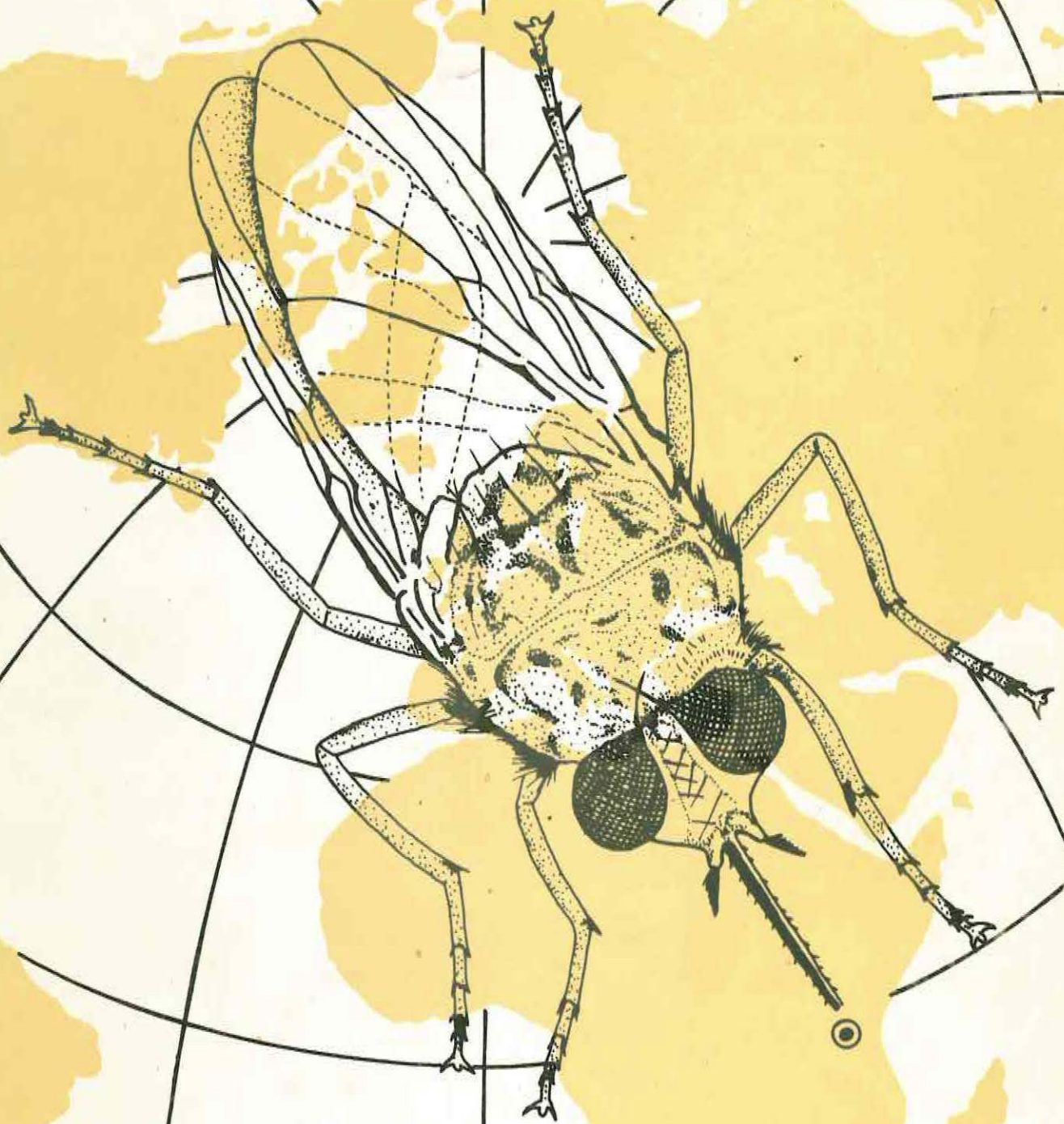


R. M. Nasson



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SIXTH ANNUAL REPORT — 1978



**THE INTERNATIONAL CENTRE OF
INSECT PHYSIOLOGY AND ECOLOGY**

SIXTH ANNUAL REPORT

1978

Nairobi, March 1979

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Professor Jan de Wilde, Chairman, ICIPE Governing Board
Professor Thomas R. Odhiambo, Director, ICIPE

VISITING DIRECTORS OF RESEARCH

Insect Ecology and Genetics

Dr. W. A. Sands (1973), Centre for Overseas Pest Research, London, United Kingdom

Insect Sensory Physiology and Behaviour

Professor J. W. S. Pringle (1970), Department of Zoology, University of Oxford, United Kingdom

Professor L. M. Schoonhoven (1975), University of Wageningen, Netherlands

Insect Pathology and Pest Management

vacant

Insect Histology and Fine Structure

Professor M. Locke (1977), University of Western Ontario, London, Canada

Insect Endocrinology

Professor W. S. Bowers (1977), Cornell University, Ithaca, USA

Professor M. Lüscher (1970), University of Bern, Bern, Switzerland

Insect/Hostplant Relations

Professor K. N. Saxena (1977), University of New Delhi, New Delhi, India

Vector Biology

Professor R. Galun (1970), The Hebrew University of Jerusalem, Jerusalem, Israel

Dr. J. Mouchet (1975), ORSTOM, Bondy, France

Dr. A. R. Njogu (1975), Trypanosomiasis Research Organization, Muguga, Kenya

Insect Biochemistry and Natural Products Chemistry

Professor O. L. Chapman (1977), University of California, Los Angeles, USA

Professor J. H. Law (1977), University of Chicago, Chicago, USA

PROGRAMME LEADERS

African Armyworm Research			Appointed
	Dr. D. J. W. Rose (Honorary position)	United Kingdom	1.7.77
Livestock Tick Research			
	Dr. M. P. Cunningham	United Kingdom	1.10.77
Tsetse Research			
	Dr. A. Challier	France	1.9.78
Medical Vectors Research			
	Dr. R. Subra	France	1.11.78

RESEARCH SCIENTISTS

African Armyworm Research			Appointed
	Dr. S. Khasimuddin	India	1.12.73
	Dr. B. I. P. Persson	Sweden	1. 9.77
Bases of Plant Resistance			
	Dr. R. C. Saxena	India	1.7.77

Mosquito Research			
	Dr. A. W. R. McCrae	United Kingdom	1.11.77
	Dr. F. W. Mosha	Tanzania	1. 3.78
Sorghum Shootfly Research			
	Dr. J. R. Clearwater	New Zealand	11. 7.75-17.4.78
	Dr. A. G. L. Delóbel	France	1.10.78
	Dr. A. K. Raina	India	1. 9.77
	Dr. G. C. Unnithan	India	1. 9.78
Termite Research			
	Dr. M. A. Arshad	Canada	15. 7.77
	Ir. O. H. Bruinsma	Holland	1.11.74-28.2.78
	Dr. G. Bühlmann	Switzerland	1. 4.75
	Dr. J. P. E. C. Darlington	United Kingdom	1.10.74
	Dr. M. G. Lepage	France	24. 7.75
	Dr. G. W. Oloo	Kenya	1. 5.74
	Dr. D. B. A. Ruyooka	Uganda	1. 9.78
Livestock Tick Research			
	Dr. R. M. Newson	United Kingdom	1. 9.74
	Dr. F. D. Obenchain	USA	6.10.76
Tsetse Reproductive Physiology			
	Dr. M. F. B. Chaudhury	Bangladesh	1. 3.74
	Dr. K. Endo	Japan	1. 6.77
Tsetse Salivary Gland Physiology			
	Dr. T. K. Golder	USA	1. 4.78
	Dr. F. L. Lambrecht	United Kingdom	1. 4.78
	Dr. M. B. A. Nyindo	Tanzania	1. 5.78
	Dr. J. O. Olobo	Kenya	1. 5.78
	Dr. L. H. Otieno	Kenya	1. 2.73
Tsetse Population Diversity			
	Dr. J. van Etten	Holland	31. 1.74
	Dr. W. F. Snów	United Kingdom	1.12.77
Bioassay			
	Dr. T. Gebreyesus	Ethiopia	14. 3.78
Chemistry and Biochemistry			
	Dr. A. Maradufu	Tanzania	1. 3.74-30.6.78
	Dr. C. K. Wilkins	USA	25. 3.77-30.11.78
Histology and Fine Structure			
	Dr. E. D. Kokwaro	Kenya	1.12.75
	Dr. A. Massalski	Canada	1.10.78
Sensory Physiology			
	Dr. J. V. Clark	United Kingdom	1. 1.77
	Dr. J. H. MacFarlane	Canada	25. 4.77
	Dr. S. Waladde	Uganda	18. 9.78
Insect and Animal Breeding			
	Dr. A. Basu	India	5.10.76-5.10.78
Insect Mass Rearing			
	Dr. R. S. Ochieng	Kenya	1.11.77

Insect Pathology	Dr. G. P. Kaaya	Tanzania	1. 7.78
	Dr. W. Otieno	Kenya	8. 2.78

SCIENTIFIC OFFICERS

Sorghum Shootfly Research	Mr. K. Ogwaro	Uganda	Appointed 1. 9.73
Termite Research	Mr. B. M. Okot-kotber	Uganda	13. 2.76
Livestock Tick Research	Mrs. C. K. A. Mango	Kenya	1. 7.71
	Mr. D. K. Punyua	Kenya	1. 9.73
Tsetse Reproductive Physiology	Mr. T. S. Dhadialla	Kenya	1.10.73
	Mr. J. Kawooya	Uganda	1. 9.73
Tsetse Salivary Gland Physiology	Mrs. N. Y. Patel	Kenya	1. 3.75
	Mrs. M. Vundla	Kenya	1. 2.75
Histology and Fine Structure	Mr. J. Owor	Uganda	1.12.73
Sensory Physiology	Mr. R. K. Saini	Kenya	2. 6.76
Mbita Point Field Station	Mr. J. B. Okeyo-Owuor	Kenya	1.10.78

RESEARCH ASSISTANTS

African Armyworm Research	Mr. B. L. Otindo	Kenya	11.1.76
Livestock Tick Research	Mr. J. W. Chiera	Kenya	9.10.76
Tsetse Reproductive Physiology	Mrs. R. W. Kunyiha	Kenya	18. 5.76
Tsetse Salivary Gland Physiology	Miss N. F. Darji	Kenya	1.10.74
	Mrs. J. A. Kongoro	Kenya	16. 4.74

TECHNICAL STAFF

African Armyworm Research	Mr. J. Igunza	Technician	Kenya	6.11.72
	Mr. J. T. Kilori	Technician	Kenya	1.11.72
	Mr. G. M. Kinyanjui	Technical Assistant/Driver	Kenya	11. 3.77
	Mr. M. Lubega	Junior Technician	Uganda	1. 3.74
	Mr. D. N. Mathenge	Junior Technician	Kenya	1. 3.74

	Mr. G. N. Mburu	Subordinate Assistant	Kenya	15. 1.74
	Mr. R. Okello	Technical Assistant	Kenya	1. 3.73
	Mr. C. Were	Technical Assistant	Kenya	1. 1.77
	Mr. J. Yarro	Graduate Trainee	Tanzania	23. 5.77
Mosquito Research				
	Mr. E. Mkuzi	Technical Assistant	Kenya	1. 6.76
	Mr. M. M. Ngunnzi	Graduate Trainee	Kenya	16. 5.77
Sorghum Shootfly Research				
	Mr. G. O. Amala	Technical Assistant	Kenya	1. 5.76
	Mr. G. M. N. Bizoza	Principal Technician	Uganda	15. 9.77
	Mr. K. E. Kidega	Senior Technician	Uganda	1.10.77
	Mr. J. C. Olela	Senior Technician	Kenya	30. 9.77
	Mr. S. M. Othieno	Technician	Kenya	1. 4.73
Termite Research				
	Mr. L. B. Busharizi	Senior Technician	Uganda	1.10.77
	Mrs. R. Kariuki	Subordinate Assistant	Kenya	1.10.74
	Mr. J. N. Kaseleweu	Subordinate Assistant	Kenya	1. 5.77
	Mr. D. T. Kasino	Subordinate Assistant	Kenya	1. 8.76
	Miss M. N. Mambea	Junior Technician	Tanzania	1.10.74
	Mr. J. K. Muli	Subordinate Assistant	Kenya	1.10.78
	Mr. H. M. Nayeni	Technician	Kenya	1. 8.76
	Mr. J. O. Onyango	Technical Assistant	Kenya	12. 5.77
	Miss M. Wanjiru	Technical Assistant	Kenya	1.12.75
Livestock Tick Research				
	Mr. A. Bwire	Technical Assistant	Kenya	1. 9.75
	Mr. G. M. Hindi	Subordinate Assistant	Kenya	1. 3.74
	Mr. A. O. Mongi	Graduate Trainee	Tanzania	3. 3.75
	Mr. J. G. Mugane	Subordinate Assistant	Kenya	1. 8.73
	Mr. J. N. Ndungu	Subordinate Assistant	Kenya	1. 8.73
	Mr. R. Ojowa	Junior Technician	Kenya	15. 2.72
	Mr. F. M. Thuo	Technician	Kenya	14. 6.77
	Mr. K. C. Wainaina	Subordinate Assistant	Kenya	1. 3.74
Tsetse Reproductive Physiology				
	Mr. F. Mukunza	Junior Technician	Kenya	14.11.73
	Mr. P. A. Osula	Junior Technician	Kenya	1. 3.78
Tsetse Salivary Gland Physiology				
	Miss R. Chesang	Junior Technician	Kenya	1. 3.72
	Mr. J. Likhanga	Technical Assistant/Driver	Kenya	1.11.74
	Mr. E. Mpanga	Technician	Uganda	1. 3.78
	Mr. P. Onyango	Technician	Kenya	1.12.74
Tsetse Population Diversity				
	Mr. J. O. Apale	Junior Technician	Kenya	1. 5.76
	Mr. F. Kathuli	Technical Assistant/Driver	Kenya	1. 1.77
	Mr. J. Kiilu	Subordinate Assistant	Kenya	7. 6.76
	Mr. A. Makau	Technician	Kenya	1. 6.76
	Mr. D. K. Mungai	Technical Assistant/Driver	Kenya	1. 6.78
	Mrs. M. Owaga	Graduate Trainee	Kenya	1. 7.77
	Mr. D. F. Uvyu	Technical Assistant	Kenya	1.12.74
Bioassay				
	Mr. G. Achieng	Technical Assistant	Kenya	1. 9.76
	Mr. L. Moreka	Subordinate Assistant	Kenya	1. 9.76
	Mr. B. N. Odero	Principal Technician	Kenya	21.10.76

Chemistry and Biochemistry			
Mr. A. Chapya	Principal Technician	Kenya	16. 4.74
Mr. N. Juma	Junior Technician	Kenya	1. 4.74
Histology and Fine Structure			
Mr. M. Chimtawi	Technician	Tanzania	15. 1.74
Mr. P. Lisamulla	Technician	Kenya	1. 2.73
Sensory Physiology			
Mr. H. M. Kahoro	Technician	Kenya	1. 5.75
Mr. M. Nabil	Technician	Kenya	1. 2.78
Insect and Animal Breeding			
Mr. J. Atema	Technical Assistant	Kenya	1.10.75
Mr. E. Awuoch	Technical Assistant	Kenya	1.12.73
Mr. H. Banda	Junior Technician	Kenya	16. 2.72
Mr. J. M. Birir	Technical Assistant/Driver	Kenya	1. 2.78
Mr. A. Ikhunyalo	Junior Technician	Kenya	16. 2.71
Mr. J. Kagoiya	Junior Technician	Kenya	1.10.73
Mr. J. Ongudha	Technician	Kenya	1.10.73
Mr. J. Wanyonje	Senior Technician	Kenya	1. 2.70
Insect Mass Rearing			
Mr. P. O. Odinga	Junior Technician	Kenya	1. 3.78
Miss A. A. Ragot	Technical Assistant	Kenya	1. 4.78
Mr. S. H. Okech	Technician	Kenya	1. 8.77
Workshop			
Mr. H. Gichinga	Junior Technician	Kenya	1. 3.73
Mr. J. M. Maina	Technician	Kenya	1. 3.75
Mr. J. N. Mtei	Junior Technician	Tanzania	12.10.76
Mr. P. O. Nyachieo	Senior Technician	Kenya	1.12.73
Mr. J. N. Omondi	Technical Assistant	Kenya	15. 7.74
Mr. J. B. Omulo	Junior Technician	Kenya	3. 9.73
Field Stations			
Mr. P. M. Mwamisi	Junior Technician	Kenya	1. 3.78
Mr. J. Mwandandu	Technical Assistant	Kenya	1. 1.77
Mr. S. W. Wanjohi	Subordinate Assistant	Kenya	1. 6.76
Communications and Information			
Mr. J. F. Shikhubari	Technician	Kenya	15. 8.75

SENIOR MANAGEMENT STAFF

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Manager for Communication Systems	Mr. J. M. Ojal
Administrative Manager	Mr. C. O. Angoma
Financial Manager	Mr. L. Z. Moshia
Accountant	Mr. R. M. P. Okura
Senior Administrative Officer	Mrs. R. A. Odingo
Senior Training Officer	Dr. L. O. Abe
Training Officer	Miss R. Washika
Communications Officer	Mrs. J. Hartley
Librarian	Mr. D. R. Kigera
Controller, Technical Services	Mr. A. Mando
Controller, Insectary Services	Dr. A. Basu (resigned 5.10.78)
Agronomist, Mbita Point	Mr. E. O. Omolo
Farm Controller, Mbita Point	Mr. B. S. K. Masyanga

ADMINISTRATIVE STAFF

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Mrs. M. U. Arara	Senior Secretary	Mrs. M. N. Okach	Assistant Secretary
Miss M. Bugembe	Secretary	Mrs. R. A. Okoth	Assistant Secretary
Mrs. S. Kabwegyere	Secretary	Mrs. A. A. Okumali	Secretary
Mr. S. M. Kimaita	Administrative Officer	Mr. J. E. Okiri	Administrative Officer
Mr. N. Kiongo	Assistant Accountant	Mr. J. O. Omuodo	Administrative Officer
Mr. J. K. Kitur	Account's Clerk	Mrs. M. R. Opande	Secretary
Mrs. T. Lohay	Secretary	Mrs. P. Owitti	Secretary
Mr. M. P. Macohito	Supplies Assistant	Mr. C. I. Rapasi	Supplies Officer
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Miss D. A. Mbeche	Secretary	Miss R. Runno	Secretary
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DEVELOPMENT PRIORITIES

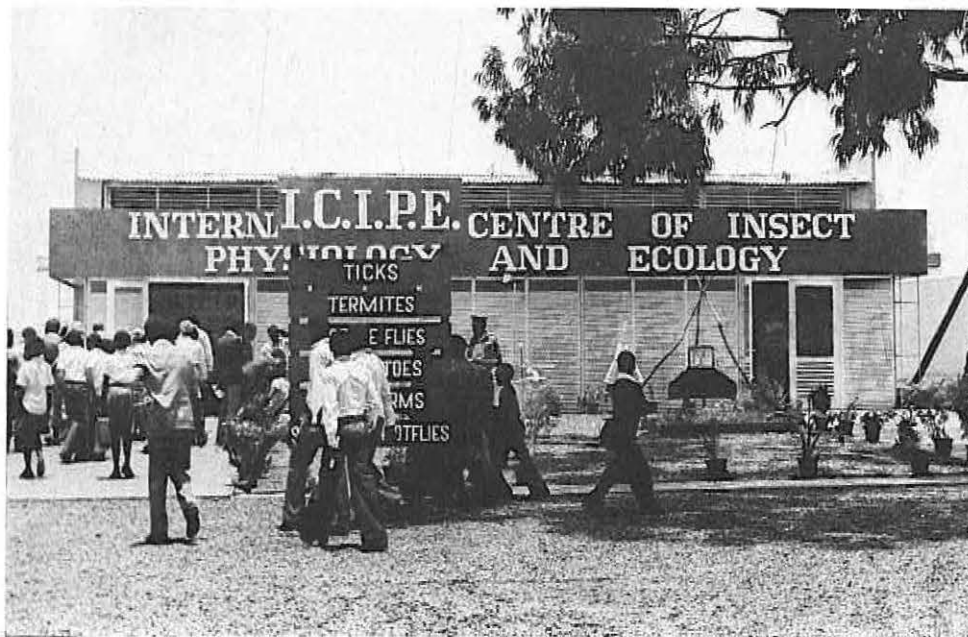
The year 1978 saw significant developments at the International Centre of Insect Physiology and Ecology (ICIPE). The Centre has established, with the collaboration of a number of international agricultural research centres—the International Rice Research Institute (IRRI) in the Philippines, the International Crops Research Institute for the Semi-arid Tropics (ICRISAT) in India, and the International Institute of Tropical Agriculture (IITA) in Nigeria—projects which will enhance the development of a Programme of research on the Bases of Plant Resistance to Insect Attack at ICIPE. This Programme will include projects for the experimental mass-rearing of insects, the screening of cultivars for resistance to insect attack, and the detailed investigation of the factors responsible for resistance phenomena. The Programme aims to make a considerable contribution to increasing food production in the tropical regions of the world by intensive study of insect/hostplant relations of pests of specific food crops such as sorghum, millet, maize, rice and grain legumes. The identification of factors which cause resistance or tolerance in particular crops to specific pests, the causes and mechanisms for the development of insect biotypes, and the consequent design of suitable methods for the long-term control of agricultural pests will also feature predominantly. With this Programme in action, ICIPE will be able to make a considerable contribution to the work of the international agricultural research community, at the same time aiding in training of research personnel from the developing world in problems of particular concern to those involved with agricultural improvement in tropical areas.

A particularly vital strategy of research on plant resistance by ICIPE staff is a major focus on pest biology within farmers' fields. This type of approach, which involves an understanding of the agronomic environment of the smaller farmer, opens a new horizon in the entomological field.

Training at the Centre proceeds with continued emphasis. There is an ever increasing need to educate and inform aspiring young scientists in problems which are of major concern to the developing countries. ICIPE offers a series of programmes at varying levels, perhaps most important of which are the Group Training Courses in Pest and Vector Management Systems, which have been pioneered by the joint action of ICIPE and UNEP. Among these a Science Bursar Scheme is offered annually to about ten successful Kenyan high-school graduates who plan to go on to university to study in a scientific field. This training programme lasts six months each year, and gives individual students an opportunity to become acquainted (by a process of 'osmosis') with varying aspects of entomological research at the Centre and, hopefully, to encourage them to orient their degree work towards some aspect of development-oriented scientific endeavour. At the graduate level, ICIPE offers research associateships to developing-country scientists who are already involved in pest management research, and who return to their country and institutions periodically over a period of three years to continue with their normal professional career; ICIPE offers post-doctoral research fellowships to gifted young scientists worldwide on a competitive basis for periods of up to three years; and it also offers graduate training to those who have already successfully completed a basic degree in science (or agriculture, or veterinary medicine) and wish to become professionally competent in the field of insect science. Technical training at the Centre itself, and in conjunction with the Kenya Polytechnic, continues to staff already employed at the ICIPE Research Centre.

A feature of the graduate training programmes is the co-operation and exchange of personnel and ideas with other developing countries, for example, the University of Ibadan, Nigeria, and IRRI in the Philippines, where for instance, the Ford Foundation are at present sponsoring an ICIPE technician in the area of rice production technology.

On a different level to the usual training schemes offered at ICIPE, the Centre put on a display at the 1978 Nairobi International Show, the annual agricultural show sponsored by the Agricultural Society of Kenya. The objective of this enterprise was to make work at the Centre known to a wider public in Kenya, particularly those from the rural farming



The ICIPE Stand at the Nairobi Agricultural Society of Kenya Show, September 1978

Development

areas who come to Nairobi for this event, and Nairobi's school-children. Approximately 30% of showgoers visited the ICIPE stand, a most encouraging number, most of these being high-school students accompanied by their teachers, farmers, members of the diplomatic corps and a number of professional scientists. In all, ICIPE's first visit to the Show was considered a major success, and the Centre's presence there was regarded with enthusiasm by the Show Committee and the community as a whole. Research, training, and communication are three facets of ICIPE's mission-oriented goals which the Centre is committed to synchronising in its efforts to make discoveries that would lead to practical pest and vector management systems widely disseminated and effectively utilized by the developing regions, the majority of whose people live in rural communities.

Planning efforts in the year 1978 were devoted largely to a re-examination and review of total ICIPE research commitments. The result is a compact, and clearly focussed research package which has been consolidated into seven core programmes (bases of plant resistance to insect attack, crop borers, African armyworm, grassland termites in semi-savannah areas, livestock ticks, tsetse, and medical vectors of tropical diseases) and four research units (chemistry and biochemistry, histology and fine structure, sensory physiology and bioassay). This leaner programme package will take on its new face in 1979. At the same time a similar exercise will be launched to re-examine and review the training activities of the Centre in much the same manner so that 1980 can usher in 1981 as the year for consolidating ICIPE's training activities.

Thomas R. Odhiambo
Director, ICIPE

March 1979.

TRAINING AT ICIPE, 1978

The year under review saw impressive developments in the training activities at ICIPE. Training gained momentum not only for ICIPE scientists but also for staff in the technical, administrative and secretarial services.

Research Associateship Scheme

Dr. J. Adebayo Odebiyi, lecturer in the Department of Agricultural Biology, University of Ibadan, continued his second phase of research at ICIPE from July to September 1978, on ecological studies on cowpea podborers in Kenya.

Dr. M. J. Mutinga, senior lecturer in the Zoology Department, University of Nairobi, joined the ICIPE scientists for a year of collaborative research on:

- (i) Dynamics of Leishmaniasis epidemiology and biological control.
- (ii) Some aspects of trypanosomiasis research.
- (iii) The role of coprophagous arthropods in the dissemination of taeniasis.

Both Dr. Odebiyi and Dr. Mutinga participated in the ICIPE/UNEP Group Training Course in Pest Management, July/August 1978.

Graduate Training Scheme

In collaboration with university institutions in Africa, ICIPE places high priority on research training. At the University of Nairobi, Miss Elizabeth Opiyo continued with her Ph.D. research on "Studies on trypanosomes in tsetse flies." Mr. J. Mwega, an economics graduate, embarked on M.Sc. degree work on the "Economic analysis of damages and other losses caused by ticks, especially in relation to East Coast fever." Mr. A. O. Mongi, from Tanzania, has continued with his Ph.D. research on "The feeding behaviour on immune and susceptible hosts of the Ixodid tick, *Rhipicephalus appendiculatus*." From the University of Dar es Salaam, Mr. J. Yarro continued with his Ph.D. work at ICIPE on eco-genetics of the African armyworm, concentrating on the larval food plant and phenotypical variations in these insects.

Mrs. Mary Owaga (Ministry of Tourism and Wildlife), and Mr. M. M. Ngunzi (Ministry of Health), two of the trainees sponsored by the United Nations Environment Programme (UNEP), continued their second year of training in tsetse ecology and mosquito ecology respectively. The third trainee, Mrs. Therese Aloo (Ministry of Natural Resources), completed training on termite ecology in September 1978.

From Malawi, Miss Harriet Thindwa has embarked on a research project on the fecundity and development of the sorghum shootfly—*Atherigona soccata* (Rond) on six promising cultivars of sorghum, under the supervision of ICIPE scientists, as part of her training towards an M.Sc. degree at the University of Nairobi.

Mr. C. M. Mutero, a post-graduate student in the Zoology Department, University of Nairobi, undertook a year's training in mosquito ecology from December 1978, under the supervision of Dr. R. Subra, Programme Leader of mosquito research at ICIPE.

Mr. K. Ogwaro, of the Sorghum Shootfly Research Programme, continued with his Ph.D. research on oviposition behaviour and hostplant preference in the sorghum shootfly, under the supervision of Professor Khamala of the University of Nairobi.



Members of the UNEP/ICIPE group training course visit a maize farm in the Kitale area

Miss Lucy Irungu and Miss Lucy Oketch proceeded to the Liverpool School of Tropical Medicine in October 1978, for a year's M.Sc. course in Applied Parasitology and Medical Entomology. Their work will be supervised by Dr. M. W. Service, of the same institution.

Mr. John Kawooya commenced his second year of research work on insect endocrinology at the University of Illinois (Urbana).

Technical Training

At the Polytechnic. Thirteen technicians continued their training in biological laboratory technology. Four of these—Miss Mercy Mambea, Mr. Arphaxad Bwire, Mr. J. M. Maina and Mr.

Training

Phillip Osula—completed their course in July 1978.

In-house Training. Designed to increase the working knowledge and efficiency of ICIPE technicians, this course was conducted in two parts:

- (i) the basic course and
- (ii) the advanced course—both parts taking a total period of twenty-nine weeks.

These courses covered areas in the basic biology of insects, and specific skills used in scientific research at ICIPE.

Overseas Training. Mr. S. H. Okech, technician in the Bases of Plant Resistance Programme, left for a six-months' training course at the International Rice Research Institute (IRRI) in the Philippines. His course will cover areas of rice production technology with special emphasis on the study of the brown planthopper. He joined another ICIPE scientist, Dr. R. C. Saxena, working at IRRI on the planthopper. Mr. Okech received a travel-study award from the Ford Foundation.

ICIPE Science Bursars Scheme

The fifth course in the series of annual motivational training for high school leavers, was offered for a period of six months, from February to July 1978. Ten students from various parts of Kenya trained and worked alongside scientists and technicians in the different research programmes at ICIPE.

ICIPE French Course

Organized in collaboration with the French Cultural Centre in Nairobi, this course was offered from February 1978, and will continue to mid-1979. Twelve members of the scientific and administrative staff are participating in this course, which is designed to provide a working knowledge of the French language.

Training for Administrative and Secretarial Staff

Mr. W. Ogallo (Planning Officer), left for the University of Nebraska at Omaha in August 1978, for a two-year training course in Business Administration.

Mr. J. E. Okiri started a seven-month course in Public Management Development at the University of Connecticut in September 1978.

Mrs. Rosemary Okoth, Miss Margaret Wafula and Miss Dorothy Mbeche, undertook advanced secretarial training in one of the reputable secretarial colleges in Nairobi.

Mr. John Kitur, Accounts Clerk, continued with his correspondence course for Certified Public Accounts (CPA).

Other Training

In June 1978, ICIPE scientists from the Tsetse Population Diversity Programme, participated in a special course on the use of horizontal starch gel electrophoresis equipment, conducted by Dr. van de Geest from the University of Amsterdam.

Dr. A. K. Raina (Sorghum Shootfly Programme), visited the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), and the Agricultural Institute (ARI) for a period of six weeks. He received a travel-study award from the Ford Foundation.

Members of the Tick Programme—Dr. R. M. Newson, Dr. F. D. Obenchain, Mr. A. O. Mongi, Mrs. C. K. Mango and Mr. D. K. Punyua—attended the 5th International Congress of Acarology, which was held in East Lansing, Michigan, from 6th to 12th August 1978. All presented papers on areas relevant to their tick research. After the congress they visited various laboratories across the United States.

Four ICIPE scientific staff participated in a one week course on Radiation Protection organized by the Ministry of Health and coordinated by the National Council for Science and Technology. The course covered the use of radiation sources, detection of ionizing radiation and radiation protection. It was conducted by an expert from the International Atomic Energy Agency in Geneva.

Group Training Course

The second ICIPE/UNEP sponsored course on Components Essential for Ecologically Sound Pest and Vector Management Systems, was organized by ICIPE from 16th July to 6th August 1978.

Thirty-two professional scientists and technologists from developing countries participated in this course: Rwanda (1), Zambia (1), Malawi (1), Mauritania (1), Zaire (2), Nigeria (3), Ghana (1), Ivory Coast (1), Togo (1), Taiwan (1), Sudan (1), Saudi Arabia (1), Lebanon (1), Jordan (1), India (1), Thailand (1), Colombia (2), Tanzania (3), Egypt (1), and Kenya (7). Training was conducted by twenty-two lecturers from universities and scientific institutes in Europe, West Africa and Kenya.

The course programme, which was aimed at acquainting young scientists with the most recent advances in ecologically sound management systems, included: lectures on the philosophy, development, and utilization of pest management techniques; practicals and field observations; consideration of the impact of the chemical management of pests and vectors; case studies.

Study Workshop on Information Transfer

An International Study Workshop dealing with Science and Technology Information Transfer, was organized and hosted by ICIPE from 3rd to 6th May 1978. The workshop, held at the Kenyatta Conference Centre, Nairobi, attracted delegates from Uganda, Tanzania, Botswana, Malawi, Lesotho, Sweden, West Germany and Kenya. Originally intended to investigate means of improving transfer of information on insect science amongst researchers in Africa, the workshop was modified to examine general problems relating to science and technology information transfer in Africa. The workshop was largely funded by SAREC and involved the University of Nairobi and the National Council for Science and Technology in Kenya.

AFRICAN ARMYWORM RESEARCH

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

Honorary Programme Leader
Dr. D. J. W. Rose (1977)

Research Staff
Mr. J. Igunza (1972) Technician
Dr. S. Khasimuddin (1978) Research Scientist
Mr. J. T. Kilori (1972) Technician
Mr. M. Lubega (1974) Junior Technician
Mr. D. N. Mathenge (1973) Junior Technician

Mr. G. N. Mburu (1974) Subordinate Assistant
Mr. R. Okello (1973) Technical Assistant
Mr. B. L. Otindo (1975) Research Assistant
Dr. B. I. P. Persson (1977) Research Scientist
Mr. C. Were (1977) Technical Assistant
Mr. J. Yarro (1977) Graduate Trainee

Collaborators
Dr. J. V. Clark, Sensory Physiology

Introduction

D. J. W. Rose

During the year there was only one report of a small outbreak of armyworm caterpillars in Kenya, and very few *Spodoptera exempta* moths were captured in the network of pheromone and light traps. Consequently, field studies were limited to negative surveys for low density populations in suitable *Cynodon* grasses and the other planned population studies were postponed.

Field populations of *Spodoptera exempta* throughout East Africa were at their lowest since regular records were started in 1961, and this may be associated with the unusually long periods of wet weather that occurred in Kenya, Tanzania and Uganda. The field cage studies reported by Dr. B. Persson show that the greatest caterpillar mortality, largely due to virus disease, occurred during the wet spells. Consideration is now being given to an investigation of the ecology of *Spodoptera exempta* viruses and their possible significance to fluctuations in armyworm populations.

Studies on the 'off-season' biology and survival of the armyworm, *Spodoptera exempta*

S. Khasimuddin

Studies on some aspects of the 'off-season' survival strategy of *Spodoptera exempta* have recently been initiated. The aspects under study include (i) investigations on the effect of nutrients on developmental times; (ii) effect of temperature on developmental times; (iii) phase-variation in the larval stage and hormonal implications in phases-variation and the phenomenon of diapause. Preliminary results obtained from these studies are reported here.

Effect of nutrients on developmental times

The larvae are reared on a semi-synthetic diet (Appendix I) in the normal breeding. To check the effect of the protein content in the diet on the developmental period of the larvae, this diet was prepared in three batches, one without the casein, the second without casein or Brewer's yeast and the third as normal diet (control). Newly hatched first instar larvae were introduced on these three diets individually in plastic vials. Results are presented in Table 1.

It was seen that exclusion of casein from the diet (II) did not have any noticeable effect to the developmental times of individual stages or the total time up to eclosion of adults from the pupae. However, exclusion of casein and Brewer's yeast (III) resulted in a marked prolongation of developmental periods of larvae of 1st through 5th instars. It also resulted in heavy mortality among all instars and none of the test insects survived beyond the 5th instar. Further investigations are in progress.

Effect of temperatures on developmental times

Experiments were set up using maize leaves as diet for larvae reared individually as well as under crowded conditions, under two regimes of temperature—one being $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ kept constant over the 24 hour cycle and the other being $22^{\circ}\text{C}\pm 2^{\circ}\text{C}$ during day hours (12 hr) and $10^{\circ}\text{C}\pm 2^{\circ}\text{C}$ during night hours. Results are presented in Table 2.

It was observed that larvae reared individually (therefore representing the 'solitary' phase) took slightly longer times than those reared crowded (representing 'gregarious' phase) under both the temperature regimes. However, the important result seems to be the marked increase in larval durations under lower temperatures, for individually reared as well as crowded larvae. Further investigations are needed with different temperature regimes. Investigations are underway to find out the critical life history stage in terms of triggering the onset of delayed development in response to temperatures.

Table 1. Time taken (days) by various instars of *Spodoptera exempta* under 3 different diets at 25°C±2°C and 65-75% RH

	I*	II*	III*
1st instar	3.562±0.727	4.333±0.816	6.937±2.351
2nd instar	2.062±0.573	2.133±0.639	8.222±6.996
3rd instar	2.437±0.629	2.133±0.516	7.70 ±3.973
4th instar	2.666±1.162	2.333±0.723	9.25 ±4.803
5th instar	3.250±0.577	3.133±0.639	12.0 ±1.414
6th instar	5.066±1.032	5.20 ±1.264	No survival
Pre-pupae	1.866±0.351	1.666±0.487	No survival
Pupa	10.60 ±1.681	10.60 ±1.992	No survival
Total-time	31.109±0.841	31.53 ±0.884	

*I = normal diet (Appendix I)

*II = normal diet minus casein

*III = normal diet minus casein and Brewer's yeast

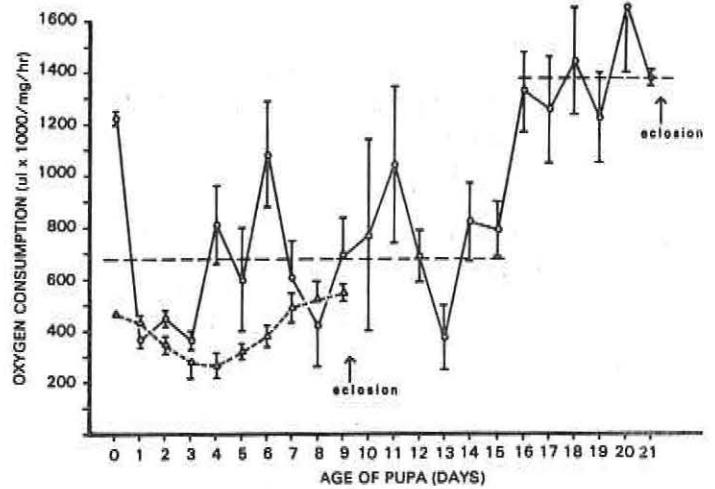
Table 2. Developmental times of growth stages of *Spodoptera exempta* at two different temperature regimes

	25°C+2°C (2±S.D.)	22°C+2°C(day) & 10°C±2°C(night) (x±S.D)
<i>Individually Reared</i>		
1st instar to pupation	17.00 ±1.188 days (n=52)	26.266±3.463 days (n=30)
1st instar to eclosion	29.043±1.186 days (n=23)	50.411±2.895 days (n=17)
<i>Reared crowded</i>		
1st instar to pupation	14.00 ±1.749 days (n=50)	23.681±2.412 days (n=50)
1st instar to eclosion	27.716±2.178 days (n=41)	48.136±1.964 days (n=45)

Phase variation in the larvae

The phenomenon of phase variation in larvae is well documented for *S. exempta*. Studies have begun to investigate the relative importance of 'solitary' and 'gregarious' larvae on the overall survival strategy of the insect. Results obtained to date include a comparison of oxygen consumption of pupae from 'solitary' as against 'gregarious' larvae. Figure 1 presents this comparison where it is seen that pupae from 'solitary' larvae consume much more oxygen than those from 'gregarious' larvae at almost all stages of pupal development. While both categories show an increased oxygen consumption just before eclosion, 'solitary' pupae take much longer to eclose than 'gregarious' pupae. More investigations in this respect are underway.

Investigations have also been started on the juvenile hormone (JH) levels of the last instar 'solitary' as well as 'gregarious' larvae at various ages after moulting into the last instar. This study is being undertaken with the collaboration of the bioassay unit. Results obtained to date are somewhat preliminary and not ready for presentation. Concurrently, experiments are underway to check the effect of applications of JH to last instar larvae of various ages, in terms of the role of JH in diapause, if any. Results will be presented in future reports.

**Figure 1.** Oxygen consumption by pupae from gregarious and solitary larvae

○—○ solitary
 ——— mean values solitary
 ▲—▲ gregarious

Appendix I

Composition of the normal diet on which the larvae are reared-Bot. 1967. J. Ent. Soc. S. Afric. 29 157-60

Wheat germ	26 gm
Vitamin free casein	4 gm
Finely ground Brewer's yeast	25 gm
Powdered agar	6 gm
Ascorbic acid	2.5 gms
Inositol	0.1 gm
Cholesterol	0.1 gm
Choline chloride	0.2 gm
Nipagin (Methl 4-hydroxy benzoate)	1.3 gm
Water	250 gm

Rearing of the armyworm in three different climatic regions

B. I. P. Persson

The fact that armyworm moths and larvae appear in large numbers during the outbreak season and then virtually disappear for the rest of the year has given rise to a number of hypotheses about how and where the armyworm survives between outbreaks. It is well established that migration plays a major role for the dispersal of armyworm populations, but where the migrant swarms come from is still largely unknown.

In order to test how the armyworm can survive in different parts of Kenya, a long term experiment was started in October 1977 involving continuous rearing attempts in large outdoor screen netting cages on three different locations, each with different climates, constituting a rough profile across the country and also across the path of the migrant swarms. These locations are:

- (i) Msabaha Research station on the coast south of Malindi
- (ii) ICIPE Head Station in Nairobi
- (iii) Mbita Point Field Station on Lake Victoria.

Armyworm Research

The experiment started in Nairobi in the beginning of October 1977, in Mbita Point in the middle of February 1978 and on the coast at the end of March 1978. The larvae are reared in large plant pots covered with nets and containing either maize or stargrass (*Cynodon dactylon*). Ten pots, five with maize and five with grass, each containing a hundred first instar larvae are used for each generation. In addition, a dual choice cage containing both maize and stargrass is used for host preference observations. On all three locations weather factors are recorded.

The larvae are exposed to rain and sunshine and the plants are not watered. Observations are made on the length of the developmental period, on the average number of generations per year, on the relative number of adults emerging, on mortality in the egg, larval and pupal stages and on adult longevity and egg laying and mating behaviour. The following results have been obtained so far.

Length of developmental period

The length of the total developmental period (from laying of eggs to emergence of adults) varies strongly between the three locations as would be expected due to differences in temperature. On the coast the average length for the six months covered in this report has been 25.5 days with a minimum in April of 23.1 days and a maximum in July of 28.0 days. The corresponding mean night and day temperatures are 26.0°C and 23.4°C respectively. In Mbita Point the variation has been stronger; from 25.3 days in February to 48.3 days in July-August (average 32.9 days). In Nairobi the shortest period was recorded in January-February with 43.2 days and the longest in June-August with 77.3 days. The average here was 56.2 days. Thus the generation turnover on the coast is more than double that of Nairobi.

Average number of yearly generations

Only in Nairobi has a full year been completed. The possible yearly number of generations is six to seven. In Mbita point six generations have been completed in seven months which means a yearly average of 10 to 11. On the coast seven generations have appeared in less than six months thus giving a probable yearly total of 14 to 16.

Relative number of adults emerging

The relative number of adults emerging has in most generations and on all three locations been low. The best overall survival has been noted on the coast where all generations, both on maize and stargrass, have produced adults (average 3.8%). On the other hand no generation has produced a large number of adults (maximum 6.0%).

In Mbita Point the overall survival has been lower than on the other two localities. The best result was obtained in May-June with 2.6% of the larvae resulting in adults. In two generations no adults emerged on

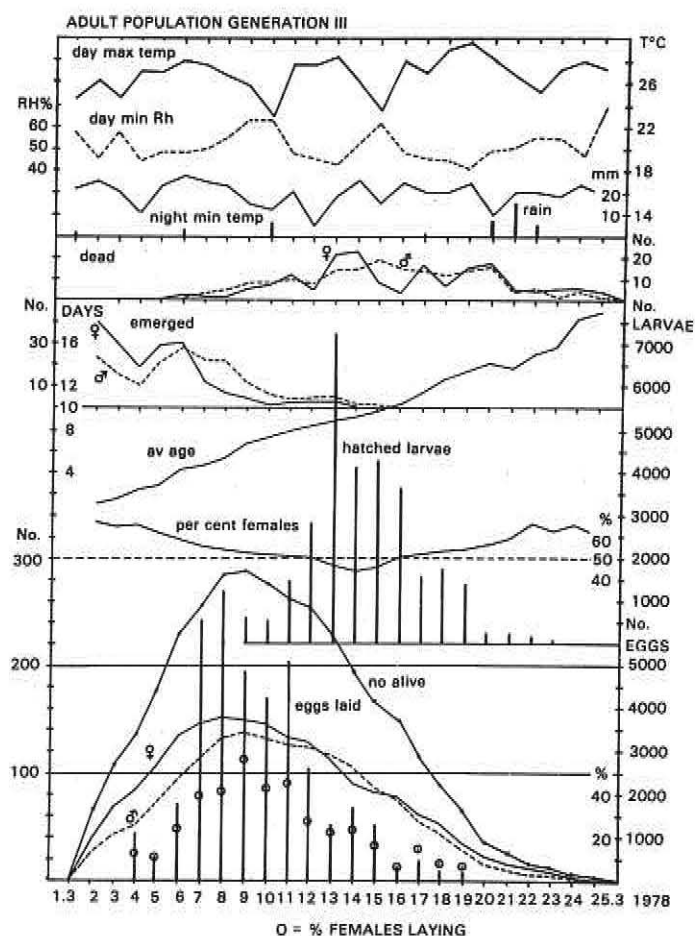


Figure 2. Rearing results, generation 3, Nairobi
Adult population, generation 3, Nairobi

maize. In Nairobi very strong variations in the relative number of adults have been recorded. In most generations the survival has been as low as in Mbita Point and in generation V (in May) the whole culture was wiped out. However, generation III in January-February produced a very high relative number of adults (18.6%). Generally the best survival and emergence has been recorded when the larval periods have coincided with sunny weather and low or moderate rainfall.

Mortality factors

The highest mortality in all generations has been recorded in the larval stage, particularly in the first and last instars. Larval mortality has been high during rainy and cloudy periods and low during sunny and dry. In the absence of predators the most important mortality agent has been a virus. This virus has been present in all generations and on all three locations. The larvae die hanging upside down in the characteristic manner of a larvae attacked by a nuclear polyhedrosis virus. The virus is particularly effective against last instar larvae just before pupation. An in-depth study on how mortality caused by this virus is correlated to rainfall and sunshine is planned for 1979. Other pathogens such as bacteria or fungi have only occasionally appeared

in the cultures and have had only a small influence on the overall mortality.

Other preliminary results

In the dual choice experiments involving maize and stargrass, a strong preference for stargrass was found on all three localities. Also, in most generations on all three localities survival on stargrass has been higher than on maize, but the developmental period longer. In generation III in Nairobi, where a large number of adults emerged, the difference in developmental period was significant (Table 3). Generally, females, both on maize and stargrass, have a shorter developmental period than males.

In addition to the above presented results a large number of data on adult longevity, reproduction and sex ratio has been obtained. The rearing experiments will continue during 1979.

Adult behaviour

The large number of adults emerging in generation III in Nairobi made possible some observations on the emerged adult population (Figure 2). The peak in egg-laying occurred approximately one week after the first moths emerged, and the highest number of larvae hatched after approximately two weeks. Females emerged before males (Table 3) but during the peak of the population the sex ratio was nearly 1:1. The total longevity of the adult population was around three

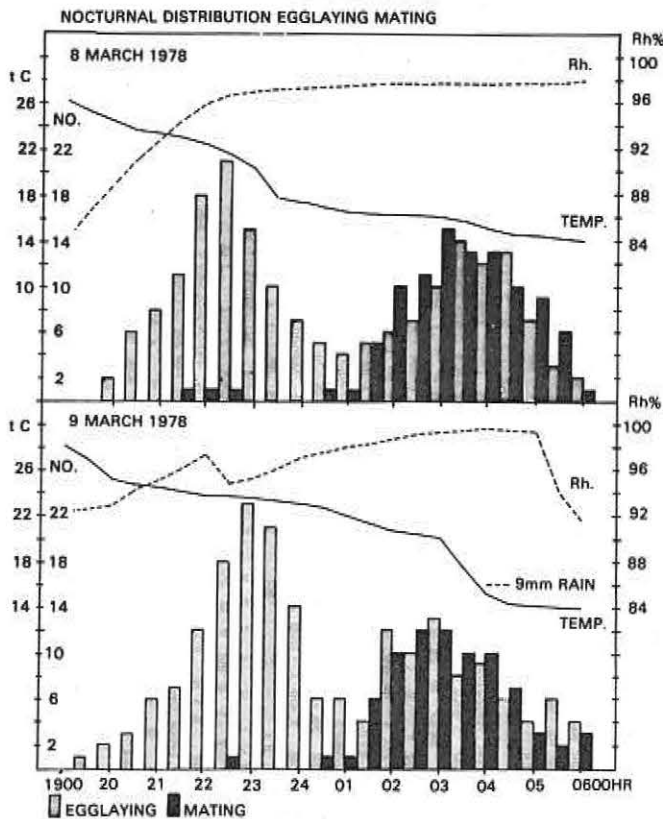


Figure 3. Adult population, generation 3, Nairobi
Distribution of egg-laying and mating during 2 nights

Table 3. Adult emergence generation Nairobi

Date	MAIZE			STARGRASS			MAIZE + STARGRASS			TOTAL		
	♂♂	♀♀	Sum	♂♂	♀♀	Sum	♂♂	♀♀	Sum	♂♂	♀♀	Sum
2.3.1978	20	28	48	2	4	6	1	8	9	23	40	63
3.3.1978	15	16	31	0	3	3	2	10	12	17	29	46
4.3.1978	2	11	13	3	4	7	5	3	8	10	18	28
5.3.1978	8	10	18	6	10	16	6	4	10	20	24	44
6.3.1978	11	2	13	11	32	2	2	4	24	24	25	49
7.3.1978	5	2	7	13	10	23	2	1	3	22	11	33
8.3.1978				22	7	29	0	1	1	22	88	30
9.3.1978				10	4	14	1	0	1	11	4	15
10.3.1978				6	1	7				6	1	7
11.3.1978				3	2	5				3	2	5
12.3.1978				4	2	6				4	2	6
13.3.1978				4	2	6				4	2	6
14.3.1978				1	0	1				1	0	1
15.3.1978				1	0	1				1	0	1
Sum	61	69	130	86	70	156	19	29	48	168	166	334
% female		53.0			44.9			60.4			49.7	
No larvae		800			800			200			1800	
% emerged		16.3			19.5			24.0			18.6	

Difference distribution emergence maize-stargrass $X^2(13) = 142.0 P < 0.001^{***}$
 Difference distribution emergence males-females $X^2(13) = 33.2 P 0.01 < 0.001^{**}$

weeks. For two nights when the population was at its largest the time of egg laying and mating was observed. The results confirm earlier observations. The peak in egg laying occurred in the first part of the night with a second weaker peak in the later part. In between mating took place (Figure 3). Multiple mating was observed and it was found that females can both lay eggs and mate in the same night.

Larval behaviour

No extensive studies on larval behaviour were carried out in 1978, but such studies will have a high priority during 1979. These will be carried out on the coast where the climate, particularly for nightly observations, is more suitable than in Nairobi. Some temperature preference studies on gregarious and solitary larvae were, however, carried out in the laboratory. A temperature gradient giving a linear increase from 8°C to 38°C was used. In both solitary and gregarious larvae the optimal temperature for feeding activity was found to be around 23°C. However, just before moulting most larvae moved to the high extreme. The significance of this for movement on the host plant will be further studied.

Fore wing length measure as a size index of the armyworm, *Spodoptera exempta*

J. G. Yarro

Previous studies have shown that there are differences in larval survival, developmental rates, pupal weights, adult weights, wing lengths as well as wing areas between populations reared on different grass species.

Some of these differences, for example the pupal weight and adult weight, might be used as indicators of the nature of the environment in which the armyworm developed, and so be used to check possible sources of moths collected in light and pheromone traps with the pupae collected at previous outbreaks. In order to do this, it is necessary to work out precise relationships between pupal weight or adult weight and other parts of the insect. This investigation deals with the relationship between the pupal weight and the fore wing length.

The larvae were reared on *Zea mais* L., *Cynodon dactylon* (L.) Pers., *Pennisetum clandestinum* Chiov., *Setaria plicatilis* (Hochst) Hack. and *Panicum maximum* Jacq. Throughout larval and pupal life the insects were kept in an insectary maintained at 25°C and 70% R.H. Pupae were weighed to the nearest 0.1mgm within 12 hours of pupation, and each of them was sexed and placed separately in a plastic vial. After emergence, adults were killed in chloroform. The fore wings of well formed moths were detached and the wing lengths of both right and left fore wings were measured using a vernier rule reading to 0.05mm. The mean of the two wing lengths was calculated for each moth.

The results show that the fore wing length is linearly related to the pupal weight Table 4.

Table 4. The regression values (b) for the relation between the pupal weight and the fore wing length in *Spodoptera exempta*

Food Plant	Sex	b	R ²	n
<i>C. dactylon</i>	♂	0.023	.69	39
	♀	0.024	.56	39
<i>Z. mais</i>	♂	0.018	.60	39
	♀	0.021	.63	39
<i>P. clandestinum</i>	♂	0.028	.75	30
	♀	0.024	.79	30
<i>P. maximum</i>	♂	0.013	.31	30
	♀	0.019	.45	30
<i>S. plicatilis</i>	♂	0.027	.58	39
	♀	0.029	.68	39

In all cases the regressions are significant ($p < 0.01$). In four of the cases the regressions for females are greater than those for males but these differences are not significant. Except for moths reared on *P. maximum* more than 50% of the variation in the wing length is accounted for by the variation in the pupal weight.

Percentages of variation in one variable accounted for by another are based on coefficients of determination, r^2 .

The pooled results (Table 5) show that 77% of the variation in the fore wing length is accounted for by the variation in pupal weight. The regression value for females is again slightly greater than that for males and this difference is significant ($F(5) = 4.00 > F_{0.05}(1, 60) = 3.82$; $p < 0.05$).

Table 5. The regression values for the relation between the pupal weight and the fore wing length in *Spodoptera exempta* (pooled results)

Sex	Regression	R ²	n
♂	0.023	.77	177
♀	0.026	.77	177

The combined results give a relationship between fore wing length and pupal weight of

$$\text{and } Y = 10.73 + 0.023X \text{ for males}$$

$$\text{and } Y = 10.59 + 0.026X \text{ for females}$$

where Y is the wing length (mm) and X the pupal weight (mgm).

Further studies on these relationships are in progress and will possibly explain the slightly greater regression for females than for males of equal pupal weight.

**The determination of the physiological age of
armyworm moths, *Spodoptera exempta***

B. L. Otindo

A network of light and pheromone traps distributed throughout eastern Africa is used to detect the occurrence and movements of armyworm moths and to forecast the possibility of armyworm outbreaks in different regions. As an aid to the analysis of trap data it will be helpful if the ages of male and female moths can be determined.

A study of possible techniques that may be used has been started. The literature has been reviewed and a start made with the dissection of moths of both sexes and a series of ages to obtain information on the fat body, crop sizes, and the condition of the reproductive systems. Possible 'ageing' techniques to be investigated include estimation of lipid contents, cuticle and resilin studies (growth layers), and electrophoretic methods.

MOSQUITO RESEARCH

Visiting Director of Research
Professor J. Mouchet (1975)

Programme Leader
Dr. R. Subra (1978)

Research Staff

Dr. A. W. R. McCrae (1977) Research Scientist
Mr. E. Mkuzi (1976) Technician
Dr. F. W. Mosha (1978) Postdoctoral Research Fellow
Mr. M. M. Ngunzi (1977) Graduate Trainee

Anopheline ecology

A. W. R. McCrae

The aim of this project is to select and pursue research topics from which the most promising could be developed and applied in the field as a basis for the control of malaria vectors, or at least for its refinement. This is no mean task in a field in which so much has been done before, and yet in which improvements in methods of control, especially in rural areas, are so badly needed. If at the same time advances can be made in sampling techniques or in basic understanding of the regulating processes of anophelines, these may be applied in assessment of control or its feasibility.

In 1978 the project has been largely concerned with establishing itself, conducting anopheline surveys in coastal Kenya and in sifting and testing ideas. While there is a clear need to improve our abilities to attack vector mosquito populations in their aquatic stages, the dispersal of control agents presents great logistic problems against species such as the freshwater members of the *Anopheles gambiae* complex, which exploit, principally, small and transient bodies of water. Four approaches to control or assessment of control of these species, therefore seem most valid in the following order of priority:

- (i) To seek new methods of control through investigation of relatively neglected aspects of anopheline ecology.
- (ii) To work towards refinements in definition of breeding sites not only in which these species are found, but from which optimal production takes place, thus offering better operational efficiency for source control.
- (iii) Defining the more permanent dry season breeding sites for possible approaches to reducing the duration of seasonal transmission.
- (iv) To conduct descriptive studies as a basis for population models of predictive power. This has not been embarked upon, as existing models allowing for extinction-repopulation in patchy environments are too simplistic and require too many assumptions to offer clear guidelines for the exact data required.

Work commencing in 1978 has so far achieved only preliminary results, which may be summarized in the context of the above priorities as follows:

Oviposition site selection. This topic is fundamental to understanding the distribution and population dynamics of all aquatic stages. For the African malaria vectors it has barely been touched upon in the past. Since it involves the mobile adult, now control methods based on attraction to treated waters offer possibilities. Tentative findings on *An. gambiae* are:

(i) Experiments using types of natural surface waters in which larvae were or were not occurring indicated that selection involves the presence of attractants rather than the absence of repellent factors. Methods of vector control or its assessment may thus possibly be based on manipulation of attractancy.

(ii) Conventional larvicidal oiling of water does not appear to inhibit oviposition in large (1m³) cages. Surface tension *per se* would not therefore be tested for by the ovipositing female.

(iii) Using wild-caught females, also in large cages, oviposition activity does not appear to be concentrated in the first 3-4 hours of the night (contrary to findings of the only previously published study, which used a long-established insectary strain of *An. gambiae* s. str.) but is more similar to activity patterns of blood feeding, building up in the second half of the night. This has important implications for range of dispersal between resting and oviposition sites, and for field sampling.

Larval ecology

Through observations on 'atypical' dry season breeding sites, shallow-water refuges have been discerned as a possibly important parameter of larval survival. This is currently under study in terms of larval tropisms. *Vorticella* attached to larvae is not a counter-indicator of pupal production in a breeding site.

Adult feeding activity on sugars. Studies by the writer in other parts of Africa gave rise to the hope that sugar baits might provide the basis for supplementary control of new sampling methods. In the case of *An. gambiae*, however, only one natural source of plant sugars is known, and that a weakly attractive one, the extra-floral nectaries of *Acacia macrostachya* (personal observation). Under extreme dry season conditions, adult *An. gambiae* s. str. from irrigated breeding sites may show as high a proportion as 30% with sugar solution in the crop (personal observation), but under the normal conditions of breeding seasons a far lower

proportion is usual. In insectary cages, both sexes of *An. gambiae* s.l. feed freely on sugar solutions.

Four all-night catches have been conducted using crushed sugar cane (sucrose content 9% and/or wild honey on a fibre matrix). Some 700g of sugars were present in each bait. Concurrent man-baited catches were also conducted. Sugar, honey and man-baited catching both indoors and outdoors was used in the final catch of the series, followed by collections of resting mosquitoes from all houses of the compound after dawn. Few of the mosquitoes taken on the sugar or honey baits were anophelines. Despite taking full precautions by using drop-nets to isolate the catchers while sampling, the mosquitoes from the sugar or honey baits (many of which had evidently fed on those baits) appeared to represent an opportunistic rather than a specifically sugar-feeding element of their populations. Few males of any species were taken, and age incidence of the commonest mosquitoes was similar to that in the man-baited samples as judged by the relative incidence of parasitic larval water mites.

These disappointing results mean that this approach appears to offer little immediate prospect for control applications. The study will be wound up with a few more catches, including further sugars as bait.

Work in the immediate future will concentrate primarily on oviposition and egg ecology of vector anophelines.

Ecological studies on *Anopheles gambiae* sibling species

F. W. Mosha

Anopheles gambiae complex which is the main vector of malaria and Bancroftian filariasis in Africa, comprises six sibling species of which three, namely *Anopheles merus* Donitz *Anopheles gambiae* (sensu stricto) Giles and *Anopheles arabiensis* Patton have been reported along the East African coast. Studies aimed at finding out about the ecology and role of each sibling specie in Bancroftian filariasis transmission, commenced in August 1978 in Jimbo village along the south Kenya coast bordering Tanzania (Figure 1). Mosquitoes were collected weekly from 2 houses (indoor/outdoor) by human night bait, and also by morning hand catch from 12 houses.

Species composition

An. gambiae complex was the most abundant species, accounting for 75.9% and 66.2% of a total of 2030 mosquitoes collected outdoors and indoors respectively (Table 1). Other mosquito species collected by human night bait included *Culex pipiens fatigans* (= *C. quinquefasciatus*), *Culex sitiens*, *Aedes pemaensis*, *Anopheles funestus* and *Mansonia uniformis* (Table 1).

A total of 227 *An. gambiae* complex mosquitoes were separated into sibling species using a criteria based on

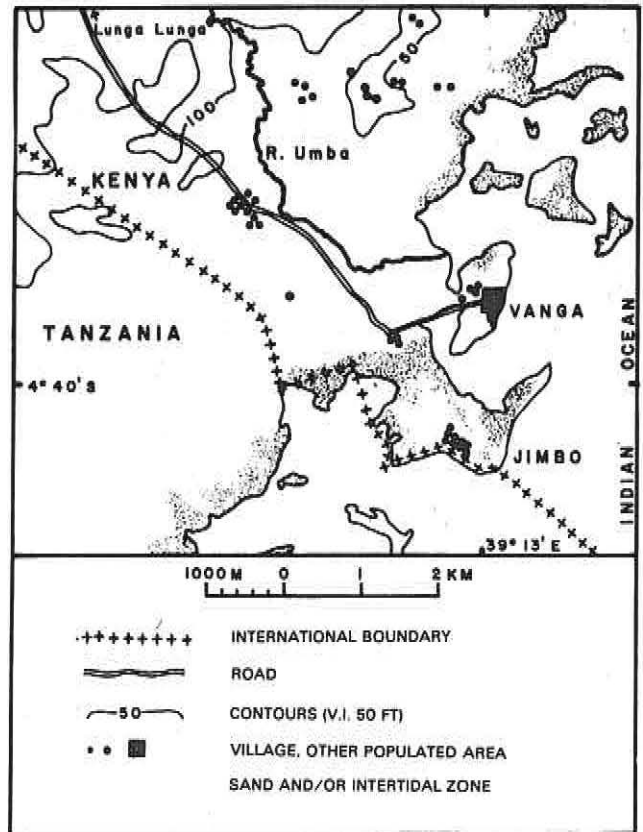


Figure 1. Sketch map of the southeast Kenya coast showing location of Jimbo village

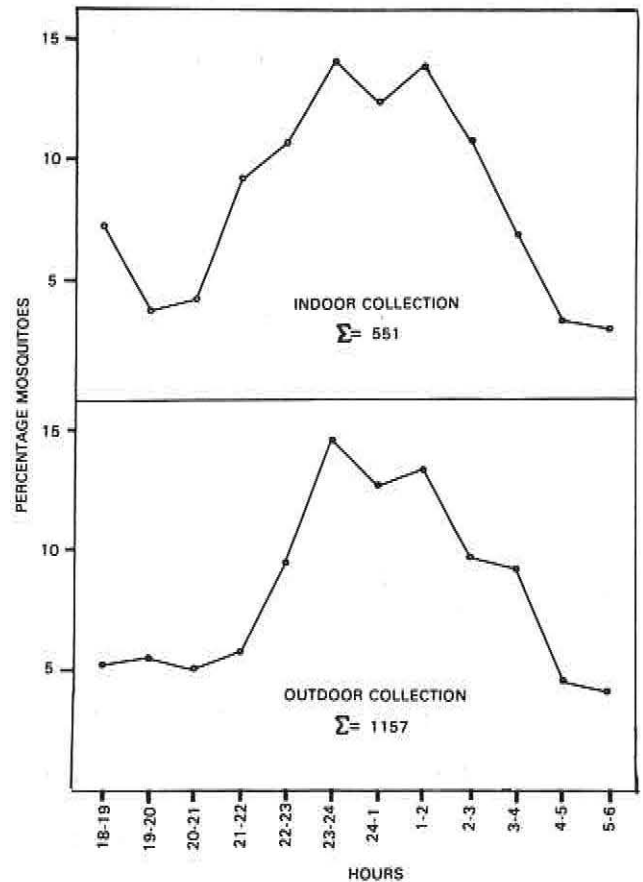


Figure 2. Indoor and outdoor biting cycle of *Anopheles gambiae* complex from 19 human bait collections carried out in Jimbo between 12.9.78 and 14.11.78

palp index and coeloconica sensillae. 68.7% ¹⁵⁶/₂₂₇

An. gambiae complex was identified as *An. merus* and the remaining as a mixture of *An. gambiae* s.s. and *An. arabiensis*. Identification of 50 *An. gambiae* complex, by examination of x-chromosome banding pattern showed that 12.0% were *An. arabiensis* while the remaining were a mixture of *An. merus* and *An. gambiae* s.s. Thus, among the *An. gambiae* complex in Jimbo, the most predominant sibling species appears to be *An. merus* followed by *An. gambiae* s.s., and *An. arabiensis* last.

***An. gambiae* complex resting and biting habits**

An. gambiae complex accounted for only 4.8% of total hand collection as compared to 69.6% of indoor human night bait collection carried out during the same period (Table 1). This exophilic behaviour is typical of *An. merus* which is a predominant species in Jimbo village. Figure 2 shows that the peak activity period for *An. gambiae* s.l. was between 23 and 24 hours both indoors and outdoors.

Infection with filarial larvae

Out of 415 *An. gambiae* complex dissected, 4 *An. merus* were infected with filarial larvae (3 with immature stage larvae and 1 with infective stage *W. bancrofti* larva).

The overall infection rate was 1.0% ⁴/₄₁₅ while the infectivity rate was 0.2% ¹/₄₁₅.

Table 1. Mosquito species collected during 22 human night bait catches carried out in Jimbo between 15.8.78 and 14.8.78

Species	Outdoor		Indoor	
	no.	%	no.	%
<i>An. gambiae</i>	1307	75.8	723	66.2
<i>An. funestus</i>	13	0.8	77	7.1
<i>C.p. fatigans</i>	118	6.8	173	15.8
<i>C. sitiens</i>	189	11.0	70	6.4
<i>Ae. pambiaensis</i>	66	3.8	49	4.5
<i>M. uniformis</i>	25	1.5		
Other	4	0.2		

Larval ecology of *Culex pipiens fatigans* (Diptera, Culicidae) on the Kenya Coast

R. Subra

Environmental management and health education are part of alternative control methods against disease vectors. Both methods should play an important role in the case of domestic mosquitoes, like *C. p. fatigans*, one of the major filariasis vectors on the East African coast. The development of such methods requires the following information:

(i) when and where does man create mosquito breeding sites?

(ii) when breeding places already exist, which factors, including human factors, play a major role on preimaginal population dynamics?

The work performed in 1978 was mainly an attempt to answer the first of these two questions. It has been complemented by some preliminary observations on the regulation of preimaginal populations.

on the Kenya coast, *C. p. fatigans* develops in 3 main types of breeding place:

- (i) pit-latrines when they are deep enough to reach the watertable
- (ii) cesspools which collect used water
- (iii) *birika* (cement water containers of about 1 cubic meter) where people keep water for ritual ablutions

The distribution of these breeding places is different in Moslem and non-Moslem settlements. Two areas, each belonging to one of these types were selected for this study. Vanga on the coast south of Mombasa was chosen as a representative Moslem village. It has about 350 houses, belonging to 3 different types:

- (i) traditional houses with mud walls and palm-leaf roofs
- (ii) modern houses with cement walls and corrugated sheet roofs
- (iii) an intermediate type with both traditional and modern features

Most of the inhabitants have traditional activities such as farming or fishing. There are also traders, civil servants and employees who usually have an income level higher than farmers and fishermen.

During the present study, differences between different types of houses and the social level of the inhabitants were noted; and related differences in the distribution of the breeding places were looked for inside the village.

Traditional houses are the most numerous (78% of the total) and form the majority of housing for farmers, fishermen and artisans (Table 2). Modern houses represent only 6% for this social category. For the other category which has higher incomes traditional houses

Table 2. Distribution of breeding-places in relation to social class and house structure in Vanga village

House Structure	Social Class No. 1 Farmers + Fishermen		Social Class No. 2 Others (Traders, Civil Servants)		Total Houses
	No. of houses	With breed. places	No. of houses	With breed. places	
Tradit.	241 84%	37	25 49%	7	266
Interm.	30 10%	14	12 24%	8	42
Modern	17 6%	9	14 27%	13	31
Total	288	60	51	28	339

represent only half of the total and modern houses represent 27%. Amongst a total of 288 houses belonging to social class 1, only 60 had one of the described breeding places and 228 had none. Although in absolute terms the greatest number of breeding places was recorded in traditional houses which are by far the most numerous, it was apparent that the percentage of breeding places was only 15% as compared with 49% in the two other categories. In the second social class, the greatest number of breeding places was recorded in modern houses which represent only 27% of the total. In this class the percentage of breeding places follows the same trend as in the first category: 28% in traditional houses, 81% in intermediate plus modern houses. In comparing the two social classes, it appears that, within the same type of housing, the percentage of breeding places is higher in the second class, but the tendency for an increase of breeding places is the same in both classes and is related to improved housing.

The non-Moslem settlement studied was in the Rabai area, north of Mombasa. These villages are usually small and six were selected. Only latrines were recorded from these villages. They were very rare in houses of farmers living in traditional housing (1%) while they were more numerous in houses occupied by non-farmers in the same type of settlement (15%). No percentage calculations were made on modern and intermediate houses which were very few, but 3 breeding places were recorded from a total of 11 houses. In most of the cases in the Rabai area latrine building is very recent. With the exception of one village, all latrines have been built in the last 10 years. Thus, *C. p. fatigans* settlement in this area is very recent.

On the Kenya coast in both Moslem and non-Moslem settlements, when there is an increase in income, there is an evolution in housing. Sanitation facilities are improved, with, as a consequence, an increase in the number of mosquitoes breeding sites, even if the human population remains unchanged.

Preliminary observations on preimaginal population

dynamics were conducted in Rabai area. Four breeding places, each in a different village, were sampled once a week in April and May, ie. during the long rainy season. Larvae and pupae of *C. p. fatigans* were recorded in each of them. These observations, interrupted in June, started again in July, in 2 breeding places only. In one of them, very little water was present (a depth of 4 or 5cm). *C. p. fatigans* was the only species recorded. In the other breeding place, water was deeper. *C. p. fatigans* had disappeared and the only species present was *Culex cinereus*. Although the mosquitoes collected are still under study some data can be presented:

- (i) *C. cinereus* preimaginal densities are very high, occupying all the available space at the water surface. In the most populated areas, densities were over 200 larvae and pupae per square centimeter
- (ii) preimaginal forms at their different stages were not homogeneously distributed at the water surface. Old larvae were found in the middle of the breeding place, pupae and young larvae on the edges
- (iii) all the eggs had been laid at the edges of the breeding places in the area occupied by young larvae and pupae

Less detailed observations were made at the same time in 2 other breeding places. *C. cinereus* was the only species recorded until these breeding places dried out.

In Rabai area *C. p. fatigans* seems to be the first mosquito species to colonize breeding places. Later on *C. cinereus*, in its turn, colonizes the same breeding places and occupies all the available space, thus eliminating *C. p. fatigans*. If such an hypothesis can be checked a new way of controlling *C. p. fatigans* would be open by replacing this species by *C. cinereus* which is a non-man biting mosquito. Competition mechanisms between these 2 species have first to be elucidated in detail.

SORGHUM SHOOTFLY RESEARCH

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Sorghum shootfly work at Mbita Point Field Station

A. K. Raina

With the establishment of Mbita Point Field Station early this year, the aspects of shootfly research involving field studies were carried out there. A survey of 25 farmers' fields in Mbita Division was conducted in May 1978. Shootfly infestation based on dead heart counts in 10×10 m plots ranged from 0.3 to 13.7%. Of the 3 varieties commonly grown, 'Andiwo' had the lowest average infestation rate (1.16%).

The 1978 International Sorghum Shootfly Nursery received from ICRISAT was planted in a replicated trial. Observations were taken when plants were 14 and 28 days old and at the time of harvest. Plants with shootfly eggs ranged from 9.2% in case of IS 1082 to 55.3% for CSH-1, on 14th day. Dead heart counts on 28th day ranged from 5.5% for IS 1082 to 70.7% for CSH-1. An interesting observation was the ratio of plants with

eggs to plants with dead hearts. This ratio was highest, 3.7:1 and 3.0:1 for IS 2195 and IS 3962 respectively. Highest yield from 4, 5 meter rows was 6.7kg for IS 2146. These cultivars were also evaluated for stem borer resistance. None of them showed any resistance; however, IS 2195 and IS 3962 had least damage. Six local sorghum varieties, 'Andiwo', 'Kumba', 'Makana', 'Ochuti', 'Othuwa' and 'Serena' were also screened in 2 replicated trials for shootfly resistance. Othuwa was least susceptible. There was no significant difference in infestation between red and white seeded varieties.

Five lines from ICRISAT material along with Othuwa have been selected for studying the mechanisms of resistance. In single choice experiments, it was observed that they received none or very few eggs compared to CSH-1. Further studies are in progress.

Fecundity and oviposition on CSH-1

Basic information on the reproductive capability of *A. soccata* when raised on a susceptible variety is very

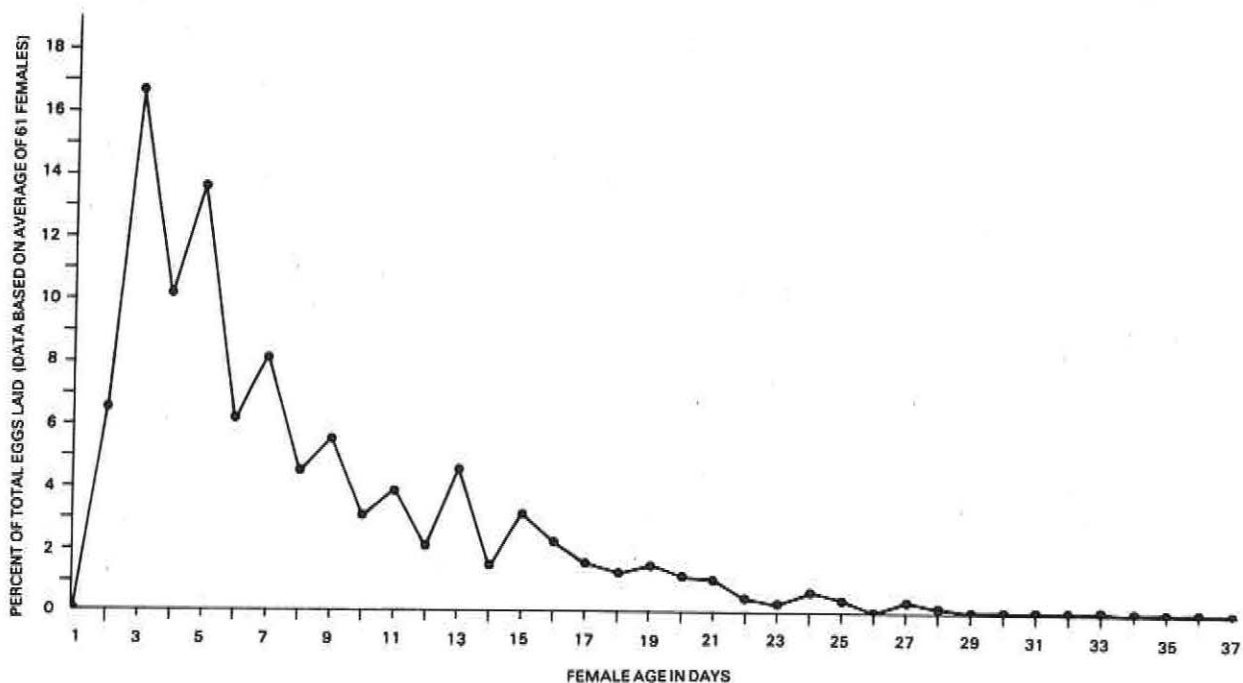


Figure 1. Average daily distribution of eggs laid by *Atherigona soccata* on CSH-1 under controlled conditions

Physiology of reproduction in *Atherigona soccata*

G. C. Unnithan

In order to undertake a detailed investigation on the physiology and endocrinology of reproduction of *Atherigona soccata* it is necessary to obtain some basic information on the structural organization of the reproductive organs, mating behaviour, and influence of mating on egg maturation and fecundity, etc. Studies have been started to gather this information. Some preliminary observations are reported below.

Organization of the internal reproductive organs of *Atherigona soccata*

Male. The internal organs of reproduction in the male (Figure 2) consist of a pair of red-pigmented testes, the vasa differentia which open into the median seminal vesicle, and the ejaculatory duct. Accessory glands are

important to determine the effect of various cultivars and environmental factors. CSH-1, a susceptible hybrid variety from India was chosen for this purpose. Flies reared on this variety were sexed and single pairs kept in oviposition cages maintained at 30°C, 65±10% R. H. and LD 12:12. Adults were fed on a mixture of brewer's yeast, glucose and water, and CSH-1 seedlings were provided for oviposition. If a male died, it was replaced with a fresh male, however, the cage was terminated if the female died. Data from 61 productive pairs was analyzed. The females laid an average of 78.4±5.49 eggs with a range of 17 to 239. Egg laying started on 2nd day after emergence (Figure 1). First batch of the eggs laid by majority of the females comprised of 27 eggs. Males lived an average of 7.3±0.7 (range 2 to 20) days and females 17.1±1.1 (range 4 to 37) days.

Under a photoperiodic regimen corresponding to the natural conditions in Kenya; it was observed that the females do not lay any eggs during the scotophase. Egg laying activity was maximum between 6.30 and 8.30 hrs, followed by the period between 14.30 and 16.30 hrs (Table 1). Almost 50% eggs were laid within 4 hrs of the beginning of the photophase. Under field conditions, where the mid-day temperatures can be higher than in the environmental chamber (30°C constant) egg laying may be more skewed towards the early mornings and late afternoons.

Table 1. Oviposition in relation to the photoperiodic cycle under LD 12:12 condition

Periods of Observation	Scotophase		Photophase				
	18:30-6:30	6:30-8:30	8:30-10:30	10:30-12:30	12:30-14:30	14:30-16:30	16:30-18:30
Percent of total eggs laid	0.0	35.8	11.8	10.2	6.1	21.4	14.7

Significant observations

(i) Molasses grass, *Melinis minutiflora* with heavy shootfly attack was collected from Nairobi. Flies reared from this were found to be *A. contigera*, a species collected in fairly large numbers in fish meal traps in Nairobi.

(ii) Preliminary observations with shootfly behaviour showed that the adults were attracted to smooth green and yellowish-green surfaces. If the surface was rough the contact was of a very short duration.

(iii) Seventeen artificial diets were tried for shootfly larvae. None of the diets sustained 1st instar larvae. However, diet number 15 was best for 3rd instar. The proportion of its various constituents is water-90.0, 'arhar' (*Cajanus cajan*) powder-3.0, sorghum shoot powder-2.0, agar-1.5, yeast-1.5, vitamin mixture-1.0, methyl-praben-0.2, and sorbic acid-0.1 parts.

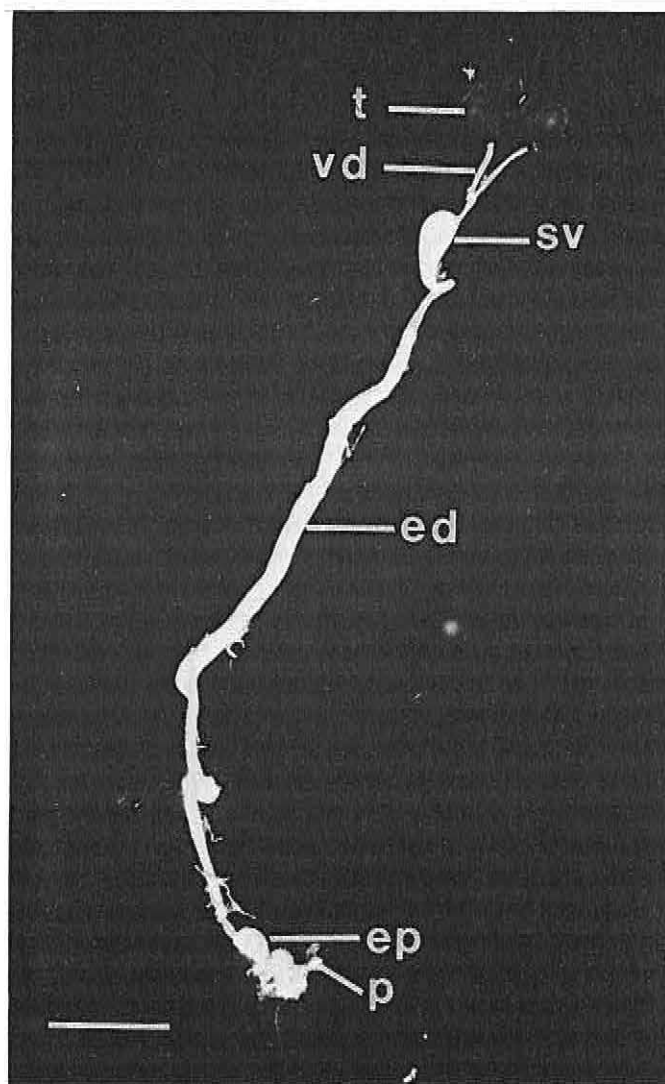


Figure 2. Internal reproductive organs of *Atherigona soccata* male. ed: ejaculatory duct; ep: ejaculatory pump; p: phallus; sv: seminal vesicle; t: testis; vd: vas deferens. Scale, 50 µm

not present. The anterior thickened portion of the ejaculatory duct appears to serve the function of the accessory gland.

Female. The internal reproductive organs of the female (Figure 3) consist of the paired ovaries, lateral oviducts, the median oviduct, three spermathecae and a pair of accessory glands. The posterior end of the median oviduct is dilated to form the genital chamber (vagina). The small oblong accessory glands, attached anteriorly to the lateral oviducts, with long narrow ducts, and the three spermathecae with long coiled ducts, open separately at the anterior part of the dorsal side of the vagina. Each ovary is composed of a variable number of ovarioles, ranging from 10–23. Examination of 42 females showed a mean of 31.3 ± 4.9 (SD) ovarioles per insect. Of the 42 females, about 43 percent had equal numbers of ovarioles in the left and right ovaries. It is not known whether environmental factors, to which the larvae and pupae are subjected to, have any influence on the number of ovarioles in the adult. This aspect is being investigated.

Ovarioles in *A. soccata* are polytrophic. Each developing follicle contains fifteen trophocytes and one oocyte. Only one oocyte will develop at one time in each ovariole. In young nulliparous females egg maturation takes place simultaneously in all the ovarioles. Whereas in older females not all the ovarioles mature eggs at the same time; besides, in these some of the ovarioles appear to be non-functional.

Effects of mating on egg production and fecundity

Maturation of at least the first batch of oocytes is not influenced by mating, although oviposition and fecundity are influenced by mating (Ogwaro, 1978). The females can store the sperms for a considerable length of time after mating, eliminating the need for multiple mating and continuous presence of males. About 80 per cent of all the eggs produced by 12 females, which were allowed to mate only once, hatched. Most of the remaining eggs were also fertilized. These females laid a mean number of 94.1 ± 28.7 (SD) eggs and lived for 21.6 ± 10.6 (SD) days. Total number of eggs produced or the number of fertilized eggs obtained from equal numbers of females kept with males all the time were no greater than those of females which were allowed to mate only once.

Mating behaviour

Previous observations have suggested that the trifoliate organ of the male is involved in mating (Clearwater, 1977). Experimental studies to assess the role of the trifoliate organ are in progress. Preliminary observations showed that males devoid of the trifoliate organ can still mate. All of the 8 males whose trifoliate organs were cut off on the day of emergence mated successfully with unmated females; and the females laid fertilized eggs. However, the courtship in males devoid of the trifoliate organ appeared to have been prolonged considerably.

Sensory receptors on the head capsule of sorghum shootfly larva *Atherigona soccata*

K. Ogwaro

The head capsule was examined to identify sensory structures which might play a role in the larval behaviour. This is part of a larger research programme of study on the sorghum shootfly and hostplant relationships. It provides some useful information on the types and arrangement of sensory receptors which may lead to a better understanding of the insect/hostplant relationship.

External morphology of the third instar head capsule was studied using light and scanning electron microscopes. (Figures 4 and 6). The internal structures were studied using classical histological techniques.

The larval sensory receptors on the head capsule consist of six dome-shaped sensillae, two styloconic sensillae, two companiform sensillae, and eight basiconic sensillae. (Figure 5).

Near the dorso-anterior margin on each side of mid-line is a single dome-shaped sensillum contained in a rim of 6 microns diameter. This is a basiconic sensillum having a thin membranous wall. Situated above the mouth hooks are four basiconic, one styloconic and two dome-shaped sensillae on each side of the

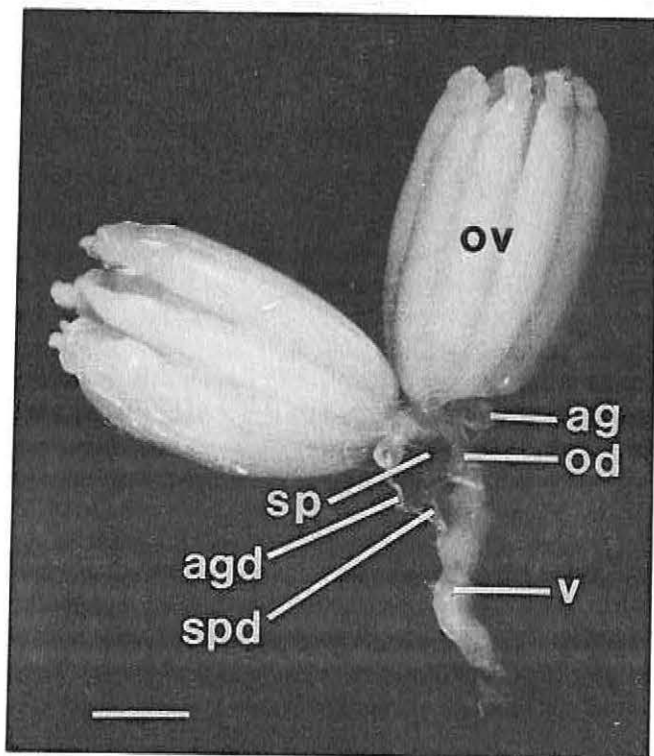


Figure 3. Internal reproductive organs of *Atherigona soccata* female. ag: accessory gland; agd: accessory gland duct; ov: ovary (with gravid oocytes); sp: spermatheca; spd: spermathecal ducts; v: vagina. Scale, 50 μ m

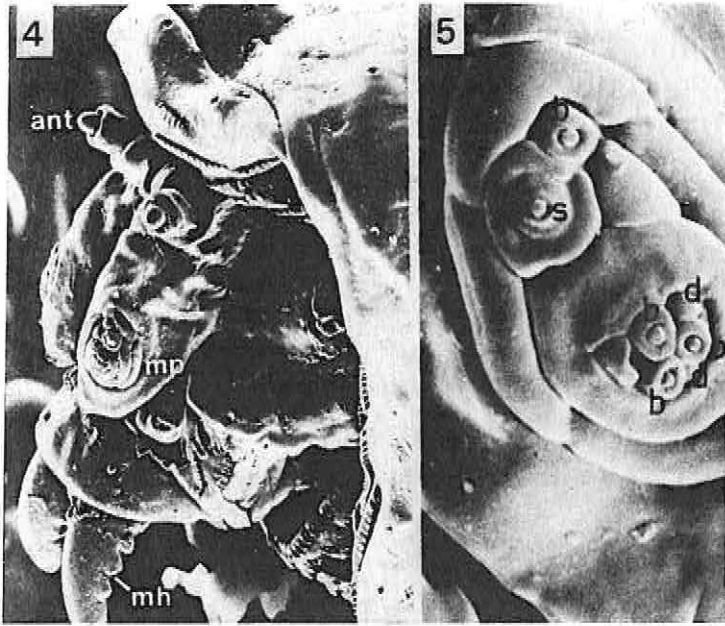


Figure 4. Side view of the head capsule. Ant: antenna, mp: maxillary ring, mh: mouth hook ($\times 750$)

Figure 5. Maxillary ring showing the arrangement of the dome-shaped (d) basiconic (b) and styloconic (s) sensilla ($\times 7,500$)

Figure 6. The antenna ($\times 7,500$)

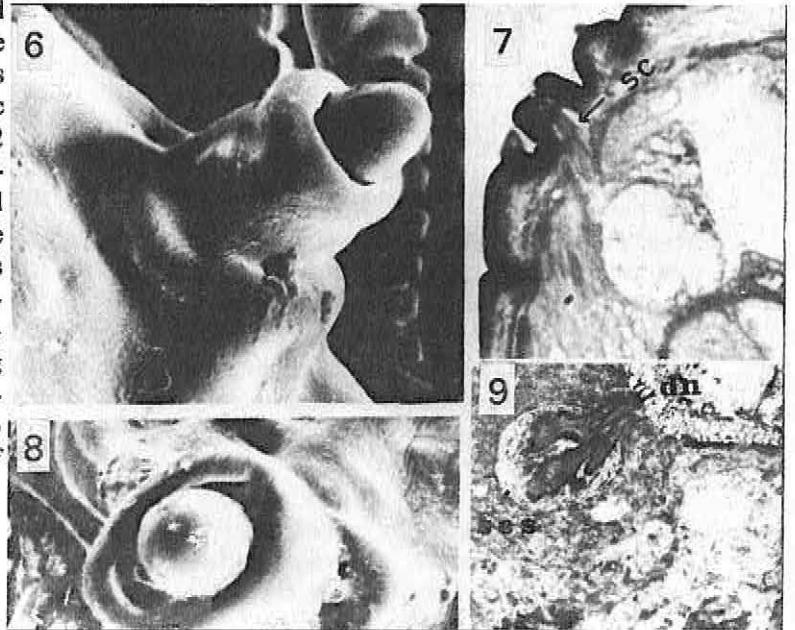
Figure 7. Longitudinal section through the styloconic sensillum showing the sense rod (scolopale) ending in the dome-shaped area within the cuticle ($\times 35,000$)

Figure 8. The styloconic sensillum ($\times 25,000$)

Figure 9. Oblique section through the maxillary ring showing a group of three dendrites (dn) within the scolopal sheath (scs) ($\times 56,000$)

cephalic lobe. The sensillae are contained in elevated cuticular rings. The basiconic and styloconic sensillae are 0.8 microns in diameter and are arranged in groups of twos and threes. The group of three are all basiconic and have two dome-shaped sensillae which are 1.2 microns in diameter. The group of two is situated dorso-laterally to the three and consists of one styloconic and one basiconic sensillae. A transverse section of the large basiconic sensillum on the dorso-anterior margin shows groups of three dendrites (Figure 9) in each of these receptors. A longitudinal section through the styloconic sensillum shows the terminal filament from the sense cell ending in a sense rod (scolopale), which is inserted into a dome-shaped area of a relatively thin cuticle. The two campaniform sensillae are situated one on each side of the head capsule immediately above the mouth hooks, (Figures 7 and 8).

Ryan and Behan in 1973, mentioned successful recording from similar receptors on the cabbage-root fly, *Erioischia brassicae* and the carrot fly, *Psila rosae* (F). Further investigation, possibly electrophysiological recording, is required for determining the functions of these receptors. It is nevertheless possible to speculate on the role of these receptors in determining the suitability of the host plant. The larger dome-shaped sensillae possibly function as odour receptors as suggested by the thinness of the wall of the external dome. The 8 basiconic receptors may serve as contact chemoreceptors. They may also function as humidity and thermoreceptors. Contact chemoreception and odour reception seem to be the most important ones in the shootfly larvae. The two styloconic receptors possibly respond to water, salt and sugars and also to temperature.



TERMITE RESEARCH

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The role of *Macrotermes* in soils

M. A. Arshad

The purpose of this study is to assess the role of termites in soil development and soil productivity in the semi-arid grassland ecosystem. The investigation is expected to yield results which might be applicable in the management of the rangeland soils. With this overall objective in mind the following two projects were undertaken.

- (i) The effect of various termite species on soil properties in order to assess whether or not there are any differences in sorting out soil constituents by different species.
- (ii) The role of termites in soil erosion and soil productivity.

Soil properties as affected by termites

The 'open' and 'closed' mounds (built by the two species—*Macrotermes subhyalinus* and *michaelseni* respectively) occurring side by side as well as the adjacent soil were sampled. The samples were air dried and ground to pass through 2 mm sieve for various analyses.

The data on particle size distribution through the depth of the mounds and the surrounding soil profile shows that the clay content is higher in the mound samples than the surrounding soil (Table 1). The royal cell has the maximum clay content and much less sand than any other part of the mound. Furthermore, there is a preferential selection of finer particles to construct the royal cell. Total absence of very coarse sand (2–1mm) in the royal cell indicates that particles of this size are probably not ingested by the workers while constructing this chamber. This is true for both species. Also, there is very little incorporation of coarse sand (1–0.5mm) either in the royal cell or the nursery. The water holding capacity consistently increases with increase in clay content.

The pH is slightly higher in the case of closed mound than open mound samples particularly below the royal chamber. This is attributable to the higher base saturation of these samples. Organic carbon is somewhat higher for the outer casings of the mounds but values are low for the samples below the mounds and the subsoil. The C/N ratios vary from 5.7 to 13.0 for soil and were close to 10 for outer casings of mounds while for royal chamber they vary from 8.1 to 8.3; the ratios are much lower for sub-soil samples. Low C/N ratios near the royal chamber appear to result from rapid mineralization of organic matter in a termite-active environment.

Role of termites in soil erosion and soil productivity

The experimental plots were set up in February, 1978 to study the role of termites in soil loss, soil productivity storm runoff and infiltration. The plots are located about 8km southeast of Kajiado on natural slopes with similar vegetation, landscape and climatic conditions. A set of three plots include active mounds (with termite activities) and another set of three plots have mounds inactivated by fumigation (without termite activities and thus serves as control). Each plot is further divided into two subplots.

Each plot measures 10m × 40m and has one mound within this area. It is enclosed by an impervious asbestos sheeting extending 15cm below and 15cm above ground on each side to prevent run-off entry from outside the plot. After each rain-storm the eroded soil and run-off are collected and measured in the sediment tanks installed at the lower end of the plot.

Because of unusually high rains in 1977 (650mm as compared to 305mm in 1976) each plot had a very dense cover of vegetation. Thus the 1978 long rains did not cause much soil erosion. In fact, most of the soil washed down in the run-off was the material disturbed during the construction of the plots and thus did not represent the actual eroded material. The data on soil loss for

the first rainy season was therefore discarded. Now that the vegetation along the walls of the plots and sub-plots has been re-established and the soil and vegetation conditions have come to a stable equilibrium, the first set of data on soil loss and run-off will be obtained during the short rains in November-December, 1978.

Apart from the data on soil loss and run-off, other changes in soil properties and vegetation conditions are being monitored. A distinct pattern of vegetation as influenced by the termite modified soil has been observed. There is a vigorous and luxuriant growth of grasses forming a distinct pattern of green ring around the mound. This is true for all the six plots although the spheres of influence vary directly with the size of the

mound. Later in the dry season this ring splits up into two rings: one consisting of the dry grasses immediately around the mound and the other with unaffected area of green grasses. Average yield of grasses in various plots with active and inactive mounds shows that the grass biomass decreases with increasing distance from the mounds (Figure 1). This may partly be attributed to the more desirable moisture status resulting from the high contents of fine soil separates (Figure 2) near the mounds. Preliminary data also indicate that the pH and the available major nutrients (calcium, magnesium and potassium) are higher in the surface soil around the mounds and their level decreases with increasing distance from the mound.

Table 1. Some physical and chemical characteristics of 'open' and 'closed' mounds and surrounding soil

Depth from top of mound (cm)	Sand Fractions				Sand 2.0-0.05	Silt 0.05-0.002	Clay 0.002	W.H.C.	pH	Organic C(%)	Total N(%)	C/N
	V.C.S.* 2.0-1.0	C.S. 1.0-0.5	M.S. 0.5-0.25 -mm-	F.&V.F.S. 0.25-0.05								
'OPEN'** MOUND												
0-5	4.3	11.4	11.8	14.8	43	9	48	39	5.3	0.762	0.077	9.9
25-50	4.4	11.4	10.3	14.5	42	10	48	40	5.2	0.058	0.082	10.4
90-120	1.6	9.0	10.4	13.0	35	16	49	42	5.5	0.804	0.079	10.2
120-150 (Nursery)	0.2	2.4	9.0	13.6	28	12	60	47	5.7	0.719	0.083	8.7
150-155 (Royal Chamber)	0.0	1.0	6.4	13.3	22	16	62	55	5.7	0.636	0.076	8.3
180-200	7.2	13.0	10.0	13.1	44	5	51	40	5.2	0.386	0.056	6.8
230-250	6.5	10.5	8.4	12.5	37	11	52	42	5.7	0.302	0.053	5.7
'CLOSED'*** MOUND												
0-2	3.2	8.9	9.2	13.3	36	12	52	42	5.5	0.672	0.069	9.7
2-35	2.6	8.7	8.6	13.1	33	14	53	42	5.4	0.693	0.070	9.8
40-75	2.6	8.4	9.4	13.1	35	15	50	44	5.6	0.787	0.077	10.1
115-140 (Nursery)	1.1	6.6	9.4	13.7	31	12	57	45	5.7	0.887	0.086	10.3
140-145 (Royal Chamber)	0.0	0.8	7.7	18.8	28	12	60	54	5.8	0.743	0.092	8.1
150-170	1.5	7.0	9.2	12.7	31	11	58	45	5.6	0.910	0.091	10.1
230-250	4.4	10.1	9.3	12.5	36	22	41	42	6.6	0.680	0.067	10.2
250-260	3.7	10.6	8.5	13.0	38	12	50	43	6.7	0.387	0.045	8.6
SURROUNDING SOIL												
0-7.5	3.4	11.6	11.6	14.6	42	22	37	39	5.4	2.155	0.166	13.0
7.5-35	3.8	11.8	12.3	15.6	44	20	36	29	5.5	1.108	0.196	11.5
35-60	3.7	12.1	10.3	12.3	39	15	46	35	5.7	0.724	0.064	11.2
60-90	7.2	14.3	10.4	12.9	45	17	38	30	5.9	0.395	0.046	8.4
110-120	3.7	10.9	9.9	10.3	36	9	55	43	5.7	0.336	0.053	6.3

* V.C.S.—Very coarse sand, C.S.—Coarse sand, M.S.—Medium sand, F.&V.F.S.—Fine and very fine sand.

** Height from ground level: open mound—130cm, closed mound—135cm.

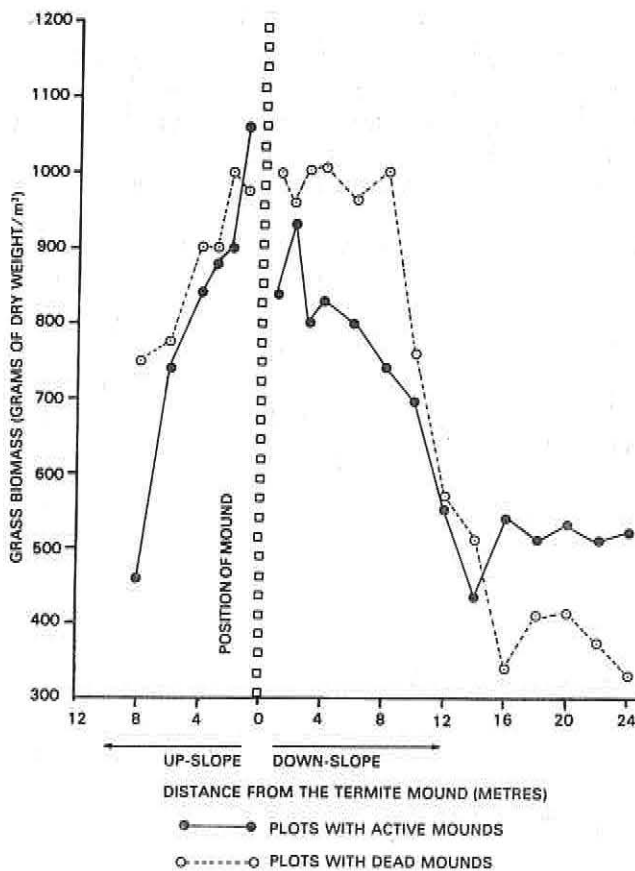


Figure 1. Grass biomass (grams of dry matter/m²) in relation to the proximity of termite (*Macrotermes*) mounds

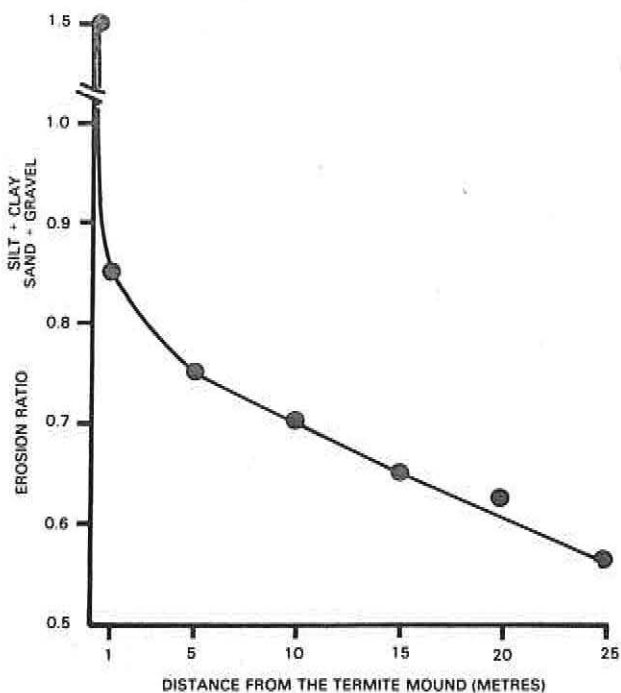


Figure 2. Textural variation in run-off plots in relation to the proximity of termite (*Macrotermes*) mounds

Populations of nests of *Macrotermes* species in Kajiado and Bissell

J. P. E. C. Darlington

Kajiado

After two years of particularly good rainfall (793mm in 1977 and 744mm in 1978) and a continuously high standing crop of grass, the *Macrotermes* population in Kajiado is flourishing. Many colonies which had been inactive or moribund during the preceding drought (only 259mm of rain fell in 1976) are now active and enlarging their mounds by new building. Young nests are also appearing in unusual numbers. Production of alates does not, however, appear to have been particularly high in either of the past two years.

Work on the populations of the closed, Kajiado-type mounds (thought to be of *Macrotermes michaelsoni* (Sjöstedt)) has been more or less completed by the addition of two very large nests to the series. One of these (no. 397) had a population of 5½ million individuals, 40% of them larvae, produced by a royal 'oligarchy' of seven queens (total fresh weight 55.9g) and a solitary king. The second nest (no. 401) contained 4 million individuals, 33% of them larvae, produced by a pair of queens (total fresh weight 31.5g) and one unusually heavy king. The nests were sampled at a time when the annual alate brood was still in the late nymphal stage. The numbers of nymphs found were not exceptional, 30,000 and 71,000 in the two nests respectively. Total dry weights of fungus comb in these nests were 37 and 26kg.

Using data for nest populations and external measurements of 28 mounds covering the whole known size range, some preliminary computer analyses were made (using a programme for stepwise linear multiple regression). The results indicate that the external measurements of mounds can be used to predict the total population of the nest beneath with an accuracy of about 70%. The reason for this very close relationship is presumably that the mound acts as a ventilation system for the nest beneath it, and its dimensions will be continually adjusted to the requirements of the population in the nests. The external measurements in question require only about five minutes per mound, and the greatest loss of time in making a mound survey is in getting from one mound to the next.

One interesting and unexpected discovery made in the course of the year is that if the royal pair is removed from a nest at a time when there are nymphs or unflown alates in the nest, a new replacement king and queen or queens can be produced. This is not possible at other times of the year, when a nest deprived of its royal pair normally stays healthy for 3 or 4 months until all the larvae have matured, then rapidly declines.

Bissell

Work has started on comparative population assessments for the open-chimney Bissell-type mounds (of *Macrotermes subhyalinus* Rambur) using the methods already developed (see ICIPE Annual Report, 1975). Because of the different internal structure of the mounds the fumigation technique has had to be modified and is slightly less satisfactory.

Two large healthy mounds (nos. 400 and 409) were each found to have populations of just under 2 million individuals. This is about half of what would be expected in a Kajiado-type mound of comparable size. The proportions of larvae were rather low, 23% and 28%. Numbers of alates were 22,000 and 10,000 respectively, and the total dry weights of fungus comb were 12 and 20kg. The caste ratios in the sterile adults were slightly different, with minor workers 1.3 to 1.5 times as numerous as major workers, instead of twice as numerous as in *M. michaelsoni* nests. However, it will be necessary to sample many more nests of *M. subhyalinus* before definite conclusions can be drawn.

Macrotermes foraging populations

M. G. Lepage

Work accomplished in 1978 carried on experiments done on *Macrotermes*' foraging and food consumption outlined in ICIPE Annual Reports 1976 and 1977—the assessment of the role of these fungus-growing termites as grazing animals and their possible competition with large herbivores within a semi-arid ecosystem.

Furthermore, a study was initiated this year of predation on foraging populations, in order to obtain some insight into the role of termites in the food chain.

Foraging and food consumption

Foraging activity was measured for one more year and observations confirmed what was known for the two previous years: there are two peaks of activity, before and after the long rains. But activity was lowered due to unusually abundant rains between November 1977 and May 1978. During these 7 months, rain and temperature explained 66% of foraging activity.

After the long rains foraging showed the same overall

activity (May to October) as that in 1976 and 1977. On closer observation it was seen there were oscillations with a periodicity of 4–5 and 15–20 days, which probably corresponded to a constant adjustment of the foraging to the needs of the colony.

As stated in the previous report, large herbivores were checked daily. Over 30 months (April 1976 to September 1978) their average daily biomass was 28.3 tons. A 'correcting' factor (1/150 to 1/225) was calculated to assume a density per hectare. Thus the daily abundance was 125 to 187 kg/ha. The yearly consumption of large mammals (2.3% of their live weight per day) ranged between 1050 and 1550 kg/ha.

Predation on the foraging populations

The Ponerinae ant *Megaponera foetens* is a specialized predator of the termites' foraging populations. This ant forages in columns raiding on foraging parties. To determine the predation the following information was collected on 5–10 nests selected for sampling: the number of raids per day, the number of ants in a raiding column, the number of ants returning with termites (and the species of termite) and the number of termites per ant.

This information is presented in Table 2 for 7 months, April to October 1978. Main prey were *Macrotermes* and *Odontotermes* spp. (94% of total prey). As seen from the table, predation increased from April to reach its maximum in August, then decreased thereafter. There was at the same time an increase in the total number of ants carrying termites and the average number they carry. Recruitment was probably more intense for *Macrotermes* since the total ants per column and the total ants carrying termites were higher than for *Odontotermes*.

Data for April to September show the estimated predation (termites taken per day and per m²): to be 0.1363 *Macrotermes*, 0.1063 *Odontotermes* and 0.0145 *Synacanthotermes*. As far as *Macrotermes* is concerned, this offtake (500,000 termites/ha/year) probably represented less than 10% of the total standing crop of worker and soldier castes (J. Darlington, personal communication). But, since the soldiers (major and minor) accounted for 20% of prey, their predation could have been more significant.

Table 2. Predation by *Megaponera foetens* on termites' foraging parties (April to October 1978)

Month	Total per raid all prey	Ants with termites all prey	<i>Macrotermes</i>			<i>Odontotermes</i>		
			Total per raid	Ants with termites	Termites per ant	Total per raid	Ants with termites	Termites per ant
IV/V	419.46	111.84	475.45	139.36	1.70	332.50	49.25	2.78
VI	385.21	120.48	435.94	160.29	1.80	313.33	64.08	2.72
VII	473.21	209.46	486.11	210.39	2.66	450.00	207.80	3.67
VIII	537.50	239.98	636.36	310.55	2.85	400.00	160.06	3.61
IX	355.45	81.42	381.90	79.23	1.92	305.45	88.27	2.52
X	291.25	93.25	373.00	133.29	1.99	176.80	37.20	2.17

Studies on caste differentiation in incipient *Macrotermes* colonies

G. Bühlmann

Our work is focused on polymorphism in the neuter castes of the incipient *Macrotermes* colonies, ie. the reasons why larvae develop into major workers, minor workers or minor soldiers. Descriptive and experimental methods are used to study the mechanisms involved in attaining and maintaining respective caste proportions.

Normal development

Under controlled conditions, early colony development follows a remarkably rigid schedule. At a given age, there is very little heterogeneity in the ratio minor workers plus minor soldiers versus major workers. The ratio minor soldiers versus minor workers is frequently found to be significantly homogeneous, ie. better balanced than would be expected by mere chance. Furthermore, the frequency of presoldiers and soldiers per colony does not follow Poisson distribution: colonies with excessively many or excessively few are very rare.

The ratio minor workers plus minor soldiers versus major workers is obviously genetically controlled, since major workers are characterized by rudimentary testes whereas the other two castes show rudimentary ovaries. The ratio between minor workers and minor

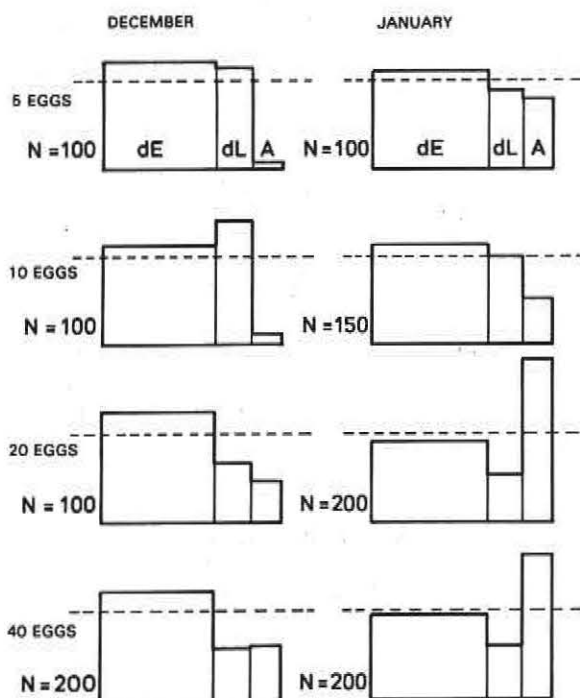
soldiers however, is of particular interest because extrinsic factors must be responsible for the determination of a young female larva for a career as a worker or soldier. Before a young colony has produced soldiers, a relatively high number of those larvae moult into presoldiers. Later on, the proportion of presoldier moult markedly decreases.

Presoldier formation

The presence of presoldiers or soldiers has an inhibitory action on the formation of new presoldiers. If newly formed presoldiers are systematically removed each week, the net presoldier production is more than twice as high as under normal conditions. If additional presoldiers are introduced net presoldier production slows down, but this is apparently not a specific inhibition since it takes place in concert with a general slowing down of the young colony, which undergoes heavy nutritional stress because of the additional presoldiers.

A bioassay was developed to assess possible inhibitory action on the presoldier production of incipient colonies. Young colonies were deprived of their presoldiers every week, the presoldiers were substituted with an experimental agent. The relative number of presoldiers removed after a given period of time was used as the criterion. Other proportions were used to assess the general healthiness of the colonies under particular experimental conditions.

TWO MALES



TWO FEMALES

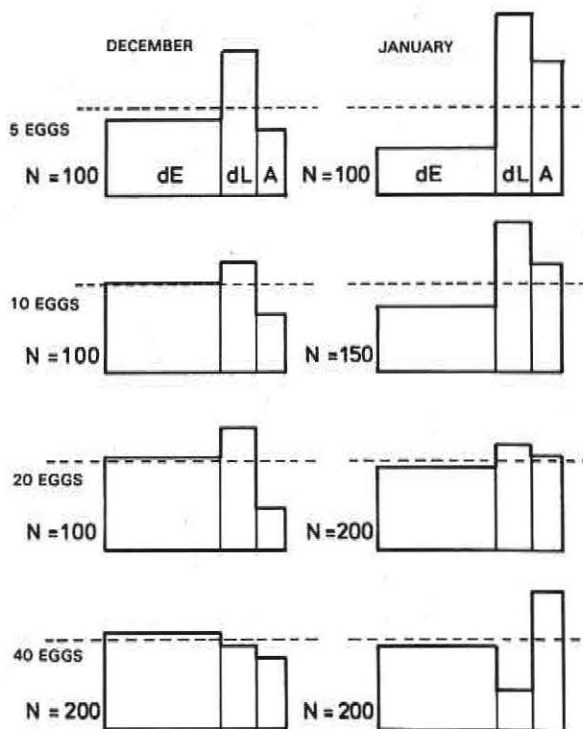


Figure 3. Survival in egg-fostering experiments. Eggs from nursery of a mature field mound given to male or female fosterparents in December or January; 5, 10, 20 or 40 eggs per box. dE: eggs which did not hatch; dL: larvae which disappeared before final moult; A: final instars (workers and soldiers). Area of every three-column set is unity; area of columns is proportional to observations under given conditions; column width is proportional to overall proportions in the experiment; dotted line indicates expected column height under the hypothesis of independence

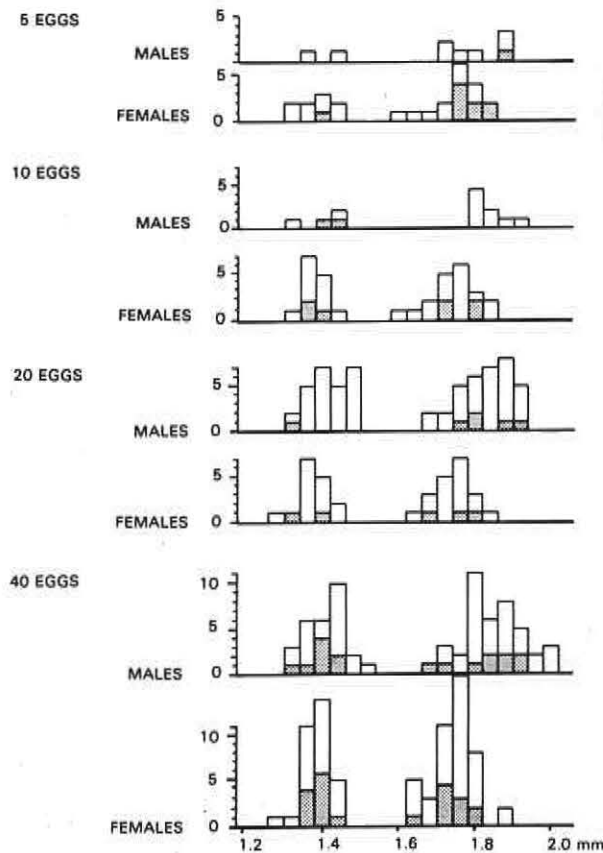


Figure 4. Frequency histogram of head width. Minor and major workers raised from eggs by female or male fosterers in December or January; 5, 10, 20 or 40 eggs per box. Shaded columns: December; white: January

Extracts of soldiers in saline, acetone or hexane were prepared and added on pieces of filter paper 1cm². The equivalent of one minor soldier per colony per week was assessed for inhibitory activity. There was no significant effect. It is possible that the contact of termites with the filter paper is not good enough. Another experiment using the dead bodies or parts of bodies as possible inhibitory agents had some effect when the abdomens of freeze-killed soldiers were offered. Although these effects were significant at the 0.05 level only, it is hoped that future experiments using modified techniques will give indications as to a possible chemical factor to control presoldier production in incipient colonies.

Experiments involving the interchange of reproductives

Attempts to witness the mating process have so far proved unsuccessful. Mating is required for the production of offspring. Mating can be delayed for prolonged periods by keeping males and females apart. If the sexes are then joined, eggs start hatching into larvae about 40 days later (at 29°C). Incipient colonies obtained this way do not really develop differently, although there is some slight variation in instar duration and in the size of the resulting workers and soldiers.

It is possible to initiate incipient colonies by using males which are older than females. Bachelors which have been kept with other males for over one year are

successful fathers. Males from year old colonies or from mature field mounds are able to fertilize young females but do not do very well as fathers. They frequently become cannibals eating their own larvae and eventually die. This is probably because internal reserves in their fat bodies are exhausted and they starve under incipient colony conditions.

Colonies where males are removed and replaced by a virgin female show that females alone are perfectly able to bring up their young. It is essential, however, that two reproductives are present, otherwise heavy mortality seems inevitable (probably because no mutual grooming is possible and infectious diseases are more likely to spread). Couples comprising two males are also able to raise young colonies as long as they are provided with fertilized eggs. Increasing the period of time a male is allowed to stay with a female improves the ability of the female to raise an incipient colony. It is very likely that even in starting colonies multiple mating takes place.

Fostering of eggs or larvae

The capability of de-alates, irrespective of their sex, to care for young was used in fostering experiments. Eggs of known origin were given to pairs of males or pairs of virgin females of known age. Success rate, instar duration, caste proportion and size of the resulting workers and soldiers were analyzed.

Eggs found in the nursery of a mature field mound seem to be very well blended. If given to fosterparents, the first larvae always appear in the first week. This seems to be consistent for all nurseries investigated so far. Nursery eggs are generally larger and have a much larger variation in volume than eggs which are laid recently by the physogastric queen (like those found in an isolated queen's chamber a few hours after extraction from its mound). Eggs from incipient colonies show an even greater amount of volume variation. They are generally larger than eggs from the field.

Larval instars are shorter in incipient colony material and the resulting soldiers and workers are smaller. The soldier/worker ratio is more in favour of soldiers than in material originating from field colonies.

There is also significant interaction with the fosterparents. If very young de-alates are used, caring for the larvae cannot be taken for granted and losses during larval development are heavy, although the hatching rate is reasonably good. Females are generally more efficient in terms of the survival of larvae but the soldiers and workers raised by males are significantly larger in size. The data, which are based on weekly observations, indicate that postembryonic development is slightly faster with female fosterers than with males.

The number of eggs offered to a pair of foster parents also affects the success rate. In general, 20 or 40 eggs are cared for better than only 5 or 10 eggs per box. Interestingly, the number of eggs or larvae raised in a box has no effect on the size of the workers and soldiers

which result.

During the months of April and May, the mature *Macrotermes* in Kajiado area lay a certain percentage of eggs which develop into winged larvae and alates. It was hoped that some eggs removed from these field mounds during the critical period would develop into winged larvae if fostered by males, females or added to young colonies in the laboratory. Eggs collected at the critical period from three to six different mounds, were placed in two hundred boxes with different fostering systems but not a single second instar larva with wingpads was observed. If, as it is believed, alate development is already determined in the egg, it seems that young termites on the reproductive line are exterminated before they can be recognized by the experimenter. In fact, larvae with wingpads never occur in incipient colonies.

Fostering of larvae is also possible. Larvae from the field develop into much larger soldiers or workers than termites raised from eggs by comparable fosterers. These are much smaller though, than soldiers and workers from the mound the larvae are taken from. Heterogeneity in groups of termites raised from larval instars is considerable and is much higher than in groups raised from eggs. This is certainly due to the heterogeneity of the experimental material, which is already heterogenous in cases where larvae are used. Mortality is relatively high when larvae are fostered. If larvae are brought up by couples which produce their own fertile eggs, it may be observed that the large

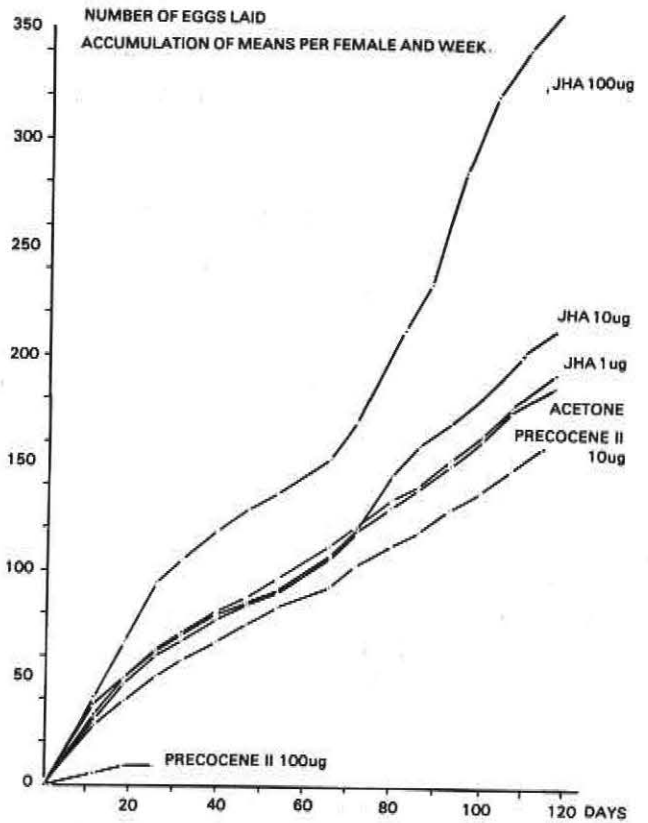


Figure 5. Egg production of *Macrotermes* couples treated on day 1 and day 69. Eggs were removed weekly

COLONY DEVELOPMENT REPRODUCTIVES
TREATED WITH:

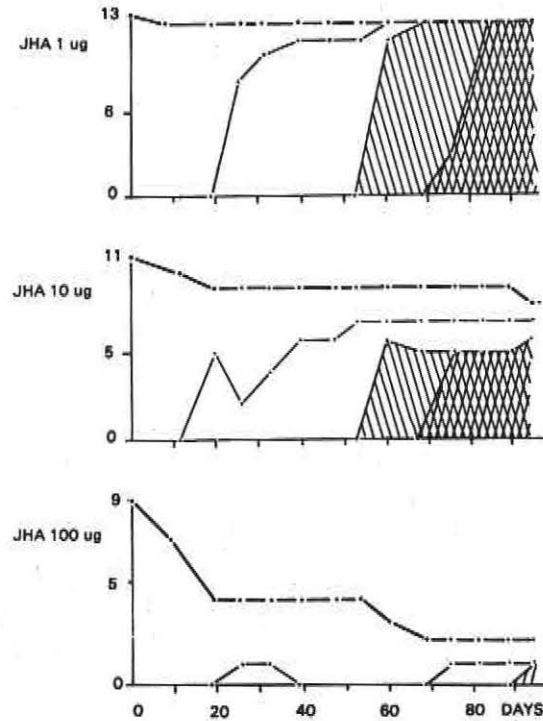
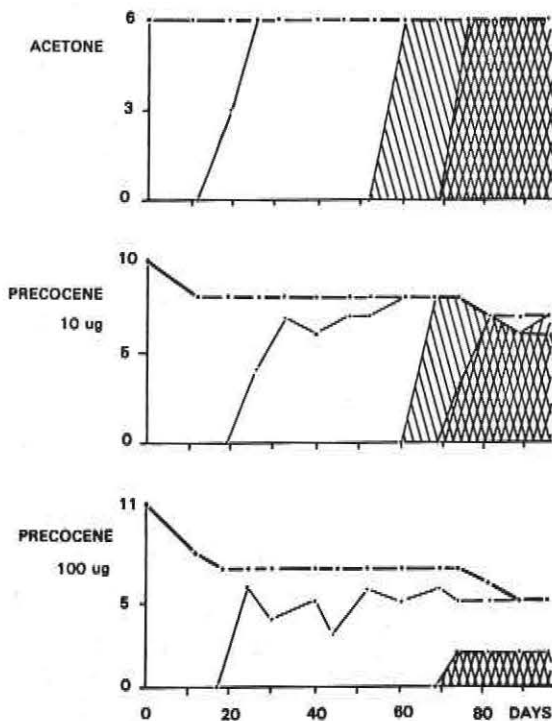


Figure 6. Development of incipient colonies. Reproductives were treated on day 1. Bold line: colonies alive; thin line: colonies with larvae; hatched to the left: colonies with workers; hatched to the right: colonies with soldiers; cross-hatched: colonies with workers and soldiers.

(introduced) larvae survive well and the true offspring of the parents are fewer in number. It is not possible as yet to determine whether there is cannibalism or not, nor who is actually depleting the young larvae.

Experiments using JHA or precocene

Experiments were conducted where the juvenile hormone analogue ZR 515 (JHA) or precocene II was topically applied to reproductives or fosterparents. Egg production, hatching rate, larval development and final caste proportions were then observed.

JHA exerts a gonadotropic effect in *Macrotermes* females. Egg production is significantly increased. The eggs produced under the influence of a high dose (100 µg), however, do not hatch, even if they are fostered by untreated males. On the other hand, a certain amount of eggs from an untreated female hatch, fostered by treated males. The chemical most likely affects embryonic development. Its effect on the ovaries is reversible, since eggs laid five weeks after treatment are again fertile. The appearance of the first larvae is delayed, if only the female is treated by the high dose, but if only the male is treated this way, colony development does not seem to be affected. If males fostering eggs from untreated females are treated with JHA, the soldier/minor worker ratio, is slightly increased but the effect is only significant at the 5% level.

Experiments using precocene II have not given any indication of an 'anti-JH' effect regarding incipient *Macrotermes* colonies so far. 100 µg topically applied in acetonic solution were lethal to young reproductives a few days after the flight. Three week old reproductives however survived the treatment but showed low egg production and signs of general weakness. Fosterparents treated with this dose were able to raise soldiers and workers from eggs. Caste proportions do not seem to be affected by precocene.

Conclusions

Caste differentiation in *Macrotermes* is controlled by a variety of factors. Most obviously, genetic mechanisms

decide the sex of the young termite. Whether a female larva becomes a soldier or a minor worker depends very much on the social environment. Factors which keep down production of presoldiers are connected with the relative number of soldiers and presoldiers already present in the colony. They are most probably of a chemical nature. It is very likely that juvenile hormone plays an important role in caste differentiation. Its level of action seems to be within the individual termite. Our experiments suggest that the speed of development i.e. the larval instar duration, is also an important variable in the complex mechanism of caste differentiation. The duration of the early instars appears to be under the control of the actual mother of the eggs and relatively independent of the nutritional conditions of the larvae. Since rapid larval development means a shortening of the time when a larva is susceptible to extrinsic factors a direct relation between developmental speed and caste differentiation could be postulated.

Recent findings on mechanisms of caste differentiation in *Macrotermes* species near *subhyalinus*

B. M. Okot-kotber

Studies on caste differentiation are being continued in our laboratory. In last year's report (ICIPE Annual Report, 1977) we reported some data on polymorphism and some hormonal aspects of caste differentiation. More data on polymorphism have become available, therefore we will now have a comprehensive picture on the developmental pathways of all the three castes. Workers (minor and major), soldiers (minor and major) and reproductives. Changes in endocrine glands of reproductives and soldier castes during development will also be dealt with in this report. The development of incipient colonies and the influence of juvenile hormone analogue (JHA) will be examined as well.

The fate of larvae in the sixth group described last

Table 3. Measurements of head capsule width, posterior tibia length, wing-pad length and the number of antennal segments of nymphs and alates

Nymphal Instar	Sample size (n)	Head capsule width (mm ± SE)	Posterior tibia length (mm ± SE)	Tibia length/head capsule width	Wing-pad length (mm ± SE)	No. of antennal segments
1st.	10	0.77 ± 0.01	0.64 ± 0.01	0.83	0.20 ± 0.00	14
2nd	36	1.21 ± 0.01	1.19 ± 0.00	0.98	0.46 ± 0.01	16
3rd	30	1.66 ± 0.02	2.03 ± 0.02	1.22	1.43 ± 0.02	18
4th	48	2.26 ± 0.02	2.88 ± 0.02	1.27	3.11 ± 0.04	19
5th	18	3.03 ± 0.03	3.57 ± 0.04	1.18	6.68 ± 0.10	19
ADULT	35	3.54 ± 0.01	4.41 ± 0.02	1.25	37.90 ± 0.01	19

year is now confirmed and these larvae have been designated as fourth instar larvae. Biometric work carried out on nymphs from the field (Table 3), shows that nymphs go through five instars before imaginal moult and that they emerge from first instar larvae which they are morphologically identical with those giving rise to sterile castes. A complete scheme of post-embryonic development for sterile and reproductive castes is now available (Figure 7).

Endocrinological investigations carried out on the reproductives (nymphs and adults) was an attempt to find out the role played by corpora allata (CA) and prothoracic glands (PG) in their differentiation. This was accomplished by histological investigations on the changes that occur in these glands during poste-

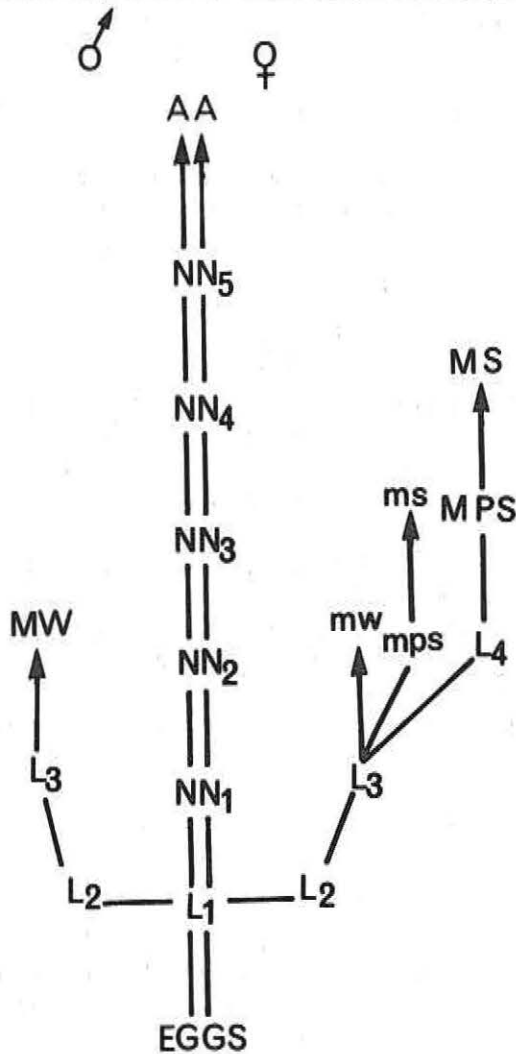


Figure 7. Possible post-embryonic developmental shunts of larvae and nymphs of a species of *Macrotermes near subhyalinus*

- L₁ — L₄ — Larval instars
- N₁ — N₅ — Nymphal instars
- MW — Major workers
- mw — minor workers
- MPS — Major presoldiers
- MS — Major soldiers
- mps — minor presoldiers
- ms — minor soldiers
- AA — Adults (imago)

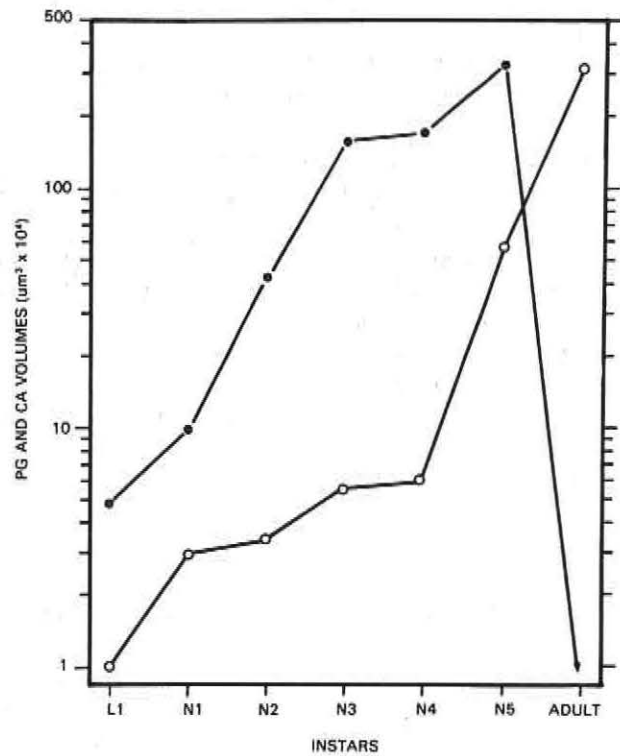
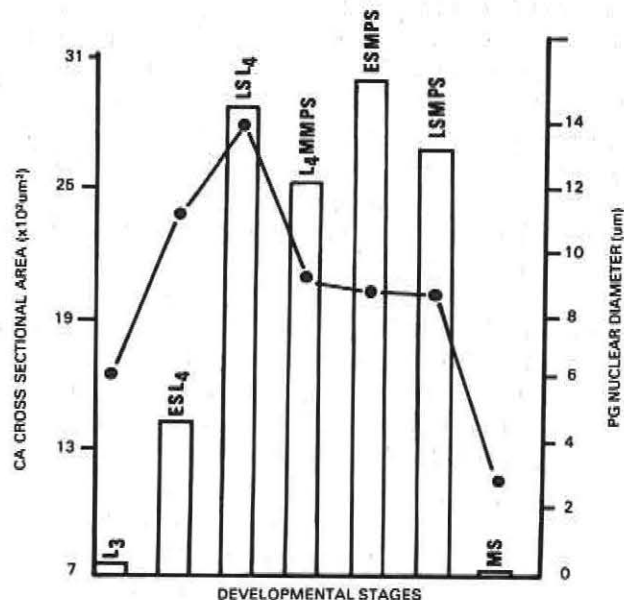
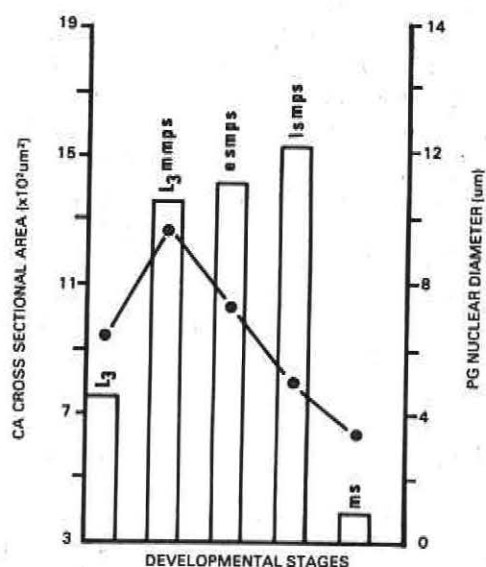


Figure 8. Changes in the volume of PG and CA during nymphal development. L₁—First instar larvae N₁—N₅—Nymphal instars
 ● — Prothoracic gland volume
 ○ — Corpora allata volume
 Each point is represented by measurements from between 3 and 10 individuals

embryonic development. The results are summarized in Figure 8. From the graph it is evident that the increase in the volume of CA is sluggish but steady during the first four nymphal instars.

However, during the late stage of fifth instar and after the adult emergence, there is a very sharp increase in the size of the CA. Data on the number and size of nuclei within cross-sectional areas of these glands show that the enlargement of the glands during nymphal development is more associated with proliferation of cells than other processes. It seems therefore that CA do not play a major role in the differentiation of the reproductives. The prothoracic glands increase in size more drastically than the CA during the development. This increase is dramatic right from first nymphal instar, implicating PG in the differentiation of the reproductives. The PG however, could attain this activity for some other physiological role, especially growth which is of a high magnitude during nymphal development.

Investigations were also carried out to establish whether CA play any role in the development of minor and major soldiers after determination. The results are expressed in Figures 9a and b. They show that apparently there is a continuous demand for JH even during the presoldier stage of development as exem-



Figures 9a and b. In these figures changes in the size of CA and the diameter of PG nuclei during different stages of soldier formation are illustrated.

- (a) L₃ — Female third instar larvae
 L₃mmps — Third instar female larvae moulting into minor presoldiers
 esmps — Early stage of minor presoldiers
 lsmps — Late stage of minor presoldiers
 ms — minor soldiers

- (b) ESL₄ — Early stage of fourth instar larvae
 LSL₄ — Late stage of fourth instar larvae
 L₄MMPS — Fourth instar larvae moulting into major presoldiers
 ESMPs — Early stage of major presoldiers
 LSMPs — Late stage of major presoldiers
 MS — Major soldiers

The column graphs represent changes in CA volume and the line graphs represent changes in the diameters of PG nuclei. Each point is represented by between 4 and 18 measurements in Figure 9a and by between 6 and 40 in Figure 9b

plified by persistent large size of the glands at this stage although a little smaller than in the fourth larval instar.

During the year, work was carried out on the determination of larval instars and their duration in incipient colonies. The colonies were kept at about 30°C and checked daily following the establishment of paired male and female to find out the exact onset of oviposition and subsequently the incubation period of eggs

laid and the duration of each larval instar. The results show that the incubation period of eggs is about 36 days. First instar larvae take about 5½ days before moulting into either female (smaller) or male (larger) second instar larvae. It takes female second instar larvae about another 5 days before moulting into third instar larvae, whereas the male second instar larvae require

Table 4. Soldier production in incipient colonies following topical application of JHA (ZR 515) on larvae compared with untreated larvae

Amount of JHA applied (µg)	Total No. of survivors	No. of pre-soldiers formed n (%)	BY 12 DAYS AFTER TREATMENT		BY 25 DAYS AFTER TREATMENT		TOTAL n (%)	
			No. of individuals with pre-soldier characteristics n (%)	TOTAL n (%)	Total No. of survivors (n (%))	No. of soldiers formed n (%)		No. of individuals with soldier characteristics n (%)
0	110	16(14.6)	0(0)	16(14.6)	95	12(12.6)	0(0)	12(12.6)
1.25	126	30(23.8)	19(15.1)	49(38.9)	83	23(27.7)	2(2.4)	25(30.1)
2.5	91	23(25.3)	12(13.2)	35(38.5)	75	15(20.0)	13(17.4)	28(37.4)
5.0	35	6(17.1)	3(8.6)	9(25.7)	24	4(16.7)	2(8.3)	6(25.0)
10.0	22	5(22.7)	2(9.1)	7(31.8)	8	3(37.5)	1(12.5)	4(50.0)

BY 12 DAYS AFTER TREATMENT

BY 25 DAYS AFTER TREATMENT

a longer period, 8 days before moulting into a larger third instar. The majority of female third instars moult into minor workers within 10 days and the minority of them take about 14½ days before moulting into minor presoldiers. Third instar male larvae take 14 days before moulting into exclusively major workers which are larger than minor workers. The first presoldiers that differentiate require about 12 days before moulting into minor soldiers.

In summary, from the time of hatching, female larvae go through three instars with a total developmental duration of about 25 days to develop into minor workers and about 33 days for minor soldiers to emerge. A major worker requires an intermediate length of developmental time between these two extremes which is about 27½ days to complete its development.

Having established the number of larval instars that prevail in incipient colonies, an attempt was made to study the influence of JHA on caste differentiation. The juvenile hormone analogue, ZR 515, was topically applied on the dorsal part of the abdomens of third instar female larvae. As a control acetone alone was also applied on a group of individuals. The dosages used ranged from 1.25–10.0µg JHA/animal in acetone. High mortality of larvae was observed among groups which were treated with 5µg of the analogue per animal or more. The results of the soldiers or soldier like individuals (inter-caste) formed following JHA treatment are presented in Table 4.

From these results it is apparent that JHA stimulates soldier production and in cases where there has been no complete effect of the analogue on a larva an intermediate form between a soldier and a worker is realized. It seems that the effect of the hormone analogue is stage specific and may also depend on the concentration and the number of applications administered. These conditions remain to be investigated.

Specificity of pheromonal trails in *Trinervitermes bettonianus*

G. W. Oloo

Unlike social Hymenoptera which utilize visual as well as chemical cues, the blind foraging termites rely almost entirely on chemical signals for orientation outside the nest throughout foraging activity. In the field situation one often finds nests of *T. bettonianus* close to one another and foraging columns extending a few metres from the nest. The question arises as to how a stray termite determines its way home in the event of finding itself in an alien trail network. Is a termite capable of discriminating between its own and another colony's natural trail in a foraging situation? If so, is such colony-specific information also contained in extract trails? For practical purposes, apart from investigating the selective or non-selective nature of

trails, it would also be useful to know the relative attractiveness of extract as compared to natural trails.

Materials and methods

Field colonies of *T. bettonianus* were collected from different ecological areas where the termite occurs (ie. Ruiru, Machakos and Narok) for laboratory experiments. To test for specificity of natural trails two neighbouring colonies were connected by a 'Y' shaped feeding bridge to a common food chamber, as illustrated in Figure 10. The food chamber was partitioned with a thin plastic wall to prevent termites from the two colonies being mixed during foraging, the wall extended up to the junction of the tunnel on the feeding bridge to keep the two trails separate but close to each other. A paper substrate with a pencil-drawn 'Y' maze with line was placed in the tunnel so that foraging termites passing through could deposit their trails (NT) along the pencil lines on the paper. Initially, termites from the nest gathered at the two chambers marked (C); the gates (G) were then opened to allow them to proceed to the food source (FS), and both outgoing and homing workers were counted as they passed the 'Y' junction until equal numbers had been recorded across the foraging routes (ie. until trails of approximately equal strength had been deposited on the paper). The paper was then removed and tested with an inexperienced termite from one of the two experimental colonies—the test termite (T) thus having to choose between following its own colony's and another colony's trail (Figure 10). Each pair of trails was tested once with a single termite, the process being repeated several times. The same procedure was used to test pairs of trails of remotely situated colonies from the same locality, and of colonies from different populations. To test the specificity of extract trails, whole body extracts were prepared from workers of a given colony

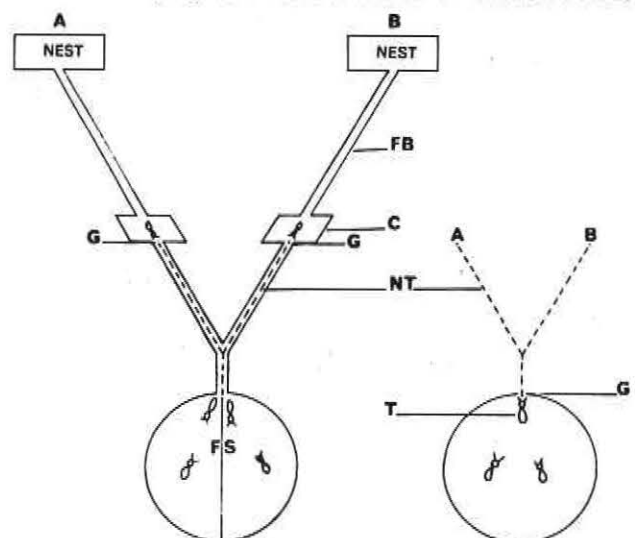


Figure 10. The device used for testing specificity natural pheromonal trails in *Trinervitermes bettonianus*. A and B trails of the colonies tested; FB foraging bridge; C collecting chamber; FS food source; G gates; NT natural trails; T test termite

Table 5a. Specificity of pheromonal trails in *Trinervitermes bettonianus*, natural trails (competitive trail-following tests with termites from different localities using 'Y' assay design shown in Figure 10.)

Source of trail (30-100 workers)	Source of test termites	Total no. choices between termite's own and other colony's trail	No. choices of termite's own trail
M ₁ vs M ₂	M ₁	22	10
M ₁ vs M ₂	M ₂	19	14
R ₁ vs R ₂	R ₁	20	14
M ₁ vs R ₁	M ₁	18	10
N ₁ vs R ₃	N ₁	20	18 (P<0.01)
N ₂ vs R ₄	N ₂	18	16 (P<0.01)
N ₃ vs M ₃	N ₃	22	18 (P<0.01)

M—Machakos colony R—Ruiru colony N—Narok colony

(2,500 workers/25ml hexane at 4°C for 17-24hrs). The extracts were assayed for trail activity and subsequently adjusted to comparable strength by diluting the stronger extracts. For each pair of colonies, extracts of comparable activity were applied on a 'Y' maze on paper at the rate of 2.5 µl/5cm and presented to the test termite to choose between the extract trail prepared from its own colony and the extract trail from the other colony, in a manner similar to that tried with natural trails.

Before comparing natural and extract trails in competitive trail following tests, the activity of a natural trail was estimated using extracts of known activity. Termite workers were collected into a food chamber by connecting the chamber to the nest. The termites were transferred with the box to a testing device similar

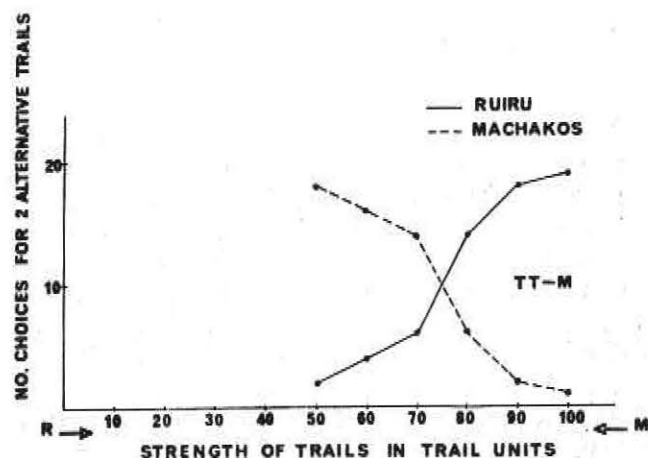


Figure 11. Competitive trail following tests using extract trails in *Trinervitermes bettonianus*, showing the influence of trail strength. R, M, trail extracts prepared from Ruiru and Machakos termites respectively; TT-M test termite from Machakos

Table 5b. Specificity of pheromonal trails natural trails at full foraging activity

Source of trail	Source of test termite	Total no. choices	No. choices of own colony's trail
M _a vs M _b	M _a	24	23 (p<0.01)
m _a vs M _b	M _b	22	20 (p<0.01)
M _a vs N _a	M _a	25	24 (p<0.01)
M _a vs R _a	R _a	22	19 (p<0.01)
M _a vs R _a	R _a	22	22 (p<0.01)
M _a vs R _a	M _a	22	17 (p<0.05)

to that illustrated in Figure 10 (ie. a 'Y'-shaped tunnel in a perspex block with a paper substrate for trail-laying, and a thin wall partitioning the tunnel lengthwise to restrict deposition of natural trails to one side of the 'Y' maze). Two workers were released from the feeding chamber to pass through and lay trails on one half (branch) of the 'Y' maze on paper, the block was removed and an extract of known activity was immediately applied on the other half or branch of the maze, the paper was then presented to a test termite for competitive trail-following. The process was repeated several times with 2, 4 and 20 workers to determine at what concentration the extract and natural trails exhibited comparable trail-following activity. The estimate was used to determine the number of workers that would be required to contribute a natural trail of equal activity to an extract trail of known activity in a foraging situation.

Results

For relatively weak trails (ie. trails contributed by 30-100 foraging workers) colonies from the same

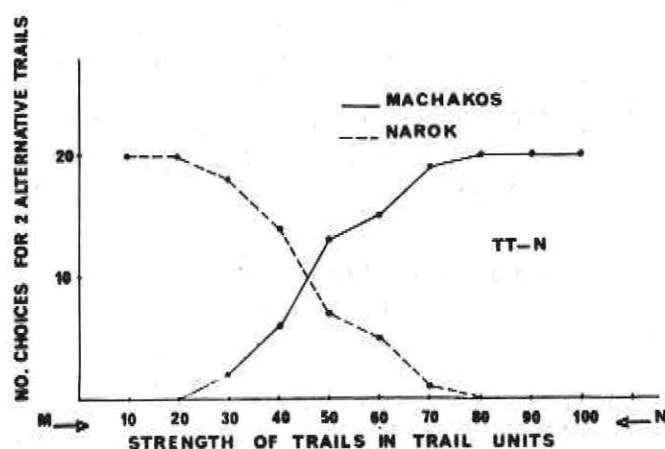


Figure 12. Competitive trail following tests using extract trails in *Trinervitermes bettonianus*, showing the influence of trail strength. M, N, trail extracts prepared from Machakos and Narok termites respectively; TT-N test termite from Narok.

Table 6. Activity of worker's trail (determination of the strength of worker's natural trail using trail pheromone extracts of known activity)

No. workers contributed natural trail	Strength of extract trail in trail units (TU)	Source of test termites	Temp. °C	Total no. choices between natural and extract trail	No. choices of natural trail
2	1TU $\left(\frac{1}{300}\right)$		26	20	19(p<0.01)
2	4TU	N	22	20	16(p<0.05)
4	16TU	N	23	20	16(p<0.05)
4	20TU	N	25	40	26
4	24TU	N	23	20	11
20	100TU	M	—	20	9
4	24TU	M	—	20	8

N—Workers from Narok colony
M—Workers from Machakos colony

locality did not seem to recognize their natural trails (Table 5a). However, well established foraging trails laid over the whole foraging period of about 1–2 hours were recognized by members of the same colony in a choice situation (Table 5b). Populations showed preference for natural trails of colonies from the same population. For example, termites from Narok showed strong preference for trails of colonies from Narok when the alternative choice was a Ruiru or Machakos trail, even for relatively weak trails (Table 5a).

In these experiments, no preference was observed with extract trails of comparable activity, preference for trail-following being dependent on trail strength (Figures 11 and 12). The strength of a single worker's trail was estimated to be about 5–6 trail units (Table 6), a trail unit being defined here as the minimum concentration of an extract that elicits trail-following

Table 7. The attractiveness of natural trails as compared to extract trails of same colony

No. workers contributed to natural trail	Strength of extract trail in trail units (TU)	Source of test termites	Total no. choices between natural and extract trail	No. choices of natural trail
30	300 TU	N	20	20(p<0.01)
30	100 TU	M	40	39(p<0.01)

when applied at the rate of 2.5µl/5cm on a filter-paper substrate. Applying this estimate to a foraging situation, the natural trail was found to be much more attractive than an extract trail of the same colony, in a competitive choice between the two types of trails (Table 7).

Discussion

The observed specificity of established natural foraging trails suggest that these trails contain or acquire some colony-specific information (possibly of chemical nature) analogous to 'colony odour' which enables members of a colony to distinguish their own trail. It could be that this component is unstable or more volatile and so not detectable in weaker trails contributed by a few worker termites. On the other hand, extract trails exhibit little or no colony specificity within a population possibly because some information is lost in the process of extracting the pheromone or in storage. This is supported by evidence to the effect that natural trails are more attractive than extract trails of the same colony. This would, of course, complicate the possibility of applying trail pheromones for manipulating the termite's foraging activity in the field situation. The apparent trail specificity recorded for the Narok population in tests with either Ruiru and Machakos populations is probably the result of adaptive behaviour influenced by local environmental factors. In this connection it is interesting to note that population related differences had earlier been demonstrated with defensive secretions of termites from these localities—i.e. the Narok defensive secretions had a slightly different chemical composition from either those of Ruiru or Machakos. But further work on pheromonal trails is necessary to establish the differences more conclusively, and to show whether the differences are qualitative or simply quantitative.

Fungi associated with *Macrotermes* near *subhyalinus*

D. B. A. Ruyooka

A species of *Macrotermes* (related to *Macrotermes subhyalinus* Rambur, Macrotermitinae) found in Kajiado district, Kenya, which builds closed mounds, is symbiotically associated with fungus of the genus *Termitomyces* Heim. Because of the strong relationship existing between this species of termite and the fungus, not only is it necessary to investigate the physiology of the former, but also that of the latter.

Abo-Khatwa (see ICIPE Annual Report, 1976) investigated *Termitomyces*, using a biochemical approach. Despite this effort, the role of this fungus in *Macrotermes* and other species is still little known. For this reason new approaches are being made in order to understand further this fungus-termite inter-relationship.

Fungi isolated from the gut of *Macrotermes* near *subhyalinus*

Preliminary work was started using workers and soldiers. Major soldiers (MS), minor soldiers (ms), major workers (MW) and minor workers (mw) were surface-sterilized, using 0.35% sodium hypochlorite (NaOCl₂) for c.2min, and thoroughly washed in sterilized distilled water. Following an incision in the abdomen, they were then plated onto a malt extract (2%) and agar (2%) medium in 9cm diameter Petri dishes and incubated at 29±2°C for 3 days. Six replicates were used.

Fungal growth was observed to commence from the termite inoculum (see Table 8 and Figure 13).

Table 8. Fungi growing from termite inoculum after 3 days

Caste	Fungi
Major soldier	Green moulds (Fungi Imperfecti)
Minor soldier	Nil
Major worker	Luxurious cotton-like mycelium, possibly <i>Termitomyces</i> (<i>Basidiomycete</i>); also greenish yellow moulds (Fungi Imperfecti)
Minor worker	White mycelium but showing less aerial growth than that from MW; also greenish yellow moulds (Fungi Imperfecti)

The results indicate that this species of *Macrotermes* carries fungi in its gut, as has been shown by other workers using different termite species. In a previous experiment, I have isolated the cotton-like fungus from the major workers of this species. It is not known in what form (conidia or hyphae) the fungus is carried in the gut. The question may be asked: what role do such fungi play in the physiology of this and related termite species? Subsequent work is aimed at trying to answer this and similar questions.

Acknowledgement

The assistance of the Department of Botany, University of Nairobi, in providing me with mycological facilities is gratefully acknowledged.

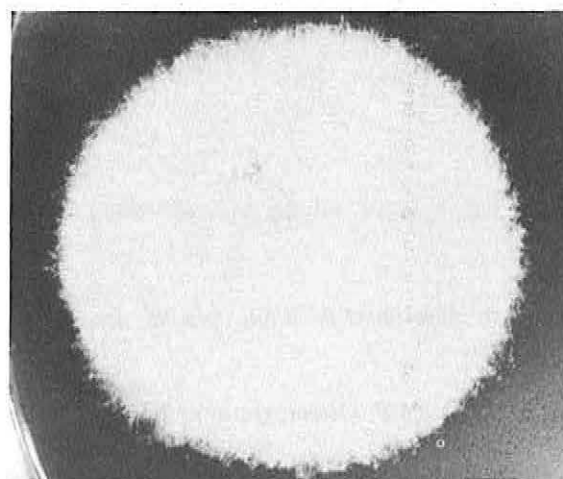
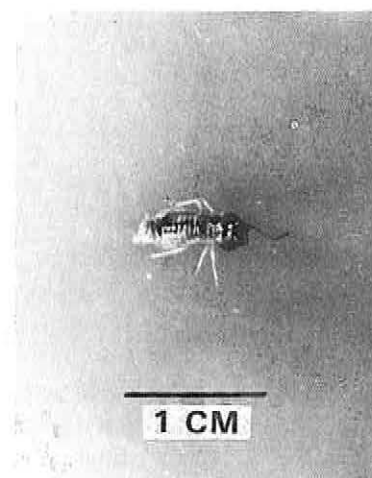


Figure 13. Major worker with ruptured abdomen on malt extract agar on the first day (above) and three days later

LIVESTOCK TICK RESEARCH

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ECOLOGY

Studies on experimental *Rhipicephalus appendiculatus* populations

R. M. Newson, M.P. Cunningham and J. W. Chiera

Work continued on the joint project between ICIPE and the Veterinary Department of the Kenya Agricultural Research Institute at Muguga on three experiments of which details were given in the 1974-77 ICIPE Annual Reports. Two of these experiments were concluded in 1978.

(i) *Duration of East Coast fever infectivity in Rhipicephalus appendiculatus*

The decline and eventual extinction of two *Rhipicephalus appendiculatus* populations that were infected with *Theileria parva*, the causative organism of East Coast fever, was monitored by means of brief introductions of ECF-susceptible cattle to pick up samples of ticks, and by collections of unfed ticks from the vegetation by blanket dragging. These ticks had not been allowed to feed or breed since September 1976.

The *T. parva* infection in the tick population (B) which had earlier been allowed to feed only on immune cattle appeared to have died out during 1977, and further introductions of cattle in 1978 gave the results shown in Table 1; no infections developed when the cattle were subsequently held in quarantine. The tick

population (A) which had continued to carry and transmit virulent ECF during 1977, showed a decrease in the severity of the disease transmitted and it, too, became extinct in 1978 (Table 1).

(ii) *Daily collections of ticks from isolated quadrats*

This experiment started in January 1976. The larvae and nymphs of *R. appendiculatus* died out during 1977 and the daily yield of adults fell to a low level by the end of that year. Daily collecting continued January-April 1978. In fact the total yield was only two adult ticks and there was no upsurge in the activity in rainy season as had been in the two previous years.

The conclusions to be drawn from these experiments are that under the favourable ecological conditions prevailing in ungrazed pasture in this important dairy-ing area of Kenya, disease-carrying adult ticks readily survive for more than one year, with a small proportion remaining for 1½-2 years. Also, the daily observations and collections on the quadrats showed that some of the adults must remain quiescent near the ground for many months before ascending the vegetation to seek a host. Alternatively, they might make more frequent, but brief, trips up the grass so that most avoided detection, but we have no evidence of this.

The results for larvae already take the approximate form of a survivorship curve. Those for the adults, and to some extent the nymphs, are so much affected by seasonal variations in activity that supplementary

Table 1. Numbers of adult *Rhipicephalus appendiculatus* obtained by (i) blanket dragging (means of 10 samples) (ii) mean counts on cattle after 6 days exposure (iii) the East Coast fever responses engendered. Part A previously harboured virulent ECF; part B had the ECF challenge reduced by grazing immune cattle during 1975-1976. In every case 2 cattle were exposed, except December 1977, A(1), B(3)

	A			B		
	(i) per 100m ²	(ii) per host	(iii) ECF	(i) per 100m ²	(ii) per host	(iii) ECF
December 1977	21.0	>1600	1D	27.3	820.7	3N
January 1978	18.9	—	—	2.2	—	—
February	6.7	—	—	1.5	—	—
March	4.5	—	—	2.0	—	—
April	4.1	285.0	1D, 1M	1.3	50.5	2N
May	2.8	29.5	2M	0.1	—	—
June	0	3.5	1M, 1N	0	—	—
July	0	0.0	2N	0	0.5	2N
August	0	0.5	2N	0	0.0	2N
September	0	0.5 ¹	2N	0	0.0 ²	2N
October	0	0.0	2N	0	0.0	2N

D=death; M=mild reaction, recovered; N=no reaction,

1 plus 12 more ticks picked up in an additional 15 days exposure

2 plus 8 more ticks picked up in an additional 15 days exposure

Table 2. Estimated total *Rhipicephalus appendiculatus* population on the cattle from each plot, based on half-body collections. Plot 2 was an unstocked control of 1000m² (mean grass height 550mm)

Plot no.	1	3	4	5	6
Plot size	1000m ²	1000m ²	4,000m ²	4,000m ²	12,000m ²
Mean grass height	54mm	74mm	291mm	371mm	304mm
January 5, 1978	28	1093	160	34	251
February 6, 1978	66	930	155	31	143
March 6, 1978	34	409	112	14	84
April 3, 1978	22	187	111	5	35
May 3, 1978	0	14	39	4	21
June 12, 1978	6	203	12	19	12
July 10, 1978	15	403	4	18	7
August 7, 1978	10	144	3	0	7
September 4, 1978	4	146	13	0	0
October 9, 1978	11	56	14	0	3
November 13, 1978	0	22	16	2	4
December 11, 1978	12	16	22	1	32
Median } 1978	11.5	166.5	19.0	4.5	16.5
Range } 1978	64	1079	157	34	251
Median } 1977	95.0	959.0	301.0	633.0	342.5
Range } 1977	369	3822	2685	3926	658

measurements of survival of ticks in the laboratory, and of confined groups in the field, must now be made.

(iii) *Reproduction and survival of Rhipicephalus appendiculatus at different host stocking densities* Observations and sampling have been maintained since June 1976 and the interpretation of the results needs modification.

From initial introductions of larvae and nymphs tick populations developed which reached peak numbers of adults on the cattle hosts in March-May 1977. The cattle were removed from the two smallest plots during August-December 1977 to allow the grass to recover.

It seemed at first that an annual cycle of numbers might emerge, similar to the normal field situation. However, there is one important difference in our experiments. The tick populations have each fed exclusively on one individual host since the experiment began, whereas a field population would have used several, or many, hosts of differing age and tick experience.

For the first 1½ years of the experiment the ground collections yielded large numbers of larvae and nymphs in recognisable cohorts. A peak of adults in March-May 1977 produced high counts of larvae on the grass in

the second half of the year. These had almost disappeared by the end of the year, due to removal on the host and by natural mortality of those which did not find a host (as observed in experiments (i) and (ii)). However, except in the case of plot no. 3, the larvae were not followed by new cohorts of nymphs and then adults, and therefore very few more larvae have appeared again either. This strongly suggests that those larvae picked up in the late 1977 did not feed successfully on the hosts, leading to severe disruption of the population cycle.

It now appears that each host has become resistant to the tick population that is compelled to feed on it, consequently the numbers of adult ticks have generally decreased throughout the past year (Table 2). The level of numbers per host in 1978 averaged only 8% of that in 1977. On only one plot (no. 3) were the numbers at all comparable to those in 1977.

The rainfall in 1978 was again well above average, and the grass height (Table 2) on the three larger plots (nos. 4-6) varied little throughout the year. In the case of the small plots (nos. 1 and 3) grass heights were similar to those of the other plots when the cattle were re-introduced, but were grazed down to the low state of the previous year by the end of the rainy season in June. There was, in fact, no correlation between habitat conditions and the observed decreases in the tick populations.

Rejection by the bovine host is well-known in the case of the one-host tick *Boophilus microplus*, but has not been seen so clearly before in the case of *R. appendiculatus*, a three-host tick. The implications for possible methods of tick control are obvious and we are preparing a field experiment to amplify these results. We have also begun laboratory observations on the feeding success of *R. appendiculatus* as resistance develops in the host.

The distribution of *Rhipicephalus appendiculatus* ticks on grass stems

J. W. Chiera

Ticks were counted on the grass in a paddock heavily infested with adult *Rhipicephalus appendiculatus* to see if their distribution was random or clumped. A number of transects were randomly laid out and the *Setaria sphacelata* flowerheads occurring along each transect were examined for ticks. In all, 9,019 flowerheads were inspected and 746 ticks counted. The distribution of these ticks on the flowerheads was found to deviate significantly ($P > 0.001$) from an expected Poisson distribution (Table 3), indicating an aggregated distribution. The unfed ticks showed no sexual aggregation, and the proportion of males and females in various combinations showed no significant departure from a binomial distribution, confirming that the associations were random. The total number of females collected (431) was, however, significantly higher than the number of males (315) if the sex ratio was equality. Results from sampling by blanket dragging showed a similar bias in this paddock. It was observed while sampling that the majority of ticks were on old dry flowerheads while very few were on young flowerheads. Some tests were therefore done to investigate the behavioural responses of *R. appendiculatus* to the physical nature of the grass stems.

Tests in the laboratory on the distribution of ticks offered old and young *S. sphacelata* flowerheads revealed that there is a very marked preference for old flowerheads (Table 4). Notice that when offered equal proportions of old and young flowerheads, 88% of the ticks on the flowerheads chose old flowerheads. This would obviously enhance clumping in a field

Table 3. The distribution of *Rhipicephalus appendiculatus* on *Setaria sphacelata* flowerheads compared with a Poisson distribution

	No. of ticks per flowerhead						Total
	0	1	2	3	4	5	
Observed no. of cases	8397	527	74	14	6	1	9019
Expected Poisson	8303.3	686.8	28.4	0.8	0.0	0.0	9019
χ^2	1.06	37.18			150.2		188.74

$P < 0.001$

Table 4. The percentage of *Rhipicephalus appendiculatus* ticks climbing when offered old and young *Setaria sphacelata* flowerheads in the laboratory at 90% R.H. and 24°-26°C

Flowerheads offered	Climbing old	Climbing young	Not climbing
old only	64.5	—	35.5
young only	—	23.3	76.7
old: young=1:1	42.3	5.7	52

containing both types of flowerheads. Young and old flowerheads offered separately to ticks showed no significant degree of clumping, but showed a highly significant degree of clumping when offered together.

Activity behaviour of *Rhipicephalus appendiculatus*

D. K. Punyua

Rhipicephalus appendiculatus, the brown ear tick, is a three-host tick requiring three different hosts to complete its life-cycle. Climbing activity is therefore the prerequisite for the success of the tick in finding a host.

The factors involved in the activity behaviour are being investigated and some of the findings are reported here.

Materials and methods

(i) Daily and seasonal activity behaviour of *Rhipicephalus appendiculatus* adults in the field

One month and six month old ticks were used in this experiment. Ticks were treated to three different levels of hydration, ie. 100%, 85% and 65% in the laboratory, after which they were marked with different colours of fluorescent powder which was sprinkled over them. The ticks were then released in the field in protected plots with 0.25m² wooden frames and a thick layer of 'tangle-foot' smeared at the top edges to prevent ticks escaping. The comparative climbing behaviour of released ticks was made in relation to the prevailing temperature and relative humidity conditions at two-hourly intervals.

The field observations were carried out during the three seasons of the year (hot rainy, hot dry, and cool dry). Open and tree shaded sites of habitat were compared. The rate of water loss/uptake under field conditions was also investigated by weighing groups

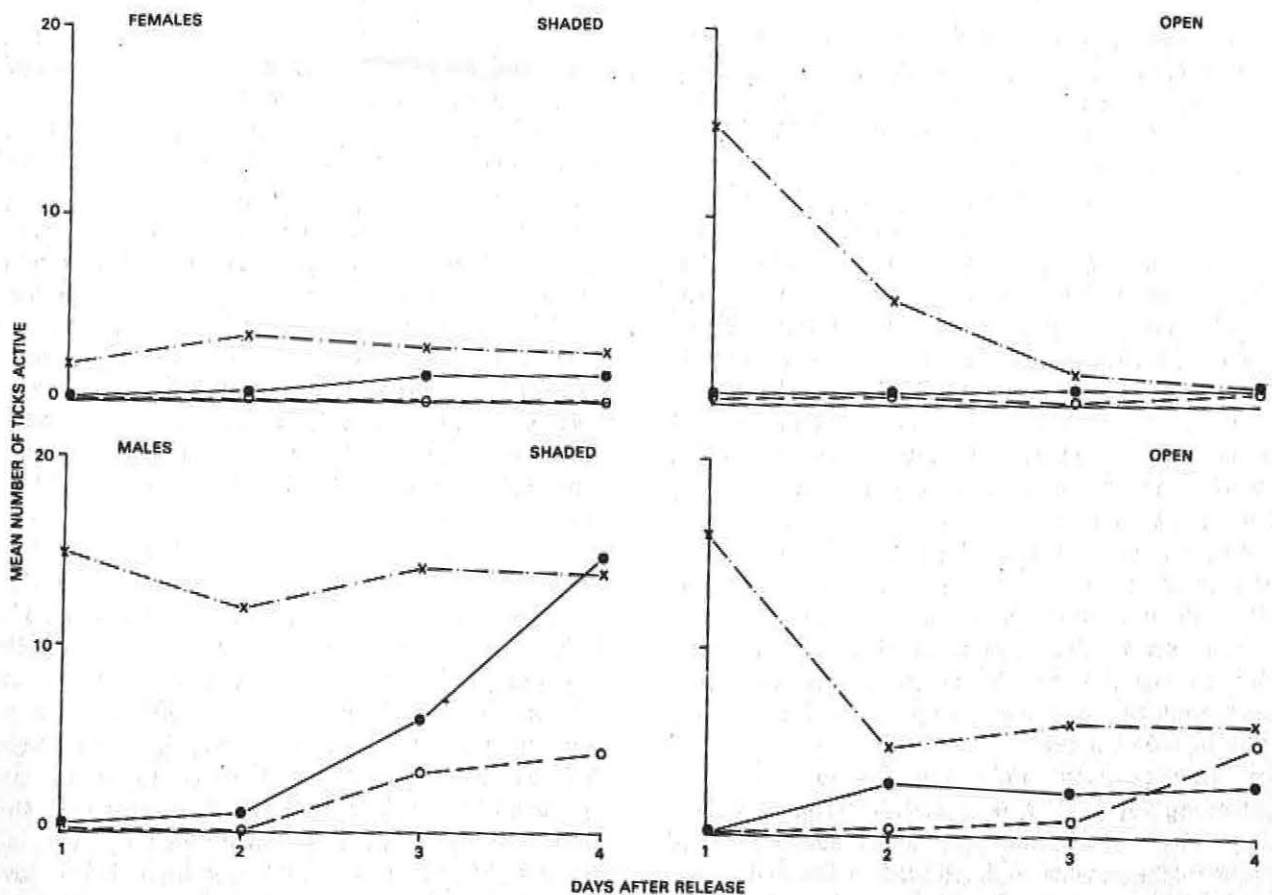


Figure 1. Mean number of males and females *Rhipicephalus appendiculatus* active per day during the cool dry season for 4 days of exposure, in relation to their hydration states. Open and shaded sites were compared for each sex

- 65% hydration
- 85% hydration
- × 100% hydration

Table 5. Analysis of variance on the effect of four factors (season, water content, site and age) on the activity of *Rhipicephalus appendiculatus* adult ticks in the field

Source of variation	df	SS	MS	F
Season	2	455.72	227.86	0.34 NS
Water Content	2	14,612.05	7,306.03	11.04 ***
Site	1	2,898.02	2,898.02	4.38 *
Age	1	14,440.03	14,440.03	21.81 ***
Season X Water Content	4	8,531.62	2,132.91	3.22 *
Season X Site	2	2,799.73	1,399.37	2.11 NS
Season X Age	2	172.72	86.36	0.13 NS
Water content X Site	2	590.74	295.37	0.45 NS
Water Content X Age	2	3,804.06	1,902.03	2.87 NS
Site X Age	1	1,236.70	1,236.70	1.87 NS
Error MS = Residual MS	16	10,591.25	661.95	

*** = $P < 0.001$ ** = $P < 0.01$ * = $P < 0.05$

NS = Not significant

of the exposed ticks, twice daily, in the early morning and late evening.

(ii) *Activity behaviour of partially fed adults of Rhipicephalus appendiculatus*

Theileria parva, which is vectored by the tick *R. appendiculatus*, is transmitted transtadially and not transovarially, (Arthur, 1962). Male ticks are known to remain on the host for a long period (up to three weeks). During this period they first feed and after a certain period detach to look for females, with whom they can copulate repeatedly. (Arthur, 1962, Balashov, 1972). During this period of searching the ticks stand a chance of being brushed off mechanically and drop to the ground. Similarly, due to the immune response of the host, a tick may, feed for some time to detach and fall to the ground. The purpose of this experiment was, therefore, to investigate the possibility of a partially fed tick being dropped off, becoming active again to look for a new host, thus giving the ingested parasite a chance to develop in the tick and being transferred to a new host without the tick necessarily going through any morphological changes.

For this experiment, ticks were fed on rabbits and after feeding for 1, 2, 3, 4, 7 and 14 days, they were forcibly removed from the host, immediately powdered with fluorescent powder and released in the field. Activity was compared between hydrated, unfed ticks and partially fed virgin ticks under the same conditions.

At the end of the experiment, the plots were thoroughly searched, the released ticks recovered again, and the level of their distribution recorded. Two levels of habitat were searched, soil level and grass level.

Results

Daily and seasonal activity behaviour of Rhipicephalus appendiculatus adults in the field

As shown in Figure 1, the most hydrated ticks were immediately active on release to the ground, and could remain highly active for another 2-3 days before the dehydrated groups increased in activity to equal the hydrated group on the 4th day. The level of activity depends on the site. The open site showed a higher, but brief level of activity whilst the tree shaded site showed a lower, but prolonged level of activity.

On a daily basis (Figure 2) it can be seen that the open site had a brief high peak of activity between 1230 hours and 1430 hours, whilst the shaded site had a prolonged lower level of activity between 1230 hours and 1630 hours.

Although the humidity on average was the same for both sites, the temperature readings were different, with the open site reaching its maximum of 30°C at 1430, corresponding with the drop in tick activity at this site, while the shaded site never exceeded 20°C.

When all these factors were subjected to a four-way analysis of variance (Table 5) it can be seen that water content of the tick, or hydration status, and age of the tick were highly significant ($P \leq 0.001$) while sites also appear to be important, being significantly different ($P \leq 0.05$). Seasonal differences did not appear to be important, but this may be due to the fact that the experiments were designed for 3 days only, which is not enough to see the effect of different seasons on tick behaviour, although they had some slight effect on the hydration status of the tick when their interaction was tested, ($P \leq 0.05$).

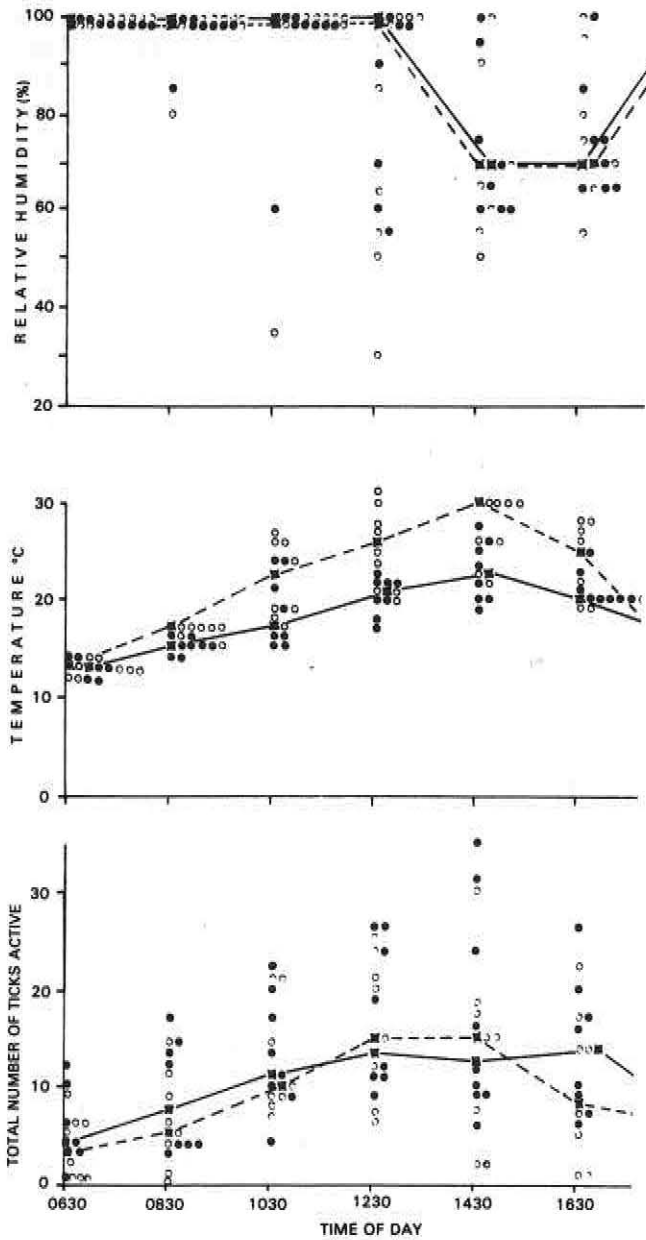


Figure 2. Daily distribution of activity of *Rhipicephalus appendiculatus* adults in relation to daily distribution of air temperature and relative humidity in the field during the rainy season

—x— median number of observations at each time of day
 ● open site
 ○ shaded site

When ticks were exposed in the field it was shown that they were taking up water at night and losing it during the day. It was however, shown that the ticks were able to replenish water lost during the day, at night during the wet seasons, but unable to recover it during the dry seasons. Thus during the dry seasons, there is more water loss than uptake and the reverse is the case during the wet seasons.

It was also observed that there were more ticks on vegetation during the rainy seasons, while during the dry seasons most ticks were down at soil level.

In summary the following can be concluded. A tick has got to be hydrated to a certain level (probably to full hydration) before it can be responsive to the optimal temperature for climbing, which appears to be somewhere in the range of 20°C and 30°C. Thus, temperature seems to be the cue for tick activity. Older ticks are more active than younger ticks.

Due to poor replenishment of water during the dry seasons, resulting in lower levels of hydration, ticks may follow the humidity gradient in the vegetation until they find the most favourable levels of habitat, in most cases at soil level during the dry seasons. This level, however, becomes saturated during the wet season and with temperatures reaching the optimum, ticks are made to leave soil level and move upwards, resulting in the increase in tick activity, hence, an increase of tick incidence on their hosts.

Table 6. Comparison between fed and unfed males and females *Rhipicephalus appendiculatus* released in the field for 3 days mean percentage of ticks active each day

DAYS	MALES						FEMALES					
	ON	DAY 1		DAY 2		DAY 3		DAY 1		DAY 2		DAY 3
HOST	FED	UNFED	FED	UNFED	FED	UNFED	FED	UNFED	FED	UNFED	FED	UNFED
1	27.0	9.0	9.7	5.7	10.0	1.7	24.7	16.0	33.3	7.0	20.3	4.7
2	40.0	1.3	29.3	0.7	32.3	1.0	4.3	13.0	8.0	15.0	9.0	12.0
3	30.0	3.0	15.0	1.3	21.7	4.3	0.3	11.7	0.0	12.3	0.0	8.7
4	52.0	13.3	37.3	9.0	30.7	13.7	0.0	20.3	0.0	18.0	0.0	19.3
7	37.3	4.3	30.3	2.0	29.7	10.0	0.0	27.7	0.0	25.0	0.0	14.3
14	25.0	6.0	12.0	3.7	18.3	2.7	—	—	—	—	—	—

Activity behaviour of partially fed adults of Rhipicephalus appendiculatus

The released, partially fed ticks showed a higher degree of activity than those unfed (Table 6). This was especially evident in males when all of the groups showed very high numbers of active ticks in the 1 day fed group, and the rest of the groups showed either no activity at all or only a slight activity as in the case of 2 day fed ticks. The level of activity was highest however, on the first day of release but decreased on subsequent days.

On recovery, most of partially fed males were found in the vegetation whilst unfed ticks were mostly found at soil level. One and 2 day fed and the unfed were mostly found in the vegetation. The 3, 4 and 7 day fed females were found mostly at soil level, while unfed ticks were located mostly at vegetation level.

PHYSIOLOGY

Aggregation—attachment pheromones in *Amblyomma eburneum* from the Kenya Coast

F. D. Obenchain and R. Ojowa

In a number of *Amblyomma* species, including *A. variegatum* and *A. gemma* which are also found in Kenya, males which have fed and matured sperm are known to produce a pheromone(s) which induces unfed females to aggregate and attach to a host in the immediate vicinity of the fed male. Previous experiments have shown that the pheromone(s) extracted from *A. hebraeum* males which had fed for 10 days did not significantly induce the attachment of *A. variegatum* or *A. gemma* females. Nevertheless, when the unfed females of either of these two species were placed with fed males of the other species in a scrotal bag bioassay (cattle hosts) they showed significantly increased attachment rates as compared to the controls (1977 ICIPE Annual Report). Indeed, when the females of these species had an opportunity to choose between fed males of both species, they did not appear to be able to discriminate between the males of their own or the other species under the conditions of the scrotal bag bioassay. Those data brought into question an earlier hypothesis that the pheromone(s) of the *Amblyomma* aggregation—attachment system was species specific.

The experiments reported here were performed with the offspring of a single engorged *A. eburneum* female collected from the Shimba Hills on the Kenyan coast in early 1978. Larvae and nymphs were successfully reared on chicken hosts at the Chiromo laboratory and adult stages were tested for the presence of the male produced aggregation—attachment pheromone mechanism. Bioassays of males were performed in stockinette—sleeve arenas (7.6cm diameter) glued to

the shaved backs of laboratory rabbits. Adult males were introduced to the arenas, allowed to attach and to feed for 2 or 10 days before the introduction of females. Control bioassays were performed in the absence of males. The attachments of *A. eburneum* females to males of *A. variegatum* are summarized in Table 7. In all cases where attachment was observed, the females were never more than 1cm from experimental males.

These initial data demonstrated that the aggregation—attachment mechanism is present in *A. eburneum* ticks and also show that unfed females of *A. eburneum* are able to discriminate between males of their own species and males of *A. variegatum* which are known to be producing their own pheromone(s). The data support the existence of species specificity within this pheromone system.

Females of *A. eburneum* and *A. variegatum* were carefully observed in order to determine the parameters of their behavioural responses to pheromone producing males. Under the condition of the bioassay, the first female appeared to reach the male by chance as a result of increased, but non-directed locomotor activity, probably induced by host proximity. Upon contact with the male, the first female to arrive immediately assumed a venter to venter orientation to the male and attachment to the host began within 2 seconds of contact. Other females arriving later showed increasingly prolonged periods of exploratory behaviour in the vicinity of the fed male. These females (2–4) usually attached with their venters orientated toward the male. While these data do not indicate the actual sites of pheromone release by males, they seem to support the hypothesis that detection of the active components of the pheromone(s) may only occur over short distances. Further studies on *Amblyomma* pheromones are planned in order to determine their actual nature and potential for control.

Table 7. Pheromone induced inter- and intraspecific attachment responses of unfed female *Amblyomma eburneum*

Ticks tested for response	Ticks tested for pheromone activity	Successful attachment responses (24h)
Male <i>A. eburneum</i>	None	4 of 5
Female <i>A. eburneum</i>	None	0 of 5
" " "	Male <i>A. eburneum</i> , 2df	0 of 5
" " "	" " " , 10df	4 of 5
Female <i>A. eburneum</i>	Male <i>A. variegatum</i> , 10df	0 of 5
Female <i>A. variegatum</i>	" " " " "	5 of 5

2df= 2day fed, 10df= 10day fed

Moulting hormone activity in adult females of the tick *Ornithodoros porcinus porcinus*

C. K. A. Mango, L. Moreka and A. Bwire

Note. Disregard actual quantifications of ecdysone titres—*Musca* bioassay data has been misinterpreted.

In previous experiments we determined the changing titres of moulting hormone activity as related to the moulting cycle of blood fed nymphs of the soft tick *Ornithodoros porcinus porcinus* Walton, 1962 (previously recognized in ICIPE Annual Reports as *Ornithodoros moubata* Murray, 1877). These titre changes were determined by the use of the *Musca* bioassay. Similar procedures were followed to investigate the presence of moulting hormone activity in the haemolymph of adult females which had fed *in vitro* on defibrinated pig blood (and could be expected to lay eggs after mating) and in the haemolymph of adult females which had fed *in vitro* on pig blood containing 4 µg/ml of added beta-ecdysone (and could be expected to supermolt). Following the blood meal, half of the ticks from each feeding regimen were placed with males and the other half were placed individually in test tubes. Beginning on day 1 post-feeding and on every day thereafter until day 14 post-feeding, 100 µl of haemolymph was collected from no fewer than five females and extracted in 1 ml of 10% ethanol. Aliquots of 2 µl of extract were injected into ligated *Musca* larvae and their response was converted into µg equivalents of beta-ecdysone per µl of tick haemolymph by use of the reference dose response curve.

The results of the *Musca* bioassays for moulting hormone activity in the haemolymph of *O. p. porcinus* females in the development period following a blood meal are shown in Figure 3. In mated females fed on defibrinated pig blood the activity fluctuated between 1×10^{-8} and 1×10^{-6} µg equivalents of beta-ecdysone per µl of tick haemolymph, with a peak of activity between days 5 and 8 post-feeding. Unmated females

showed similar levels of activity but with a more erratic pattern of fluctuations. The activity titres of putative moulting hormone in the haemolymph of these female ticks is equivalent to the concentrations of ecdysteroids observed in adult female *Pisaura mirabilis* spiders with the radio-immunoassay by Bonaric and DeReggi (1977, Experientia, 33 1664–1665). The peak of moulting hormone activity in the mated females was coincident with the onset of vitellogenic uptake

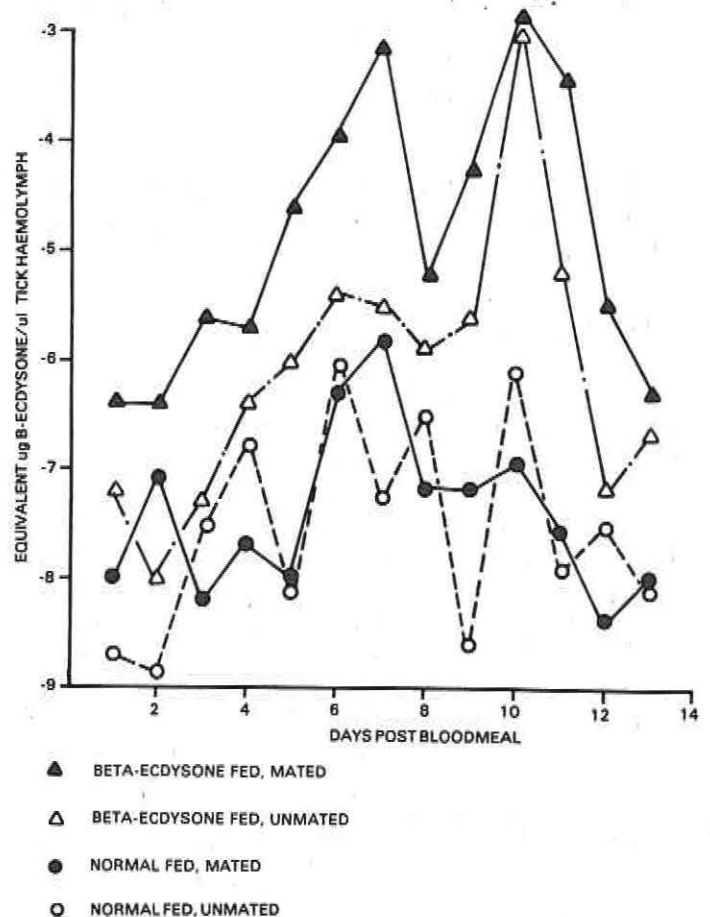


Figure 3. *Musca* bioassay determinations of the changing titres of moulting hormone activity as related to oogenesis or supermoulting in adult female *Ornithodoros p. porcinus*

(Balashov's growth stage III) by developing eggs in their ovaries.

Ticks fed on defibrinated pig blood containing 4 µg/µl of added beta-ecdysone showed substantially elevated titres of moulting hormone activity. Both mated and unmated females in this feeding group showed two peaks of activity. The first peak between days 5 and 8 was higher in mated than in unmated ticks and corresponded to the activity peak of normally fed and mated females. Since ovarian development and vitellogenesis begins in these ticks (even though the developed eggs will be resorbed during supermoulting) this first peak may be related to the initiation of some stage of ovarian maturation, although its titre is considerably higher than in normally fed and mated females. The second peak in moulting activity in both mated and unmated beta-ecdysone fed ticks exceeds 1×10^3 µg/µl equivalents of beta-ecdysone. This resembles the pre-ecdysial activity titres of fifth stage nymphs just prior to their moult as we have recorded recently. Radio-immunoassays of last stage nymphal *Pisaura* spiders show peak titres of ecdysoteriods at this level just prior to their adult moult. The ecdysone fed females in this set of experiments began to moult on day 14 post-feeding and it appears that titres on the order of 1×10^{-3} µg/µl equivalents of beta-ecdysone are necessary for the coordination of events in the last few days preceding ecdysis in nymphs and supermoulting *O. p. porcinus* ticks and in other normally moulting arachnids as well.

Precocene induced moult inhibition in nymphal *Ornithodoros porcinus porcinus* and its antagonism by beta-ecdysone

F. D. Obenchain, C. K. A. Mango and A. Bwire

It has been previously demonstrated that prolonged exposures of post-fed nymphs of ixodid and argasid ticks to the vapours of precocene-2 will induce a substantial and long term inhibition of moulting (Leahy and Booth, personal communication). Similar results were reported for insects exposed to high doses of the compound in the original paper describing the effects of precocene on insect development, but it is still not known if the inhibition of moulting is related to the demonstrated anti-allatal effects of the compound. In previous experiments in this laboratory it was shown that the pre-moulting periods of fed nymphal *Ornithodoros porcinus porcinus* could be significantly shortened by the incorporation of 4 µg/ml of added beta-ecdysone to the blood meal of *in vitro* fed ticks. In light of the above information a series of experiments were performed on replicates of 50 nymphal ticks to determine whether feeding on blood containing beta-ecdysone would substantially alter the subsequent

inhibition of moulting of nymphal ticks exposed for various periods of time to the vapours of 2mg of precocene-2 applied in acetone to the covers of 6mm petri dishes. All precocene exposures began immediately post-feeding.

In general, nymphal ticks of all instars except the 5th showed substantial delays in moulting following exposure to precocene-2 vapours for periods ranging from 3 to 64 hours. Longer exposure times were associated with both increased mortality and increased pre-moult periods, but increasingly longer exposure times were also needed for induction of similar levels of moult inhibition in successively older nymphal instars. The responses of 4th stage nymphs to increasing periods of precocene-2 exposure are plotted in Figure 4.

The effects of 4 µg/ml of beta-ecdysone added to the *in vitro* blood meal of 4th instar nymphs, together with the opposite effects of different periods of exposure to the vapours of precocene-2, are plotted in Figure 5. By comparison to Figure 4 it can be seen that the non-precocene treated nymphs moulted much more rapidly when beta-ecdysone was incorporated into the blood meal. This acceleration of moulting appears to be relatively unaffected by shorter periods of exposure to precocene-2, but after longer periods a large fraction of the precocene treated ticks began to show prolonged premoulting periods.

Observations on the effects of precocene treatment on precocious adult development and sterility have not been completed, but the increased moulting success of precocene treated ticks previously fed on blood with added beta-ecdysone has provided considerably more material for such observations.

The processing of lipovitellins in the brown ear tick *Rhipicephalus appendiculatus*

F. D. Obenchain, T. S. Dhadialla, Z. Ahmad and R. Ojowa

This study reports the presence of two major storage lipo-haemo-proteins from the eggs of *Rhipicephalus appendiculatus* ticks as determined by disc electrophoresis. In all respects, these proteins appear similar to those reported by other workers for such ticks as *Dermacentor andersoni*. Some other workers have also used immunochemical techniques to demonstrate the presence of circulating proteins in the haemolymph of engorged female ticks which are antigenically related to the storage proteins of the eggs, but there is no evidence of the roles of tissues outside the ovary in the conversion of blood meal proteins to lipovitellins. Until recently the midgut epithelium was considered the most likely source of lipovitellin-like proteins which occur in the haemolymph. Obenchain and Oliver (1973, *J. Exp. Zoology*, 186 217-236), however, showed

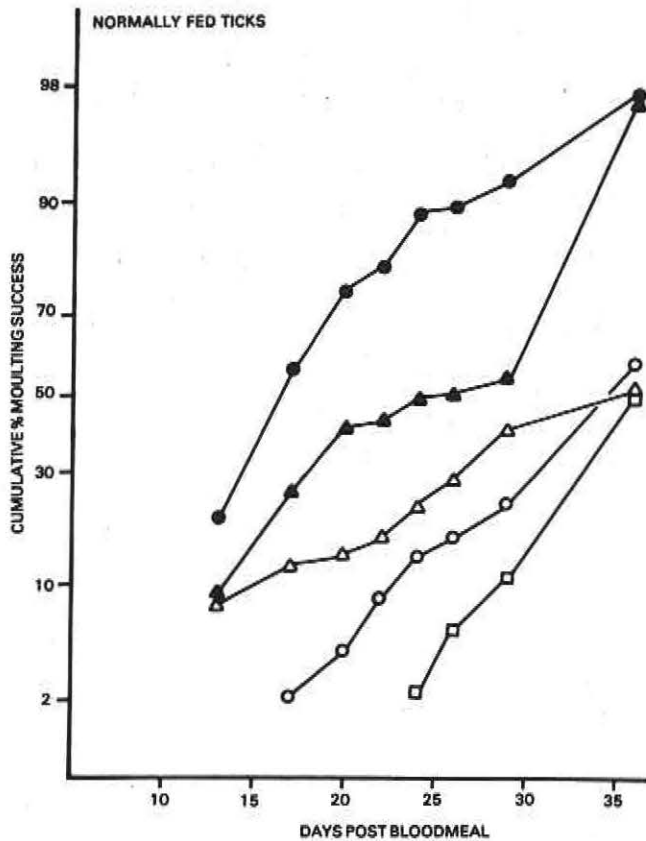


Figure 4. The effects of increasing exposures to the vapours of 2 mg of precocene-2 on moult inhibition in normally fed 4th instar nymphal *Ornithodoros p. porcinus*

that ticks, like insects, have fat body tissues. In ixodid ticks the fat body undergoes a cycle of differentiation during the period of adult engorgement and, after the tick drops from the host, individual fat body cells show a marked hypertrophy just before the peak of oogenesis. Fine structural observations also show that the trophocytic fat body cell is rich in endoplasmic reticulum, Golgi complex and mitochondria at this time. These observations strongly implicate the tick fat body in the synthesis or processing of lipovitellin or its precursors. This study further reports the results of studies using immuno-diffusion, immuno-electrophoretic and indirect immuno-fluorescent techniques on the aqueous extracts of various tissues or the tissues themselves, on the presence of proteins which are antigenically related to tick lipovitellin in the midgut epithelium, haemolymph, fat body, Gene's organ, central nervous system, developing ovary and mature eggs of *R. appendiculatus*.

The two lipo-haemoproteins which constitute the major storage proteins were separated from freshly laid eggs by ammonium sulphate fractionation and chromatographic separation on Sephadex G-200. Fractions with significant absorbances at 278 and 398 nm were pooled and used for antibody preparation. The rabbit antisera were raised by intramuscular injections of 0.6mg of protein solution (1 mg/ml) emulsified in

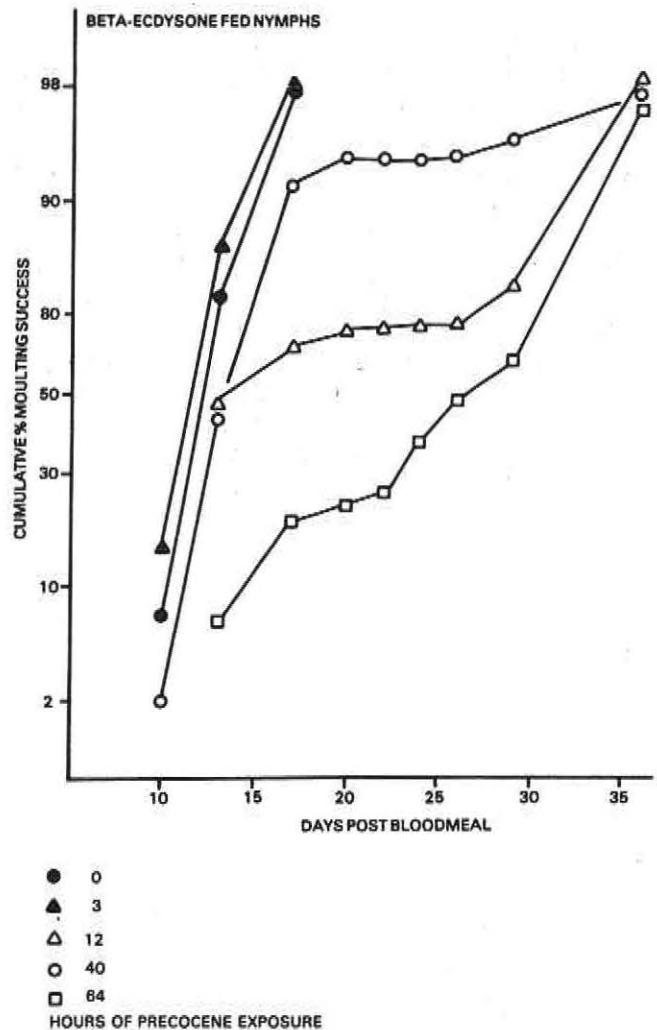


Figure 5. The effects of increasing exposures to the vapours of 2 mg of precocene-2 on moult inhibition in 4th instar *Ornithodoros p. porcinus* fed on pig blood with 4 µg/ml added beta-ecdysone

0.6ml of Freund's complete adjuvant, with booster injections of 0.6mg of protein solution in 0.6ml of incomplete adjuvant three weeks later. Rabbit antisera was used in Ouchterlony double diffusion analyses, immunoelectrophoretic and rocket immunoelectrophoretic analyses of tissue extracts. Additionally, frozen sections of candidate tissues from cattle fed ticks were incubated with the rabbit antisera, washed and re-incubated with goat anti-rabbit sera for indirect immunofluorescent demonstration of the tissue localization of lipovitellins.

The results of the Ouchterlony experiments demonstrated the strong immunological identity of proteins in the aqueous extracts of eggs and tick ovary to the standard lipovitelline preparation. Proteins in the tick haemolymph and fat body also showed strong partial identities. Extracts of the midgut epithelium gave extremely weak partial identity reactions, while there were no antigen-antibody reactions between the rabbit antisera and the extracts of the midgut contents, Gene's organs or the central nervous system. Electro-

phoretic experiments showed that the major immunologically similar proteins of the eggs and ovary extracts had the same mobilities as the lipovitellin standard protein mixture. The reactive proteins from the haemolymph and fat body extracts, however, had lower but similar electrophoretic mobilities. The indirect immunofluorescence studies showed the localization of lipovitellin or immunologically related proteins only in the developing oocytes of the ovary, in the trophocytic cells of the fat body and slight reactions in the external extracellular spaces of the midgut epithelium. The latter observation may be due to immunological reaction with haemolymph proteins contained within those extracellular spaces. While these observations do not rule out the involvement of the midgut

epithelium in rapid turnover synthesis and release of lipo-haemoproteins it seems more likely that the tick fat body is a major site of synthesis and or processing of lipovitellogenic proteins which will be eventually sequestered and stored in the developing oocyte.

Other experiments have shown that proteins in the crude homogenates of eggs from three other Kenyan *Rhipicephalus* species and from *Boophilus decoloratus* show strong partial immunological identity with the standard lipo-haemoproteins of *R. appendiculatus*. Weak partial immunological identities were also observed with proteins from the eggs of *Haemaphysalis leachi* and *Hyalomma truncatum*, but no reactions were observed with crude extracts of eggs from two *Amblyomma* species or from *Ornithodoros p. porcinus*.

TSETSE RESEARCH

REPRODUCTIVE PHYSIOLOGY

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Tsetse flies reproduce by adenotrophic viviparity, giving birth at regular intervals to fully developed larva nourished within the mother by a secretion from modified accessory glands. Following larviposition the larva pupariates almost immediately without further feeding. Cyclical events of the reproductive cycle such as egg maturation, ovulation, larval development, milk gland activity and larviposition are regulated with marked accuracy by the female tsetse fly. Earlier work from this laboratory has shown that some of these events are under hormonal control. The present report describes some studies on the control of egg maturation and further observation on the regulation of ovulation. In addition, findings in relation to possible mechanism of the regulation of larviposition and abortion are reported. *Glossina morsitans* was used in all experiments.

Control of egg maturation

Each of the paired ovaries of the tsetse fly contains two polytrophic ovarioles. Only one oocyte is matured per pregnancy cycle and thus only one oocyte enters and completes vitellogenesis at any one time. The oocytes undergo sequential development in which the first oocyte is matured in one ovariole of the right ovary, the second oocyte is matured in one ovariole of the left ovary, the third is matured in the alternate ovariole of the right ovary and the fourth is matured in the alternate ovariole of the left ovary, repeating the cycle from the next oocyte. Oocyte maturation continues in virgin females although at a slower rate.

In *G. morsitans morsitans* the process of vitellogenesis begins about 5–6 days before the eclosion of the adult female. The corpus allatum (CA) has been found to be essential for the completion of egg maturation in many insects. However, the role of CA in the egg maturation in the tsetse fly does not seem to be well defined. Females allatectomized at 12h following emergence (before or after blood meal) larvipos-

sited for 4–5 cycles before revealing under-developed or abnormally developed oocytes. Females allatectomized 10 min after emergence produce at least one larva, the second egg being only partially developed. Preliminary experiments combining allatectomy and CA-reimplantation in virgin females seems to produce at least two eggs in 22 days in comparison with only one egg in the allatectomized ones during the same (or a longer) period. Although oocyte maturation continues for some time in the allatectomized female, the surgery appears to slow down the process of vitellogenesis significantly.

Effect of Precocene II on CA and on egg maturation in the tsetse fly was also studied. Various doses of Precocene II was applied to freshly deposited larvae as well as pregnant females. Results of these experiments indicate that Precocene II did not adversely affect the egg maturation of the treated individuals or their offspring. Histological examination of the CA tissue from a few of the treated females revealed no abnormality.

Regulation of ovulation

The female tsetse fly will ovulate only after she has successfully mated. Results of our previous experiments suggest that the act of mating triggers ovulation by stimulating the female to release a neurohormone from the brain that is conveyed by the haemolymph to its target organ, the ovary.

Results of our recent experiments show that ovulation can be induced in virgin mature females with dibutyl cyclic AMP, cholera toxin (a cyclic AMP generator) or a phosphodiesterase inhibitor. The dibutyl cyclic AMP (25µg/fly) caused 55% of the virgin females to ovulate. Ovulation was induced in 45% of the females injected with aminophylline (10µg/fly), a phosphodiesterase inhibitor, possibly by preventing the degradation of cyclic nucleotides and thus elevating endogenous levels of cyclic AMP. A role of cyclic AMP in

tsetse ovulation is also suggested by the effectiveness of cholera toxin. Cholera toxin, a potent stimulant of adenylate cyclase in insects as well as in vertebrates caused 33% females to ovulate within a 24-hour test period. Although we cannot eliminate the possibility that cyclic AMP exerts its effect by stimulating the release of ovulation-stimulating hormone from the brain, it is more likely that the ovulation stimulating hormone, like many other neurohormones, uses cyclic AMP as a second messenger in triggering its response within the ovary.

Control of parturition and abortion

Starvation of the pregnant female leads to abortion, as does application of juvenile hormone analogues (JHA) to the pregnant females. In such cases expulsion of the uterine contents is probably controlled by the female fly. It is not known as to what extent the fully developed larva plays a part in normal deposition of larva. We have been studying the role of maternal endocrine system in the mechanism of parturition. Our preliminary results show that surgical removal of neurohemal organ (aorta and corpus cardiacum along with the CA) following ovulation in many cases (46%) prevents the larva from being deposited which pupariates within the uterus, eventually killing the mother. The results indicate that the brain neurosecretory cells release a parturition factor which is responsible for larviposition activity in the uterus. Moreover, larviposition can be induced in decapitated pregnant females by extracts of pars intercerebralis. Intact females in which the abdominal nerves have been

severed do not respond to the injection of extract. From these results it appears that the release of neurosecretion from the pars intercerebralis brings about the parturition or premature parturition, but that nervous factors are also important.

In our experiments with cyclic AMP, we noted that in several virgin females eggs were not only released into the uterus but also expelled from the uterine following injection of either cholera toxin or dibutyryl cyclic AMP. Moreover, pregnant females injected with 0.5µg cholera toxin during the second pregnancy cycle had a high incidence of premature parturition: 57% of the females aborted within 2 days. An injection of 25µg dibutyryl cyclic AMP caused 37% abortion. Our preliminary evidence thus suggests that elevation of cyclic AMP may be involved in parturition as well as ovulation in tsetse flies, where a neurohormone uses cyclic AMP as a second messenger in triggering its response within the uterus.

If we consider the neurosecretion to be responsible for ovulation, parturition and abortion, we must assume that the following conditions are fulfilled to cause the release of the neurosecretion resulting in the appropriate event:

- (i) The female must be mated, *and*
- (ii) A mature egg must be present (for ovulation and parturition) or
- (iii) An appropriate external factor must be applied on the pregnant female to create a "stress" in order to release neurosecretion (for abortion).

SALIVARY GLAND PHYSIOLOGY

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Morphogenesis of *Trypanosoma brucei* in the presence of crude tsetse midgut contents

L. H. Otieno, M. Aruwa and N. Darji

It has been known for some time that bloodstream *Trypanosoma brucei* placed into suitable media *in vitro* transform and multiply as culture stages morphologically identified with some forms characteristically observed in the insect vector. The factors which trigger these changes have been sought for many years but have so far remained elusive. In this brief summary of the year's work, attention has been focused upon the tsetse midgut contents and the part it may play in the transformation process.

Three *T. brucei* isolates (EATRO 999, 1416 and 1969) were used. Bloodstream form trypanosomes from mice infected with the organisms were separated from mouse blood by means of DEAE-52 cellulose anion-exchanger method according to Lanham and Godfrey 1970. The eluted trypanosomes were washed six times in phosphate buffered saline glucose and finally suspended in the same medium at 0°C.

Trypanosomes suspensions prepared in this way when mixed with crude midgut contents from *Glossina morsitans* and incubated at room temperature for 30 min transformed into forms morphologically resembling those characteristically found in the insect vector. Transformation took place within 10 min. Examination of stained preparations by light microscopy confirmed that many bloodstream form trypanosomes had indeed transformed into forms morphologically indistinguishable from insect vector forms. Examination of transformed trypanosomes under EM revealed that many of these trypanosomes had lost their surface coating. (Figure 1).

The transformation was found to be affected within 5 minutes and prolonging the incubation period for up to 3 hours did not appreciably increase the number

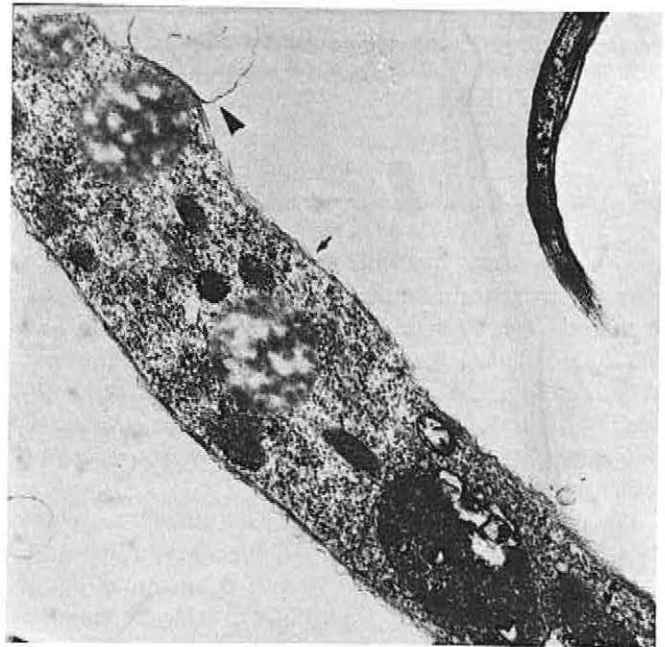


Figure 1. TEM section of a bloodstream form of *Trypanosoma brucei* undergoing transformation into insect vector form. Thick arrow shows surface coat peeling off. Thin arrow shows a denuded surface

of trypanosomes transforming into the insect forms. Transformation, however, is not induced by incubating the mixture (trypanosome/tsetse midgut contents) at 56°C.

Infectivity tests carried out on the transformed trypanosomes revealed that, although up to 91% transformation was observed within the short time of 5 minutes, these organisms appeared not to have lost their infectivity to mice. However, big decline (630-fold) in the number of infective organisms was recorded after 30 minutes incubation (Table 1). This observation indicated that morphological change preceded physiological change.

Six proteolytic enzymes (Gooding and Rolseth, 1976) have previously been isolated from the digestive

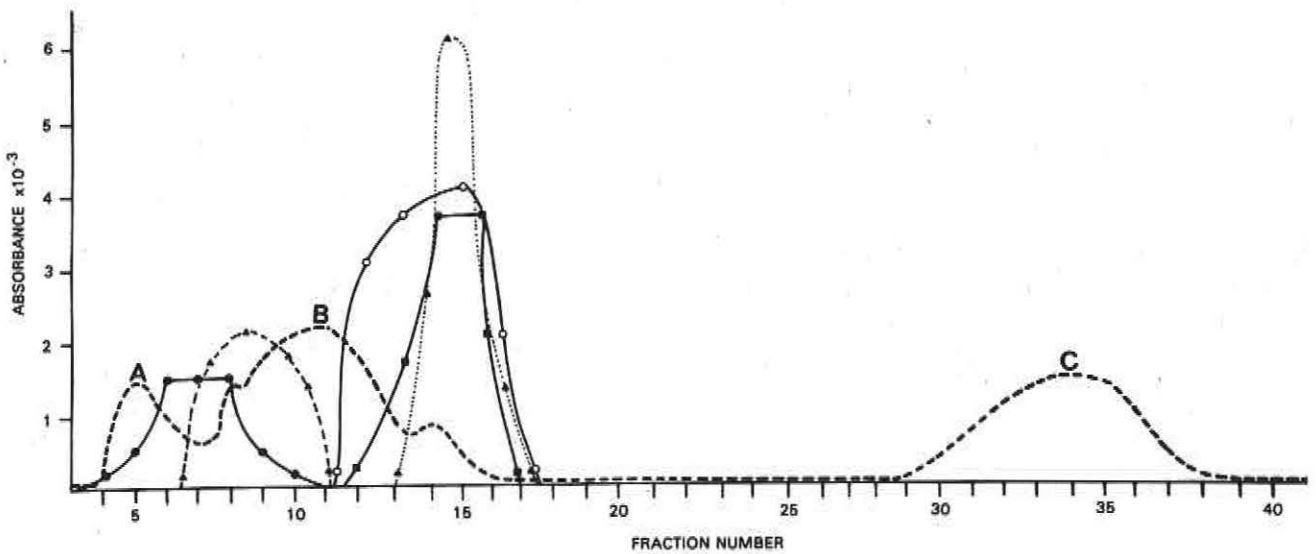


Figure 2. Sephadex G-100 chromatography of midgut homogenates from female *Glossina morsitans*. Protein (A_{280}) is shown by the plain curve. The absorbance due to enzyme activities at the respective wavelengths are:

- AP
- ▲ Trypsin/Proteinase VI
- CPA
- CPB

The fractions were pooled as follows: Fraction 1, tubes 1-7; fraction 2, tubes 8-10; fraction 3, tubes 11-17; fraction 4, tubes 29-38

portion of adult *G. morsitans* midgut. These were identified as trypsin, carboxypeptidase A and B, aminopeptidase a trypsin-like enzyme designated 'proteinase vi' and chymotrypsin-like enzyme designated 'proteinase vii'. In our study, these enzymes were isolated from 24 hour old female *G. morsitans*, their activities examined and later tested for possible effects on trypanosome transformation.

The protein pattern obtained for the effluent fractions (A_{280}) is shown in Figure 2. All specific enzyme activities were present in peaks A and B and none could be detected in peak C. Caseinolytic activity similarly was observed in peaks A and B and not in C. The fractions having the highest activities for each enzyme were pooled and each pool tested for the various enzymes. The results are summarized in Table 2. There

was considerable overlap. CPB was demonstrated in fraction 3, which had the highest activities $2.1667 \mu\text{moles}/\text{min}/\text{mg}$ protein. Trypsin/proteinase vi was present in two of the fractions designated 1 and 3, being higher in the latter ($4.727 \times 10^{-1} \mu\text{moles}/\text{min}/\text{mg}$ protein). CPA was also demonstrated in fraction 3 with activity of $6.429 \times 10^{-1} \mu\text{moles}/\text{min}/\text{mg}$ protein. Aminopeptidase was found in fraction 1 1.277×10^{-1} and $5.450 \times 10^{-2} \mu\text{moles}/\text{min}/\text{mg}$ protein respectively. Proteinase vii could not be detected in any of the fractions. When blood stream form trypanosomes were incubated with aliquots from the three peaks A, B and C, no transformation was observed in any of these fractions. Commercially prepared trypsin and pronase were also tested on these trypanosomes and in both cases there was no transformation.

Table 1. Infectivity titrations of bloodstream form *Trypanosoma (T.) brucei* after incubation with *Glossina morsitans* midgut contents. The samples were tested 5 and 30 minutes after incubation

Method of enumeration	Incubation period (min)	Control				Test Samples				
		BSF	MF	Total	Infective Dose ID63/ml	BSF	MF	Total	% Transformed	Infective Dose ID63/ml
Haemocytometer tryps/ml	5	2.50×10^5	—	2.50×10^5	6.6 ± 0.3	6.25×10^4	2.00×10^6	2.50×10^6	80.0	6.8 ± 0.3
	30	2.50×10^5	—	2.50×10^5	6.8 ± 0.3	6.25×10^4	1.50×10^6	1.75×10^6	85.7	4.0 ± 0.3
Stained preparations 20 (fields)	5	41	—	41		5	52	57	91.2	
	30	35	—	35		11	65	76	85.5	

BSF — Bloodstream forms
MF — Transformed trypanosomes

Table 2. Enzyme activities in pools of the various fractions of midgut contents from *Glossina morsitans* after DEAE chromatography

Fraction	1	2	3
Protein Content	0.005 mg/ml	0.068 mg/ml	0.010 mg/ml
Trypsin/Proteinase VI (BAPNA)	1.182×10^{-1}	0.00	4.727×10^{-1}
CPB (HA)	0.00	0.00	2.1667
CPA (HP)	0.00	0.00	6.429×10^{-1}
AP(LpNA)	1.227×10^{-1}	5.450×10^{-3}	0.00
Proteinase VII (ATEE)	0.00	0.00	0.00

Specific activities are expressed in $\mu\text{moles/minute/mg protein}$

Initial studies on the establishment of *Trypanosoma brucei* in the tsetse fly, *Glossina morsitans morsitans*

R. M. W. Vundla, L. H. Otieno and E. Mpanga

Following the establishment of proteolytic enzyme levels in newly emerged female flies, the effect of trypanosomes on the enzyme activities *in vitro* and *in vivo* have been studied. Two inhibitors, soy bean trypsin inhibitor (STI) and 1-chloro-3-tosyl amido-4-phenyl-2-butanone (TPCK) have been shown to have no effects on the respective enzymes *in vivo*, at concentrations of up to 10 micrograms/millilitre (10 $\mu\text{g/ml}$).

The work on proteolytic enzyme levels at different time intervals after feeding (reported in the Annual Research Conference 1978) was continued in order to obtain more consistent results, especially with regard to trypsin (EC.3.4.4.4.), and also to establish enzyme levels in unfed, newly emerged flies.

The results obtained in these studies are summarized in Table 3. Proteinase VII, was shown to be present in unfed flies. This enzyme has so far not been detected in fed flies.

Following the standardization of the proteolytic enzyme activity of the gut, an attempt is being made to: (i) Investigate the effect(s) of trypanosomes on the activities of the proteolytic enzymes (ie. to see if ingested trypanosomes affect the host fly in any way) by determining enzyme activities after incubation with trypanosomes both *in vitro* and *in vivo*.

ii) Effect(s) of the tsetse proteolytic enzymes on the establishment of the trypanosomes in the fly. To date, two inhibitors, STI, an inhibitor specific for trypsin and TPCK have been investigated.

For the *in vitro* experiments, trypanosomes (*T. brucei*) (strain 1969) were separated from infected mouse blood at peak parasitemia by the method of Lanham (1972). The washed trypanosomes were incubated with tse. tse gut homogenates (diluted 10 times) for 1, 2 and 3

Table 3. Proteolytic enzyme activity ($\mu\text{moles/gut}$) in tsetse midguts at 4 and 24 hours after feeding, compared to unfed flies

ENZYME	UNFED FLIES	FED FLIES—HOURS AFTER FEEDING	
		4	24
Carboxypeptidase A	0.1600 ± 0.07 (n=6)	0.2700 ± 0.15 (n=3)	0.0420 ± 0.19 (n=4)
Carboxypeptidase B	0.1500 ± 0.05 (n=9)	0.1000 ± 0.03 (n=3)	0.3700 ± 0.11 (n=6)
Aminopeptidase	0.0600 ± 0.02 (n=9)	0.1000 ± 0.05 (n=6)	0.1300 ± 0.04 (n=5)
Trypsin	0.1700 ± 0.02 (n=9)	0.3100 ± 0.04 (n=4)	0.6000 ± 0.18 (n=5)
Proteinase VIII	0.0713 ± 0.05 (n=9)	0.00	0.00

Each value is the mean \pm SD

The degrees of freedom (n) for each value is shown

Table 4. The effect of trypanosome suspension on the activities of tsetse midgut aminopeptidase and trypsin

ENZYME	TRYPANOSOME/ENZYME RATIO					
Aminopeptidase	0.0454±0.009 (n=4)	0.0400±0.009 (n=4)	0.0398±0.004 (n=4)	0.0432±0.009 (n=4)	0.0320±0.004 (n=4)	0.0454±0.009 (n=4)
Trypsin	0.0700±0.006 (n=4)	0.0795±0.002 (n=4)	0.0807±0.002 (n=4)	0.0753±0.005 (n=4)	0.0779±0.005 (n=4)	0.0787±0.006 (n=4)

Each value is the mean ± SD

The degrees of freedom (n) for each value is shown in brackets

hours at 4°C, after which the enzyme activities (trypsin and aminopeptidase (AP), EC. 3.4.1.2. only) were determined. A control was run in which gut homogenate diluted with phosphate buffered glucose (PSG) to the same degree as the tests was also incubated prior to enzyme assays. A blank in which trypanosomes were incubated in PSG was also incorporated.

For the *in vivo* tests, previously unfed flies, 24 hours after emergence were membrane-fed on, a) infected and b) non-infected mouse blood. The flies were dissected 24 and 48 hours later and their trypsin and AP activities determined.

On incubation with gut homogenates, trypanosomes showed no effect on the activities of trypsin and AP (Table 4). Likewise, the *in vivo* tests, though still in the preliminary stages, so far show no differences in the enzyme activities of infected and non-infected flies.

Table 5. The effect of soybean trypsin inhibitor (STI) on tsetse midgut trypsin activity

	HOURS AFTER FEEDING	
	4	24
Control	0.2560±0.100 (n=4)	0.5975±0.120 (n=4)
STI <i>in vitro</i>	0.0037±0.003 (n=4)	0.0037±0.003 (n=4)
STI <i>in vivo</i>	0.3252±0.03 (n=4)	0.5746±0.120 (n=4)

Each value is the mean ± SD

The degrees of freedom (n) for each value is shown in brackets.

Previously unfed flies, 24 hours after emergence were membrane-fed on mouse blood containing 10 micrograms/millilitre (µg/ml) of the respective inhibitor. At this concentration, STI blocked 90% of the trypsin activity while TPCK removed about 50% of the AP

activity-*in vitro*. A control in which there was no inhibitor in the blood was also included. Trypsin and AP activities in the STI-fed flies were determined 1, 4 and 24 hours after feeding. Activities of the two enzymes in the TPCK-fed flies were determined 24 hours after feeding.

The results of trypsin inhibition by STI are summarized in Table 5. Although used at concentrations which showed very high inhibitions of the respective enzymes *in vitro*, both inhibitors had no effect on the respective enzyme *in vivo*. STI-fed flies actually showed a slight increase in trypsin activity.

Tsetse midgut proteolytic activity is more consistent when expressed as a function of the number of midguts, as opposed to the protein concentration. This is because the protein content of the midgut varies considerably, depending on the size of the bloodmeal. The failure of STI to effect enzyme inhibition *in vivo* is probably due to:

- (i) The inhibition of trypsin by STI is almost totally in favour of formation of the enzyme-inhibitor complex. The inhibitor is therefore likely to be exhausted, the used up trypsin being replaced by the fly.
- (ii) STI being a protein is undoubtedly destroyed by the gut proteases.

Many enzymes when inhibited tend to increase in their activity to levels that are above normal once the inhibitor is removed. This could explain the slight increase observed in the trypsin activities of STI-fed flies.

TPCK, being a non-protein and having no ester bond in its molecular structure is unlikely to be attacked by the gut proteolytic enzymes. Its inability to inhibit AP *in vivo* is therefore probably due to the same reasons as those described for trypsin in 'a'. The search for an *in vivo* inhibitor of one or more of the proteases, efficient enough to be used in further studies is being continued.

Chemical composition of salivary secretions of infected glands of *Glossina morsitans*

N. Y. Patel

During the year 1977-78, chemical analysis of secretions of the infected salivary glands of *G. morsitans* were carried out to provide further insight into the nutritional and developmental requirements of the metacyclic trypanosomes.

It is well known that the percentage infected salivary glands in nature is very low (0.1%).

In order to carry out chemical analysis of infected saliva it is necessary to have large numbers of gland infected flies. Under laboratory conditions the percentage of infected flies was increased according to a modification of Jenni's method (1977). About 10-12% of mature infections were produced by the use of this modified method of feeding and maintenance.

Each infected fly was allowed to salivate 15-20 times on a clean microscope slide and the saliva was stored at 4°C until required for analysis. About 500 probes were used for amino acid, carbohydrate, lipid and phospholipid analysis. For disc gel electrophoresis 50 probes were used.

The secretion from an infected fly is a whitish milky fluid with an approximate pH of 8.0 as compared to the clear transparent, viscous fluid of clean saliva (pH 7.0-7.5). This indicates that the presence of parasites produce an alkaline effect on the pH of the gland environment.

Proteins from infected saliva were separated electrophoretically on acrylamide gel in (1 mm diameter) capillary tubing into 2 very faint bands as compared to 11 fractions in clean saliva. (Figure 3).

Amino acid analysis of both clean and infected saliva was carried out using an Auto-Analyser (at the Kenya Industrial Research Organization). Both the samples showed the same number of ninhydrin positive peaks with a very slight variation in the size of the peaks. Since it is difficult to measure the actual amounts of clean and infected saliva, it was not as yet possible to quantify these results.

Sugar analysis performed by descending paper chromatography showed an absence of both inositol and glucosamine in infected saliva. At the same time the concentration of glucose and arabinase was reduced.

Lipids from infected saliva were separated into four classes, namely, phospholipid, free fatty acid, free cholesterol and a sterol ester. Clean saliva in addition contained a triglyceride.

The chromatogram for phospholipids showed faint spots corresponding to phosphatidyl ethanolamine and lecithine for both saliva. Sphingomyelin was present in infected saliva but not in clean saliva.

Whether or not these substances are responsible for the various developmental and physiological changes



Figure 3. Electrophoretic protein patterns stained with Coomassie Brilliant Blue G (i) clean tsetse saliva (ii) infected tsetse saliva. The shading is proportional to the staining

that take place in these trypanosomes in the salivary gland is not yet known.

Measurement of molecular weight by electrophoresis on SDS-acrylamide gel

Sodium dodecyl sulfate (SDS) dissociates proteins into their constituent polypeptide chains. The subsequent electrophoresis in polyacrylamide separates the polypeptides according to their molecular weights. The size of the polypeptide chains of a given protein can be determined by comparing their electrophoretic mobilities of marker proteins with well characterized polypeptide chain molecular weights.

Saliva from the microscope slides dissolved in 20µl of sample buffer containing 0.1% SDS and 0.1% mercaptoethanol. The sample was carefully boiled for 4-5 minutes in a 100°C water bath.

Polyacrylamide gels (7.5%) were made up with 1%

SDS. The buffer solution for electrophoresis contained: 7.8g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 38.6g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 2.0g of SDS per litre, it was adjusted to pH 7.2. Gels were prepared in 1mm bore capillary tubing. A standard Canalco Electrophoresis Chamber was used at 1.0 mA/gel. Electrophoresis was stopped when the marker reached 1–2mm from the bottom of the gel tube. The gels were removed from the tubes by freezing them for a few minutes. The tips of the gels were cut off at the distal end of the marker using a sharp razor blade. The gels were stained with Coomassie blue and were destained in 7.5% acetic acid.

The protein bands on the experimental gels were compared directly to those characteristic of known MW compounds subjected to electrophoresis simultaneously with the unknown.

The saliva sample showed 10 bands, corresponding to the ones obtained by normal polyacrylamide electrophoresis. Examination of these bands, compared to known MW standards established that the major peaks had an approximate MW of 168,000 and 120,000, 71,500 and 57,200.

Trypanosoma brucei: cultivation in vitro of salivary gland forms from *Glossina morsitans*

M. Nyindo, N. Patel, N. Darji and R. Chesang

Two strains of *T. brucei* (EATRO, 999, EATRO 1969) were propagated at 28°C and 38°C from the salivary glands of 5 *G. morsitans* for more than 6 months. Salivary glands from these flies were obtained 30 days after the flies had fed on rats infected with *T. brucei*. The parasites were grown on a bovine embryonic spleen cell feeder layer in RPMI 1640 buffered with 25mM HEPES and supplemented with 20% heat-inactivated bovine fetal serum, 1% lactalbumin hydrolysate and antibiotics in standard concentrations. In the first 2 to 3 weeks of cultivation the density of parasites in the salivary glands and culture medium remained constant, probably due to defective binary fission as shown in the electronmicrograph (Figure 5) where one parasite (a) is undergoing the classical longitudinal binary fission, while the other parasite (b) possesses an appendage constituting defective binary fission.

The parasites had their kinetoplasts situated between the nucleus and posterior end (Figure 4) and internally ribosomal material, Golgi complex and few mitochondria were seen (Figure 5). No surface coat was detected. The doubling time interval at 38°C and 28°C was 4 hours and 12 hours respectively.

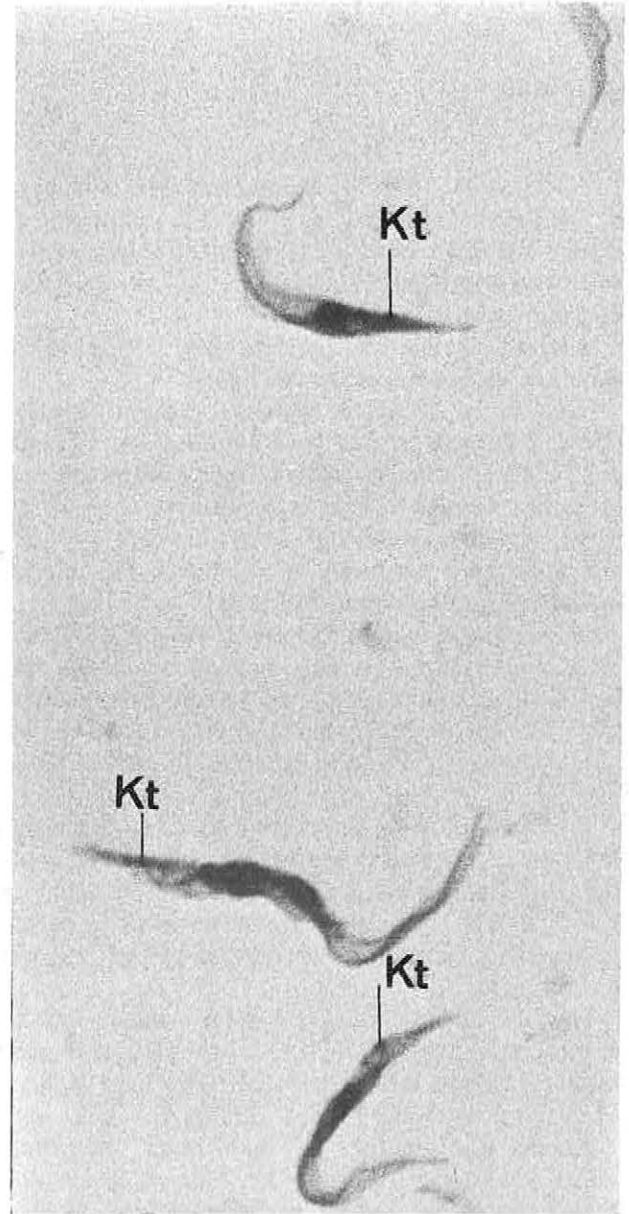


Figure 4. Immature metatrypomastigotes of *Trypanosoma brucei* from the salivary glands of *Glossina morsitans* on day 24 of propagation. The Kinetoplast (Kt) lies between the nucleus and posterior end. ($\times 2,500$)

Rats and mice were inoculated with the cultured parasites from day 17 and day 25 for EATRO 999 and EATRO 1969 respectively. The inoculated animals become positive in 6 to 8 days for parasites inoculated on day 17 and day 25 only.

Results obtained from this experiment suggested that, (1) in the first 3 weeks of propagation of *T. brucei* from the salivary glands of *G. morsitans* infected for 30 days parasites as metacyclic forms have very limited success of increasing in number when the logarithmic phase of other forms is reached, (2) the parasites which successfully grow in continuous culture are the immature metacyclic forms.

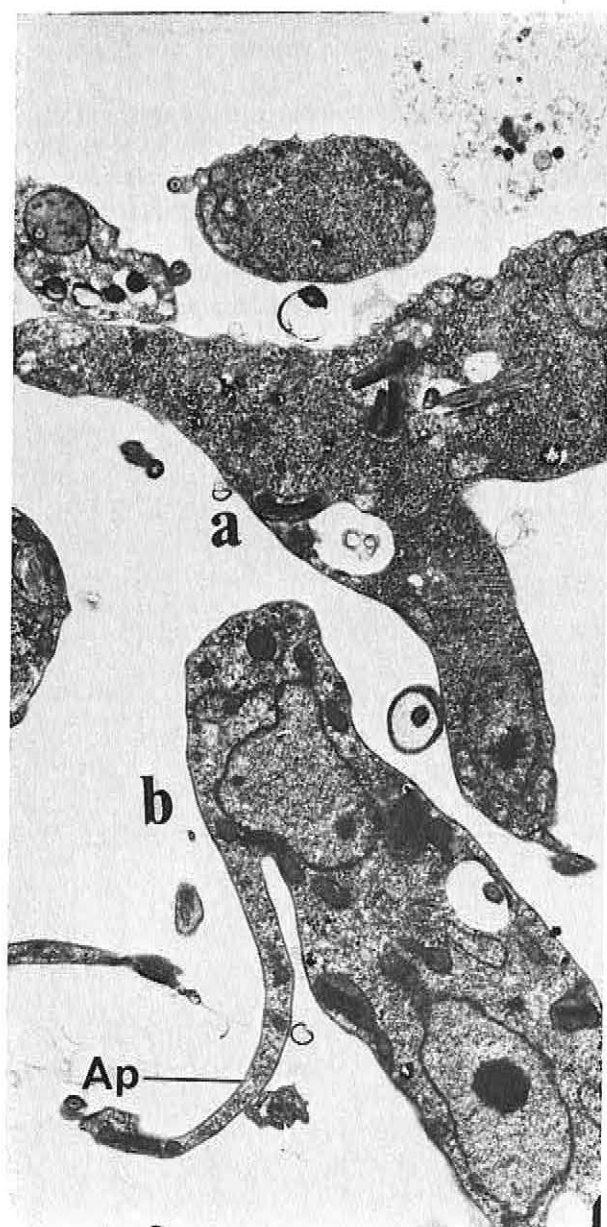


Figure 5. Electron micrograph of immature metatrypomastigotes of *Trypanosoma brucei* from salivary glands of *Glossina morsitans* on day 18 of cultivation. One parasite (a) is undergoing eques longitudinal binary fission while the other parasite (b) possessal an appendage (Ap). The appendage is unable to establish as a daughter cell. The parasites are covered by thin plasma membrane. ($\times 35,000$)

Field studies on vectorial capacity of *Glossina pallidipes*

L. H. Otieno, N. Darji, N. Patel and J. Kongoro

The transmission and epidemiology of human and animal trypanosomiasis are dependent upon a great number of biological and ecological factors involving the vertebrate hosts, the tsetse fly vector and the trypanosome organism. Among these factors, the biting activity and the behaviour of the fly is imperative

towards acquiring the trypanosome infection and to subsequent transmission. Recent studies have shown that salivation behaviour of the infected fly may be quite important in the transmission of *Trypanosoma brucei*. For example, the first salivary secretions contained larger number of trypanosome organisms compared to the subsequent probes. Furthermore, a clear cut difference was observed between the numbers of the various pathogenic African trypanosomes (*T. brucei*, *T. congolense* and *T. vivax*) secreted by *Glossina pallidipes*. Flies infected with *T. vivax* secreted saliva infected with very few trypanosomes and some of them invariably failed to show trypanosomes in the saliva, while some of them were shown to have thrown off the infection altogether. Another important finding is that there is no significant difference between the number of trypanosomes secreted by males and female *G. pallidipes*, but highly significant differences between the mean numbers of the different trypanosome species present in saliva.

These studies are being extended to various contrasting areas of Kenya where populations of *G. pallidipes* are resident.

Tsetse and trypanosomiasis research: host preference and infection rates

F. L. Lambrecht

A start was made in organizing the programme concerning host preference and infection rates in *Glossina* in various flybelts in Kenya.

A first survey was made in May of the following areas: Embu, Meru, Isiolo, Samburu and Marsabit. A more detailed survey inside Meru National Park was carried out at the end of June during which the presence of *Glossina pallidipes* and of *longipennis* was established, and the use of the biconic (Challier-Laveissiere) trap assessed. This was followed up in September, by a third visit during which the habitats of *G. pallidipes* were found and the biconic traps used for the collection of flies intended for dissection. A total of 160 tsetse were dissected for infection rates. Table 6 below summarizes the findings.

A rough vegetation map has been drawn showing the broad division in three basic vegetation types: *Acacia* woodland, *Combretum* woodland, and *Acacia-Commiphora* woodland.

G. pallidipes habitats were located in the *Combretum* woodland in the northwestern part of the park. *G. longipennis* seems to be scattered in most areas of the park.

Having found good-yielding trapping site for *G. pallidipes*, it is expected that the next surveys will allow large numbers of flies to be dissected and to include other data, such as age-grading. Both *G. palli-*

Table 6. Trypanosome infection rates

Glossina pallidipes

	Females	Males	Total
Number of flies	53	45	98
Proboscis	2	1	3
percent	3.8%	2.2%	3.0%
gut	3	0	3
percent	5.7%	0	3.0%
sal. gl.	0	0	0
percent	0	0	0
Total	5	1	6
percent	9.4%	2.2%	6.1%

Glossina longipennis

	Females	Males	Total
Number of flies	19	41	60
proboscis	0	0	0
percent	0	0	0
gut	1	0	1
percent	5.2%	0	1.6%
sal. gl.	0	0	0
percent	0	0	0
Total	1	0	1
percent	5.2%	0	1.6%

dipes and *G. longipennis* will be studied.

Each survey has been the subject of a separate report to the Director.

In order to sample flies under various seasonal conditions, surveys will be spread over 1979 at a rhythm of about 8 per year. For comparison, similar surveys are planned in the coastal area in collaboration with other ICIPE tsetse research programmes.

According to findings and available personnel, other research objectives will be added in view of being integrated into a more comprehensive study programme on the tsetse ecology.

POPULATION DIVERSITY

Research Staff

Mr. J. O. Apale (1976) Junior Technician
 Dr. J. van Etten (1974) Research Scientist
 Mr. F. Kathuli (1977) Technical Assistant/Driver
 Mr. J. Kiilu (1976) Subordinate Assistant

Mr. A. Makau (1976) Technician
 Mr. D. K. Mungai (1978) Technical Assistant/Driver
 Mrs. M. Owaga (1977) Graduate Trainee
 Dr. W. F. Snow (1977) Research Scientist
 Mr. D. F. Uvyu (1974) Technical Assistant

Population diversity in the tsetse fly *Glossina pallidipes* Aust.

J. van Etten

Over the past year, research has been focused on laboratory studies which could help in understanding some aspects of the diversity which was found between two populations of *Glossina pallidipes*, Nkruman and Mwalewa (see previous ICIPE Annual Reports). In addition, a study on genetic variation and degree of heterozygosity at macro molecular level by means of electrophoresis has been initiated, and has been focused on three populations of *G. pallidipes* in Kenya: Nkruman, Mwalewa and Kibwezi.

Diurnal activity patterns

The difference in diurnal activity patterns in flies from the two study areas has previously been reported (ICIPE Annual Report, 1976). The pattern of flies from Nkruman shows an afternoon peak which is most pronounced with males, and the pattern of flies from Mwalewa consists of two peaks, a morning peak and a much smaller afternoon peak with a clear mid-day depression. Last year, the influence of temperature on the pattern was discussed (ICIPE Annual Report, 1977).

Studies on the spontaneous activity of males of at least the second laboratory generation of flies ori-

ginating from the two populations, have been started this year. Preliminary results at $24 \pm 1^\circ\text{C}$ and $70 \pm 10\%$ R.H. show that with 12hr light, 12hr dark, the total activity increases with increasing hunger (Table 1), but also that the pattern changes with increasing hunger. On day 1 and 2 after feeding, the patterns of Nkruman and Mwalewa are nearly the same: an increase in activity in the morning to a level which is more or less constant from 10am till 5pm after which activity declines sharply. Males from Mwalewa exhibit more or less the same pattern on day 3 also. Males from Nkruman, however, show a high activity peak between 4 and 6pm on day 3, which is similar to the pattern of males from Mwalewa on day 4. The pattern with the high activity peak between 4pm and 6pm seems to be the pattern of very hungry flies. This stage is reached in the Nkruman males one day earlier than in the Mwalewa flies. The lower number of signals on day 4 for Nkruman males (Table 1) is mainly caused by the poor conditions of the flies.

The pattern found under low temperature conditions in the field are in both areas similar to the pattern obtained in the laboratory 3 days after feeding. However, before further suggestions on the meaning of these data could be made, more data are required, especially at higher temperatures.

Fat reserves

The study on building up of fat reserves under labo-

Table 1. Spontaneous activity of *Glossina pallidipes* males originating from two populations, expressed as numbers of recorded signals per day, measured over 4 days after feeding

No. of days after feeding	Mwalewa		Nkruman	
	n	Av. no of signals	n	Av. no of signals
1	14	34.2 ± 17.8	15	22.2 ± 16.7
2	15	82.4 ± 66.3	16	85.3 ± 70.2
3	13	202.2 ± 156.8	14	212.0 ± 158.7
4	12	382.0 ± 260.9	9	270.9 ± 121.6

Table 2. Allele frequencies of one loci of three enzymes, which show variation in three population of *Glossina pallidipes*

	EST			AO					LAP						
	n	0.95	1.00	het	n	0.95	1.00	1.05	het	n	0.95	1.00	1.05	1.10	het
Nkruman	153	0.09	0.91	0.15	207	0.09	0.73	0.18	0.31	214	0.02	0.85	0.12	0.004	0.22
Kibwezi	136	0.08	0.92	0.14	127	0.03	0.78	0.20	0.33	128	0.05	0.76	0.20	0.004	0.33
Mwalewa field	163	0.06	0.94	0.07	152	—	0.99	0.01	0.02	176	0.04	0.86	0.10	—	0.14
Mwalewa lab. colony	45	0.01	0.99	0.02	48	—	0.94	0.06	0.08	45	—	0.90	0.09	0.01	0.11

ratory conditions is, due to lack of material, still in such a preliminary stage that results cannot yet be discussed.

Breeding

Attempts to breed *G. pallidipes* from Nkruman have failed so far. The attempt to breed *G. pallidipes* from Mwalewa, however, has resulted in a small closed colony, which has been maintained for over 3 years (Figure 1). The flies have been maintained single at $24 \pm 1^\circ\text{C}$ and $70 \pm 10\%$ R.H. After an initial increase, a marked decrease occurred, due to a decrease in fecundity and an increase in the pre-mating death rate from the 5th generation onwards. The 10th and 11th

generations had the worst performance, but from the 12th generation onwards fecundity increased and pre-mating death rate decreased, resulting in the increase of the number of mated females in the colony (Table 1).

Genetic variation

Preliminary studies on the genetic variation on a macromolecular level via iso-enzyme patterns obtained with horizontal starch-gel electrophoresis (ICIPE Annual Report, 1976) have resulted in the detection of three enzymes which show variation: leucine amino peptidase (LAP), aldehyde oxidase (AO) and non-specific esterases (EST). LAP showed variation at the

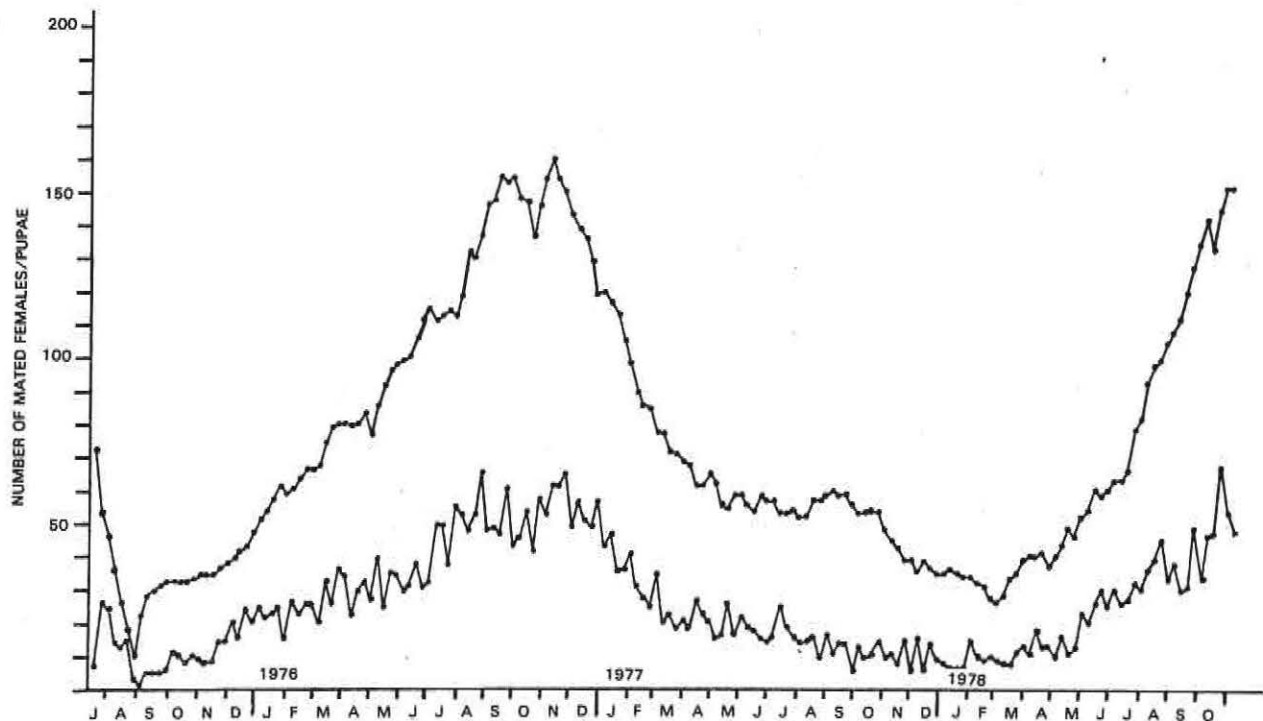


Figure 1. Mwalewa colony. The total numbers of females surviving at the end of each week (upper line) and the total number of pupae produced in each week (lower line) all generations included

LAP-3 locus, aldehyde oxidase has only one locus, while EST showed variation at the EST-1 locus. All three enzymes also show variation in *G. morsitans*. Only a limited number of other enzymes have been tested in small number, but so far no more enzymes with variation have been found.

The frequency of the alleles of the enzymes which show variation and the frequency of heterozygotes in the samples of three populations of *G. pallidipes*, Nkruman, Mwalewa and Kibwezi, which have so far been studied, have been summarized in Table 2.

The following conclusions can be drawn:

(i) At the LAP-3 locus 4 alleles, and at the EST-1 locus 2 alleles have been found in all three populations. At the OA-locus, however, 3 alleles are found in the Nkruman and in the Kibwezi populations, but the Mwalewa population misses the 0.95-allele.

(ii) The frequency of the heterozygotes in the three enzymes does not differ much in the Nkruman and Kibwezi population. However, the Mwalewa population has a much lower frequency of heterozygotes in all three enzymes. This might suggest that the size of the gene pool in Mwalewa is rather small. However, no data on this aspect are available yet.

(iii) The frequency of heterozygotes in the laboratory colony seems slightly reduced compared with the field populations, but the sample size is too small to draw final conclusions.

Tsetse ecology on the Kenya Coast

W. F. SNOW

During 1978 a major programme on the ecology and behaviour of *Glossina pallidipes*, based at the ICIPE Coastal Field Station, Mombasa, has been initiated on the south Kenya coast. The seasonal fluctuations, structure and characteristics of *G. pallidipes* populations are being studied, although it will be some time before meaningful results can be presented as many of the investigations are on a long time-base or require the analysis of a great deal of preserved material.

Monthly samples, using Challier traps, are being taken at Muhaka, 35km south of Mombasa where moderate numbers of *G. pallidipes* occur in a small, isolated area of forest. Wild hosts are rare and domestic stock may represent the main food source for tsetse in this area. This, incidentally, is the proposed site of the definitive ICIPE Coastal Field Station which will give the opportunity for really long term monitoring of the tsetse populations. Quarterly collections coinciding with the large and small rains, intermediate period and dry season are being taken in the Shimba Hills National Reserve and Mwalewa Forest near the Tanzania border, for comparison with Muhaka data. These localities contain extensive areas of *G.*

pallidipes habitat and support relatively high population densities of fly. In Shimba Hills particularly wild hosts such as antelope, buffalo, pig and elephant are abundant but domestic animals are absent from both Shimba and Mwalewa. In addition, a 1000 acre farm, 25km south of Mombasa at Diani, with a history of cattle losses due to trypanosomiasis, has been investigated as an example of the epidemiological problems still existing on the Kenya coast. Fly distribution, numbers and mortality patterns have been assessed.

Major aspects of the work in progress or due to start in the near future are outlined below.

(i) The age structure of the female component of the samples from Muhaka, Shimba and Mwalewa is being evaluated using ovarian age-grading techniques. The apparent mortality patterns are being compared with seasonal density fluctuations and the relative population densities at different localities. The project has not been in operation long enough to attempt a detailed comparison of mortality patterns with seasonal density changes, although mortality rates seem to have increased progressively since the observations began at Muhaka in May. It also seems that the high density populations at Shimba and Mwalewa may be living longer than those at Muhaka.

These results, when complete, should indicate how far adult mortality, considered alone, can account for the seasonal fluctuations in tsetse numbers and may also indicate the relative importance of density dependant and density independent factors in the natural regulation of tsetse numbers.

(ii) Predation as a factor in adult mortality is being considered although it is suspected that, on the south Kenya coast, it is an infrequent event which may be difficult to detect in the field. Age structure and apparent mortality is being related to absolute population numbers and habitat area to quantify tsetse deaths per day per unit area of habitat. The precipitation test will be used against wild-caught potential predators and to measure digestion rates in captive predators.

Considering the extreme longevity, slow reproductive rate and general stability of tsetse populations, predation and parasitism may be of marginal importance in the natural regulation of fly numbers. However, it is felt that some fundamental observations are needed to evaluate these factors which may be eliminated incidentally in the course of attempted insecticidal control of tsetse.

(iii) The application of age-grading techniques based on measurement of apodeme growth layers, thoracic weight/size ratios and ovariole lengths will enable the age of young (particularly female) flies to be estimated very accurately. These techniques are still being evaluated and will require 'calibration' by the analysis of the flies of known age. It will eventually be possible to determine the age at which female flies are inseminated, take their first blood meals and the du-

ration of their first reproductive cycle.

(iv) As soon as possible after permission is obtained to take animals to Shimba Hills National Reserve, it is planned to initiate a series of catches using goats as bait. Attack patterns in relation to diet activity cycles will be investigated. Dissection of freshly fed flies from the bait, with measurement of ovariole lengths, will allow accurate ageing within each reproductive cycle and indicate how frequently female flies are feeding. The reproductive state of males will also be assessed.

(v) Studies are being made of the general ecology and distribution of tsetse, including *G. austeni*, *G. brevipalpis* and the epidemiology of trypanosomiasis in livestock on the south Kenya coast. Even in the limited area being investigated there are considerable complexities in the ecology of the tsetse which occupy a wide range of ecotypes. Where the natural vegetation cover has been cleared and the classic tsetse habitats are now absent, fly utilize secondary vegetation communities and trypanosomiasis remains a problem, with occasional high cattle mortalities.

The study at Diani mentioned above is part of this aspect of the programme. In this situation, although the major breeding locus of *G. pallidipes* was a small

area of relict forest, tsetse were found throughout the farm. It is probable that dispersal from this focus was achieved by flies following cattle as they moved around the farm grazing. Scrub clearance and pasture improvement is now in progress and it is anticipated that tsetse members will be drastically reduced.

Two important points are emerging from the observations on tsetse distribution and general ecology:

(i) Settlement and agricultural development often proposed as a method of consolidating areas from which tsetse have been eradicated. The south Kenya coast supports a high human population density (40–100/km²) and much of the area is intensively cultivated, yet tsetse persist. In such situations there is no alternative to continued painstaking surveillance and small-scale, local control measures.

(ii) In all *G. pallidipes* habitats which have been examined in the more developed areas of the coast, *Lantana camara* is a major component. The control of this weed, perhaps by biological methods, could contribute significantly to tsetse control. Similar situations occur in western Kenya. In any case the clearance of scrub and thicket in the more populous areas of the south Coast could eradicate tsetse on a local scale without resort to pesticides.

BIOASSAY RESEARCH UNIT

Research Staff

Mr. G. Achieng (1976) Technical Assistant
Dr. T. Gebreyesus (1978) Senior Research Scientist

Mr. L. Moreka (1976) Subordinate Assistant
Mr. B. N. Otero (1976) Principal Technician

Services provided by the unit

B. N. Otero

The Bioassay Research Unit was organized in November 1977 with the main purpose of providing routine bioassay services to other programmes and units. The Bioassay services currently provided include:

- (i) Galleria wax test for juvenile hormone, using wax moth (*Galleria mellonella*) pupa.
- (ii) Musca test ecdysones using house fly, *Musca domestica* larvae.

The following methods are being developed:

- (iii) Radio-immunoassay for ecdysones and juvenile hormones.
- (iv) Tenebrio test as supplementary bioassay for juvenile hormones using flour beetle (*Tenebrio molitor*) pupa.
- (v) Chilo dipping test for ecdysones using stem borers (*Chilo zonellus* or *partellus*) larvae.

- (vi) Gastromargus test for anti-juvenile hormones (precocenes) using grasshoppers (*Gastromargus africanus*).

In addition, reliable bioassay methods for repellants and larvicides are being developed to help in the screening of plant products.

Besides the bioassay services, the unit also provides insects for those who wish to carry out their own tests. The unit has rendered its services to the following programmes:

- (i) Tick physiology — ecdysone bioassay to determine the ecdysone activity in ticks.
- (ii) African Armyworm — juvenile hormone bioassay to determine the effect of juvenile hormone on migratory behaviour.
- (iii) Termite — to observe the effect of juvenile hormone on caste differentiation.

Although the primary purpose of the unit is to provide bioassay services on a routine basis, the staff have also started original as well as collaborative research work.

CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT

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Mr. N. Juma (1974) Junior Technician

Dr. A. Maradufu (1974-87) Research Scientist
Dr. C. K. Wilkins (1977-78) Research Scientist

Collaborator
Dr. T. Gebreyesus (1978) Bioassay

Termite resistance in the African Pencil Cedar

C. K. Wilkins

For centuries man has made selections of wood for building purposes based on his observations of its durability. Information has been handed down in the form of customs which are evident in all parts of the world. Wood for building must be able to withstand weather and fungal and insect attack. In tropical areas insect attack is particularly severe and termites are among the most serious pests in wood destruction.

In Kenya many indigenous woods are known to show termite resistance. Among these is the heartwood of the African Pencil Cedar, *Juniperus procera* which is widely used as fenceposts.

The aim of this research was to elucidate the chemical bases of termite resistance of *J. procera* heartwood. Infrared spectra were measured on a Perkin-Elmer spectre photometer model 720.

Juniperus procera was collected from the Rift Valley escarpment beside A-104. Bark and sapwood were removed, the heartwood reduced to shavings and the shavings were extracted with methanol at 4° for two days. The solvent was decanted and evaporated to leave a brown gum. The brown gum (10g) was chromatographed on a column (55×3.2cm) consisting of Machery Nagel silica gel 60 (<200 mesh) and calite (5:1) starting with chloroform and using gradient elution with ethylacetate and then methanol until the column material and the eluate were devoid of material. The fractions (100ml) were monitored using TLC on silica gel developed with 5% ethylacetate in chloroform and visualised using iodine or 1% vanillin and 10% p-toluene sulfonic acid in methanol. Major constituents appeared in fractions I, II, IV, V, VI, VIII and IX.

Fractions I and II (combined) and fraction IV were further chromatographed on silica gel columns impregnated with silver nitrate (7%). Fraction I-II yielded a fast moving component (I-IIA) while fraction IV gave the major compound of the extract IV E. These appeared as single spots on TLC silica gel/10% silver

nitrate-toluene/chloroform 4:1 and 5% ethyl acetate in chloroform respectively. Comparison of infrared spectra of fractions I-II A and IV E with those of α -cedrene and α -cedrol from the Aldrich collection revealed their identity with these two compounds respectively, with a 2ml hexane solution of the compound under examination.

Compounds with repellent activity were tested at 0.01, 0.1 and 1mg levels while those which showed no repellent activity were tested at 1mg only (0.01, 0.1 and 1mg per half sheet of filter paper corresponded to approximately 0.003, 0.03 and 0.3% respectively).

The pieces of filter paper were placed so they covered the bottom of petri dish, moistured, 50-60 *Odontotermes badius* were added (25 on each side) and the dish covered and placed in the dark for thirty minutes. Pictures of the dish were taken at 2 minute intervals for 20 minutes. Distribution of the termites were tabulated and averaged. Distributions for α -cedrol (were treated/untreated) 2/47, 3/47, 24/36 at 1, 0.1 and 0.01/mg respectively. α -cedrene showed a distribution of 33/27 at 1mg.

Results

α -Cedrol showed at best a weak attractive activity while α -cedrol was strongly repellent to *O. badius*. The 0.3% level was comparable to that reported in *J. procera* of 0.5% for both compounds. α -Cedrol is at least partially responsible for the termite resistance of *J. procera* in areas where *O. badius* are found.

Suggestions for future research

Other compounds can be isolated and their repellence tested on *Odontotermes* species and other termite species.

Notes on other termite resistant woods

Rawsonia lucida, *Markhamia platycalyx* and *Euphorbia tirucalli* were screened for repellent activity but no suitable bioassay was devised for non-volatile materials so these woods were not investigated.

HISTOLOGY AND FINE STRUCTURE RESEARCH UNIT

Director of Research
Professor T. R. Odhiambo

Visiting Director of Research
Professor M. Locke (1977)

Research Staff

Mr. M. Chimtawi (1974) Principal Technician
Dr. E. D. Kokwaro (1975) Research Scientist
Mrs. J. A. Kongoro (1974) Research Assistant
Mr. P. Lisamulla (1973) Senior Technician
Dr. A. Massalski (1978) Research Scientist
Mr. J. Owor (1973) Associate Scientific Officer

During the year under review, the HFSRU made important supportive contributions to ICIPE in many aspects of the biology of the target insects, some of which are summarized below.

Sensory organs on the antenna of the pigeon pea aphid (*Sitobium nigrinactaria*)

A. Odebiyi and E. D. Kokwaro

Ultrastructural studies on the distribution and structure of sensilla on the adult antenna, and cornicle (Figure 1) of several aphid species have provided data useful in the classification of this genus.

Trypanosoma brucei derived from the midgut and salivary glands of *Glossina morsitans*

M. Nyindo and M. Chimtawi

Transmission electron microscopy (TEM) has been used to study the morphology of cultured forms of *Trypanosoma brucei* derived from the midgut and salivary glands of *Glossina morsitans*. The study has indicated more clearly the characteristic morphology of the infective parasites.

Ultrastructural studies on the uterus of *Glossina morsitans*

J. A. Kongoro

Light microscopic observations indicated the presence in the uterus of *Glossina morsitans* of two types of secretory cells, besides the squamous cells which compose the general epithelium of the organ (ICIPE Annual Report 1975). The first secretory cell type were the choriothete cells, found on the ventral part of the uterus. The second secretory cell type were cells observed in the anterior dorsal region of the uterus which is known as the oviductal shelf.

As a basis for further studies, the aim of the present work was to study the organelles and cell inclusions that characterize the different cell types in the uterus of *G. morsitans*.

Materials and methods

The flies which were obtained from the ICIPE laboratory colony were dissected in 2.5% Glutaraldehyde buffered with 0.05M Sodium Cacodylate and 5% sucrose at pH 7.5. Teneral flies were used in this study. The uteri were kept overnight in the fixative.

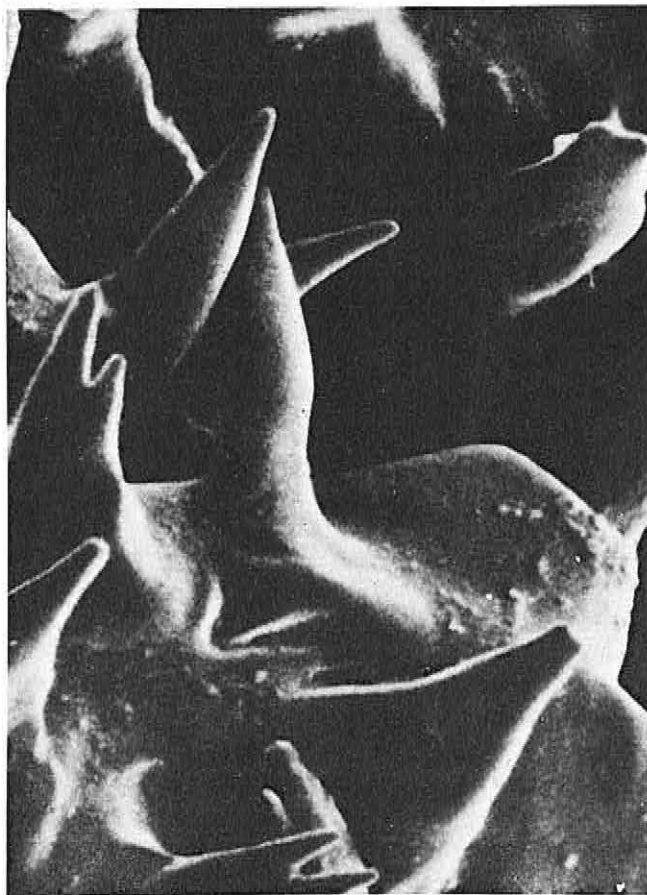


Figure 1. SEM of sensory structures on the cornicle of *Sitobium nigrinactaria* ($\times 9,000$)

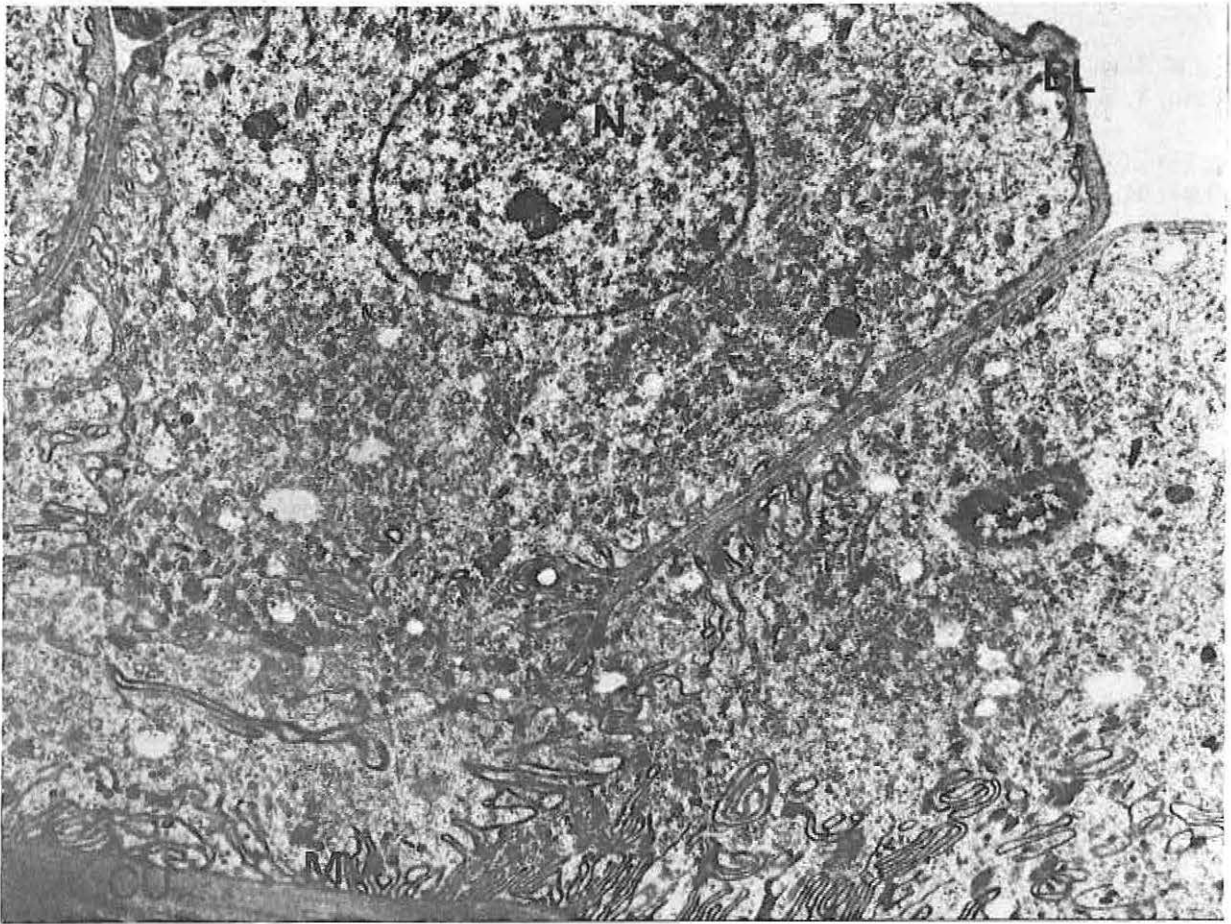


Figure 2. Longitudinal section through a choriothete cell, showing nucleus (N), basal lamina (BL), microvilli (MV), cuticle (CU) ($\times 11,000$)

The tissues were osmicated, dehydrated in the graded alcohols and were then embedded in Araldite. Thick (1 micron) and ultrathin (500\AA – 700\AA) sections were obtained using an LKB III ultratome. The sections were contrasted with uranyl acetate and lead citrate prior to being examined with a Phillips 201 transmission electron microscope.

Results and discussion

The choriothete cells, the oviductal shelf cells and the uterine epithelial cells were observed to have different ultrastructural characteristics.

The choriothete cells had a thin basal lamina (BL) (Figure 2) and the apical membrane was bounded by cuticle (CU) which had secretory granules in it. Beneath the cuticle the apical membrane was arranged into numerous microvilli (MV) (Figure 2). Mitochondria (M) and many groups of microtubules (Mt) were observed (Figures 3 and 4). Autophagic vacuole-like (AV) and lysosome-like structures were also observed. Secretory granules observed in the apical region were occasionally in very close association with the microvilli. The nucleus (N) which was basal was round

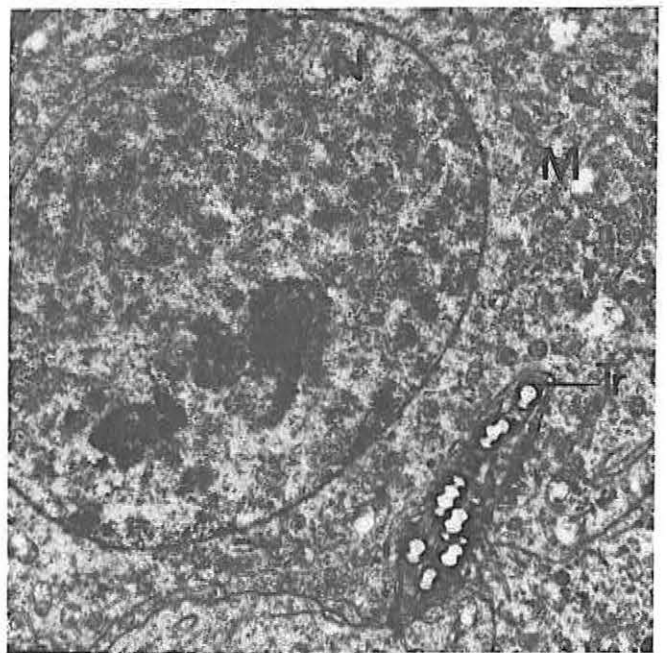


Figure 3. An oblique section of a choriothete cell, showing the nucleus (N), tracheole (Tr), mitochondria (M) ($\times 20,000$)



Figure 5. A portion of a cell from the oviductal shelf of the uterus showing numerous parallel microvilli (MV) ($\times 35,000$)

and had a well developed nucleolus. The cells of the oviductal shelf had a thin basal lamina and the apical membrane was arranged into numerous parallel microvilli (Figure 5). The apical membrane was bounded by cuticle which had secretory granules in it. Microtubules were observed and occasionally these were in close association with microvilli. Numerous mitochondria were observed. The nucleus had a well developed nucleolus. The oviductal shelf region was well supplied with circular and longitudinal muscles. Occasionally, axons containing neurosecretory granules were observed. This is proof for the innervation of this region.

The general uterine epithelium is composed of squamous cells. The cells are bounded by a thin basal lamina. The apical membrane was arranged into microvilli which were fewer than those of the two cell types described above. Microtubules were fewer than those in the other two cell types and they were mainly in the apical region, where they seemed to be associated with the microvilli. Mitochondria were observed in the cytoplasm and very few autophagic vacuole-like structures were observed.

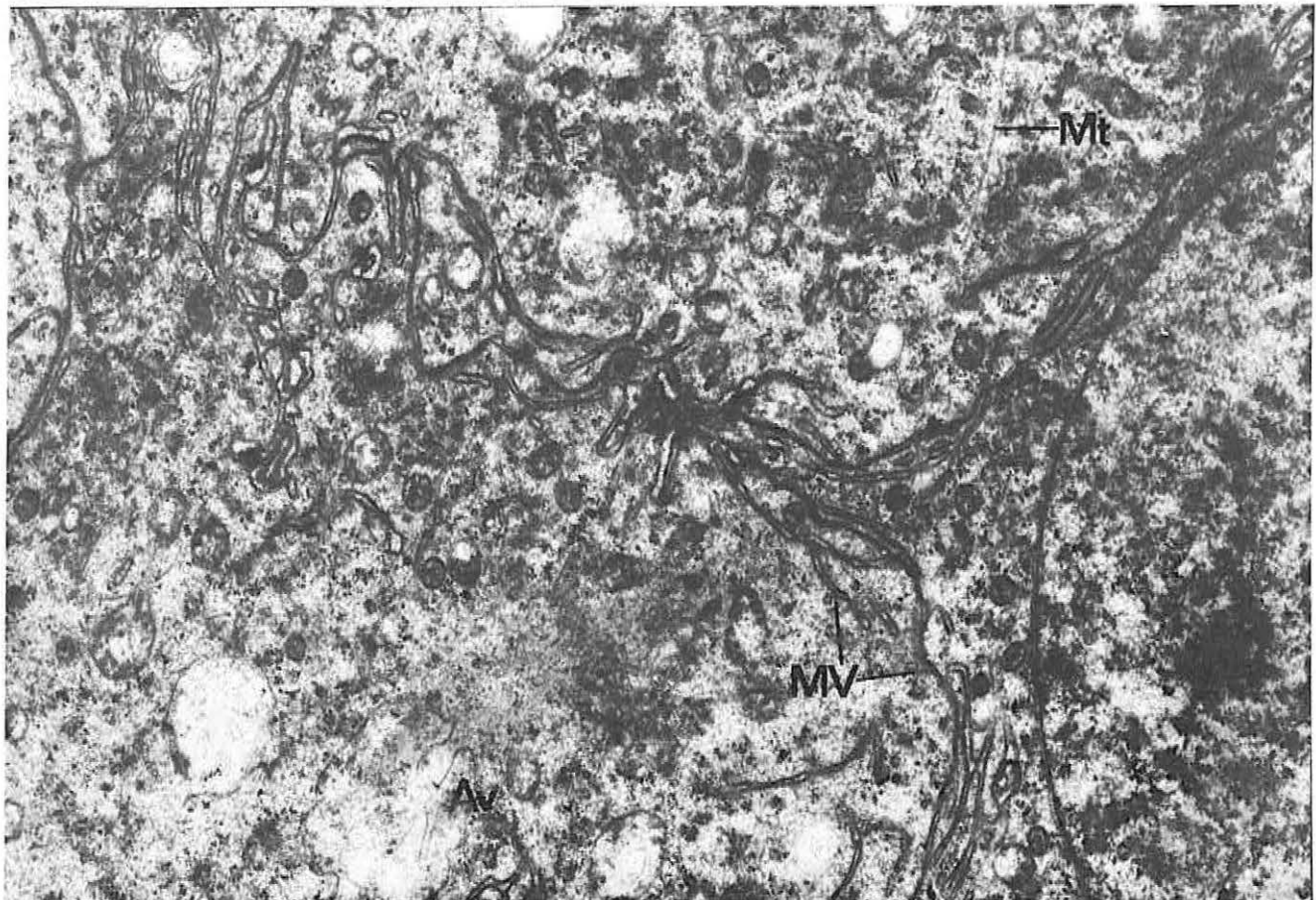


Figure 4. Portion of a choriothete cell showing microtubules (Mt), microvilli (MV), autophagic vacuole-like structures (Av) ($\times 30,000$)

The presence of microtubules in the three cell types is interesting. The structures could be skeletal, conductive, or they could even be involved in motion.

Summary

The three uterus cell types of *G. morsitans*, namely, the choriothete cells, the oviductal shelf cells and the uterus squamous epithelial cells had different ultra-structural characteristics. Both the choriothete cells and the oviductal shelf cells had secretory characteristics but the oviductal shelf cells had more microvilli and mitochondria than the choriothete cells. The numerous microtubules observed in the choriothete cells could probably enable the cells to be capable of some motion.

The role of corpora allata in *Macrotermes* during soldier development

J. Owor and B. M. Okot-kotber

Experiments to determine the right fixation procedure of the 4th instar larva in soldier development are in progress. The following fixatives were tested with the objective of achieving the best preservation of the corpora allata:

- (i) Karnovsky's (paraformaldehyde-glutaraldehyde) followed by OsO_4
- (ii) Double fixation, Glutaraldehyde followed by OsO_4 , both in Sodium Cacodylate buffer
- (iii) OsO_4 in $\text{K}_2\text{Cr}_2\text{O}_7$ buffer
- (iv) Mixture of glutaraldehyde and OsO_4

None of these fixatives was fully satisfactory as far as the overall preservation of the tissue was concerned, although fixative (iii) seems to give the best preservation (Figure 6). The most prominent feature, which appears to be common, regardless of fixative used, is the presence of a 'cavity' within the nucleus (Figure 7). It is premature to determine, at the present state of study, whether the cavity is a fixation artefact or a natural phenomenon occurring in the nuclei of corpora allata of the 4th instar larva during soldier development. Further studies necessary to elucidate this problem are envisaged. These will include trials of several fixatives and comparative studies of the corpora allata of larvae during soldier development.

Secretion in the *Glossina* gut after feeding

J. Owor

The midgut is separated from the haemocoel by a basal lamina. The epithelium is surrounded by two layers of muscle arranged in circular and longitudinal bands each surrounded by a further basal lamina. The laminal surface is protected by a continuous peritrophic membrane.

Three different cell types were identified per morphological evidence and secretory activity patterns:

- (i) The 'secretory' cell types. This cell type before a meal has few RER, some apical Golgi complexes, and very dense secretory vesicles. After a meal these cells show more Golgi complex secretory activity at the apical face. Many membrane-bound Golgi complex-

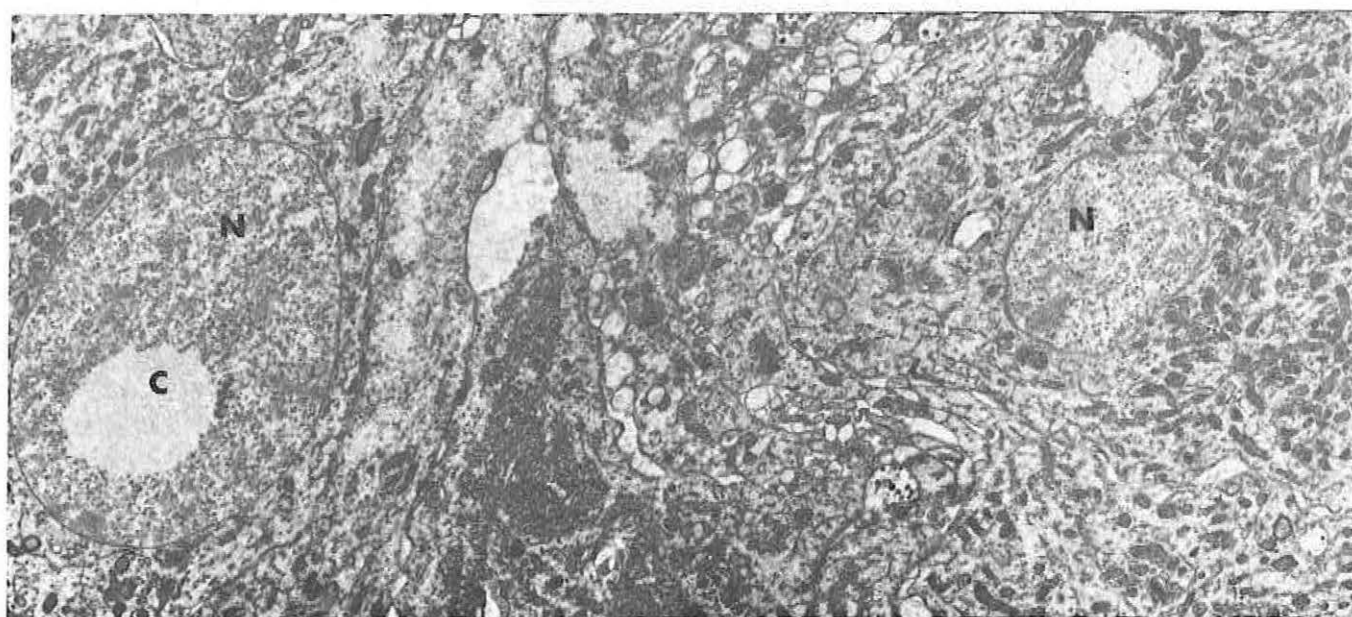


Figure 6. Low power micrograph of *Macrotermes* 4th instar larva showing two types of nuclei (N). The 'cavity' (C) is thought to contain labile substance ($\times 10,000$)

associated secretory vesicles are observed about to be discharged into the gut.

(ii) The 'absorptive' cell-type are more complex than the preceding ones. The basal and lateral surfaces are infolded and are often associated with mitochondria. They have a structure appropriate for transport and secretion. The presence of a nuclear 'halo' associated with activity, was observed.

(iii) The presence of 'crypt' cells are being investigated.

Secretions in the accessory reproductive glands of *Glossina morsitans*

E. D. Kokwaro

The male reproductive accessory glands of *G. morsitans* secrete a complicated mixture of components described according to their ultrastructural appearance as follows:

- (i) the non-membrane bound, electron-opaque bodies (Figure 10)
- (ii) the ribbon-like fibre structures (Figure 10)
- (iii) the loosely arranged matrix (Figure 10)
- (iv) aggregations of densely packed, electron-opaque granules (Figure 11) and
- (v) the uniformly homogeneous, electron-opaque material

The possibility of these secretory material contributing to the formation of at least part of the spermatophore is being investigated.

The ultrastructural organization of spermathecae and their ducts in the tsetse fly *Glossina morsitans*

E. D. Kokwaro

Light and electron microscope studies of the cytology of the spermathecae and their ducts in *G. morsitans* has revealed two principal cell types: (i) epithelial cells which surround the cuticular lined receptacle and the cuticular lining of the spermathecal duct (Figure 12), and (ii) secretory cells of the spermathecal receptacle (Figure 13). The secretory cells are characterized by a mass of long microvilli converging into a secretory cavity. The secretory cells appear to discharge their secretion through cuticle-lined ductules into the lumen of the spermatheca. The lumen of virgin females contains electron-opaque substance.

For the future it is envisaged that the relationship between the structure of the spermathecae to the function of sperm-storage will be investigated.

Transport of proteins across tick gut

F. Obenchain and M. Chimtawi

Transmission electron microscopy has been used to study the mechanics of protein transport across the tick gut.

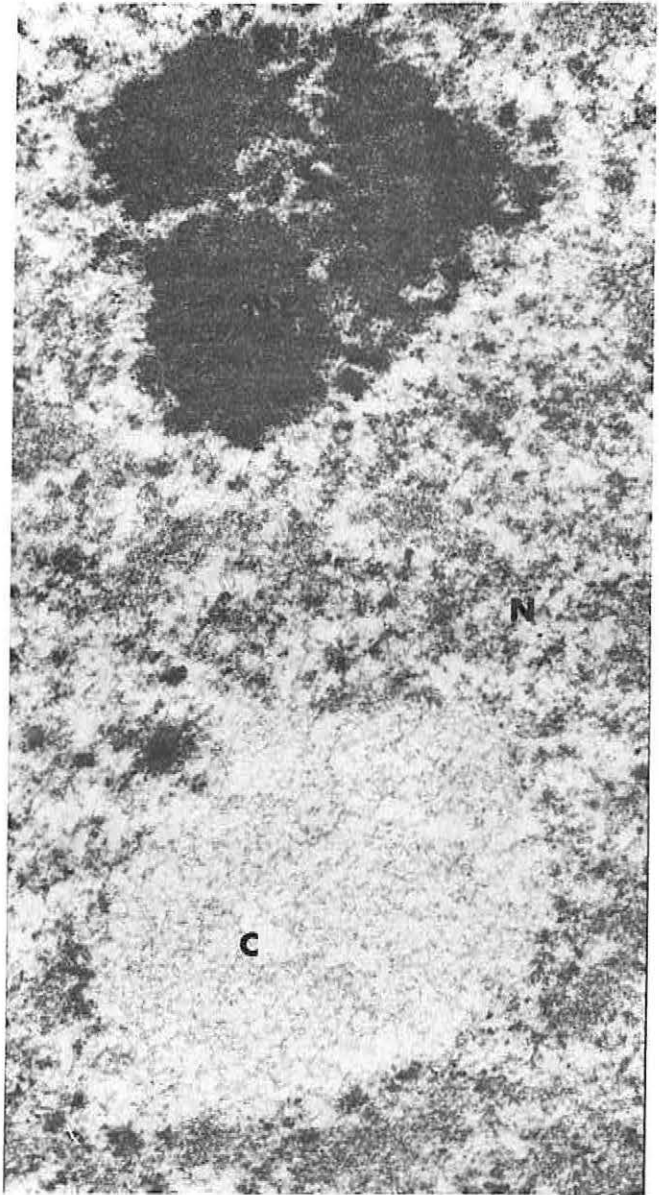


Figure 7. The cavity (C) shows perhaps the residue of the contents. The specimens in Figures 7 and 8 were fixed in 1% OsO₄ buffered in K₂Cr₂O₇ pH7.2 with 0.85% NaCl. N: nucleus, NL: nucleolus (× 52,000)

Histology and Fine Structure

Concerning support services outside the ICIPE, the following were continued:

- Ultrastructural studies on the parental barrier in the cane rat elephant shrews (Dr. Oduor-Okelo, University of Nairobi)
- The fate of *Trypanosoma congolense* in tsetse gut (Mr. John Kaddu, East African Trypanosomiasis Research Organization)

- Effect of drugs on mammalian muscle contraction (Dr. J. Mugo, University of Nairobi)
- Fungicidal drugs (Dr. V. Mbaya, University of Nairobi)
- Morphological studies of the ova of *Spodoptera* species in East Africa (Dr. Dewhurst, Desert Locust Control Organization for Eastern Africa).

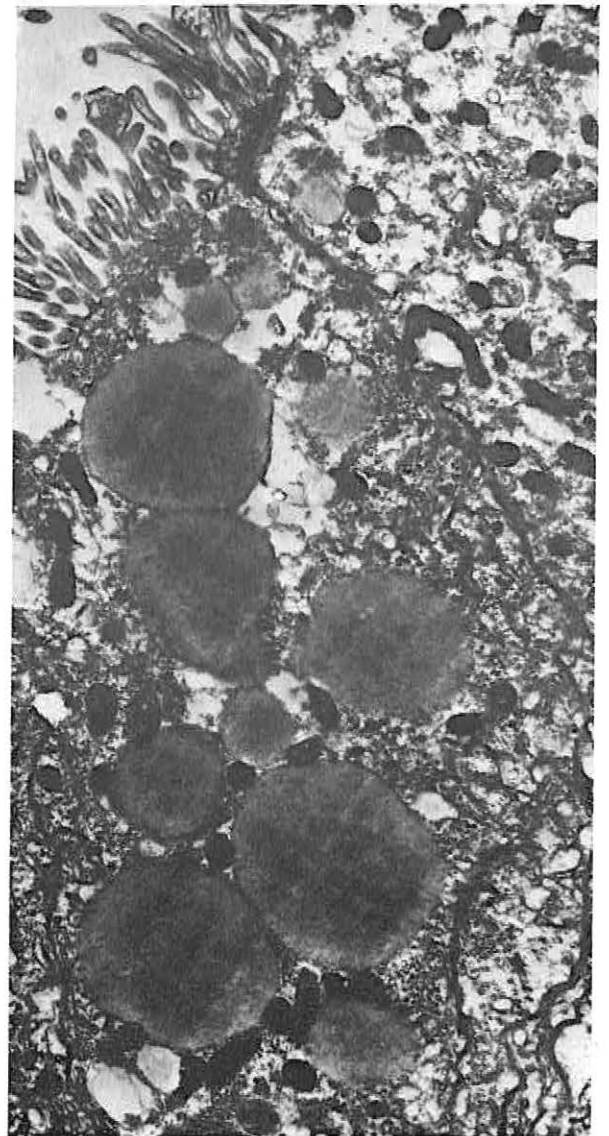
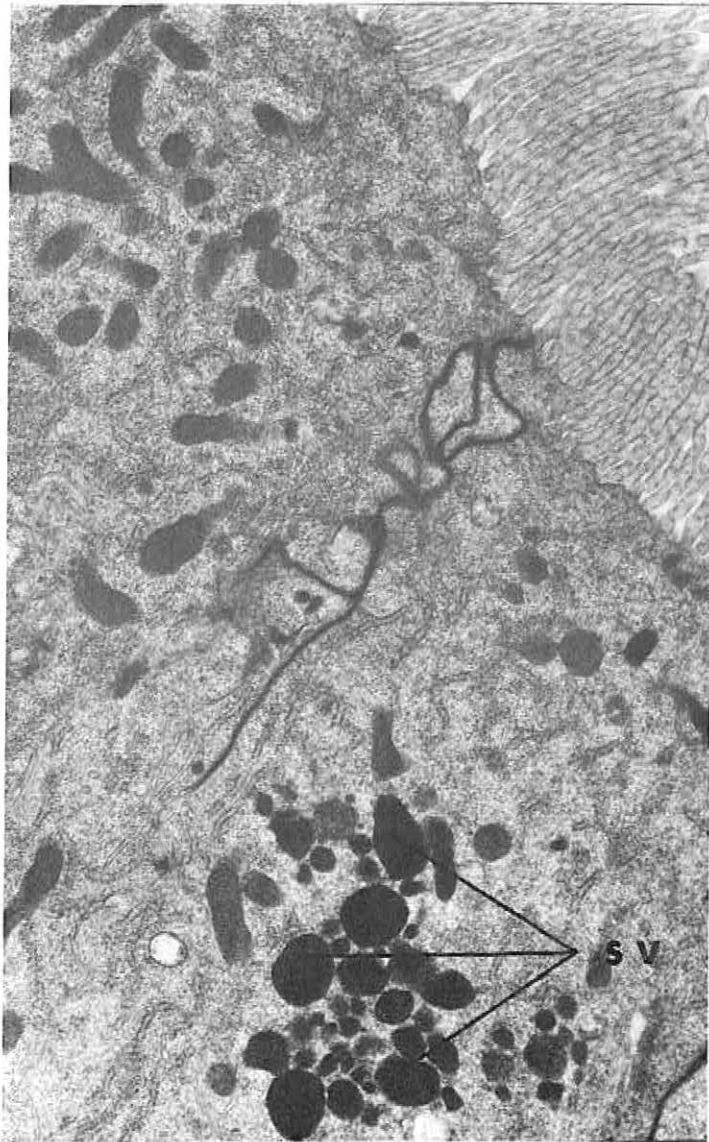


Figure 8a and 8b. Low power micrograph of 'secretory' cells of the midgut of *Glossina*. SV: secretory vesicles. Figure 8a—unfed; Figure 8b 24 hours after feeding

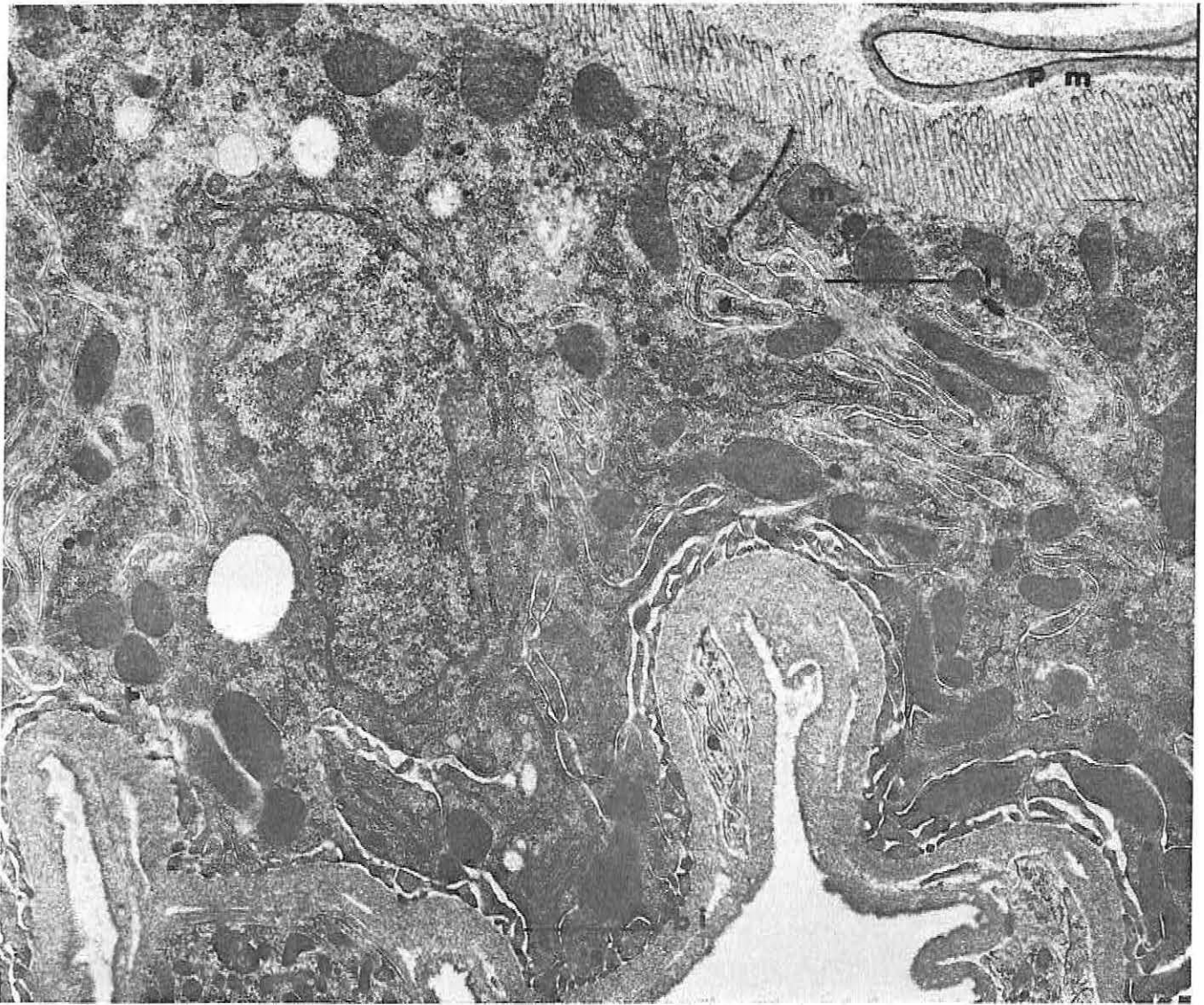
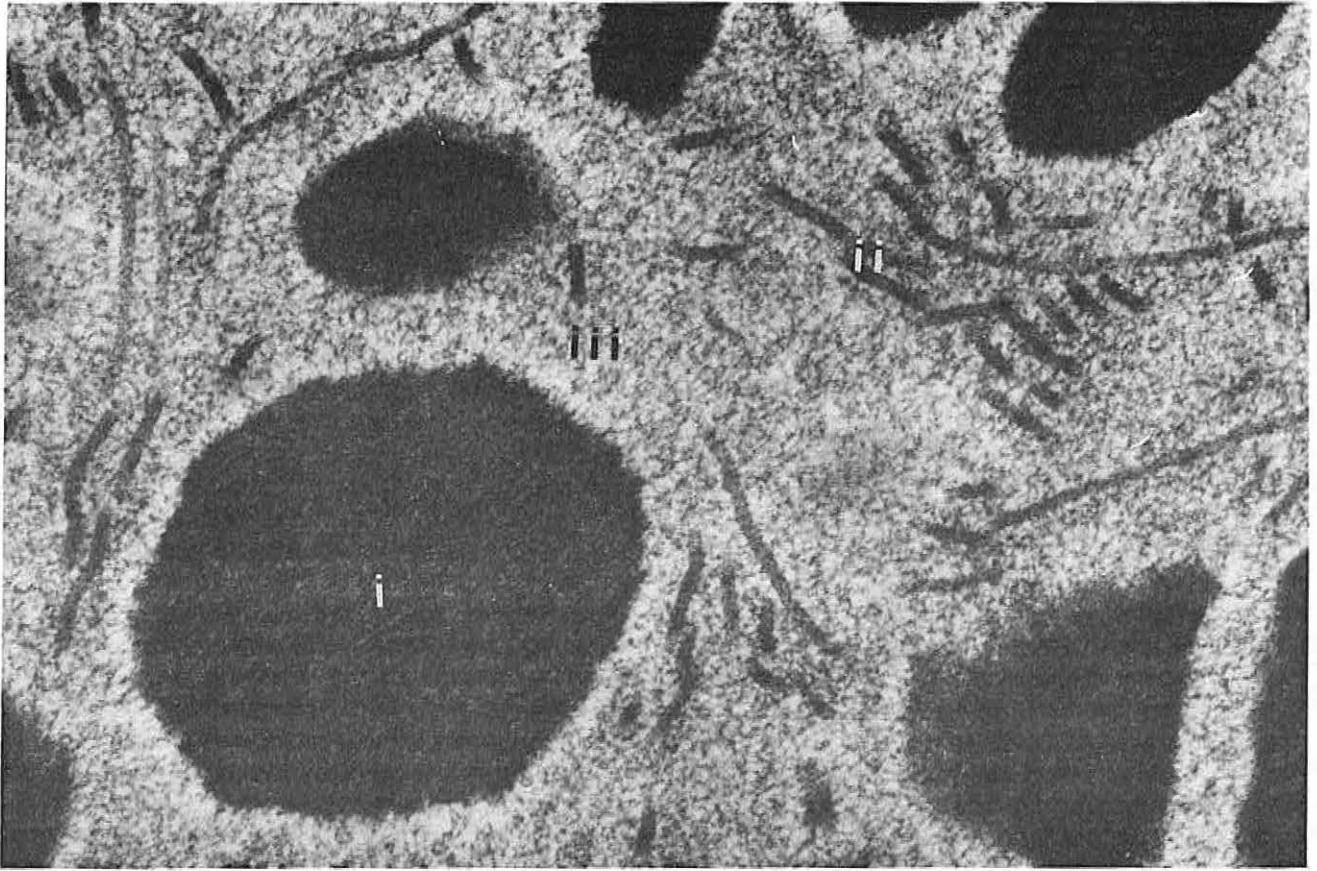
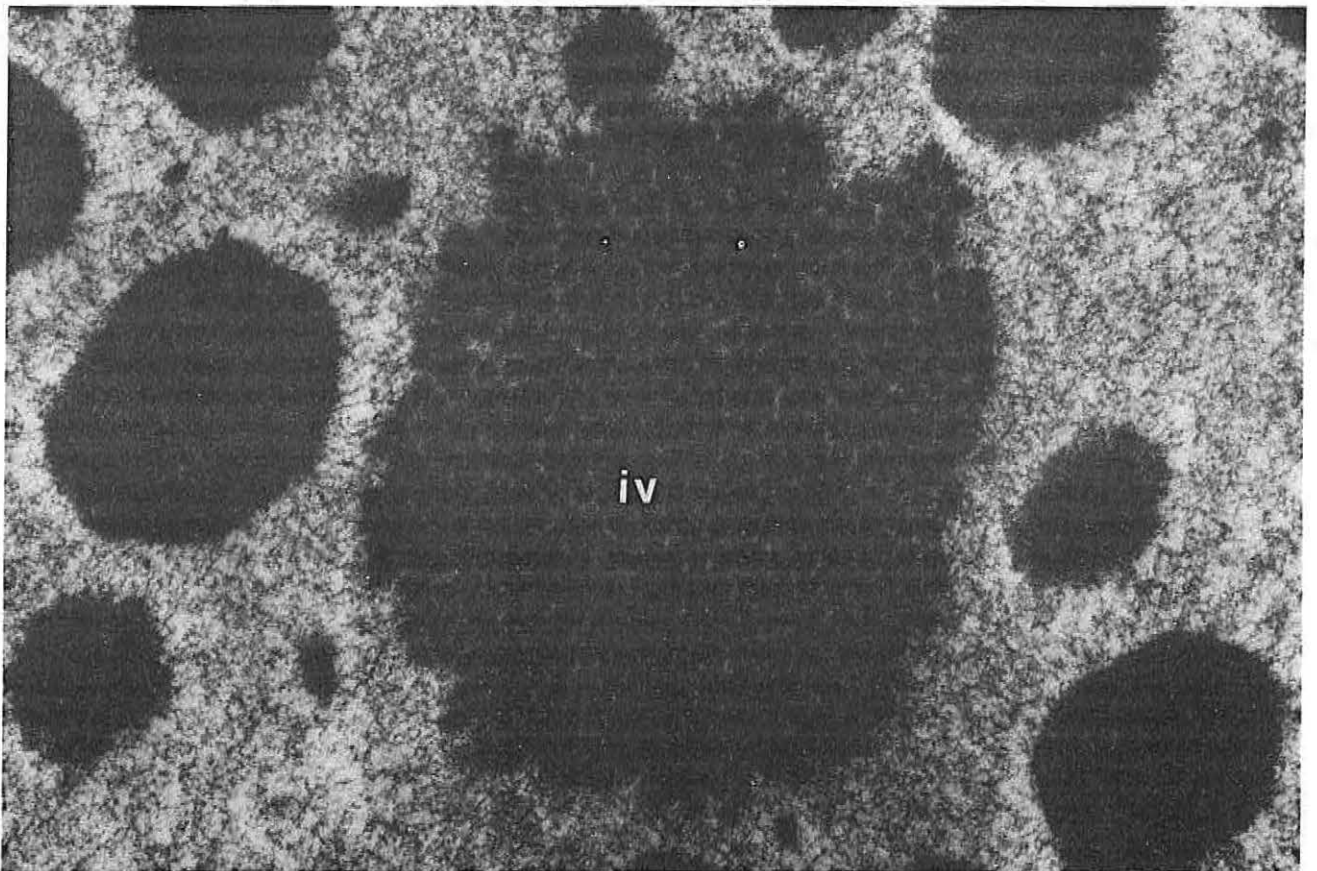


Figure 9. Survey micrograph of 'absorptive' midgut cell. Typical are apical mitochondria (m). Unlike secretory cells the mitochondria (m) especially in the basal region, are often associated with the lateral infolding (li) and basal infoldings (bi). pm: peritrophic membrane



Figures 10 and 11. Types of accessory gland secretions ($\times 70,000$)



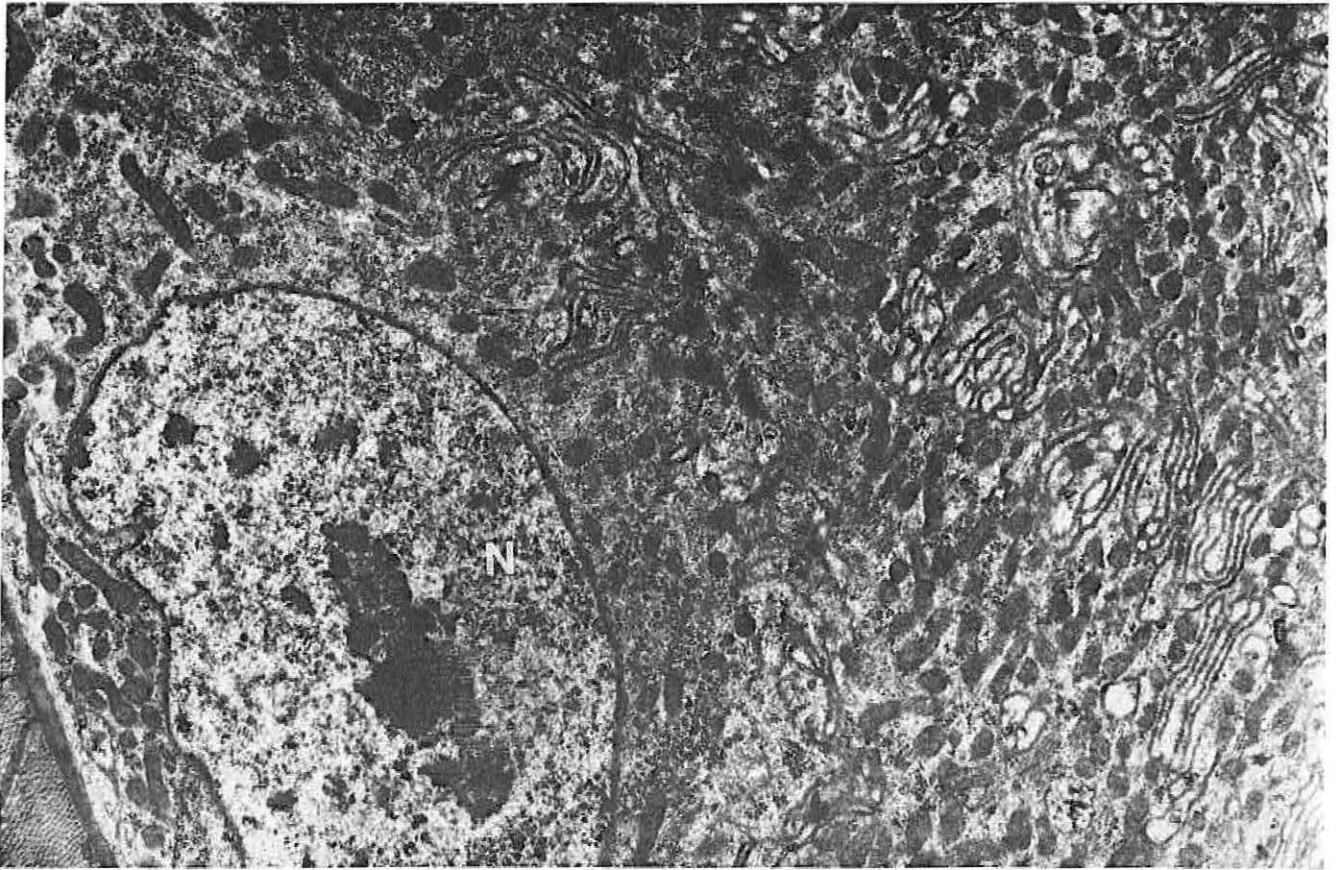


Figure 12. Portion of spermathecal duct in a male virgin adult *Glossina morsitans* illustrates cell type I with a distinct nucleus (N) with the surrounding cytoplasm ($\times 15,700$)

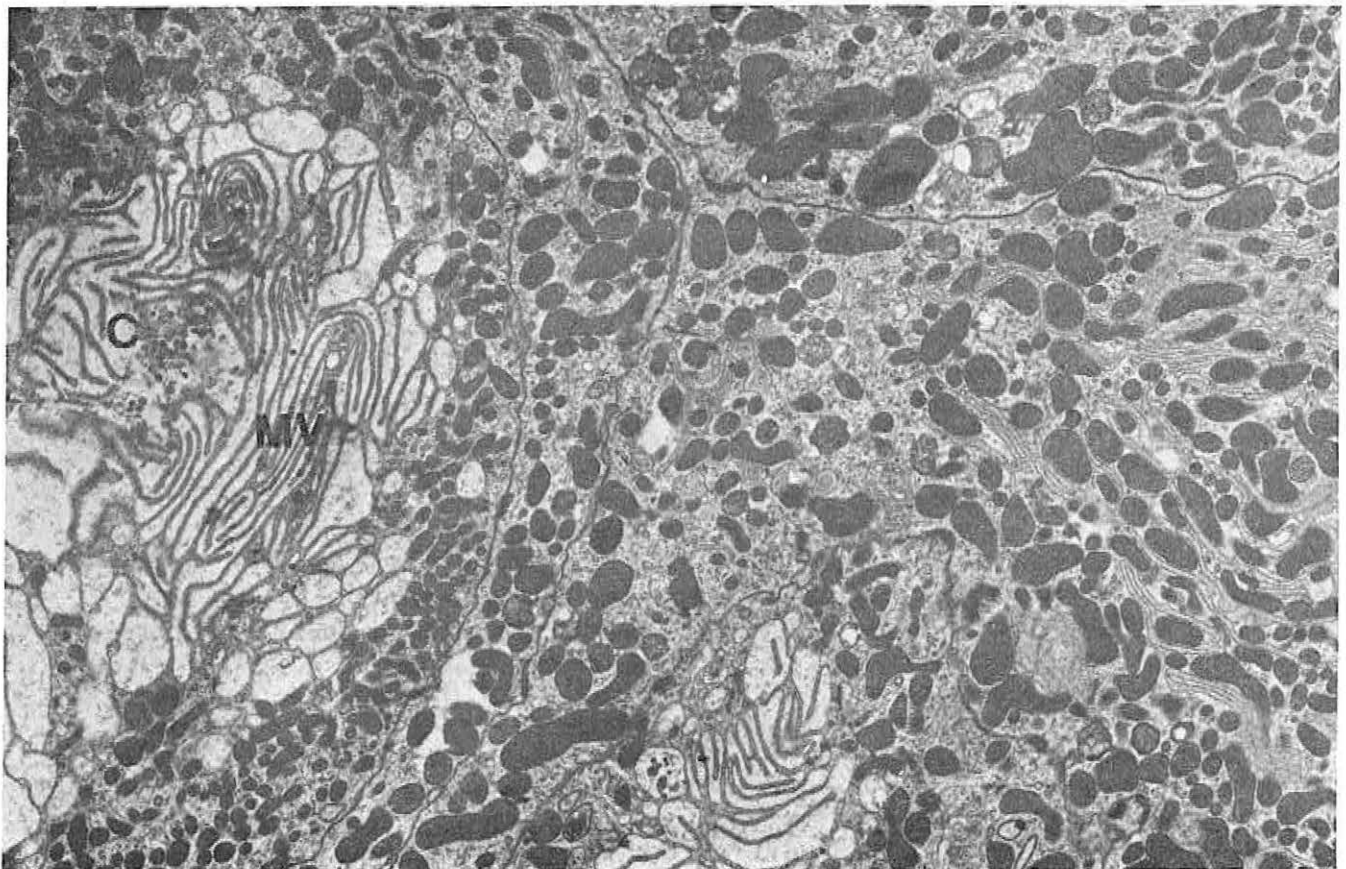


Figure 13. Details of cell type II at the level of microvilli (MV) radiating from a secretory containing cavity (C) ($\times 10,120$)

SENSORY PHYSIOLOGY RESEARCH UNIT

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Visiting Director of Research
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Dr. J. MacFarlane (1977) Research Scientist
Mr. M. Nabil (1978) Technician
Mr. R. K. Saini (1976) Associate Scientific Officer
Dr. S. Waladde (1978) Postdoctoral Research Fellow

Gustatory responses of *Spodoptera exempta*

J. V. Clark

The responses of the sucrose receptors in the styloconic sensilla of *Spodoptera exempta* have been studied in the fifth and sixth instars, and as previously reported (ICIPE Annual Report, 1977) the thresholds found are below 10^{-6} M sucrose for the lateral sensillum and between 10^{-2} M and 10^{-3} M sucrose for the medial sensillum in sixth instar larvae (Figure 1). The threshold for fifth instar larvae seems to be slightly higher for the medial sensillum than in sixth instar larvae, the impulse frequency at 10^{-2} M sucrose being significantly different from the control level at only 10% (sum of squares test) (Figure 2). The impulse frequency values for the receptors have been determined using a data scoring interval of 100msec after a 20msec delay to allow for the contact artefact caused by the first contact of the electrode with the sensillum: longer scoring intervals have revealed a raised threshold for the lateral sensillum, but it would seem that the 100 msec scoring interval is the closest of those tried to the contact period of the sensilla with the leaf during feeding.

The diminution of the receptor response observed for sixth instar larvae at the highest stimulus concentration (10^0 M sucrose) was found not to be due to increased osmotic pressure, as shown by using various concentrations of a non-stimulating chemical (mannitol) to raise the osmotic pressure of the stimulating solution, which consisted of 10^{-1} M sucrose with 10^{-1} M NaCl as electrolyte.

Bioassay studies of feeding activity

Feeding experiments using sucrose incorporated into an agar/acellulose medium (for details see ICIPE Annual Report, 1977) place the threshold of the feeding response, as measured by faecal pellet production, at between 10^{-2} M and 10^{-3} M sucrose. This does not disagree with the reported concentration of at least 6mM

sucrose in leaves of *Zea mays*. However, using glass fibre filter paper discs dipped in varying concentrations of sucrose as the feeding substrate, experiments have shown that the feeding threshold as determined by faecal pellet production is one order of magnitude higher than that determined using the amount of substrate eaten. This suggests that the threshold shown in Figure 3 may be misleading.

The agar/acellulose assay has been used to test for feeding activity with the levels of sucrose and adenosine reported for *Zea mays* of 6mM and 2mM respectively. Feeding levels significantly different from control were found with 6mM sucrose, but not with 2mM adenosine (Table 1). This latter result may be due to the use of the faecal pellet production as the index of feeding activity—with the agar/acellulose method the use of weight of substrate eaten is not a practicable index as the loss of weight of the substrate is comparable to the weight lost through feeding at near threshold concentrations. Adenosine and sucrose at the concentrations used were not synergistic when

Table 1. Feeding activity with various feeding stimulants. Mean dry faeces weight column shows means with standard deviations

Feeding stimulant	Substrate composition (w/v)	No. of larvae	Mean dry faeces wt. (mg)	Mean larval wt. change
Control	4% agar 4% alphacel	13	0.4 ± 0.1	-13%
2mM Adenosine	4% agar 4% alphacel	17	1.1 ± 0.5	-9%
6mM Sucrose	4% agar 4% alphacel	16	2.5 ± 0.6	-11%
2mM Adenosine 6mM Sucrose	4% agar 4% alphacel	21	2.2 ± 0.5	-9%
2mM Adenosine 6mM Sucrose	2% agar 20% alphacel	21	2.6 ± 0.6	-3%
11% w/v Maize Leaf Powder	3% agar	25	44.0 ± 4.1	×49%

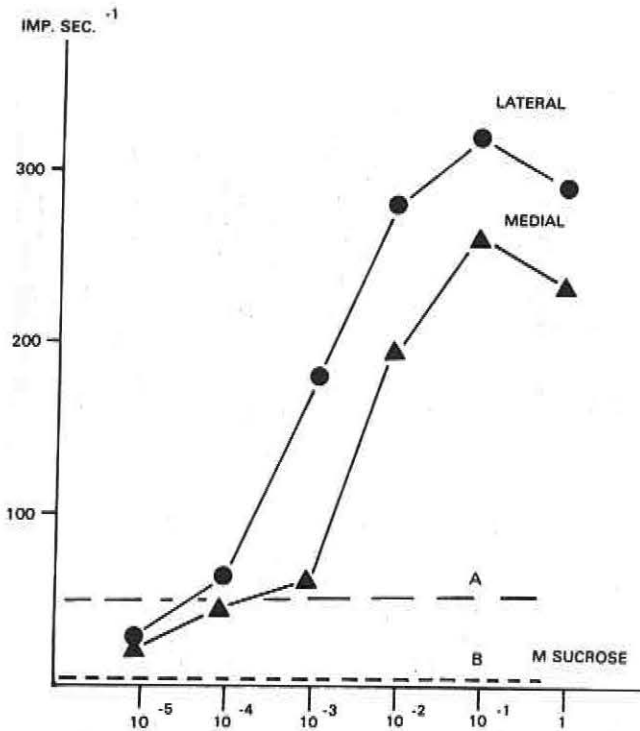


Figure 1. Impulse activity at different sucrose concentrations for the lateral and medial styloconic sensilla of sixth instar larvae. Data averaged from five preparations. Electrolyte (10^{-3} M to 10^{-2} M NaCl) spiking levels shown by lines A and B (medial and lateral receptors respectively)

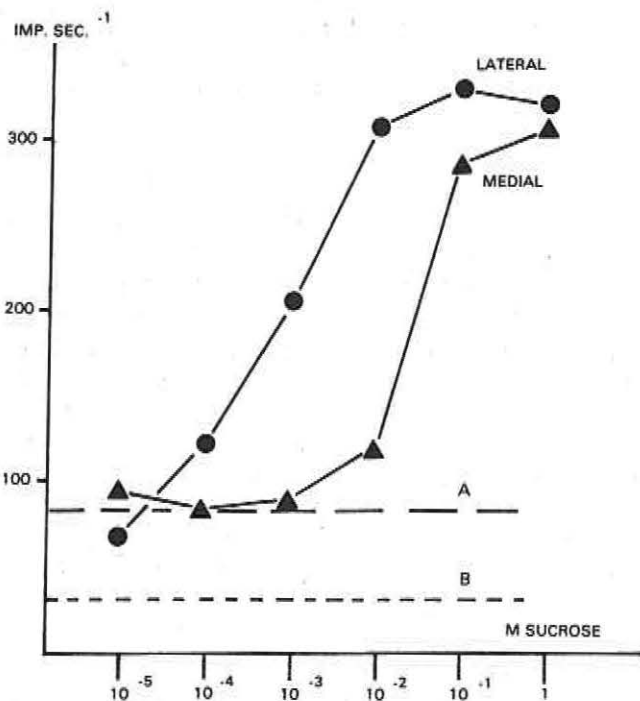


Figure 2. Impulse activity at different sucrose concentrations for lateral and medial styloconic sensilla of fifth instar larvae. Data averaged from five preparations. Electrolyte (10^{-3} M NaCl) spiking levels shown by lines A and B (medial and lateral receptors respectively)

tested together, and an increase in the dry matter content of the medium, making it more comparable in this respect to maize leaves, did not produce any significant increase in feeding. It was found that feeding activity on a medium incorporating dried maize leaf powder produced a level of feeding activity far above that on sucrose medium alone, suggesting that phagostimulants other than sucrose and adenosine are present in dried maize leaf powder. Using a glass fibre filter paper bioassay, in which discs of glass fibre filter paper dipped in test solutions are used as the feeding substrate, significant feeding activity has been demonstrated both with water and acetone extracts from *Zea mays* leaves.

The agar/ α cellulose bioassay has been used also to study the feeding response to muzigadial, an anti-feedant extracted from *Warburgia ugandensis*. The larvae of *Spodoptera exempta* were tested at concentrations of 0.1ppm to 100ppm of muzigadial, at two levels of sucrose in the substrate, 10^{-1} M and 10^{-2} M. The muzigadial was first dissolved in 2.5ml of ethanol,

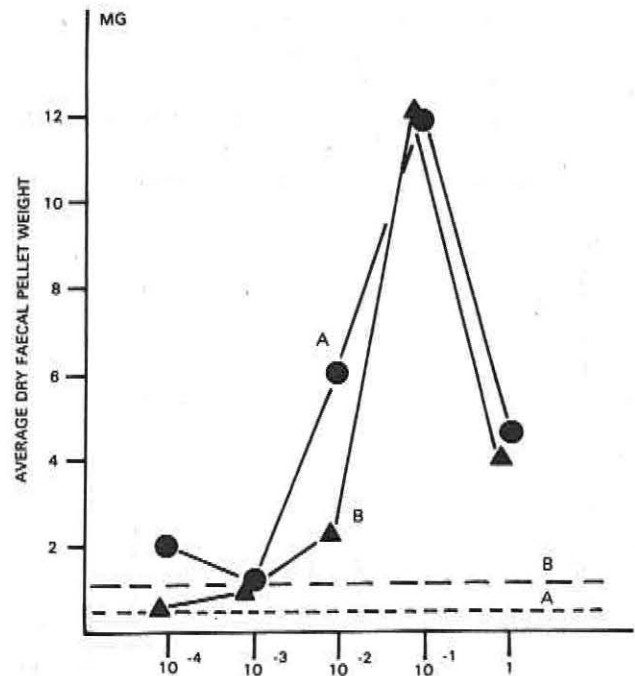


Figure 3. Faecal pellet production over 24 hours of sixth instar larvae feeding on an agar/cellulose medium with different concentrations of sucrose. Results from two different assays. Control levels shown by the two lines A and B

and then the solution made up to 50ml with deionised water. Care was taken to keep the temperature below 50°C when incorporating it into the agar/ α cellulose medium. No feeding responses significantly different from control were found at any of the concentrations tested. These results seemed at first to be consistent with electrophysiological recording, which showed that bursting activity elicited by muzigadial from the styloconic sensilla and galeal palps occurred only at a con-

centration of 1,000ppm. However, this same solution, when painted on two quadrants of a square of maize leaf, did not prevent sixth instar larvae from feeding on the treated sectors as much as on the untreated sectors. Thus it seems, at least under the conditions of the experiment, that electrophysiological activity was not correlated with feeding activity.

Behaviour of *Macrotermes*

J. MacFarlane

Gallery building by *Macrotermes*

It has been proposed that the concentration of trail pheromone on the foraging trails of termites determines the size (height and width) of the galleries constructed over these trails.

The effect of increasing the number of termites on the trail (traffic density) on the gallery

It is assumed in these experiments that each termite using the trail reinforces the concentration of trail pheromone, that is the concentration of trail pheromone is directly related to the traffic density on the trail. The width of the gallery in these studies was confined to 1.0cm. The height of the gallery increased as the traffic density increased (Figure 4) at most of the traffic densities tested. The only exception was

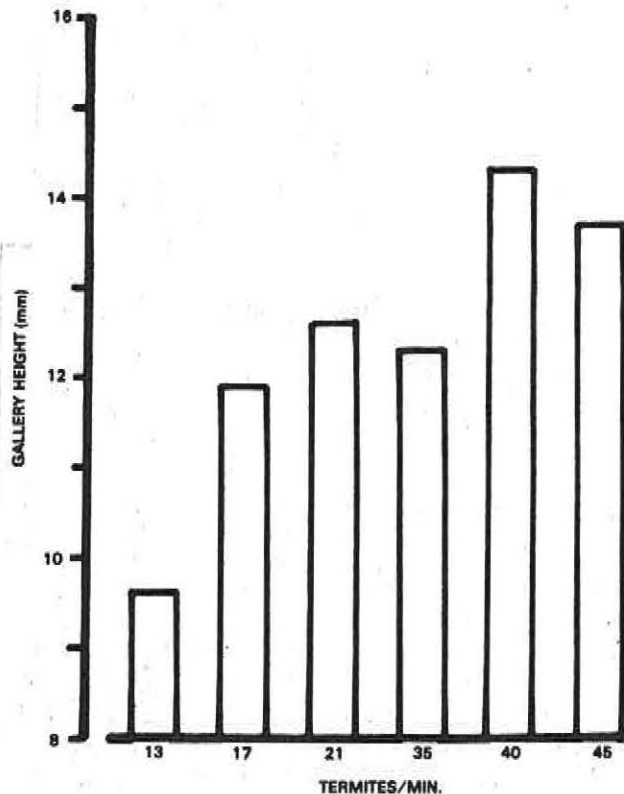


Figure 4. The effect of the number of termites using the trail (traffic density) or trail pheromone concentration on gallery height. Gallery width 1cm

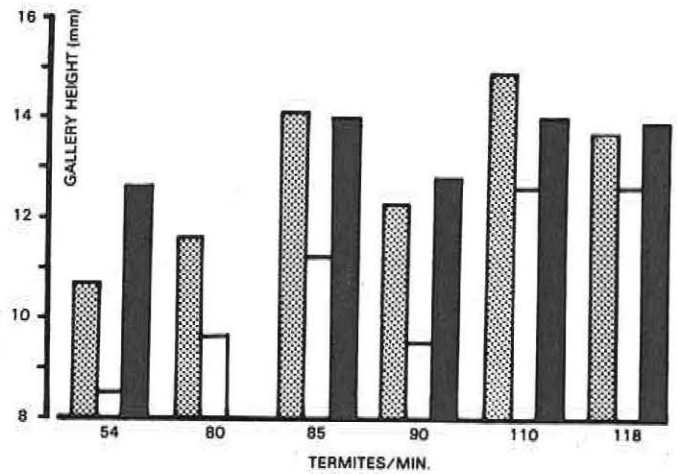


Figure 5. The effect of reducing trail pheromone concentration by a moving strip of paper (4 cm/min) in part of the trail on gallery height. Gallery width 2cm

stipled area — height of gallery before moving paper
white area — height of gallery over moving paper
black area — height of gallery after moving paper

at a traffic density of 45 termites/min using termites 24hr after collection, whose gallery was lower than that of termites 3–6hr after collection at both lower and higher traffic densities (Figure 4). It also has been observed that termites more than 48hr after collection build very slowly and rarely complete the construction of a gallery. The lowest traffic density and therefore the lowest trail pheromone concentration for the construction of a gallery is less than 15/min.

The effect of reducing trail pheromone concentration in part of the foraging trail

The pheromone concentration was reduced in an 8cm section of the 24cm foraging trail by a continuously moving (4cm/min) strip of paper. The gallery width was fixed at 2.0cm. The height of the gallery constructed was reduced over the moving paper at all traffic densities tested (Figure 5). It was also observed that the height of the gallery increased at traffic densities up to 85/min and then remained relatively constant. The trail pheromone concentration on the trail reaches saturation at traffic density of 85/min when the maximum height of the gallery is reached. Termites at traffic densities of 90/min and 118/min (24hr after collection) constructed lower galleries than termites 3–6hr after collection at lower and higher traffic densities.

Trail pheromone extracts

Trail pheromone activity has been demonstrated in hexane extracts of major workers, female alates and male alates. This is the first demonstration of trail pheromone in male and female *Macrotermes*. The relative amount of trail pheromone in these castes is at present unknown. The other castes, minor worker, major

soldier and minor soldier have not been tested for trail pheromone activity.

Natural trail pheromone and trail laying: In some initial studies a trail laid by a single major worker was effective for approximately 2-3 min while trails of 5-10 major workers lasted for approximately 6 min. The major problem in this study is to determine when the termites are actually laying trails. In some initial studies there is an indication that the abdomen does not touch the ground as in *Zootermopsis nevadensis*. It appears that only the area of the sternum where the sternal gland, source of trail pheromone, is located, is partially lowered so that the hairs touch the ground. These hairs then spread the pheromone on the grounds

but at present it is unknown if the trail is continuous or discontinuous as with some ants.

Histology of the sternal gland: A well developed sternal gland is found in all castes of *Macrotermes*. Although trail pheromone activity has been demonstrated only in female alates, male alates and major workers, on the basis of presence of the sternal gland, it is probably present in the other castes although not as pronounced. An early estimate of the size of the sternal gland and hence the amount of trail pheromone is of the following order: female alate < male alate < major worker < minor worker < (?) minor soldier < major soldier.

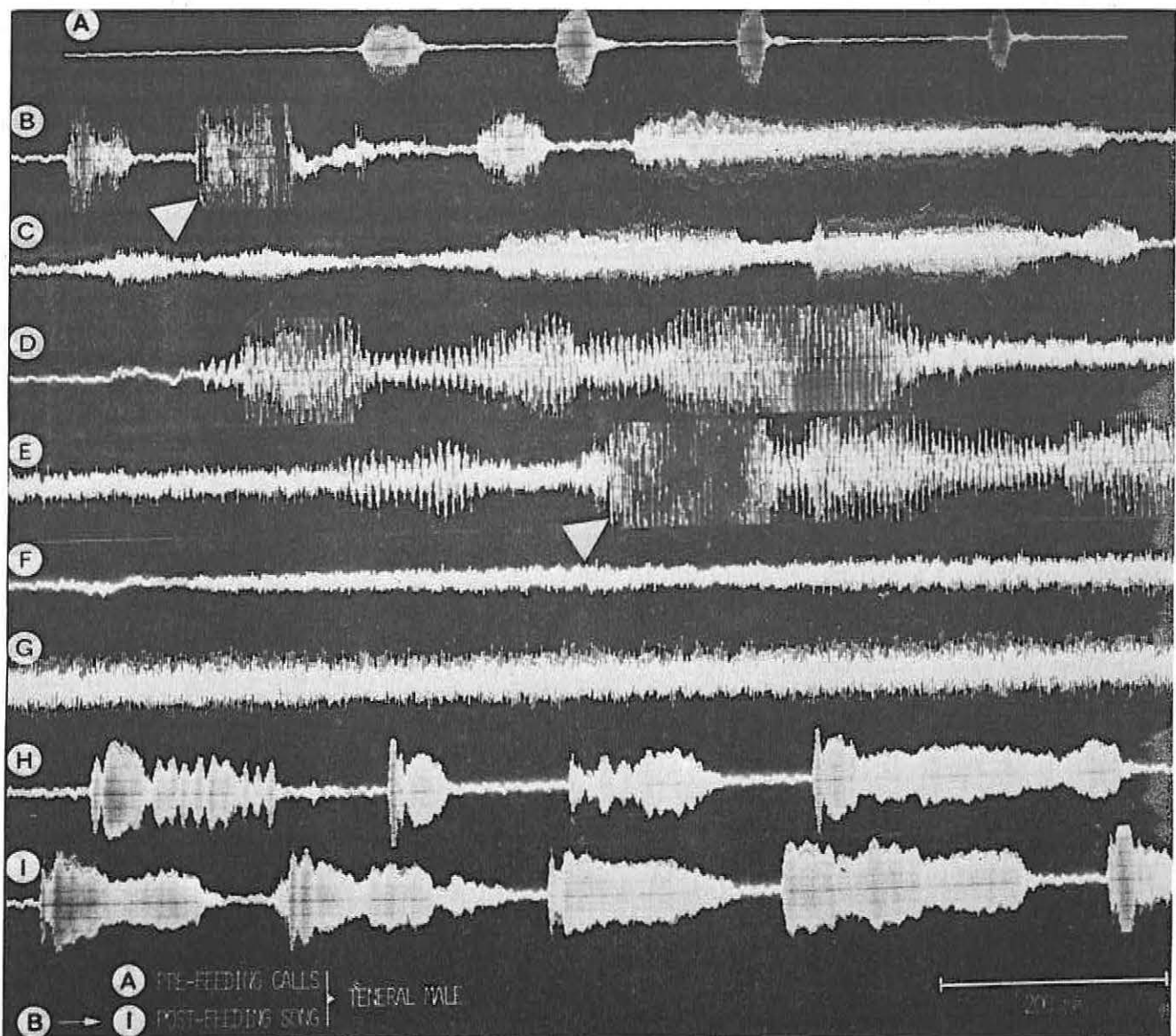


Figure 6. An oscillograph of the pre and post feeding songs of a teneral male
 A — pre feeding calls near the host (rabbit)
 B — post feeding song immediately after feeding

B — E post feeding song immediately after feeding
 F — I post feeding song half an hour after feeding
 ▲ wing fluttering

Acoustic component of tsetse fly communication

R. K. Saini

It has been known for a long time that various species of tsetse flies produce modulated sounds which have consequently been suggested as a means of communication. They are closely related to the vital functions of the community, namely, hunting, feeding and mating. So far, no experimental proof has been put forth showing that sound is used by any species of tsetse for communication. Preliminary studies conducted by Erickson and Møller (ICIPE Annual Report, 1974) show that the spectrum of the sounds produced by the tsetse flies *G. morsitans morsitans* extends to about 80 KHz. They hypothesize that sound may constitute a means of communication between the tsetse flies and that the ultrasonic components of the sounds i.e. between

20—70 KHz, likely carry the most important part of the information.

After the establishment of a fully fledged acoustic laboratory at ICIPE, studies have been initiated on *G. morsitans* to determine:

- (i) What are the characteristics of the sounds produced as related to the various activities of the flies
- (ii) How do the flies produce these sounds

A tsetse fly of whatever age or sex emits modulated sounds, in solo or in concert. Singing can be elicited by external movements of their vials, movement to and from a light source or even by the calling of other flies. Complete darkness inhibits singing. When singing flies (especially gorged flies) are brought near a number of flies in separate vials in darkness, the flies in darkness start to sing. When singing in concert the flies often start and stop together.

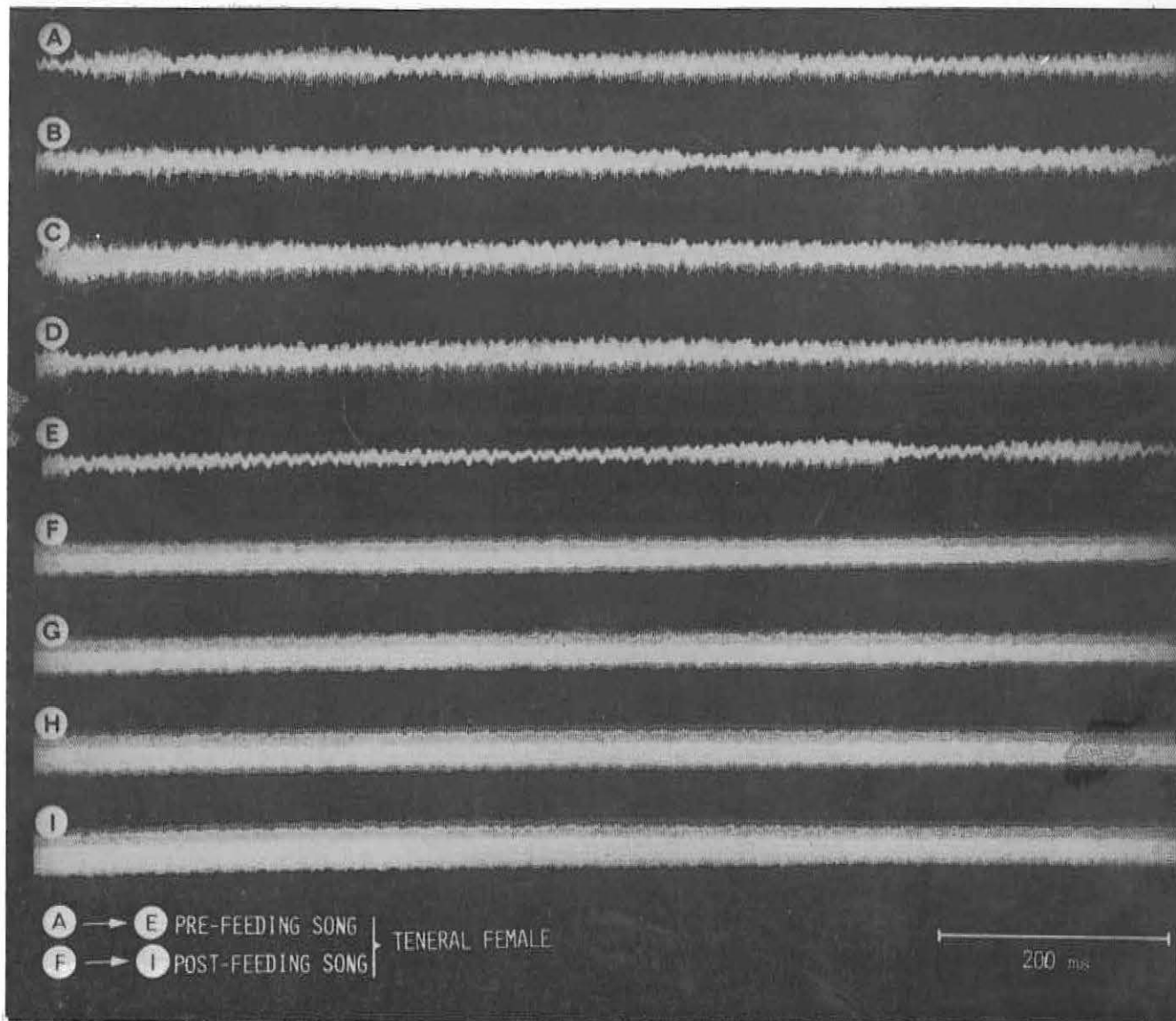


Figure 7. An oscillograph of the pre and post feeding of a teneral female A — E pre feeding song near the host F — I post feeding song after the host has been removed

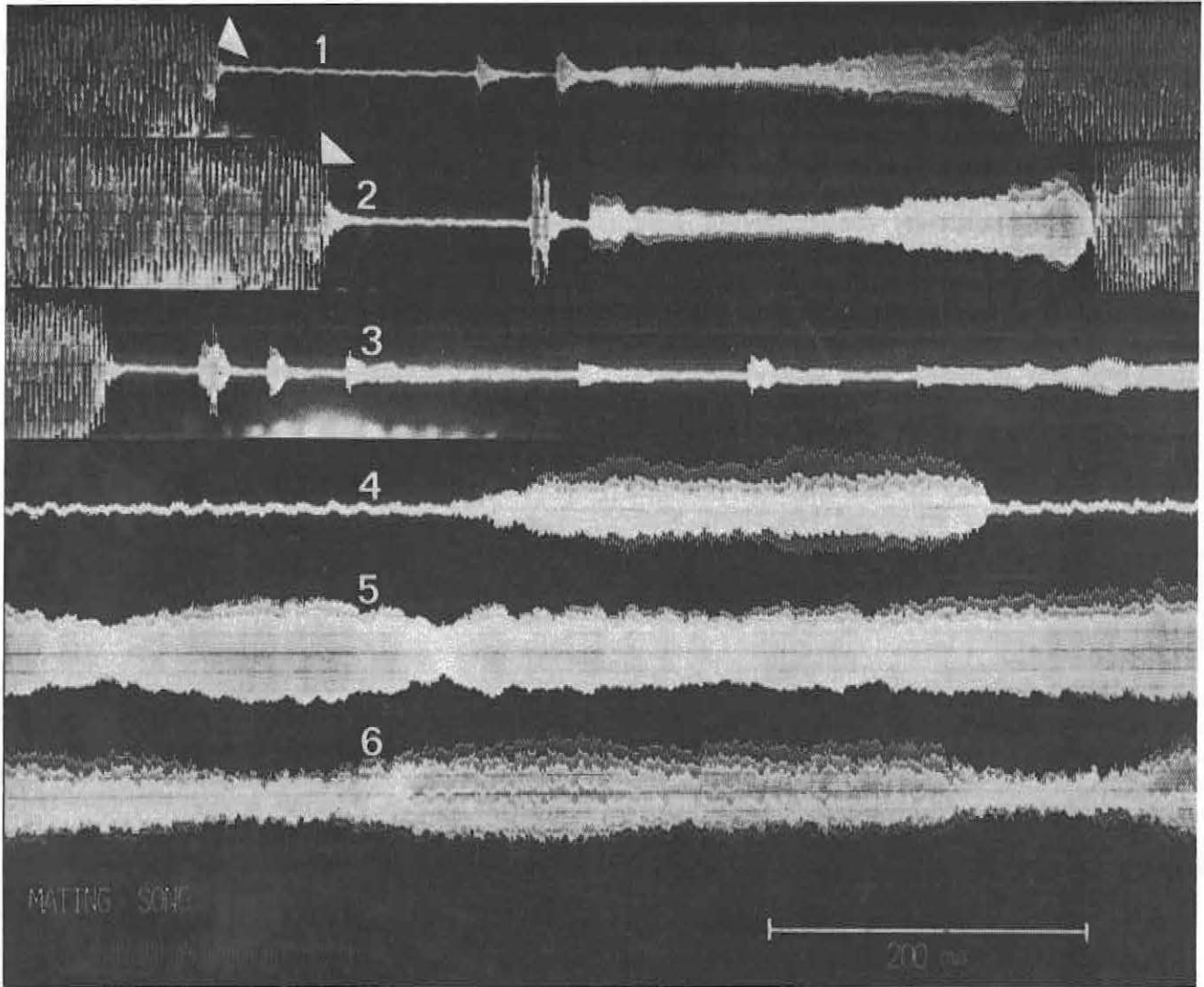


Figure 8. An oscillograph of the mating song between a 2 day old female and a 2 week old male
1 to 3 singing when male is trying to introduce the adeagus 4 to 6 mating song in copula
▲ indicates flight

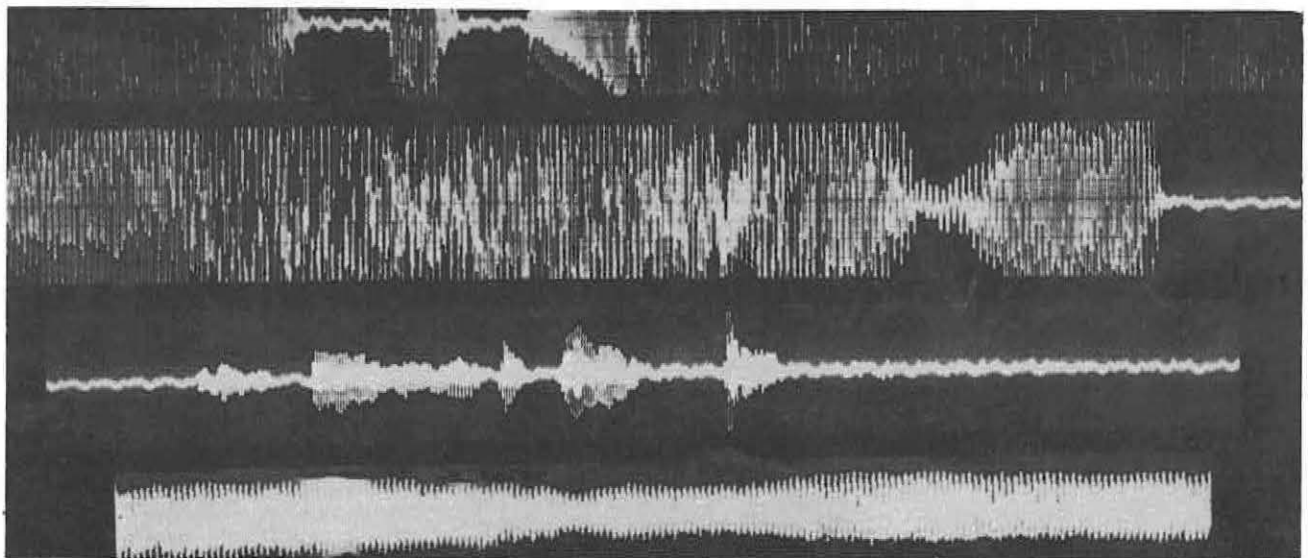


Figure 9. An oscillograph of the distress song made when a 2 week old male tries to mate with a 1 week old female

Sensory Physiology

Basically two sound patterns can be recognized, an interrupted call and a continuous song (Figures 6, 7 and 8). The sounds vary in pitch and amplitude, while those of a teneral female usually have a warbling quality. The ability to sing improves with age, perhaps due to the better development of the thoracic musculature. The calls vary considerably in pitch, tone, pattern and duration and are often followed by one or more bursts of wing fluttering. The distress call is heard whenever the fly is handled. Similarly, when the female resists the male's copulatory efforts, distress calls may be heard. These calls usually develop into a song and are almost always interrupted by violent wing fluttering. (Figure 9) The pre-feeding song is usually followed by wing fluttering. Post-feeding songs are produced as and after the proboscis is withdrawn when flies are gorged with blood. Semi-gorged flies seldom produce sounds. The post-feeding song usually lasts longer and terminates without any wing fluttering. The female song usually lasts longer than the average male's.

Sound production

It is generally accepted that the flies are capable of producing sounds of two types. The first type of sound is a chirping and/or whining which is audible to man and the second type of sound is a complex pattern in the ultrasonic frequencies. The methods by which these sounds are produced are under investigation.

Preliminary results show that sound is primarily produced by muscular contractions which cause rapid vibratory movements of the pteropleural area, perialar membranes and one or sometimes both wings. Amputation of both wings and/or halteres and blocking of the thoracic spiracles with vaseline does not inhibit sound production. Neither does amputation of the abdomen of a singing fly halt sound production. Hence it is the thorax of these flies which produces these complex sounds. The thoracic mechanism which produces these modulated sounds however, is not yet known. Although the wings are not required for actual sound production, they probably do aid in transferring the sounds to the air since their amputation changes the tone slightly.

Tick sensory physiology

S. M. Waladde

As shown in the ICIPE Annual Report, 1973, it has been a prime concern of the tick, project to elucidate on the sensory basis underlying host selection, successful feeding engorgement and reproduction. In order to continue with this programme, more emphasis is being directed towards examining the behavioural and chemosensory studies of Ixodid ticks, especially

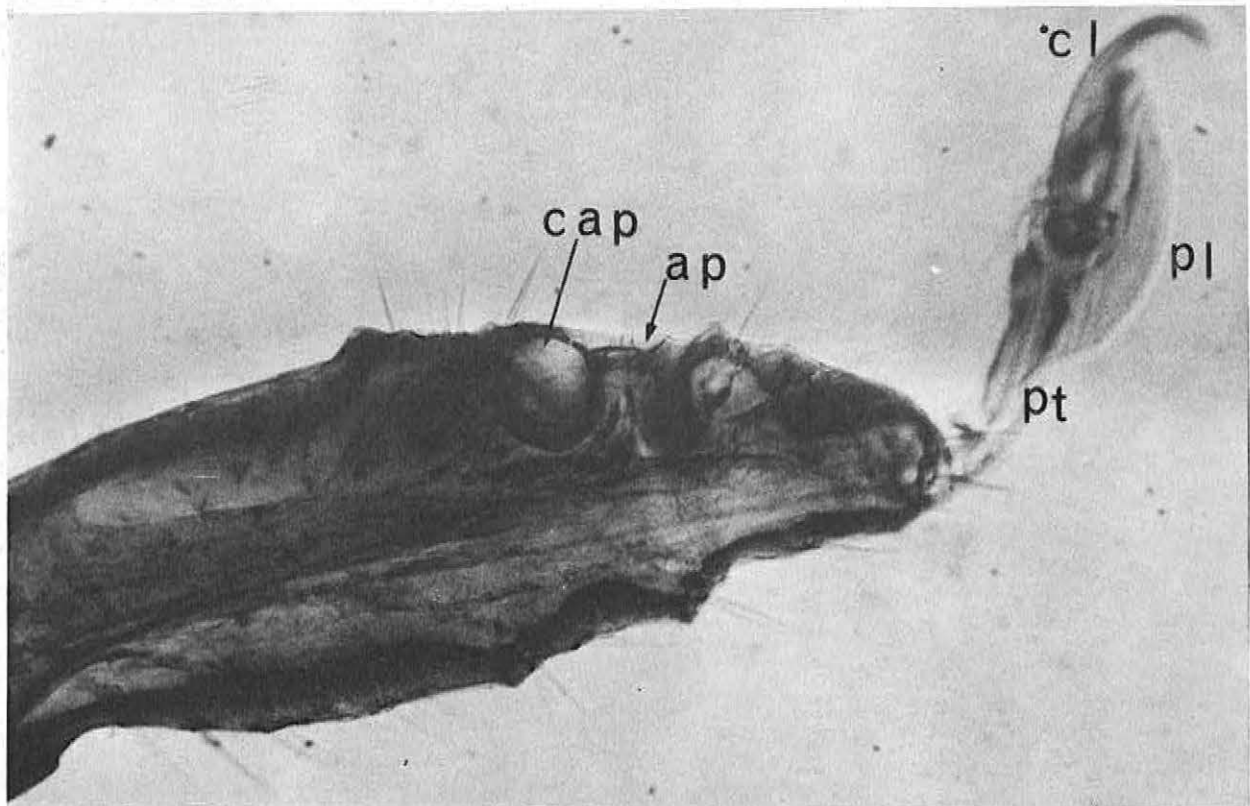


Figure 10. Whole mount of tarsus I from the adult *Rhipicephalus appendiculatus* showing nerve processes to the sensilla stained with methylene blue: note Haller's organ consisting of anterior pit (ap) and posterior capsule (cap), pretarsus (pt), pulvillus (pl) and claw (cl)

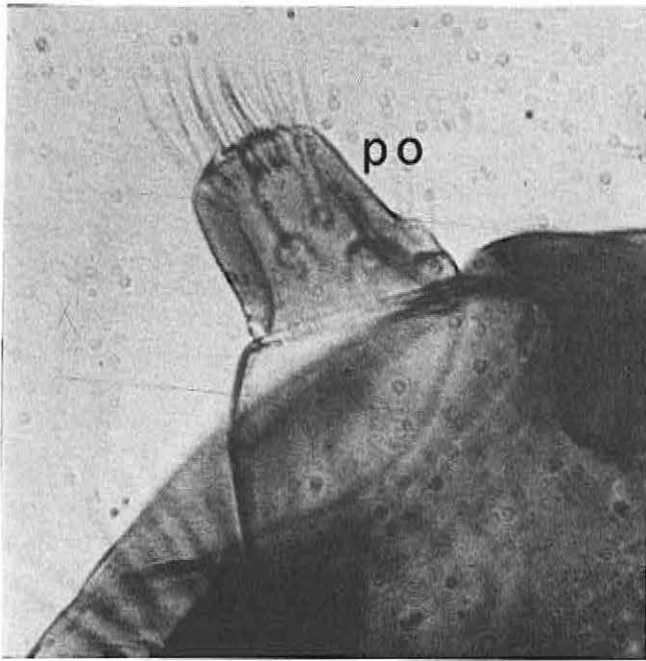


Figure 11. Whole mount of the third segment of the palpal unit bearing a pseudosegment known as the palpal organ (po) from the adult *Rhipicephalus appendiculatus*

Rhipicephalus appendiculatus and *Amblyomma variegatum*.

This work covers three main areas of research:

(i) To examine tick responses to natural stimuli under laboratory conditions. This work will be divided into three phases whereby larvae, nymphs and adults will be observed separately. Observations on what happens to certain patterns of tick behaviour when one or groups of target sensilla are ablated will be carried out. This approach may lead to the identification of biologically active compounds (tick pheromones, sex pheromones kairomones) which govern tick behaviour on and off the host.

(ii) Electrophysiological recordings from groups of sensilla or single dendrites within a particular sensillum will be done in order to identify the effective stimuli, (physical and chemical) of the target sensilla. When this is done in connection with ultrastructural studies, it will be possible to characterize the functions of several sensilla especially those on the tarsi and mouthparts.

(iii) Other disciplines such as biochemistry and organic chemistry may provide advice on how to obtain relatively pure forms of the natural chemical stimuli or their analogs. These compounds will be used in behavioural studies and electrophysiological tests on either gustatory or olfactory receptors. Field studies might be designed so that laboratory observations are verified under field conditions.

Preliminary observations carried out on the tick species mentioned above, have shown that the pattern of sensory receptors on the mouthparts and the forelegs are possibly similar to those found on other tick species (Figures 10 and 11). However, electrophysiological records obtained from certain sensilla show responses which have not been reported before (Figure 12). As the three areas of research outlined above mature, a comprehensive explanation for the observed phenomena may emerge.

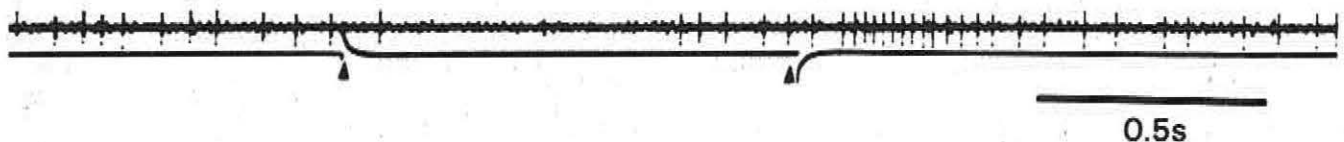


Figure 12. Recent olfactory responses from a sensilla (to be known as md 7) situated on the dorsal surface posterior to the posterior capsule. The onset and end of the air stimulus application is indicated by the position of the triangular spots below the bottom trace. Note the inhibitory effect of air and the transient increase in the impulse frequency after the application of the stimulus

INSECT AND ANIMAL BREEDING UNIT

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Dr. A. Basu (1976-1978)

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Breeding experiments

G. Basu

Experimental breeding of *Glossina morsitans* using goats as host animals

With the idea of developing a colony of *Glossina morsitans* using goats as host animals, as well as to compare this performance with the original colony, feeding of flies on goats has been undertaken. If suitable a separate colony will be established using goats as hosts.

Six healthy goats (5 castrated, 1 un-castrated) were procured from a farm near Longonot (Naivasha). They were kept under observation and quarantine for 3 weeks. During this period they were checked regularly for any external parasite, body weight was checked, each one was numbered, stool was examined for any parasitic infection and blood was examined for trypanosome infection and for haemoglobin %, which varied between 9.5 to 10.2g%.

They were cleaned with chloroform and were washed with Teepol. Hairs were clipped from flanks with a hair clipper. A surgical instrument trolley was modified to a trevids for restraining the animal during feeding of flies. Canvas belts and elastic bands were used to restrain the animal and two cages of flies were fixed on each flank at a time.

A hundred each of newly emerged male and female flies were introduced to each goat. Flies were fed six days a week (excluding Sundays). Thus each goat was used once a week. The number of flies per goat were increased to 240 females and 200 males in subsequent weeks. The first batch of flies were mated after 7 days. Mated flies were separated after 5 days and the females kept in a larviposition tray with 10 females in each cage and six cages in one tray.

The first batch of females started laying pupae 4 days after separation, all the pupae collected were weighed and kept in pools as a single day's produce. A hundred and fourteen produced between 18/8/78 and 28/8/78 were transferred to an emergence cage on 12/9/78. The first batch of flies (2 females) emerged on 17/9/78.

Preliminary observations

The newly emerged flies from the rabbit colony fed well on goats. The goats, though restless during feeding, have not yet shown any skin reaction.

Goat's blood was checked every week for Hb% and has not so far shown any significant difference, it is also negative to trypanosomes.

Two hundred and eighty-five pupae have been produced between 18/8/78 and 19/9/78. Total weight of these pupae is 8.5288gm with a mean weight of 29.29.9256mg.

Experimental breeding of *Glossina pallidipes*

Work has been started for establishing a colony of *G. pallidipes* as follows:

- (i) A small wire netted structure with grass roof will be constructed at Mbita Point Field Station.
- (ii) Two adult goats will be kept in this structure.
- (iii) *G. pallidipes* will be collected from the Lambwe Valley at regular intervals and will be released into this structure from time to time. It is assumed that the flies will feed on these animals and larviposit there.
- (iv) These pupae will be collected and brought to ICIPE, Chiromo, at regular intervals.
- (v) Flies emerging from these pupae will be fed on rabbit ears and further breeding will be tried in the method used for *G. morsitans* breeding.
- (vi) Side by side with the goats some rabbits will also be kept at the Field Station. Wild flies kept in PVC cages will be fed on rabbit ears and further breeding will be tried in the normal laboratory climate conditions of Mbita Point.

Work has already been started with a visit to the Lambwe Valley and a preliminary survey of the population. Olando, Ruma and Guasi areas in the Valley were selected for setting up Langridges traps. Twenty-four hours' collection was checked every morning. Highest number of flies were collected at the Ruma area. On an average, 55% were matured flies and the rest were

tenerals. People at Sindo Tsetse Camp expect a higher population by the middle of October.

A site has been selected at Mbita Point Field Station where the proposed holding structure will be set up. We are getting 6 Langridge's traps made in our workshop as the traps at Sindo Tsetse Camp are quite

old and flies are escaping through leakages. We expect to visit the Lambwe Valley again in the second week of October taking these traps and some rabbits with us. During this visit the holding structure will also be erected. This work is being carried out in collaboration with Dr. R. S. Ochieng'.

INSECT MASS REARING

Research Staff

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Mass rearing work in progress

R. S. Ochieng'

To facilitate research in many aspects of entomology, it is necessary to rear insects under controlled conditions. Successful insect colonization is a basic necessity for efficient and productive research of virtually every aspect of entomology. Colonized insects have been used in studies of the development and behaviour of insects in an effort to devise new ways to control them and to utilize them if they are beneficial. Insects in large numbers may be needed to control others by releasing laboratory sterilized ones, by using caged insects to attract and destroy their own kind, for production and use of attractants, for the production and use of insect pathogens, insect parasites and predators, and for producing growth-relating substances for insect control.

This project has therefore embarked on laboratory rearing of the following insects: sorghum shootfly *Atherigona soccata*, *Chilo zoinnelus* and *Maruca testulalis*. We have also started a survey of the prevalence of *Busseola fusca* in South Nyanza.

Work in progress

At Mbita Point Field Station work has started on sorghum shootfly, *Atherigona soccata*, the legume pod-borer, *Maruca testulalis* and the maize and sorghum stem borer, *Chilo zoinnelus*. Work is done in the field and laboratory. Preliminary field observations at the station show that *Maruca testulalis* is available in reasonable numbers all the year round, provided humidity is sufficiently high. Collections of larvae from the field have been made, and young raised in the laboratory. Young larvae, probably second instar larvae, do survive on artificial diet. Adult moths can

be successfully maintained on glucose with very little mortality. These preliminary observations have now enabled us to locate the oviposition site of the moth and also to identify the structure and type of egg masses produced. Detailed studies of the biology of the moth will be made.

Similar field observations on *Chilo zoinnelus* have shown that at Mbita Point this stem borer is probably the most important pest of sorghum. Field populations have built up high enough to facilitate work on artificial methods of breeding. Cooperative work is also in progress on sorghum shootfly between this project and the sorghum shootfly project. A self sustaining colony has been established by Soto's method.

It is probably pertinent at this stage to point out that the Field Station at Mbita Point is in its early stages of development. At the time we moved into the station, there were no crops at all in the vicinity of the station. A garden, the "Convent Garden" was therefore cleared and sown with maize, sorghum, cowpeas, sweet potatoes, cassava and Canadian Wonder beans. This was aimed at encouraging the insect population to build up. The first crop last year had very low populations of insects, but the second crop sown this year had a good build-up of *Maruca*, *Chilo* and *Atherigona*. The insect populations can be sustained at high level on fresh crops grown roughly twice a month.

The problems that were encountered in the early stages are now being minimized. Insect populations in the field have grown reasonably high and preliminary observations are now being made. The fields have now also been fenced and permanent bird scarers employed.

An irrigation pump has been installed and as a result, crops can now be grown at any time. In the laboratory, power requirements have been met by the replacement of the generator, and water, although untreated, is sufficient for limited working purposes.

INSECT PATHOLOGY

Research Staff

Dr. G. P. Kaaya (1978) Postdoctoral Research Fellow

Dr. W. Otieno (1978) Research Scientist

Insect pathology project

W. Otieno

The Insect Pathology Project has been initiated to:

- (i) carry out field surveys to establish the occurrence and distribution of pathogens, parasites and predators affecting ICIPE's target insects; and to conduct laboratory studies on isolated pathogens. Initial emphasis is being placed on the mosquitoes;
- (ii) function as a supporting service unit to other ICIPE projects in carrying out a regular disease diagnostic service on the target insects reared in the laboratory or collected from the field;
- (iii) evaluate and determine the performance of promising pathogens (originating from other laboratories or agencies) upon local insect disease vectors or pests.

Field surveys

In our field surveys, we have determined the presence of:

- (i) six bacterial species of *Bacillus* to be associated with mosquito larvae collected so far. Tests are underway to determine their true *pathogenicity* or *saprophytic* nature;
- (ii) two pathogenic fungi, namely *Beauveria* species and *Coelomomyces* species have now been isolated from our field sampling. Work is in progress to get these to grow on artificial culture medium;
- (iii) two protozoans, namely, *Vorticella* species and *Rotifer* species. The relationship between these two named Protozoan species and the larval mosquito

host is under investigation.

Service unit

- (i) A regular laboratory disease diagnosis of sorghum shootfly larvae has revealed that a fungus, *Entomophthora* sp. is a persistent pathogen of the larvae. The workshop on the sorghum shootfly research programme has recommended that this collaborative effort be intensified in the search for parasites, predators and pathogens for sorghum shootfly. (Dr. A. Delobel, Collaborator)
 - (ii) A parasite, tentatively identified as a *Trichopria* species (Diapriidae) has been isolated from the ICIPE tsetse colony in Nairobi. Its potential value is under assessment. (Dr. L. H. Otieno, Collaborator)
 - (iii) Over the last six months, the insect Pathology Laboratory has provided diagnostic services to diseased or dead insects from the following ICIPE Programmes, the Termite Programme, the Insect Mass Rearing and Armyworm Programmes.
- ### Pathogens from other world laboratories
- (i) With the assistance of the World Health Organization, the insect Pathology Laboratory received two batches of a promising nematode, *Romanomermis culicivorax* for experimentation.
 - (ii) It is hoped that we shall soon be able to obtain a virulent strain of *Bacillus thuringiensis* from Dr. Margalit for laboratory experimentation. We plan to conduct tests to determine the performance of this promising bacterial pathogen on Dipterous larvae of ICIPE's target insects.

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Note: Figures in parenthesis at the end of each entry indicate the ICIPE serial number. The above publications, including earlier editions of this Annual Report, are available from the Librarian, ICIPE.

CONFERENCES ATTENDED

7th May to 13th May 1978

Conference on the Regulation of Insect Reproduction (II), Zinkovy, Czechoslovakia

Dr. M. F. B. Chaudhury (Tsetse Reproductive Physiology) "Regulation of reproduction in tsetse flies"

6th August to 12th August 1978

Vth International Congress of Acarology, Lansing, Michigan, USA

Dr. R. M. Newson

"The development of *Rhipicephalus appendiculatus* populations at three different host stocking densities"

Dr. F. D. Obenchain

Invitation symposium paper: "Non-acaricidal chemicals for the management of acari of medical and veterinary importance" Contributed paper: "The neuroendocrine complex of *Ornithodoros moubata*"

Mrs. C. K. A. Mango and Mr. L. Moreka

"Moulting hormone activity in the 5th nymphal instar of the tick *Ornithodoros moubata*"

Mr. D. K. Punyua

"Diurnal activity behaviour of *Rhipicephalus appendiculatus* Neumann 1901 in the field"

Mr. A. O. Mongi

"Water relations of the ticks *Rhipicephalus appendiculatus* Neumann 1901 and *Rhipicephalus pulchellus* Gerstaecker 1873"

19th August to 26th August

IVth International Congress of Parasitology, Warsaw, Poland

Professor Thomas R. Odhiambo

Dr. L. H. Otieno (Tsetse Salivary Gland Physiology)

"Rapid transformation in vitro of bloodstream form *Trypanosoma (Trypanozoon) brucei* on incubation with *Glossina morsitans* midgut contents"

28th August to 1st September 1978

Eastern and Southern African sub-Regional Conference on Cooperation in the Control of Animal Health and the Promotion of Livestock Production, Gaborone, Botswana

Dr. M. P. Cunningham (Tick Programme)

Dr. L. H. Otieno (Tsetse Salivary Gland Physiology)

18th September to 22nd September 1978

Research Coordination Meeting of FAO/IAEA on the Use of the Sterile Insect Technique for Tsetse Fly Eradication or Control, Antwerp, Belgium

Dr. M. B. A. Nyindo (Tsetse Salivary Gland Physiology)

"*Trypanosoma brucei*—cultivation in vitro of infective forms derived from the midgut of *Glossina morsitans*" published in J. Parasitol. 64 (3) 469-474