toward these two fatty acid substrates as compared to ovarian mitochondria. This finding suggests that queen termite fat body could be a major tissue site for the metabolism of fatty acids. Moreover, the oxidiziable endogenous pool of fat body mitochondira was lesser (about one third) than that of ovarian mitochondria. These results indicate that queen termite ovarian mitochondria possess extremely active oxidative phosphorylating and TCA — enzymic systems in a manner similar to that of the cockroach fat body.

For further work on mitochondrial metabolism it was of interest to develop a practical method to preserve isolated mitochondria for prolonged periods. This was necessary because isolated mitochondria particularly from insect tissues are known to be biochemically labile i.e. unstable. The mechanism of mitochondrial instability has been recently discussed (2). Mitochondrial instability i.e. aging causes severe limitations to mitochondrial researchers. Apart from the daily tedium of mitochondrial isolation, it limits the range of experiments and prevents a close cooperation between distantly located scientists. For these reasons, a simple and practical method was developed to restore and preserve isolated mitochondria. This is the first reported successful attempt to accomplish this with insect tissues. A detailed investigation on this subject (2) revealed that isolated insect mitochondria from various tissues can effectively be preserved functionally and structurally if kept frozen at liquid N_2 temperature (-196°C) in the presence of certain cryoprotective agents such as dimethyl sulfoxide (DMSO).

Table 6 shows the successful application of this method in preserving mitochondrial coupling activities of insect fat body, ovaries and flight muscles.

The presence of DMSO was essential in preserving the mitochondrial coupling activities of flight muscles which suggests that flight muscle mitochondria are more sensitive and labile to this freezing method than fat body and ovarian mitochondria.

The extreme stability of insect fat body and ovarian mitochondria when frozen at liquid N_2 temperature in the absence of DMSO, for at least 3 weeks, is an uncommon phenomenon. To our knowledge no reports have been published on preserving mitochondrial functions at liquid N_2 temperature in the absence of DMSO. In this regard insect mitochondria from these tissues could provide a useful model, of a longer-lived organelle, in assessing the similarity or difference between the functions of isolated mitochondria and those in their natural environment.

Table 6: Preservation of Mitochondria Isolated from Various Insect Species and Tissues by Freezing and Storing in Liquid N_2 Temperature

Insect Species	Tissue	±DMSO (10% v/v)	Substrate	Minimum Preservation Period
Macrotermes subhyalimus	Fat body	—	Succinate	2 months
(Queen termite)	Ovaries	—	Succinate	2 months
Odontotermes Sp	Flight	†	DL-α glycero-	3 weeks
(♀ termite alate)	Muscle		phosphate	
Nauphoeta Cinerea	Fat body	—	Succinate	3 weeks
(♀ 7-8 D.O.)	Ovaries	—	Succinate	3 weeks
Glossina Morsitans	Flight	†	L-Proline	3 weeks
(♀ tsetse flies)	Muscle			

2) Influence of Juvenile Hormones and β-Ecdysone on Oxidative Metabolism of Fat Body and Ovaries of *Macrotermes subhyalinus* and *Nauphoeta Cinerea*

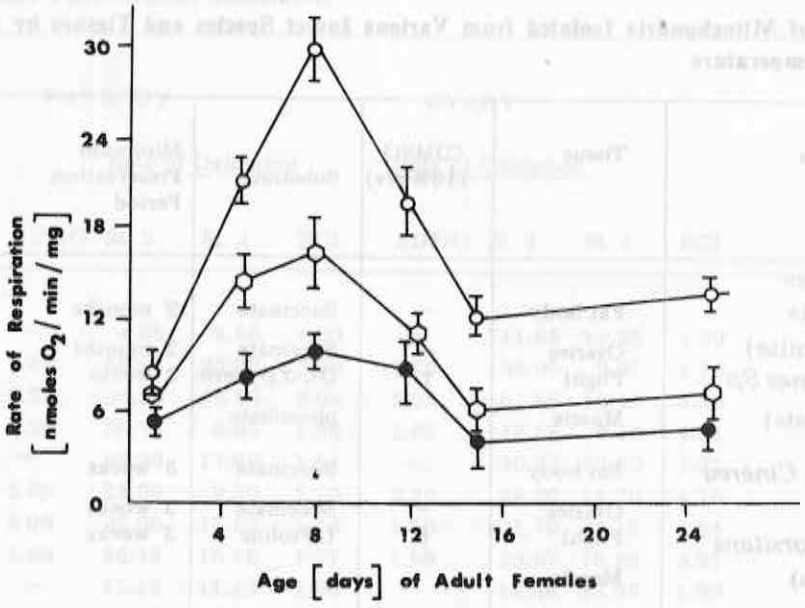
Since every aspect of termite development is mediated by chemical signals of pheromonal and hormonal nature, biochemical studies on the mode of action of these chemicals at cellular and subcellular levels are of great importance for a greater understanding of the biochemical mechanisms of termite development.

It has been suggested by Luscher, in a series of publications, that in lower termites, juvenile hormone (JH) plays an important role as a pheromone in the termite colony by regulating the production of different castes. It is possible that in higher termites, JH may be involved as well. Luscher has suggested that the synthesis of the large quantity of protein needed for the enormous egg production of the termite queen *Macrotermes subhyalinus* could be under the influence of corpora allata (c.a.), the source of JH (s). The increase in the biosynthetic activity of insect tissues requires an increase in energy supply which is often accompanied by an increase in the oxidative metabolism. Many researchers have reported that removal or implantation of the c.a. significantly alters the respiratory rate of living insects as well as of isolated organs such as the fat body. For this reason the c.a. hormone has been qualified as a "metabolic hormone". However, our knowledge on the metabolic effects of JH is limited, being based mostly upon experiments in which the c.a. is removed or implanted. Furthermore, despite the recent availability of pure juvenile hormones and ecdysones, experiments designed to demonstrate the direct action of hormones on oxidative metabolism are lacking. Therefore, we studied the influence of both hormones on isolated organs (fat body and ovaries) of the termite queen *Macrotermes subhyalinus* and as well of as the cockroach *Nauphoeta cinerea*.

MATERIALS AND METHODS

Tissue homogenates and mitochondria were prepared from the fat body and ovaries of both *Macrotermes subhyalinus* and *Nauphoeta cinerea* as described previously. During the course of respiration small aliquots (5 µl of 3 x 10^{-5}m final concentration) of each juvenile hormone (JH_3) (methyl-10, 11-epoxy-3, 7, 11 trimethyl-trans 2, 6-dodecadienoate; C-16) or B-ecdysone, dissolved in ethanol were added by means of a long-needle Hamilton microsyringe. The rate of oxidation was determined before and after the addition. All other determinations were carried out as described previously.

Fig. 1: Age influence on basal and oxidative respiration rate of fat body homogenates of the Cockroach *Nauphoeta cinerea*



RESULTS AND DISCUSSION

Fig. 1 shows the oxidation rate of fat body homogenate of the cockroach *Nauphoeta cinerea* in relation to the age of females after adult ecdysis. The three curves obtained, representing basal metabolism and oxidative metabolism of a-glycerophosphate and succinate, show progressive increase in both basal and oxidative metabolism as adults get older, reaching a maximum oxidative rate at 7-8 after adult ecdysis. This is the period where ovarian development and yolk incorporation into the oocytes are at maximum. This period is followed by a decrease in both basal and oxidative metabolism reaching its minimum at day 15, and coinciding with the oocyte reaching its full maturity and readiness for pregnancy. During pregnancy, insects stop feeding and their activity significantly ceases which is accompanied by low rates of basal and oxidative metabolism as shown in Fig. 1.

Fig. 2 shows the influence of JH_3 on the basal metabolism of cockroach fat body homogenate. The addition of JH_3 (40 nmoles/mg protein resulted in a significant stimulation of the respiratory rate of basal metabolism during early ovarian development (i.e. from day 1 to 8) and not thereafter. Maximum stimulation (about 65% increase) was achieved at day 8 after adult ecdysis. Fig. 2 also shows that during oocyte maturation (i.e. from day 1 to 12) c.a. becomes inactive and secretes JH which is required for yolk incorporation (3). Upon cocyte maturation (about day 15) the c.a. becomes inactive and remains so during the whole gestation period (45 days from adult ecdysis). During late cocyte maturation and pregnancy (12-25 days) basal metabolism of the fat body becomes minimal and no longer responds to the addition of JH_3. It should also be noted that B-ecdysone (at 40 nmoles/mg protein) produced a similar stimulatory effect on basal metabolism to that of JH_3. This shows that the fat body is not at all times competent to react with JH_3.

Fig. 2: Influence of exogenous JH_3 upon the basal metabolism of fat body homegenate of the Cockroach *Nauphoeta cinerea* during adult development.

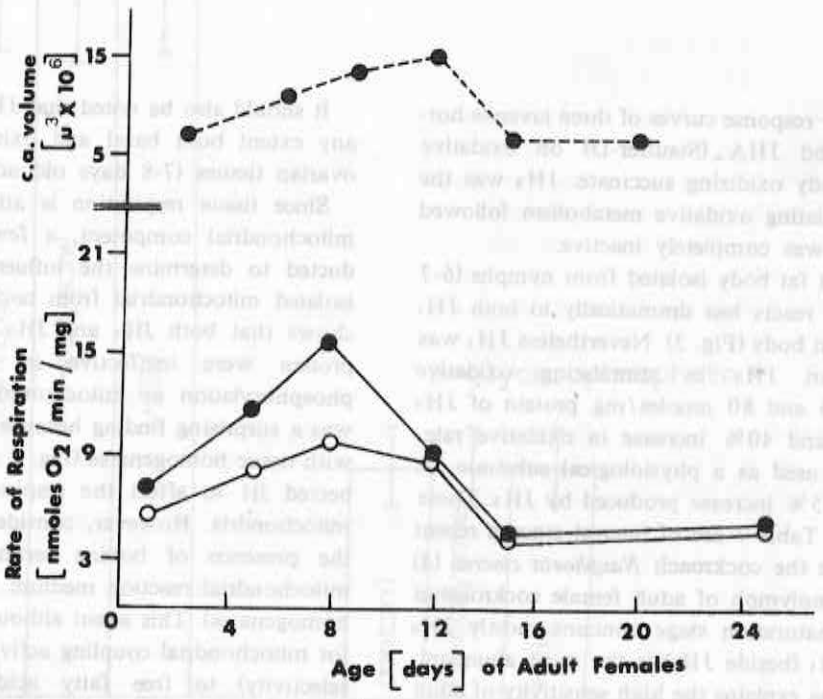


Fig. 3: Dose-response curves of JH_3, JH_1 and JHA against fat body homogenate oxidising succinate of 7-8 days old females of the Cockroach *Nauphoeta cinerea*.

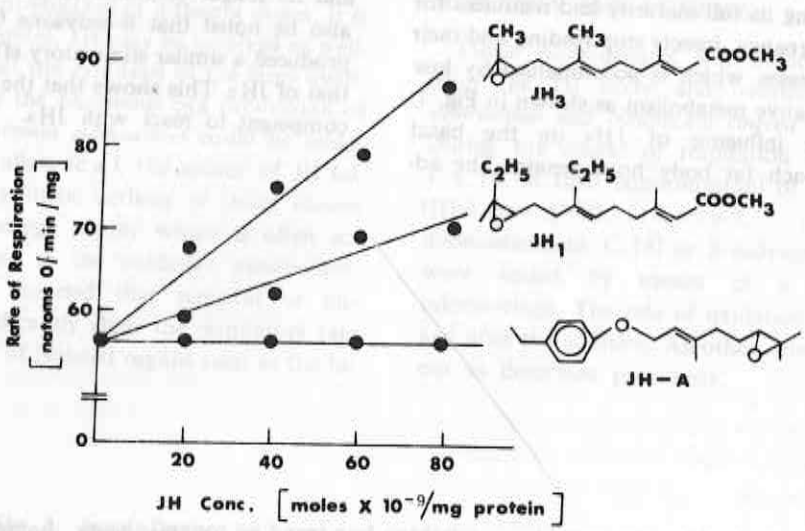


Fig. 3 shows dose – response curves of three juvenile hormones JH_1, JH_3 abd JHA (Stauffer-D) on oxidative metabolism of fat body oxidizing succinate. JH_3 was the most potent in stimulating oxidative metabolism followed by JH_1 while JHA was completely inactive.

Table 7 shows that fat body isolated from nymphs (6-7 days) of cockroaches reacts less dramatically to both JH_1 and JH_3 than adult fat body (Fig. 3). Nevertheless JH_1 was more effective than JH_3 in stimulating oxidative metabolism. Thus 20 and 80 nmoles/mg protein of JH_1 produced about 20 and 40% increase in oxidative rate, when succinate was used as a physiological substrate, as compared to 0 and 25% increase produced by JH_3. These results of Fig. 3 and Table 7 are of interest since a recent report on JH titer in the cockroach *Nauphoeta cinerea* (4) reveals that the haemolymph of adult female cockroaches during the cocyte maturation stage contains mostly JH_3 while in nymphs JH_1 (beside JH_2) is the most abundant. This important finding explains the high sensitivity of adult fat body to JH_3 and that of nymphs to JH_1. This indicates that both differentiated tissues (fat body of adults and nymphs) are so programmed that they react selectively to JH_3 in a tissue specific way.

It should also be noted that JH_1 or JH_3 did not alter to any extent both basal and oxidative metabolism of the ovarian tissues (7-8 days old adults).

Since tissue respiration is attributed exclusively to its mitochondrial compotent, a few experiments were conducted to determine the influence of JH_1 and JH_3 on isolated mitochondrial from cockroach fat body. Table 2 shows that both JH_1 and JH_3 at 40 and 80 moles/mg protein were ineffective in altering respiratory-chain phosphorylation or mitochondrial respiratory rates. This was a surprising finding because from our previous studies with tissue homogenates (Fig. 2 and 3) one would have expected JH to affect the respiratory activity of fat body mitochondria. However, consideration should be given to the presence of bovine serum albumin (BSA) in the mitochondrial reaction medium (BSA was absent in tissue homogenates). This agent although an essential component for mitochondrial coupling activity is known to bind (non-selectivity) to free fatty acids and many other antimitochondrial agents thus protecting mitochondrial function (5). But whether or not the presence of BSA was responsible for the lack of effect of JH on mitochondrial activities can only be determined after further studies to

develop a reaction medium for insect mitochondrial preparations lacking BSA.

Similar experiments to those of cockroaches were conducted with fat body and ovarian homogenates of the termite queen *Macrotermes subhyalinus*. Fig. 4 shows that neither JH_3 nor B-ecdysone (at a concn of 40 nmoles/mg each) had a significant effect on the oxidation rate of termite queen fat body oxidizing succinate (this is in contrast to cockroach fat body homogenates, see Fig. 2 and 3). This lack of response of both hormones is similar to that of the cockroach ovaries. The failure of JH to elicit a stimulatory effect on queen termite fat body could well be attributed to the presence of an extremely high titer of endogenous JH circulating with the blood. The JH titer in the haemolymph of one single queen termite exceeds that of any other insect studied so far. As a result of the presence of this extremely high titer of JH in the haemolymph, surrounding tissues could be so conditioned or so saturated that they might no longer be responsive to external JH. Termite ovary homogenate, on the other hand, responded to the addition of B-ecdysone.

Fig. 5A shows O_2 electrode traces obtained from termite queen ovarian homogenate. Addition of B-ecdysone (40 nmoles/mg protein) during the course of respiration, in presence of various physiological substrates, elicited instant and significant increase in respiratory rates (note the increase in the slope of O_2 electrode tracings). This stimulatory effect varies between 70-85% increase in the respiration rate depending on the type of rate depending on the type of substrate being oxidized. The subsequent addition of JH_3 (40 nmoles/mg) inhibited the B-ecdysone-stimulated respiration rate by 20-50% but in no case was this inhibition complete. When the sequence of addition was reversed (Fig. 5B) i.e. JH_3 was added first, followed by B-ecdysone, the same pattern prevailed. JH_3 itself was ineffective in changing the respiratory rate but it inhibited B-ecdysone-stimulated respiraiton. There is little literature showing that JH can act inhibitively, at the cellular membrance level, to B-ecdysone-induced-pysiological reactions such as the puffing of salivary glands and cuticle synthesis. Both processes are known to be inhibited by JH.

The precise role of B-ecdysone in stimulating the oxidative metabolism of both the termite queen ovaries and the cockroach fat body is not known. However, it could be indirectly related to the high protein synthetic activity of these tissues, which in turn could be induced by B-ecdysone increasing tissue demand for energy supply.

Recent reports have shown that not only can ecdysone in-

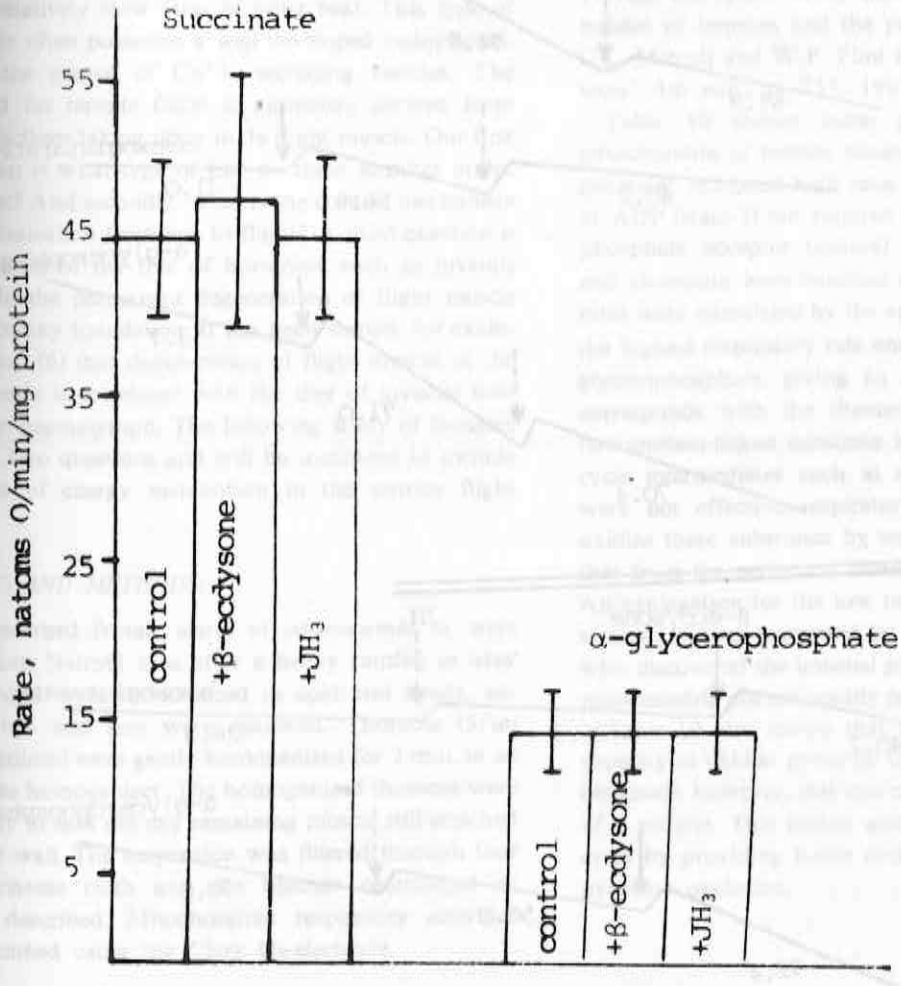


Fig. 4: Influence of JH_3 and β-ecdysone on oxidative metabolism of fat body homogenate of the termite queen *Macrotermes subhyalinus*.

duce ovarian development in mosquitoes but that it is also present in high titers in some female adult insects such as locusts, silkmoths, and milkweed bugs even though the prothoracic glands, the principal source of ecdysone, are degenerated. Moreover, high titer of ecdysone has been recently (5) recovered from the ovaries and eggs of adult silkmoths. For this reason we looked at the presence of B-ecdysone in the ovaries of queen termites using high pressure LC of 70% methanolic extract. Preliminary results showed the absence of B-ecdysone (at 1 ng sensitivity) from this tissue.

We conclude from papers 1 and 2 above the following:-

1. Basal and oxidative metabolism of cockroach fat body were significantly higher (6-12 times) than those of the ovaries.
2. The oxidative rate of the termite queen ovaries were higher (2-4 times) than that of the fat body, although basal metabolism of both tissues was similar. Based on the high oxidation rate of the fatty acid palmitate and its carnitine ester, the termite queen fat body could be a major tissue-site for fatty acid and lipid metaboli
3. The differences in the oxidation rates of ti homogenates has been confirmed by using isol mitochondria.
4. JH_1, JH_3, and B-ecdysone but not a JH analogue physiological concentrations, stimulate both basal oxidative metabolism of cockroach fat body during early cocyte maturation and not thereafter. was more potent than JH_1 in its stimulatory ef
5. JH_1 was more effective than JH_3 in stimulating b and oxidative metabolism of fat body of cockroach nymphs.
6. Neither JH_3 nor B-ecdysone was effective in stimulation of oxidative metabolism of termite q fat body.
7. B-ecdysone induced both basal and oxida metabolism of the termite queen ovaries while JH ted antoganistically.

We are now conducting experiments to find out precise mode of metabolic activation of these various mones at both extra and intramitochondrial levels.

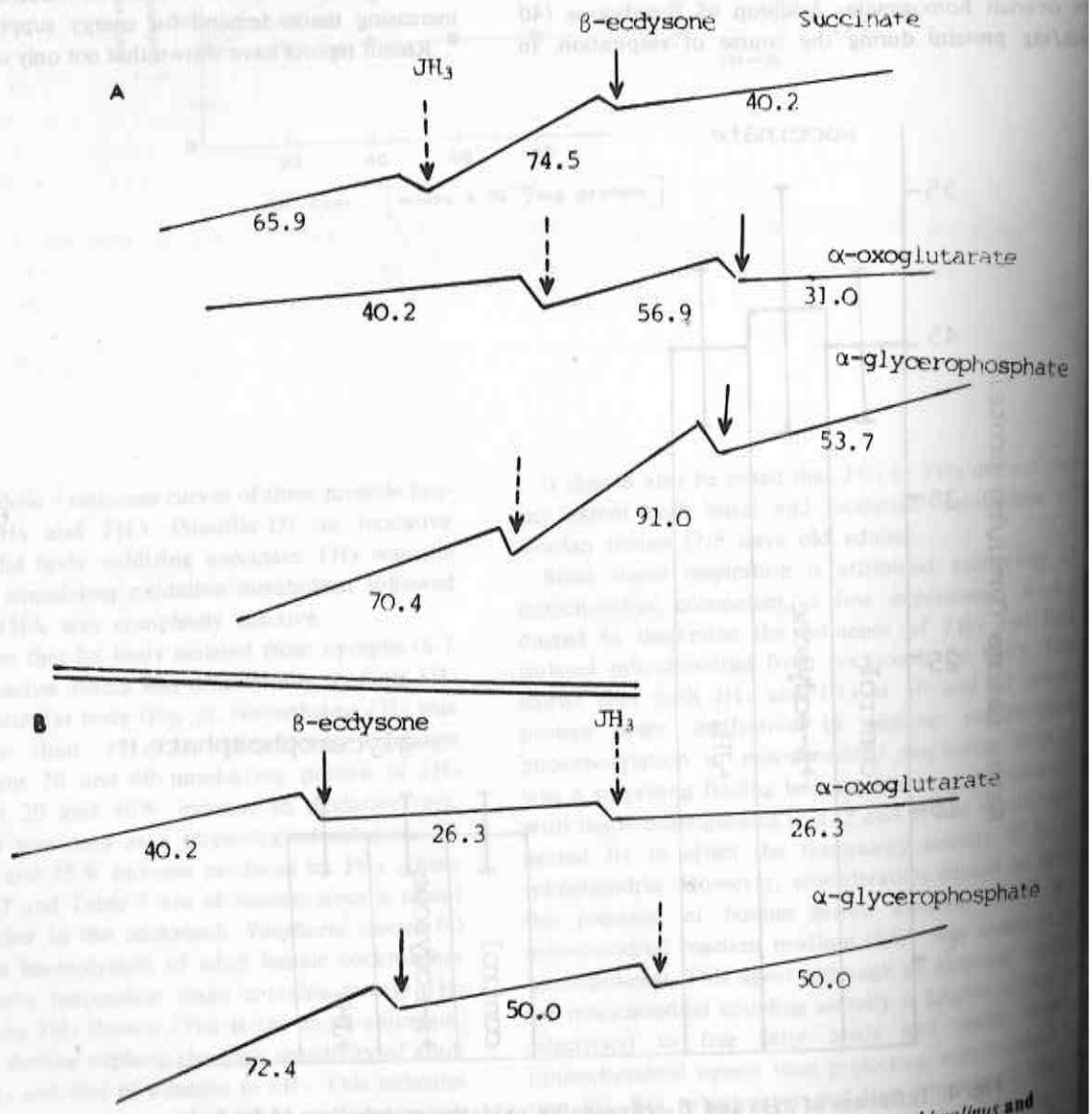


Fig. 5: O_2 – electrode tracings of ovaries homogenate of the termite queen *Macrotermes subhyalinus* and the influence of β-ecdysone and JH_3 on its respiratory rates.

3. Biochemical Characterization of Flight Muscle Mitochondria of Termite Alates

In recent years there has been a considerable broadening of our knowledge and understanding of the energy metabolism of insect flight muscle. Some insects such as flies and honeybees utilize carbohydrate during flight and their flight muscle mitochondria oxidize the end products of glycolysis; α-glycerophosphate and pyruvate. Others such as moths utilize lipids and their flight muscle mitochondria oxidize long-chain fatty acids. Still others such as cockroaches and locusts derive flight energy from both sources and possess mitochondria capable of oxidizing long-chain fatty acids and its carnitine ester as well as α-glycerophosphate and pyruvate. However, there are still gaps in our knowledge. For instances, there is no information about the energy metabolism of the flight muscles of termite alates despite the fact that behavioural studies on termite flight and colony foundation have received some attention.

One distinct feature of caste differentiation in termites is the development of flight muscle in those reproductives with the potential capability to establish new colonies. It has been known that Isoptera, to which termites belong possess a synchronous or closed-packed type of flight muscle of relatively slow rates of wing beat. This type of flight muscle often possesses a well developed endoplasmic reticulum, the source of $Ca^{2}+$ secluding vesicles. The energy used for termite flight is ultimately derived from chemical reactions taking place in its flight muscle. Our first question then is what type of fuel do these termites utilize during flight? And secondly, what is the control mechanism during the transition from rest to flight? A third question is the significance of the role of hormones such as juvenile hormones in the permanent degeneration of flight muscle soon after colony foundation. It has been shown, for example by Dekort (6) that degeneration of flight muscle of the colorado beetle is correlated with the titer of juvenile hormone in the haemolymph. The following study of focusses on the first two questions and will be continued to include other facets of energy metabolism in the termite flight muscle.

MATERIALS AND METHODS:

Newly emerged female alates of *odontotermes sp.* were collected from Nairobi area after a heavy rainfall in May 1975. Termites were immobilized in cold and heads, abdomen, wings and legs were removed. Thoraces (5/ml isolation medium) were gently homogenized for 2 min. in an ice-cold glass homogenizer. The homogenized thoraces were stirred gently to ease out any remaining muscle still attached to the body wall. The suspension was filtered through four layers of cheese cloth and the filterate centrifuged as previously described. Mitochondrial respiratory activities were determined using the Clark O_2-electrode.

RESULTS AND DISCUSSION:

Table 8 shows three types of insect flight muscle mitochondria depending upon the type of substrate they utilize. The first type is presented by termite alates, periodical cicada and houseflies. Their mitochondria oxidize carbohydrates as indicated by their high respiratory activity toward d-glycerophosphate; an end product of glycolysis. Only housefly mitochondria was able to oxidize pyruvate at a maximum rate. The failure of termite and periodical cicada mitochondria to oxidize pyruvate indicates that the limiting factor in pyruvate dehydrogenase, but that the control could be at the TCA cycle level (see Table 2). The second mitochondrial type is presented by the colorado beetle which oxidizes the amino acide L-proline at a maximum rate. The role of L-proline as an energy-furnishing reserve is found also in the tsetse fly. The third mitochondrial type is that of the locust which efficiently utilizes carbohydrates α-glycerophosphate), lipids (fatty acids carnitine esters) as well as amino acids (L-proline).

The aforementioned variations in the oxidative properties of the three types of flight muscle mitochondria seem to depend on the duration of flight (short or long) as well as on the type of flight muscle (synchronous or asynchronous) which these insects possess.

It is of interest to note from Table 9 the similarities between the mitochondrial oxidative properties of both the termite and the periodical cicada, the latter is known to have the longest development period of any known insect (13 and 17-year life cycle). More similarities in the behaviour and habitat of termites and the periodical cicada do exist (see C.L. Metcalf and W.P. Flint in "Destructive and useful insects" 4th edit., p. 737, 1962).

Table 10 shows some properties of flight muscle mitochondria of termite alates. Flight muscle mitochondria generally, exhibited high rates of respiration in the presence of ADP (state-3) but respired slowly in the absence of the phosphate acceptor (state-4) α-Glycerophosphate, proline and glutamate were oxidized at appreciable rates and these rates were stimulated by the addition of ADP. In particular, the highest respiratory rate and control were found with α-glycerophosphate, giving an ADP/O ratio near 2 which corresponds with the theoretical value obtained when a flavoprotein-linked substrate is oxidized. In contrast, krebs cycle intermediates such as succinate and α-oxoglutarate were not effective respiratory substrates. The failure to oxidize these substrates by termite mitochondria resembles that from the periodical cicada and housefly (see Table 9). An explanation for the low rates of oxidation of these substrates has been suggested by Van den Bergh and Slater (7) who discovered the unusual phonomenon that flight muscle mitochondria are not readily permeable to these compounds.

Table 10 also shows that termite mitochondria lost the capacity to oxidize pyruvate (no. ADP/O and RCI could be obtained), however, this loss can be reversed by the addition of L-proline. This amino acid is known to "spark" TCA-cycle by providing Krebs cycle intermediates necessary for pyruvate oxidation.

Table 7: Influence of JH_1 and JH_3 on the oxidation rate of fat body homogenates of cockroach nymphs (6-7 days old) oxidizing succinate.

Condition		Oxidation rate patoms 0/min/mg	Significance (p)
Control	(Et-OH)	34.77±3.91 (12*)	—
	$+JH_3$		
	(20 moles)	35.10±0.44 (3)	>0.45
	(80 moles)	43.73±2.90 (3)	>0.01
	$+JH_1$		
	(20 moles)	42.20±1.60 (3)	>0.01
	(80 moles)	48.33±1.50 (3)	>0.0025

Table 8: Influence of JH and JH on energy-linked functions of fat body mitochondria of adult female cockroaches (7-8 days old)*

Condition		ADP/O	State 3	State 4	RCI
Control	(Et-OH)	2.0±0.3	90.6±3.8	24.4±2.6	3.71
	$+JH_3$				
	(40 moles)	2.0±0.3	90.6±3.8	26.0±3.8	3.48
	(80 n moles)	2.0±0.2	87.7±2.2	25.0±1.8	3.50
	$+JH_1$				
	(40 n moles)	1.9±0.1	88.7±2.1	24.3±3.5	3.65
	(80 n moles)	1.9±0.1	85.9±3.8	25.0±1.7	3.44

*In presence of BSA (0.3%)

Table 9: Respiratory activity of flight muscle mitochondria of various insect species

Substrate	*Odontotermes Sp.*	*Colorado beetle*[b]	*Periodical Cicada*[c]	*Musca* domestica	*Locusta* Migratoria[d]
α-Glycerophosphate	308.0	123.6	680.0	1000.0	280.0
Pyruvate	11.8	—	13.0	1008.0	—
L-Proline	64.6	988.9	35.0	—	—
L-Glutamate	60.0	90.6	133.0	32.0	340.0
Succinate	47.0	41.2	52.0	32.0	98.0
α-oxoglutarate	17.6	57.7	17.0	22.0	148.0
Palmitate	4.2	—	—	—	—
Palmitoyl Carnitine ester	4.0	—	—	—	358.0

[a]Respiratory rates are expressed as natoms 0/min/mg mitochondrial protein, in presence of ADP.

[a]Leptinotarsa decemlineata: from De Kort, C.A., Bartelink, A.K.M., and Schuurmans, R.R., Insect Biochem. 3, 11, (1973).

[c]*Magicicada septendecim;* from Hansford, R. G. *Biochem. J. 121*, 771 (1971)

[d]from Van den Bergy, S.G. *methods* in *Enzymology*, 117 10, (1967).

Table 10: Properties of *Odontotermes* Sp. flight muscle mitochondria

Substrate	ADP/0[b]	Respiration rate State-3[c]	Respiration rate State-4[d]	RCI[e]
Endogenous	—	0.0	0.0	—
α-Glycerophosphate	1.70	308.0	88.4	3.48
Pyruvate	—	11.8	11.8	1.00
L-Proline	1.62	64.6	35.2	1.84
Pyruv. † Proline	2.84	68.8	32.4	2.12
L-Glutamate	2.62	60.6	20.6	2.94
Succinate	—	47.0	47.0	1.00
α-oxoglutarate	—	17.6	17.6	1.00
Palmitate	—	4.2	4.2	1.00
Palmitoyl Carnitine ester	—	4.0	4.0	1.00

[a]Values given are means of a great number of polorographic experiments at ρH 7.4 and 30°

[b]The ration of nmoles of ADP added to the natoms of oxygen utilized, induced by the addition of ADP.

[c]Rate expressed as natoms O/min/mg; after ADP addition.

[d]Rate expressed as natoms O/min/mg; before ADP addition.

[e]Respiratory control index; defined as the ratio of oxygen uptake in the presence of added ADP to the rate of respiration after the added ADP has been completely utilized.

The amino acids L-proline and L-glutamate, were oxidized by termite mitochondria at appreciable rates giving net ADP/O ratios of 1.62 and 2.62 and RCI 1.84 and 2.94 respectively. In contrast, fatty acids such as plamitate and its carnitine ester were not oxidized to any extent.

We conclude that alates of the termite *Odontotermes sp.* derive their flight energy primarily from carbohydrate metabolism and that the "α-glycerophosphate shuttle" is highly operative. Some energy could also derive from the oxidation of the amino acids L-proline and L-glutamate. But it is unlikely, that the oxidation of these amino acids is a prime source of flight energy, because their oxidation rates are far less than of α-glycerophosphate.

The oxidation of α-glycerophosphate by termite flight muscle mitochondria showed high affinity to the phosphate acceptor ADP. This affinity can be measured by determining the Km (a value inversely related to affinity) which is defined as the substrate concentration (ADP) at which the velocity (respiration rate) is half maximal. Usually the km for a substrate roughly equals its cellular concentration. Therefore, Kinetic studies of this sort, *in vitro*, have been used successfully to predict factors which control the activity *in vivo*.

Fig. 1 shows the dependence of termite mitochondria, oxidizing α-glycerophosphate, on the concentration of ADP. The apparent km value was 20×10^{-5}M. This value, obtained from α-glycerophosphate oxidation, was found to be considerably smaller than these values calculated for the oxidation of other substrates (km values were in the range of 2×10^{-5} to 10^{-3} M). Similar km value from α-glycerophosphate oxidation by blow fly mitochondria has been reported.

These findings suggest that as the nucleotide in the resting muscle is predominantly ATP, the small change in ADP levels will lead to a considerable enhancement in the rate of α-glycerophosphate oxidation during which energy is produced to support flight. Increase in the respiratory activity of termite mitochondria due to ADP addition, however, could be insufficient in magnitude to satisfy the requirements for a physiological control mechanism in a termite just beginning flight. So other control systems such as the Ca^{2+} role has been investigated.

Table 11 shows the affinity of mitochondira, isolated from various tissues of termites and cockroaches, to ADP. Maximum rates of oxidation (in the presence of ADP) were achieved by termite flight muscle mitochondria oxidizing a-glycerophosphate (V max = 320). Succinate was poorly oxidized by flight muscle and showed no response to ADP. On the other hand, ovarian motochondria of termites showed the highest rates of succinate oxidation the termite (V max = 140) followed by the fat body of the cockroach (V max = 88) and of the termite (V max = 80). Cockroach ovarian mitochondria were the least sensitive to the addition of phosphate acceptor which indicates that this tissue is metabolically inactive as far as oxidative metabolism is concerned. Furthermore, these oxidation rates were closely correlated with km values. Thus the highest affinity to ADP were the mitochondria isolated from flight muscle and the least were those isolated from the cockroach ovaries. These affinities give a good indication of how metabolically active these tissues are.

Fig. 1: The dependence upon ADP Concentration of the rate of *a*-glycerophosphate oxidation by *Odontotermes* flight muscle mitochondria. Incubation mixture contained α-glycerophosphate and an excess of Pi.

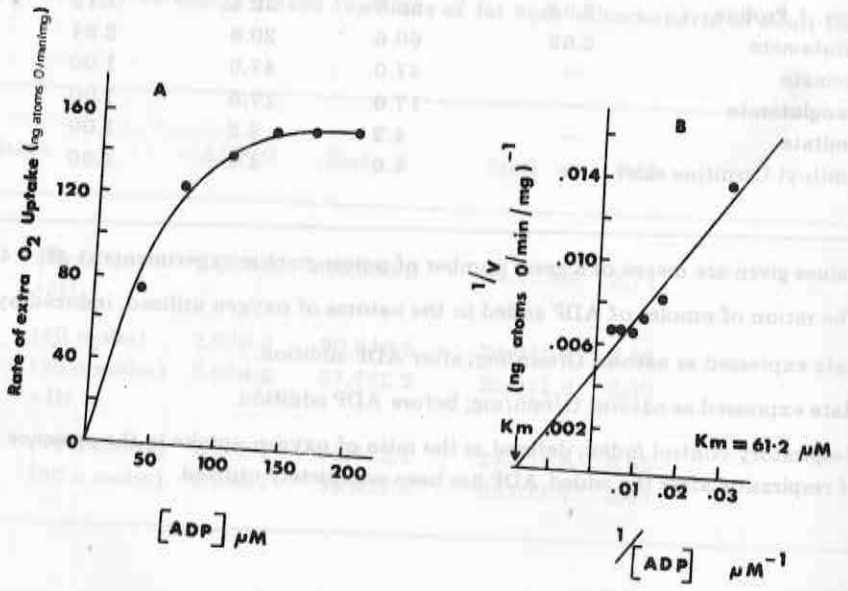


Fig. 2-A shows that several cycles of respiratory activities of termite mitochondria could be achieved by ADP addition (200 nmoles). In the presence of oligomycin (10^{-3}M), an inhibitor of energy transfer reactions, ADP no longer was effective in the stimulation of respiration. On the subsequent addition of 2, 4-Dinitrophenol (2,4-DNP; 5 x 10^{-5}M), an uncoupling agent, oligomycin – induced inhibition was reversed and respiration proceeded, at a higher rate, without ADP control. Furthermore, addition of ADP in the presence of 2,4-DNP (no oligomycin) was ineffective in stimulation of respiration. These findings suggest that the site of the nucleotide ADP is not α-glycerophosphate oxidase, rather the mitochondrial respiratory chain system.

Fig. 2 – B and C show that a-glycerophosphate oxidation by termite mitochondria, unlike L-proline oxidation is controlled not only by ADP but also by Ca+ ions. Addition of Ca^{+2} (10^{-4} gm ion litre $^{-1}$) to mitochondria oxidizing α-glycerophosphate gave cycles of enhanced oxygen uptake. L-proline and other physiological substrates show no enhancement of oxygen uptake upon Ca^{+2} addition. This finding is similar to the Ca^{+2}-induced respiration of flight muscle mitochondria of the periodical cicada (see reference given in Table 1). Blowfly mitochondria, on the other hand, show no effect of Ca^{+2} on the rate of α-glycerophosphate oxidation (8). It is suggested that the difference in response to Ca^{+2} of the two types of mitochondria, reflects the presence or absence of Ca^{+2} "permease" and is related in someway to the different physiology of synchronous and asynchronous flight muscle.

Therefore, it appears that α-glycerophosphate oxidation by termite flight muscle mitochondria is controlled by both ADP and Ca^{+2} levels. The release of divalentions such as Ca^{+2} is known to be accompanied by nervous stimulation of the muscle at the intitiation of flight. Further work will be conducted to determine the precise role of Ca^{+2} in termite flight muscle, and d-glycerophosphate oxidation will be used in future studies as a convenient marker for studying flight muscle development and degeneration.

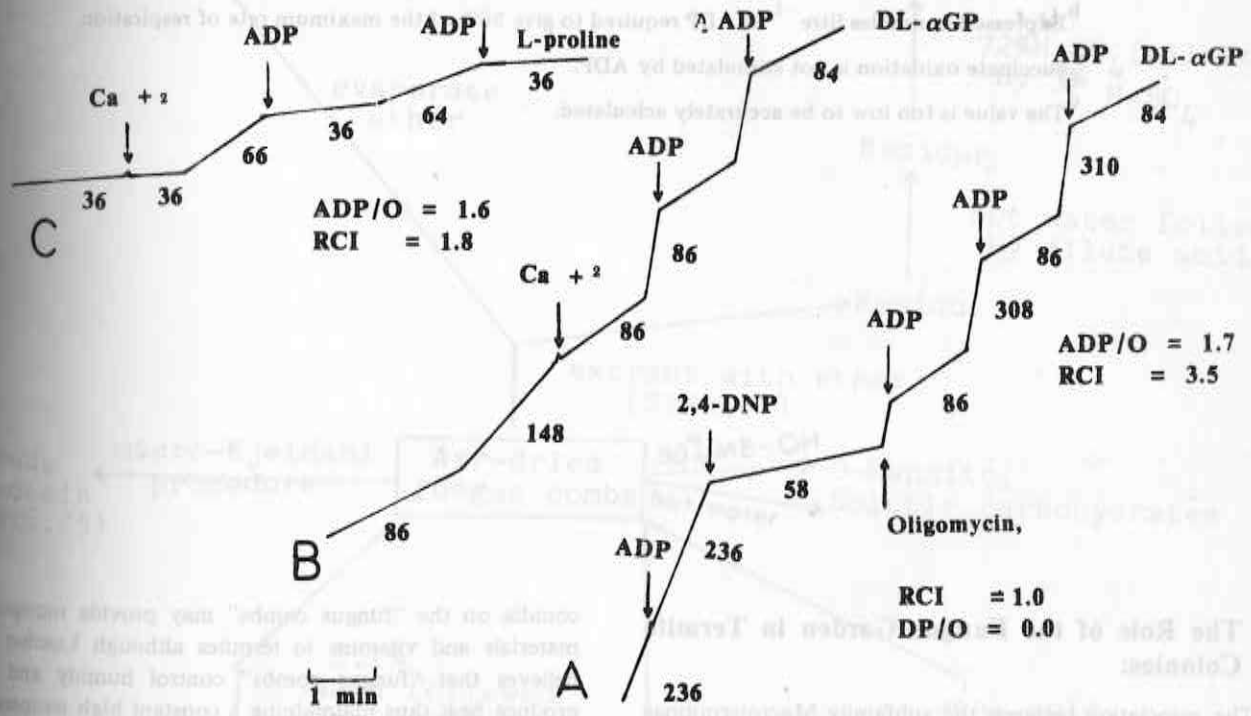


Fig. 2: Oxygen electrode tracings of flight muscle mitochondria of the termite *Odontotermes sp.* oxidizing α glycerophosphate and L-proline and the influence of Oligomycin, 2, 4-DNP and Ca^{+2}.

Table 11: Affinity of Mitochondria from various tissues and species to the phosphate acceptor, ADP

Species	Tissue	Substrate	V max[a]	Km[b]
Odontotermes sp	Flight-muscle	α-Glycerophosphate	320	20.0
		Succinate	—[c]	—[c]
Macrotermes subhyalinus	Ovaries	Succinate	140	23.3
	Fat Body	Succinate	80	46.7
Nauphoeta cinerea	Ovaries	Succinate	18	—[d]
	Fat Body	Succinate	88	26.7

[a] Maximum rate of respiration, upon the addition of 5 various concentrations of ADP; expressed as natoms O/min.

[b] Expressed as nmoles litre $^{-1}$ of ADP required to give 50% of the maximum rate of respiration.

[c] Succinate oxidation is not stimulated by ADP.

[d] The value is too low to be accurately acIculated.

4. The Role of the Fungus Garden in Termite Colonies:

The association between the subfamily Macrotermitinae and the fungal genous *Termitomyces* has reached its highest degree of specialization (9). It is generally agreed that the fungus *Termitocyces* which grows in the "fungus combs" a special structure constructed by termites, serves to convert the cellulose-lignin complexs into easily digestible compounds so that it can be utilized by termites. Whether the "fungus combs" are constructed from faecal material or from undigested plant material is a matter of interesting debate (9). Sands (1969) believes that "fungus combs" are constructed primarily from faecal material produced by termites while Grasse and Noirot (10) believe that they are constructed from masticated plant material. Furthermore, it is not known for certain to what extent "fungus combs" are essential in the diet of termites. It has been suggested that the white nodules, which are made up of conidiophores and conidia on the "fungus combs" may provide nitrogenous materials and vitamins to termites although Luscher (11) believes that "fungus combs" control humidy and also produce heat thus maintaining a constant high temperature within the nest. But it is possible that the function of "fungus combs" are partly nutritional and partly concerned with environmental control in the nest though direct proof is lacking. We therefore tackled this problem by first conducting a detailed chemical analysis of the "fungus combs" to assess their nutritional value to termites. Second by carrying out some metabolic studies on both *Termitomyces* conidia and workers of termites to assess the oxidative capabilities of these species in the utilisation of various physiological substrates, including some of those found in "fungus combs" and third, we conducted some feeding experiments where various extracts of *Termetomyces* conidia and of "fungus combs" were fed to workers to determine their survival rate. This last problem is discussed under "Caste Formation".

MATERIALS AND METHODS:

a. Collection of samples:

"Fungus combs" of *Macrotermes subhyalinus* were obtained from Kajiado, Kenya. Parts of "fungus combs" from individual mounds were collected during April - August of 1975. The material was air-dried at room temperature and in some cases drying was done at 105-110°C. To determine moisture, parts of "fungus combs" *in locu* were placed in a plastic box containing water-soaked cotton and separated from the sample by a wire-net. The dried material was ground into a fine powder and this material was used for extraction and chemical analysis. No attempt was made to separate outer and inner comb material or freshly deposited material from old ones.

b. Chemical analysis:

Moisture, pH, legnin and crude fibre content were determined by standard methods (12). Total lipids were determined by using anhydrous diethly ether and a continuous extraction method (Sexhlet). D-mannitol was extracted by 80% methanol followed by crystallization in aqueous ethanol. This sugar was identified using infra-red spectroscopy, n.m.r., and gas-liquid chromatography (5% SE-30, 3mm by 75 mm column, 190°n N_2 carrier gas at 25 ml/min) after synthesizing the hexa-acetate derivative using acetic anhydride/pyridine mixture. Melting points of crude and crystallized D-mannitol and those of a standard material were determined. Quantitative estimates of D-mannitol content were made on the basis of the yield of crystallized sugar in aqueous ethanol. Nitrogeon content (crude protein) as well as cellulose, uric acid and soluble carbohydrates were determined by the National Agricultural Laboratories, Nairobi, Kenya. Fig. 1 outlines a diagram showing the various stages of organic analysis of "fungus combs".

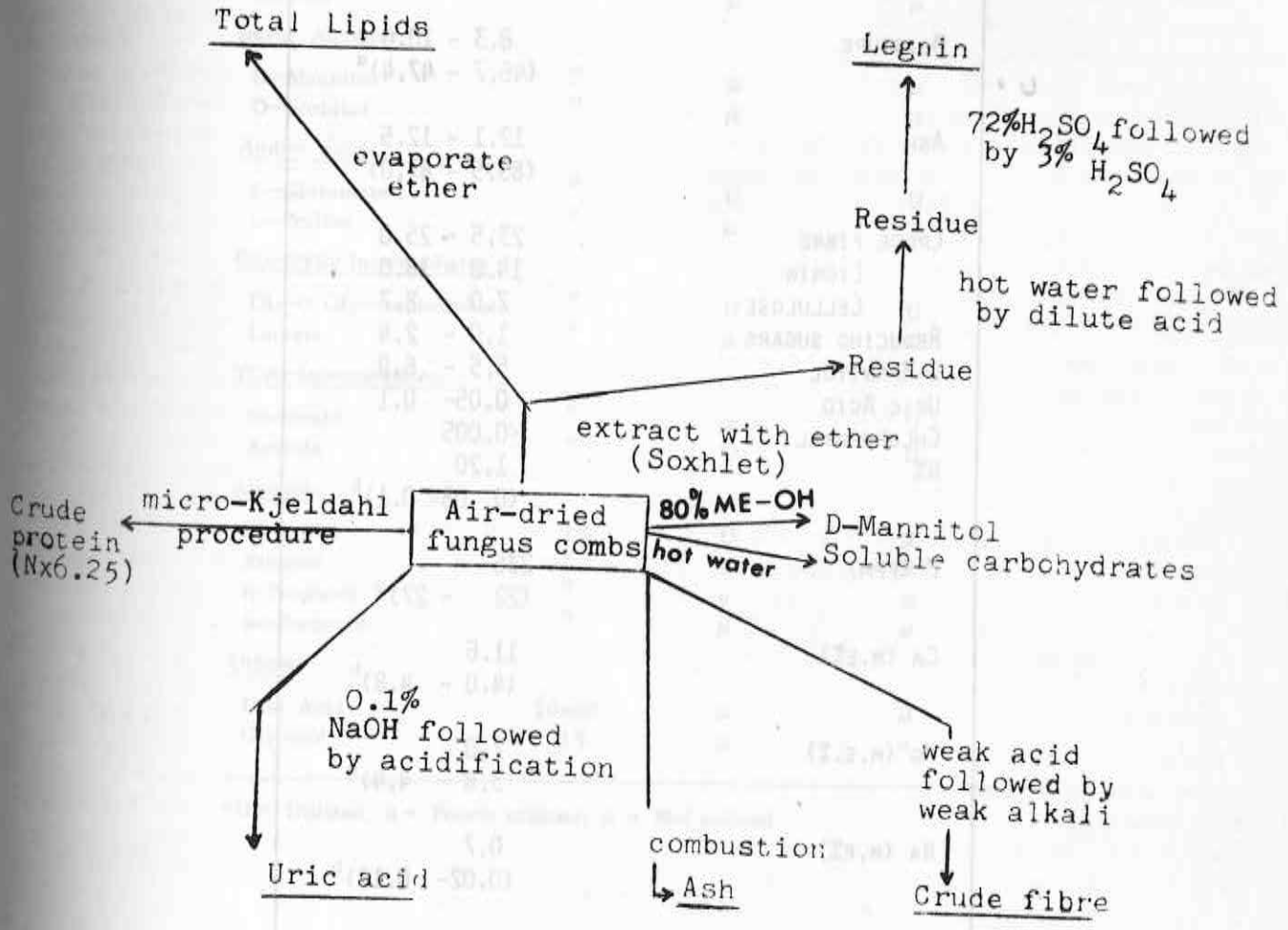


Fig. 1: An outline diagram showing the various stages in the proximate analysis of "fungus combs".

c. Biological Work:

Freshly collected conidia of *Termitocyces* were homogenized in saline (0.9% KCI), centrifuged at 800g. and the supernatent was used for polarographic studies (homogenized conidia gave a very strong smell of yeast). Minor workers of *Macrotermes subhyalinus* were homogenized in 0.25 M sucrose and 2nM EDTA, pH 7.4 The homogenate was filterd through four layers of cheesecloth, the filterate was centrifuged at 800g and the clear supernatent was used directly for metabolic studies.The reaction media for both *Termitomyces* conidia and termite workers consisted of 0.25 M sucrose, 30mM KH_2 PO4, 15mm KCI, 2mM EDTA, 5mM $MgCI_2$, 50 mM Tris-RCI; PH 7.4 and a physiological substrate in a given concentration as indicated in Table 13. The oxidation rate of various substrates was followed polarographically at 30°C using Clark-O_2 electrode connected to a recording system.

RESULTS:

Table 12 shows the chemical composition and some physical properties of a "fungus comb" sample. The given figures represent the average composition of various samples of the same mound. However, it should be noted that variations could exist within the colony depending on the maturity of the "fungus combs" and the time of sample collection.

The analytical figures presented in Table I show that the

Table 12: Chemical Composition of 'fungus combs' of *macrotermes subhyalinus*

CONTENT	% OF AIR - DRY WEIGHT
MOISTURE	8.3 - 10.0 (46.7 - 47.4)[a]
ASH	12.1 - 12.5 (85.5 - 87.0)[b]
CRUDE FIBRE	23.5 - 25.0
LIGNIN	14.8 - 18.0
CELLULOSE	7.0 - 8.7
REDUCING SUGARS	1.0 - 2.4
D-MANNITOL	5.5 - 6.9
URIC ACID	0.05- 0.1
CHLOROPHYLL	<0.005
N%	1.20 (0. 08- 0.1)[b]
P (PPM)	236 (22 - 27)[b]
CA (M.E%)	11.6 (4.0 - 4.8)[b]
MG (M.E.%)	7.0 3.8 - 4.4)[b]
NA (M.E%)	0.7 (0.02- 0.18)[b]
PH	4.2 - 4.7 (6.1 - 6.5)[b]

[a]% OF MOISTURE IN FRESH WET WEIGHT.

[b]VALUES CORRESPOND TO % CONTENTS OF A SOIL MATERIAL COLLECTED FROM THE SAME MOUND.

moisture content of "fungus combs" was about half of the wet weight of a freshly obtained sample. The ash and lignin contents (12.84 and 18.01% respectively) agree very closely with those reported for the related species *Macrotermes natalonsis* (5) (13% ash and 15% lignin). The nitrogen compounds consisting of crude protein and non-protein nitrogen were 18.56% of the dry weight. Of this value 5% of uric acid was present. The total lipid fraction was relatively low (0.87%) and we are in the process of fractionating and identifying this fraction by using TLC and GLC techniques. The crude fibre fraction which contains most of the cellulose and part of the hemicellulose and lignin amounted to 24%. Lignin alone accounts for 18% and cellulose was about 6%. The soluble carbohydrate fraction containing mono, di-and tri-saccharides, oligosaccharides, polyhyroxy and acid-sugars was shown to be 36.74% of which only 6.9% consisted of D-mannitol sugar.

The pH of the sample was relatively acidic 4.6. This acidity could be due to the high organic matter present in the "fungus combs" in addition to presence of polyphenolic compounds found in the alkali-soluble material (possibly humic and fulvic acids) after extraction in 0.1% NaOH.

Table 13 shows the oxidative ability of homogenates of *Termitomyces* conidia and of minor workers of *Macrotermes subhyalinus* toward various physiological substrates including D-mannitol and uric acid which were found in "fungus combs". Simple sugars such as glucose, mannose and galactose were oxidized efficiently by termites while fructose and the disaccharide sucrose were not utilized. The same pattern prevailed for the *Termitomyces* conidia. Exception, however, was D-glucose which was not oxidized by this fungus.

Sugar alcohols such as D-mannitol and D-sorbitol were not oxidized by both fungus conidia and termites. The amino acids L-glutamate and L-proline, glycolytic intermediates (α-glucerophosphate and lactate), and TCA-intermediates (succinic and acetric acids) were utilized efficiently by termites but not by the fungus conidia with the exception of L-glutamate and L-glycecophosphate.

It is interesting to note that *Termitomyces* conidia, showed high oxidative ability to utilize low-molecular weight alcohols such as methanol and ethanol. Longer-chain or branched alcohols such as n-propanel and iso-propanol were not oxidized. Termites were not able to utilize any of these alcohols. Uric acid and glecerol were not oxidized by both species.

DISCUSSION

Very little is known about the chemical composition of "fungus gardens" of the subfamily *Macrotermitinae*. Most researches agree that they consist mainly of lignin and cellulose but detailed study on its composition is lacking. For this reason, we carried out a thorough chemical analysis of the organic constituents present in the "fungus garden" which might contribute to the knowledge of the very complex problem of termite nutrition.

The moisture content of "fungus combs" was relatively high (47.03%) compared to that of the surrounding soil. Moore (14) commented that termites, in general, are very susceptible to desiccation, because their cuticle is exceptionally soft and its water-retaining properties poor. For this reason "fungus combs," provided by their large surface area, couid contribute to the maintenance of a high humidity level in the mounds particularly in the central nest, in the vicinity of nurseries and the royal chamber. Hesse (15) considered that fungus gardens function primarily to control humidity by taking up excess moisture or by releasing water to a drying atmosphere within the mound.

Table 14: The Ash, Lignin and Cellulose Composition of "fungus combs" of Various species of *Macrotermitinae*

Species	% Ash	% Lignin	% Lignin/Cellulose ratio	Difference
Odontotermes redemanni	10.7	—	0.49	5
O. badius	27.7*	23.8*	—	8
O. obesus				
Macrotermes subhyalinus	12.84	18.01	3.04	Present Study
M. Natalensis	13.00	15.00	—	5
M. Falsciger	23.9	—	—	7
M. Goliath	10.1*	43.3*	—	8

*Average values of two samples.

And the metabolic heat of the fungi are an additional advantage because they contribute to temperature control.

The ash and lignin contents and lignin/cellulose ratios of the sub-family Macrotermitinae "fungus combs" are reported in Table 14.

As shown in Table 14 the ash content of "fungus combs" *Natalensis, M. goliath* and *O. redemanni* (10.1%) but differ *Natalensis*, M. goliath and *O. redemanni* (10.13%) but differ significantly from those reported for *M. falciger* and *O. badius*. It is possible, however, that the low ash content of the first group of termites originated from the mineral content of the plant and fungus tissues, while in the second group the high ash content could be a result of the presence of a certain amount of soil being used in constructing the "fungus combs".

The lignin/cellulose ratio present in "fungus combs" of *M. subhyalinus* was found to be considerably higher (3.0) than those of other species. Undigested woody material normally contains 20-30% lignin and 40-60% cellulose (i.e. lignin/cellulose ratio 0.33 - 0.75) (1). According to Becker and Seifert (13), these percentages are roughly reversed in the faeces of many termites and the same probably applies to the newly deposited combs of Macrotermitinae (1). Thus the high lignin/cellulose ratio in *M. subhyalinus* "fungus combs" suggests that digested plant material is used for constructing these combs. However, according to Noirot (17), the only group of termites that does not appear to use excrement for building "fungus combs" is Macrotermitinae. But according to our results it is difficult to accept this explanation because of the relatively high lignin/cellulose ratio present in "fungus combs."

The view that digested materials are used in constructing "fungus combs" of *M. subhyalinus* is further supported by the presence of a relatively high content of uric acid in the combs. Uric acid is a main end product of nitrogen metabolism in most insect species. Some insect species from Diptera, such as tsetse fly, produce over 70% uric acid in their excreta (18). Moreover, uric acid has been isolated from bodies (19) and faeces (20) of termites. We also recorded the uric acid content in major workers of *M. subhyalinus* and found an average of 440 + 40 mg uric acid per animal. Termite and fungi homogenates were unable to oxidize uric acid (Table 2) which suggests that this waste product is of little use to the metabolism of both organisms. Uric acid determinations will be carried on in future studies to find out the stage of maturity of "fungus combs" as well as the age of the various castes of *M. subhyalinus*.

Some of the valuable nutritional ingredients present in "fungus combs" was the sugar D-mannitol (6.9%). Whether this sugar is formed by *Termitomyces* as a metabolic product of fungal metabolism, or originates from plant material is not known. D-mannitol is a very common constituent of many fungi belonging to basidiomycetes and fungi Imperfecti (21). It is also found in many Spermatophyta plant families and is often present in exudates of trees. The nutritional value of D-mannitol to *Termitomyces* and termites is not certain. It is known however, that some fungi, yeast, bacteria and some few insect species can utilize D-mannitol. Nevertheless, this sugar was not oxidized by homogenates of *Termitomyces* and termites (Table 2), indicating that the oxidative step is not the factor limiting the rate in D-mannitol utilization. It is possible, however, that some initial reactions such as sugar phosphorylation take place prior to oxidation. Further metabolic studies using ^{14}C-mannitol are necessary to determine the fate of mannitol metabolism in both fungus and termite tissues. On the other hand, some preliminary results have shown that the presence of D-mannitol in the "fungus combs" could be important in allowing only monocultures of *Termitemyces* to grow while suppressing the growth of others.

It is interesting to note that D-mannitol is widely used in industry in making artificial resins (chewing gums) and plasticizers because of its water-retaining properties. Whether or not the presence of D-mannitol in "fungus combs," in such a high content, has a similar function remains to be investigated.

Acetic acid was found to be oxidized (presumably to CO_2 and water) by termite homogenates (Table 2) and not by the fungus conidia. Acetic acid is a product of cellulose fermentation which is believed to take place anaerobically within the gut of higher termites by their bacterial symbionts. The presence of acetic acid in gut contents of termites has been reported (22, 23). Hungate (23) estimated the amount of acetic acid produced by intact termites and found that the amount of oxygen necessary to oxidize the estimated amount of acetic acid agreed fairly closely with the measurements of oxygen consumption by termites. This indicates that acetates produced from the digestion of cellulose could provide an energy source for termites.

The pH of *M. subhyalinus* "fungus combs" was relatively acidic (4.6) and agrees with those reported (15) for *M. falciger* (4.3), *M. bellicosus* (4.4), *M. subhyalinus* (4.5). This acidity may be associated with the high organic matter present in these "fungus combs". The relative acidity of these "fungus combs" could be suitable for the growth of monocultures of *Termitomyces* but not other species which are not often found in "fungus gardens".

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The Permanent Secretary, Mr. S.D. Gathuini officially opening the ICIPE Kajiado Field Station in January 1976. On his left are Professor Thomas R. Odhiambo, Professor Martin Luscher and the Swiss Ambassador to Kenya Dr. Richard Pestalozzi.

TICK RESEARCH

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Resident Supervisor of ICIPE/EAVRO Joint Project on *R. appendiculatus.*

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INTRODUCTION

During the year the staff of ICIPE were saddened by the untimely death of our colleague Dr. Tawfik Hefnawy. Not only was his death a great personal loss to us all, but the work on the physiology of hard ticks was suddenly interrupted and, at the date on which this is written, has not been resumed.

Three main lines are being pursued in the research on ticks. First we are investigating certain aspects of the physiology and behaviour of hard ticks, in particular *Rhipicephalus appendiculatus*, the vector of East Coast fever. We have obtained evidence of the existence of pheromones, in this, and other ticks, that act as attractants between the sexes and that cause aggregations among unfed adults. And we have done some preliminary work on the reactions of the ticks to certain climatic factors, especially moisture. Secondly we are continuing our long-term study of the growth and persistence of natural populations of *R. appendiculatus* on large experimental plots made available to us by the Director of the East African Veterinary Research Organisation at Muguga. New plots have recently been established where various components of the ticks' environment can be manipulated, in particular its likelihood of finding food. Finally we are continuing our study of the physiology of the argasid tick *Ornithodoros moubata*, paying special attention to the role of endocrine secretions in growth and development.

A summary of the main research findings during the year follows.

PHYSIOLOGY OF HARD TICKS

1. Sex Attraction in *Rhipicephalus appendiculatus*

INTRODUCTION

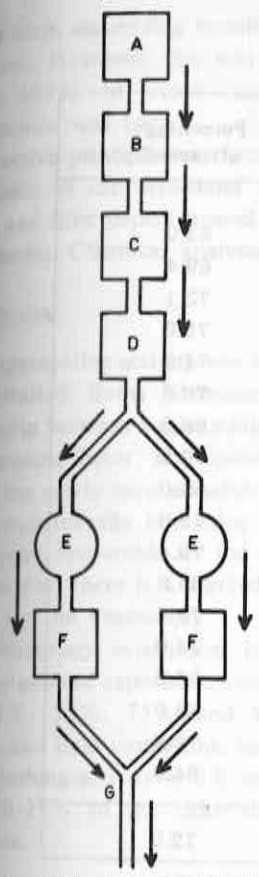
The adults of *R. appendiculatus* do not mature sexually until after feeding on the host for a few days. The males then detach and seek receptive females. At sexual maturity the females are only slightly engorged (see section 5) and can wait 2-3 weeks to mate. After mating they rapidly become fully engorged and drop off the host. Normally they mate immediately they are ready and spend in all only 7-10 days on the host.

It was shown (ICIPE Annual Report, 1974) that the females of *R. appendiculatus* after feeding on the host for six days contain phenol and *p*-cresol in nanogram quantities and that males after feeding are attracted to these substances. Apart from the fact that they were obtained by washing whole ticks in ether and so were available in the integument, their origins were known. Two likely sources, the dermal gland secretions (DGS) and the excreta of feeding ticks, were therefore examined. The report below describes attempts to repeat and confirm our initial results and to examine in greater depth the mechanism utilised in species and sex recognition.

MATERIALS AND METHODS

A Y-shaped olfactometer (Figure 1) was built and tested. It has two advantages over the T-tube used in the earlier bioassays: (a) substances of low volatility can be tested and, (b) diffusion gradients cannot develop. Each tick was tested alone and scored for which arm it chose to enter and the total time spent in it during three minutes. Every test situation was replicated 25-175 times.

Ticks were obtained from the colony at EAVRO, Muguga and had moulted 1-3 weeks previously. Sexually active males (SAMs) and partially engorged virgin females (PEVs) were prepared by feeding ticks of each sex separately on the ear of a rabbit. After the required period of feeding those that had not already detached were gently removed. Males were fed for the same length of time as the females against which they were to be tested.



Apparatus used for bioassaying sex pheromones of *Rhipicephalus Appendiculatus*.

A: Air pump; B: Charcoal filter; C: Trap; D: Humidifier; E: Flowmeter: F: Test chamber; G:Y-shaped olfactometer; arrows show the direction of air flow.

DGS was collected with a fine pipette from the droplets which appeared when the ticks were irritated by gentle pressure. The DGS was completely soluble in water and aliquots for testing were diluted with deionised water. In each case, 50 males were checked individually for their responses to air passing through the test solution compared with a water blank in the olfactometer.

The dry granular excreta of ticks feeding on rabbits were collected and stored in corked tubes at +4°C until used dry in the olfactometer.

Finally an experiment was carried out on the movement of fed adults on a normal bovine host. Seventy five males were placed on one ear and 75 females on the other in separate bags on each of two calves. After 24 h the ear bags were removed, and the ticks were reduced to 50 attached specimens on each ear. Their subsequent movements were checked by daily observation for 14 days.

RESULTS

The first bioassays in the olfactometer showed that the SAMs could not distinguish PEVs (fed for 4-15 days) from blank controls. Nor did they show significant preference for females of their own species from nymphs of *O. moubata*.

The DGS of unfed males and females were both found to be colourless and have a similar smell. The DGS of the PEVs was yellowish and smelt different. Nevertheless, when aliquots of 0.0025 – 1.15μl were tested on SAMs which had fed for 5-16 days, in a total of 14 tests there was no consistent attraction compared with a water control. However, the total results showed, a significant ($P < 0.01$) attraction of 55.0%. In addition, the detachment was investigated of SAMs attached on a host and exposed to a stream of air carrying the odour of PEV DGS. No consistent effect was seen.

The results given in Table 1 were obtained with the olfactometer, and showed that males were significantly attracted to the excreta of PEVs when both were fed 3-20 days compared with blank controls. In addition, the following other results were obtained:

(i) SAMs were significantly attrated ($P < 0.01$) to the excreta of PEVs compared with the PEVs themselves.

(ii) SAMs showed a significant preference ($P < 0.01$) for the excreta of PEVs rather than those of SAMs (their own).

(iii) SAMs were indifferent to their own excreta (no attraction or repulsion).

(iv) Unfed males showed a significant preference ($P < 0.01$) for the excreta of PEVs compared with SAMs.

(v) Unfed males were neither attracted nor repelled by the excreta of PEVs offered with a blank control.

(vi) Unfed males were repelled significantly ($P < 0.01$) by the excreta of SAMs compared with a blank control; so (iv) is really repulsion by excreta of SAMs, rather than attraction to PEV excreta.

(vii) Unfed females were significantly attracted ($P < 0.01$) to the excreta of SAMs compared with PEVs.

(viii) Unfed females were significantly repelled ($P < 0.01$) by the excreta of PEVs compared with a blank control.

(ix) Unfed females were indifferent to the excreta of SAMs; so (vii) is really repulsion by PEV excreta not attraction to SAM excreta.

In another experiment, the ticks had not all settled on the calves to feed when the ear bags were removed on day 1 and further movements occurred during the next 24 h. Ticks attaching elsewhere than on the ears were therefore removed, leaving 72 males and 44 females on the calves' ears on day 2. The results were as follows:

(i) All males detached from the ears between days 7-14 (maximum 35% on day 10); 12 females (27%) also did so.

(ii) Only 4/72 males migrated directly to the females on the other ears and copulated with them.

(iii) Another 43 males (60%) migrated to other parts of the body and attached there. There was a tendancy to keep changing places of attachment and 8 males eventually reached the ears with females and copulated with them. Three females (7%) also reattached on the neck and shoulders. They were removed to prevent any affect on male migration.

(iv) A total of 12 females (27%) therefore copulated and 19 others (43%) were still attached to the ears on day

Table 1: Attraction of fed unmated males of *Rhipicephalus appendiculatus* to the excreta of partially engorged virgin females.

No. of days of feeding	No. of males tested	Reaction +ve	Reaction −ve	P	Percentage attracted
2	50	31	19	N.S	62.0
3	160	111	49	<0.01	69.4
4	250	183	67	<0.01	73.1
5	260	195	65	<0.01	75.0
6	315	236	79	<0.01	74.9
7	325	230	95	<0.01	70.8
8	200	136	64	<0.01	68.0
9	150	106	44	<0.01	70.6
10	100	66	34	<0.01	66.0
11	175	126	49	<0.01	72.0
12	175	132	43	<0.01	75.4
13	125	86	39	<0.01	68.8
14	150	115	35	<0.01	76.7
15	100	74	26	<0.01	74.0
17	25	20	5	0.01	80.0
18	75	51	24	<0.01	68.0
19	25	18	7	0.05	72.0
21	25	16	9	N.S.	64.0
Total	2685	1932	753	<0.01	72.0

15 but had not mated.

(v) After their initial detachment from the ears 25 males and nine females disappeared, having presumably left the hosts.

(vi) By day 15 only seven males were still left on the calves; one female had died.

DISCUSSION

At the concentrations tested female DGS is not active in attracting SAMs. The possibility that DGS has a defensive role in both sexes, fed and unfed, needs to be investigated and chemical analysis may also help in assessing its biological function.

Observations on response show that SAMs are highly attracted to the excreta of PEVs but are indifferent to their own. Unfed adults of both sexes are indifferent to the excreta of feeding ticks of the opposite sex, but are repelled by the excreta produced by their own sex. This probably enables ticks searching for a feeding site to avoid others of the same sex, and thus most likely attach beside a member of the opposite sex.

The experiment with ticks on calves showed that the males feed for some time before they detach and seek the females. Only a small proportion appeared to move directly to the attached females, but considering the large proportion wandering about these could have reached their target by chance.

Under field conditions males usually outnumber females by at least two to one on the host and the adults' feeding site (the ears) is very restricted. After a few days feeding males detach and move about and then attempt to mate with any object simulating a tick, even with each other. It seems therefore that there is a high probability of a male finding a female without the need for chemical attraction. Sexual attraction if present must be very weak or active over very short distances. The function of the excreta in attracting or repelling the ticks is still open to question. The excreta might enable ticks of the same species to identify one another in mixed infestations.

2. Assembly, or Holding, Pheromone of *Rhipicephalus appendiculatus*

INTRODUCTION

Engorged nymphs excrete a fluid which dries to white deposits, as well as dry whitish excreta. Adults excrete the same two substances for one to two days after moulting. Later (six to nine days), they produce solid excreta. The influence of the various excreta on the behaviour of unfed adults was investigated.

MATERIALS AND METHODS

Aqueous extracts of all five types of excreta were bioassayed by placing a group of five adult ticks in a petri dish with two semi-circular filter papers covering the floor of the dish. The two filter papers were treated with faecal extract and water respectively. The ticks were then incubated in darkness at 28° C for 17 h before those on each piece of paper were recorded.

RESULTS

The results of the bioassay are given in Table 2. It can be seen that there was very marked aggregation, with excreta

of both sexes assembling members of the same and the opposite sex. However, this was only true during January-March 1975. In April and May the aggregation phenomenon was lost.

The active principle was found to be insoluble in ether, very stable (it can withstand heating for 30 minutes at 75° C), and filter papers treated with it remained active for two months. Chemical analysis is in progress.

DISCUSSION

The aggregating activity was lost in April, when the rainy season started. Some laboratory experiments suggested a relationship between aggregation and relative humidity. The term "holding factor" is proposed because it could function to keep the newly moulted adult at the site of the nymphal-adult moult (usually concealed on the ground) until conditions were favourable for the adult to seek a host. When the rains start there is a marked increase in adult climbing activity on the vegetation.

A preliminary experiment in which adults of *R. appendiculatus* were exposed to constant relative humidities of 0%, 33%, 55%, 75% and 95% under constant temperature and light conditions, has shown that there is little or no climbing activity at 0% and 33% RH, and generally about 20-25% of the ticks climb at the other relative humidities.

3. Stem-climbing and Aggregation in *Rhipicephalus pulchellus*

INTRODUCTION

In the field *R. pulchellus* can often be found on the tops of flowering stems of grasses in groups of two or more, whilst there are none on the majority of stems. For instance, counts of ticks on random quadrats in Nairobi National Park gave results of which those in Table 3 are typical. A single plant with 16 stems was observed in which 13 had no ticks, while three stems each had one, two and 14 ticks respectively. These figures show that *R. pulchellus* are not randomly distributed at the tops of grasses. Experiments were set up to try and determine how such aggregations come about.

MATERIALS AND METHODS

Three types of experiments were undertaken.

(a) An arena was constructed in a glass dish 34x22x6 cm with a layer of sand on the bottom in which 5x10 fine glass "stems" 40 cm tall were stood up. An electrified barrier near the rim prevented the ticks from escaping. Strips of moist paper towelling provided shelter on the surface of the sand. Thirty-four individually marked adult *R. pulchellus* were released at random into the arena, and those which settled at the tops of the stems were recorded daily. The experiment was repeated twice.

(b) Another arena was built in a circular glass dish 9.5 cm in diameter near the edge of which a single "stem" was placed. After two ticks had climbed and settled, 15 similar stems were placed in a ring around the periphery and 16 ticks added, together with a circle of damp filter paper.

(c) The original arena was used and 24 small rectangles of filter paper on the surface of the sand were substituted for the "stems"; 100 ticks were put in and the numbers under the paper counted.

Table 2: Assembly of unfed *Rhipicephalus appendiculatus* adults on filter papers treated with water extracts of nymphal and adult excreta.

Form of excreta	Ticks tested		No. of ticks assembled			Significance	% assembled on test papers
	Sex	No.	T	C	0		
Nymphal excreta							
Fluid	Males	160	132	21	7	<0.01	82.5
	Females	159	115	36	8	<0.01	72.3
Dry	Males	60	48	9	3	<0.01	80.0
	Females	60	44	14	2	<0.01	73.3
Adult excreta							
Fluid	Males	60	53	4	3	<0.01	88.3
	Females	60	40	14	6	<0.05	66.7
White dry	Males	60	44	12	4	<0.01	73.3
	Females	60	39	18	3	<0.05	65.0
Black dry	Males	60	48	10	2	<0.01	80.0
	Females	60	40	17	3	<0.05	66.7

T: Test papers; C: Control papers; O: Ticks assembled neither on T nor C.

Table 3: Counts of *Rhipicephalus pulchellus* on grass stems in Nairobi National Park on random quadrats approximately 60 cm square

Quadrat no.	No. of ticks per stem					
	0	1	2	3	4	< 4
1	13	2	—	—	1	—
2	59	2	—	1	—	—
3	23	—	—	1	—	—
Total	95	4	0	2	1	0

Index of dispersion, 4.35 $\quad x = 11.22 \quad P < 0.001$

Table 4: Distribution of 34 *Rhipicephalus pulchellus* in an area with 50 "stems", after intevalls of 8–14 days

Replicate	No. of ticks per stem								No. of ticks on ground
	0	1	2	3	4	5	6-9	10	
1	42	3	3	1	0	1	0	0	17
2	41	8	0	0	0	0	0	1	16
3	39	5	4	1	1	0	0	0	14

RESULTS

The results of the first experiment are shown in Table 4, and there is no doubt that under the conditions of this experiment the ticks tend to aggregate at the tops of stems.

The results of the second experiment showed that ticks do not necessarily climb a stem that other ticks have climbed.

In the last experiment ticks aggregated very markedly under the filter papers, for instance:

No. of ticks under a paper 0 1 2 3 4 5
Frequency 1 6 3 3 5 6

DISCUSSION

Ticks were shown to aggregate on the stems they climbed and it seemed possible that a tick left some kind of "mark" on the stem that attracted other ticks to climb it too. However, the second experiment disproved it. The last experiment showed that ticks aggregated under cover, and it seems likely, therefore, that it is this prior aggregation which results in some clumping of the ticks when they later climb the nearest stem (Browning, 1976).

4. Phenolic Compounds from Hard Ticks

INTRODUCTION

As already described (ICIPE Annual Report, 1974) up to four phenolic compounds have been identified from the ether washings of 6-day fed virgin females of six species of hard ticks, and they were found to attract the males of the species in the two species which were tested by bioassay (Wood *et al*, 1975).

Further experiments were carried out to determine the reasons for the production of phenolics. Larvae and nymphs were also examined because it was felt they might be products of cuticle formation, which occurs in the feeding process of larvae, nymphs and adult females.

MATERIALS AND METHODS

All ticks were fed for the requisite number of days, then frozen until extracted using the technique described by Wood (ICIPE Annual Report, 1974).

RESULTS

(a) No phenolic content at the level of ca. 0.05 ng/tick was detected in 21,500 fully engorged larvae of *R. appendiculatus* examined.

(b) Only phenol and *p*-cresol were detected in nymphs of *R. appendiculatus* fed for 5 days and mostly fully engorged. The two were detected after a re-examination following the finding of a phenol with m/e 138 - a methymethoxyphenol (Table 5).

(c) Batches of *R. appendiculatus* females were fed for up to 12 days without mating and a progressive increase in phenolics was noted (Table 6). None were present before feeding and none were present in males even after six days of feeding.

DISCUSSION

Small amounts of phenol and *p*-cresol were detected in the nymphs, but it should be remembered that the weights of unfed females and nymphs of *R. appendiculatus* are in the ratio of 20:1 and the other species are similar. Nymphal and larval weights are in a similar ratio and therefore phenolics, even if present in the larvae, could be below the threshold of detection.

It is still not clear if the phenolics in adult female ticks after feeding are by-products of such metabolic processes as cuticle formation or digestion and are only secondarily employed as pheromones.

Table 5. Phenolic compounds in four species of hard ticks in nanograms per tick

Species	No. of nymphs	m/e 138	Phenol	p-cresol
R. *appendiculatus*	4,800	4.3	0.10	0.10
R. *appendiculatus*	5,000	—	0.22	0.58
R. *pulchellus*	13,400	—	0.16	0.58
R. *compositus*	6,500	—	0.22	0.12
Amblyomma variegatum	2,800	—	0.45	0.52

5. Factors of Mating which Stimulate Rapid Feeding in Female *R. appendiculatus*

INTRODUCTION

The importance of mating on the rapid feeding of female *R. appendiculatus* has been indicated (ICIPE Annual Report, 1974). Females left virgin on a host do not feed to repletion and have an average weight of 15 mg even after 18 days of attachment, whereas females 1-3 days after mating complete engorgement and increase 20-fold in weight (average 347 mg). The influence of various mechanical and chemical factors of the mating act upon rapid feeding of female *R. appendiculatus* has been investigated.

MATERIALS AND METHODS

Unfed adult ticks were obtained from a colony maintained at EAVRO, Muguga. Since females have to be partially fed, and males fully fed, for successful mating to take place, each sex was separately fed on a rabbit. After 7-8 days of feeding fully fed males and partially engorged virgin (PEV) females were pulled off the host, washed in water, dried and then used for various treatments followed by reattachment to a new host.

Mechanical stimulation due to mating

(a) Fed males and PEV females were stuck on their backs on masking tape. The legs of the males were strapped down with narrow strips of masking tape. The genital openings of males and females were blocked by placing small drops of molten balsam-paraffin (1:1 ratio) on the genital opening and then spreading it evenly with a heated probe. Following this treatment, males and females continued to attempt to mate on reattachment onto new hosts. Some of the males, however, managed to remove the blocking material from their own genital openings, as well as some of those of females, and inseminated them successfully. These treated females which thus were inseminated were considered as a set of controls.

(b) In order to investigate the effect of agitation or contact among females themselves, over 100 unfed adult females were put on a host to feed and then collected, weighed and the number of days spent on the host recorded.

Mechanical stimulation due to presence of spermatophore or spermatophore-like objects

Insertion of G-25 Sephadex beads (particle size 20-80μ) into the seminal receptacles of PEV females was attempted but proved difficult since the female genital aperture is extremely small and chitinous. Sephadex beads were, however, inserted into the seminal receptacle of a very few females.

Injection with male accessory gland (AcGl) and/or testis extracts into the haemocoel of virgin females

Extracts of AçGl and/or testis were prepared by homogenizing 20 AcGl, 20 testes or 20 AcGl plus testes from fully fed unmated males in 40μl of 0.9% NaCl solution (saline). The homogenates were centrifuged for 5 minutes, twice, and the supernatants were used for injection.

Two μl extracts were injected postero-laterally into PEV females using a "Hamilton" micro-syringe with an almost blunt needle. Of the three experimental groups, the first and second groups were injected with AcGl and testis extracts, respectively, and the third group was injected with an extract of both AcGl and testis.

Table 6: Phenolic compounds from virgin *Rhipicephalus appendiculatus* females and males during feeding

Days fed	Ticks	Sex	Phenol	p-cresol
0	3000	F	*	*
2	1300	F	1.0	0.2
4	1500	F	2.1	2.7
6	1000	F	3.8	4.2
8	950	F	4.2	3.3
10	850	F	8.6	6.2
12	800	F	11.0	5.8
6	3000	M	*	*

* none detected

Four virgin control groups were (1) untreated PEV females, (2) PEV females injured by inserting the microsyringe needle the same way as for the experimental group, (3) PEV females injected with 2 Ul saline and (4) PEV females injected with 2ul distilled water. A group of injured PEV females reattached along with fed unmated males for mating was also used as a control.

For all the above experimental and control PEV females, the average weight of each group was determined before reattachment onto the host. Weights of individual females from different groups were then recorded as they dropped and presented as a mean percentage increase for the group. These females were dissected to check insemination. The average weight with blocked genitalia before re-attachment was based on two groups of 35 and eight with mean weights of 9.3 mg and 110 mg respectively. The weight increase of virgin females left on the host (Table 7) was given as a percentage of the weight of fully engorged mated females (347 mg).

RESULTS

In treatments with both mechanical and chemical stimuli where PEV females were detached and then put back on the host for mating with normal fed males, the full feeding response of 347 mg of normally fully engorged mated (FEM) females was never obtained. This may be an effect of forcibly detaching the PEV females from the host. Since blocking the genitalia or injection of PEV females was not possible while these ticks were still attached on a host, detachment of the PEV females could not be avoided.

Mechanical stimulation due to mating

For reasons not known, the weight increase of untreated PEV females on reattachment and mating with normal fed males was significantly less ($P > 0.05$) than the weight increase of females which were inseminated despite blocking the genitalia of both males and females (Table 7). Females from the latter group were considered as having received the full stimulus of mating. This group of females constituted the 100% rapid feeding response (Table 7). Hence the rest of the results were calculated on the feeding response of this group. Attempted copulation by males unable to inseminate females because of blocked genitalia of both males and females nevertheless induced 60.8% engorgement in these females (Table 7). This increase was significantly less ($P < .05$) than both the feeding response of the 100% control group and significantly more than that of the reattached untreated PEV females (19.4%). PEV females removed from a host and reattached with males on a new host for copulation, had an almost 8-fold increase in weight over 1-3 days whereas a fully engorged mated (FEM) female normally has about a 20-fold increase over the weight of a PEV female in the same time.

When overcrowding was created by placing more than 100 unfed females on one rabbit ear, most fed, and averaged 43% (149.0 ± 9.2 mg) of a FEM females feed (Table 7). This increased feeding as compared with 19.4% for normal virgin females is assumed to result from agitation in the absence of males.

Mechanical stimulation due to presence of spermatophore like objects

The four females which received G-25 Sephadex beads in their seminal receptacles failed to engorge and were not significantly different from untreated PEV females.

Table 7: Effect of various contact treatments on the weight of female *Rhipicephalus appendiculatus*

Treatment	Number	Days of attachment	Av. wt. before attachment (mg)	Av. wt. after dropping (mg ± S.E.)	X Increase ± S.E. *	Percentage of increase
Normal males and females PEV	23	1-3	20.5	160.0 ± 17.6	7.7 ± 0.86[a]	
Males and females with blocked genitalia: inseminated	19	6-12	28.6	55.6 ± 6.9	1.9 ± 0.24[b]	19.4
Males and females with blocked genitalia: not inseminated	19	2-7	67.1	291.2 ± 23.3	10.0 ± 1.65[c]	100
Virgin females left on the host	24	1-8	67-1	156.4 ± 60.2	6.1 ± 0.67[d]	60.8
	< 100	12-15	—	149.0 ± 9.2	— >	ca.43

* Differences significant at 5% level: 5 b:c, b:d. c:d.

Injection with male AcG1 and/or testis extracts into the haemocoel of virgin females

There was no significant difference in the increase in weight of females receiving AcG1 extract (28.7%), testis extract (24.4%), saline or distilled water (23.9%) over a period of 2-11 days of reattachment (Table 8). However, when AcG1 and testis extract were injected together into PEV females, not only did they detach within 4-7 days of reattachment but also increased significantly ($P < 0.01$) 48.3% in weight. AcG1 and testis extracts seem to stimulate feeding in PEV females only when received together.

Injury of PEV females seems to suppress feeding since these gained only 13.5% in weight compared to 19.4% for uninjured PEV females (Table 8). The 6.6 times increase in weight of injured females mated with normal males (Table 8) was not significantly different from the increase in weight of normal PEV females (7.7 times) mated with normal males (Table 7).

Finally, both mechanical (blocking genitalia) and chemical (injection of AcG1 and testis extracts) treatments were given to PEV females. Five out of 11 such treated PEV females were inseminated and reached an average weight of 115.4 ± 38.3 mg. The other six females which were not inseminated averaged 118.7 ± 66.1 mg. on dropping off the host. Moreover, all 11 engorged and dropped off within 1-2 days after treatment and reattachment onto the host. Normal PEV females mated with fed unmated males engorged and dropped within 1-3 days of reattachment.

DISCUSSION

Females with blocked genitalia showed an average weight increase of 60.8%, even though stimulation of the females by either the males' mouth parts or the transfer of a spermatophore and/or its contents was prevented. It was observed that when PEV females attached alone on a rabbit ear they still gained an average of 43% of the weight gain of a FEM female. The stimulus which induced feeding, though not rapid, was presumably entirely due to the constant contact and/or agitation of the females.

AcG1 or testis extracts injected into PEV females, caused no significant difference in increase of weight from that of PEV control; females injected with the same amounts of saline or distilled water. But an enhancement effect on the feeding of females injected with AcG1 plus testis extract was achieved. A similar effect was indirectly demonstrated by Purnell *et al* (1973) when newly emerged unfed adult males were irradiated with 4 Krad and allowed to mate with untreated PEV females. It may safely be assumed that these males were aspermic. The mean engorged weight of untreated females mated by irradiated males was 169.0 mg This constitutes 49% of the weight of a FEM female. No eggs laid by such females hatched. In these matings mechanical stimulation and contact between male and female, which is a prerequisite to the transfer of a spermatophore (presumably containing only seminal fluid) took place. It has already been demonstrated that mechanical stimulation of female ticks, in which sperm and spermatophore transfer have been prevented, will induce 60.8% feeding.

Table 8: Effect of various chemical treatments on the weight of female *Rhipicephalus appendiculatus*

Treatment	Number	Days of attachment	Av. wt. before re-attachment (mg)	Av. wt. after dropping (mg ± S.E.)	X Increase ± S.E. *	Percentage increase
Injured females + normal male	24	2	31.2	205.1 ± 13.30	6.67 ± 0.43[e]	65.8
Injured females	26	5-11	30.0	40.5 ± 1	1. 4 ± 0.14[f]	13.5
PEV females	19	6-12	28.6	55.6 ± 6.9	1. 9 ± 0.25[g]	19.4
AcG1 injected females	13	2-11	46.0	131.4 ± 18.0	2. 9 ± 0.39[h]	28.7
Testis injected females	17	2-11	34.3	83.6 ± 10.2	2. 4 ± 2.9[i]	24.4
0.9% NaCl injected females	17	1-9	42.6	109.0 ± 17.7	2. 6 ± 0.41[j]	26.4
Distilled H_2O injected	21	2-9	29.3	69.9 ± 10.4	2. 4 ± 0.35[k]	23.9
AcG1 and testis injected females	14	4-7	36.8	177.3 ± 21.0	4. 8 ± 0;56[l]	48.3

* Significant at 1% l—f. g. h. i. j. k.

Significant at 5% f:g

6. Water Relations and Behaviour of *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus*

INTRODUCTION

Many species of ticks, especially the adults, can starve for at least one year under suitable conditions. There is a critical equilibrium humidity (CEH) for each species, below which water is gradually lost to the atmosphere, whilst above it, moisture is absorbed by the tick from the surrounding atmosphere (Lees, 1946; Knulle, 1966). Some insects can metabolise fat reserves and use the metabolic water to replace losses via transpiration (Edney, 1966; Noble-Nesbitt, 1969).

Ticks have well developed systems for maintaining their internal water balance. It is important, therefore, to study the responses shown by ticks to humidity differences and comparison has been made between two closely related species with identical life cycles, but complementary ecological niches (Walker, 1974).

MATERIALS AND METHODS

Unfed adults, 2-3 weeks old, of both species were supplied by the EAVRO tick colony, and kept at 96% relative humidity (RH) for 3-4 days before use.

(a) Water loss and mortality

Groups of 10 females of each of the two species were weighed and then exposed in batches of 10 to RHs of 0%, 20%, 55%, 62%, 85% and 96% in gauze-covered vials. The required humidities were maintained by the use of saturated salt solutions with excess solid salt (Winston and Bates, 1960) in sealed laboratory desiccators. All experiments were carried out at approximately 23° C. Weighing was done daily on a Mettler H64 analytical balance to the nearest 0.01 mg. The number of dead ticks was counted each day.

(b) Water content

To determine water content before dehydration a group of 10 females of each species, in the same state of water balance as those used in the previous experiment, were weighed and were then completely dehydrated by incubating at 50° C until no further loss of weight occurred.

(c) Humidity preference

The aggregation behaviour of *R. appendiculatus* under different humidities was studied in a miniature arena, as described in Section 3, above. The arena was placed in a tray of water to trap any escaping ticks. The floor of the arena was covered with a layer of sand in which were embedded 25 vials, 2.5 cm in diameter, covered with nylon gauze which was flush with the surface of sand. The vials contained saturated salt solutions to provide RHs of 0%, 32%, 75%, 96% and 100%. A disc of filter paper 4 cm in diameter was placed over each vial. The ticks were randomly scattered into the arena at the start of the experiment. The vials were arranged in 5x5 array and the 5 humidities were distributed in a latin square layout. The number of ticks under the paper discs was counted twice daily. The ambient temperature was 23° C, and the ambient RH 62%.

RESULTS AND DISCUSSION

(a) Water loss and mortality

The results are shown in Figure 2. RHs of 0% and 20% showed very little difference for both curves plotted, and the same trend was observed for 55% and 62% RHs for *R. pulchellus*. For *R. appendiculatus*, at these two humidities, there was a marked difference. Despite an almost identical pattern of water loss for the two species, it is evident from the curves for *R. appendiculatus* (Fig. 2) that it loses more water to the atmosphere than *R. pulchellus* under the same conditions.

Mortality curves were drawn for each species (Fig. 2) and show that *R. appendiculatus* is more sensitive to water loss than *R. pulchellus* with a slightly higher mortality at all RHs from 0%-62%. No deaths were observed in either species at 85% and 96% RH during the period of observations. These results suggest why the two species are ecologically separated; the former prefers moist conditions, while the latter is a dry country tick.

(b) Water content

The mean water content of 10 female *R. appendiculatus* was found to be 56.1%. The corresponding figures for the males are similar. Ticks kept at 96% RH made slight gains in weight over 14 days, so that their water content rose to 57.2% and 58.0% RH respectively, showing that they could not have been fully hydrated initially. Similarly, both species made a minute loss of weight at 85% RH and then remained steady, and the CEH for both must therefore be very close to 85% at 23° C, but this should further be investigated as it may also be influenced by the age of the tick (Lees, 1946, 1964).

Desiccation at 0% and 20% RH caused weight losses of 62% in both species by day 14. When the ticks lost water during exposure to humidities below the CEH, compensatory production of water by fat metabolism may have occurred, which would therefore have caused a reduction in dry weight and thus influenced a direct attempt to measure water content.

(c) Humidity preference

This experiment was only carried out for female *R. appendiculatus* and the analysis of the first set of observations is presented here (Table 9). Humidity itself was the only significant factor, ($P < 0.01$), and the ticks showed a significant preference for RHs of 96%-100% compared with 0% and 32% RH. Furthermore, there was significant difference in preference between 75% and 100%.

Work on *Ixodes ricinus* (Lees, 1946) and *Hyalomma asiaticum* (Balashov, 1960) has shown that the response is determined by the state of the water balance of the tick. However, in testing the response of several tick species to different humidities Sonenshine (1963) found only one

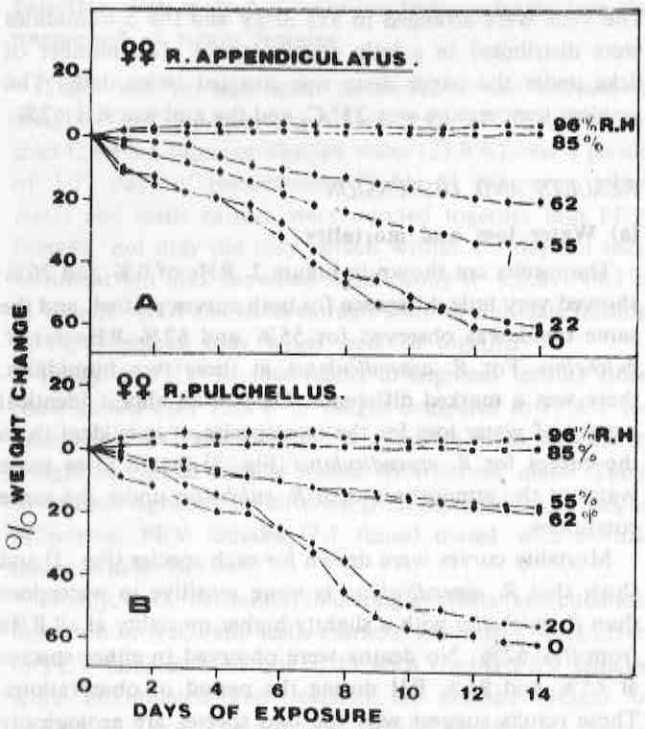


Fig. 2A & B Wt. changes of females of *R. appendiculatus* and *R. puchellus* after exposer to 6 different relative humidities (R.H.). There was no mortality at 85% R.H. and 96% R.H. The bars indicate 1 S.D.

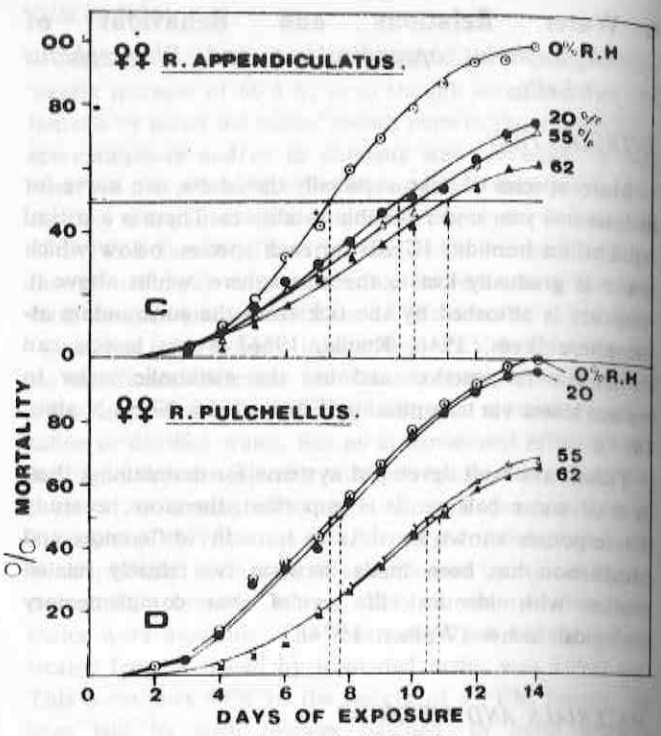


Fig. 2C & D Mortality curves for *R. appendiculatus* and *R. pulchellus* during exposure to different humidities at 23° C.

Table 9: Preferences shown by female *Rhipicephalus appendiculatus* for five relative humidities, offered in a latin square design; initial observations; 100 ticks (68 under filter pages, 26 on the arena periphery, 4 in the water trap)

% R.H.	Columns I	II	III	IV	V	Treatment total	Treatment mean	Row total
0	1	0	4	1	1	7	1.40	15
32	1	1	1	1	1	5	1.00	9
75	3	2	3	2	2	12	2.40*	14
96	6	3	7	3	2	21	4.20*	15
100	8	3	5	2	5	23	4.60*	15
Total	19	9	20	9	11	68	13.60	68

* Least significant difference of 5% devel of significance = ± 2.05

Summary table of analysis of variance

species that was sensitive to the different RHs offered.

It should be noted that in each case the ticks were exposed to a gradient of relative humidity under the filter papers ranging from the indicated humidity over the centre of the vial of salt solution to the ambient humidity (62%) at the edge.

ECOLOGY OF HARD TICKS

The ecological studies have centred on the tick-proof paddock on the estate of the East African Veterinary Research Organisation (EAVRO) at Muguga, into which was introduced in October, 1973, a population of *Rhipicephalus appendiculatus* infected with the causative organism *(Theileria parva)* of East Coast fever (ECF). A laboratory, tick-rearing rooms and pens for cattle have been built at Muguga and used by the staff of the joint ICIPE-EAVRO project since October, 1974.

The tick colony of the Division of Protozoology, EAVRO, has continued to provide all the hard ticks used in the physiological studies, as well as many of the rabbits for feeding hard and soft ticks at ICIPE.

1. Studies of Experimental *R. appendiculatus* Populations and ECF Transmission.

INTRODUCTION

The initial seeding of ticks and ECF into a paddock of 2 ha at Muguga, and the development of the tick population during the next 12 months, has been described (ICIPE Annual Report, 1974) and it was shown that a strong disease challenge was produced (Purnell *et al*, 1975) and since then the opportunity has therefore been taken to administer closely monitored challenges to groups of immunised cattle (e.g. Radley *et al.* 1975). Studies continued on the dynamics of the tick population, the role of other hosts for the ticks, and the duration of development on the ground. Observations on the tick population in an adjacent paddock of 2 ha, not stocked with cattle and not artificially infested with ticks, continued in parallel, whilst observations were also made on a paddock under the control of the Division of Protozoology, EAVRO, seeded with *R. appendiculatus*, and stocked with two African buffalo (*Syncerus caffer*), and cattle in experiments.

MATERIALS AND METHODS

Groups of cattle susceptible to ECF and others immune to the disease were grazed in the main paddock as required. Ticks were counted *in situ* on the ears during days 11-15 of exposure. The mean of these counts was used to give an index of adult tick abundance. With certain very heavy infestations the ears were cut off the cattle immediately after death and then treated with 20% aqueous potassium hydroxide solution to dislodge the ticks for separation and counting.

Regular live-trapping around the paddocks for carnivores and rodents continued to provide specimens to examine for ticks but in February 1975 kill-trapping of the rodents began.

Samples of ticks, mainly larvae and nymphs, were obtained thrice weekly by dragging a cloth over the vegetation (Milne, 1943) using one plot in each paddock drawn at random. In the buffalo paddock a sample was obtained every 1-2 months while the animals were being examined under anaesthesia and the same three representative plots were checked each time.

Batches of engorged larvae (4x50), nymphs (4x50) and females (1x10) were exposed every month in gauze - covered vials put out in grass at the edge of the tick-infested paddock in an open and in a tree-shaded, but well vegetated, site accompanied by a maximum and minimum thermometer, read daily. The females were checked daily for oviposition, whilst larval and nymphal moulting and egg hatching were checked weekly.

The ICIPE staff were also responsible for all cattle during their time in the paddock and for the collection of all blood samples, blood smears and lymph node biopsies required by collaborators studying the ECF situation.

In March, 1975, the tick-infested paddock was divided into two parts for an experiment on the effect on tick infectivity of grazing immune cattle permanently in the ECF-infected paddock. The smaller part is to be used only by susceptible cattle to confirm the continued passage of ECF.

RESULTS AND DISCUSSION

Four immune cattle survived a heavy tick and disease challenge during 60 days in October - December, 1974, whilst six susceptible cattle in two successive groups all died of ECF in an average of 16.3 days.

The increase in the population of ticks described in the first year has continued, as shown in Figure 3 by counts of adults on the ears and by counts of larvae and nymphs collected from the vegetation. By December, 1974, the adults were so numerous on the ears that it was difficult to count them and no more cattle were exposed until the long-term experiment on immunity was started in April, 1975. In both 1974 and 1975 there was a marked increase in the number of adults on the cattle in April coincident with the start of the rainy season, and in 1975 so many ticks were picked up that seven out of eight cattle receiving unlimited tick challenge died with symptoms of acute tick toxicosis within 8-14 days. These animals had 7,700-14,500 adult ticks on the ears alone at death, approximately 10 times the level found in heavy field infestations. A repeat of this experiment was postponed until November, 1975. Meanwhile the paddocks were used four more times for observations on tick pick-up, ECF challenge and a deliberate attempt in June to repeat the tick toxicosis for closer study. The latter was unsuccessful as the cattle picked up fewer ticks than in April.

Larvae have shown four well-defined peaks of numbers following peaks of adult feeding activity. Nymphal numbers were building up rapidly in late 1974. However, they decreased gradually during 1975, since there was no apparent seasonal effect on activity and the opportunities in 1975 for larval feeding to produce further nymphs were limited.

The larval and nymphal collections from the paddock containing buffalo are from a tick population established in 1972. During the period of sampling (December 1974 - September 1975) larvae and nymphs gradually increased, both roughly doubling in number, with larvae about ten times as numerous as nymphs. There was no clear relationship bet-

ween the changes in numbers in this paddock and in that described above, which reinforces the assumption that under the favourable climate and habitat at Muguga, the life cycle of *R. appendiculatus* is not greatly influenced by the weather and variations in the number of ticks observed are caused mainly by such factors as host availability.

The ticks collected from various non-bovine hosts are listed in Table 10 which shows that despite the abundance of *R. appendiculatus* in the area, the involvement of these host species in the life cycle of the tick was slight, although the presence of nymphs could be important in the dispersal of ECF if they found such hosts unacceptable, dropped to the vegetation again without feeding, and were subsequently picked up by cattle outside the paddock. In fact the monitoring of the adjacent unstocked paddock produced no *R. appendiculatus* throughout the period under review, although a small number of *Haemaphysalis leachi* adult was collected.

Table 10: Ticks collected from wild animals captured at Muguga, October 1974–September 1975

Species	No. caught	No. infested	*R. appendiculatus*			*Haemaphysalis sp.*			*Ixodes sp.*		
			A	N	L	A	N	L	A	N	L
White-tailed mongoose *(Ichneumia albicauda)*	9	8	0*	36	120	5	4	1	3	7	7
Large-spotted genet *(Genetta tigrina)*	2	2	0	0	0	30	1	2	0	3	18
Zorilla *(Ictonyx striatus)*	2	2	0	27	0	0	0	2	0	0	0
Domestic cat *(Felis cattus)*	1	1	0	0	0	37	0	0	0	4	0
Groove-toothed rat *(Otomys irroratus)*	29	2	0	2	2	0	3	0	0	0	1
Four-striped grass mouse *(Rhabodomys pumilio)*	61	11	0	0	1**	0	14	11	0	0	0
Unstriped grass mouse *(Arvicanthis bhyssinicus)*	1	0	0	0	0	0	0	0	0	0	0
Mouse *(Mus sp.)*	1	0	0	0	0	0	0	0	0	0	0

A = adults, N = nymphs, L = iarvae; *plus 1 adult *Rhipicephalus hurti*, **plus 3 larvae *R. hurti*

Table 11: Duration of development of *R. appendiculatus* on the ground in an open and in a shaded site at Muguga

		July 1974	Aug. 1974	Sep. 1974	Oct. 1974	Nov. 1974	Dec. 1974	Jan. 1975	Feb. 1975	Mar. 1975	Apr. 1975	May 1975	June 1975	July 1975	Aug. 1975
Mean daily maximum °C	open	—	—	—	—	—	—	—	31.3	34.5	34.8	27.1	22.3	22.5	23.9
	shaded	—	—	—	—	—	—	—	27.3	29.8	30.1	22.9	19.9	19.6	18.3
Mean daily minimum °C,	open	—	—	—	—	—	—	—	15.5	13.9	12.3	11.9	12.4	11.2	10.6
	shaded	—	—	—	—	—	—	—	12.7	11.9	10.8	10.8	11.4	10.8	10.6
Pre-oviposition (days), 10 females	open	25.6	19.0	15.9	19.9	9.5	9.3	6.0	5.6	8.6	14.7	15.2	16.5	21.4	15.2
	shaded	—	23.0	21.9	19.4	19.4	15.2	6.3	5.7	9.6	15.3	20.2	25.3	21.6	18.7
Incubation (weeks), 10 egg-batches	open	9.2	6.8	6.0	10.4	11.6	10.4	9.4	12.1	10.5	11.4	—	16.4	9.6	8.5
	shaded	—	7.2	10.4	11.0	10.0	10.3	10.0	12.3	12.0	12.4	—	—	13.7	—
Larval-nymphal moult, (weeks), 200 larvae	open	7.8	4.8	4.9	—	6.6	3.5	2.9	2.2	*	—	4.4	—	—	5.7
		*	5.2	3.9	—	2.9	3.5	1.7	2.1	5.9	—	4.7	—	—	7.3
Nymphal-adult moult, (weeks), 200 nymphs	open	7.1	6.8	6.7	3.9	2.4	3.7	3.0	2.9	5.7	4.2	4.9	10.4	9.6	6.8
	shaded	6.7	5.3	6.4	3.7	2.6	4.6	3.4	3.6	6.0	4.5	6.1	13.7	9.5	8.7

* all died before completing development.

The results of the development studies are given in Table 11. For the period for which temperature data were available the shaded site was consistently cooler. The duration of the pre-oviposition period showed marked seasonality that can be inversely related to environmental temperature (Branagan, 1973). Oviposition occurred most quickly in January-March (hot season) with a mean of 7.7 days compared with 21.2 days in the cold season (June-August). Oviposition also occurred sooner in the open site in eight of the observations, not significantly so in five, and in one case the females in the shaded site died before ovipositing.

The rate of development of the eggs has so far shown little variation either with site or season, and has averaged 10.5 weeks from oviposition to hatching. The duration of larval-nymphal moult has also shown no clear pattern of variation. The duration of the nymphal-adult moult has shown seasonal variation, being shortest in the warmer part of the year from October to February.

2. Activity and Behaviour of *R. appendiculatus*

Use has been made of the dense tick population available in the tick infested paddock to carry out a number of experiments.

(i) Efficiency of Sampling from the Vegetation

The number of larvae and nymphs collected was compared by covering the same areas by blanket dragging and by beating the vegetation with an entomological sweep net. There was no difference for larvae, but a small (up to x 2) and irregular gain in nymphs by using the net. All subsequent sampling has therefore been done by blanket dragging as this is less fatiguing and easier to standardise.

A series of comparisons was made as shown in Table 12. Standard sampling plots (see section 1) were used. Where replicates were used, one of each was sampled in long, medium and short grass. The final experiment with 22 collections in one day was made on the same plot as that used for four collections per day, after an interval of five days, in a vain attempt to collect to depletion.

The once-daily collecting showed a fall in larval catches to one-third and one-sixth respectively, whilst the only other definite depletion was in the final experiment where the mean counts in the last four samples compared with the first four showed a halving of the nymphal count and a two-thirds reduction in the larval count. It can be concluded that a single sample may collect 1-2% of the larvae and nymphs present. Nevertheless the results obtained in the tick infested paddock over a two year period have given a coherent picture of population changes. It seems also that when sampling is done more frequently than once per day the technique itself stimulates the ticks and makes more available for capture.

Table 12: Comparisons of frequency of sampling nymphal and larval ticks from the vegetation by blanket dragging and the yield obtained at the last sample, compared with the first

Frequency	No. of days in succession.	Replicates	Result	
			NN depleted	LL depleted.
1/week	5*	3	No	No
1/day	10	3	No	Yes
1/day	7	3	Yes	Yes
5/day	1	3	No	No
4/day	7	1	No	No
13/day	2	1	No	No
22/day	1	1	Yes	Yes

* 5 weeks in this case.

(ii) The Effect of Rainfall on Activity

It was known already from other field observations that rainfall after a dry spell causes a marked and rapid increase in the number of adult *R. appendiculatus* on grazing cattle.

At the end of the dry season in March, 1975, a latin square layout of 5 x 5 1 m^2 quadrats was made in the tick-infested paddock at Muguga. Water was applied from a watering can to give the equivalent of single falls of 10, 20 and 40 mm of rain and 10 mm on 4 successive days, with five unwatered controls. There was also a total of 14mm of rain on the night the water was applied and the next one. Eleven days later the 40mm applications were repeated and 40mm was applied to the plots which previously received 10 mm. Adults were collected by hand off the grass each day during a standard 3-minute search; larvae and nymphs by drawing a piece of cloth over the tips of the grass.

The results for adults are shown in Figure 4. The initial water plus rain undoubtedly produced an immediate increase in the number of ticks on the top of the grass, but an analysis of variance showed no significant difference between treatments, presumably due to the effect of the rain, so the results are presented together. Detailed examination of results from the later additions of water showed no discernible effect, indicating that the potential for rapid response to rain must build up in dry conditions, but once released does not soon reappear.

Collections of larvae and nymphs were started one day before the first application of water, and these were the highest numbers obtained. In both cases the daily collections thereafter declined, and had become almost zero by mid-April, when there was heavy rain. There was no response either to the water or the rainfall.

These cumulative collections showed that the mean density of ticks in March-April 1975 was at least 64 adults, 24 nymphs and 990 larvae per m^2.

The collections from the plots were continued during April-May from the time when the cattle which subsequently died of tick toxicosis (see above) were first introduced, and went on in the presence of the sole survivor. There was a very large increase in the number of ticks collected within a few days of the introduction of the experimental cattle, and it occured before the heavy rains started. It was then observed that the majority of the males, at least, were recognisably partly fed, and also that the cattle were attracted to the experimental plots and were rubbing their heads on the marker stakes, to alleviate the irritation of the ticks. Normally it is impossible to tell whether partly fed ticks are on the host for the first time or not. The results show that ticks which are disloged are able to repeat their host-finding behaviour and re-ascend the grass. Such a possibility is not normally considered, but it could have an important effect on disease transmission.

Fig. 4: Total number of the ticks collected daily by hand from quadrats of 1 M_2. Rainfall is also shown.

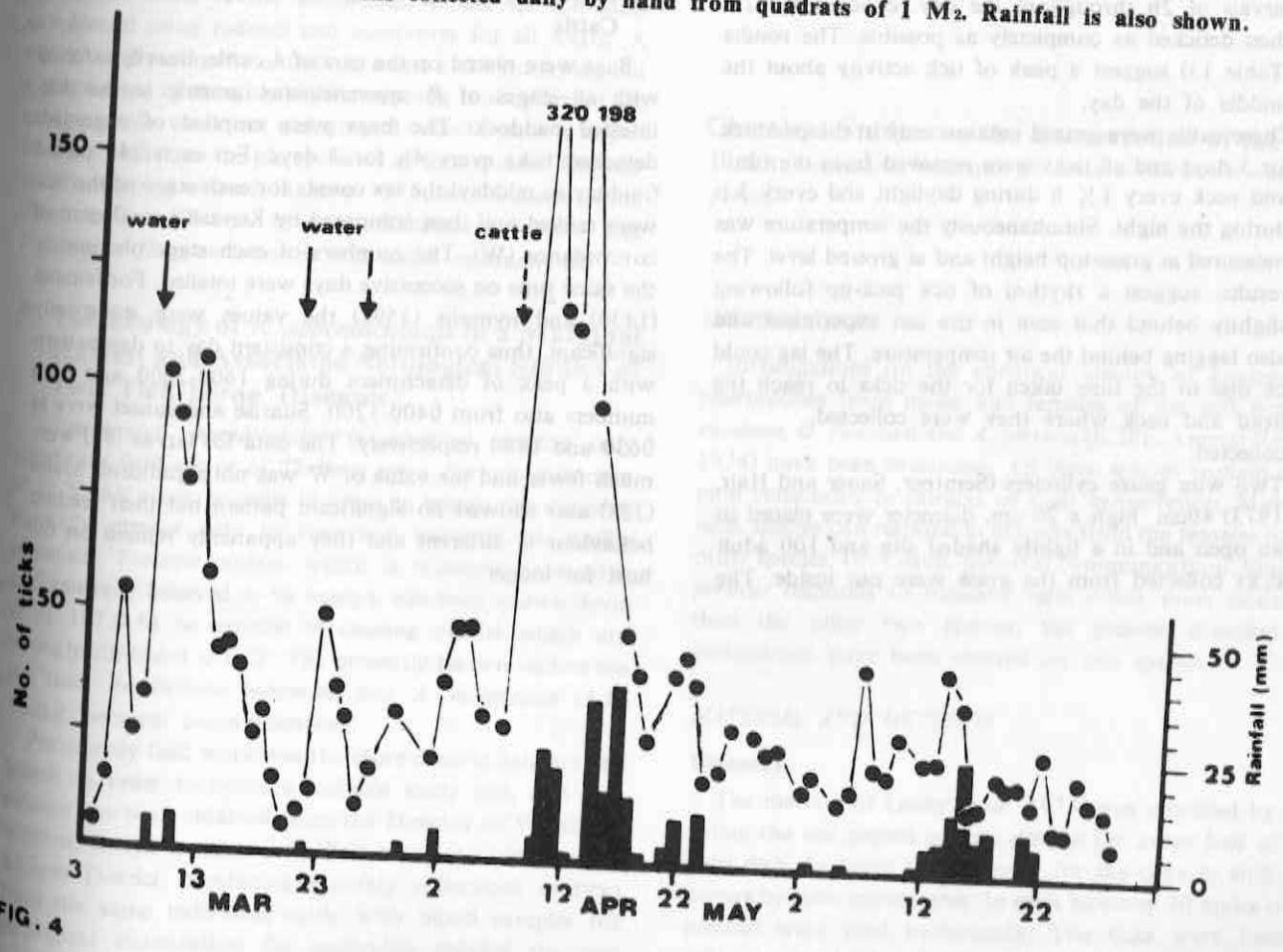


FIG. 4

Table 13: Ticks collected from cattle exposed whilst grazing for two hours at different times of day

Time of exposure	Mean no. of ticks/animal/hour
0815 — 1000	104.5
1000 — 1200	194.5
1200 — 1400	345.0
1400 — 1600	202.0
1600 — 1800	168.5

(iii) Circadian Rhythm of Activity

A series of observations and experiments was made in the tick infested paddock at Muguga.

(a) Counts and collections of adult ticks from 1 m^2 quadrats at 0800 and 1100 showed that the net increase in ticks on the grass 0800-1100 was at a rate of 6 times that over the remaining 21 hours, i.e. most of the ticks that appeared in the morning were later returning to the ground.

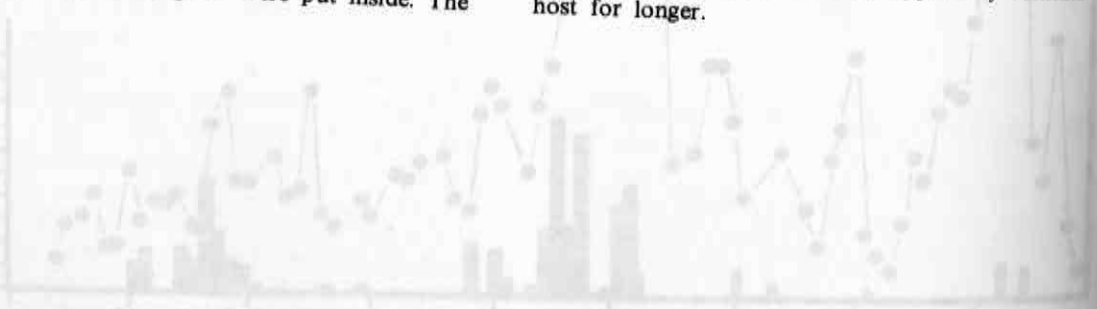
(b) Pairs of cattle were introduced to the paddock at intervals of 2h throughout the day, exposed for 2h, then deticked as completely as possible. The results (Table 13) suggest a peak of tick activity about the middle of the day.

(c) Three cattle were grazed continuously in the paddock for 3 days and all ticks were removed from the head and neck every 1 ½ h during daylight and every 3 h during the night. Simultaneously the temperature was measured at grass-top height and at ground level. The results, suggest a rhythm of tick pick-up following slightly behind that seen in the last experiment and also lagging behind the air temperature. The lag could be due to the time taken for the ticks to reach the head and neck where they were collected.
collected.

(d) Two wire gauze cylinders (Semtner, Sauer and Hair, 1973) 40cm high x 20 cm. diameter were placed in an open and in a lightly shaded site and 100 adult ticks collected from the grass were put inside. The ticks visible on the grass and the sides and lids of the cylinders were counted at intervals of 2h during the day and 4h at night. The results again indicated a circadian rhythm of activity that was related to the ambient temperature. In this experiment and the previous one, relative humidity was measured continuously in the grass and remained above 90%, which is higher than the equilibrum humidity value indicated in Figure 2.

(iv) Periodicity of Engorged Ticks dropping from Grazing Cattle

Bags were placed on the ears of 4 cattle, heavily infested with all stages of *R. appendiculatus*, grazing in the tick-infested paddock. The bags were emptied of engorged, detached ticks every 4h for 7 days. For each 24h period (midday to midday) the six counts for each stage of the tick were ranked and then compared by Kendall's coefficient of concordance (W). The numbers of each stage obtained at the same time on successive days were totalled. For females (1430) and nymphs (3591) the values were statistically significant, thus confirming a consistent day to day pattern with a peak of detachment during 1600-2000 and high numbers also from 0400-1200. Sunrise and sunset were at 0630 and 1830 respectively. The data for larvae (91) were much fewer and the value of W was not significant. Males (328) also showed no significant pattern but their feeding behaviour is different and they apparently remain on the host for longer.



3. Reproduction and Survival of *R. appendiculatus* at Different Host Stocking Densities

Permission has been obtained from the Director of Veterinary Services, Kenya, and land made available at Muguga, for a long-term study of *R. appendiculatus* at different stocking densities. Double-fenced enclosures are being constructed which will be seeded with ticks at a density equivalent to that used for the existing paddock, but the areas are adjusted so that with one cow in each the effective stocking will be in the ratio of 1 animal: 0.1, 0.4, 1.2 ha respectively, plus unstocked plots with and without ticks.

Preliminary exposure of 2 ECF-susceptible cattle for 68 days confirmed that the area is free of this disease, and is carrying only a very sparse, non-breeding population of stray individuals of *R. appendiculatus*. The principal tick species at present are *Rhipicephalus hurti* and *Haemaphysalis leachi* (see below).

4. Comparison of the Life Cycles of Four Species of Ticks Occurring Together at Muguga

On the land available to the project at Muguga four species of ticks commonly occur: *R. appendiculatus* which depends mainly on cattle as the host for all three stages of the life cycle; *R. hurti* which uses rodents as the larval and nymphal host, and cattle mainly for the adults; *Haemaphysalis leachi* which uses rodents for the immatures and various wild and domestic carnivores as host for the adults and some of the immatures; *Ixodes* sp. (probably *nairobiensis*) using rodents and carnivores for all stages. A variety of experimental situations exists and by carrying out a programme of monthly rodent tarpping to supplement the existing cattle, carnivore and ground collections, it is hoped to gain insight into the life cycles of all four species. Regular rodent trapping started in June 1975, and material from three species (*Otomys irroratus*, *Rhabdomys pumilio* and *Lophuromys flavopunctatus*) is still under examination.

5. The Ecology of *R. appendiculatus* in a Marginal Habitat and Associated Serological Studies of Two Tick-borne Diseases

Rhipicephalus appendiculatus is accepted as the only significant field vector of *Theileria parva*. Serious epizootics of ECF can occur in cattle in areas in which the vector is normally present only in very low numbers. A similar organism, *Theileria mutans*, which is widespread in cattle and generally believed to be benign, has been shown (Irvin *et al.* 1972) to be capable of causing deaths which are wrongly attributed to ECF. The presently known vectors are the ticks *Amblyomma cohaerens* and *A. variegatum* (A.S. Young, personal communication).

Preliminary field work was therefore done in January and March in order to locate a suitable study site, and permission has been obtained from the Director of Veterinary Services, Kenya to carry out a field study at Lolngoswa in Kajiado District, combining monthly collections of ticks from the same individual cattle with blood samples for serological examination for antibodies against the two *Theileria species*. Thin blood smears are also made. The site chosen is ecologically marginal for R. *appendiculatus*, but favoured by R. *pulchelus* and A. *gemma*.

The preliminary work indicated that many animals in the area carried antibodies against *T. mutans* and a few against *T. parva*. There is also recent veterinary evidence of ECF. Field work started in July 1975 and data are being obtained on the life cycles of both *R. appendiculatus* and its virtual replacement species *R. pulchellus*. The latter is little known and makes a valuable comparison. Regular collections of immature ticks are also being made from the vegetation at seven sites in the area. Blood samples for serological examination and blood slides are being collected as planned.

6. Population Study of *R. appendiculatus* on the Kenyan Coast

Weekly collections of ticks have been obtained from 5 marked cattle continuously since March 1973 at Ukunda on the Kenyan Coast, as well as daily rainfall records. Temperature and humidity records were collected on the site by thermohygrograph throughout the first year. It is hoped to continue the observations for a total of 3 years. The cycle of *R. appendiculatus*, the principle species, is in marked contrast to that observed at Muguga, with generally low numbers and marked seasonality. The species appears to be severely restricted by a long period of the year with little or no rain and high temperatures, even though the relative humidity is high for much of the time.

Chemical Studies on the Exosecretion of the Soft Ticks *Ornithodoros moubata, Ornithodoros tholozani and Argas persicus*

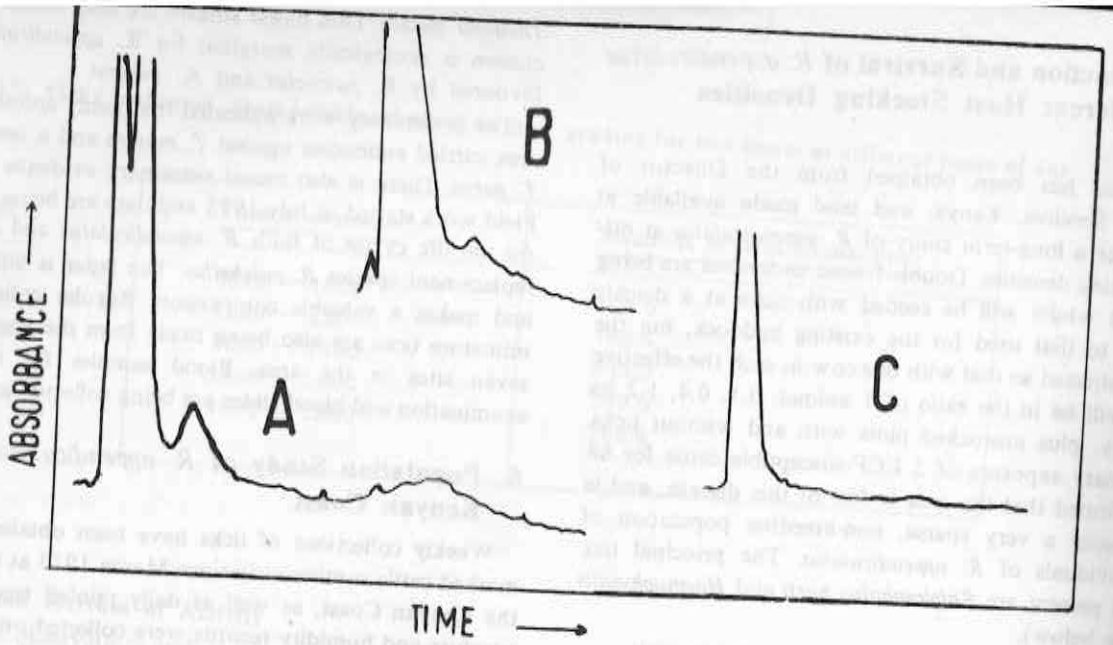
INTRODUCTION

Investigations on the chemical identity of aggregation pheromones from males and females of the soft ticks *O. moubata*, *O. tholozani* and *A. persicus* (ICIPE Annual Report, 1974) have been continued. All three species contain common substances in extracts of their exosecretion. Males of each species can respond to extracts from the females of the other species. (R. Galun, personal communication). Since *A. persicus* responds to bioassay tests much more positively than the other two species, the present chemical investigations have been centred on this species.

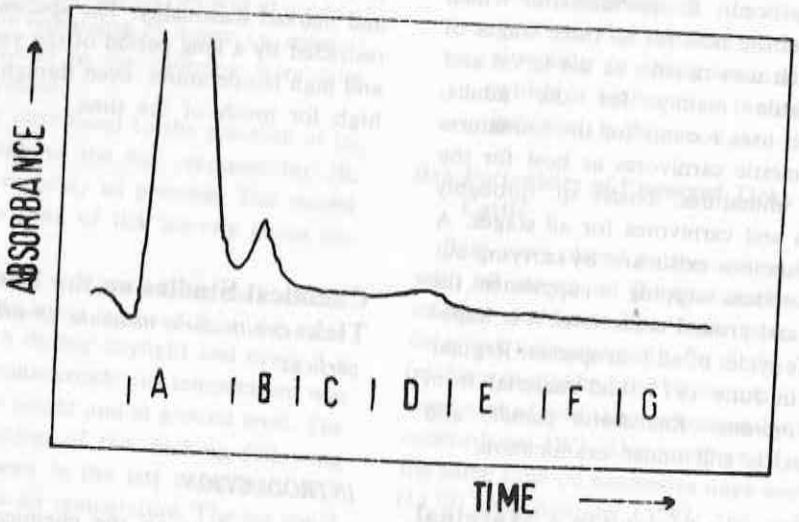
MATERIAL AND METHODS

Bioassay

The method of Leahy *et al.* (1973) was modified by impaling the test papers on two pins in the lower half of the petri dish, to avoid the tendency for the ticks to shift the papers by their movements. In each bioassay 30 males of *A. persicus* were used individually. The ticks were from a colony maintained in Professor Galun's laboratory in Israel.



5A: High prossure liquid chromotography separation of the aqueous tick washings, using an ultra-violet detector, A = *Ornithodoros moubata*, B = *O. tholozani* C = *Argas persicus.*



5B: HPLC fractionate of *A. Persicus* extracts into different zones for bioassay.

Batches of 100 ticks were soaked in 5.0ml of distilled water, and aliquots of 0.2ml of this liquid were applied to semi-circular filter papers 5.5cm diameter, which were then air-dried at room temperature. Single ticks were tested for preference for these papers against blank controls, by holding them at 23° C for 12 h then examining. Results in which the ticks showed a statistically significant preference ($P < 0.01$) were regarded as showing activity.

Chemical methods

Tick extracts were clarified by centrifugation and were concentrated *in vacuo* at 40° C before being injected in the high pressure liquid chromatograph (HPLC). Columns for HPLC separation were packed with Aquapack-A440 (Water Associates) and water was used for elution throughout. The HPLC was equiped with a u.v. detector and both u.v. absorbing fractions were collected and bioassayed.

Preliminary chemical functional group analysis was done on tick washings and the reactions monitored in the HPLC. The presence of the vicinal hydroxyl group was tested using periodate (Sweeley, 1959; Bishop and Cooper, 1960). Sodium borohydride, lithium borohydride and 2,4 - dinitrophenylhdrazine were used to test for the presence of aldehydes and ketones. Permanganate-periodate mixture was used to test for the presence of unsaturation (Lemieux and von Rudloff, 1955). Acetylations were achieved with acetic anhydride-pyridine at room temperature.

RESULTS AND DISCUSSION

Separation by HPLC shows that all three species give ex-

tracts of similar chemical content (Fig. 5a). Washings from female *A. persicus* when tested against males were found to be active. However, on HPLC fractionation (Figure 6b) only those fractions corresponding to part A were found to be weakly active. When eluants from the HPLC were pooled the aggregation was still only weak – 21/30 ticks attracted ($P = 0.05$). It is probable that the column retains the vital ingredient, and the use of another HPLC absorbent is therefore necessary.

Preliminary chemical functional group analysis indicated the absence of d, β - unsaturated aldehydes, simple ketones and vicinal glycols. Potassium permanganate eliminated parts of the area of peak A in the HPLC (Figure 5b) that correspond to weakly active fractions. The non-volatile nature and the u.v. activity of these weakly active fractions suggest that they are properly constituents of hydroxylated aromatic compounds. However, it is important first to isolate the most active fraction before a full chemical investigation is carried out.

PHYSIOLOGY OF SOFT TICKS

1. Comparison of Reproduction and Development in *Ornithodoros moubata* on Different Hosts.

INTRODUCTION

In the field, *Ornithodoros moubata* has been reported to feed on aardvarks, warthogs and porcupine, (Heisch, 1954). Walton (1957, 1958a, 1958b) studied the habitats of *O. moubata* and distinguished four forms namely, the "warthog form", the "man eating form," the "chicken eating form" and the "mixed feeding form". Because *O. moubata* seems to be so complex, several laboratory hosts were tested and their suitability established before a laboratory colony could be reared. This work which began in 1973 (ICIPE Annual Report, 1973) has been completed.

Galun's and Kindler's feeding method (1968) was modified by the adoption of artificial bat wing membrane instead of the calf gut membrane because ticks feed more readily through it. This facilitated studies on the effects of blood of various hosts on reproduction and development of the tick to be carried out without the use of live hosts.

MATERIALS AND METHODS:

Laboratory bred hungry, virgin females were weighed and fed on adult and newly born rabbit, chicken, guinea pig and rat. The ticks were weighed again immediately after feeding and mated. Observations on the fed body weight, size of bloodmeal taken, number of eggs deposited, preoviposition and egg incubation periods, number of nymphs that hatched and the resulting percentage hatchability were recorded. In another experiment, using the same parameters reproduction of ticks fed on the four hosts was compared with ticks collected from the field when fully engorged on an unknown natural host. The object was to find out whether there is a correlation between the size of bloodmeal and egg output and the comparative effects on reproductive capacity of the natural host and the laboratory host.

The effects of different hosts on the rate of development was also investigated by feeding laboratory bred nymphs on rabbit, chicken, guinea pig and rat. The rate of development was assessed by recording the number of nymphs that moulted, the period required for moulting and the percentage of the resulting adults. The ticks were kept in a room at a constant temperature of 28° C and 84% R.H.

2. Comparison of Two Types of Artificial Membrance Feeding on Reproduction and Development of *O. Moubata*

Two large abattoirs for pigs and cows are situated near the ICIPE and so porcine and bovine blood can be cheaply obtained for the mass rearing of ticks. The possibility of breeding ticks without having to feed and maintain them on live hosts was therefore examined. First the bat's wing membrane (BWM) and calf gut membrane (baudruche) feeding techniques were compared for O. *moubata*. The BWM technique proved superior and was therefore chosen to test the effects of bovine, porcine and rabbit blood on the reproduction and development of *O. moubata*. The bat's

Table 14: Effects of different types of laboratory hosts on reproduction of *Ornithodoros moubata* fed in vivo.

Mean values	Rabbit	Chicken	Guinea Pig	Rat	Newly born Rabbit
Unfed weight (mg)	67.4	66.2	67.2	71.7	74.0
Fed weight (mg)	249.4	245.9	248.3	203.5	266.0
Weight of blood meal (mg)	183.8	173.2	180.5	128.5	192.0
Number of eggs laid	226.3	191.6	119.2	117.7	194.5
Preoviposition period (days	10.3	11.6	12.8	14.4	10.7
Incubation period (days)	16.3	14.6	14.5	13.9	12.9
No. of nymphs produced	176.4	160.4	92.9	101.3	164.0
Hatchability (%)	78.7	83.4	78.9	80.5	90.2

*20 ticks were fed on new born rabbit whereas 60 were fed on the other four hosts.

wing membrane also lasts longer with regular use (up to 6 months).

As Table 14 shows, ticks fed on adult and newly born rabbit, chicken and guinea pig took comparable sizes of bloodmeal and the mean number of eggs deposited by ticks fed on both adult and newly born rabbit and chicken were high while those deposited by ticks fed on guinea pig and rat were fewer. The mean number of nymphs resulting from adult and newly born rabbit and chicken fed ticks were higher compared to those produced by females fed on either rat or guinea pig. The percentage hatchability did not differ much in all cases except in case of ticks fed on newly born rabbit.

The results (Table 15) show the nutritional superiority of the natural host over the laboratory hosts on reproduction of *O. moubata.*

Ticks fed on chicken had adults emerging after the 3rd nymphal stage, while for those fed on rabbit, rat and guinea pig adults emerged after the 4th nymphal instar. At the 4th moult, 49.3%, 76%, 12.7% and 34% emerged on rabbit, chicken, rat and guinea pig respectively.

Table 15: Effects of blood of unknown natural host, rabbit, chicken, guinea pig and rat on reproduction of *O. moubata* in vivo.

	Unknown Natural host*	Rabbit	Chicken	Guinea Pig	Rat
Mean weight of 50 unfed ticks (mg)	—	72.4	70.2	64.9	54.8
Mean weight of 40 fed ticks (mg)	492.0	354.5	239.2	202.2	201.7
Mean size of bloodmeal ingested (mg)		282.3	176.9	95.2	147.1
Mean number of eggs deposited	517.0	271.4	171.0	59.6	96.7
Mean preoviposition period (days)	—	10.0	11.0	12.0	14.0
Mean incubation period (days)	14.0	12.0	13.0	15.0	15.0
Mean number of nymphs	498.0	240.0	156.4	46.8	63.6
Mean % hatchability	96.2	88.2	90.9	75.5	75.4

*20 ticks collected from a warthog burrow in Nairobi National Park but the source of the bloodmeal was not identified.

Table 16a: Proportion of nymphs of *O. moubata* feeding on bovine and porcine blood, feeding through two types of membranes.

	BOVINE BLOOD		PORCINE BLOOD	
	Bat's wing	Baudruche	Bat's wing	Baudruche
Mean % Feeding	91.0	74.0	88.6	73.6
S.D.	± 7.4	± 10.8	± 3.8	8.2
t.	4.37		5.58	
P	< .001		< .001	
No. of Ticks Tested	900	900	900	900

Table 16b: Effect of bovine and porcine blood on reproduction of *O. moubata* fed through the bat's wing membrane

	Bovine	Porcine
Mean weight of 50 unfed ticks (mg)	59.9	55.1
Mean weight of 40 fed ticks (mg)	288.3	284.8
Mean size of bloodmeal ingested (mg)	233.1	234.6
Mean no. of eggs deposited	173.8	283.1
Mean preoviposition period (days)	10.8	11.8
Mean incubation period (days)	16.3	13.2
Mean number of nymphs	145.2	249.7
Mean % hatchability	85.9	84.7

Table 17: Effects of rabbit, bovine and porcine blood on the reproduction of *O. moubata* fed through bat's wing membrane

	Rabbit	Porcine	Bovine
Mean weight of 50 unfed ticks (mg)	100.5	75.1	73.1
Mean weight of 40 fed ticks (mg)	236.4	228.5	238.1
Mean size of bloodmeal ingested	136.4	203.4	165.1
Mean number of eggs deposited	210.0	224.0	151.1
Mean preoviposition period (days)	9.6	13.3	15.4
Mean incubation period (days)	14.2	15.2	14.6
Mean no. of nymphs produced	185.0	178.3	112.3
Mean % hatchability	87.9	76.4	66.4

The results (Table 16a) show that O. *moutaba* fed much more readily through bat's wing membrane on both bovine and porcine blood than they did through calf gut membrance.

The results (Table 17b) show that ticks of comparable unfed weights had comparable bloodmeals on bovine and procine blood. However, the ticks fed on the latter blood laid more eggs and had more nymphs indicating nutritional superiority of porcine blood. Similar observations were made on *Glossina* at the Tsetse Laboratory in Bristol (Jordon, personal communication).

Table 17 shows ticks artificially fed through membranes on rabbit, porcine and bovine blood; the mean number of eggs produced by females fed on rabbit, and on porcine blood did not differ much but was higher than that of ticks fed on bovine blood. The same applied to the mean numbers of nymphs resulting from rabbit and porcine blood. The adult offspring of ticks fed on porcine blood began to emerge at 4th moult and about 20% adults, mostly males, were obtained by that time. But the offspring from females fed on bovine blood had extended development, and emergence was after the 5th nymphal stage. Moreover, the percentage of ticks maturing on porcine blood (62.2%) was greater than that on bovine blood (12.6%) and this was a significant difference.

The development of the offsprings fed on porcine, bovine and rabbit blood was compared. For all the three hosts, adults began to emerge at the 4th moult with 36.5%, 21.5% and 37%, and 85%, 51.5% and 72.3% adults at the 5th bloodmeal respectively.

From the above results, it can be concluded that rabbit and chicken are better hosts than rat or guinea pig for laboratory rearing of *Ornithodoros moubata*, and can be used to maintain colonies.

Also, *O. moubata* could equally well be maintained on porcine and rabbit blood by membrane feeding.

Table 18: The effects of type of laboratory host on development

	Baby Rabbit		Adult Rat		Baby Rat		Baby Mouse	
	Lab.bred	Field Coll.	Lab.bred	Field Coll.	Lab.bred	Field Coll.	Lab.bred	Field Coll.
Time for 1st Moult (days)	18	20	26	29	15	28	22	22
% of 1st Moulting	31 (100)	58 (100)	62 (100)	91 (100)	9 (100)	33 (100)	9 (100)	11 (100)
% Survival	99	100	90	98	100	99	96	100
Time for 2nd moult (days)	15	16	17	19	21	19	16	11
% of 2nd stage moulting	42.2(99)	61 (100)	42.2(90)	20.4(98)	49 (100)	42.4(99)	24 (96)	20 (100)
% Survival	96.9	100	100	84.7	97	100	100	100
Time for 3rd moult (days)	23	22	23	21	23	16	15	22
% of 3rd stage moulting	68.8(96)	81 (100)	54.4(9)	69.9(83)	37.1(89)	47.4(95)	17.9(95)	45.4(97)
% Survival	83.3	98	88.9	100	100	96.8	95.8	96.9
Adults	M F	M F	M F	M F	M F	M F	M F	M F
	11 0	23 2	15 8	17 8	1 1	2 0	0 0	0 0
Time for 4th moult (days)	25	20	23	21	26	31	19	20
% 4th stage moulting	73.5(68)	86.4(67)	27.3(55)	27.5(80)	19.6(92)	19.6(92)	11 (91)	6.4(94)
% Survival	100	97	95.5	100	91.3	95.7	100	91.5
Adults	M F	M F	M F	M F	M F	M F	M F	M F
	5 26	17 13	3 6	1 4	2 2	1 0	0 0	0 0
Time for 5th Moult (days)	32	29	31	31	18	18	16	22
% 5th stage moulting	63.4(41)	75.7(37)	40 (55)	16 (55)	42.9(77)	41.4(87)	11.0(91)	22.1(86)
% Survival	97.6	97.3	85.5	52	89.6	97.7	89.0	75.6
Adults	M F	M F	M F	M F	M F	M F	M F	M F
	9 12	11 10	10 10	6 0	8 8	6 2	0 0	0 0
	25 28	56 25	28 24	24 12	11 11	9 2	0 0	0 0
Total	63	81	52	36	22	11	0	0
% Matured	63	81	52	36	22	11	0	0

3. Mass Rearing of *O. moubata*

An attempt to find a suitable laboratory host for mass-rearing of *O. moubata* was made. It had been suggested that newly born rat and mice about a week old be tried as possible hosts since these were easy to handle and therefore involved less labour.

It has been established that *Ornithodoros moubata* could reproduce and develop well on adult rabbit and reproduce well on new born rabbit (See Table 14). Also investigated were the effects of new born rabbit, adult and new born rat and mouse on the development of *O. moubata.*

MATERIALS AND METHODS

Materials and methods as described in the earlier part of this report were adopted. Both the new born rabbit and adult rat were immbolized in wire mesh cones and placed in containers with nymphs while baby rats and mice were placed in Kilner jars with nymphs until the animals died and were then removed. Moulting was used, as a parameter for assessing engorgement. The nymphs were fed five times at the end of which the percentages of ticks that had matured were calculated.

RESULTS

The results of Table 18 show that *O. moutaba* developed best on new born rabbit followed by adult rat with 63-81% and 36-52% ticks maturing on the two hosts. The poorest host was the new born mouse followed by new born rat which resulted in zero and 11-22% adults emerging.

4. The Effects of Edysone, Juvenile Hormone and their Analogues on *O. moubata* and *R. appendiculatus*

Ecdysone and its analogues have been reported to cause inhibition of larval development and egg production when ingested by the boll weevil, *Anthonomus grandis*, Boheman (Earle, Padovani, Thompson and Robbins, 1970), the house fly *Musca domestica* (Robbins, Kaplains, Thompson, Shortino, Cohen and Joyner, 1968; Morgan and Labrecque, 1971) and when applied topically to *Culex tarsalis* after a bloodmeal (Ittycheriah, Marks and Quraishi, 1974).

However, very little information exists on the role of ecdysone, juvenile hormone and their analogues on ticks. Ecdysone and phytoecdysones were reported to have caused supermoults and deaths among adults and moulting in partially engorged nymphs of *Ornithodoros moubata* (Kitaoka, 1972) and to have induced or terminated diapause in *Dermacentor albipictus* (Wright, 1969).

One juvenile hormone analogue was shown to induce termination of diapause, vitellogenesis and oviposition in female *Argas arboreus* (Bassal and Roshdy, 1974). Another juvenile hormone analogue ZR512, completely blocked embryonic development of eggs of *Hyalomma dromedarii* when topically applied to females on the first day of oviposition. (Bassal, 1974).

MATERIALS AND METHODS:

Groups of adults and nymphs of *O. moubata* were fed on blood-meal to which varying doses of ecdysterone (Beta ecdysone) and ponasterone A had been added. They were then observed for super-moulting and egg laying in case of adults and accelerated moulting in case of nymphs. ZR512 a juvenile hormone analogue prepared by Zoecon Corporation was topically applied to both half and fully engorged mated *R. appendiculatus* females. Control ticks were treated with equal amounts of the solvents only.

RESULTS:

Adult *O. moubata* fed on bloodmeal containing ponasterone A and ecdysterone supermoulted into normal adults capable of laying viable eggs. The supermoulted adults attained larger body sizes, took larger bloodmeals and laid more eggs than normal ticks. (Fig 6 and 7). In case of nymphs treated with ecdysone, they showed accelerated moulting compared to normal ticks. When *R. appendiculatus* were topically treated with ZR512 while still feeding, and on the day they dropped from the host, most of them laid viable eggs but when treated two days after dropping from the host, the eggs were not viable.

DISCUSSION

When ingested, ecdysone and analogues inhibited larval development and caused sterility in such insects as the boll weevil, and house fly (Earle *et al.*, 1970, Robbins *et al.* 1968 and Morgan and Labreque 1971), and when applied topically to *Culex tarsalis* (Ittycheriah *et al.*, 1974). Insect growth with juvenile hormone activity also show sterilising effects (Staal, *et al.*, 1973 Nassar *et al.*, 1973). In contrast, adult *O. moubata* has been shown in the present study and by Kitaoka (1972) to supermoult and to be capable of laying viable eggs.

Ecdysone and analogues do not seem to impair the ability of *O. moubata* to reproduce.

The supermoulted ticks increased to nearly twice their normal size. They took larger bloodmeals and laid more eggs proportional to their sizes. So far, ticks have been induced to supermoult up to three times on separate bloodmeals and probably may continue supermoulting into giant adults. The supermoulted ticks increased their body sizes with subsequent moults. 3rd and last stage nymphs treated with ecdysone had accelerated moulting. The mean number of treated nymphs that moulted within ten days was 73% compared to 22% in controls.

This induced continuous growth and moulting in adults of *O. moubata* seems to be a primitive behaviour similar to that of many crustaceans which continue to moult and grow after reaching sexual maturity, in some cases throughout the whole life span. Morphological changes can take place after the animal has become mature. Crustaceans share with insects not only the intermittent replacement of integument but also its control by neuroendocrine mechanisms (Scharrer and Scharrer 1963). But some crustaceans such as *Maja* and *Carcinus*, have terminal growth (Prosser and Brown 1966).

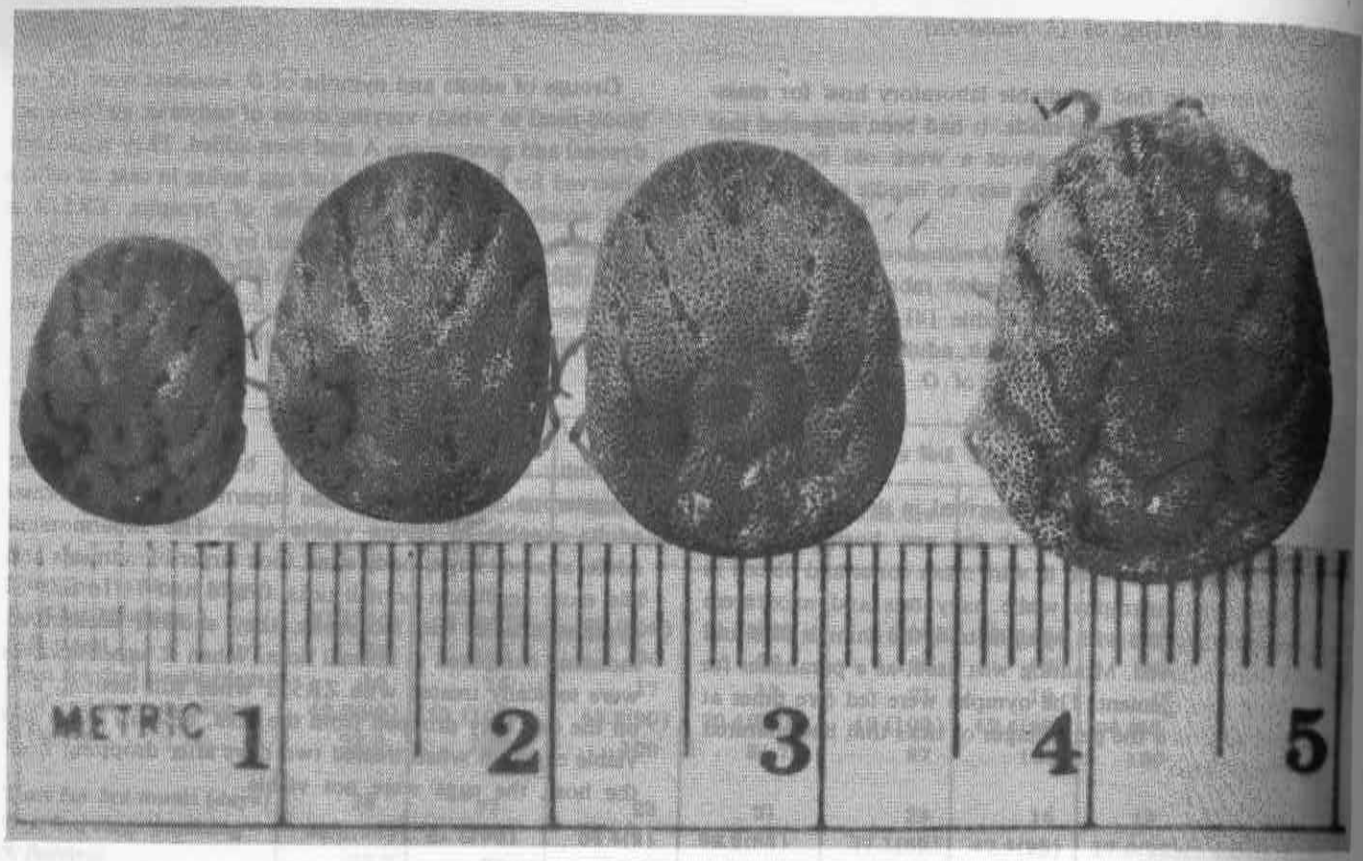


Fig. 6 Normal and Super Ticks

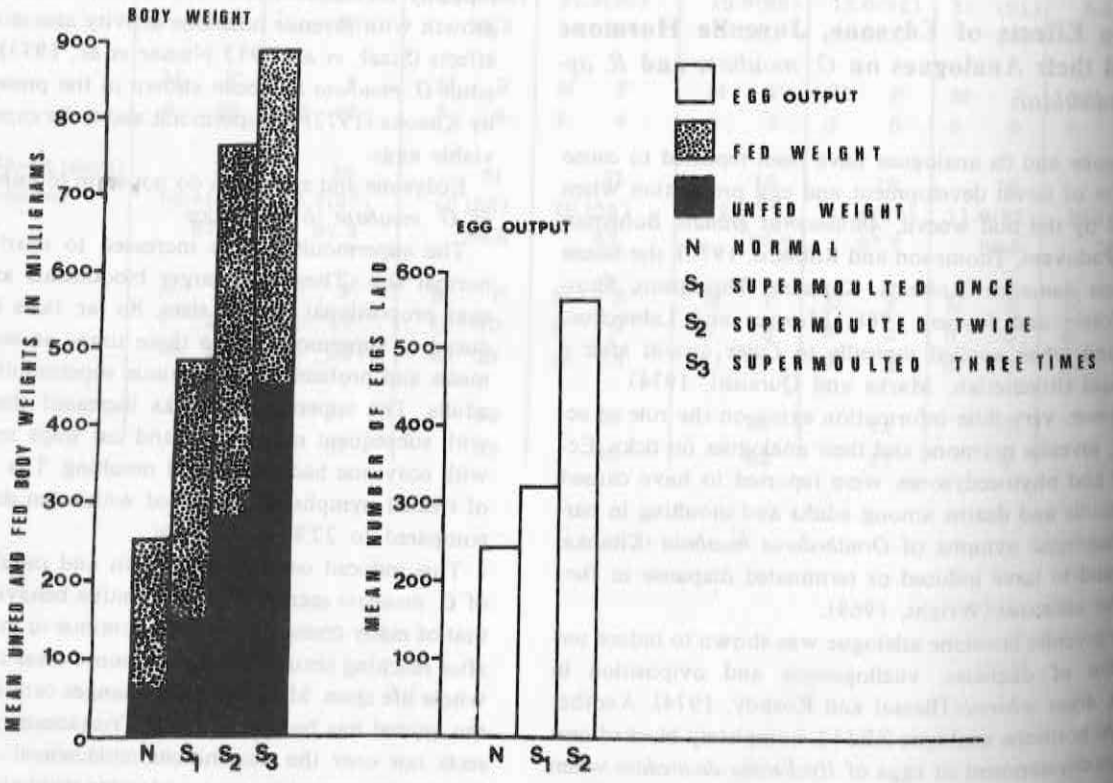


Fig. 7 The effects of Ecdysone on body weight and Egg output of female Ornithodoros moubata

O. moubata seems therefore to occupy an intermediate position between crustaceans and insects. Like crustacean, the adult tick continues to grow and moult without losing its reproductive ability when given ecdysone, but like insects, growth and moulting stops at maturity.

In the present study, interference with embryonic development in *R. appendiculatus* has been achieved, a similar result to that of Bassal, (1974) on *Hyalomma dromedarii*. Further work is underway.

SENSORY BEHAVIOUR

1. Receptor Sites for the Sex Pheromone in *Ornithodoros moubata*

INTRODUCTION

Chemoreception is well known in several groups of insects, but in ticks only the ixodids have been studied. (Lees, 1948; El-Ziady, 1958; Chow, 1970; Totze, 1933). Moreover, information about the chemoreceptor sites is still scanty and often contradictory. Thus, various investigators have attributed the sense of smell to Haller's organ which is believed to be reinforced by the palpal organ, while others do not attribute olfaction to the palpal organ at all (Foelix and Wang, 1972). Until recently no sex pheromone had been demonstrated in the argasid tick, *Ornithodoros moubata*, but studies in our laboratory have shown that one does exist and that the perception of this pheromone may have an olfactory component. The object of this preliminary study therefore was to determine receptor sites for the pheromone, as well as to describe morphologically the types of receptors on such sites.

MATERIALS AND METHODS

(a) Recently fed virgin male and female adult ticks were used. Material to be tested for sex pheromone activity were obtained by keeping a 2 cm diameter filter paper disc in contact with virgin female ticks for known periods of time. The tarsal segments on the first pair of legs, or the mouthparts, or both, of males were painted with black paint. In another experiment the tarsi on the same pair of legs or palps were amputated. Ten male ticks so treated were introduced into a petri dish divided into four sectors, one of which contained the paper disc impregnated with the female pheromone; the other sectors contained clean discs. The pheromone activity assay method has already been described (ICIPE Annual Report 1974). Unoperated male ticks were similarly exposed to the sex phermone source as controls.

(b) In order to see whether the pheromone collected on the paper dics was a sex pheromone (i.e. an attractant released by the female that increases the chance of mating) the following procedure was adopted. Virgin males which had been treated as above were exposed to virgin females at $27 \pm 2^{\circ}$ C in 2.5 x 7 cm glass vials, one pair per vial, and observed for mating. Untreated males were used in control experiments.

(c) The distribution, types and morphology of the palpal (or mouth parts) receptors were investigated by means of whole mount preparations, by examination of histological sections of paraffin embedded material under the light microscope, of gold coated specimens in a JEOL 15 scanning electron microscope (SEM) and thin sections on a transmission electron microscope (TEM).

RESULTS

(a) **Sex pheromone receptor sites:** The results given in Table 19 show that amputation of the palps destroys the ability of the male *O. moubata* to locate the pheromone impregnated paper discs, and that amputation of the first pair of tarsi only impairs it. Painting in each case produces a lesser effect than amputation. This may mean that there were receptors left uncovered. On the other hand, amputation of the tarsi on the second pairs of legs does not give a significantly different result from the controls.

Results from mating experiments showed close agreement to those from sex pheromone bioassays. Thus up to 80% of the controls mated, but in males with the first tarsi or palps amputated only 5% and 20% mated respectively. From this it can be implied that the female releases a chemical which attracts the male for the purpose of mating, and is detected primarily by the palpal receptors. (Fig. Ia).

(b) Sex pheromone receptor types and morphology

As Figure 8 shows each palp is composed of 4 segments. From whole mount preparations and SEM four receptor types were recognised, based on size characteristics, and their distribution is illustrated in the figure.

(i) Long basiconic sensilla:- these are blunt-tipped receptors 100-130 μ long, 10 μ in diameter at their bases and occur only on the dorsal surface of segments 2 and 3 and are usually 3 and 2 in number respectively.

(ii) Medium size basiconic sensilla:- they are blunt-tipped receptors 45μm long, 7.5μm in diameter at the base and found only in segment 1.

(iii) Short basiconic sensilla:- They are blunt-tipped receptors 10 - m long, occur on all segments and up to 12 are usually found on the tip of each palp.

(c) The results obtained by light and electron microscopy show: that both the large and small receptors are innervated and have similar basic cellular constituents as found in insect receptors. Some of the small type (iii) have dual innervation. One dendrite ends in the cuticle at the base of the receptor and has typical properties of a receptor responding, among other things, to mechanical stimuli. The second dendrite continues into the receptor lumen, branching as it does so. The big type (i) is also innervated but the number of sensory cells is not yet certain. The cell bodies lie within the epidermis and from there they give off their axons to the central nervous system. Their cytoplasmic contents are often less dense than those of the surrounding cells. The cuticular wall of both the big and small receptor types is pierced by pores as encountered in other chemoreceptors of known function.

Table 19 Effect of amputation of tarsi (T) and/or palps (P) on the assembly of male ticks within 1 hr at paper with female pheromone (No. 4) or at control papers (No. 1-3)

Test paper	00 tested		No. ticks in contact with papers Sector				%% 00 assembled at paper in sector 4
	No. 00	Condition of 00	1	2	3	4	
1	40	P and 1st T painted	2	2	1	4	10
1	40	Normal males	0	0	0	34	85
2	30	P & 1st T amputated	0	0	0	0	0
2	30	Normal males	0	0	0	24	80
3	30	1st T painted	0	0	0	11	36
3	30	Normal males	0	0	0	26	86
4	60	1st T amputated	0	0	0	31	52
4	60	Normal males	0	0	0	50	83
5	50	2nd T amputated	0	0	0	38	76
5	50	Normal males	0	0	0	44	88
6	60	P amputated	0	0	0	0	0
6	60	Normal males	0	0	0	52	87

The SEM results also reveal other types of structures that have not yet been described in ticks, and which look like "slit organs" or "companiform sensilla". These are always found on the ventral aspect of the two basal palpal segments. The findings of the Haller's organ basically agree with what is described in other ticks except in two respects. One is that the posterior capsule contains a uniform population of thousands of cuticular structures instead of the usual 7-8 sensilla; and the other is an additional pair of "slit organs" or "pit organs" to the other structures ont he anterior pit.

DISCUSSION

There is increasing evidence that among the arthropods, pheromone perception is by chemoreceptors. During the present study a pheromone mediated behaviour was demonstrated in *Ornithodoros moubata* and it could be destroyed by blocking the palps. On the other hand, absence of Haller's organ could not completely impair the ability to locate the female sex pheromone by the male *O. moubata*. From these results it can therefore be inferred that the receptors involved in detection of the sex pheromone, in this tick species, are located on the palps. However, recently Berger *et al* (1971) demonstrated sex pheromone in 3 species of ixodid ticks and obtained evidence that Haller's organ might be involved in its perception. But whatever contribution Haller's organ might make to the detection of the pheromone, it does not on its own enable the male *O. moubata* to detect the female sex pheromone if the palps have been removed. In no instance did aggregation on the pheromone source occur in their absence. Thus, it is the palps that appear crucial to pheromone perception in *O. moubata*.

The structural characteristics of the palpal receptors which have been described here, correspond to those of other sensilla of proved chemosensory function in many arthropods. It is therefore possible that these receptors are indeed involved in chemosensory functions mediating the behaviour reported here.

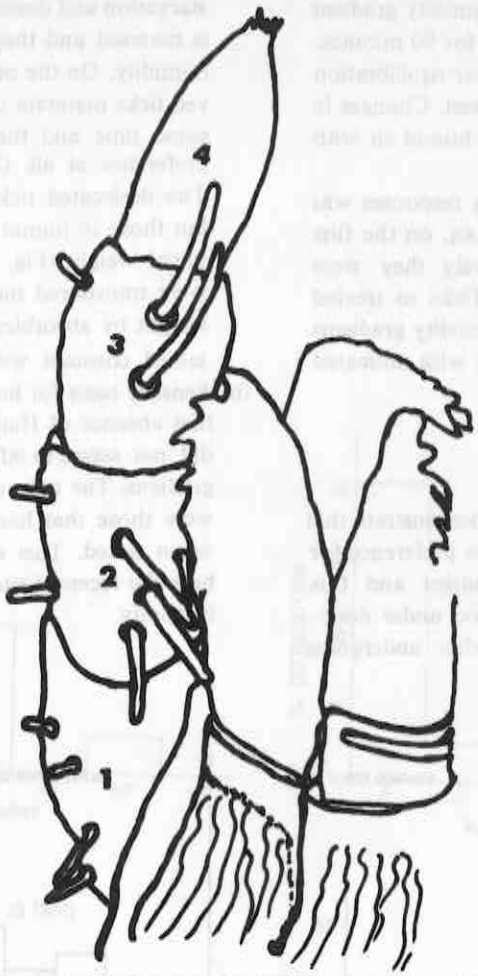


Fig. 8: Distribution of Receptor organs on the Palp of *O. moubata* (Dorsal View)

2. Response to Humidity in *Ornithodoros moubata*

INTRODUCTION

Water balance is known to be a critical issue in the survival of all arthropods, and avoidance of dehydration is one way of maintaining this balance. The soft tick *O. moubata* occurs in fairly arid environments throughout East and Central Africa and the problem of water loss and uptake is therefore particularly acute. Resistance to desiccation in this tick was also found to be of high order and is obviously important in survival (Lees, 1946 and 1947; Browning 1954a and 1954b). These ticks also have the ability to absorb water from the atmosphere at high relative humidities (RH).

These characteristics enable the tick to survive for long periods between blood meals. Walton (1960) showed that the East African *O. moubata* is a complex of several variants with differing tolerance to dehydration, depending on habitat.

Behavioural responses of ticks to humidity are important in the maintenance of water balance. Sonenshine (1963) and Howell and George (1973) showed that responses of the different argasids they studied varied greatly and were determined by the state of water balance, feeding and age. The responses of a variety of ticks to changes in humidity should therefore, be thoroughly investigated with regard to several factors. The present study investigated:

(1) Some basic behavioural responses in one variant of the East African *O. moubata* complex (Warthog or porcupine burrow form).
(2) The likely sensory basis of such behavioural responses.

MATERIALS AND METHODS

(a) Unfed adult male ticks were obtained from nymphs collected from Nairobi National Park. Freshly moulted ticks were divided into groups of 20% and kept in 2.5 x 7.5 cm specimen tubes covered with either cotton wool or muslin cloth. Responses to humidity were tested in a circular chamber 13 cm diameter and 4cm high, comprising two halves. The lower half was divided into seven compartments designed to hold containers of different concentrations of potassium hydroxide (KOH) to give a relative humidity (RH) gradient. A fine wire mesh was stretched across three quarters up the upper half, forming a false floor for the ticks. The roof of the upper half, was of perspex with a hole in the centre, through which the test ticks were introduced. The hole was then sealed with a greased glass slide. Ticks were tested either immediately after moulting or after having been held at either 0% RH over sulphuric acid or 95% RH over solution of Potassium hydroxide exposed to a humidity

gradient. Their distribution within the humidity gradient chamber was recorded every 30 minutes for 90 minutes. A maximum of one hour was allowed for equilibration before the ticks were introduced in each test. Changes in body weights of ticks held in dry air and humid air with time were recorded.

(b) The sensory basis for humidity orienting responses was investigated by amputating Haller's Organ, on the first pair of legs, or the palps. Alternatively they were blocked with low melting point wax. Ticks so treated were then exposed in groups of 20 to humidity gradients inside the arena as before and compared with untreated controls.

RESULTS

(a) **Humidity Response:** The present data demonstrate that unfed, freshly moulted *O. moubata* shows preference for extremely dry air in a humidity gradient and this behaviour is sustained over a long period under desiccating conditions (Fig. 9). However, after undergoing starvation and desiccation for 3-7 months this behaviour is reversed and they begin to migrate to areas of high humidity. On the other hand, unfed, hydrated and starved ticks maintain their preference for low humidity for some time and thereafter do not seem to show any preference at all. (Fig. 10)

The desiccated ticks lost 58% of their initial weights, but those in humid air (85% RH) lost only 20% of the initial weight (Fig. 11). When partially desiccated ticks were transferred into a 95% RH container they gained weight by absorbing water and within 15 days they attained constant weight (Fig. 12).

(b) Sensory basis for humidity response: The results showed that absence of Haller's Organ Fig. 13 or palps Fig. 14 did not seem to affect the responses to the humidity gradiens. The ticks that were subjected to this treatment were those that had been shown to respond positively when tested. This shows that the two organs do not bear the receptor sites involved in orienting responses to humidity.

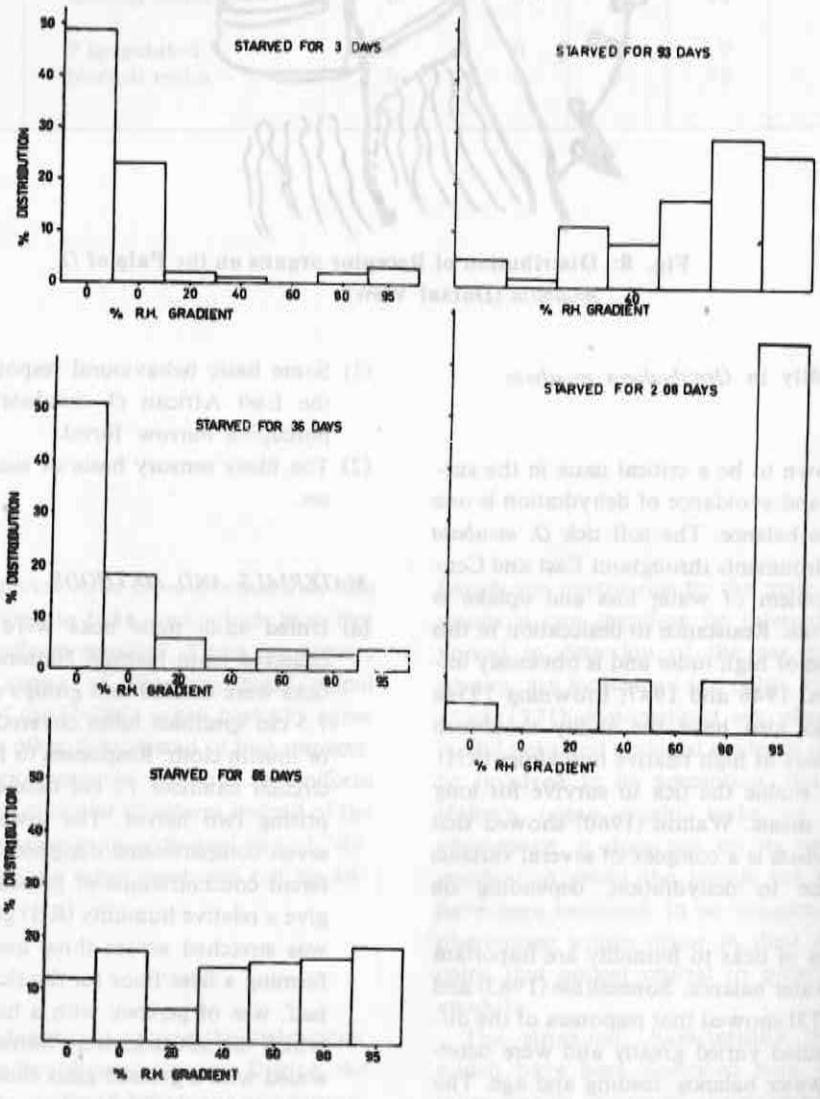


Fig. 9: Effect of desiccation on responses of *Ornithodoros moubata* to relative humidity

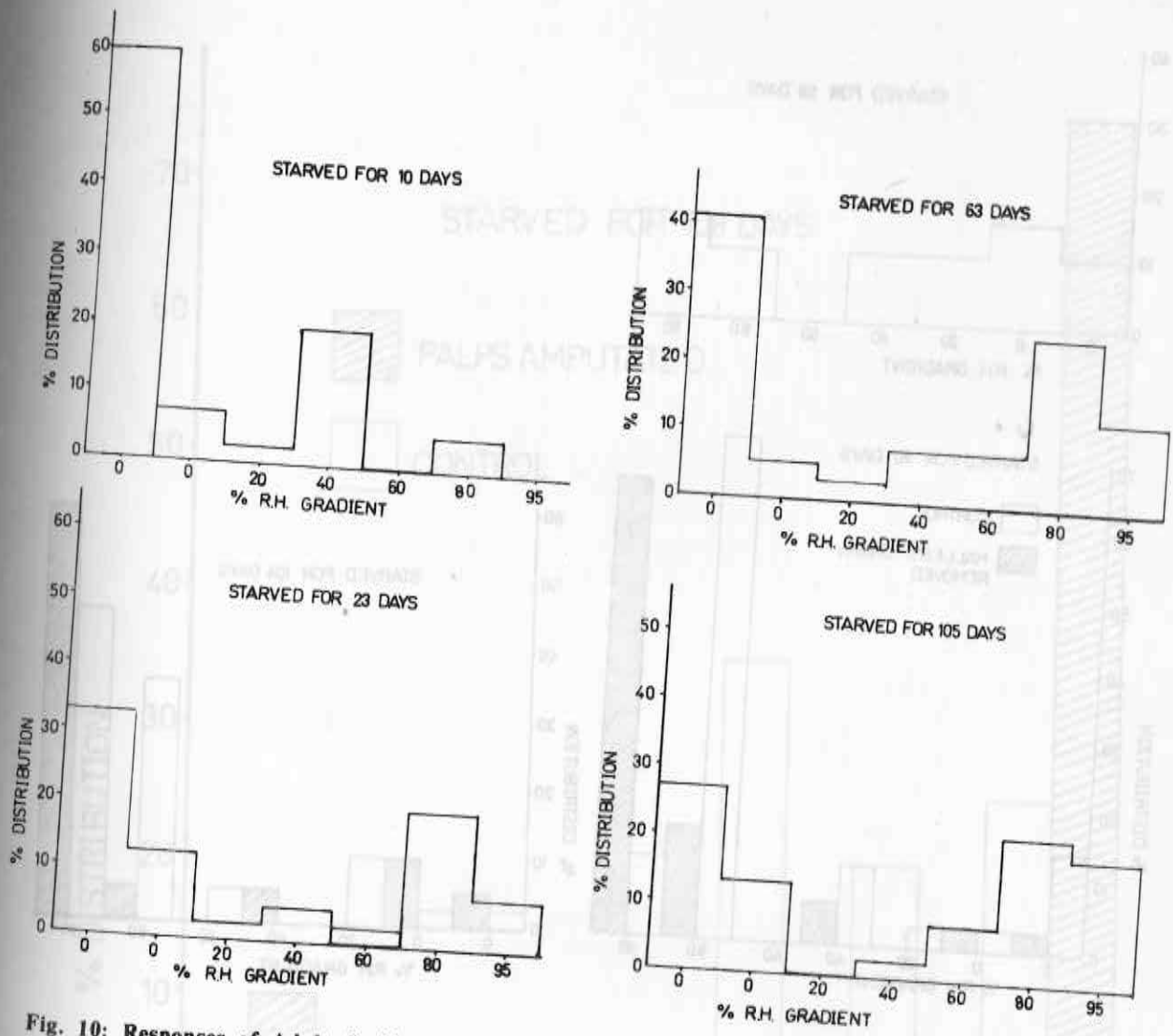


Fig. 10: Responses of Adult *O. Moubata* (starved and hydrated) to Relative Humidity Gradient

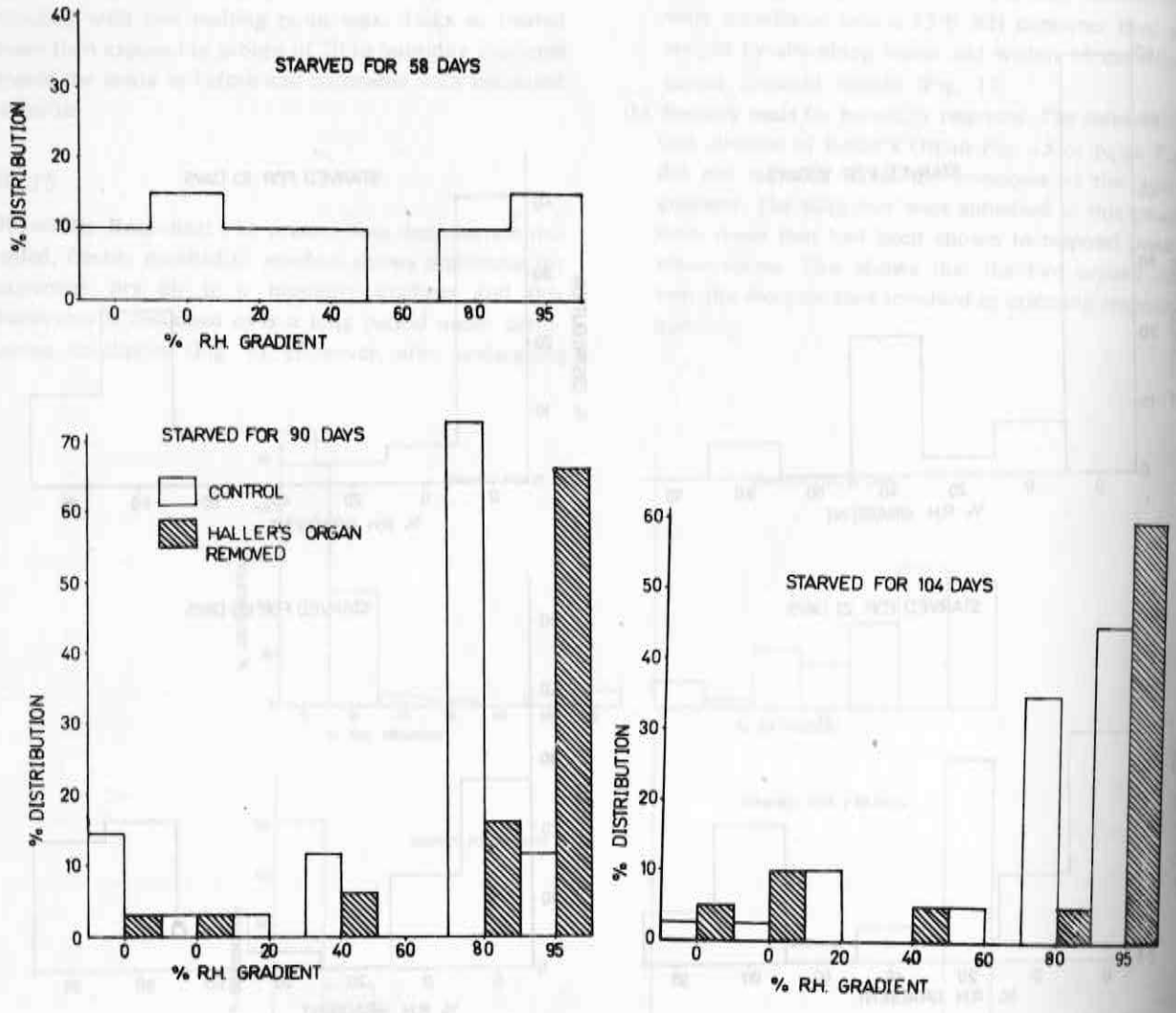


Fig. 13: Effect of Absence of Haller's organ on Responses of *0. Moubata* to humidity

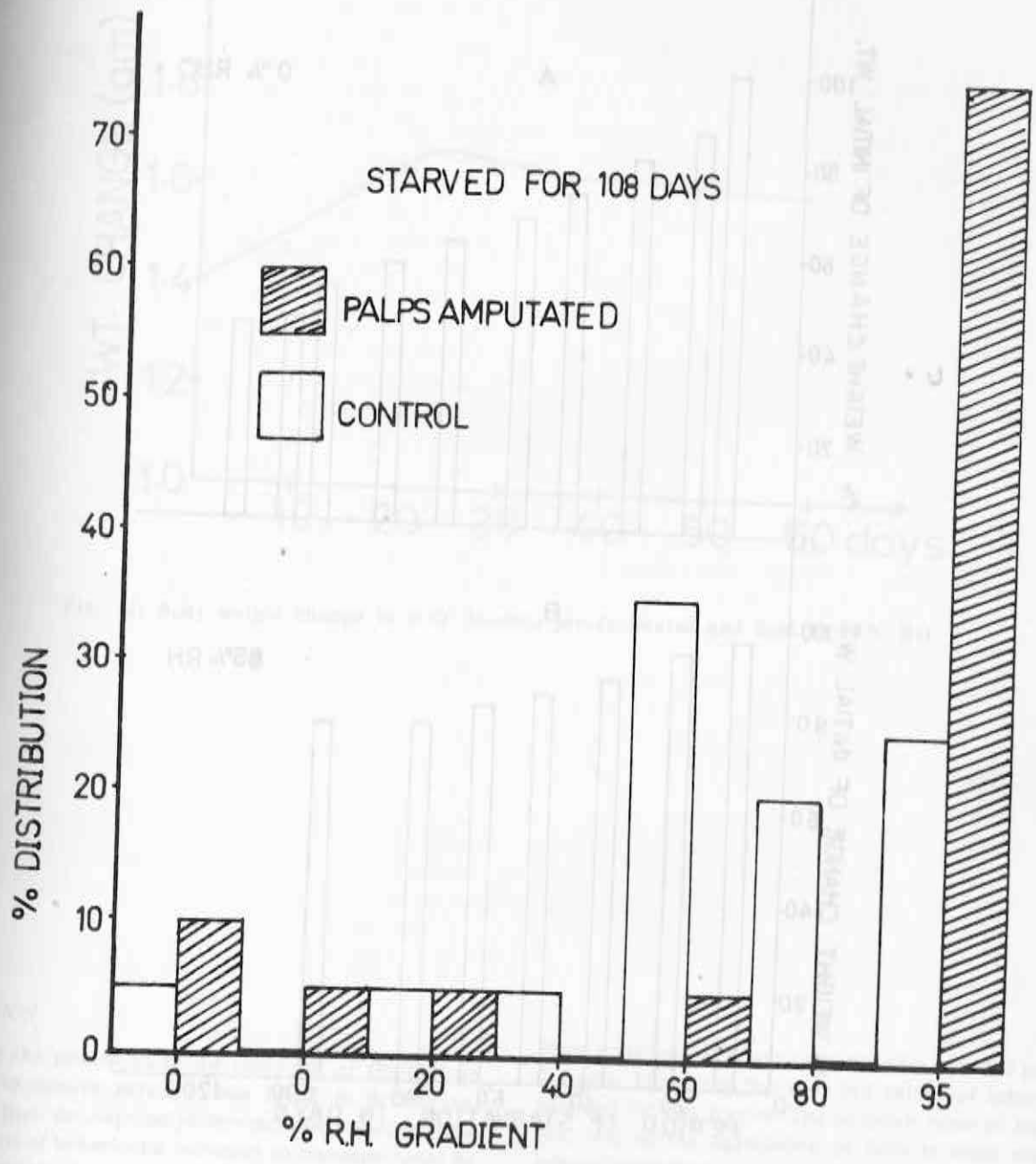


Fig. 14: Effect of Absence of Palps on Response of *O. Moubata* to Relative Humidity

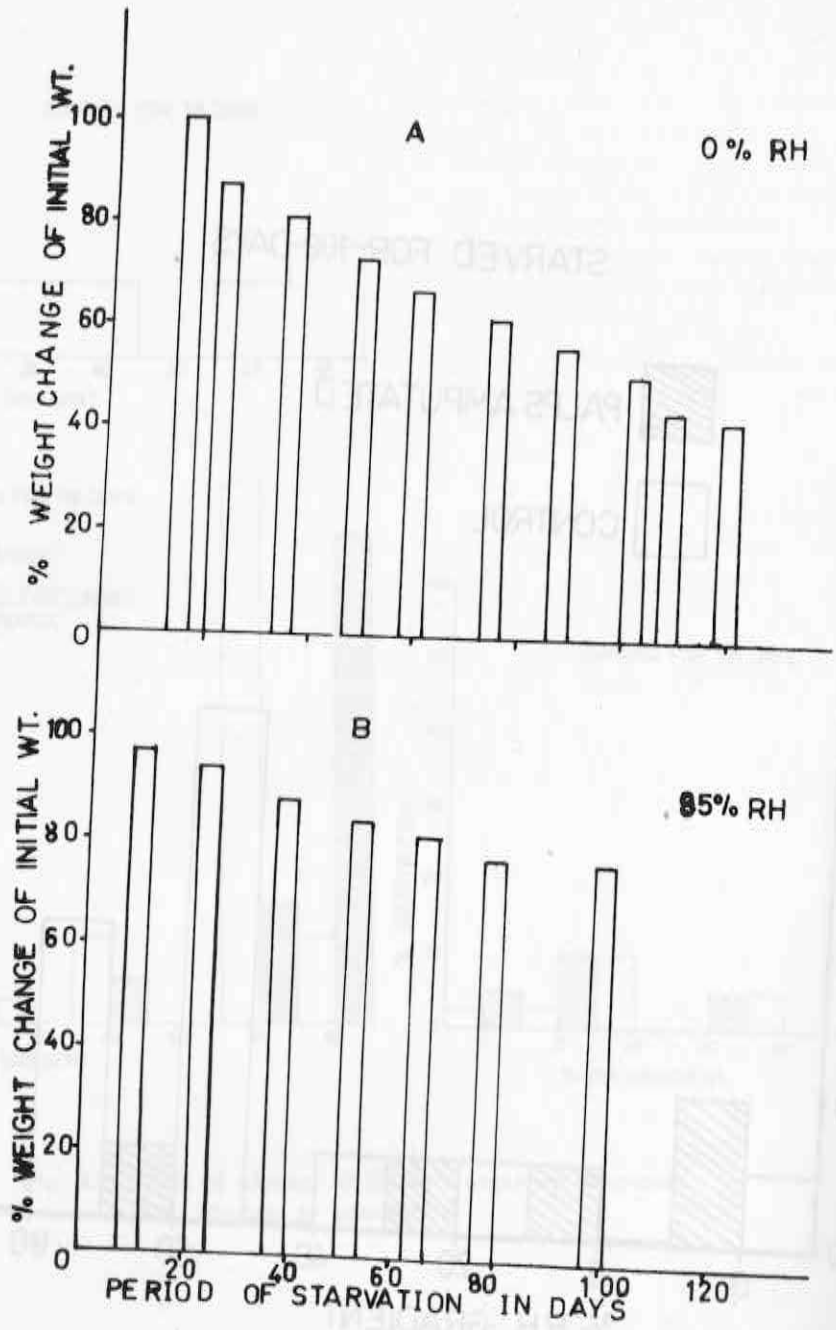


Fig. 11: Effect of desiccation on Body weight in *Ornithodoros moubata*

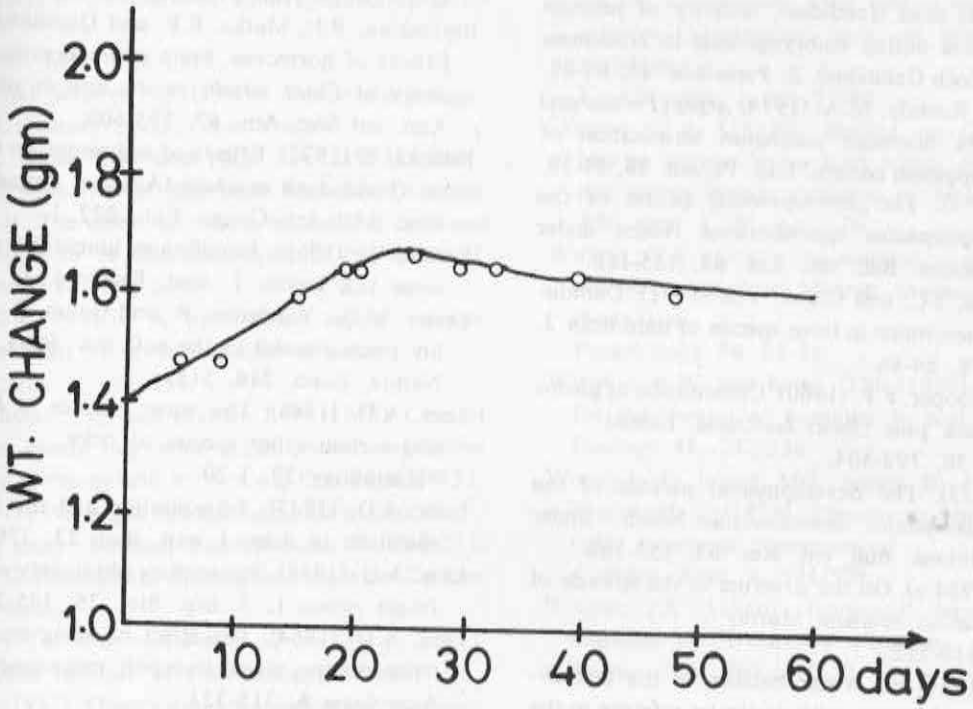


Fig. 12: Body weight change in o *O. Moubata* pre-de iccated and held at 95% RH.

DISCUSSION

During the present study the responses of *Ornithodoros moubata* to relative humidity was found to deviate appreciably from the response pattern recorded for other ticks. The pattern of behavioural responses to humidity could be divided into three categories. Unfed, freshly moulted ticks showed a strong preference for an environment close to 0% RH and could be maintained for a period of 2 months. However, this pattern could be reversed by prolonged starvation accompanied by desiccation of 3 months duration, giving a strong preference for higher humidities. This undoubtedly demonstrates the tick's adaptation for survival in periodically arid habitats, where, due to migration of host animals, it undergoes prolonged starvation between blood meals. Starved hydrated ticks, on the other hand, did not show a preference for any humidity after an initial period of 3 weeks. This is the first time this pattern of behaviour is recorded for this species. The adaptive value of responses that lead to the aggregation of ticks in areas with low relative humidities is not readily apparent.

In *O. moubata* no evidence for specific receptor organs for humidity has so far been demonstrated, in contrast to the hard ticks; and it seems probable that in this orgasid tick no specific receptor organ for humidity exists. The state of water balance might be the major influence on tick activity under different humidity conditions. This was demonstrated by one experiment in which amputation of palps resulted in considerable dehydration due to bleeding and eventually led to the ticks behaving like those that had undergone prolonged desiccation.

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A delegation of Saudi Arabian Parliamentarians who visited Kenya and took off time to see the research activities of the ICIPE. Second and third from left are ICIPE research scientists Doctors A . Maradufu and N. Abo-Khatwa.

Dr. A. Gluart, a visiting research scientist at the ICIPE in early 1976 explaining the operation of the Transmission Electron microscope to a group of students from Jamhuri High School, Nairobi.

TSETSE RESEARCH

REPRODUCTIVE PHYSIOLOGY
(Glossina morsitans)

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

Scientists:
Dr. F.B. Chaudhury (1974) – Research Scientist
Mrs. J. Kongoro – Research Assistant

Collaborators:
Mr. J. Owor – Experimental Officer

SALIVARY GLAND PHYSIOLOGY:
(G. morsitans, G. pallidipes and G. brevipalpis)

Directors of Research:
Professor Thomas R. Odhimabo (1970)

Scientists:
Miss N. Darji – Research Assistant
Mrs. N. Patel – Experimental Officer
Dr. L.H. Otieno (1973) – Research Scientist
Dr. A.Y. Youdeowei, Research Associate (1973)

ISOLATION MECHANISMS:
(Glossina pallidipes)

Director of Research:
Dr. Wim Helle (1971)

Scientists:
Mr. J. Kawooya (1974) – Experimental Officer
Dr. J. van Etten (1974) – Research Scientist

ACOUSTIC COMPONENT OF TSETSE FLY COMMUNICATION

Directors of Research:
Professor T.R. Odhiambo (1970)
Professor A.R. Moller (1973)

Scientists:
To be selected in 1976

Technical Staff:
Miss E.I. Carina Erickson,
Consultation Technician (from May 1974)

REPRODUCTIVE PHYSIOLOGY

INTRODUCTION

Tsetse flies reproduce by adenotrophic viviparity. Eggs mature one at a time in alternate ovaries and each mature egg is then released into the uterus where fertilization, embryogenesis, hatching and larval development occur. The larval stages feed *in utero* on a secretion from the "milk" glands of the mother. Following larviposition the mature larva does not feed but pupates rapidly. As each larva is deposited, the female ovulates again almost immediately and the cycle is repeated.

Mating is necessary to initiate the process of ovulation (Odhiambo 1971, Saunders and Dodd 1972). Females normally mate 2-3 days after emergence but do not ovulate until 5-7 days later. We observed (ICIPE Annual Report 1974) that the time period between mating and ovulation can be reduced to less than 24 hours in delayed mated females possessing one mature egg. Also, we have demonstrated that the component of mating which induces ovulation is not insemination or spermatophore formation. However, we observed that females subjected to repeated short "sterile" matings showed an increased rate of ovulation with the increase of cumulative *in copula* time for individual females. But whether sterile matings of short duration were capable of transferring any chemical from the male to the female to cause ovulation was not yet known.

The hormonal involvement in the control mechanism of ovulation is not clear. Foster (1974) found that the ablation of median neurosecretory cells (MNC) of the pars intercerebralis almost always inhibited ovulation in the tsetse fly, *Glossina austeni*. However, he noted that the surgical brain trauma also inhibited ovulation in control flies. Ejezie and Davey (1974) found the MNC to undergo cyclic changes of net synthesis and release in *G. austeni* and attempted to correlate these phenomena with ovulation. Because 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 purpose of the present study is to determine the exact nature of the stimulus for ovulation which the female receives from the male during copulation and to investigate the possible endocrine involvement in the induction of ovulation in the tsetse fly. *G. morsitans morsitans.*

MATERIALS AND METHODS

Rearing and Maintenance

Flies used for the present study were obtained from the ICIPE laboratory colony. Within 24 hrs of emergence, flies were sexed and placed in 18x18x4.5 cm cages of PVC tubing with terylene netting. Individual females and mating pairs were kept in vial cages of 3x6 cm plastic tubes covered at both ends with terylene netting. Flies were placed on rabbit ears for 20 min for blood meal each day except Sundays and then were kept in a controlled temperature-photoperiod room at 25± 0.5° C, 65± 5r.h. and a daily 12L:12D photoperiod with about 100 lx light intensity in the vicinity of the experimental flies.

Unless otherwise stated, dissections were routinely performed under *Calliphora* Ringer's solution.

Effect of Multiple Interrupted Matings of Shorter Duration on Ovulation

Twelve-day-old females were allowed to mate consecutively with fresh males for periods of 10, 15, or 20 min by manually terminating each mating after the required time *in copula* for each male. Each female was, in this manner, allowed to accumulate 30-90 min period of *in copula* time. Females were dissected after 24 hrs to determine whether ovulation had occurred. Two mated females were dissected immediately after the completion of mating to determine the presence or absence of spermatophore. Control females were permitted uninterupted matings with single males and ovulation was checked 24 hrs after mating was completed. Calculations of the percentages are based on the observations made on 20 females in each treatment.

Transfer of Accessory Glands Secretion Labelled with Tritiated Leucine

One μl of ICi/inI of L- {4,5-^{3}H} Leucine was injected through the thorax of each of 2-6 day-old virgin males which were allowed to incubate for various time periods to determine the period of highest incorporation in the accessory glands on day 6 post emergence (age of females routinely allowed to mate with experimental females). Males with suitable incubation periods were mated with females for 10, 20 and 30 min or were allowed un-interrupted matings. Immediately after the termination of mating the female reproductive system of each mated insect was dissected out, placed in scintillation counting vial and was prepared for counting in a Pakard Tricarb Scintillation Counter and the count per min was calculated. Reproductive systems of virgin females were used as controls. Calculation of the average is based on 11-15 individuals per time period.

Surgical Removal of Male Accessory Glands

Accessory glands were surgically removed (-AcG1) as follows: The anaesthetized fly was placed ventral side up on a wax bottom dissecting dish and was restrained with a thin band of Plasticine. An incision was made on the centre of the 4th sternite with a microscalpel. Whilst exerting slight pressure on the adjoining sternites the accessory glands were pulled out with a pair of fine forceps and exercised from the base (Fig. 1). The flies were then transferred to clean plastic vial cages. The wound sealed itself automatically within 2-3 hours after the operation. Operated flies exhibited normal mating behaviour a few hours after the operation and were routinely used for mating experiments the following day.

Insertion of Glass Beads in the Uterus of Virgin Females

One or more glass beads of about 300 μ were inserted into the uterus of 10 to 12-day-old virgin females through the vaginal opening with a pair of microforceps. Treated females were placed in glass vials and kept under constant observation to determine the time of expelling the beads. Females which expelled the beads in less than 40 min after insertion were disregarded. Control females received identical surgical manipulation and handling but did not receive glass beads. Females were dissected 48 hours after the treatment to check ovulation.

Surgical Procedures for Removal of Corpus Allatum (CA) and Corpus Cardiacum (CACC) Complex of Female Flies

Prior to surgery the flies were chilled in a refrigerator (3° C) for 8-10 min. The immobilized fly was then positioned on a wax bottomed dissecting dish and was restrained with a thin band of Plasticine placed around the thoracic region of the insect. The head of the insect was deflected with 2 fine insect pins (preferably minutens) so as to gently stretch the entire cervical region. The preparation was then immersed with cold physiological saline under which the entire surgery was performed. Microforceps were used to tear the dorsal cervical membrane to expose the CA. The CA was removed either with microforceps or was 'scraped' off gently with the help of a microscalpel fashioned from the tip of a 27 gauge hypodermic needle. The CACC complex, together with the anterior portion of the dorsal aorta, was removed using a microforceps. The removed portion of tissue was routinely examined under the phase-contrast microscope to determine the success of operation. Controls for the operation consisted of females that were treated exactly in the same manner except that the CA or CACC complex area was only pinched lightly with the forceps instead of being removed.

In each of 2 series of experiments, 10 to 12-day-old females were operated under one of the three following experimental conditions: (1) operated immediately after the completion of mating; (2) operated 4 hours before mating; and (3) Operated females remained virgin throughout. In a third series, 12-day-old females were operated 0,4,8,12,16,20 or 24 hours after the completion of mating.

All operated females were held for 24 hours before checking ovulation.

Injection of Haemolymph from Mated Donors into Virgin Recipients

The haemolymph was obtained by pressing down on the thorax of the donor insect whose legs had been cut off.

The expressed 0.5-08 μl haemolymph was removed with a pre-calibrated finely pointed microcapillary pipette and was immediately injected into the recipient female through her thorax. The control recipients received haemolymph

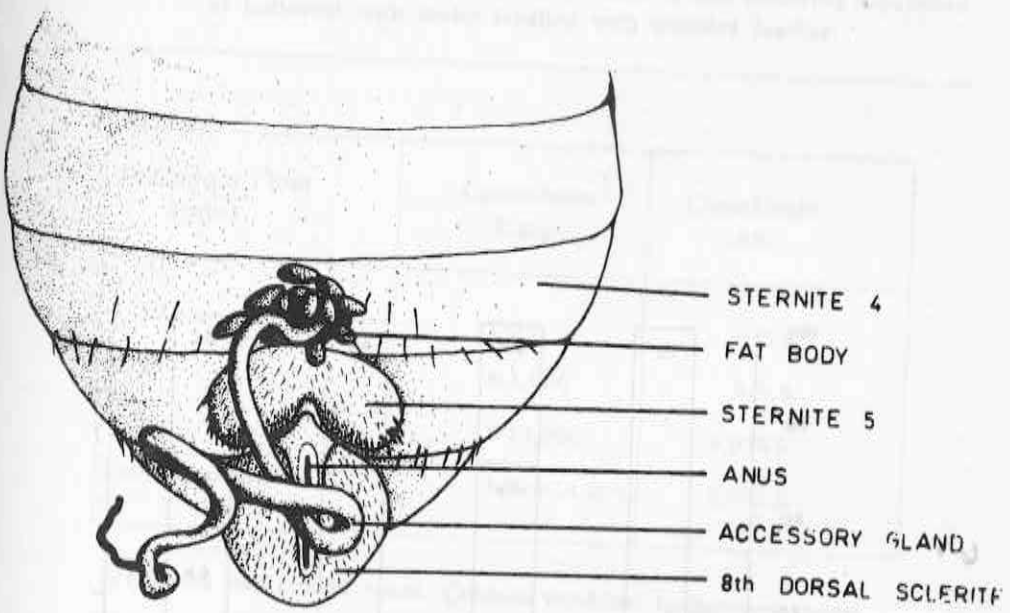


Fig. 1:

from virgin donors. Females were injected when they were 10 days old and ovulation was checked when they were 12 days old. Donors were 3 days old when mated and 4-10 days old when haemolymph was expressed from them.

RESULTS

Effects of Multiple Interrupted Matings of Shorter Duration on Ovulation

Results are presented in Fig. 2. One 15 min or 20 min mating or two 10 min matings did not result in any ovulation in mated females. However, the percentage of ovulated females increased to 100 as the cumulative *in copula* time period increased to 60-100 min for each female. Observations on females dissected immediately after such repeated short duration matings revealed no spermatophore or sperm in the females reproductive system, but the possibility of the transfer of trace amounts of the male secretion during such brief copulatory acts could not be ruled out.

Transfer of Accessory Glands Secretion During Copulation of Short Duration

Incubation time for highest incorporation of tritiated proline in a 6-day-old virgin male was found to be 6 hours. The reproductive systems of females mated with labelled males showed radioactivity irrespective of mating time (Table 1). As expected, the amount of radioactivity in the female system rose with the increase of *in copula* time showing maximum activity in the reproductive system of the females who experienced uninterrupted matings. Results clearly show that a male factor was transferred into the female reproductive system during copulation even when the *in copula* time was limited to 10 min.

Effect on Ovulation of the Females Mated with Males Lacking Accessory Glands

Results summarised in Table 2 show that the females that experience mating with -AcGL males for 40 min or more ovulated successfully whereas the females that mated less than 40 min did not ovulate.

Insertion of Glass Beads in the Uterus of Virgin Females and its Effect on Ovulation

Ten out of 15 females who received beads in the uterus retained them for more than 60 min (normal time for a successful copulation of *G. morsitans morsitans*). Eight of the 10 females successfully ovulated. Examination of the 2 females that did not ovulate revealed that the yolk incorporation in the first follicle was somewhat abnormal. Two out of 6 controls showed normal ovulation.

Results of Removal of CA or CACC and its Effect on Ovulation

Table 3 summarizes the results of the removal of CA from the premated, postmated and virgin females (-CA) and its effect on ovulation. They clearly show that the removal of CA does not have any effect on ovulation. Most of the premated and postmated operated females ovulated normally whereas operated virgins did not ovulate. Mortality

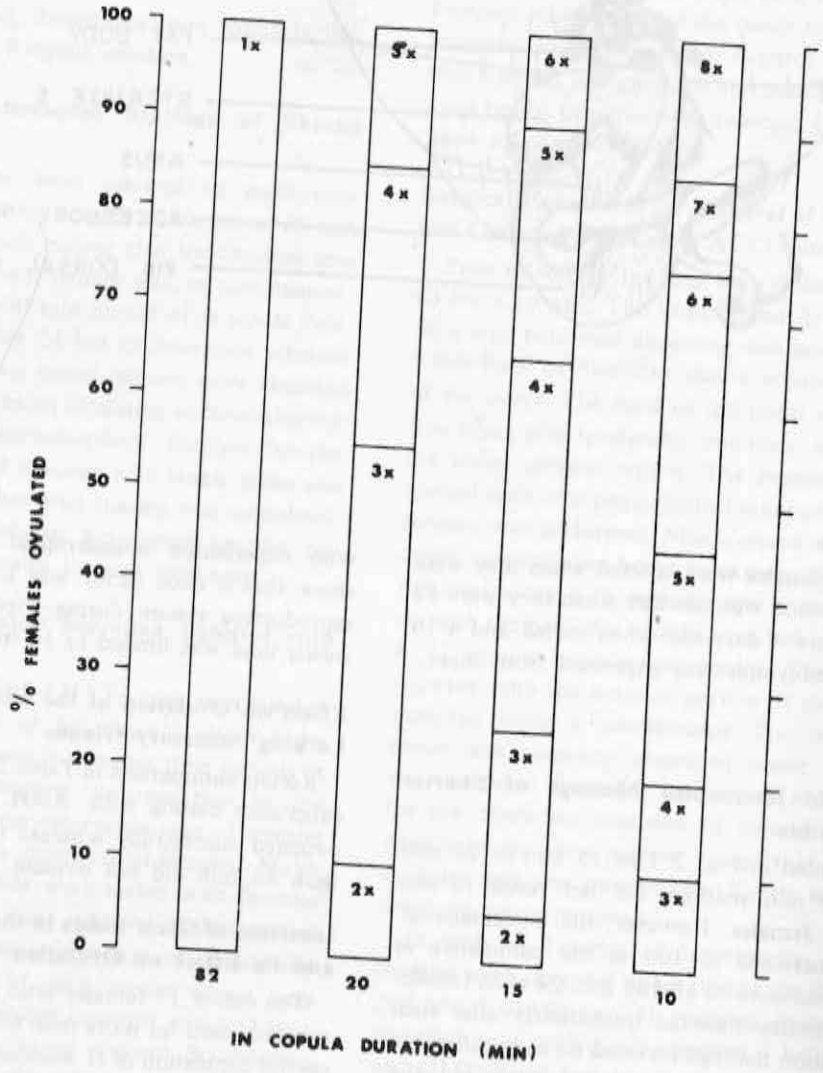

100
90
80
70
60
50
40
30
20
10
0
% FEMALES OVULATED
1x
5x
4x
3x
2x
6x
5x
4x
3x
2x
8x
7x
6x
5x
4x
3x
82
20
15
10
IN COPULA DURATION (MIN)

Fig. 2:

Table 1: Radioactivity in the female reproductive system following copulation of indicated with males labelled with tritiated leucine.

In Copula Time (min)	Count/min[1]/ Range	Count/min Av.
10	0-50	6.6
20	0-1,000	248.5
30	5-14,000	5,076.9
Uninterrupted mating[2]/	1,319-14,000	5,862.8

1/1 Above background count. Controls exhibited background counts.

2/ Complete spermatophore formation.

Table 2: Ovulation in females mated with males whose accessory glands were surgically removed.

No. Females Mated[1]/	No. Ovulated	*In Copula* Time for Ovulated Females	
		Range (min)	Av. (min)
20	12	40-110	55.3

1/ Average *in copula* time for females not ovulated was 24 min.

(due to surgery) ranged from 10-28.5% operated females and 0-10% in sham operated controls. This mortality occurred within 24 hours following surgery. A few individuals that died before the 24 hours period were found to have ovulated.

Table 4 presents the results of the removal of CACC from the premated, post-mated and virgin females (-CACC) and its effect on ovulation. The removal of the glands almost completely inhibited the ovulation in mated females whereas most of the sham operated mated females ovulated normally. Virgins, operated or sham operated did not ovulate. Mortality in -CACC females was much higher than that in -CA females (up to 60%).

Results of the CACC complex removal from 12-day-old mated females at various intervals following termination of mating and its effect on ovulation are summarized in Table 5. Ovulation was completely inhibited when CACC was removed within 4 hours following the completion of mating. However, an increase of the time interval between mating and surgery increased the incidence of ovulation. As expected, the removal of CACC 20-24 hours after mating had little or no effect on ovulation.

Table 3: Effect of the removal of CA from pre-mated, post-mated and virgin female on first ovulation

Age at Treatment (days)	No. Operated on	No. Died within 24 hrs Following Operation	No. Ovulated 1/
		A. Operated immediately after mating	
10	25	5	20 (1)
11	25	6	19 (3)
12	24	4	24 (1)
		B. Sham operated controls	
10	20	0	17
11	20	2	18 (1)
12	20	0	20
		C. Mated 4 hrs following operation	
10	30	8	18 (2)
11	35	10	25 (5)
12	35	7	28 (3)
		D. Sham operated controls	
10	25	2	20 (1)
11	30	1	29
12	25	0	25
		E. Operated virgins	
10	15	3	0
11	10	1	0
12	10	3	0
		F. Sham operated controls	
10	15	0	0
11	15	0	0
12	15	1	0

1/ **Ovulation was checked 24 hrs after the surgery. Figures in parentheses indicate number of dead females showing ovulation.**

Table 4: Effect of the removal of CACC from the pre-mated, post-mated and virgin females on ovulation.

Age at Treatment (days)	No. Operated on	No. Died within 24 hrs Following operation	No. Ovulated 1/
		A. Operated immediately after mating	
11	20	12	0
12	25	13	0
		B. Sham operated controls	
11	20	3	17 (1)
12	20	3	16 (1)
		C. Mated 4 hrs following operation	
11	30	17	0
12	30	13	3
		D. Sham operated controls	
11	20	2	17
12	20	1	16 (1)
		E. Operated virgins	
11	15	6	0
12	15	9	0
		F. Sham operated controls	
11	15	1	0
12	15	0	0

1 Ovulation was checked 24 hrs after the surgery. Figures in parentheses indicate number of dead females showing ovulation.

Table 5: Effect on ovulation, of CACC removal from 12-day-old mated females at various intervals following completion of mating.

Operated hrs after Mating	No. Operated on	No. Died within 24 hrs Following Operation	No. Ovulated 1/	% Survivals Ovulated
0	15	7	0	0
4	14	5	0	0
8	18	8	2	20
12	19	10	4	44.4
16	15	8	5 (2)	71.4
20	15	6	8 (3)	88.8
24	15	8	7 (8)	100

Ovulation was checked 24 hrs after the surgery. Figures in parentheses indicate number of dead females showing ovulation.

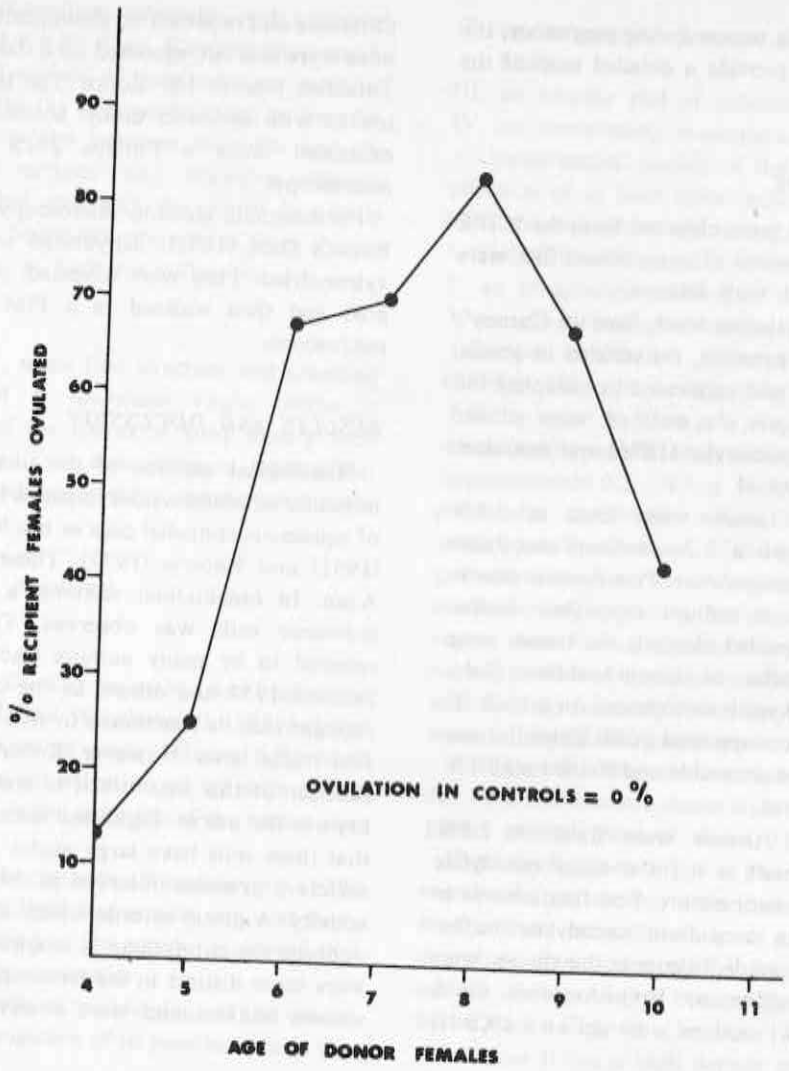


Fig. 3:

SUMMARY

Virgin female tsetse flies retain their first egg within the ovary, whereas mated females ovulate. Thus, mating is necessary for the first ovulation which initiates the reproductive cycles that follow. The component of the mating act that causes ovulation is not insemination, spermatophore formation, transfer of male accessory gland secretion or stimulus from any contact pheromone. Females subjected to 'sterile' matings show that the rate of ovulation increases with the increase of *in copula* time. These findings indicate that the stimulus for ovulation is mechanical and probably nervous. Removal of CACC complex inhibits ovulation almost completely but allatectomy does not have any effect on ovulation. Additionally, injections of haemolymph from mated females into virgins induce ovulation in the recipients; blood from virgin donors has no effect on ovulation.

Evidence suggests that afferent nervous impulses resulting from the prolonged mating as well as perhaps those resulting from the ovarian distension due to presence of a mature egg trigger the release of an ovulation-stimulating hormone from the MNC into the haemolymph, probably via the CC.

The Uterus (*Glossina morsitans*)

INTRODUCTION

The uterus in the *Glossina* species is the largest and most prominent part of the female reproductive system. Its anatomy was first described by Hagan (1951). Roberts (1972) gave an account of the anatomy of the uteri of *G. austeni* and *G. morsitans* with special reference to the ventral fold of the organ which is generally referred to as the choriothete. Roberts (1973) gave an account of the anatomy of the uterus of *G. austeni* and *G. morsitans* with respect to the processes of ovulation and fertilization.

The fine structure and detailed histology of the *Glossina* uterus has not yet been described. As a basis for future work

on changes taking place in the uterus during pregnancy, the present work is designed to provide a detailed map of the organ in a virgin female.

MATERIALS AND METHODS

G. morsitans virgin females were obtained from the ICIPE laboratory colony. The abdomens of immobilized flies were opened and quickly flooded with fixative.

For paraffin sections the tissues were fixed in Carnoy's fluid (1887) under reduced pressure, dehydrated in graded alcohols, cleared in xylene and infiltrated in paraplast (58-60)^0 C under reduced pressure, 7u sections, were stained with Heidenhain's Iron Hematoxylin (1896) and examined with a Carl Zeiss Ultraphot II.

For epoxy embedding, tissues were fixed in 2.5% Glutaraldehyde buffered with a 0.2m sodium cacodylate buffer for 3 hours at room temperature. Post-fixation was in 1% osmium tetroxide in a sodium cacodylate buffer. Following dehydration in graded alcohols the tissues were embedded in either Araldite or Epon-Araldite. Gold-coloured (500^0 A - 700^0 A) sections were cut on a LKB III ultratome and mounted on unsupported grids. Thick (lu) sections were also cut, mounted on a slide and stained with 1% Toluidine blue in 1% Borax.

For epoxy embedding, tissues were fixed in 2.5% Glutaraldehyde buffered with a 0.2m sodium cacodylate buffer for 3 hours at room temperature. Post-fixation was in 1% osmium tetroxide in a sodium cacodylate buffer. Following dehydration in graded alcohols the tissues were embedded in either Araldite or Epon-Araldite. Gold-coloured (500^0 A - 700^0 A) sections were cut on a LKB III ultratome and mounted on unsupported grids. Thick (lu) sections were also cut, mounted on a slide and stained with 1% Toluidine blue in 1% Borax.

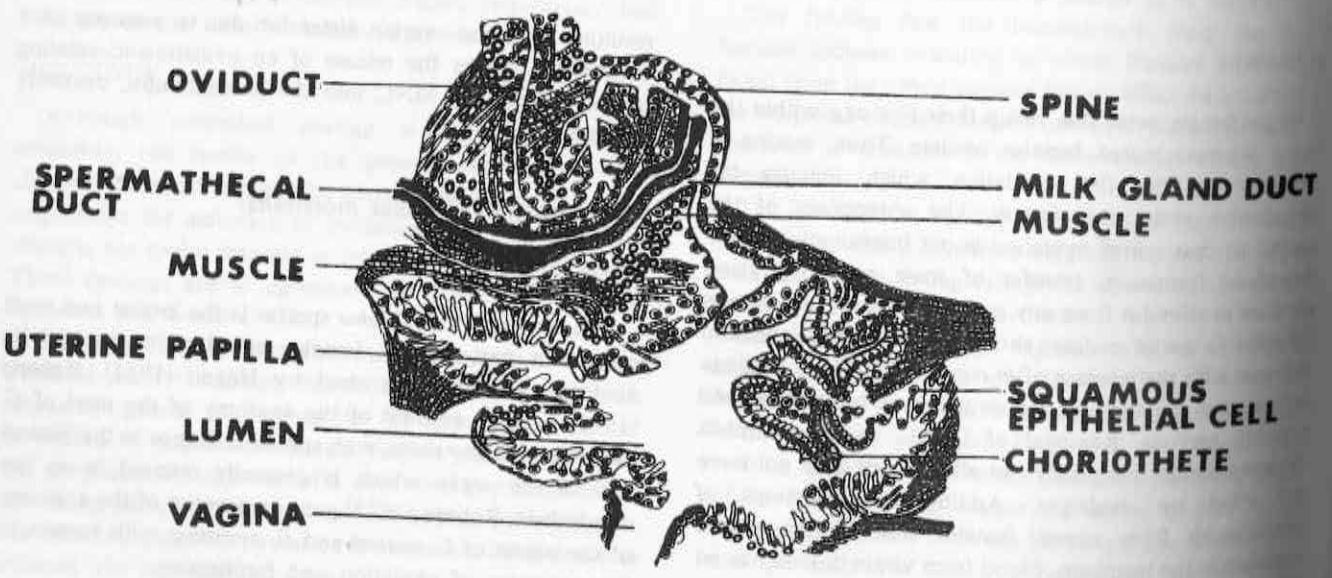
The thin sections were contrasted with saturated uranyl acetate in 50% ethanol and examined under a Phillips 291S transmission electron microscope.

For scanning electron microscopy, tissues were fixed in Bouin's fluid (1897), dehydrated in graded alcohols and xylene-dried. They were mounted, coated with carbon and gold and then scanned in a JSM 15 scanning electron microscope.

RESULTS AND DISCUSSION

Histological sections of the uterus revealed (Fig. 1) a muscular structure whose lumen is lined mostly with a layer of squamous epithelial cells as has been described by Hagen (1951) and Roberts (1972). These cells stained red with Azan. In longitudinal sections, a structure composed of columnar cells was observed. This structure has been referred to by many authors (Jackson 1948, Bursell and Jackson 1957 and others) as the choriothete because they thought that its function is to remove the chorion from the first instar larva. However, Roberts (1972) stated that the function of this structure is to support the developing embryo in the uterus. Light and electron micrographs showed that these cells have large nuclei. In electron micrographs, secretory granules observed in the cells indicated secretory activity. A group of cells which also had large nuclei were opposite the choriothete in longitudinal sections. These cells were more distinct in the Heidenhain's Iron Hematoxylin - stained sections and were observed in this region only.

FIG. I: A schematic drawing of the uterus – an oblique section of a virgin *Glossina morsitans*, Approx. x 400



The cells of the uterus are lined, internally, with a layer of cuticle which stained blue with Azan. Electron micrographs showed that the apical surface of these cells are modified into microvilli. Externally, the cells are bounded by muscles. A layer of basal lamina lies between the cells and the muscles. Histological sections and scanning electron micrographs showed that internally the uterus is greatly folded in the virgin fly. Spine-like projections from the internal wall can be seen at high magnification.

SUMMARY

The general histology, some fine structure and scanning electron microscopy of *G. morsitans* virgin uterus is described. The lumen of the uterus is lined mostly with squamous epithelial cells. Two areas seen in longitudinal sections contain cells which are possibly secretory but more work needs to be done.

The Proventriculus *(G. morsitans)*

INTRODUCTION

The proventriculus of *Glossina morsitans* was studied in detail by Stuhmann (1907) and Wigglesworth (1929) at the light microscope level. Another study (Moloo S.K. *et al* 1970) was restricted to an investigation of the cells of the annular pad and their role in the secretion of the peritrophic membrane.

Studies on digestion in the tsetse fly (Wigglesworth 1929; Olembo 1972) revealed that fluid was rapidly removed from the blood meal and that blood in the anterior region of the gut became increasingly concentrated.

The present work is a study of the histology of the whole proventriculus and an investigation of its possible role in the digestive process.

MATERIALS AND METHODS

Tenerals of *Glossina morsitans* were obtained from the ICIPE Insectary. They were anaesthetised with ether and dissected in either 2.5% glutaraldhyde containing 5% sucrose in 0.05M cacodylate buffer *p*H 7.4., or 4% glutaraldehyde without sucrose. The gut was excised, fixed for 3-7 days and washed for 48 hrs in several changes of 0.05 M cacodylate buffer. It was then divided into its different functional regions, and post-fixed in 1% OSO_4 buffered with cacodylate buffer to *p*H 7.4. This was followed by dehydration in a graded series of ethanol and the material was embedded in araldite via propylene oxide. Sections were cut with glass knives on an LKB III Ultratome, collected on 300 mesh grids, stained first with saturated uranyl acetate in 50% ethanol for 20 minutes and then for 5 minutes in Reynold's lead citrate. Sections were examined using a Philips 200 in Prof. D.S. Smith's laboratory in Miami-Florida, and Philips 201S at ICIPE - Nairobi.

RESULTS AND DISCUSSION

Light microscope studies show that the proventriculus (fig. 1) comprises:

I. definitive midgut cells,
II. an invaginated portion of the foregut (the posterior limit),
III. an annular pad of columnar epithelial cells,
IV the surrounding musculature.

Ultrastructural studies of the proventriculus confirm the presence of at least three cell-types, corresponding to the light microscopic divisions viz:

1. Definitive midgut cells – cell-type I
2. an invaginated portion of the foregut – cell-type II
3. an annular pad of columnar epithelial cells – cell-type III

Further studies on cell-type II are still essential to establish either its homogeneity or heterogeneity.

Cell - type I: This is a single layer of columnar cells whose apical membrane is arranged into regular microvilli, approximately 0.2 - 0.3 μ wide bordered by a bilaminated peritrophic membrane, which at places folds to form a multi-layered structure. Occasionally a few secretory granules are observed at the bases of the microvilli. The mitochondria appear to be more numerous at the apical region than around the basal lamina. The basal infoldings do not extend to the apical surface. The nucleus is more or less centrally located. This cell-type is bounded by circular and/or longitudinal muscles.

Cell-type II: The apical surface of this cell-type is bounded by a thin electron dense layer below which is an amorphous ground mass of about 0.01 μ thickness (Fig. 2)

Typical of this cell-type is the highly convoluted apical membrane, extending into basal infoldings. It has a pronounced intercellular space which contains course strands of granular material. It was observed that the apical surface of this cell-type became re-arranged, forming membrane stacks (Fig. 3).

Cell-type II has a high density of microtubules. The role of these could be skeletal and/or conductive.

In a transitional region this cell-type merges into cell-type III and is ligated by a double layer of perhaps epicuticular material. The microvilli of cell-type III in this region lack the bilaminated peritrophic membrane both in fed and unfed flies. This would suggest that cell-type III cells in this region do not participate in the formation of this membrane and that the peritrophic membrane is not pushed toward this area. This 'open' end could be a possible site for the infiltration of the trypanosomes into extoperitophic space.

Cell-type III: are epithelial cells, ending in microvilli, which tend to conform to the curve between cell-type II and cell-type III. The lengths of the microvilli vary. The rough endoplasmic reticulum is well-developed.

There are signs of activity even in unfed flies as is evidenced by the presence of lysosome-like granules. This activity is greatly increased after a blood meal. After feeding there is a more than fourfold increase both in the Golgi-secretion complexes and lysosome-like granules. The nature and role that these lysosome-like secretory granules play in the digestion of the blood meal is being investigated. This is considered a likely site for the synthesis and secretion of material for the lysis of the blood corpuscles in preparation for digestion. The presence of neurosecretory granules indicates that the proventriculus and cell-type II and III are innervated. The site of release and possible function of these granules in relation to digestion are being investigated and a

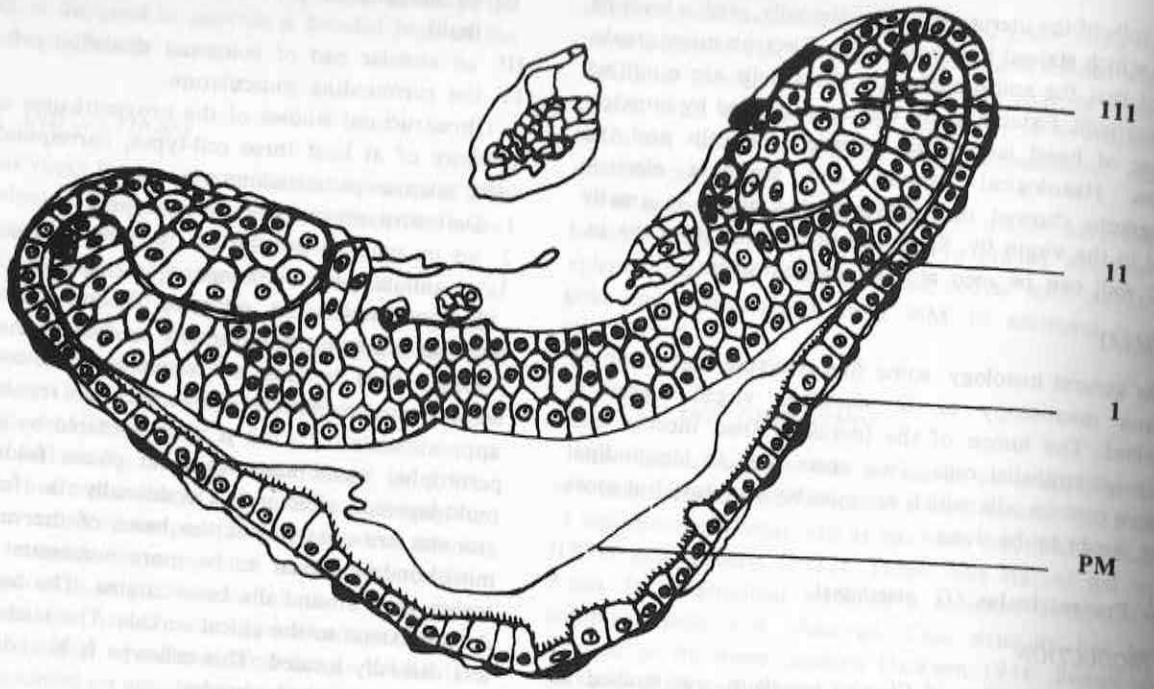


Fig. I. Schematic diagram of a cross-section of the Proventriculus of *G. morsitans* showing the three cell-types 1, 2 & 3 and the (p.m.) peritroptic membrane.

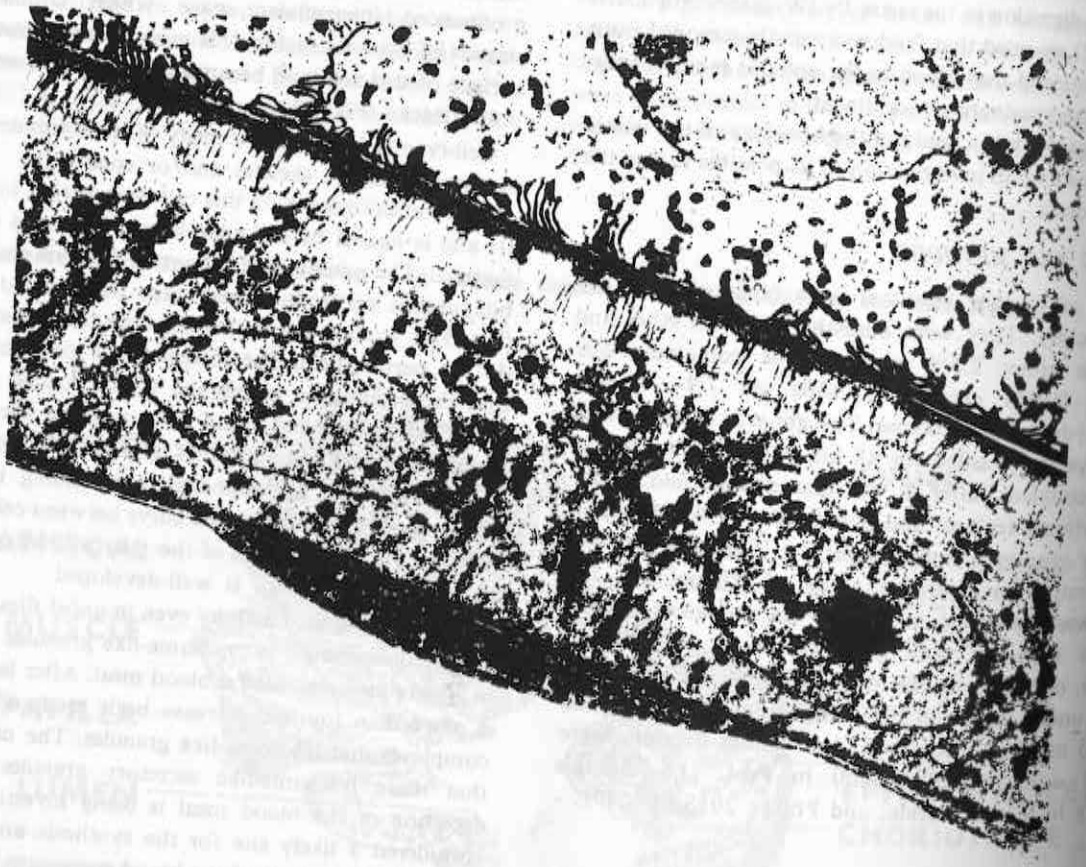


Fig. 2: Transmission Electron micrograph x 8,500 showing two adjacent cell-types 1 & 2. The apical surface of cell-type II end in amorphous ground mass bounded by electron dense layer. Note the microvilli, on the apical surface of cell-type 1.

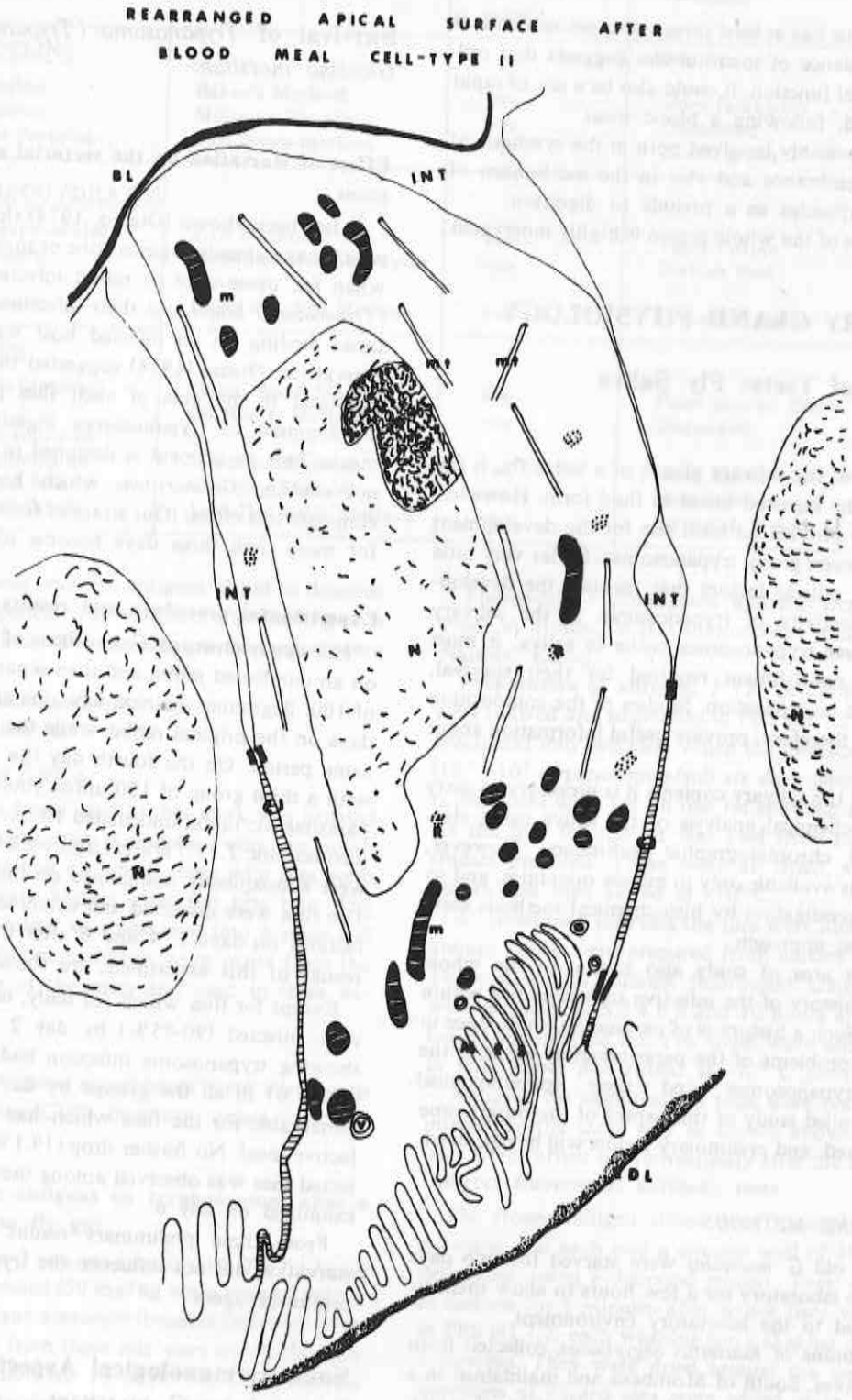


Figure 3: Diagramatic representation of rearranged processes MES, noted in cell-type II after a blood-meal. R-ribosomes, mtmicrotubules, m-mitochondria, int intercelluslar space, EDL- electron dense layer, BL - basal lamina, V vesicles, CJ - cell-junction & N - nucleus.

study to ascertain the origin of this nerve is currently underway.

SUMMARY

The proventriculus has at least three cell-types named I, II and III. The abundance of microtubules suggests that cell-type II has a skeletal function. It could also be a site of rapid absorption of fluid, following a blood meal.

Cell-type III is possibly involved both in the synthesis of the peritrophic membrance and also in the mechanism of lysis of blood corpuscles as a prelude to digestion.

The musculature of the whole orgain is highly innervated.

SALIVARY GLAND PHYSIOLOGY

Biochemistry of Tsetse Fly Saliva

INTRODUCTION

The chief role of the salivary glands of a tsetse fly, is the maintenance of the ingested blood in fluid form. However, these glands also provide an ideal site for the development of the infective *brucei* group trypanosomes. So far very little is known of the critical factors that regulate the development of the infectivity of trypanosomes in the salivary glands. Since these trypanosomes bathe in saliva, it must contain all the nourishment required for their survival, development and multiplication. Studies of the composition of saliva would, therefore, provide useful information about these factors.

For a study of the salivary contents it is necessary to carry out detailed biochemical analysis of the saliva using electrophoretic and chromatographic techniques. However, tsetse fly saliva is available only in minute quantities, and so a preliminary investigation by histochemical methods seem to offer the best approach.

An important area of study also is that of the whole developmental history of the infective trypanosomes within the tsetse flies. Such a history is of paramount importance in unravelling the problems of the parasitological status of the tsetse-borne trypanosomes and their immunological response. A detailed study of this aspect of the programme has been launched, and preliminary results will be discussed.

MATERIALS AND METHODS

Two weeks old *G. morsitans* were starved for two days and kept in the laboratory for a few hours to allow them to get acclimatised to the laboratory environment.

Wing membrane of *Rousettus aegyptiacus*, collected from the Similani caves, South of Mombasa and maintained in a bat house at ICIPE were used for the collection of saliva. Salivary secretions were obtained from these flies using the technique developed at ICIPE by Youdeowei (1973) and analysed using thin layer chromatography and acrylamide electrophoresis.

Preliminary results (Table 1) show that tsetse saliva contains basic proteins and electrophoretic analysis suggests the presence of six proteins.

Besides proteins; lipids, fatty acids and phospholipi have been identified in the saliva.

However, of particular interest is the identification carbohydrates, mainly polysaccharides and glycogen.

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

Effect of starvation on the vectorial capacity of *G. mor- sitans*

It has been shown (Otieno, 1974) that *Glossina morsitans* which have already ingested one or more clean blood meals when fed upon mice or rabbit infected with *Trypanosoma (Trypanozoon) brucei* lose their infections more rapidly than those feeding on an infected host within 24 hours after emergence. Otieno (1974) suggested that a toxic substance produced in the guts of such flies prevents the normal development of trypanosomes ingested with subsequent meals. This experiment is designed to find out whether or not starving *G. morsitans* which have been fed would eliminate this effect. (Our strain of *G. morsitans* when starved for more than three days become too weak to feed).

Experimental procedure and results

200 newly-emerged *G. morsitans* of both sexes were fed on an uninfected rabbit and then separated into two groups of 100. Beginning the next day, one group was fed for two days on the original rabbit while the other starved for the same period. On the fourth day the two groups, together with a third group of 100 unfed young flies, were fed on a parasitaemic rabbit (inoculated 10 days earlier with a highly pleomorphic *T. (T.) brucei* Lambwe strain). The three groups were subsequently maintained on the original clean rabbit. The flies were dissected and examined for trypanosome infections on days 2, 4 and 6 after the infective meal. The results of this experiment are shown in Table II.

Except for flies which fed daily, nearly all the other flies were infected (90-95%) by day 2. The number of flies showing trypanosome infection had dropped considerably (19-38%) in all the groups by day 4. But the drop was remarkable for the flies which had starved before the infective meal. No further drop (19.1%) in the number of infected flies was observed among these flies when they were examined on day 6.

From these preliminary results it would appear that starvation did not influence the trypanocidal effect of this presumed agent.

Some Immunological Aspects of Trypanosomes Ingested by *G. morsitans*

The transformation from bloodstream to culture or midgut forms *T.T. brucei* trypanosomes involves significant structural and physiological changes (Vickerman, 1971). These changes include the loss of surface coat and consequently the variable antigens. The present study was undertaken to examine whether the

Table 1: Summary of Results from histochemical analysis of tsetse saliva

Constituent	Staining Method	Result (+ve) −ve)	Remarks
PROTEINS			
Tyrosine	Baker's Method	+ve	Very faint pink
Tyrosine	Million's Reagent	−ve	Unstained
Basic Proteins	Fast green method	+ve	Bluish green
CARBOGYDRATES			
Polysaccharides	PAS Method	+ve	Faint Purple
Glycogen	Carmine—Haemotoxylin Method	+ve	Pinkish Red
Glycogen	Lugol's Iodine Method	+ve	Brownish black
LIPIDS			
Lipids (general)	Sudan Black Method	+ve	Faint greyish Black
Fats	Oil Red O Method	−ve	Unstained
Fatty acid and Phospholipids	Nile Blue Method	+ve	Light Blue
Neutral fats	Nile Blue Method	−ve	Unstained
Phospholipids	Acid-Haematin Method	+ve	Brownish Black

loss of surface coat (variable antigens) could be detected in organisms ingested by *G. morsitans* after feeding on a parasitaemic animal. Two serological techniques were employed.

1. indirect fluorescent antibody test
2. direct agglunation test

MATERIALS AND METHODS:

Organisms. *T. (T.) brucei* used in this work was isolated from *G. pallidipes* caught from Lambwe Valley, in South Nyanza, Kenya. The rabbit on which these wild flies were fed became parasitaemic 8 days after the first bite. The blood of the infected rabbit was passaged into 4 mice and six days later, trypanosome stabilates were made from the infected blood of one of the mice and used in these experiments.

Flies

G. morsitans used in these experiments were all tenerals bred and reared at the ICIPE insectary, unless indicated otherwise.

Detection of variable antigens on trypanosomes after a brief sojourn in tsetse fly gut

Preparation of sera: 2 rats inoculated with *T. (T.) brucei* Lambwe strain were treated (50 mg/kg body weight) with a single dose of diminazene aceturate (berenil) five days after inoculation. Tail blood from these rats were examined daily for trypanosome infection for 13 days. No trypanosomes were seen in these animals and on the 14th day, the rats were challenged with the same stabilate materials. But no infection was detected when immune sera was collected seven days later. The serum collected was pooled and stored at -20° C for later serological analyses.

Normal sera was collected from the above rats through the retro-orbital plexus some four days before they were inoculated with trypanosomes.

Fluorescein – conjugated antisera were obtained from Prof. V. Houba, WHO Immunology and Training Centre, Nairobi, Kenya.

Preparation of antigen. *T. (T.) brucei* stabilate materials were thawed and suspended in PBS pH 7.2 and immediately inoculated into two rats. When the infection reached a peak (10^6 - 10^7 trypanosomes/ml) six days later, newly emerged *G. morsitans* were fed on one rat showing heavier infection. As the flies fed the rat was bled from the tail and blood smears prepared. These were air dried, wrapped in filter papers and then stored at -70° C.

At given time intervals the flies were killed in groups and antigen slides were prepared from smears of gut contents. These slides for indirect fluorescent antibody tests (IFA) were prepared at 0,2,4,6,8 and 10 hours after the flies had fed on the infected rat. The slides were dried and wrapped in filter paper and stored at -70° C until ready for use.

Antigens for agglutination tests were obtained from flies infected in the same way as shown above except that the tests were carried out immediately after the flies were killed.

Indirect fluorescent antibody tests

The frozen antigen slides were brought to room temperatures and each had a circular well of 10 mm diameter delineated, using a diamond marker. They were then fixed in acetone for 5 minutes after which they were washed 3x in PBS *p*H 7.2, each washing (on a shaking machine) lasting 5 minutes. They were dried around the wells, and 25 μl quantities of diluted sera were added using a micropipette. The slides were then incubated in a moist chamber kept at 37° C for 30 minutes after which they were washed 3x in PBS with 5 minute changes. They were again dried around the wells and fluorescein-conjugated, diluted anti-rat globulin serum added onto the wells before another incubation in a humid chamber maintained at 37° C for 30 minutes. The slides were then washed and finally mounted in PBS *p*H 8.6 glycerine medium. They were examined on a

Leitz Ortholux fluorescence microscope with an Osram HBO-200 light source.

Dilution of serum samples. The frozen serum samples were thawed and then 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, dilutions made using the loop microtitration technique commonly used in virus titrations.

Agglutination tests: Direct agglutination tests on slides were carried out using the method described by Cunningham and Vickerman (1962). The same antisera used for IFA test was also used for agglutination reactions. The antisera was diluted as follows: 1:10, 1:20, 1:40, 1:80. Live trypanosomes, obtained from fresh gut contents of flies killed at 0, 2, 5, 7, 18, 25, and 48 hours after an infected meal were mixed directly with 50 ul quantities of the serially diluted antisera. The samples were then incubated in a humid chamber at room temperature for one hour. The slides were then examined under low power (16x) microscopy for direct agglutination. Samples from four flies were examined at the various time intervals.

RESULTS

IFA tests: Trypanosome smears obtained from flies dissected at 2, 4, 6, 8 and 10 hours after feeding were tested for IFA to see if they still retained the surface antigens had before being ingested. Samples from 10 flies examined during each examination interval (0, 2, 4, 6, 8 10 hours after feeding).

The results of these tests are presented in Table III. control samples using normal sera were negative. trypanosome smears obtained from the rat before the were fed, gave positive reaction (bright-green colour w examined under UV light source) at the serum dilut tested. Samples obtained from flies 2 hours after feed gave positive reactions up to 1:640 dilution and those tes at 4 to 8 hours after feeding gave progressively weaker re tions and lower end points.

Preparations of trypanosome smears from flies dissected hours after feeding gave very strong positive reactions pa ticularly around the posterior end of the trypanosomes (ca ping effect?)

These results show that ingested trypanosomes lose the antigenic constitution very soon after being ingested. Thes observations compare favourably with the results obtaine on the loss of infectivity of *T. (T.) brucei* after a brief sojour in the tsetse gut. It was (Otieno, 1973) noted in the ICIPE Annual Report 1973 that, after only a brief period in the tsetse gut (1 hour), 1 in 50 organisms was infective to mice.

Agglutination tests on ingested trypanosomes

Positive control tests were done using infected blood at

Table 11: A comparison of the rate of *T. (T.) brucei* infections in *G. morsitans* fed daily, those which have been starved after an initial food and teneral flies feeding on a parasitaemic rabbit.

Day of examination	Treatment before infective meal					
	Fed daily for 3 days		Fed ones, then starved for two days		Not fed previously (newly-emerged)	
	No. Exam.	% infected	No. Exam.	% infected	No. Exam.	% infected
2	26	65.4	23	95.7	32	90.6
4	23	30.4	21	19.1	34	38.2
6	26	11.5	21	19.1	30	30

Table III: **Tests for IFA from trypanosomes obtained from *G. morsitans*. Teneral flies had previously been fed on *T. (T.) brucei* infected rat**

Samples tested at hours after feeding	Samples tested at dilutions							
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
0	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	—	—
4	+	+	+	+	—	—	—	—
6	+	+	—	—	—	—	—	—
8	+	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—

peak infection. The infected blood was incubated with serially diluted antisera. For later tests (using trypanosomes obtained from tsetse gut), gut contents from a freshly dissected fly were tested. The results of these tests are summarized in Table IV.

The samples examined in the first 5 hours gave positive reactions but thereafter a pro-zoning effect was observed throughout the experimental period (e.g. negative at 1:20 but positive at 1:80). This phenomenon may be due to different rates at which each fly degrades these antigens. It is, however, interesting, to note that clumps of agglutinated trypanosomes could still be observed 48 hours after ingestion.

Development of *T. (T.) brucei* in *G. morsitans* Haemolymph

The life cycle of *T. (T.) brucei* in the tsetse fly as it is known to date confines (the entire cycle of development to the alimentary canal and salivary glands. Recently (Mshelbwala, 1972; Otieno, 1973) there have been reports of *brucei* trypanosomes occurring naturally in tsetse haemolymph. In order to verify this as a possible site of development we, in preliminary experiments, introduced blood forms of *T. (T.) brucei* into the haemocoel of *Glossina morsitans morsitans* (Westwood) and noted the subsequent events.

Experimental procedure and results

A highly pleomorphic *T. (T.) brucei* Lambwe strain from heavily parasitaemic mouse, was freed from blood cells and serum and then suspended in phosphate buffered saline (PBS) pH 7.2. The concentration of trypanosomes in the suspension was adjusted to $1x10^7$ organisms/ml. Aliquots (1 to 2μ) of this suspension were sucked by capillarity into glass micro pipettes with an opening measuring 4μ. These aliquots were then inoculated into the ventro-lateral side of the mesothorax just beneath the cuticle of young unfed *G. morsitans*, using a slight modification of the method described by Weathersby (1952). The flies were later fed on a clean rabbit and then transferred to our Insectary and maintained at 25° C and 60-80% Relative Humidity. They were fed daily on the same rabbit throughout the experimental period. The rabbit was found to be infected 18 days after its first exposure. At the time 43 of the 89 inoculated flies were still alive and of these one (2.33%) was found to have trypanosomes both in the proboscis and gut.

The experiment was repeated using the same strain of trypanosomes. After inoculation, the flies were separated into two groups: one group was fed daily on a clean rabbit for 10 days after which the host was changed every second day. Unfortunately the rabbits started dying from unknown cause 16 days after the beginning of the experiment. The flies which survived (59 out of 76 inoculated) up to day 20 were dissected and examined for trypanosome infection.

Table IV. Tests for agglutinins on trypanosomes harvested from the gut of recently fed young *G. morsitans*. Young fl had previously been fed on rat infected with *T. (T.) brucei*.

Trypanosomes tested at hours after ingestion	Samples tested at dilutions			
	1:10	1:20	1:40	1:80
0	+	+	+	+
2	+	+	+	+
5	+	+	+	+
7	+	—		+
18	+	+	+	+
25	—	+	+	—
28	—	—	+	+
48	—	—	+	—

Table V. The ability of *G. morsitans* to host *T. (T.) brucei* introduced through the haemolymph. The flies were inculated with blood forms of *T. (T.) brucei* and examined for trypanosome infection about three weeks later.

Trial No.	Flies inoculated	Flies examined	Flies with infection in				% infected
			Proboscis	Salivary glands	Gut	Haemolymph	
1	89	43	1	—	1	—	2.33
2	76	59	1	—	2	—	3.39
Total	165	102	2	—	3	—	2.92

Two (3.39%) had gut infection and one of these two had a proboscis infection as well. Table V ummarizes the results of both experiments.

The second group was examined in daily batches of 5 to 6 flies for haemolymph infection over a period of 10 days. Haemolymph was collected from each fly using the method described by Mshelbwala. The fluid was mixed with two drops of PBS ans successively sucked into 1 ml syringe. The entire sample was then inoculated intraperitoneally (IP) into a clean mouse. The inoculated mice were examined for trypanosome infection five days later and thereafter twice a week for one month. Parasitaemic mice and any mouse not infected by the end of the experiment were destroyed. Table VI summarizes the results of the experiments.

Infected and uninfected mice were used as the criteria for determining which flies were harbouring infective trypanosomes. As Table VI shows, infective trypanosomes were detected from inoculated flies up to day six after inoculation. Blood stream form trypanosomes were detected in the haemolymph throughout this period. The failure to demonstrate any trypanosomes in the flies after that period made it impossible to assess the length of time the blood-stream forms were capable of retaining their infectivity and morphology while in this environment. Table VII, however, shows that the ratio of blood stream forms was highest on day 1. But numbers declined as they changed into typical vector form and also into forms not usually seen in the tsetse flies.

The ability of trypanosomes to invade the proboscis of these flies strongly indicates that these organisms developed normally through an apparently abnormal environment. More evidence for this indication is the fact the rabbit on which the flies fed was found infected 18 days later. It seems therefore that migration through the haemolymph is possibly a natural route for the trypanosomes which so far has escaped the notice of many researchers.

The multiplication of tsetse-borne salivarian trypomastigotes, is generally known as occuring by equal binary fission in the trypomastigote stages. But our findings suggest that, other forms of multiplication may be taking place in the haemocoel. On day 4, after introduction of blood stream forms into the haemolymph, various morphological forms which occured were seemingly still undergoing some kind of multiplication.

Two types of reproduction were observed:

1. The cell first rounds off, forming many nuclei. The daughter cells are then flagellated and may be released as flagellated round (Sphaeromatigote) cells or may be released in the non-flagellated form. It is thought that the daughter cells elongate and assume typical trypomastigote forms.
2. The cells become very much enlarged and multinucleated with many free flagella. The daughter cells later separate and become filiform trypomastigotes.

Distribution of *T. (T.) brucei* in *G. morsitans* haemocoel

The finding that *T. (T.) brucei* naturally invaded the haemocoel of *G. morsitans* aroused interest as to how they got into this compartment. In preliminary attempts to answer this question, infected flies are being examined histologically for the presence of trypanosomes in the haemocoel.

MATERIALS AND METHODS

The trypanosome strain was same as used above. Young *G. morsitans* were fed on a rabbit infected with *T. (T.) brucei*. At various time intervals after the infective feed groups of flies were immobilized by cold exposure when wings and legs were removed prior to fixation in Duboseq Basil's modification of Bouin's fixative. After fixation the thorax of each fly was separated from the rest of the body, dehydrated and embedded in paraffin.

Serious problems have been experienced in cutting tissues embedded in paraffin. Peterfi's double embedding method is at the moment being tried.

Table VI. The ability of *G. morsitans* inoculated with blood forms of *T. (T.) brucei* to retain infective trypanosomes. The flies were examined in groups over a period of 10 days.

Days after inoculation	Flies examined	Flies with tryps in the haemolymph	Flies with infective tryps
1	5	5	5
2	6	6	6
3	5	4	4
4	5	3	3
5	5	1	1
6	5	1	1
7	5	—	—
8	5	—	—
9	5	—	—
10	5	—	—

Table VIII: A Comparison of *Trypanosoma spp.* infection rates in wild *G. pallidipes* at Lambwe Valley and Kibwezi forest.

Locality	Number of flies examined	Site of infection				Total infected	% infected
		proboscis	Gut	Salivary	Haemolymph		
Lambwe Valley	955	111	47	2	3	131	13.7
Kibwezi forest	491	11	6	—	—	17	3.5

The distribution of *Trypanosoma ssp.* among infected *G. pallidipes* was as follows:

Trypanosoma spp	Lambwe Valley	Kibwezi forest
T. congolense	29.0%	35.5%
T. vivax	35.9%	29.3%
T. brucei	35.1%	35.8%

Field Studies

As a result of some of our laboratory observations, it has been necessary to do some field studies on some aspects of the biology of *Glossina pallidipes*. Two contrasting areas in Kenya where large population of the flies are usually common were selected and the studies were carried out within a one month period (August 1975).

1. Lambwe Valley, South Nyanza, was visited during a normally dry season in Kenya but which at the time had an unusual wet period in which the thickets infested by the flies were very green at the time of study.
2. Kibwezi forest, Machakos District on the other hand was in effect in a period of prolonged drought and almost all vegetation was devoid of green leaves.

The main purpose of the field work was to study in particular:-

a. The incidence of *Trypanosoma spp.* infections in *G. pallidipes*
b. *Trypanosoma spp.* infecting these flies.
c. Incidence of haemolymph infection among infected *G. pallidipes*
d. The use of bat wing membrane as a method of isolating infected flies.

Results and Observations

Langridge traps were used at Lambwe Valley, whereas "fly-round" hand-net technique was used at Kibwezi forest. The langridge traps at the Veterinary laboratory at Kiboko were damaged and could not therefore be used at Kibwezi.

In both Lambwe Valley and Kibwezi forest flies were caught in the mornings.

Tsetse fly population

Lambwe Valley

Generally there was a high population of flies in the area and of interest was the fact that 90% of the flies caught inside the traps were females while those collected on the screen of the traps were mainly males. Males seemed to perch on the screen but did not actually crawl into the traps, unlike the females. A majority of the females caught were pregnant and when they were taken to the laboratory there, was a high rate (about 75%) of abortion of larvae at various stages of development. This was particularly striking and deserves further study to find out why mere collection and handling of pregnant wild flies resulted in abortion. According to the tsetse control officer at Sindo, no pupae have ever been collected from the field at Lambwe Valley yet the flies continue to breed successfully.

We made some attempts to search for pupae but this proved fruitless and was abandoned. Where the flies larvipost at Lambwe remains unknown.

Kibwezi forest

The population of tsetse at the Kibwezi forest was much lower than at Lambwe Valley. However, unlike Lambwe where only *G. pallidipes* were collected, two species namely *G. pallidipes* and *G. brevipalpis* were collected at Kibwezi. *G. longipennis* also reportedly occur there but we did not collect any specimens.

The sex ratio of flies collected was: Females: Males, 1:1.07. Thus, there was an equal number of the sexes collected in the fly round. Gatehouse (1972) states that "samples of many species collected by fly-rounds are found to be predominatly male (usually more than 80%)" The sex ratio of 1:1.07 obtained during our work at Kibwezi departs significantly from the above statement.

Out of the 501 flies *(G. pallidipes)* collected from the field (2%) died by the time the flies were transported to the laboratory, leaving 491 flies to be dissected and studied. This number contained 48 (20.3%) pregnant females but not a single case of abortion was recorded in striking contrast to the case at Lambwe. However, these results cannot really be compared because different methods were used in collecting the flies and at Lambwe no actual count of the pregnant females and abortions was made. Attempts to collect *G. pallidipes* pupae were unsuccessful.

Trypanosome infection rates

A total of 955 and 491 adult *G. pallidipes* were dissected at Lambwe Valley and Kibwezi forest respectively. For each fly a smear of the haemolymph was made on a microscope slide and then the proboscis, salivary glands and entire gut dissected out in PBS on a microscope slide and examined under the microscope for trypanosome infection. The haemolymph smears were fixed and stained in Giemsa for examination later. The results are presented in Table VIII.

No trypanosomes were observed in the haemolymph or salivary glands of the flies examined from Kibwezi forest. However, 0.31% of the flies examined from Lambwe Valley had haemocoelic infections and 0.21% salivary gland infections all of which were *T. brucei* infections.

Presence of trypanosome in *G. pallidipes* saliva

The bat wing membrane technique was used to test its efficiency with regard to identifying wild flies with trypanosome infection. The flies were made to salivate and the salivary drops were fixed in methanol, dried and stained with Giemsa. The slides were later examined for the presence of trypanosomes. Out of 212 flies examined at Lambwe 15 (7.1%) were found infected with either *T. brucei, T. congolense* or *T. vivax*. Only 1 specimen (0.05%) had salivary gland infection. At Kibwezi forest 229 flies were examined and 4(1.7%) were found infected with the three species of trypanosomes named; there was no salivary gland infection. In cases of *T. vivax, T. congolense* or *T. brucei* in the proboscis, few parasites were ejected with the saliva. But in the single case of gland infection, numerous trypanosomes were ejected with the saliva.

Transportation of tsetse flies

We found out that when wild *G. pallidipes* were kept in complete darkness in a moist chamber, they survived without food for over one day. But when exposed to light they were continuously active and after some time were exhausted and fell on their backs. At this stage they could neither feed nor fly and eventually died.

96 flies (51 males 45 females) were transported in the dark to Nairobi from Kiboko. The flies were collected a day before they were transported to Nairobi and only 36.5% mortality occurred. Compared with our earlier experiences this was regarded as a success particularly considering that the flies had been caught the previous day.

Saliva secretion in wild *Glossina pallidipes* austen

INTRODUCTION

The diseases, trypanosomiasis and nagana still constitute a serious threat to human life and livestock farming in tropical Africa. A recent survey by a research team from the Tsetse fly Salivary Gland Project of the International Centre of Insect Physiology and Ecology, (ICIPE), Nairobi, Kenya recorded 10 cases of human sleeping sickness and several cases of cattle disease in the Lambwe Valley area of Kenya. Inspite of considerable research into the biology of the vectors, *Glossina spp*, there seem to be important gaps in our knowledge of the biology of these dipteran vectors. For instance it is only recently that studies have commenced of the process of salivation by tsetse flies (at the ICIPE Research Centre, Nairobi, Kenya – See Youdeowei 1973, 1974, 1975). Salivation by the tsetse fly is an essential component of the transmission of tryponosome parasites from an infected fly into the vertebrate host. Work with laboratory bred *G. austeni, G. morsitans* and *G. pallidipes* showed that the quantities of saliva secreted by those flies increased with the intensity of hunger. An important finding was that the first salivary drop was 2.5 times the size of any subsequent drop, indicating that the salivation which accompanies the first probe is likely to transfer a large quantity of trypanosomes even though feeding may not ensue (for details see Youdeowei 1974,1975). It was not certain whether this observations recorded with laboratory bred flies would necessarily apply to wild collected flies and it had not yet been possible to study the salivation behaviour of infected flies due to their scarcity. The work reported here concentrates on a study of the salivation behaviour of wild flies (including infected ones) and compares the results with those obtained for laboratory bred flies. Attention has been focussed mainly on *Glossina pallidipes* which is an important vector of trypanosomiasis in Kenya.

MATERIALS AND METHODS

The Study Area

The tsetse flies used for this work was *Glossina pallidipes* collected from the Lambwe Valley. Observations were also made on specimens of the same species at the Kibwezi forest.

Trapping of Flies

The langridge traps were used in the Lambwe Valley where the fly population was high and the "fly-round" hand net used at Kibwezi where the fly population was not as high. The flies were sexed ad kept individually in plastic tubes (4.2cm x 3.2 cm) with nylon gauze at the two ends. They were then taken to the laboratory where observations were made.

Collection of Saliva

Saliva was collected on clean microscope slides from each fly by the batwing membrane technique (Youdeowei, 1975a). The saliva was fixed for 1 minute in methanol and stained for 40 minutes with Geimsa's stain and then air dried.

In some slides the number of salivary drops secreted per minute was counted and recorded. They were then drawn with a camera lucida to a magnification of X 100 and the area of each drop measured with a plannimeter and recorded.

All slides were then examined under oil immersion magnification for the presence of trypanosomes. The number of parasites in the saliva was recorded.

RESULTS

Salivation in relation to hunger

The effect of the hunger stage on the amount of saliva produced by female *G. pallidipes* was studied. The stage of hunger was determined by a simple method after Nash (1969). Three hunger stages were recognised as follows:

Stage 1. Starved; with the abdomen empty and curved ventrally.

Table IX: The effect of hunger stage on salivation in wild female *G. pallidipes*

Hunger Stage	Mean ± S.E. Area of salivary drop	Difference	t	No of drops
1	25.79 ± 1.96 cm^2	6.67	2.83 P > 001	50
2	19.03 ± 1.33 cm^2			50

Table X: The proportion of wild *G. pallidipes* not salivating at 2 stages of hunger. The number of flies examined are given in brackets.

Sex	% not Salivating	
	Stage 1	Stage 2
Males	22.9% (87)	50% (8)
Females	32.4% (71)	66.0% (50)

Table XI: The number of salivary drops secreted per minute by male and female *G. pallidipes* at Lambwe.

SEX	Mean ± S.E. no Salivary drops/min	Difference	t	No. of flies tested
Males	7.9 ± 0.63	0.59	0.168 NS	87
Females	7.32 ± 0.78			71

NS = not significant

Table XII: The Relative sizes of Salivary drops secreted by both sexes of wild *G. pallidipes* at Lambwe.

Sex	Mean ± S.E. Area of salivary drop	Difference	+	No. of drops measured
Males	19.43 ± 1.14 cm^2			100
Females	24.24 ± 1.42 cm^2	4.81	2.69 ($P < .01$)	100

Table XIII: Comparison of the sizes of salivary drops secreted by free and infected wild *G. pallidipes*. Data from Lambwe and Kibwezi pooled.

State of flies	Mean ± S.F. Area of salivary drop	Difference	t	No. of measured
Infected	20.18 ± 2.9 cm^2	1.49	0.38 NS	25
Free	21.67 ± 2.7 cm^2			25

NS = Not significant

Stage 2. Intermediate – with abdomen not distended and not empty.

Stage 3. Gorged; with abdomen distended and full of ingested blood.

In all the samples from Lambwe only hunger stages 1 and 2 were seen. For each hunger stage, 50 salivary drops were measured from flies and the data is presented in Table IX. The figures presented in table IX, XII and XIII are relative and not absolute estimates of the quantities of saliva produced. The difficulty in obtaining absolute estimate of the quantities of saliva secreted by tsetse flies have been fully discussed elsewhere (Youdeowei 1975b).

There was a highly significant difference between the relative quantities of saliva secreted by the flies at the two levels of hunger. The hungrier flies secreted the greater quantity of saliva. This confirms the results obtained with laboratory bred flies where Youdeowei (1975b) showed that the quantity of saliva secreted by tsetse flies increased with the intensity of hunger.

The proportions of males and females salivating at each hunger stage were also compared. The results (Table X) show that whereas 22.9% (males) and 32.4% (females) of the hungrier flies did not salivate, 50% (females) and 66% (males) of the less hungry flies did not salivate. This is further evidence to demonstrate that the hungrier the fly the more it secretes saliva.

Salivation in male and female *G. pallidipes*

The number of salivary drops secreted per minute and the sizes of the salivary drops secreted by male and female flies at Lambwe Valley were compared. The results are presented in tables X1 and XII for flies at hunger stage 1.

There was a highly significant difference between the relative sizes of the drops: the females, which are much larger in size, secreted larger salivary drops than the males (table XII).

Salivation in infected flies

There was no difference between the volumes of saliva produced by infected and uninfected flies (table XIII): thus it is not possible to identify an infected fly by the relative quantities of saliva produced. There is therefore no experimental evidence to support the suggestion that the presence of trypanosome parasites in the tsetse stimulates its salivation.

DISCUSSION

The results obtained with the wild flies confirm previous data regarding the influence of hunger upon salivation in that the proportion of flies salivating and the quantity of saliva produced increases with the intensity of hunger. The females tend to secrete more saliva but this is probably due to their larger salivary glands. Hunger is thus an important physiological state in the tsetse fly and favours the deposition of trypansome parasites from an infected fly into the vertebrate host. Possibly a primary unsuccessful probe carries the highest hazard for transmission since the deposited parasites are not in this instance sucked back by the insect with the blood meal/saliva mixture. Is it, in effect, unwise to disturb a probing tsetse fly?

ISOLATION MECHANISM

INTRODUCTION

The main objective of the project has been comparative study of a number of characteristics of *Glossina pallidipes* in the field and was carried out in two selected areas:

(i) The Nkruman area, about 160 km South West of Nairobi at an altitude of 2,500 ft. and

(ii) Mwalewa Forest near Lunga Lunga in the Coastal area near the Tanzania border at an altitude of 200 ft.

The Nkruman areas is composed of a mixture of three types of vegetation woodland, bushland and bushed woodland. Mwalewa forest is characterised by two vegetation types: wooded thicket and bushed grassland. There are some differences in the climatic conditions of the two areas. Rainfall occurs in Mwalewa almost all the year round with maximum precipitation in April and May, and November and December. Minimum rainfall occurs in February and October. In Nkruman there is maximum rainfall in March, April and it is almost dry the rest of the year, but total rainfall for both places was almost the same in 1974/1975.

The maximum temperature is about 3° C higher in Mwalewa forest. But while maximum temperature at the Coast occurs between 12 and 3 p.m., in Nkruman it occurs between 2 and 5 p.m. This is of particular significance with regard to diurnal activity observations. The two areas are clearly suitable for comparative studies. Not only are they geographically isolated but they also constitute completely different habitats.

In addition to this, the availability of flies in both places is about the same and with methods used, the total catches in the two areas do not differ significantly.

The second objective of the project is to develop laboratory breeding techniques for the two *G. pallidipes* populations. The results obtained so far are contained in this report.

MATERIALS AND METHODS

Field work was fundamentally orientated to the clarification of two questions: Firstly as to whether conflicting reports on *G. pallidipes* field behaviour are genuine or a consequence of differing techniques and secondly, if genuine, whether these differences are merely phenotypic or a consequence of genetic variations. With these two questions in mind, the work has concentrated mainly on the following subjects.

(i) A comparative study of the efficiency of two types of traps:- The Landridge Box Screen (LBS) and Awning Screen Skirt (ASS) (Moloo, in press). The two types of traps were used in both Nkruman and the Coastal area. Trap catches were assessed for a period of at least three consecutive days about once a month in both areas.

(ii) The comparison of two catching methods. This was done by comparing the catches of the two types of traps and the catches on a stationary car. The catching was carried out on two consecutive days about once every month in both areas.

(iii) The study of diurnal activity by means of catching in a stationary car. The catches were recorded from 0600-1900 hrs and were assessed on an hourly basis.

Breeding experiments of *G. pallidipes* were mainly confined to flies from Nkruman. These were fed on rabbits and kept in single tubes at constant environmental conditions of 24 ± 1°C, and 70 ± 10% R.H.

RESULTS AND DISCUSSION

Table 7 shows a summary of the results obtained in the study of the efficiency of the two trap models. According to Moloo (in press) who worked in Uganda with these two types of traps, the ASS is more efficient than the LBS. He caught 3-4 times more flies in the ASS. In both types of traps 70-80% of the total catch were females.

However, our results are somewhat contradictory. We found no significant difference between the two types at either of the two places. The percentage of females in both areas and in both types of traps were rather low. In Nkruman the LBS caught a significantly lower percentage of females than the ASS did. We did not find this difference in Mwalewa forest.

The total catches in the two areas (Table 7) show higher number of flies in Nkruman but the difference is not significant though the overall percentage of females is significantly higher in Mwalewa forest.

A summary of the catches in a stationary car is given in Table 8. There is no significant difference in either the total catches nor in the percentage of females.

A comparison of the catch by traps (as average per trap) with the catch in a stationary car indicates no significant difference between the two methods in Nkruman but in Mwalewa forest the car caught significantly more flies than the trap did (Table 9). In both areas there exists no significant difference in the female percentage by the two catching methods.

Diurnal activity patterns of both areas are shown in Fig. I. Our results show no correlation between the average temperature and relative humidity and the activity in either of the two places.

In Nkruman both sexes show activity throughout the day with main activity between 4.00 and 6.00 p.m. which is most obvious for the males. In Mwalewa forest the pattern shows two activity peaks and a clear mid-day depression. The morning activity is significantly higher than the afternoon peak.

Table 7: Summary of the catches in a standing car in Nkruman and Mwalewa forest.

	type of trap	0	0	0 + 0	% 0	number of trapping days	total in both types of traps	% 0 of total
	LBS	669	1106	1775	377	84	4051	43.5
	ASS	1093	1183	2276	481			
MWALEWA	LBS	834	660	1494	55.8	74	2839	53.7
forest	ASS	686	659	1345	51.0			

Summary of the catches in two types of traps in Nkruman and Mwalewa—forest.
LBS=Langridge—Box Screen.
ASS=Awning Screen Skirt.

Table 8: Summary of the catches in a standing car in Nkruman and Mwalewa forest.

	0	0	total	% 0	number of catching days	average of total per day
NKRUMAN	337	359	696	48.4	11	63.3
MWALEWA forest	220	213	433	50.8	9	48.1

Table 9: Comparison of the total catches in traps (average of 4 traps) and a standing car in Nkruman (10 days) and Mwalewa forest (8 days).

	catching method	0	0	total	% 0
NKRUMAN	trap	200	228	428	46.7
	car	337	358	696	48.5
MWALEWA forest	trap	123	87	210	58.6
	car	194	167	361	53.8

The results obtained in Nkruman are rather similar to the result Harley (1965) obtained in Uganda, while the results of Mwalewa forest are similar to the result Moggridge (1949) obtained in Kilifi, which is also in the Kenya Coastal area.

The activity pattern of flies from the Coast has a V-shaped pattern as described by Brady (1972). Vanderplanck (1948), Nash (1937) and Pilson (1967) explained this on the basis that there is a positive phototropic effect up till 30° C and a negative phototropic effect above 30° C which concides with a fall in activity. In this case the mid-day depression could be a direct response to climatic conditons.

The average maximum day temperature at the Coast was 33° C., and Nkruman 29.9° C. This could explain the difference in the pattern of the two areas.

However, Brady (1974) showed with *G. morsitans* that the mid-day depression occurs even at lower temperature. Besides this, even when maximum day temperature above 32° C were recorded in Nkruman there was still no decrease.

It will be worthwhile to repeat the work of Brady with flies from the two *G. pallidipes* populations. This may show if the differences in activity patterns found in the field are maintained under constant conditions in the laboratory. This may give information whether this difference is merely phenotypic or has a genetic base.

However, to carry out this experiment laboratory colonies are needed and we are still far from this. In our preliminary study on the rearing of *G. pallidipes* we worked mainly with flies from Nkruman. It was extremely difficult to start a colony because of the problem of finding pupae in the field and the high death-rate during transportation and in the first week of arrival of live females in the laboratory. The results obtained with our first trial are summarized in Table 4. Only the parental and the first generation have been completed. Nevertheless some important observations can be made. The number of flies involved are too small to draw conclusions but the data may be used as guidelines for future work.

Table 10: Breeding results Nkruman-colony.

generation	no ♀	puparia				mean lifetime per ♀ in days	fecundity
		no	no/♀	mean wt in mg	% emerged		
parentes I	25	57	2.3	39.7	94.7	74.7	0.35
parentes II	133	133	1.0	34.1	95.5	35.2	0.24
F1	65	102	1.7	38.8	87.3	62.7	0.29
producing F1	29	102	3.5	38.8	87.3	77.8	0.50
F2 *	34	40	1.1	39.0	62.5	53.5	0.25
producing F2 *	13	40	3.0	39.0	62.5	70.0	0.54
F3 *	8	20	2.5	41.5	45.0	54.1	0.58
producing F3 *	5	20	4.0	41.5	45.0	74.0	0.53
F4 *	4	10	2.5	43.0	40.0	49.5	0.66
producing F4 *	3	10	3.3	43.0	40.0	60.0	0.66

generation not completed

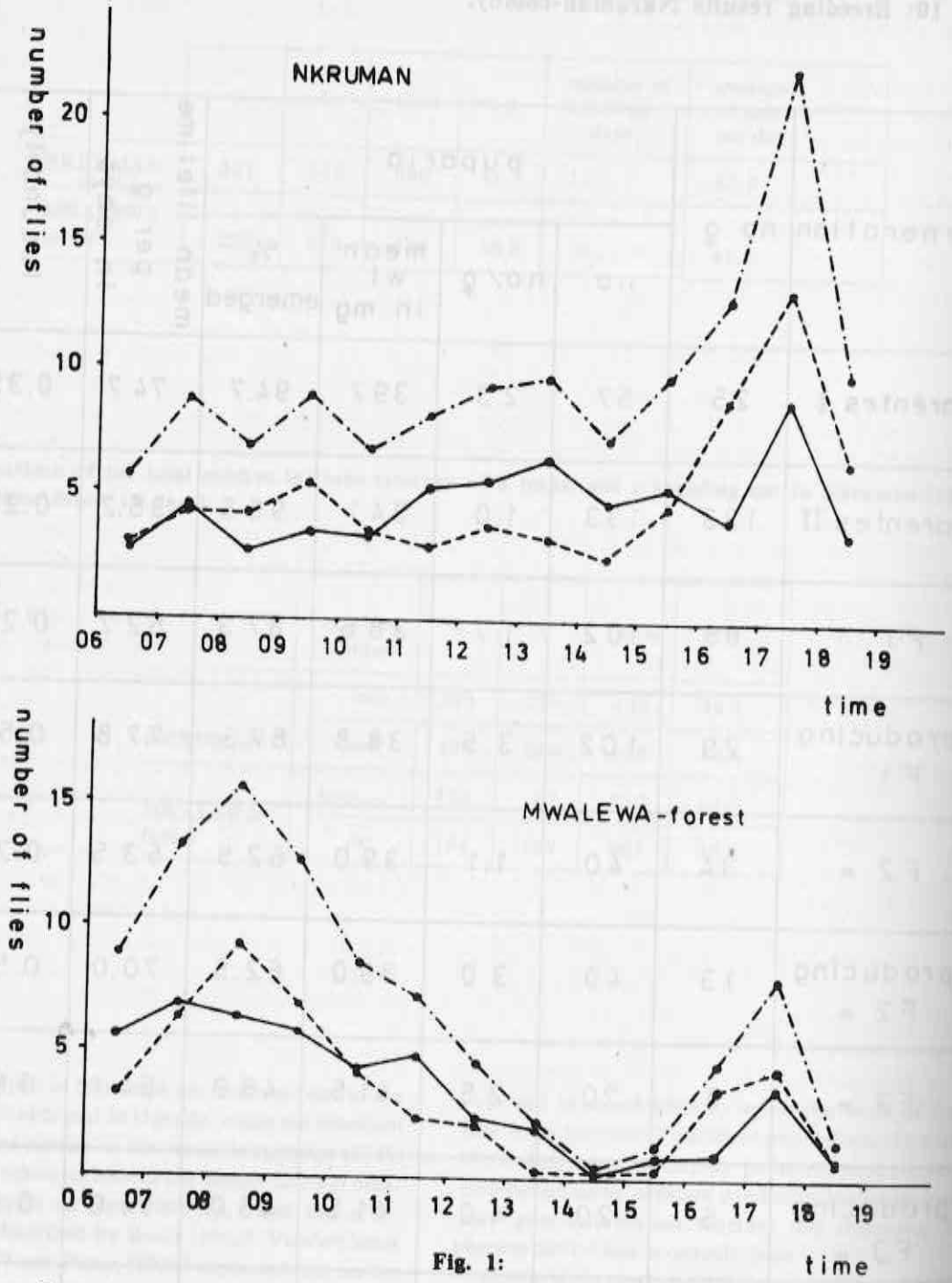


Fig. 1:

Diurnal activity pattern in Mwalewa-forest and Nkruman.
The amount of flies is indicated as twice the average catch in a standing car during every hour of the day.

——— ♀

- - - - - - - - ♂

—·—·—·— Total (♀ + ♂)

The data show that in every new generation the number of reproducing females expressed as percentages of the total females of the generation, increases. And in every succeeding generation the reproducing females and the generation as a whole tend to attain the same degree of fecundity.

The results strongly indicate that problems arising in the first two generations, like high death-rates and low reproduction, disappear in the later generations. Another thing is clear: the number of females in the parental generation have to be much higher. A lot of attention is focussed on the diurnal activity pattern because it may be possible to detect whether or not the differences in patterns are genetic. But with differences in behaviour which the we have so far found, this may be difficult or even impossible to do. However, the comparative studies demonstrate for the first time that the conflicting reports on *G. pallidipes* probably do not arise from the different methods used.

These comparative studies will be continued to obtain more data. Furthermore an experiment has been initiated to determine some physiological characteristics like dry weight, fat content, size and age.

SUMMARY

Comparative studies of some characteristics of the two *Glossina pallidipes* populations from the Nkruman and the Coastal area have indicated some obvious behavioural differences. Differences are found in the efficiency of two types of traps and a stationary car as well in the total numbers of males and females caught. There exist very obvious differences in diurnal activity patterns. The results of a first attempt to breed *G. pallidipes* from the Nkruman area have been reported but the data indicates that we are still far from a good laboratory colony.

INTRODUCTION

Although the tsetse fly has become one of the most intensively studied insect pests in Africa because of its economic importance, its genetic studies are still scarce and this mainly because of its low fecundity and rearing difficulties in the laboratory. However, genetic studies are needed to have available genetic markers and to obtain information on genetic variation in natural and laboratory populations. This knowledge may be of direct use. An important tool for such studies is the quantification of the amount of genetic variation by electrophoretic means.

In this paper, a study on the feasibility of the zymogram technique for the tsetse fly *Glossina morsitans* Westwood is presented. Emphasis is laid upon the question of which enzymes are suitable for the recognition of genetic polymorphism, and the occurrance of distinct zones of activity on the zymograms. Another aspect studied deals with technical problems encountered when working with field-collected material: such as effect of blood meals, effect of developmental stages, age, and sex. Since tsetse flies are viviparous insects producing full-grown larvae, the effect of pregnancy on the zymorgams was also considered. When carrying out field work, it is desirable to have means at hand by which the collected material can be fixed and stored safely over longer periods. For this reason, the effect of freezing in liquid nitrogen and subsequent prolonged storage at -20° C was studied.

MATERIAL AND METHODS

Pupae and adults examined in this study refer to a *G.m. morsitans* strain that is reared in our laboratory with 400 pupae, from the mass-reared colony of the Institut d'Elevage et de Medecine Veterinaire des Pays Tropicaux, Maisons-Alfort, France.

Isoenzymes were separated by means of horizontal starch gel electrophoresis, carried out on plates which were cooled at 0°. For most enzymes the discontinuous buffer system of Poulik (1957) worked satisfactorily. For lactic acid dehydrogenase and malic acid dehydrogenase, tris-citrate buffer at oH 7.0 was used for both reservoir and gel (Shaw & Prasad, 1970). Entire flies or pupae were homogenised in 0.2 ml gel buffer. The homogenates were brought into a slit in the gels by means of small pieces of 3MM Whatman chromatography paper. After electrophoreses, gels were cut into three transverse slices and incubated with specific substrates and stains for the detection of the enzymes. A total of eight different enzymes was studied: leucine-amino peptidase *(lap)* lactic acid dehydrogenase *(ldh)*, malic acid dehydrogenase *(mdh)*, xanthine dehydrogenase *(xdh)*, NADP-dependent malic acid denydrogenase *(mdh-t)*, malic enzyme *(me)*, non-specific esterases *(est)* and alkaline phosphatase *(alph)*. For the detection of lap-isoenzymes, the procedure described by Knowles & Frisom (1967) was used. Malic enzyme, alkaline phosphatase, and xanthine dehydrogenase were demonstrated by the methods described by Toledo & Maglhaes (1973), and techniques for visualising the other enzymes were found in Shaw & Prasad (1970).

RESULTS AND DISCUSSION

Six enzyme systems appeared to lend themselves very well to the purpose of analysing genetic variation, i.e., *lap, me, alph, mdh-t, ldh,* and *mdh*. These enzymes show a predictable pattern on the zymograms. The other two enzymes are not attractive for further analysis for various reasons. Zymograms of esterases showed in general considerable tailing that made it difficult to distinguish individual bands. This tailing obscured the bands on the zymograms and made the recognition of eventual variants hardly possible. Xanthine dehydrogenase is of problematic value. One single band with a variable mobility was found, in both the inbred and the randomly bred strains. The reason for this variation is not yet known, but is apparently non-genetic. If allozymes of *xdh* are present, their identity will be difficult to interpret, because of the overall variation in mobility.

In Fig. 1, zymograms of the preferable enzyme systems studied thus far are represented. Some of the enzyme patterns are very simple in that they consist of single bands *(idh, me)*. Others, however, are more complex and show

two or more distinct bands of activity. For all enzyme systems investigated, males and females exhibited similar patterns, and there are no reasons so far to assume that any differences between sexes occur.

Pregnant females were studied for each enzyme system, using a minimum of twelve flies of a definite stage of pregnancy: females with first, second, and thirs instar larvae. Examination of pregnant flies revealed that the later stages of pregnancy the staining intensity of isoenzymes is usually influenced by an increase in activity (for instance in the case of *lap*). A yellowish tailing occurring in late pregnancy may interfere with the proper recognition of the zymogram patterns. The presence of an additional band originating from the larval genotype was never observed, probably because of the small sample size and the generally low frequency of mutant allozymes.

The stage and age of the flies appeared to be an important factor enzymes like *alph*, *xdh*, and *me* were detected only in teneral and older flies, but not in pupae. All other enzymes are also present in pupae, although their activity depends largely upon the age of the pupae. In *lap*, for instance, the activity of one of the isoenzymes increases to such an intensity that the presence of the other *lap*-isoenzymes is masked. The effect of age of the adult flies is also very marked in the case of leucine-amino peptidase. In teneral flies, a single band is found in the middle zone of activity but flies which have had their first blood meal, show a distinct double band in this zone. Older flies have zymograms qualitatively similar to those of the flies which have had their first blood meal. The staining intensity of the bands tends to diminish with the age of the flies.

Freshly taken blood meals were shown to interfere with some zymograms. In some instances blood enzymes would yield extra bands with the same or different mobilities as those of the fly. In addition, blood substances cause considerable tailing. Removal of the blood by taking out the gut can also affect the electrophoretic patterns, as some of the enzymes may be located in this organ (e.g. in case of *lap*). For none of the enzymes included in this paper were adverse effects observed on the zymograms included in this paper were adverse effects observed on the zymograms of flies which had been stored in the freezer for over 6 months. Freezing in liquid nitrogen appears to be a useful tool for the field ecologist who intends to analyse his material by isoenzyme determinations.

Genetic variants were found for a number of loci involved in the different enzyme systems. This conclusion was derived from the absence of the variants in the inbred strain(s), and from the fact that these variants were usually

Table 11: Observed and calculated genotype frequencies of variants at the *Lap*–3 locus in a laboratory strain of *Glossina m. morsitans* (number of flies examined: 480)

Genotype	Observed frequency	Calculated frequency
a/a	0.004	0.0001
b/b	0.87	0.86
c/c	0.002	0.0036
a/b	0.002	0.186
b/c	0.125	0.11

Table 12: Observed and calculated genotype frequencies of variants at the *me* locus in a laboratory strain of *Glossina m. morsitans* (number of flies examined: 174)

Genotype	Observed frequency	Calculated frequency
a/a	0.006	0.0001
b/b	0.17	0.13
c/c	0.43	0.38
a/b	0.01	0.005
b/c	0.37	0.46
c/d	0.02	0.01

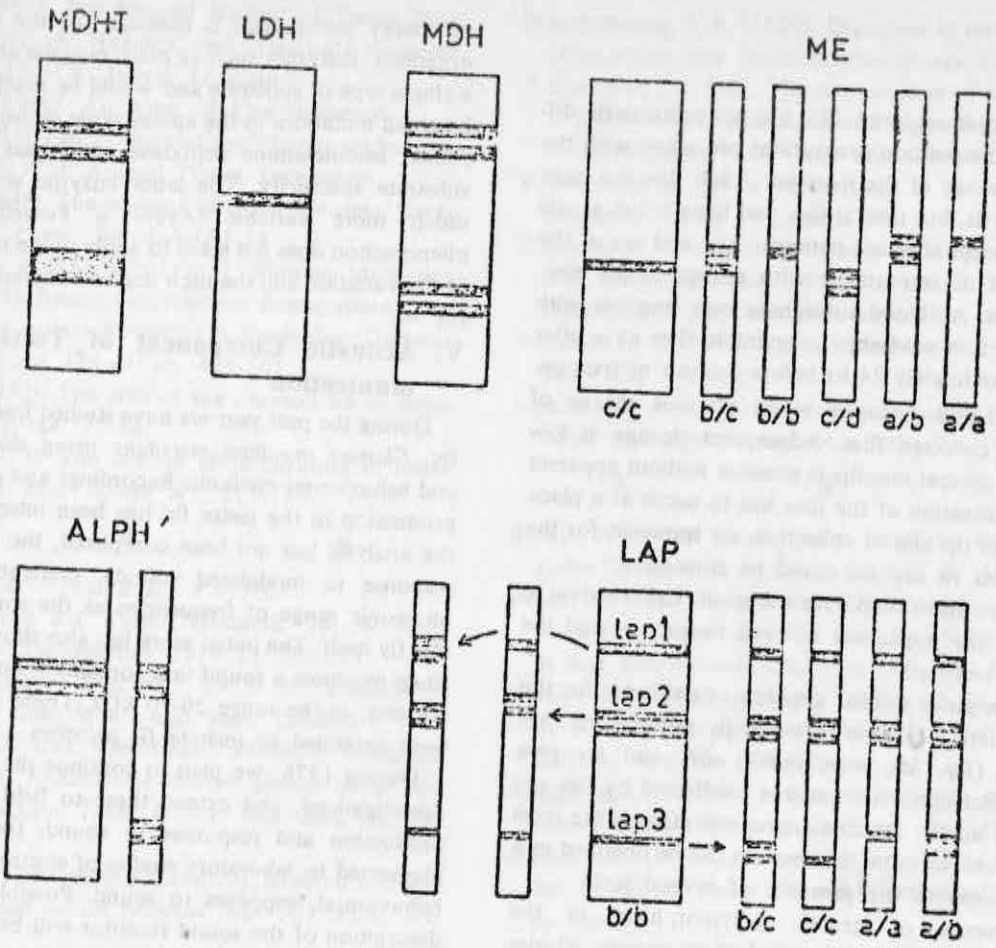


Fig. 1: Diagrammatical representation of zymograms of different enzyme systems in the tsetse fly *Glossina m. morsitans*. Symbols above the diagrams refer to the enzymes: *mdh-t* = NADP-dependent malic acid dehydrogenase, *ldh* = lactic acid dehydrogenase, *mdh* = malic acid dehydrogenase, *me* = malic enzyme, *alph* = alkaline phosphatase and *lap* = leucineamino peptidase. The wide columns represent the common types observed, the narrow columns the different mutant genotypes. For the *me* and *lap*$_3$ locus genotypes are added by symbols below the diagrams.

found as heterozygotes in co-dominance with the more common band(s).

For *alph* a total of 142 flies was examined. This enzyme is usually expressed as a double band (Fig. 1), but in two flies an additional double band was observed, in co-expression with the common type. These extra bands were not found in flies of the inbred strain.

For *me*, a total of 174 flies was tested. Four different bands (a, b, c, and d) were found. The number of bands for a particular individual is one or two. It is assumed that each band (allozyme) is encoded by a specific allele from the *me* locus. The combinations (a, b, c, bc, and cd) and their frequencies found are given in Table 1.

The situation with respect to *lap* was more complex. Three distinct zones of activity were observed on the zymograms. In the fast (*lap*$_1$) zone, only one variant was observed in 480 individuals, expressed as a double band in apparent co-dominance with the common type of *lap*$_1$ (Fig. 1). This variant (*lap*$_1$b) does not affect the mobilities of *lap*$_2$ and *lap*$_3$ thus strongly suggesting that the *lap*$_1$ zone is under control of a distinct gene locus.

In *lap*$_2$ (the middle zone) a variant was observed once. This was expressed as a double band with a mobility different from the prevailing type. Mobility of the other zones was not affected by this variant.

In *lap*$_3$ several allozymes were observed. The allozyme under control of the common allele occurs in most flies tested (*lap*$_3$b). Two variant heterozygotes were observed at different frequencies and apparently reflect co-dominance of the common and a mutant allele. The variation in the *lap*$_3$ zone neither affect the *lap*$_3$ the *lap*$_1$ nor the *lap*$_2$ zones, thus giving support to the assumption that in the lap-system at least three loci are involved. In Table II the number of different genotypes observed and calculated is given. The common allele is homozygous in the greater part of flies, and its allozyme is expressed as a single band (*lap*$_3$b in Fig. 1). The other alleles (*lap*$_3$a and *lap*$_3$c) were observed to occur at low frequencies, their presence most commonly associated in heterozygosity with *lap*$_3$b. However, the relative high numbers of flies examined for *lap* allowed the detection of *lap*$_3$a as a homozygote. a large proportion of the work on lap-isoenzymes was done by Rietveld (1974).

Observed and calculated genotype frequencies of variants at the *lap*-$_3$ locus in a laboratory strain of *Glossina m. morsitans* (number of flies examined: 480)

CONCLUSIONS

Isoenzyme studies on tsetse flies are not particularly difficult, provided that certain precautions are taken with the collection and storage of the material. Adult flies are most suitable for analysis, but teneral flies and female flies at late pregnancy may yield aberrant patterns. Age and sex of the adult flies are of no importance with respect to the electropherogrammes. As blood substances may interfere with the zymograms, it is advisable to maintain flies alive after capture for approximately 24 hr before analysis or fixation.

Freezing in liquid nitrogen is an obvious means of fixation of the collected flies. Subsequent storage at low temperature for several months is possible without apparent damage. If examination of the flies has to occur at a place situated far from the site of collection, air transport for the collected samples in dry ice could be considered.

Live pupae are also suitable for transport. Upon arrival in the laboratory, the pupae are allowed to emerge and the adults can be analysed.

Six of the enzymes studied appear to be suitable for the analysis of genetic variation of tsetse fly populations. Allt hese enzymes *(lap, ldh, mdh, mdh-t alph,* and *me* give reproducible electrophoretic patterns unaffected by the age and sex of the adults. Leucine-amino peptidase is the most attractive enzyme, as more information can be obtained in a single run, thanks to the presence of several loci.

The occurrence of genetic polymorphism in the laboratory strain is interesting and may suggest greater variation in field-collected flies. On the basis of the results presented in this report, one can make estimates of some relevant parameters of genetic variation. In doing so, it is assumed that for enzymes exhibiting one electrophoretic band, only one locus is involved *(ldh, me).*

After establishing the frequencies of the alleles a, b, c, and d, the frequencies of the various homo- and heterozygotes were calculated, according to the Hardy-.

Fig. I Diagrammatical representation of zymograms of different enzyme systems in the tsetse fly *Glossina m. morsitans.* Symbols above the diagrams refer to the enzymes: *mdh-t* = NADP-dependent malic acid dehydrogenase, *me* = malic enzyme, *alph* = alkaline phosphatase and *lap* = leucine amino peptidase. The wide columns represent the common types observed, the narrow columns the different mutant genotypes. For the *me* and lap$_3$ locus genotypes are added by symbols below the diagrams.

Alkaline phosphatase is apparently also controlled by a single gene locus, as co-dominance of both alleles was found to occur. With respect to *lap, lap*$_1$ and *lap*$_3$ are under control of distinct gene loci; for *lap*$_2$ a maximum of 2 loci may be involved. *Mdh-t* and *mdh* are controlled by at least one locus each, and at most by three and four loci, respectively. This brings the total of loci investigated maximally at 14. Genetic polymorphism was observed to occur in five loci or 35% of the investigated loci. The average proportion of heterozygous loci per individual is at least 4%.

Another interesting aspect of this study is the considerable variation in the *me*-locus. Kojima *et al.* (1970) suggested that enzymes which are involved in glucose metabolism are likely to be less variable than other enzymes, as energy metabolism is essential for the survival of the organism. Enzymes such as malic enzyme are dependent on a single type of substrate and would be much more affected by small mutations in the apo-enzyme molecule than, for instance, leucine-amino peptidase which has a much lower substrate specificity. The latter enzyme was found to be much more variable (Ayala & Powell, 1972). This phenomenon does not seem to apply to the tsetse fly in view of the variation and the high degree of heterozygosity found for *me.*

V. Acoustic Component of Tsetse Fly Communication

During the past year we have studied hearing in the tsetse fly, *Glossina morsitans morsitans,* using electrophysiological and behavioural methods. Recordings and analysis of sound production in the tsetse fly has been intensified. Although the analysis has not been completed, the initial data show response to modulated sounds, preferably in the same ultrasonic range of frequencies as the sound produced by this fly itself. The initial work has also shown that *G.m. morsitans* produces a sound that contains strong ultrasonic components, in the range 30-70 kHz. These studies have now been extended to include *G. pallidipes.*

During 1976, we plan to continue the above-mentioned investigations, and extend these to field studies of sound production and responses to sound; these will be complemented by laboratory studies of electro-physiological and behavioural responses to sound. Possible localization and description of the sound receptor will be an important objective.

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CHEMISTRY RESEARCH UNIT

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The Chemistry Unit is by nature a fluid group which interacts with other groups depending on the development of projects. Details of these investigations appear in the chapters relating to the specific projects involved; an outline of the Chemistry Unit progress is presented below.

I. Insect-controlling compounds from plants.

Our experience during the past few years at ICIPE has convinced us that the Kenyan flora is an extremely promising source to isolate highly active compounds pertaining to insects such as antifeedants, insecticides, etc. In fact, the variety and activity of compounds isolated in either pure states was beyond our expectation. Some of these, although not yet fully characterized in terms of chemical structures, fall in the category of the most active compounds known to date. The following lists these preliminary discoveries (the structures are not given due to potential patent possibilities which are currently in process).

1. Antifeedants

Bioassays are carried out by the disc choice method using *Spodoptera exempta* caterpillars. The compound is designated an "antifeedant" if effective at the level of 100 ppm. The most effective of these anti-feedants, the structure of which is known, functions at 0.1 ppm. In addition, electrophydiological studies have led to the new finding that a terminal thiol group on the receptor is responsible for the taste of insects. Some antifeedants have been found to stop the feeding in a nonreversible manner, an aspect which clearly has tremendous practical value. Twenty-five compounds have already been structurally characterized, while more are at the stage of purification.

2. Insecticides

Compounds (two) possessing kill effects stronger than natural pyrethroids against adult mosquitos are in the process of being structurally identified.

3. Feeding stimulants, biting stimulants

Two each of such compounds have been identified; they are now being investigated further by electrophysiology.

4. Insect Hormone mimics

A plant with a very high content of a certain identified insect hormone mimic has been discovered. Work is in progress to complete the isolation, structural studies and more extensive bioassays.

In addition to the plants currently under investigation, numerous other plants have been identified as sources of pest-control compounds. Another area which has been neglected due to lack of hands is the search for the pest attractants contained in host plants – this again has a direct bearing on practical applications.

II. Pheromones for control of behaviour.

A number of projects have been initiated to characterize sex attractants, aggregation substances, trail substances and alarm pheromones from various species of hard and soft ticks, and from termites. The elucidation of the chemistry of these compounds must be coupled with reliable bioassay procedures in order that the initial isolation can be continuously monitored at each step. Once structures of active compounds can be determined, synthesis of these compoundents becomes an attractive goal. Some of the specific problems to be investigated are listed below.

1. Pheromones of hard ticks

Studies at ICIPE by Wood *et al.*, have shown that in several species of hard ticks (*Rhipicephalus appendiculatus, R. pulcellus, R. compositus, Amblyomma americanum, A. variegatum*), females produce a number of simple phenols after partial engorgement. For the first two of these species, phenol and p-cresol seem to serve an attractants to male ticks. Salicyladehyde and 2, 6-dichlorophenol are also found in some of these species. At the same time there are indications that fecal material from these ticks serves for aggregation or sex attraction. The characterization of these fecal components remains to be carried out.

2. Pheromones of soft ticks

Preliminary studies on soft ticks (*Ornithodorus moubata, O. persicus,* and *O. tholozani*) indicate that a polar, water soluble, material which seems to function as an aggregation pheromone can be isolated from soft tick washings. High pressure liquid chromatography of these washings has so far failed to yield any highly active fractions. However, at-

tempts will be continued to find a suitable isolation procedure in order that the chemistry can be pursued in terms of the types and of molecular weight ranges of substances that are involved.

3. Tick aggregation pheromone

A compound which in preliminary experiments induced aggregation in ticks has been isolated from tick faeces and purified.

4. Termite Queen-chamber Building

It has been shown that ether extracts of the cuticle of *Macrotermes subhyalinus* queens will elicit the building of a queen chamber by workers. This novel type of pheromone must play a vital role in the social organization of the termite colony, and is an interesting chemical lead that should be pursued. Current indications are that the pheromone may be an unsaturated carboxylic acid.

5. Termite Trail Substances

Isolation work aimed at characterizing the trail pheromones from several soil and wood-dwelling species has been initiated. The species under study include *Schedorhinotermes lamanianus, Trinervitermes bettonianus, T. gratiosus, Hodotermes mossambicus* and *Macrotermes subhyalinus*. There are interesting examples of interspecies trail following. This is a difficult and complex subject, but one which seems promising from a chemical point of view, if sufficient quantities of active material can be obtained.

III. Insect Defensive Secretions

The production by insects of specific substances that serve to provide protection from their own insect (and other) enemies is a well-known phenomenon. The search for such insect defensive compounds has been a continuing effort at ICIPE, and this has most recently focussed chiefly on the defensive secretions of termites, which seems to represent a fertile field for future studies, since the termites have apparently evolved a variety of rather novel chemical defensive agents. Some of these compounds may be particularly interesting in combating ants, since ants are the chief competitors of termites.

1. Amitermitinae

Soldiers of this group produce a number of relatively simple straight-chain and also terpenoid defensive secretions. Characterization work using the usual gc/ms and the spectroscopic techniques is now underway.

2. Nasutitermitinae

Work was begun at ICIPE in 1972 on the defensive secretion of *Trinervitermes bettonianus soldiers*. This program has now expanded to include three Trinervitermes species and two Nasutitermes species. While the more volatile components have proven to be simply some well known terpene hydrocarbons and 3-ethloctanol-2, there are also present as major components of these defensive secretions, some resinous components that seem to be effective against ants both by mechanical and toxic effects. A number of these resinous components have now been isolated and extensive spectroscopic studies have indicated that these compounds are the first representatives of an entirely new group of tricyclic diterpenes (C_{20} compounds). Unlike any other diterpenes found to date, the skeletons are lineary arranged 8/6/6 and 7/7/6 systems, which are in turn biogenetically related in a straightforward manner. It is likely that unraveling of the relationship among these series of compounds will disclose new biogenetic knowledge. There also remains the possibility that some useful anti-insect activities may be found among them.

3. Termitinae

Initial explorations on the defensive secretion of *Noditermes wasmbariculs* and several *Cubitermes* species indicate that there are a number of new terpenoid compounds to be characterized. These compounds are hydro-carbons of lower molecular weights than the above-discussed diterpenes from nasute soldiers, and they should lend themselves to the usual structural elucidation techniques.

FINE STRUCTURE RESEARCH UNIT

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Mrs. E. Kokwaro (1975) – Experimetntal Officer

The Fine Structure Research Unit, a necessary Research Support Service in the ICIPE Structure, has become fully operational during the 1974/75 period. Space in the Northern Star Building provided the housing of the laboratory. Generous aid equipped it with modern instrumentation for high resolution work on the structural organization of cells and their products, notably one philips 2015 Transmission EM, one JEOL JSM-15 Scanning EM, one JEOL vacuum Coating Unit, one LKB III ultra-microtome, one LKB-Huxley ultramicrotome and most of the necessary ancillary equipment establishing one of the finest such laboratories in Africa.

That this Unit has, within a year, established itself not only as a functional part of ICIPE, but a fruitful centre for collaborative work with numerous laboratories outside the Centre, fulfilling the dual function envisaged in earlier planning meetings and discussions, is due to the efforts of the Research Scientist and the trained Experimental Officers and technical staff of the laboratory.

During its first year of operation, over 3,500 Transmission EM and over 800 Scanning EM PHOTOGRAPHS HAVE BEEN TAKEN. To illustrate the variety of techniques used and tissues being studied, some of these photographs are included in this report. Others, with more detailed accounts of the work, will be found in the reports of the collaborating ICIPE projects.

SUMMARY OF WORK CARRIED OUT, UP TO AUGUST 1975.

1. With ICIPE personeel (about 30%)

a) Dr. M. Kaib (Electrophysiology) Detailed TEM and SEM studies on the antenna of Paracystis integer, a termotophilic tineid.
b) Dr. N. Abo-Khatwa (Chemistry) started July 1975. A TEM study of ovarian and fat-body mitochondira from queens of *Macrotermes*.
c) Ir. P. Scheltes (Stem-borers) started July 1975. TEM studies on the cuticle, brain, and corpora allata of diapausing and non-diapausing stem-borers (Chilo).

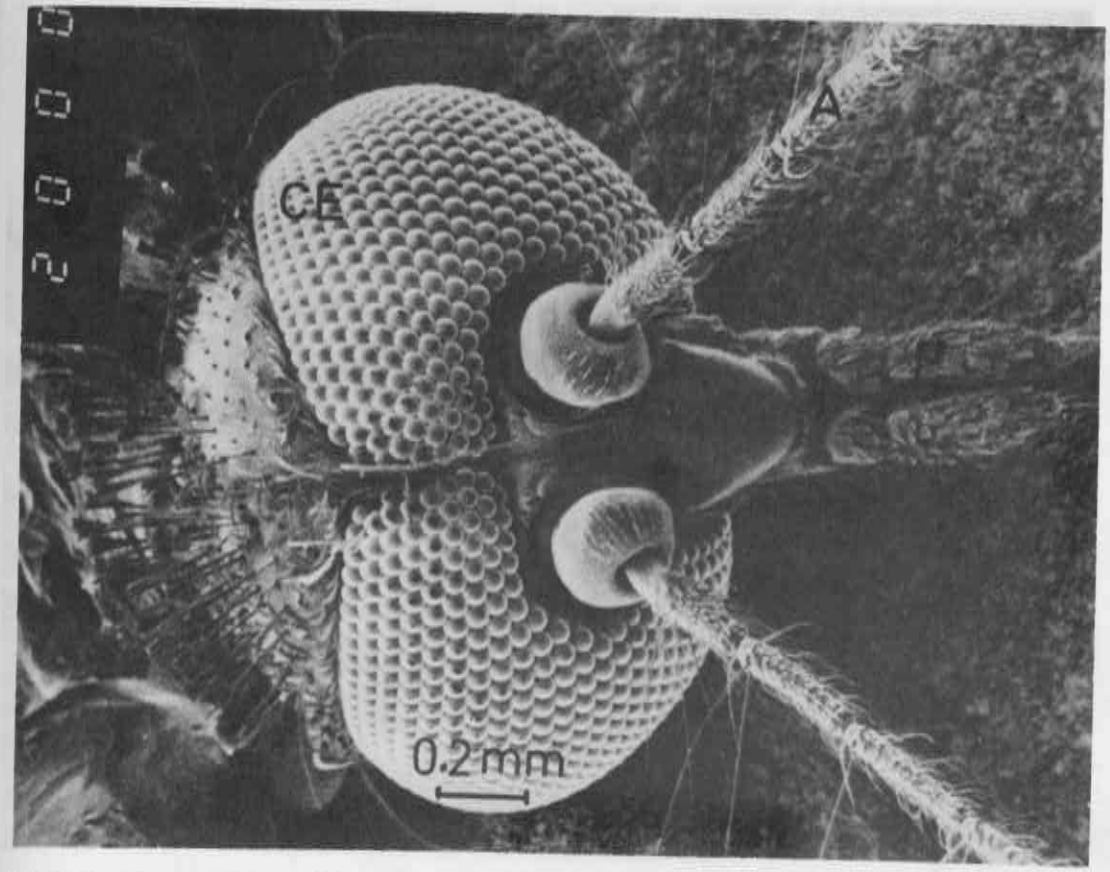
2. With other Research Institutions (about 70%)

a) Mr. M. Berry (EAVRO), Muguga) TEM studies on malignant catarrh fever virus of cattle, with the emphasis on immunological aspects.
b) Professor R. Tucker (Vet. Anatomy, University of Nairobi). TEM studies on sensory epithelia in various birds and fish.
c) Dr. B.A. Kimeto (EAVRO, Muguga) TEM studies on East Coast Fever.
d) Dr. R. Hanna (EAVRO, Muguga) TEM studies on the structure of helminths.
e) eDr. D. Metzelaar (Medical Research Centre) Pilot TEM studies on various viruses prior to a major programme of virus screening.
f) Dr. W. Mathai (Vet. Anatomy, University of Nairobi) TEM studies on salivary gland of ticks infected with *Theileria*.
g) Dr. Z. Parvez (Entomology Dept. University of Nairobi) TEM studies on viruses in various lepidoptera species.
h) Dr. T. Dolan (EAVRO, Mug
g) Dr. Z. Parvez (Entomology Dept. Univeristy of Nairobi) TEM studies on viruses in various lepidoptera species.
h) Dr. T. Dolan (EAVRO, Muguga) SEM studies on various blood parasites in wild animals.
i) Dr. G. Creasely and Dr. A. Levin (Kenyatta National Hospital) Preliminary attempts to study cells from Burkitt's lymphoma using the SEM.

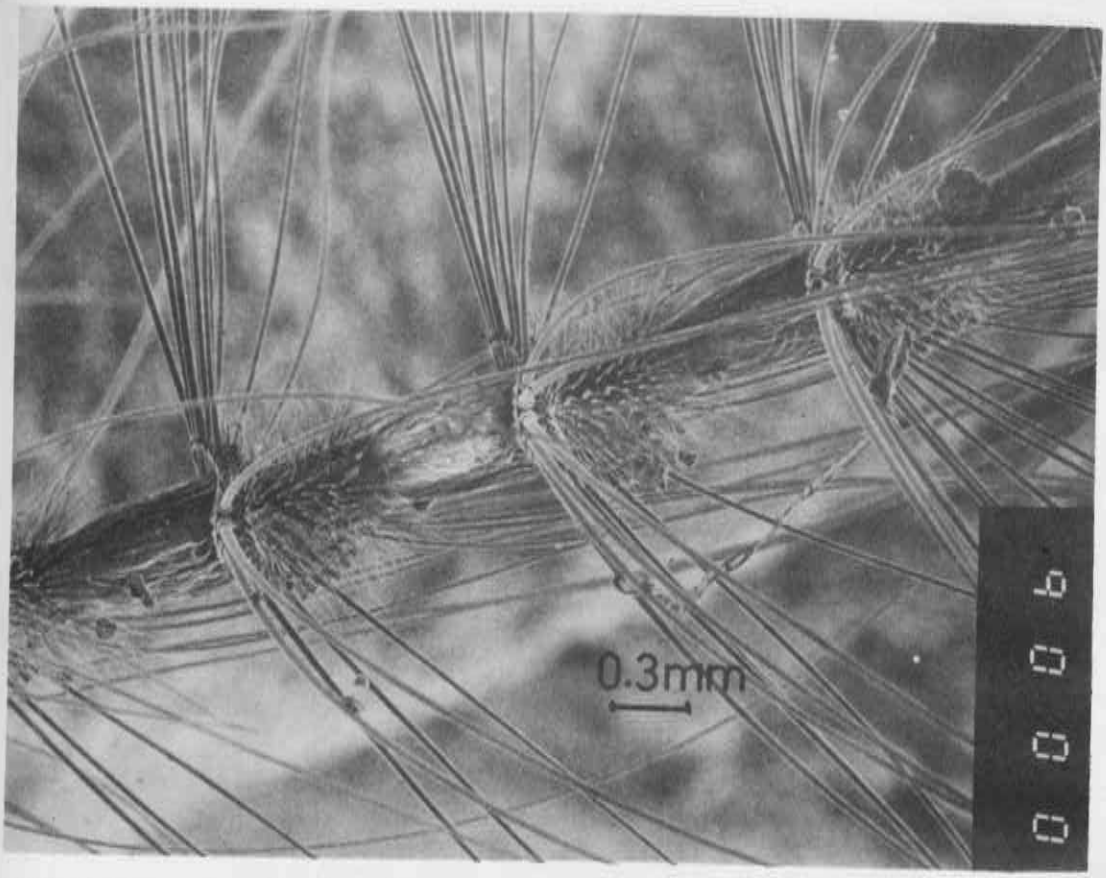
In addition, assistance has been given to other members of staff familiarising themselves with the various instruments of the Unit. A scientist from the salivary gland project has been training in basic EM techniques. Studies on the proventriculus of *Glossina morsitans*, on tick sensilla, and on the fate of *Trypanosoma congolense* in Glossina pallidipes were contained. Miss M.L. Sequeira (Experimental Officer) left the Unit in August, 1975, and her work is described elsewhere in this Annual Report.

Mr. M.B. Chimtawi (Senior Technician) spent 4 months in Professor David Smith's laboratories in Miami, Florida, undergoing further training in EM techniques, and Mr. P. Lisamulla (Senior Technician, photography) attended a 3 month course in Photographic and Microscope Techniques with Leitz, West Germany.

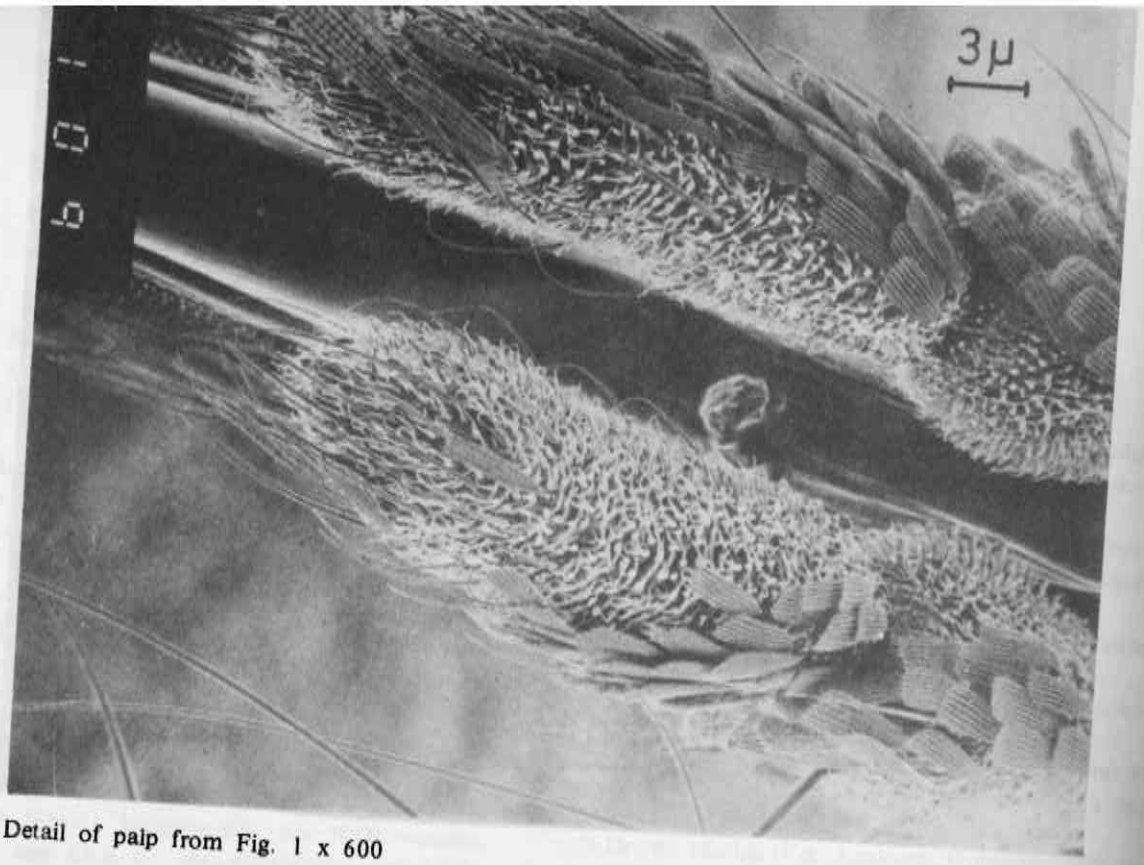
Miss Ulla Jarlfors (EM technician in Professor Smith's laboratories, Miami, U.S., visited the Unit in early 1975.



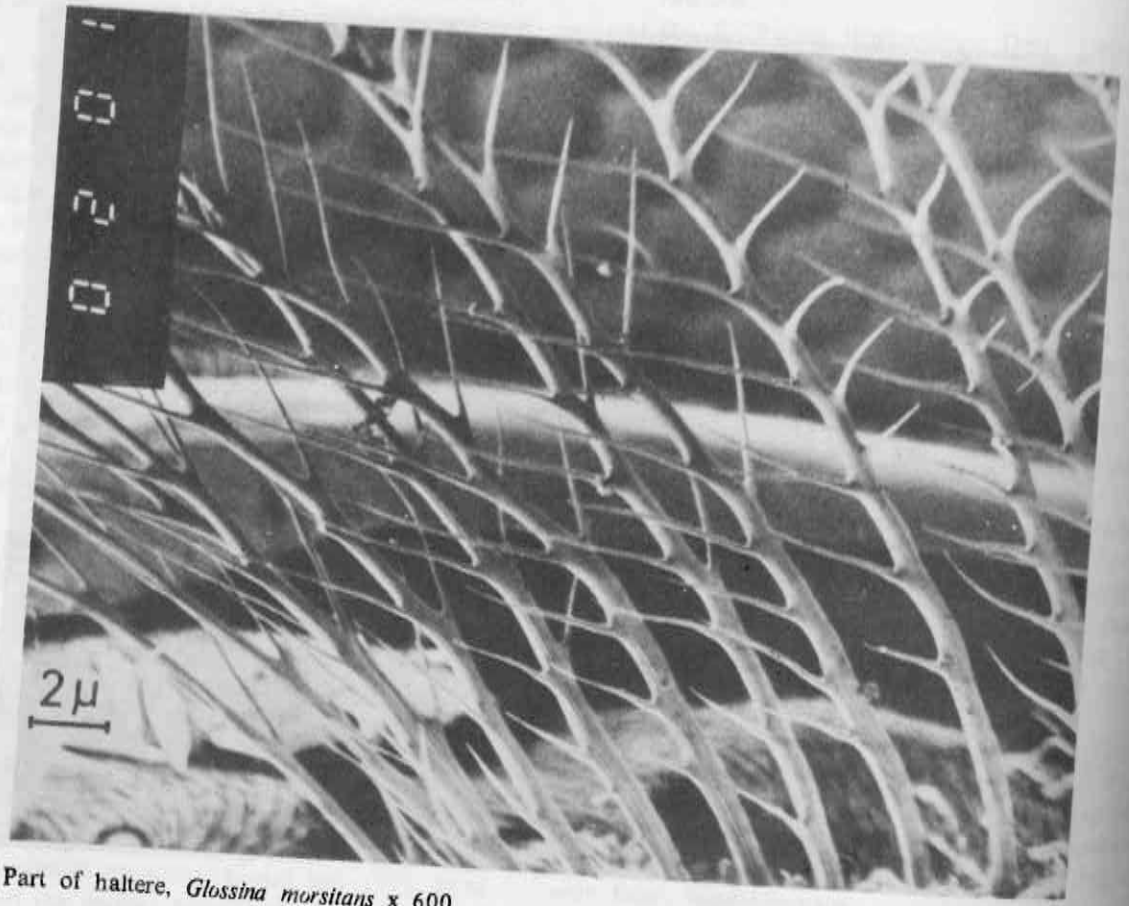
Head of male mosquito, x 50



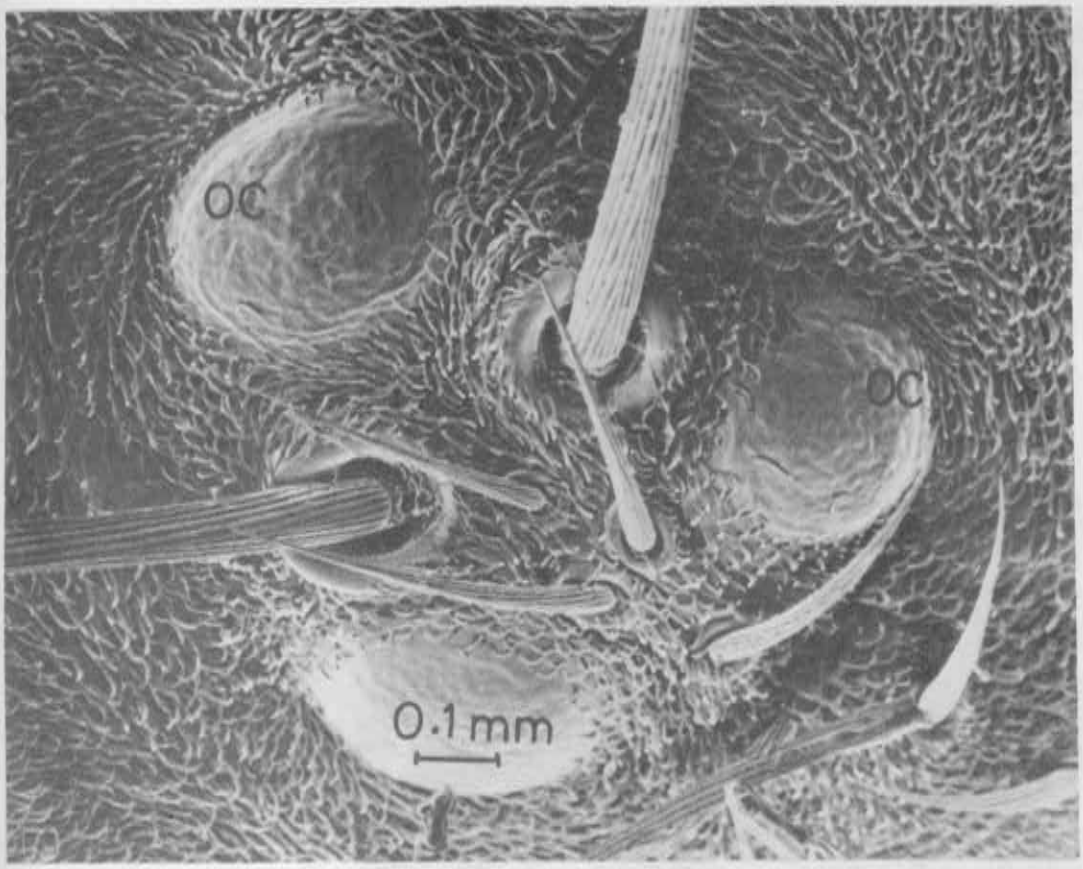
Detail of antenna from Fig. 1 x 600



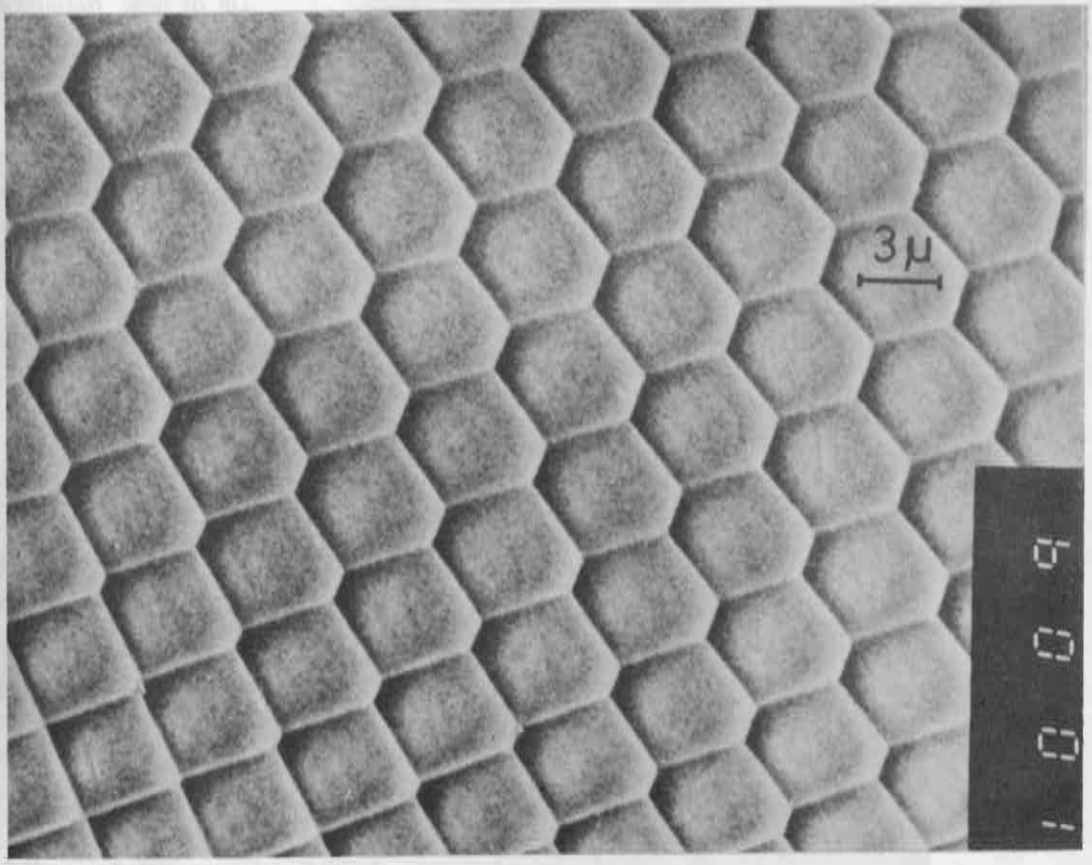
Detail of palp from Fig. 1 x 600



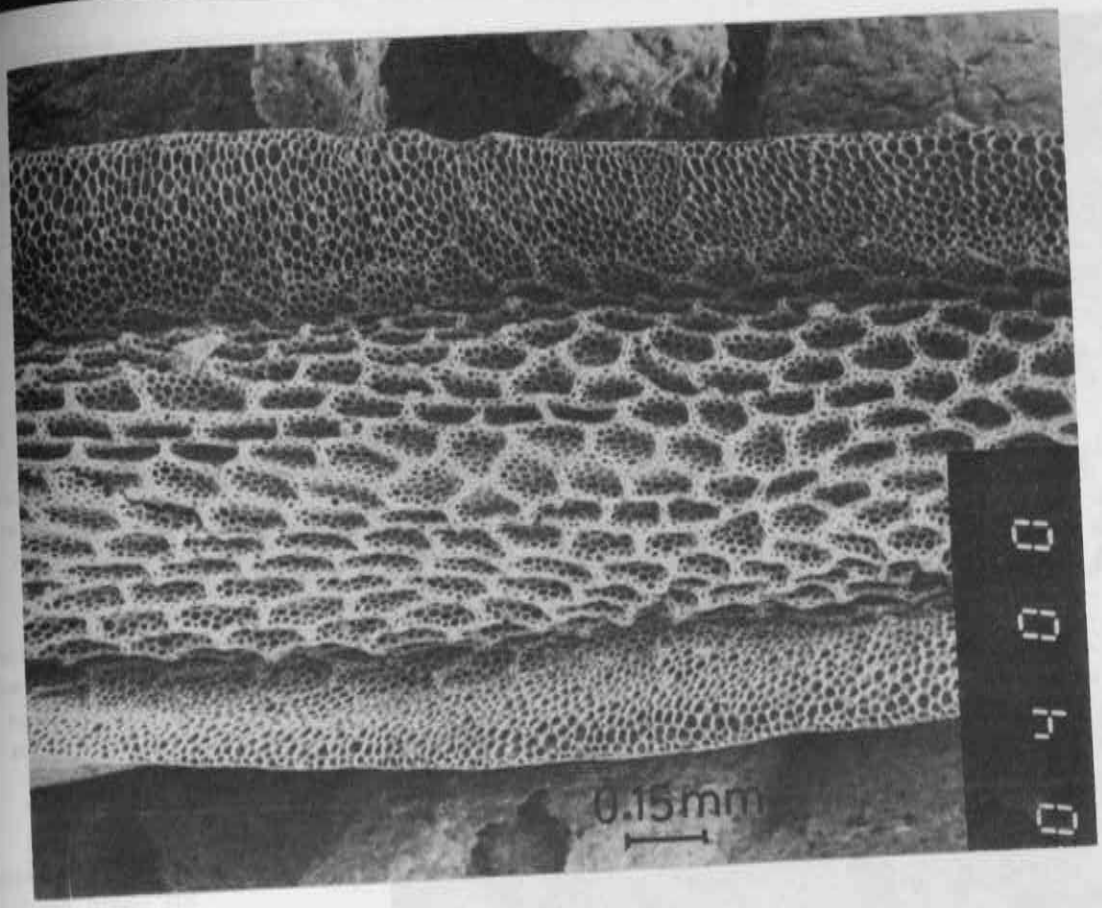
Part of haltere, *Glossina morsitans* x 600



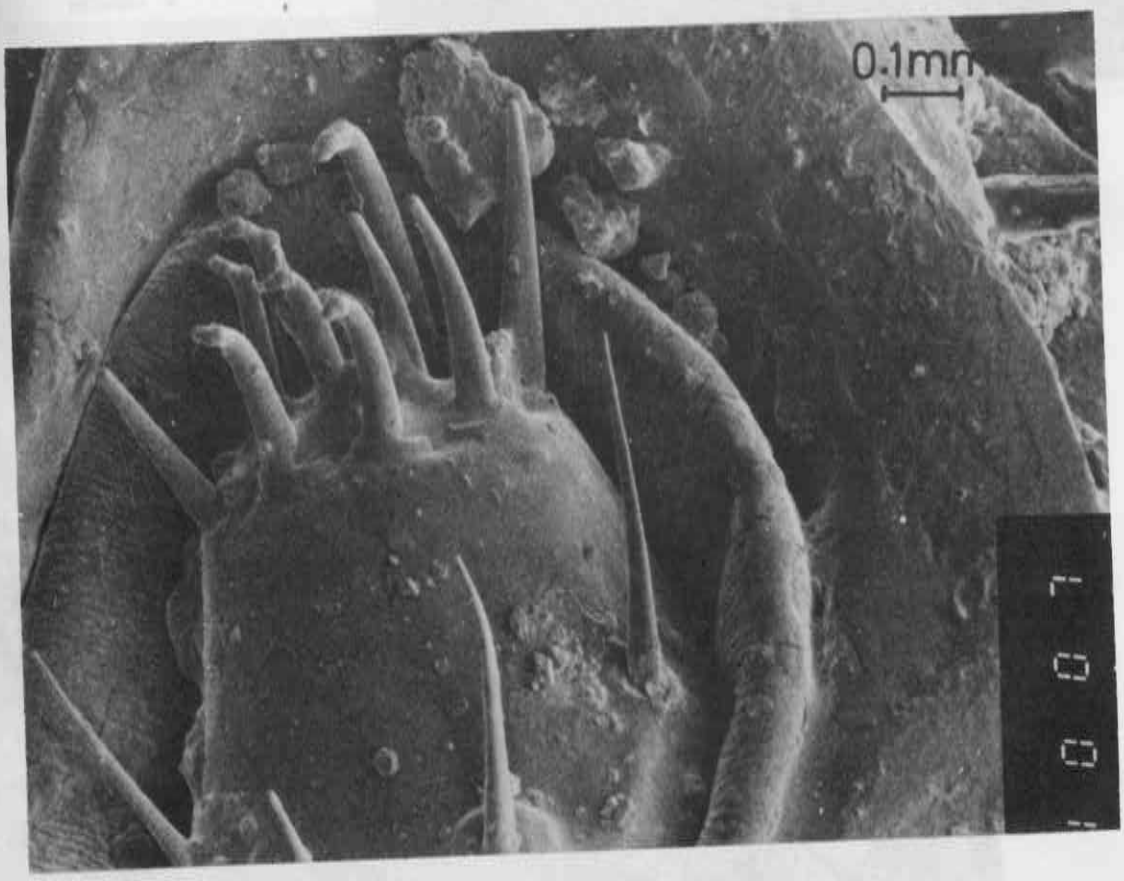
Ocelli, *Glossina morsitans* x 600



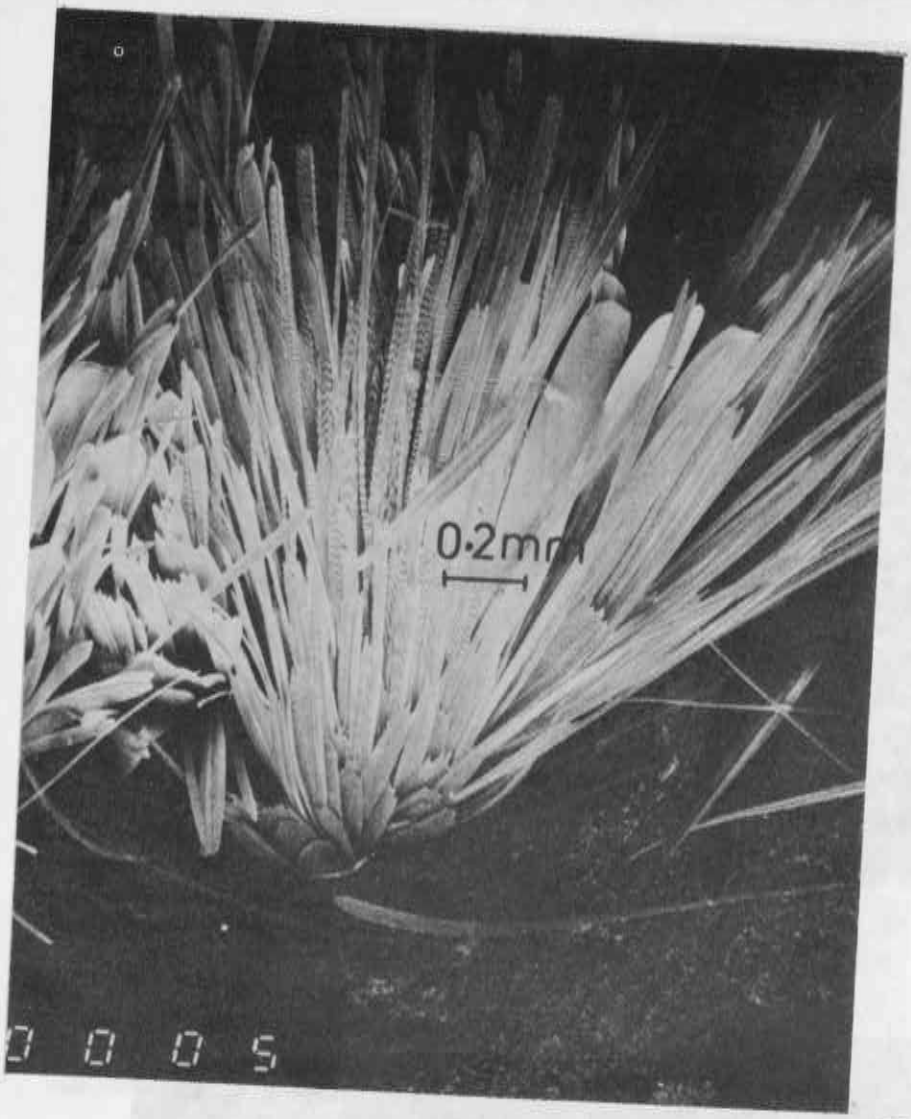
Compound eye, *Glossina morsitans* x 600



Sorghum shoot fly, egg, x 150



Sensilla, tick x 1000

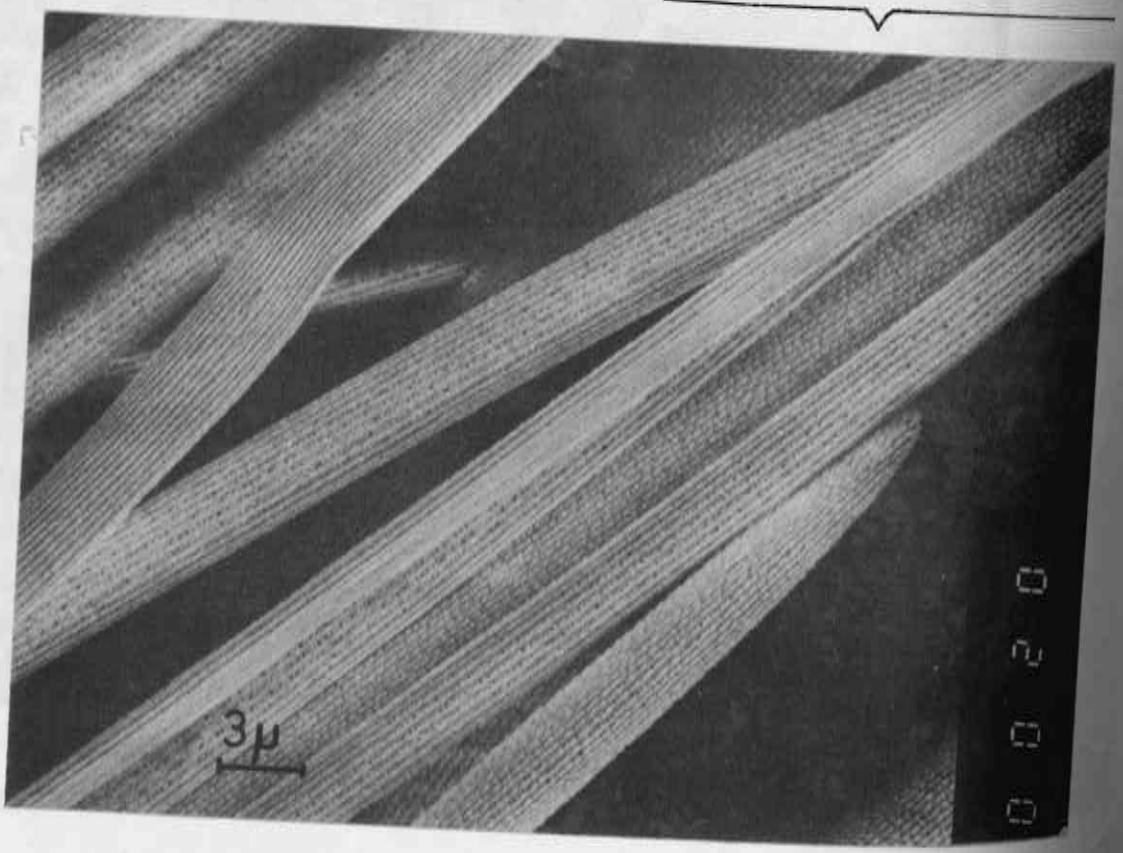


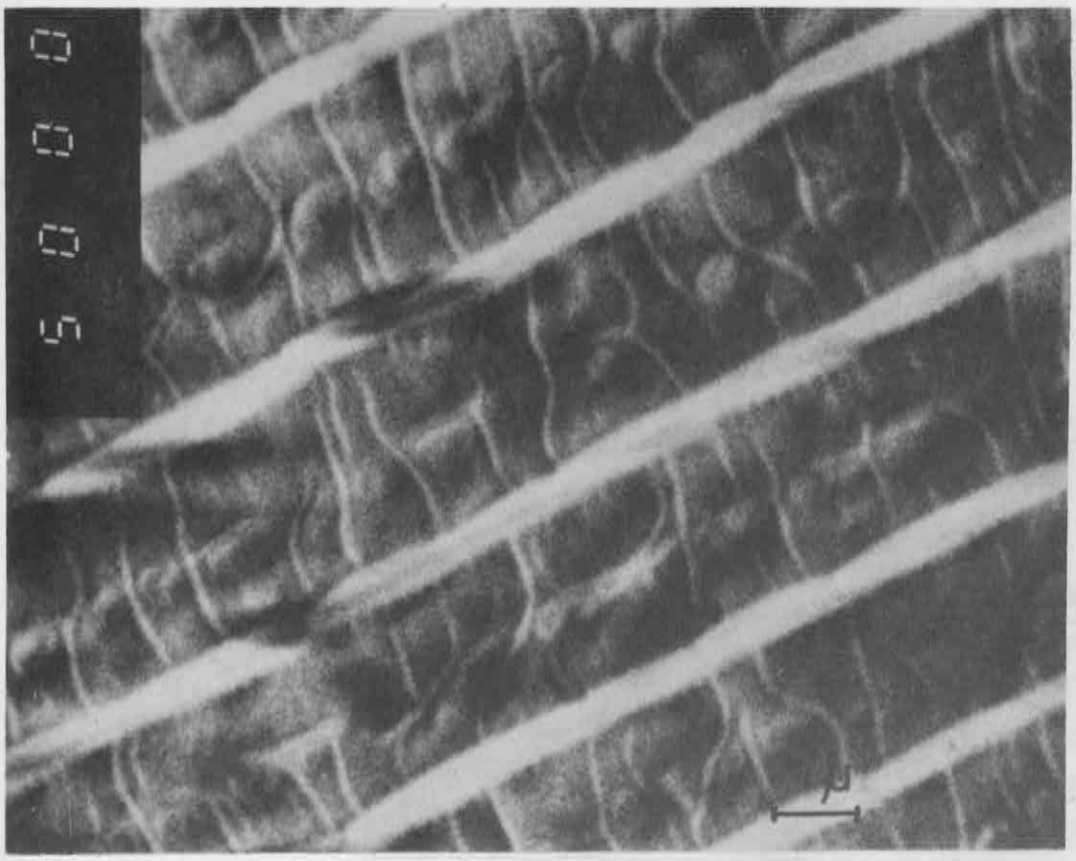
Hair pencils, *Spodoptera exempta* x 60

KEY TO LETTERING

A antenna
C collagen
CE compound eye
Ci cilia
CM cell membrane
ER endoplasmic reticulum
G golgi
Gr Granules
J junction
M mitochondrion
MS myelin sheath
N nerve nucleus
NE nerve fibres
OC ocellus
P palp
R ribosomes
SES sensory epithelium
SH sperm head
ST sperm tail
SP spines
V vesicles

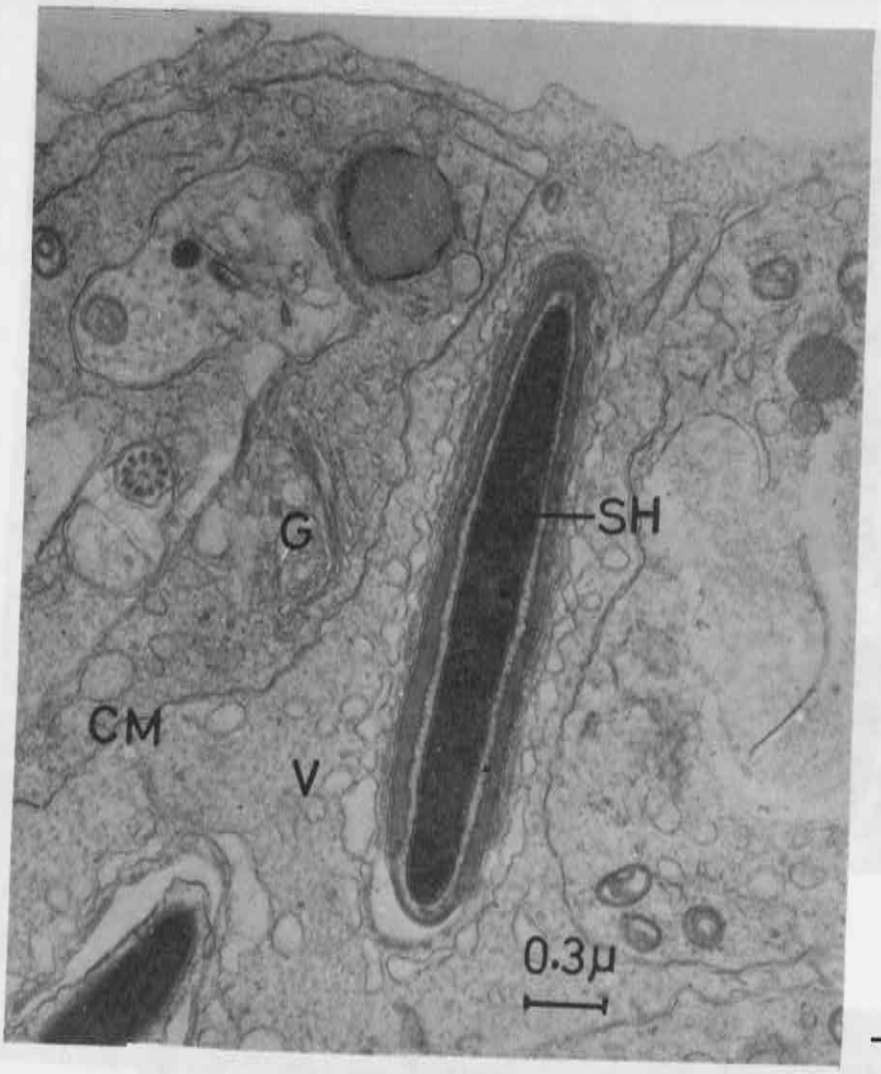
ditto, x 3000





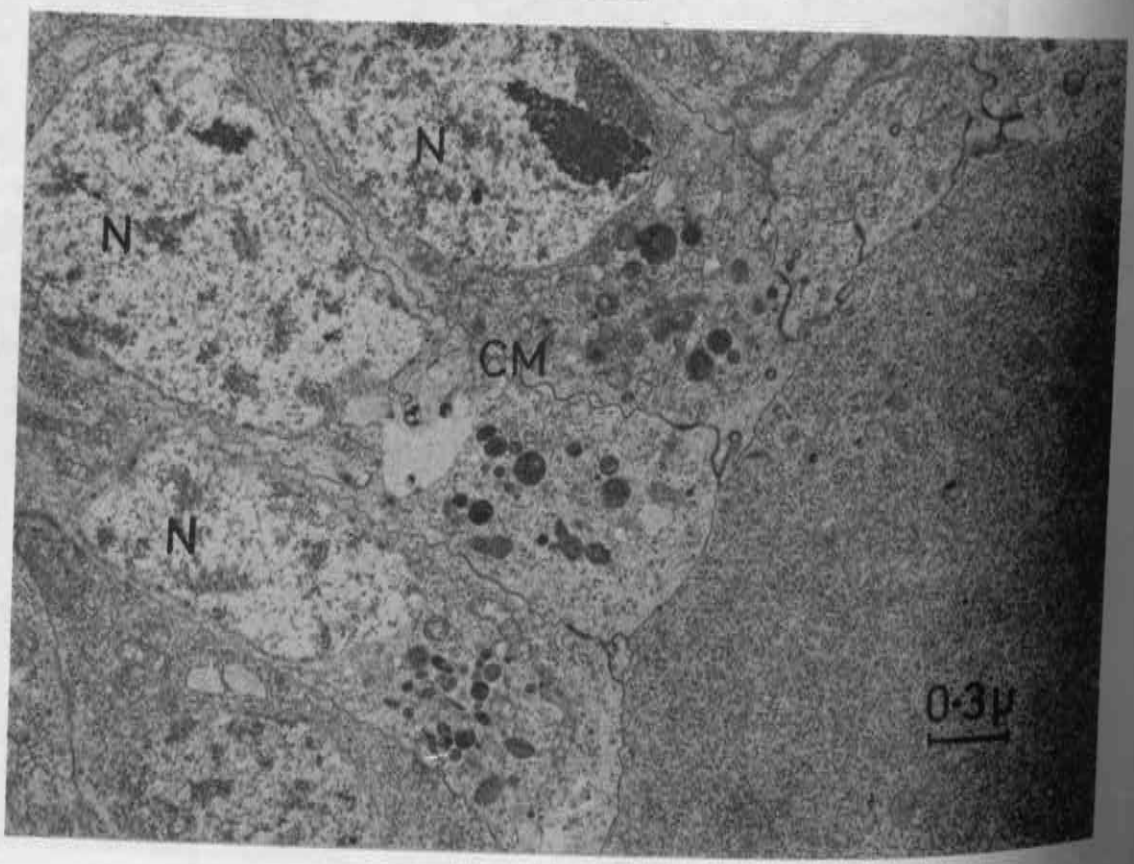
ditto x 10,000

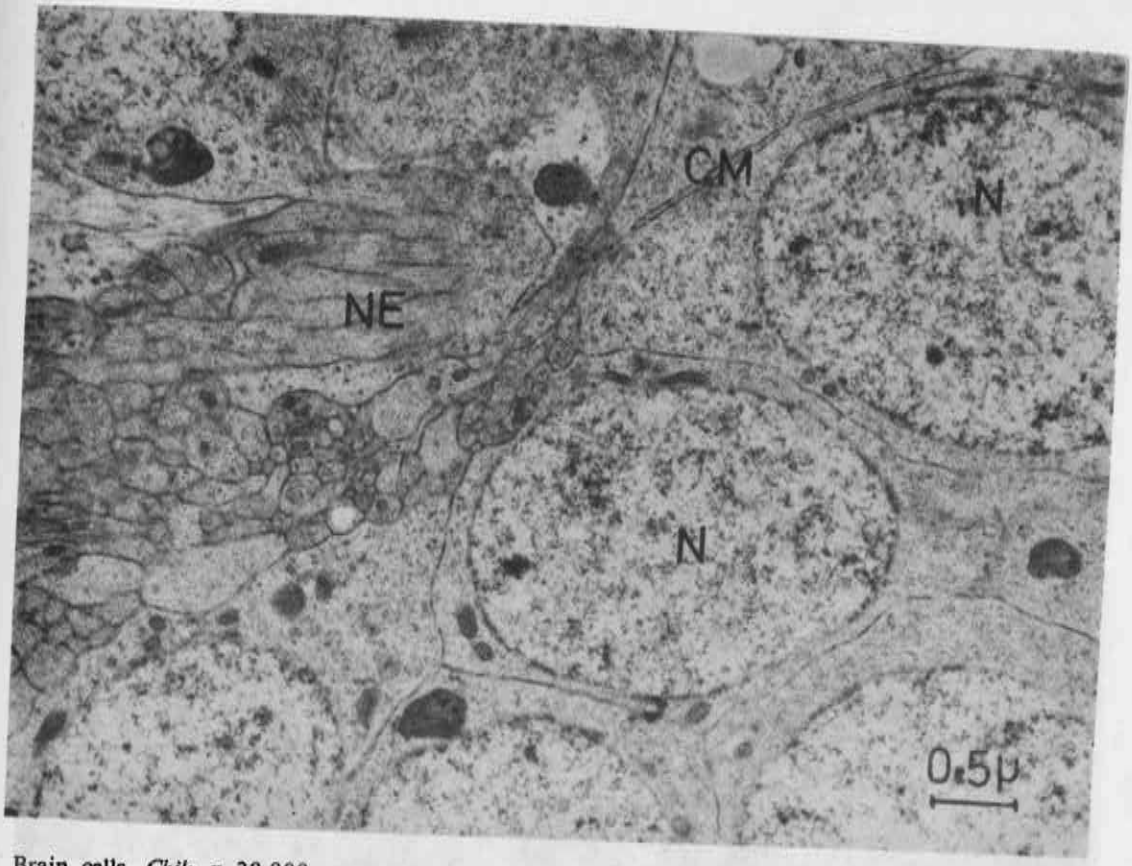
Eleutherum jaegeri (glass) x 300



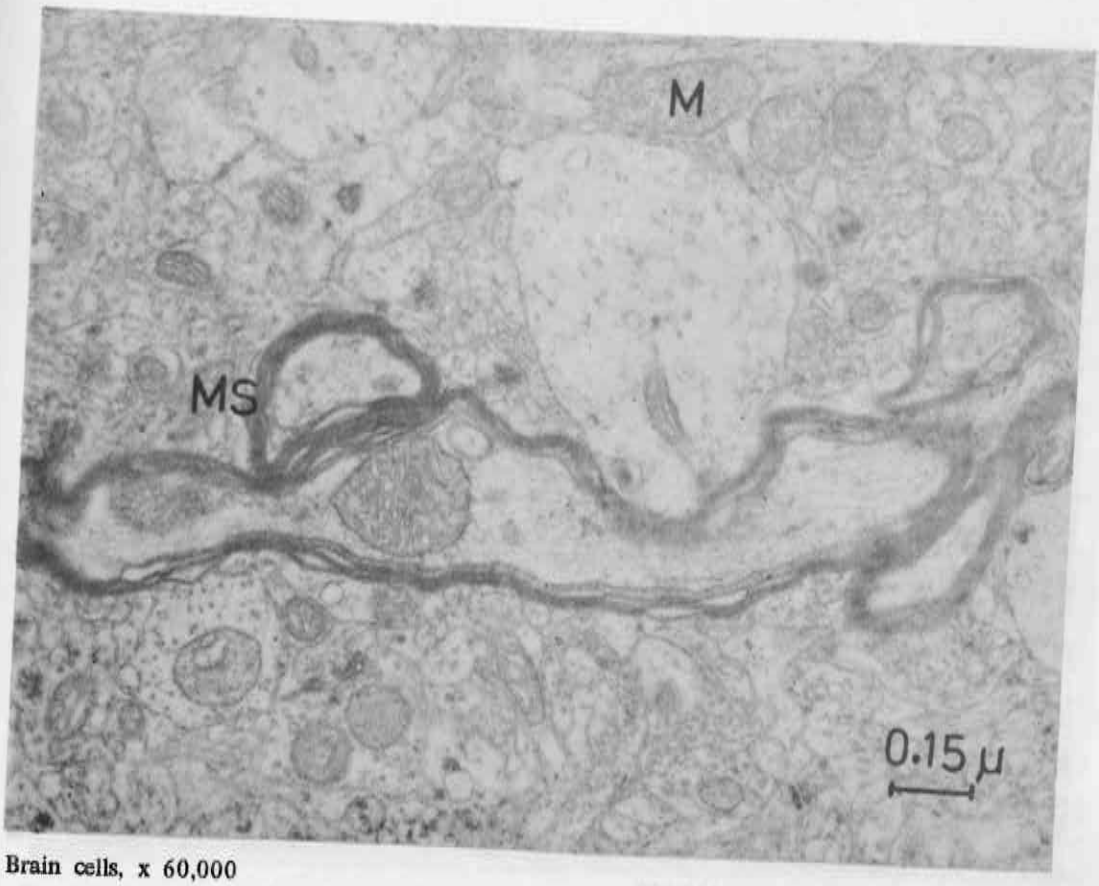
Testis, goat x 60,000

Ovary, fleshfly, x 30,000

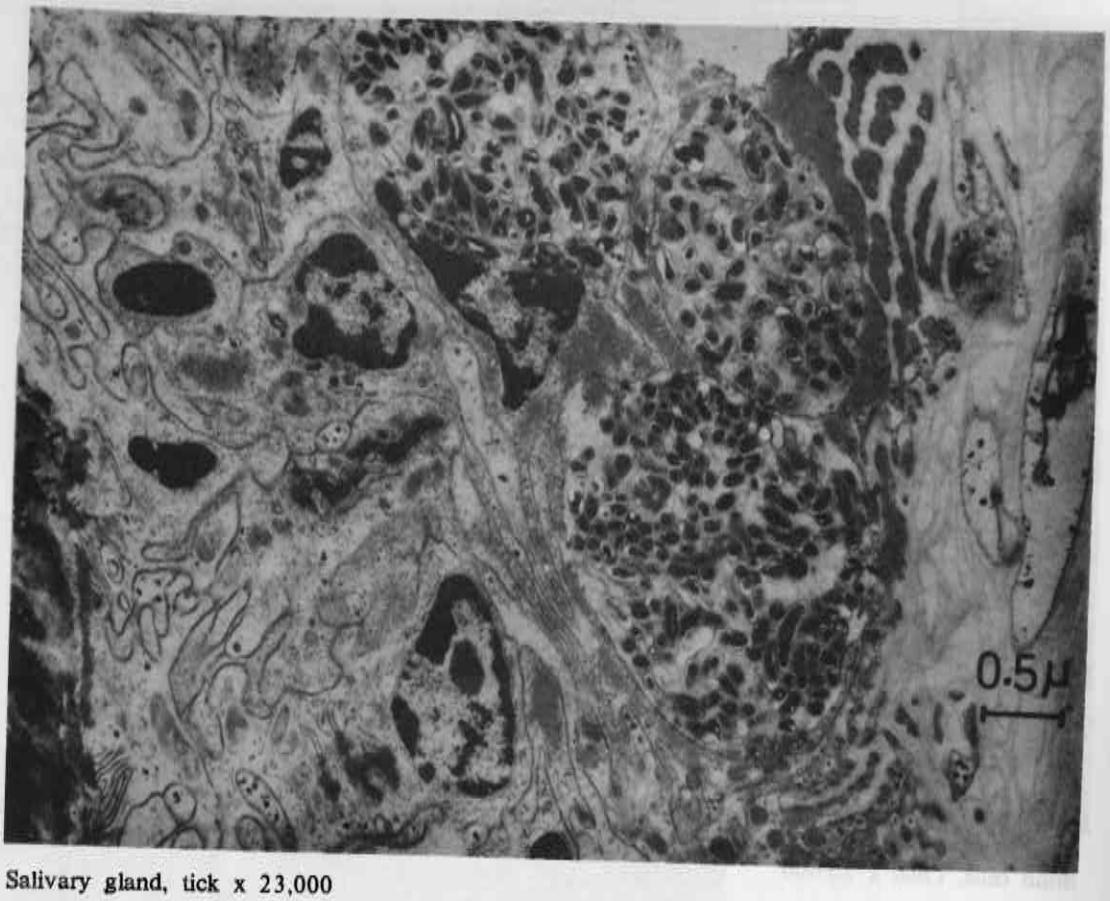




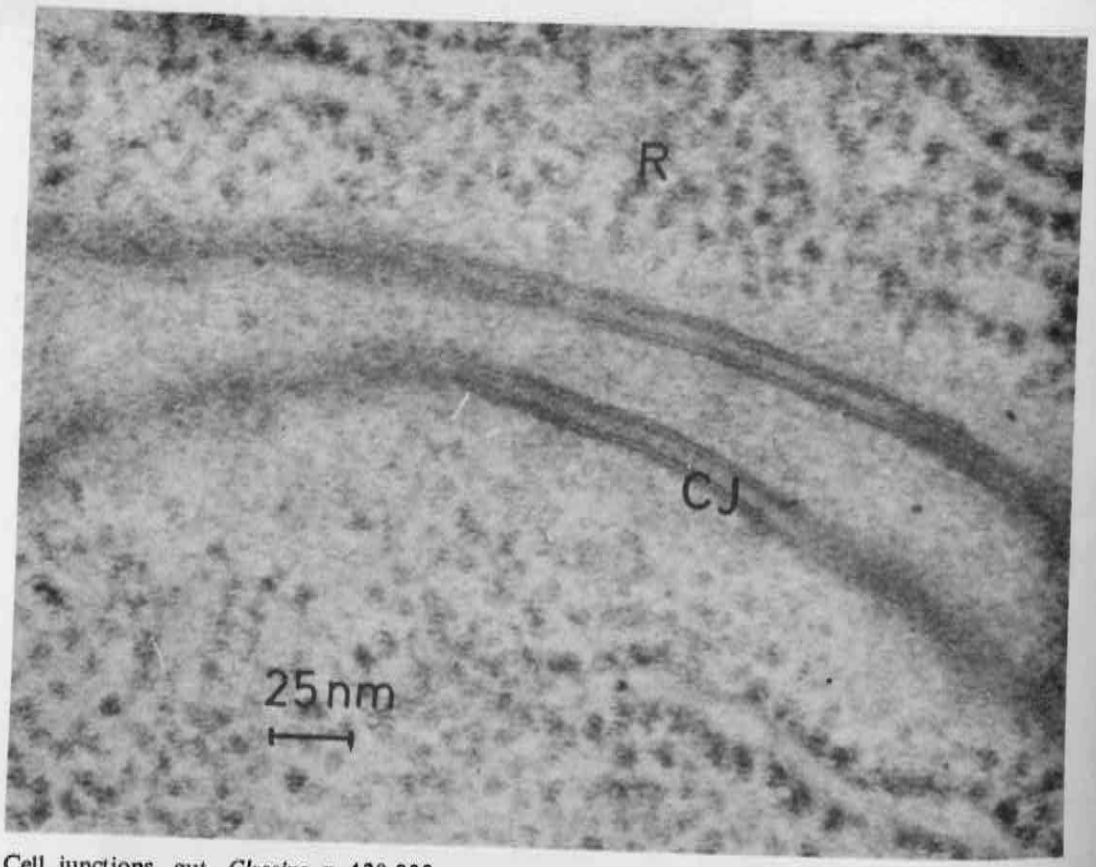
Brain cells, *Chilo* x 20,000



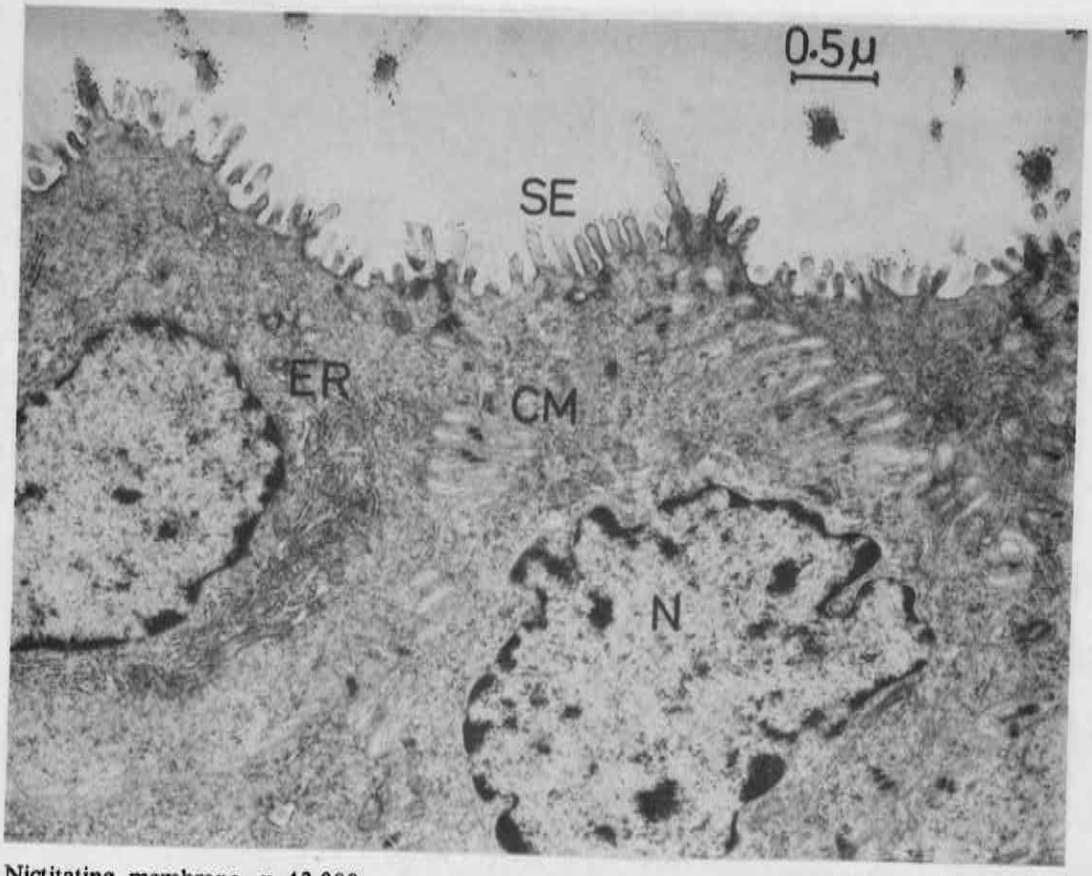
Brain cells, x 60,000



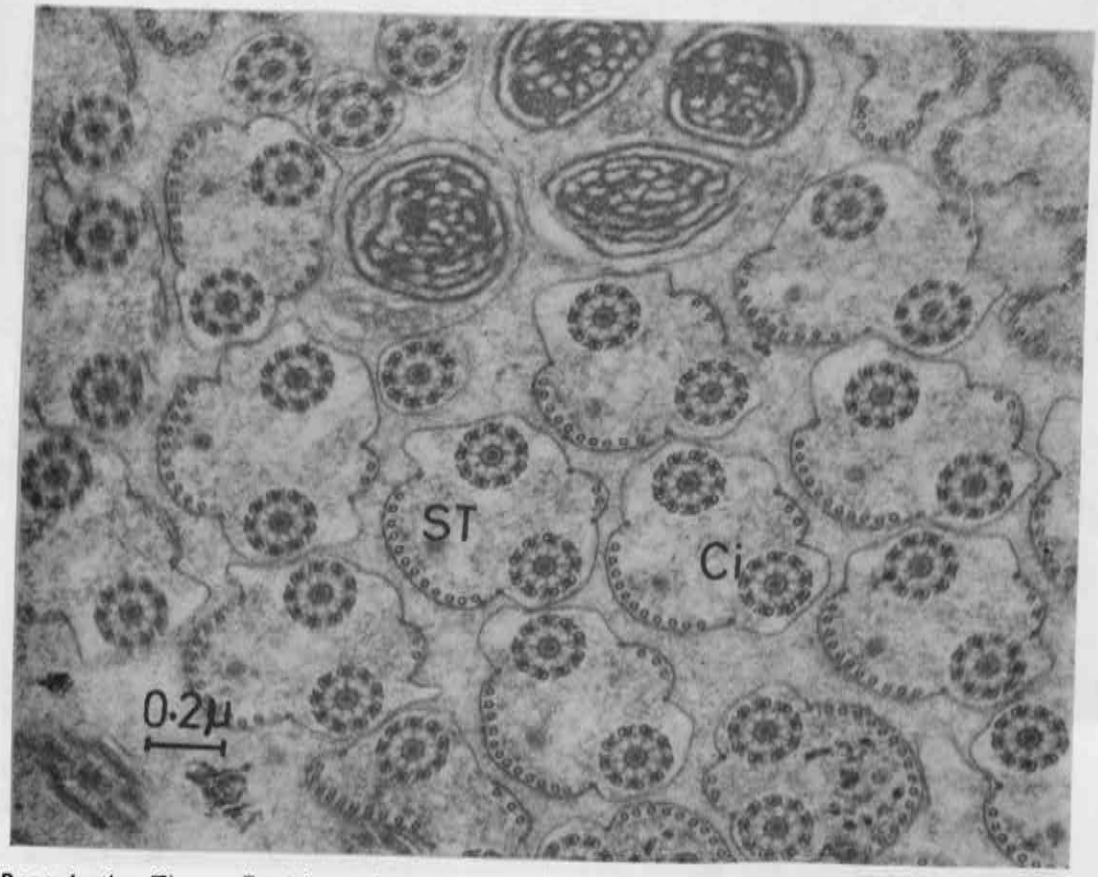
Salivary gland, tick x 23,000



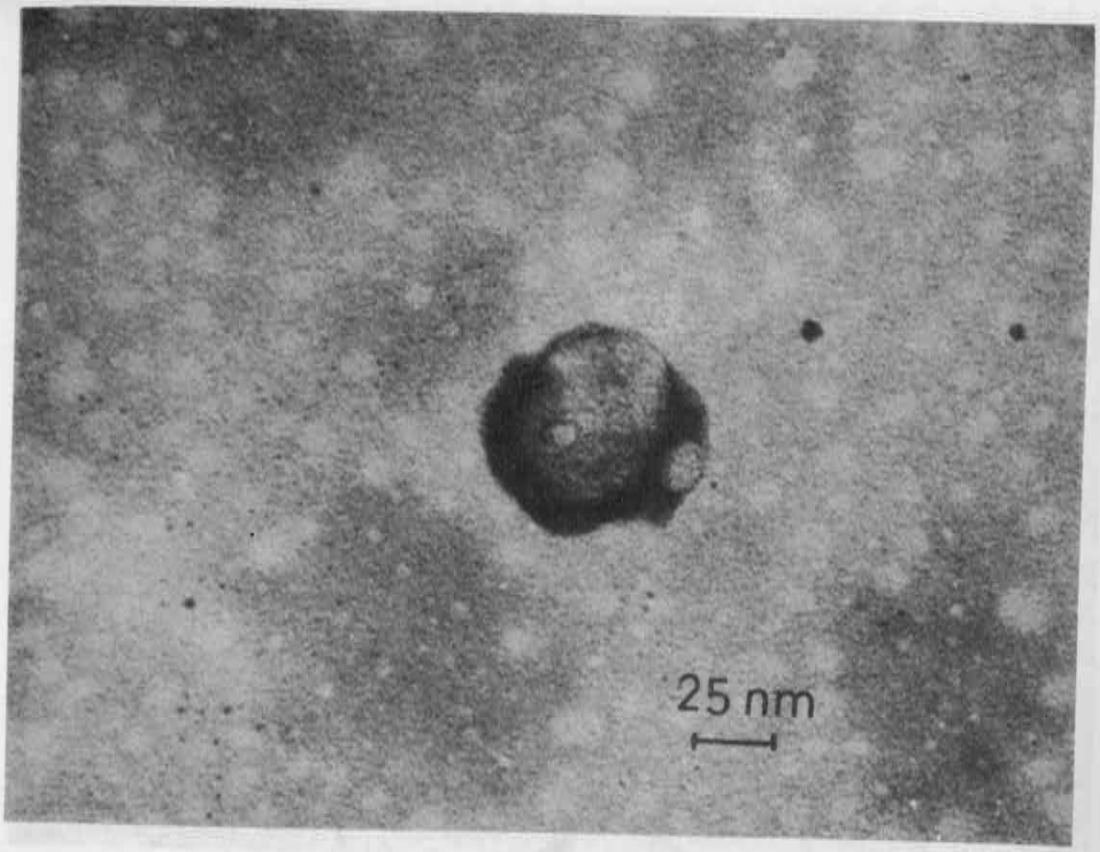
Cell junctions, gut, *Glossina* x 420,000



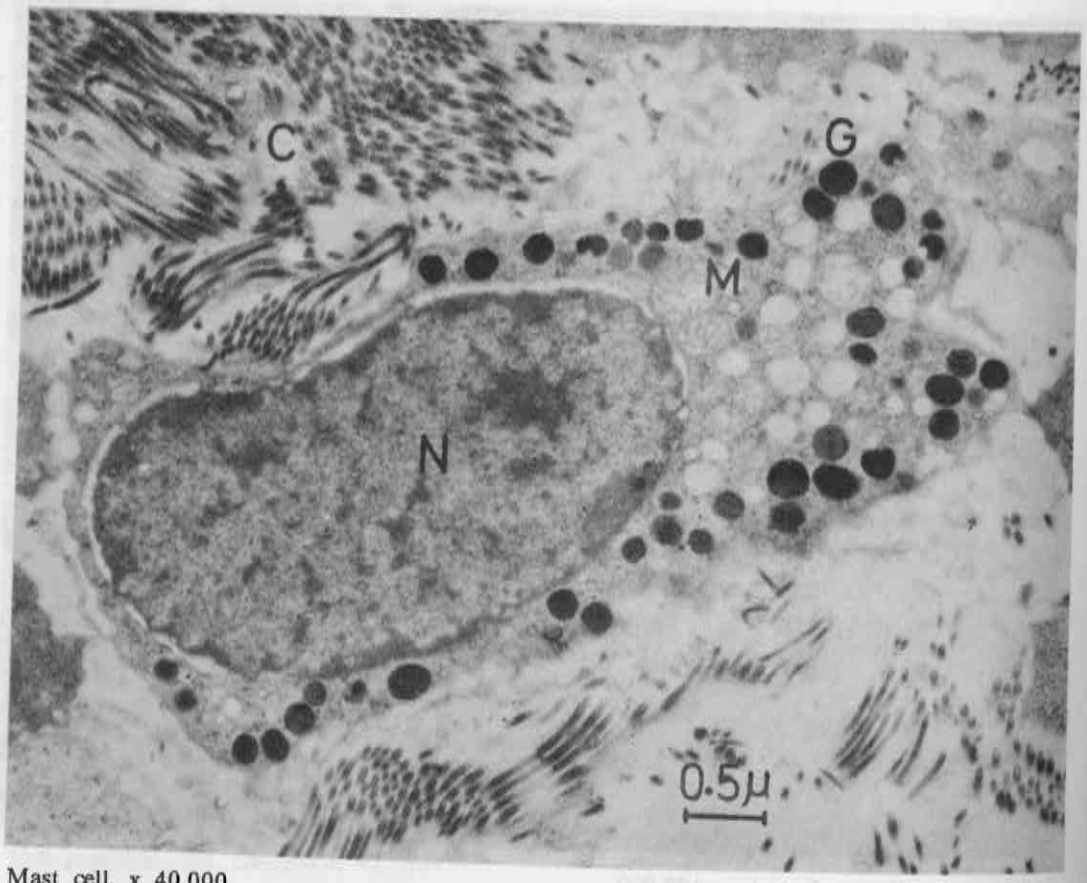
Nictitating membrane, x 42,000



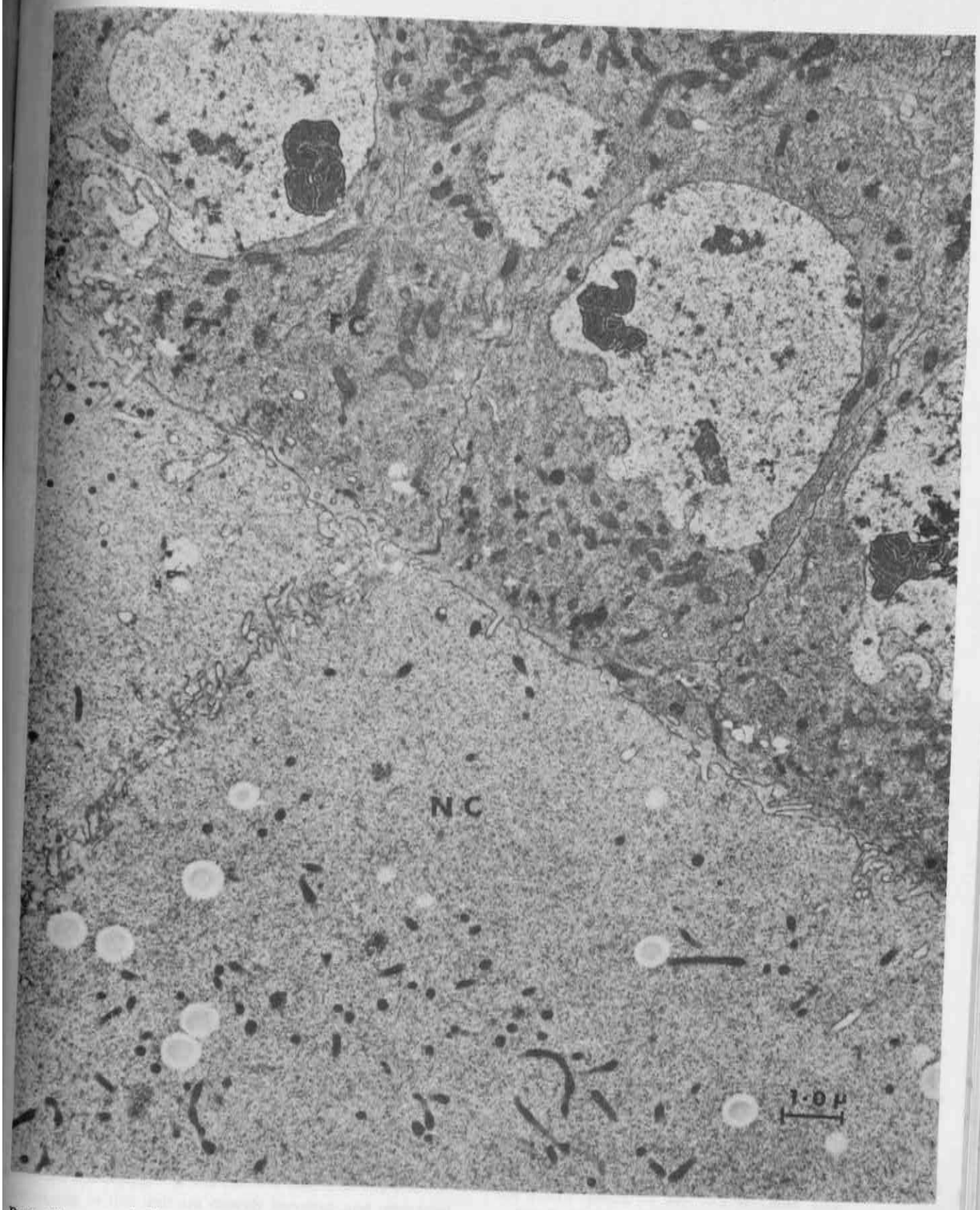
Reproductive Tissue, *Fasciola* x 120,000



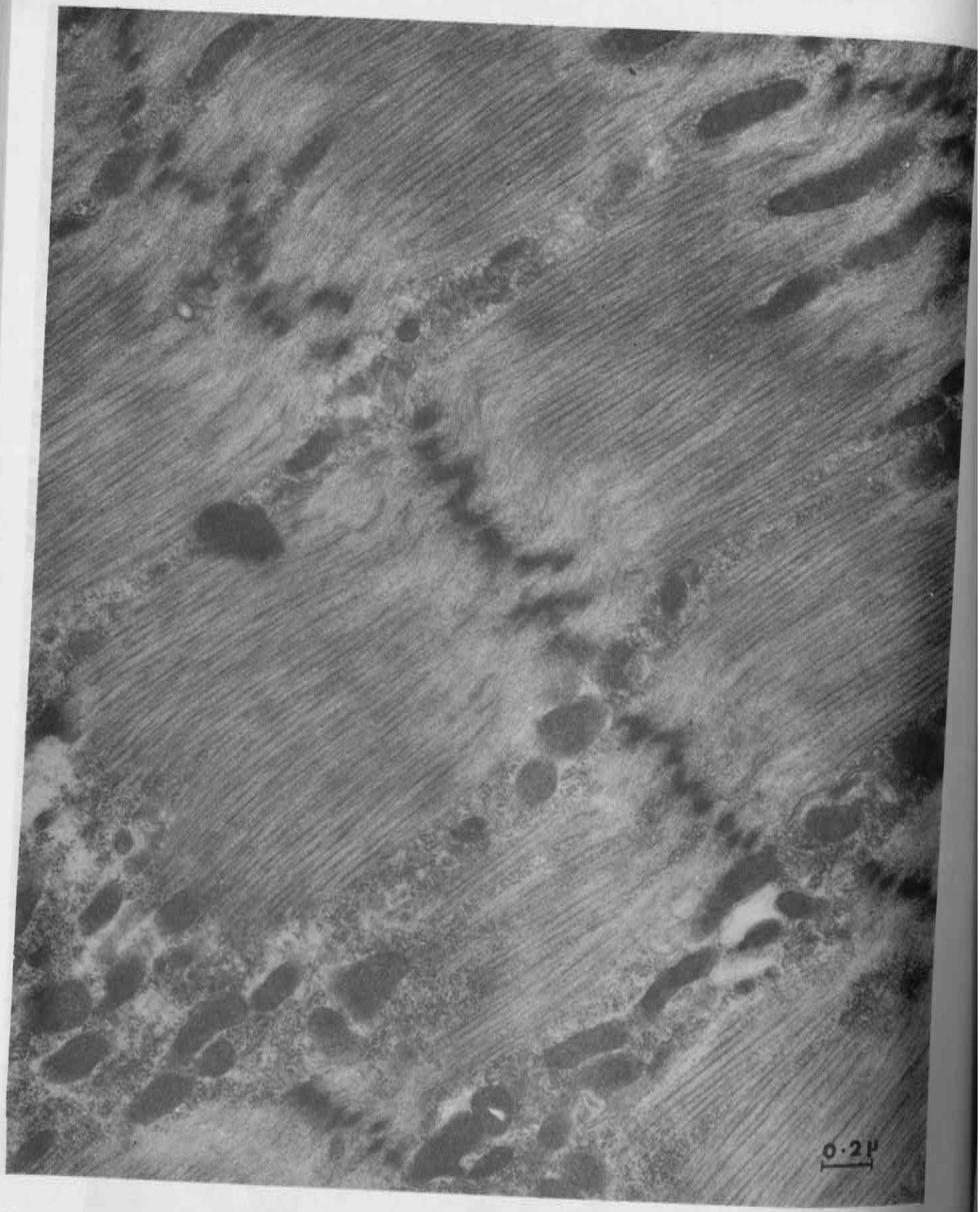
Virus particle x 420,000



Mast cell, x 40,000



Part of ovary of *Glossina morsitans* showing follicular cells (FC) and nurse cells (NC)



Uterine Striated muscle from *Glossina morsitans*

SENSORY PHYSIOLOGY RESEARCH UNIT

Directors of Research:
Professor D. Schneider (1970)
Professor F. Huber (1970-1975)

Scientists:
Dr. M. Kaib (1973-1976) – Research Scientist
Dr. G.R. Karuhize (1972-1976) – Research Scientist
Dr. R.A. Steinbrecht (1975) – Research Scientist
Dr. Ma Wei-Chun (1972-1975) – Research Associate.

This unit has formerly been called "Electrophysiology." With the understanding that electrophysiological methods are only partly covering the instrumentation necessary to study sensory physiology and behaviour, the unit was recently renamed "Sensory Physiology Research Unit." This more general field is represented by 5 Research Directors, but the two mentioned above are responsible for the unit proper and the scientists listed.

The general outline of research problems, approach and work plan are given in the 1973 Annual Report. As in the 1974 Annual Report, the findings of the individual scientists are reported in the corresponding sections dealing with the respective target insects.

From these reports it will become clear that the scientist belonging to this unit are directly involved and integrated into the research groups dealing with the respective target insects.

Dr. Ma continued his work on the analysis of taste receptors in the armyworm larva which enable the animal to select the proper food plant.

Dr. Kaib worked on the following problems:

1. Chemistry of frontal gland secretion in termite soldiers;
2. Orientation during trail following in two termite species;
3. Trail following and trail laying in a tineid moth caterpillar which lives in close contact with termites in their mounds;
4. Gallery building and repair in termites with references to trail activity of different castes;
5. Fine structure of antennal sensilla in the moth caterpillar leading to an electrophysiological study of the chemoreceptor cells which seem to respond to the trail pheromone of the host termite;
6. Caste polymorphism of termite sensilla.

Dr. Karuhize worked on:

1. Assembly pheromone of the tick *Ornithodoros moubata;*
2. The effect of relative humidity and behaviour of the same soft tick species;
3. The fine structure of receptors inside olfactory pits on the antenna of tsetse flies.

Flanking work on palpal and tarsal receptor polymorphism in soft and hard ticks and on the effect of relative humidity on the behaviour of hard ticks in comparison to the above species of soft tick is under way.

Dr. R. Steinbrecht recently joined the group and started collaboration with Dr. Kaib and Dr. Karuhize. His work will assure the continuity of the above programmes.

As it appears from the individual research reports, the scientists of the sensory physiology unit are closely collaborating with the other groups such as tick and termite research as well as chemistry and fine structure.

Miss Loving (in black) and her science class from the Swedish school in Nairobi being shown some micrographs in the Fine Structure Unit by the senior technician Mr. Mathayo Chimtawi.

ICIPE PUBLICATIONS

ICIPE Serial Nos

78. Aidley, D.J. (1974). Migratory capability of the African armyworm moth, *Spodoptera exempta* (Walk). E.A. Agric. and For. J. **40** (2) 202-205
36. Anonymous (1974). Pest Research Profile: The International Centre of Insect Physiology and Ecology. Pans **20** (2) 229-233.
23. Denlinger, D.L. and Wei-Chun Ma. Maternal nutritive secretion as possible channels for vertical transmission of micro-organism in insects the tsetse fly example Proc. N.Y. Acad. Sci. Conf. on Pathobiology of Invertebrate Vector of Disease **266** 162 -
34. Denlinger, D.L. (1974). Diapause potential in tropical flesh flies. Nature **252** (5480) 223-224.
22. Denlinger, D.L. (1975). Insect hormones as tsetse abortifacients. Nature **253** (5490) 347-348.
5. Denlinger, D.L. and Wei-Chun Ma (1974). Dynamics of the pregnancy cycle in the tsetse *Glossina morsitans* J. Insect Physiol. **20** 1015-1026.
21. Erickson, I.E. Carina and Aage R. Moller (1975). Sound production in the tsetse fly *Glossina Morsitans morsitans* J. Acoust. Soc. Am. **57** (4), 984-986.
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35. Galun, Rachel, S. Sternberg and C. Mango. The use of sterile females for the control of the tick *Argas Persicus* (Oken) Israel J. Ent. VII 109-115.
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37. Lewin, Roger 1975. International attack on insect pests. New scientist April 17th 1975. 124-25.
1. Meyer, Dietrich and M. Luscher (1973). Juvenile hormone activity in the haemolymph and the anal secretion of the queen of *Macrotermes subhyalinus* (Rambur) Isoptera Termitidae. Proc. VII Congr. IUSSI, London, 268-273.
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25. Prestwich, G. (1975). Chemical analysis of soldier defensive secretions of several species of East African termites. IUSSI Symp. on pheromones and Denfensive Secretions of Social Insects Dijon 149-153.
28. Prestwich, G.M. Kaib, W.F. Wood,a nd J. Meinwald (1975). 1, 13 Tetradeca-Dien-3-one and Homologs: New natural products isolated from *Schedorhinotermes* soldiers. Tetrahedron letters No. **52** 4701-4704.
42. Scheltes, P. (1974). The role of Graminaceous host-plants in the induction of aestivation-diapause in larvae of *Chilo Zonellus* Swinehoe and Chilo argyrolepia Hmps. Proc. of the Symposium on the host plant in reltion to insect behaviour and reproduction Tihany, Hungary, 11-14 June 1974.
31. Punyua, D.K. and R.E. Purnell, M.P. Cunningham, F.L. Musisi and R.C. Payne (1975). The establishment of an experimental field population of *Theileria Parva* – Infected ticks. Trop. Anim. Hlth. Prod. 7 133-137.
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41. **Van Der Geest and J. Kawooya (1975). Genetic variation in some enzyme systems in the tsetse fly** *Glossina Morsitans*. Ent. experiment. Applicata **18** 508-514.
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6. Wei Chun Ma, and D.L. Denlinger (1974). Secretory discharge and micro-flora of milk gland in tsetse flies. Nature 247, (5439) 301-303.
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5A. Youdeowei, Anthony (1975). Salivary secretion in three species of tsetse flies *(Glossinidae)* Acta Trop. **32** (2).

6A. Youdeowei, Anthony (1975). A simple technique for observing the saliva of tsetse flies *(Diptera Glossinidae)*. Bull. ent. Res. **65** 65-67.

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29. Chaudhury, M.F.B. and T.S. Dhadiala (1975). Evidence of hormonal control of ovulation in tsetse flies.

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INSIDE BACK COVER:

Dr. Abo-Khatwa (an ICIPE Research Scientist) thanking professor Sir Alan Hodgkin after delivering a public lecture in December 1975 at the ICIPE. Sir Alan Hodgkin is seated (cente) and Professor Odhiambo (left).

INSIDE FRONT COVER:

Dr. Olof Tandberg, Foreign Secretary of the Royal Swedish Academy of Sciences presenting a name plaque for the "Northern Star" building. Looking on are Mr. J.M. Ojal (ICIPE DEPUTY DIRECTOR) and Mr. Chris Archer.

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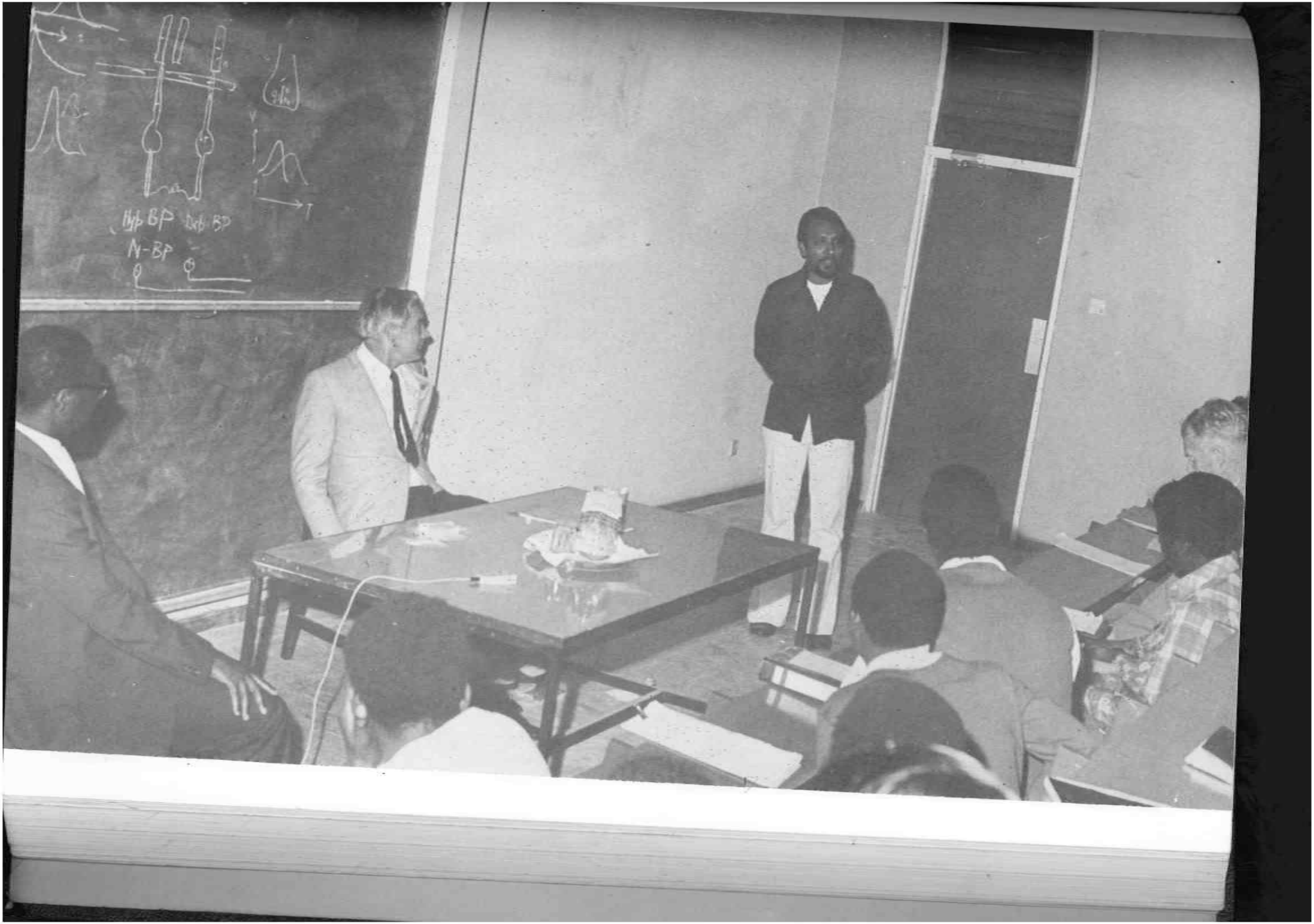
Hyp BP
N-BP

Prof. Thomas R. Gibson