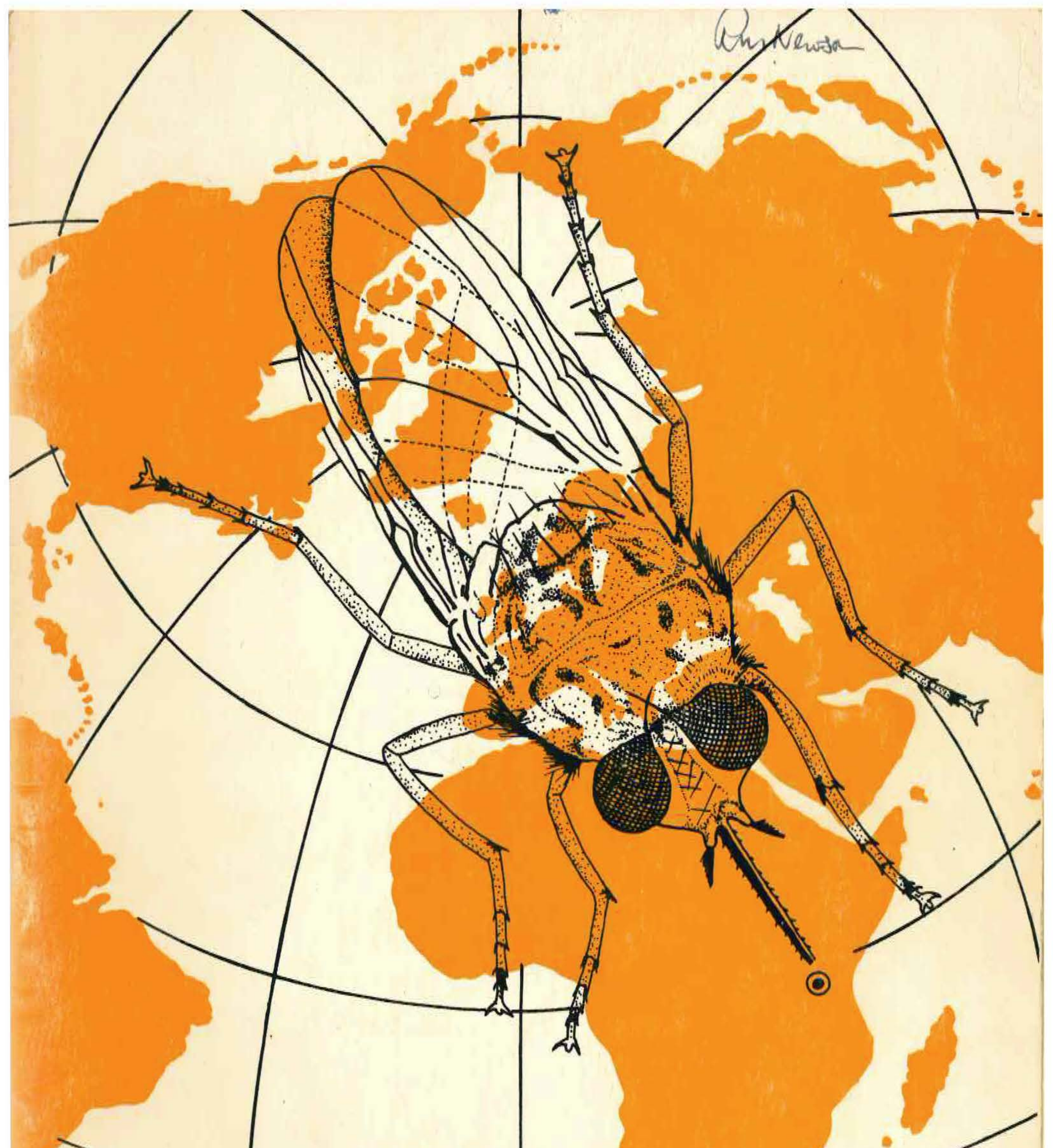

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FOURTH ANNUAL REPORT — 1976

Prof. Thomas R. Odhiambo presenting ICIPE Technicians Certificate to successful technical trainees of the Centre.

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THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

FOURTH ANNUAL REPORT

1976

Nairobi, March 1977

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Studies on interspecific pheromone communications among *Spodoptera* species

S. Khasimuddin

Spodoptera exempta (Wlk.), the African Armyworm, is by far the most prevalent and destructive species of armyworms in East Africa. There are other species of *Spodoptera* which also exist in the same geographic region. Specimens of *S. triturata, S. exigua, S. littoralis,* have been collected in the field together with *S. exempta* during the past few years. However, none of these species has ever been reported to cause any significant damage to their respective host plants.

The sex-pheromones of all these species have been identified (Campion, 1975; Beevor, *et al.*, 1975). *S. exempta* and *S. triturata* have the same pheromone with cis-9, TDA as the main attractant and cis-9, trans 12 TDDA as the secondary attractant (Campion 1975), while *S. exigua* has cis-9, trans-12 TDDA as the main and cis-9 TDA as the secondary attractant. *S. littoralis* females produce cis-9, trans 11, TDDA as the sex-pheromone. It seems therefore that these species utilize the same or similar chemical compounds as their pheromones. As these species are geographically co-existent yet presumably reproductively isolated, the interelationships in terms of pheromones could be very interesting and might throw some light on their comparative ecology.

In Lepidoptera, the phenomenon of various species sharing the same sex-pheromones is not un-common. Gaynard and Brady (1972) report species of *Plodia, Cadra* and *Spodoptera* showing attraction to each other. Klun & Robinson (1972) state that many species may use the same pheromones but avoid pheromonal confusion by responding to specific concentrations/or blends of chemicals characteristic of their own species. Kaae, *et al.*, (1973) discuss the role of sex-pheromones in maintaining reproductive isolation among ten species of Noctuidae. Similar cases of interspecific sex-pheromone related attraction and pheromone specificity in Lepidoptera have been reported by Roelofs & Comeau (1969); Evans (1952); Gaynard & Brady (1971) and Sanders (1971). In the family Aageridae, Nielson & Balderston (1973) report intergeneric sex-pheromone communication.

The sex-pheromones of *S. exempta, S. triturata* and *S. littoralis* being very similar it was of particular interest to investigate interspecific pheromone communication especially because of the possibility of using them in future as survey tools to supplement/replace light traps in the forecasting of *S. exempta* infestations in East Africa.

Three species, *S. exempta, S. triturata* and *S. littoralis* were studied, all being bred in the laboratory, *S. exempta* and *S. triturata* on maize leaves and *S. littoralis* on Caster leaves.

Pupae of all three species were sexed and separated into male and female pupae and each sex kept in separate rooms to avoid males from receiving any female odour (pheromones?) until used in the experiments. Adults were fed on 5–10% sucrose solutions immediately after emergence and all through their adult life.

Bioassays were done on 2–3 day old adults under the air flow system of bioassay described earlier (ICIPE Annual Report 1974 and 1975). Live females of each species were used as the pheromone source to test the behavioural response of groups of 10 males of each of the species at a time. The response shown by each male in any group was recorded and the overall response of the group computed as described earlier (ICIPE Annual Report, 1974 and 1975). All biossays were conducted during 00.00–06:00 h. period of the light/dark cycle.

Table 1 presents results of the laboratory biossays. It is seen that in all the three species the females are very attractive to conspecific males. While *S. triturata* females produce 60-70% response, females of *S. exempta* produce 80–85% response and those of *S. littoralis* also produce 80–85 response in conspecific males.

Males of *S. exempta* show between 18% and 44% response to females of *S. triturata* while in the reciprocal arrangement males of *S. triturata* show 4%–24% response.

S. exempta males show 4–17.5% response to *S. littoralis* females, and *S. littoralis* males show 37.27% to 45.0% response to *S. exempta* females. This means that *S. exempta* females can attract *S. littoralis* males to a greater degree than in the reciprocal case.

Between *S. littoralis* and *S. triturata* the attraction of males of one species to females of the other seems to be of a comparable order of magnitude.

It can therefore be inferred that traps baited with *S. exempta* pheromone may also attract *S. littoralis* males. In a natural situation it is not uncommon to find larvae of all three species feeding on a common host at the same place. This phenomenon in the light

Table 1. Behavioural responses of males of three species of *Spodoptera* to sexually mature females

Species of females	Species of males	No. of males used	Overall response
			%
S. triturata (n = 5)	*S. triturata*	5	8.0
		5	60.0
		5	70.0
	S. exempta	5	18.0
		5	36.0
		5	44.0
	S. littoralis	10	26.0
		10	14.0
		10	2.0
S. littoralis (n = 10)	*S. littoralis*	10	81.0
		10	88.0
		10	85.0
	S. exempta	8	17.5
		10	6.0
		10	4.0
	S. triturata	10	12.0
		12	4.0
		10	4.0
S. exempta (n = 10)	*S. exempta*	8	83.0
		10	82.0
		10	85.0
		10	68.0
	S. triturata	5	20.0
		5	24.0
		5	20.0
		5	20.0
		5	4.0
	S. littoralis	11	37.27
		10	45.0
		10	38.0

of the laboratory results discussed above suggests that there probably is at least some degree of congregation among these species. *S. exempta* is markedly seasonal while the other two species are present in the field more or less all through the year. The host range of *S. littoralis* is much more broader than *S. triturata* or *S. exempta*. It therefore follows from these facts that the three species occupy different ecological niches but coexist in the same geographical region.

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Field studies with the synthetic pheromone and related compounds

S. Khasimuddin

The sex-pheromones produced by females of *Spodoptera exempta* Wlk. have been identified by Beevor, *et al.* (1975). Studies on laboratory bioassays for pheromones were reported earlier (ICIPE Annual Report, 1974) wherein responses of males to the then suspected female pheromone were discussed. Consequently, some of the chemical compounds suspected as the female sex-pheromone and later, the identified sex-pheromone chemicals were tested in the field for their activity together with chemically related compounds.

Three trials were run in the field between June 1975 and May 1976 each time with a different set of synthetic chemicals together with live females and controls. Trial I was run near Nakuru on wheat fields and trials II and III were run at Lambwe valley on maize and sorghum fields. Each treatment was replicated 3 times. Traps used were sticky paper type supplied by the Takeda Chemical Company of Japan. These traps were mounted on wooden posts and held 1 meter above ground for the first trial and 2/3 meter for trial II and III. The treatments were randomised and traps were arranged in three rows with at least 20 meters distance between successive traps in any row and the rows themselves being at least 40 meters apart. The spread of the traps covered between 25–30 acres each time.

Counts of moths (males as well as females) trapped were taken each morning together with moths of other *Spodoptera* species that were trapped, particularly during trial I.

Trial I: The pheromones of *S. exempta* were identified by Beevor *et al.* (1975). Prior to the publication of this, ICIPE chemists suspected the pheromone to be a mixture of cis, 9-trans 11 TDDA and cis, 9-trans 12 TDDA (ICIPE Annual Report 1974). These Compounds

Table 2. Summary of moth catches of different species of *Spodoptera* in pheromone traps—Trial I—June 13–July 11, 1976

Compound	*S. exempta* Total No. of moths trapped	*S. exempta* % of ♀♀ trapped	*S. littoralis*	*S. exigua*
cis, 9-TDA	41	39.02	—	—
cis, 11-TDA	20	50.00*	2	1
cis, 9-trans 11 TDDA	20	45.00	56	2
cis, 9-trans 12 TDDA	57	26.31	—	9
cis, 9-trans 11 + cis, 9-trans 12 TDDA (1:10)	44	29.54	—	5
cis, 9-trans 11 + cis, 9-trans 12 TDDA (1:5)	12	50.00	—	2
cis, 9-trans 11 + cis, 9-trans 12 TDDA (5:1)	14	21.42	13	12
cis, 9-trans 11 + cis, 9-trans 12 TDDA (10:1)	12	16.66	18	—
Control	7	28.57	—	—

* High catches of ♀♀ to be noted.

and their combination therefore were tried at the first trial and the results are presented in Table 2. It was seen that the number of *S. exempta* moths trapped was very low inspite of a heavy population of the insect (ICIPE Annual Report, 1975). This could be due to many reasons which are not being discussed here. In general cis, 9 TDA, cis, 9-trans 11 TDDA and cis, 9-trans 12 TDDA trapped the most number of moths. An interesting feature was the high numbers of females trapped with cis, 11 TDA. This suggests that the com-pound has attraction for females, but this aspect will not be discussed here in detail.

The highest number of *S. littoralis* were trapped with cis, 9-trans 12 TDDA and this was expected because this compound is the sex-pheromone for this species (Campion, 1976). Low numbers of *S. exigua* males were also trapped with almost all treatments and no particular trend was obvious.

Trial II: The data (Table 3) indicate that among the various chemical formulations tried cis, 9-trans 12 TDDA, which happens to be the minor component of the pheromone, trapped the most number of moths while the major component (cis, 9-TDA) trapped not many more than the control. A mixture (1:10) of cis, 9-trans 11 TDDA and cis 9, trans 12 TDDA and cis, 9-trans 11 TDDA alone were of moderate attractiveness. As in trial I, cis, 11 TDA attracted as many females as males. The number of moths trapped by the rest of the compounds were not very different from the control.

Trial III: The results are summarised in Table 4. The maximum number of moths were trapped by cis, 9-TDA + cis, 9-trans, 12 TDDA (10:1) which is the pheromone identified (Beevor, *et al.*, 1975). In this trial, unlike in trial II, the major component of the pheromone (cis, 9-TDA) trapped a reasonably good number of moths. At the moment the reason for this difference in the attractiveness of cis, 9-TDA at different times is not discussed further. Moths trapped by the other treatments were too few to be of any value.

Table 3. Summary of moth catches of *exempta* in pheromone baited traps (March 26–April 11, 1976), Lambwe Valley

Pheromone Chemical	No. of ♂♂ trapped	No. of ♀♀ trapped	Total	% of ♀♀ trapped
cis 9, DDA	7	1	8	12.5
cis 9, TDA	2	2	4	50.0
cis 9, trans 11 TDDA	6	0	6	
cis 9, trans 12 TDDA	18	1	19	5.2
cis 11, TDA	2	2	4	50.0
cis 9, trans 11 TDA*	3	1	4	25.0
cis 9, trans 12 TDDA (10:1)				
cis 9, trans 11 TDDA+ cis 9, trans 12 TDDA (10:1)	8	1	9	11.1
cis 5, TDA	1	3	4	75.0
Myrestly acetate	3	1	4	25
Palymtyl acetate	2	0	2	—
Myrestyl acetate+ Palymtyl acetate (1:1)	0	0	0	—
Crude extract	2	0	2	—
Virgin females	3	0	3	—
Control	1	2	3	66.6
cis, 7–TDA	0	0	0	—

Table 4. Summary of moth catches of *S. exempta* in pheromone baited traps (May 3–12, 1976) Lambwe Valley

Pheromone Chemical	No. of ♂♂ trapped	No. of ♀♀ trapped	Total
cis 9, DDA	8	0	8
cis 9, TDA	7	0	7
cis 9, trans 11 TDDA	1	0	1
cis 9, trans 12 TDDA	0	0	0
cis 11—TDA	1	0	1
cis 9, trans 11 TDDA	1	0	1
cis 8, trans 12 TDDA (10:1)			
cis 9, trans 11 TDDA+	2	0	2
cis 9, trans 12 TDDA (1:10)	18	1	19 (5.26%) co
trans 12 TDDA (10:1)			
cis 9, TDA + cis 9,			
trans 12 TDDA (1:10)			
Myrestyl acetate	0	0	0
Palymytyl acetate	0	0	
Myrestly acetate+			
Palymytyl acetate (1:1)			
Crude extract	0	0	0
Virgin 00	0	0	0
Control	0	0	0

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Studies on field biology

S. Khasimuddin

The African Armyworm has long been established as a migrant pest (Brown and Swaine, 1966; Brown, *et. al.*, 1969). Outbreaks of larvae are restricted to the rainy months of the year and Brown and Swaine (1966) state that once a larval outbreak occurs in a given area, the moths migrate and a subsequent outbreak rarely, if ever, is experienced in the same area. On the other hand Hattingh (1941) suggests continuous presence of the insect in the same area through successive generations. Rose (1975) has monitered three successive generations in Rhodesia and reports some interesting findings on the quality changes in larvae and adults through successive generations leading to migratory or non-migratory populations. Whellan (1954) states—"although most moths migrate, some do not do so before oviposition and, on occasions, many oviposit in the area in which they emerge".

Reports on the successive generations at the same place are mainly from South Africa or Rhodesia while the work of Brown *et al.*, (1969) was mainly restricted to East Africa. No published evidence on the field biology in East Africa is available. In the ICIPE Annual Report, 1975, were discussed some aspects of the field biology where evidence of breeding of the insect through three successive generations in the Lambwe Valley was presented. This is a report of further investigations during the subsequent year (1976) carried out at the Lambwe Valley in more detail.

The larvae, pupae and adults of three successive generations were sampled in the same way as described earlier (ICIPE Annual Report, 1975). This season, samples of larvae (black and non-black) were also collected for each of the three generations in 60% alcohol and brought back to the laboratory. Measurements on the widths of head capsules were made in all samples of the larvae with the help of a binocular microscope.

Adults collected for each of the generations were marked for the date of capture and separated into respective sex. Measurements were made on the forewings and hind wings of moths from all samples using a vernier caliper. These measurements like the ones on the width of head capsules have been used to make a comparative study.

A summary of the results from sampling of various life history stages during the three generations is presented in Table 5. As can be seen, the first moths in the area were trapped during the last week of February and gave rise to the first generation which lasted until the last week of March. A second generation occurred during the month of April and the third generation during May.

During all three generations there were black as well as non-black larvae. Larval densities per unit area started out at 20–25 per square meter in the first generation, declined to 6–8 during the second generation and increased again to 40–50 during the third generation.

The widths of head capsules of larvae are presented in Table 6. The data indicate that larvae from all the

Table 5. Summary of successive generations in the Lambwe Valley—1976

	Preceeding Generation	1st Generation	2nd Generation	3rd Generation
Eggs	—	Feb. 28–March 3–4	April 2–7	April 30–May 3/4
First Larvae	—	March 1st week	April 1st week	May 1st & 2nd week
Caterpillar phase	—	Green and Black	Green and Black	Green and Black
1st Pupa	—	March 14–15	April 20	May 16/17
Moths trapped	February 26/27	March 24–April 7	April 28– May 11	May 25/26 onwards
Moths seen in field	—	March 28–31	April 27–May 6	May 25/26
Approx. Density (larvae) (m)	—	20–25	6–8	40–50

Table 6. Measurements on the width of head capsules (in mm) of larvae through three successive generations—Lambwe Valley—1976

	IIIrd Instar ($\bar{x}$ ± S.D.)	IVth Instar ($\bar{x}$ ± S.D.)	Vth Instar ($\bar{x}$ ± S.D.)	VIth Instar ($\bar{x}$ ± S.D.)
Standard (Insectary stock)	2.38±0.21 (n = 25)	4.32±0.23 (n = 25)	8.04±1.09 (n = 25)	9.65±0.49 (n = 25)
I Generation		6.71±0.63 (n = 7)	8.24±0.66 (n = 25)	9.89±0.47 (n = 40)
II Generation	4.50±0.0 (n = 14)	6.40±1.01 (n = 24)	8.70±0.68 (n = 24)	10.27±0.49 (n = 19)
Mbita	5.78±0.28 (n = 4)	8.09±0.95 (n = 12)	9.43±1.05 (n = 15)	10.53±0.21 (n = 16)
III Generation	4.44±0.31 (n = 20)	6.21±0.68 (n = 93)	7.60±0.59 (n = 25)	9.91±0.10 (n = 10)

Table 7. Comparison of Width of head capsules of black *vs* non-black caterpillars (in mm) from two locations

Place & phase of larval	IIIrd Instar ($\bar{x}$ ± S.D.)	IVth Instar ($\bar{x}$ ± S.D.)	Vth Instar ($\bar{x}$ ± S.D.)	VIth Instart ($\bar{x}$ ± S.D.)
SINDO				
Non-black	4.6 ± . .	7.48±0.79 (n = 4)	9.55±0.90 (n = 9)	10.33±0.90 (n = 13)
black		6.93±0.43 (n = 10)	8.50±0.22 (n = 14)	10.77±0.54 (n = 25)
LAMBWE				
non-black		6.65±0.07 (n = 4)	8.10±0.50 (n = 13)	10.64±0.36 (n = 15)
black	4.50±0.0	6.40±1.01 (n = 14)	8.70±0.68 (n = 5)	10.27±0.49 (n = 19)

Table 8. Measurements on wings (in mm) of moths of three successive generations—Lambwe Valley

	MALES		FEMALES	
	Forewings ($\bar{x}$ ± S.D.)	Hindwing ($\bar{x}$ ± S.D.)	Forewing ($\bar{x}$ ± S.D.)	Hindwing ($\bar{x}$ ± S.D.)
I Generation	13.03±1.14 (n = 19)	9.92±0.70	13.09±0.70 (n = 28)	10.10±0.49
II Generation	13.48±1.14 (n = 25)	10.15±0.96	13.71±0.96 (n = 39)	10.48±0.89
III Generation	14.54±1.07 (n = 27)	11.24±0.84	15.32±0.88 (n = 48)	11.73±0.78

three generations were in general larger than those reared in the laboratory. Within the three generations larvae from the second generation appear to have been the largest in size as compared to the first and the third generation. A comparison of the widths of head capsules of black larvae against non-black larvae do not differ much in their size (Table 7). This agrees with the findings of Rose (1975) in Rhodesia. The increase in numbers of larvae in the third generation after a decline in the second also agrees with the statement ..."vigour was regained in the third generation" (Rose, 1975).

Measurements on wing lengths of moths of the three generations (Table 8) indicate that moths became larger through successive generations. A gradual build-up of vigour through successive generations yielding large size moths is indicated. For a population that bred through three generations this build-up suggests a phenomenon leading to migration as larger moths were found by Rose (1975) to be mostly migrants as compared to smaller sized moths.

These monitoring results have confirmed that the insect is capable of breeding through successive generations at a given place as was indicated in the previous season (ICIPE Annual Report, 1975). Results from the past two years have also shown that generally the populations build-up in vigour gradually. The significance of such a build-up lies in the point that such populations can form supplements to a generally migratory population on the one hand or can themselves be potential sources of subsequent outbreaks as a result of build-up in numbers and size of individuals.

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Preliminary report of studies on developmental biology

S. Khasimuddin

The highly seasonal occurrence of larval outbreaks of the African Armyworm is a phenomenon which has not been fully understood as yet. To a considerable extent, adult migrations explain the redistribution of larval outbreaks during the 'outbreak season' which lasts for only a few months in any given year (Brown & Swaine, 1966; Brown, *et. al.*, 1969). However, what becomes of the insect during the rest of the year is still not known, nor is there any information on how the insect survives and where, during this "off-season". The understanding of this phenomenon calls for detailed investigations into the developmental biology and ecology of the insect. Suggestions as to the quality changes in the life-history stages of the insect (Rose, 1975) exist which indicate that adults from black and gregarious larvae are migrants while those from non-black and solitary larvae do not migrate but oviposit in the same area and give rise to non-migratory populations. Apart from this there is not much information on the differential colouration (and therefore differential behaviour) of larvae and the role, if any, of such a phenomenon in the overall survival strategy of the insect.

It was discovered earlier (ICIPE Annual Report, 1974 and 1975) that pupae from some field outbreaks exhibit a unique phenomenon of passing a prolonged period of about 6 months in the pupal stage before emerging as adults. Although the proportion of such pupae was very low—∼5% of a given population—their numbers may well be enough for what is needed by the insect to start subsequent generations under favourable conditions.

Investigations have therefore been initiated towards understanding the phenomenon of delayed pupal period on the one hand and the role of "differentially coloured" larvae in the survival strategy of the species on the other hand.

Extended pupal duration: The normal pupal life in *S. exempta* lasts between one to five weeks (Carnegi, 1975). The rate of oxygen consumption can serve as an index of the developmental period (Mansingh and Smallman, 1967), being lower in "diapausing" individuals than in the non-diapausing ones.

Investigations towards measuring the oxygen consumption rates have recently begun on pupae from the laboratory colony. It is early to report on the results obtained as more experiments are to be done, especially on pupae from field outbreaks. The oncoming 1977 season (approx. February through July) is hoped to provide material for investigations.

Development biology: The significance of the phase of the larvae (black or non-black) in the developmental biology is planned to be investigated with several variables such as temperature, humidity, moisture content of the host plants, texture of the host plant, larval density per unit area and the like.

A pilot experiment with larval density as the variable and other factors being constant, suggested that larvae raised at low densities turn out to be non-black and those raised at higher densities completely black. It was also apparent that pupae from such non-black larvae take longer to emerge than in the case of pupae from black larvae. Further detailed investigations are planned in future on these lines.

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Studies on pheromone perception and pheromone production in the African armyworm moth, ***Spodoptera exempta***

R. A. Steinbrecht

Electroantennogram (EAG) methods have been known for 20 years (Schneider and Hecker, 1956), and are increasingly used to supplement behavioural and field experiments in the study of pheromone communication. In order to record EAG responses, glass electrodes filled with Ringer solution are impaled at the distal and proximal end of the antenna of a mechanically immobilized moth. Upon stimulation by air puffs loaded with pheromone, the recording electrode at the tip of the antenna becomes negative against the reference electrode at the antennal base. This DC potential is amplified and displayed on a cathode ray oscilloscope (Fig. 1). The amplitude of the EAG increases with stimulus intensity (Fig. 2). Generally, the EAG is understood to be a summation of receptor potentials which are recorded extracellularly from a greater number of reacting receptor cells (Kaissling, 1971). The advantage of this method, once a suitable preparation has been obtained, is the standardized and graded response by which a great number of samples can be compared as to their pheromone activity. While behaviour tests usually are dependent on a number of additional parameters other than pheromone concentration (e.g. time of the day, temperature, visual cues), such factors do not play a major role in EAG recording.

In *Spodoptera exempta*, males were prepared for testing responses to the female sex attractant pheromones. Because of the delicacy of the antennae, receptors were easily damaged by fixing the antenna and inserting the electrodes. In such damaged preparations the response to pheromone stimulation was reduced or even totally abolished. It cannot be excluded, however, that some of the experimental animals had a reduced sensitivity per se which was not due to preparatory artefacts. Good preparations could be studied for up to six hours without much loss of sensitivity.

Although in behaviour experiments most male *S. exempta* do not respond fully to the female pheromone before the age of 2½ days (ICIPE Annual Report 1974), EAG responses of equal reaction characteristics were obtained from males of different ages between ½ and 3 days. Responses of high sensitivity were recorded at all times of the day, while behaviour responses could only be elicited between 0.30 am and 3 am (ICIPE Annual Report 1974). Although the age of the animal and the time of the day still cannot be excluded regarding their possible influence on the EAG, such influences certainly are not predominant, which again illustrates the value of EAG techniques in routine work.

I. Reactions to synthetic pheromone compounds

(Z)-9-Tetradecenylacetate (Z-9-TDA) and (Z)-9, (E)-12-Tetradeca-dienylacetate (Z-9-E-12-TDDA), known as pheromone components of ***Spodoptera exempta*** (Beevor *et. el.* 1975) were administered as stimuli in quantities of $10^{-6}-10\mu g$ on a piece of filterpaper over which an air volume of 80 ml was blown towards the antenna in one sec. Monitoring the airflow with a thermistor close to the antenna enabled proper adjustment of stimulus conditions so that the air flow was always the same and hence the number of pheromone molecules hitting the antenna was proportional to the pheromone load on the filter paper.

Average dose response curves for both synthetic compounds are shown in Figure 3. No significant differences between (Z)-9 TDA and (Z)-9, (E)-12-TDDA were observed. The threshold for both compounds was found with the most sensitive preparations to be at 10^{-4} µg pheromone on the filter paper, while usually only 10^{-3} µg was well above the response to a control stimulus (filter paper with solvent alone). With 10 µg pheromone on the filter paper the saturation plateau of the dose response curve was not yet reached. Such high intensities, however, are already far outside the physiological intensity range. There was therefore, no attempt to reach the saturation of the receptors with even higher concentrations.

Mixtures of the two pheromone components have so far not shown a synergistic effect as to the EAG response.

Whether the two components act on the same or two different receptor types still remains an open question, since the EAG does not differentiate among different receptor types. This can be answered by single cell recordings only, which will be the next steps of this study.

II. Reactions to extracts of female pheromone glands

An EAG preparation calibrated by the synthetic pheromone compounds can be further used to study the pheromone activity of the female pheromone gland under various conditions. Abdominal tips of females, which were ligated to evert the glands, produced about the same responses as raw extracts of glands in hexane administered on filter paper. The latter, however, gave more reproducible results and could be stored in the freezer for months without apparent loss of activity.

In Fig. 4, EAG amplitudes produced by various extracts are plotted against age of the females. Female moths of 1,2,3 and 5 days produce nearly the same response, and hence contain about the same quantity of pheromone. In behaviour assays, however, females were generally not able to elicit full courtship behaviour of males before the age of 3 days. Therefore, failure to release the pheromone rather than actual lack of production prevents courtship behaviour at earlier ages. Moreover, there was no significant difference in pheromone production between wild and insectary reared females, while in behaviour tests the latter often appeared to be less attractive (ICIPE Annual Report 1974). Apparently such differences are due to changes in calling behaviour rather than pheromone production.

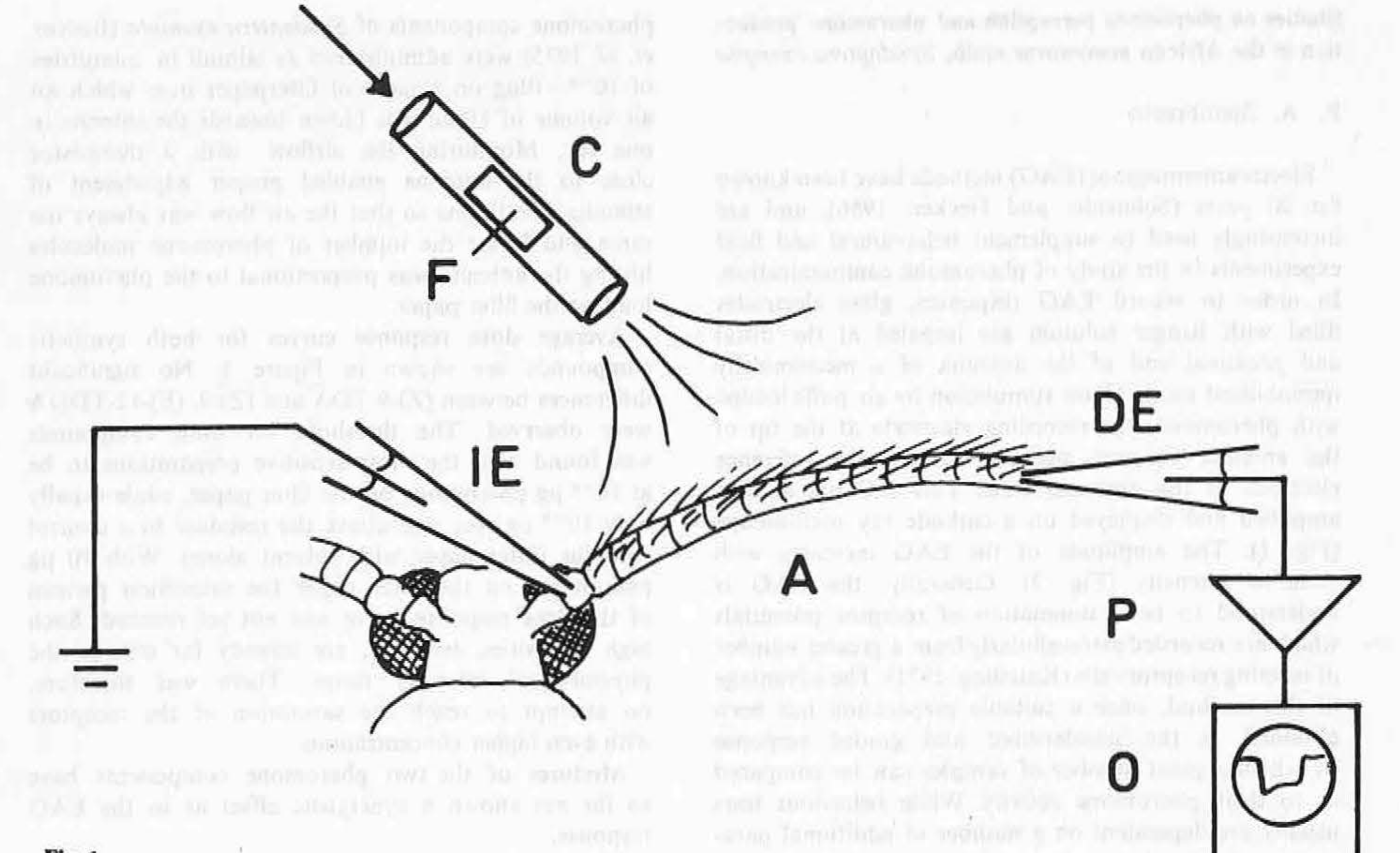
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Fig. 1.

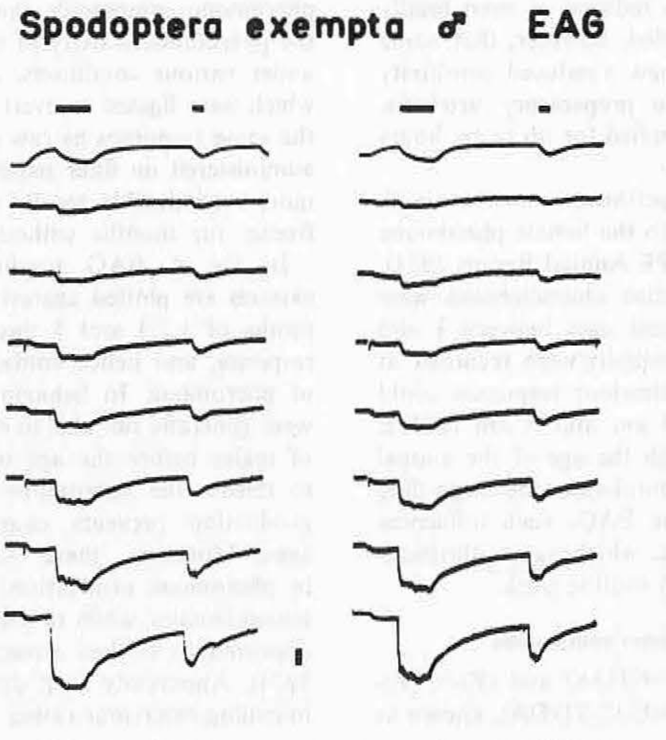
Spodoptera exempta ♂ EAG
T
C
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
10
(Z)-9-TDA
(Z)-9,(E)-12-TDDA

Fig. 2.

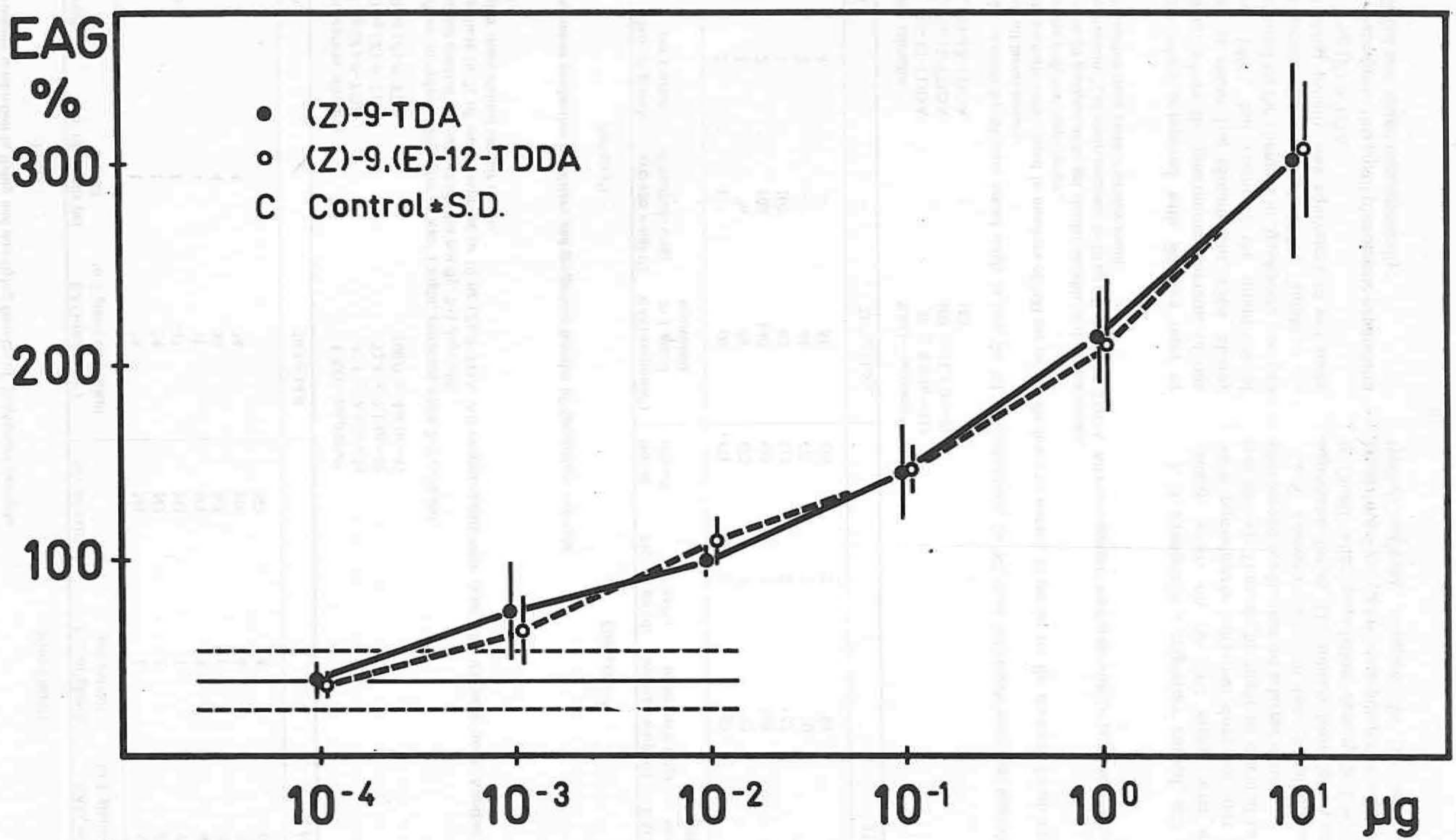
EAG %
300
200
100
(Z)-9-TDA
(Z)-9,(E)-12-TDDA
C Control ± S.D.
10⁻⁴
10⁻³
10⁻²
10⁻¹
10⁰
10¹
µg

Fig. 3.

Table 9. Pheromone production in flying and non-flying females of *Spodoptera exempta*

Fliers[1])			Non-Fliers[2])		
No. of extract	No. of glands per extract	EAG-response[3]) to 1 gland equivalent	No. of extract	No. of glands per extract	EAG-response[3]) to 1 gland equivalent
F1	1	75	N1	1	54
F2	1	54	N2	1	68
F3[4])	1	47	N3	1	71
F4	1	71	N4	1	44
F5	4	90	N5	1	44
F6	4	74	N6	4	76
			N7	4	60
Mean ± S.D.		76.3±14.0			63.5±11.6

Calibration stimulus	EAG—response[4]
0.1 ng (Z)-9-TDA	42.1 ± 8.9 (n=5)
1.0 ng (Z)-9-TDA	72.4 ± 7.1 (n=3)
10.0 ng (Z)-9-TDA	100.0 ± 4.4 (n=4)

1. Animals flewn in tethered flight for at least 1 night, extracted when 2–3 days old.
2. Normal caged control animals which did not fly, 2–3 days old.
3. EAG amplitude in % of mean response to 10 ng (Z)-9-TDA. All measurements were done with the same male. Another experiment with another male yielded similar results.
4. Poor flier.

Table 10. Pheromone production in solitary and gregarious females of *Spodoptera exempta*

Solitary[1])				Gregarious[2])			
No. of extract	No. of glands per extract	average weight[3]) of animals (mg)	EAG-response[4]) to 1 gland equivalent	No. of extract	No. of glands per extr.,	average weight[3]) of animals (mg)	EAG-response)[4] to 1 gland equivalent
S1	2	115	69	G1	2	57	67
S2	2	76	93	G2	2	72	76
S3	2	106	140	G3	2	108	53
S4	1	107	62	G4	2	57	104
K1	2	—	44	G5	2	70	79
K2	4	—	51	G6	2	88	86
Mean ± S.D.			73.7±33.6				77.5±16.5

Calibration stimulus	EAG—response[4]
1 ng (Z)-9, (E)-12-TDDA	58 ± 8.9 (n=11)
10 ng (Z)-9, (E)-12-TDDA	100 ± 11.1 (n=9)
100 ng (Z)-9, (E)-12-TDDA	183

1. Animals for extracts S1–S4 were reared singly in jars; for K1 two individuals, for K2 three individuals were kept together in one jar throughout all larval instars.
2. Gregarious animals were reared in numbers of 100 per jar for the first two instars, 50 per jar for the third and fourth instars, and 20 per jar for the fifth and sixth instar.
3. Animals were 50 grouped as to get similar weights in the same extract.
4. EAG-amplitude in % of mean response to 10 ng (Z)-9, (E)-12-TDDA. All measurements were done with the same male. Another experiment with another male yielded similar results.

This is little when compared with *Bombyx mori* or *Trichoplusia ni*, where the pheromone contents of one female gland is about 1μg (Steinbrecht, 1964, Shorey and Gaston, 1965), but confirms the quantities of pheromone found by the chemists in *Spodoptera exempta* and related species (Beevor *et al.*, 1975, Tamaki *et al.*, 1973). One gland usually was equivalent to not more than 5×10^{-3} μg (Z)-9-TDA.

Two hypotheses as to this small pheromone production were established and tested experimentally:

1. *S. exempta* is a migratory animal, and migration usually occurs on the first nights after emergence, while reproductive behaviour does not start until later (see above). Thus, flight might be essential to stimulate pheromone production on a larger scale.

2. *S. exempta* occurs in two morphologically distinguishable forms. The solitary form (green caterpillars) is found when population density is low, while the gragarious form (black caterpillars) is observed in the typical outbreak situation. In the latter situation,

moths are found in extremely high population density, and pheromone communication appears unnecessary to enable the finding of mates. This would be in agreement with the relatively low catch rates of pheromone traps in outbreak situation this and earlier Annual Reports. Therefore, it might be possible that only solitary females produce substantial amounts of pheromone, whereas in gregarious females the pheromone production could be suppressed.

Hypothesis 1 was tested with females suspended on the thorax and exposed to a gentle air stream. Under such conditions, moths may fly throughout the night after removal of tarsal contact. One moth was even flown for the consecutive night after diurnal rest and feeding. Pheromone production in the flying moths appears to be slightly higher than in the controls (Table 9). The difference, however, is not significant ($p > 10\%$).

For testing hypothesis 2, army worms were reared singly, and in numbers of 2–3 per jar from the first instar, and without exception showed the typical green colour of solitary army worms. Controls of the same lot were reared in groups of 100, later 50, of and then 20 animals (standard procedure of the insectary) and showed without exception the colour of gregarious armyworms. Pupal and imaginal weight was recorded, and extracts were prepared of females two or three days after emergence. Although some females showed extraordinary high pheromone contents (equivalent to more than ten times the amount usually found), such highly attractive females were found among the gregarious females as well as among the solitary ones, irrespective of animal weight (Table 10).

This high individual variation of pheromone production is so far not understood, but may well explain the highly varying results of behavioural assays (this and earlier Annual Reports). It is intriguing that the average response to extracts containing one female gland equivalent is not much above the EAG threshold. However, the threshold of the behaviour response may be several logarithmic steps below that of the EAG response, as shown for *Bombyx mori* (Schneider *et. al.*, 1967), and thus there should be enough range even for of greater distance communication under suitable environmental conditions.

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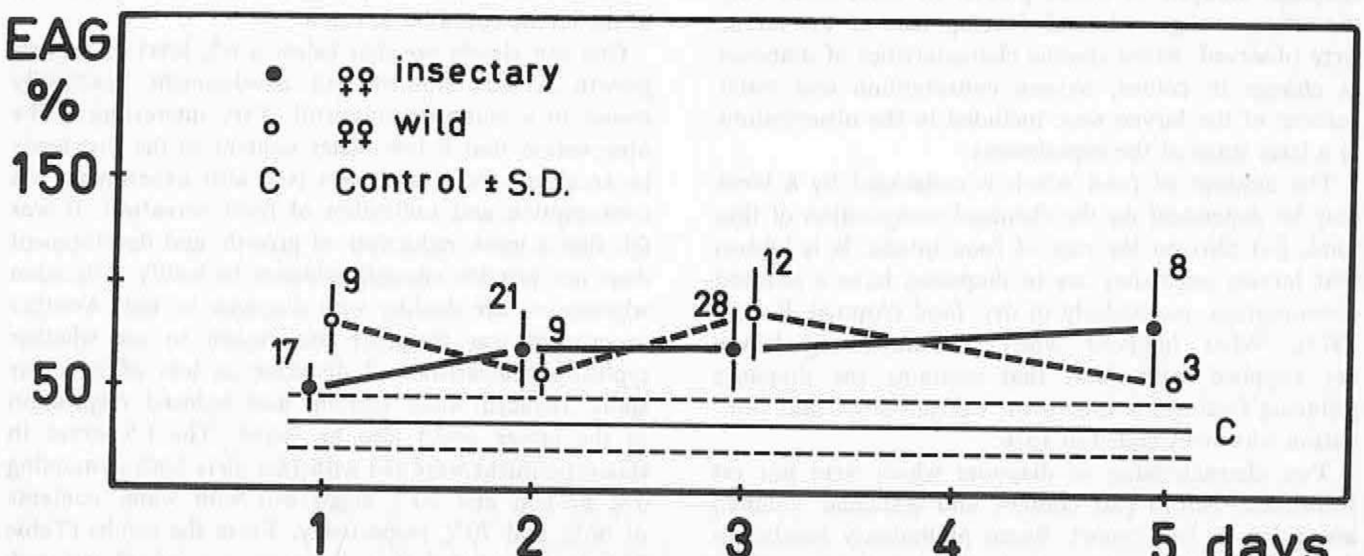


Fig. 4.

MAIZE STEM-BORER AND SORGHUM SHOOTFLY RESEARCH

Directors of Research:
Professor Jan de Wilde

Scientists:
Dr. J. R. Clearwater (1975) Research Scientist
Mr. K. Ogwaro (1974) Experimental Officer
Dr. P. Scheltes (1972–1976) Research Scientist

Maize Stem-borer Research

Aestivation-Diapause in *Chilo partellus* (Swinhoe) and *Chilo orichalcoeciliella* (Strand)

Paul Scheltes

Introduction

From the results mentioned in the ICIPE Annual Report 1975, it was concluded that diapause in the Spotted Stalk-borer, *Chilo partellus* (Swinhoe) and the Coastal Stalk-borer, *Chilo orichalcociliella* (Strand), was primarily induced by the hostplant, in this case maize. Indications were that a maize plant develops its diapause-inducing factor(s) when exposed to drought. A reduced water content in the water stressed stem was related to the incidence of diapause. No evidence could, howe. :r, be found to consider water content as a factor of similar importance in the induction of diapause as—for example—short day length and/or low temperature as is the case in the temperate regions. Research on the influence of the water content in the food on diapause induction was nevertheless continued and will be described in this report. Also other plant factors (sugars, proteins) which may act as token stimuli inside the maize plant under water stress will be dealt with. These experiments were—as usual—done with artificial diets. Changes in these diets were based on chemical analysis of maize plants. In most cases only the effects on growth and development of the larvae were observed. More specific characteristics of diapause as change in colour, oxygen consumption and water content of the larvae were included in the observations in a later stage of the experiments.

The amount of food which is consumed by a larva may be dependent on the chemical composition of that food, but also on the rate of food intake. It is known that larvae, once they are in diapause, have a reduced consumption, particularly of dry food (Annual Report 1974). What happens when non-diapausing larvae are supplied with food that contains the diapause inducing factor(s) is unknown. Consumption and utilization was investigated in 1976.

Two characteristics of diapause which were not yet mentioned before (fat content and testicular volume) are given in this report. Some preliminary results on the juvenile hormone titer of diapausing larvae were reported in the Annual Report 1975: only intermediate levels could be detected. More information along these lines will be given hereafter. This includes experiments on the endocrinological background of the termination of the aestivation-diapause.

Chemical analysis of maize stem

Contents of crude protein, total sugars and water were determined in maize stems at various stages of growth according to standard methods. Results are summarized in Table 1.

From the results it is clear that the major differences in a maize stem take place after the formation of the tassels: a large decrease in protein sugar content and a less drastic but nevertheless significant reduction in water. After these changes values remain rather constant for a fairly long period. An experiment carried out to follow the composition of maize stems which were completely disconnected from their water supply after maturation, revealed that the protein level in such plants does not significantly decrease over a period of at least one month. The sugar content however decreased gradually as well as—of course—the water content.

Induction of aestivation-diapause by artificial diet.

The previous results were used in the preparation of series of artificial diets to be tested for its diapause inducing qualities. Proteins (casein) in these diets ranged from 1.5–18% (dry weight), sugars (glucose+sucrose) from 10–40% (dry weight) and the water content was either 86% or 70%. It was found earlier that the most suitable stage to induce diapause (with maize material) was the just moulted fifth larval instar. The same instar was used with the diet experiments. Some results, which are representative for the total package of experiments are shown in Table 2. Results on the various sugar levels are omitted since no major differences in the response of the larvae could be detected.

One can clearly see that below a 6% level of protein growth is very limited and development practically comes to a complete standstill. Very interesting is the observation that a low water content of the diet tends to accelerate both processes (see also experiments on consumption and utilization of food hereafter). It was felt that a mere reduction of growth and development does not provide enough evidence to justify a decision whether we are dealing with diapause or not. Another experiment was therefore undertaken to see whether typical characteristics of diapause as loss of cuticular spots, reduced water content and reduced respiration of the larvae could also be found. The L5–larvae in this experiment were fed with two diets both containing 3% protein and 20% sugar but with water contents of 86% and 70% respectively. From the results (Table 3) it was concluded that diapause was indeed induced. Moreover the diapause of the larvae on the diet with the water content of 70% seemed to be more intense.

Protein levels found in diapause inducing maize stems were usually slightly higher than 6%. Those in diapause inducing diets on the contrary had to be below 6%. At first sight this looks contradictory. One should however remember that the proteins in maize stems are "crude proteins", based on "total nitrogen." Only part of this nitrogen can be used by the larvae. But proteins in the diets are almost exclusively casein, which can fully be utilized.

Consumption and utilization of food

Larvae reared on diet with a relatively low water content of 70% attain—within the same period—a higher weight than similar larvae reared on the same diet with 86% water. This can be seen in Table 2 and has without any exception also been observed for all the other 30 artificial diets which were tested. Detailed studies revealed that the higher growth rate of larvae on the diet with 70% water is the consequence of a very significant higher consumption rate (expressed in dry weight) of that diet.

When the consumption rates of larvae were expressed in wet weight of the diet, no significant differences between the "86% water diet" and the "70% water diet" could be found. This suggests that water is the factor in the diet that regulates the consumption rate of the larva.

Analysis of the utilization of the two different types of food showed that (a) the Approximate Digestibility (A.D.) of the diet with 70% water is only half as high as the A.D. of the diet with 86% water. (b) the Efficiency of Conversion of Digested food (E.C.D.) is significantly higher (about 1½ x) for larvae on the drier diet and c) the Efficiency of Conversion of Ingested Food (E.C.I.) is slightly lower for larvae on the drier diet. Research on this is continued and includes the natural maize plant.

Table 1: Average composition of hybrid maize at different stages of growth

stage of growth	Length of stem still point of growth (cm)	Number of plants analysed	Crude protein (% of dry weight)	Total sugars (% of dry weight)	Water (% of wet weight)
6–8 leaves	5	5	21.4	6.7	91.5
8–9 leaves	25	5	16.0	15.1	90.8
9–10 leaves; no tassel visible	60–80	3	13.4	15.1	90.3
Tassel well developed; pollen shed; cobs developing	110–130	5	6.9	35.8	80.2
Plants 4–5 weeks beyond maturity. Leaves dry, stem green "diapause inducing stem")	160–200	5	6.4	39.7	79.7

Table 2: Effect of various diets on growth and development of newly moulted fifth instar larvae

diet			average weight (mg) per larva after a 15 days feeding period	percentage pupation after a 25 days feeding period
protein*	sugar†	water %		
1.5	20	86	16.2 (27†)	0
3	20	86	32.5 (25)	4.3
6	20	86	66.2 (25)	48.3
9	20	86	84.8 (24)	62.5
18	20	86	—	78.6
1.5	20	70	39.9 (25)	0
3	20	70	64.4 (24)	12.0
6	20	70	87.4 (24)	80.0
9	20	70	115.5 (26)	85.7
18	20	70	—	94.7

*in % of dry weight.
†number of larvae observed.

Characteristics of aestivation-diapause

Apart from colour change, reduced oxygen consumption and reduced water content, two more characteristics of diapause were found:

a. The fat content (expressed in % of dry weight) which is in non-diapausing larvae 36–40%, rises under dapause conditions gradually to a maximum of 54% and then slowly goes down. These observations were made from larvae collected in an experimental field during one season.

Table 3: Some characteristics of diapause in larvae after a 36 days feeding period on diets of different protein in water contents

Diet		%immaculate and transitional larvae in the larvae population	water % of larvae	respiration ($\beta l\ O_2$/mg dry weight/hr)
Protein (% of dry weight)	Water %			
3	86	38.1	72.3±5.2	2.34±0.46
3	70	80.0	66.0±5.6	2.01±0.35
normal diet*:				
29.3	86	—	80.8±2.55	4.26±1.20

*normal diet, used breeding *Chilo* in insectary. Data were obtained half way the normal time required for 5th instar larvae to pupate (< < < < < 36 days).

b. The maximal testicular volume of non-diapausing larvae is about 0.2 mm^3. In diapausing larvae the volume remains below 0.05 mm^3 till termination of the diapause.

Endocrine involvement in the aestivation-diapause phenomenon

Although we never really managed to induce diapause with juvenile hormone applications (Annual Report 1975), there are nevertheless a number of reasons to believe that aestivation-diapause is governed by this hormone:

a. Extracts made from the haemolymph of *Chilo*-larvae in various stages of their development were checked for the presence of JH. For this purpose we used the Galleria bio-essay.

Results were somewhat confusing. In diapausing larvae we could almost always observe an intermediate level of 350–500 (Galleria) U(units)/ml haemolymph. Extremes down to 100 G.U. and up to 2500 G.U. have been found but are rare. No explanation for these extremes can as yet be given. Titer determinations in non-diapausing larvae are more difficult since we are dealing with larvae with continuing development (as opposed to arrested development of diapausing larvae). A more or less generalized over all titer as in diapausing larvae can therefore not be expected. This is not only due to the normal moulting processes (as known to be related to JH) in active larvae, but also to the fact that these moults are usually not very much synchronized. This leaves us with a rather heterogeneous group of larvae at the moment of extraction (25–50 larvae are required per extraction), even when this group was initially very homogeneous. Variations ranged from non-detectable levels below 50–100 G.U. (depending on the quantity of haemolymph extracted) to a maximum of about 1000 G.U. These differences could not always be explained by the developmental stage of the insect. It may be significant that JH-levels in diapausing larvae never came as low as the minimum in non-diapausing larvae.

b. The titer of JH was followed in the haemolymph of diapausing larvae after exposure of the larvae to diapause terminating conditions (moist filterpaper). Two different experiments were undertaken; in one case the titer dropped within three days from 750 G.U./ml haemolymph to 80 G.U. In the other case a decrease was registered from 2500 G.U./ml. haemolymph on day 0, to 225 G.U. (day 3) and 130 G.U. (day 8). It was concluded that termination of diapause goes together with a rather rapid decrease in JH-content.

c. Earlier experiments (Annual Report 1974) showed that diapause could be terminated by ecdysterone injections provided that the larvae had been ligated (head/thorax). In non-ligated larvae ecdysterone could only evoke stationary ecdysis (larva-larva). From recent experiments we now know that this statement is only true if there is a certain period between the ligation and the application of the hormone. In our experiments we induced a stationary ecdysis if the ecdysterone was applied one day after ligation, but a progressive ecdysis (larva-prothetelic larva) if the application was done 7 days after the ligation. These results suggest that the titer of JH in the haemolymph of larvae is, even one day after ligation, still sufficiently high to prevent a termination of the diapause.

Histology on the endocrine system of diapausing and non-diapausing larvae is in progress in collaboration with the Histology and Fine Structure Research Unit of the ICIPE.

Sorghum Shootfly Research

Taxonomy of five species of *Atherigona*
John Clearwater

Introduction

The purpose of this portion of the study is to examine the endocrinology of reproduction, development, diapause and seasonality of *Atherigona soccata* Rond. It was first necessary to establish rapid methods of identifying this insect, and its distribution in time. Four other species were studied for comparison. Work on reproduction system morphology and mating behaviour must be completed before a sufficiently strong base is laid for experiments in endocrinology which may be begun in 1978.

The genus *Atherigona* is a large one, and unfortunately many of the species are difficult to determine. Pont (1972) Deeming (1971), (1972) and van Emden (1940) use the shape of the trifoliate process and the hypogial process to determine males, and their figures and keys have been used to identify many of males captured at Nairobi.

The females are more difficult. The last tergite of the ovipositor is a valuable character, but insufficient in my experience to afford a firm identification. The pattern on the abdomen and fore leg give an indication but are very variable. Most of the workers in India e.g. Kundu, Kishore, and Jotwani (1971) have neatly sidestepped the problem, and in studies of the temporal distribution of *Atherigona soccata* at Udaipur have measured variations in the numbers of dead hearts in sorghum crops rather than variations in the number of insects.

The female *Atherigona soccata* Rondani is fairly robust insect with triangular or circular spots on two or three abdominal tergites (Fig.1). Two regular cones dominate the centre of the eighth tergite (T8). The tergite is often uniformly black, though the posterior portion in some insects are lighter. The free sclerite is narrow and long. A fine dark line characterises the seventh tergite (T7) with a lighter brown area surrounding the posterior half ("cricket bats"). The sixth tergite is small, square and without sharp edges.

The female *Atherigona conigera* van Emden is a small insect with circular, triangular or crescent shaped on the abdomen (Fig.2). The marking on the fore-femur is usually small. The dorsal surface of the head forward of the ocellar triangle is yellow—a feature shared with only a few other species. The central portion and anterior lateral arms of T8 are black, rounded and are very sharply set off from the remainder of the tergite which is yellow. A black triangle tips the fine black line on T7 and the posterior area extends laterally.

The female of *Atherigona trapezia* van Emden is a little larger than *A. conigera.* The dorsal surface of the abdomen is black usually with a small yellow patch on T4. The anterior-lateral arms of T8 are straight, with a faint spur directed caudally. The centre of T8 is also straight and moderately well set off from the remainder of the tergite. T7 resembles *A. conigera* (Fig. 3).

The female of *Atherigona laevigata* Loew is striking and the only one that may be readily identified in the field. The abdomen is cigar shaped in contrast to the rounded form of the other species. The rich black dorsal surface of the abdomen is sharply set off from the remainder. This species is unique among those caught

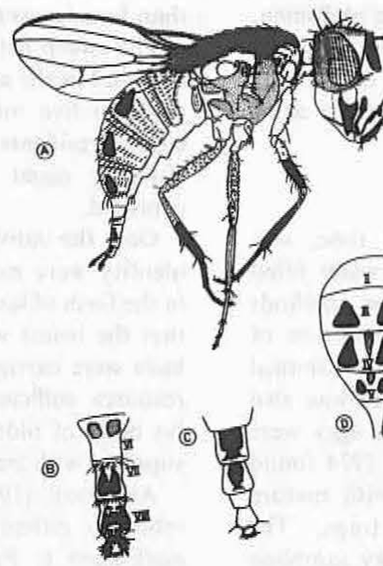


Fig. 1. ***Atherigona soccata.***
(a) Female. Note black antennae and Maxillary palp.
(b) Ovipositor tergite. The broad double central cone of TVIII and the "cricket bats" of TVII are unique features of this species.
(c) Ovipositor sternites. The "crown" of the cephalad portion of SVII is a useful distinguishing characteristic.
(d) Abdominal Pattern. The black spots may be triangular or circular, but never crescent shaped. The median vittae is often present in TII and TIII. One of both of the black spots of TIII may be absent.

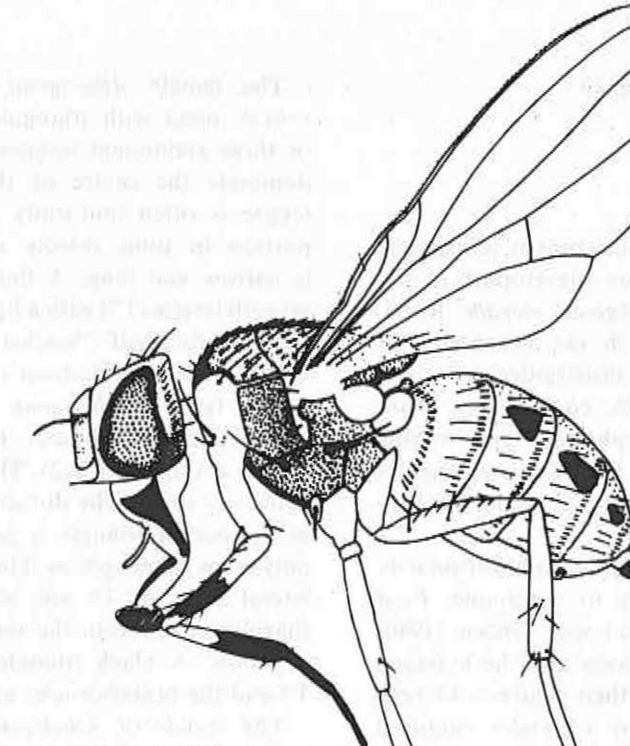


Fig. 2. *Atherigona conigera*
(a) Female. Note thin yellow antennae, yellow base to antennae.

at Nairobi in having a yellow meso– and meta pleuron– usually it is grey or black. Another unique feature is a completely split T8 (Fig. 4).

The female of *Atherigona* (*Acritochaeta*) *orientalis* Schiner has two lines of blurred spots on the abdomen, and the posterior tip of the thorax is yellow. The T8 has a deep anterior cutout, with no central differentiation. Two black masses are on the anterior arms (Fig. 5).

The temporal distribution of these species

The distribution of the five species in time, was measured with three types of trap—baited, water filled trays, sweep-nets and suction traps. Three methods were necessary as each sampled a different portion of the fly population. The traps baited with fishmeal captured many more females than males. It was also suspected that females with fully developed eggs were under-represented in these traps. (Hughes 1974 found that females of *Musca vetustissima* Wlk. with mature eggs were under—represented in baited traps. The traps, working over the whole of the 3—4 day sampling period were likely to capture flies passing briefly the area, as well as residents. This method is most suitable for examining low population densities as catches on sorghum with baited traps were consistently highest compared with sweep and suction samples.

Suction traps captured insects in the air, and gave a measure of the relative flight activity of each species. These traps, active one day of the week for each area, were more likely to capture residents. Many more males than females were obtained with this method.

The sweep net removed insects resting on the vegetation and in the adjacent air. Taking the sample occupied three to five minutes out of the week for each area, so that residents were much more likely to be captured. Roughly equal numbers of males and females were captured.

Only the individuals that could be assigned a specific identity were examined in detail. The fat body, when in the form of large balls free in the haemocoele, indicated that the insect was a maximum of 2-4 days old. These balls were carried over from the pupae, and are a food resource sufficient to mature one batch of eggs. The fat body of older flies was in the form of sheets richly supplied with tracheas.

Anderson (1964) used the fat body and follicular relics to differentiate between young and old *Fannia canicularis* L. Parous females could be identified by the yellow follicle remnants (corpora lutea) at the base of the ovariole. The first corpus luteam is faint, so in common with Anderson, the field caught females with easily visible corpora lutea were considered to have completed at least two gonotropic cycles.

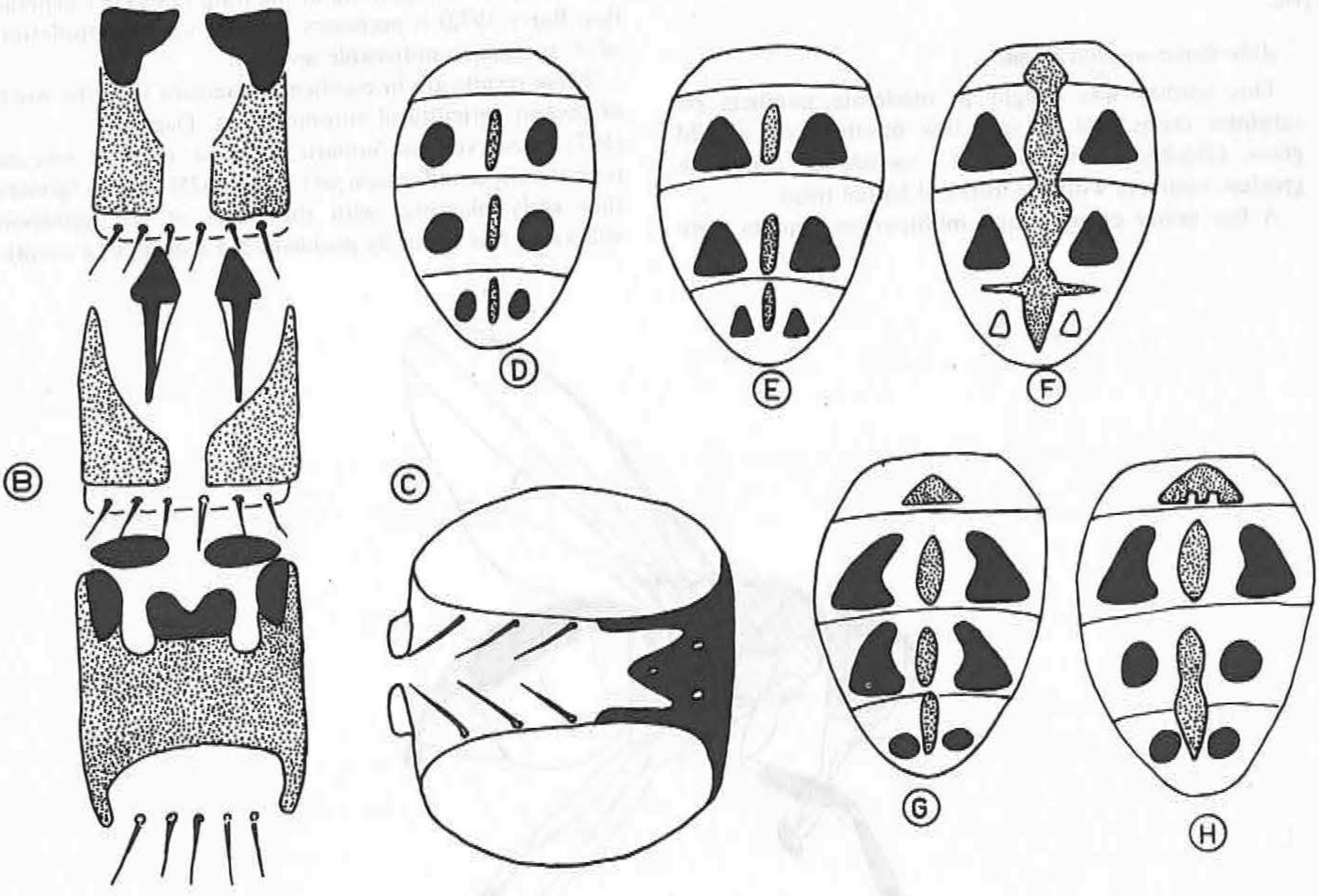


Fig. 2. ***Atherigona conigera***
(b) Ovipositor tergites. The black areas of TVIII are sharply distinguished from the yellow surrounding.
(c) Head. The dorsal surface of the head, between the ocellar triangle and the antennae base is yellow.
(d)—(H) Abdominal Patterns. Only (H) is uncommon.

Total catches of *Atherigona* with baited traps at Nairobi, show a similar pattern to total catches at Kibos (ICIPE Annual Report 1975). The number of insects is very low during the dry season (Fig. 6). A sharp rise in numbers coincides with the onset of the long rains. The populations remained at a moderate level till the beginning of the short rains when further sharp rises were seen (mid October). This pattern is the sum of at least two forms of variation in population rise.

Atherigona soccata **Rond.**

This species was caught in moderate numbers on sorghum crops and in very low numbers on a wild grass, (*Digitaria scalarum*). *A. soccata* is caught in greatest numbers with the fishmeal baited traps.

A few newly emerged and multiparous females were captured during the dry season this year (Fig. 7) May, the month following the beginning of the long rains (April 1976 at Nairobi) saw very little increase. Not until July was there a substantial increase in numbers with the maximum population found in September.

The recruitment of newly emerged adults remains roughly constant throughout the population growth phase. Accumulation of multiparous females results in substantial growth of the population (Fig. 7 & 8.) Increasing mortality of these individuals as they come to the end of their lifespan or die, due perhaps to the adverse affects of high temperature and low humidity of this time of the year, results in a decline in numbers (Fig. 8). A substantial number of these females are actively developing and laying eggs throughout this period (Fig. 9).

These results suggest that there is a very small carryover of insects onto the new crop. A period of at least one month after the beginning of the long rains (= 1 generation Barry 1972) is necessary to build up the population of *A. soccata* to noticeable levels.

These results are in excellent agreement with the work of several agricultural entomologists. Deeming (1971) observes in Samaru, Nigeria that *A. soccata* is essentially a mid season pest. Rao (1975) advises farmers that early planting, with the onset of the monsoon will avoid this shoot-fly problem, but a delay of a month may lead to losses of over 50%. Ponnaiya (1951) in Siruguppa India found that the Hingari (October) crop is heavily hit, while the Mungari (June) almost entirely escapes.

A fortituitous group of measurements suggest that *A. soccata* survives the dry season as an aestivating third instar larva, (Table 4). A sample of 125 plants taken from a crop planted during the 1975 short rains contained 70 pre-adult *A. soccata*. Two samples taken two days later, after the first heavy shower of the 1976 long rains

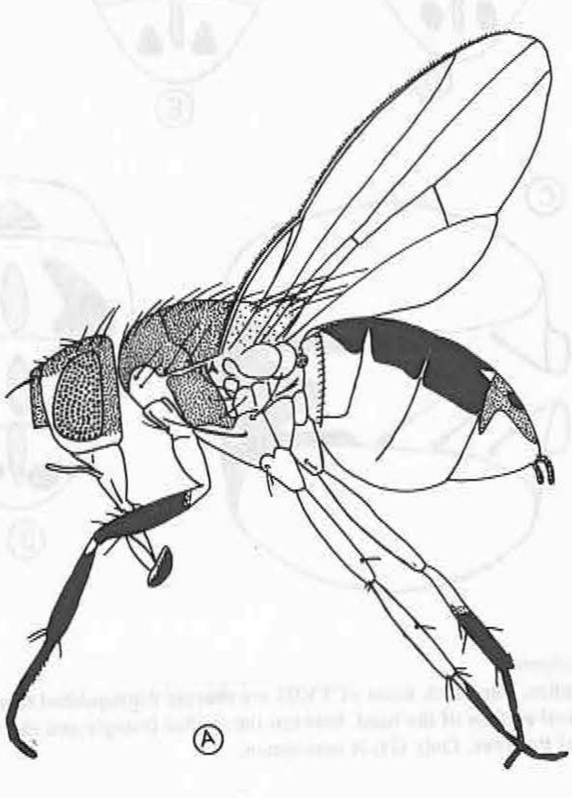


Fig. 3. *Atherigona trapezia.*

(a) Female. Note thin yellow antennae, and largely black foreleg. A fine comb is found at the tip of the metathoracic tibia.

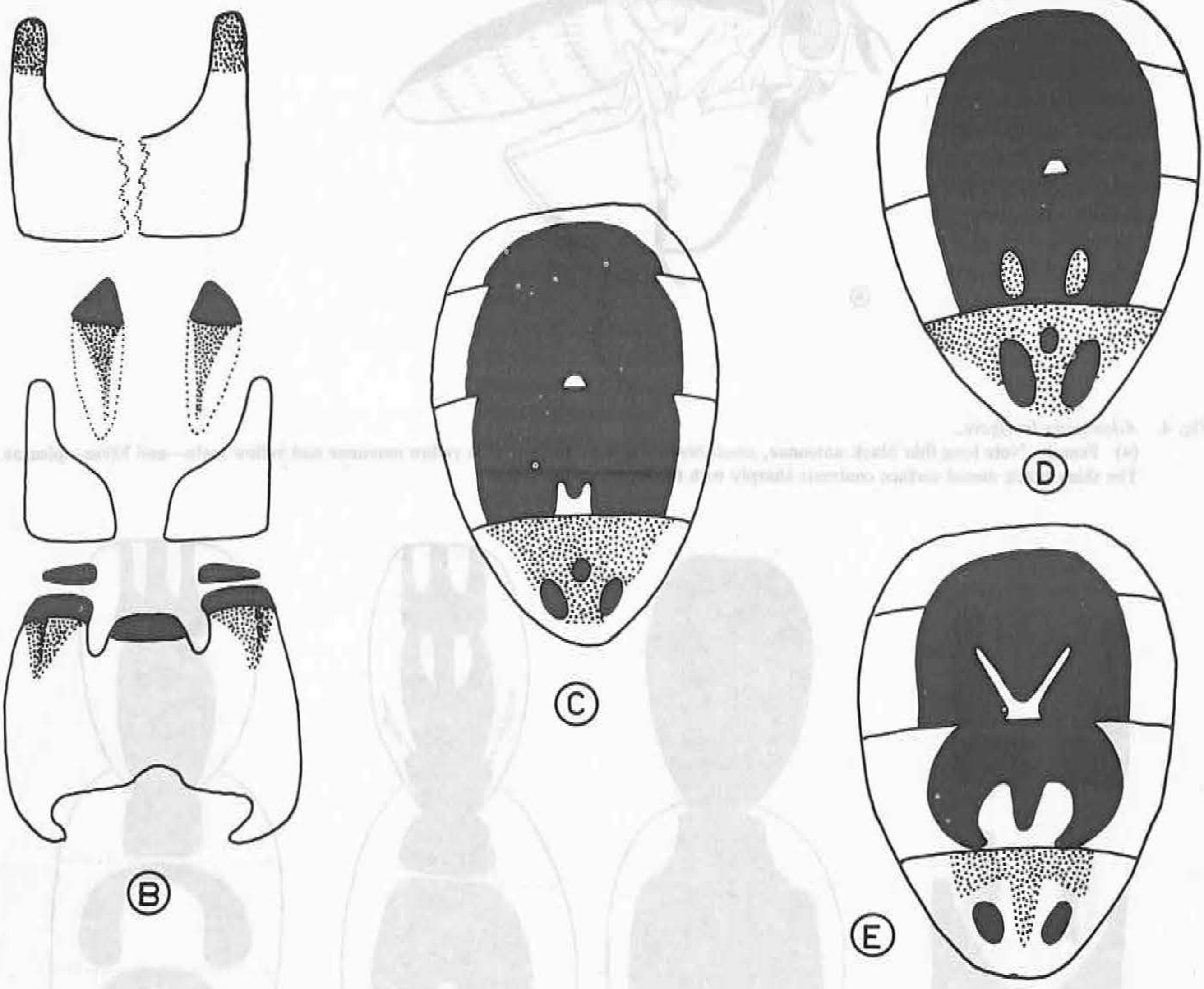


Fig. 3. *Atherigona trapezia.*

(b) Ovipositor tergites. The thin lines at the side of the last tergite are due to fusion of two plates.

(c) —(E) Abdominal patterns (C) is the most common (E) is the least.

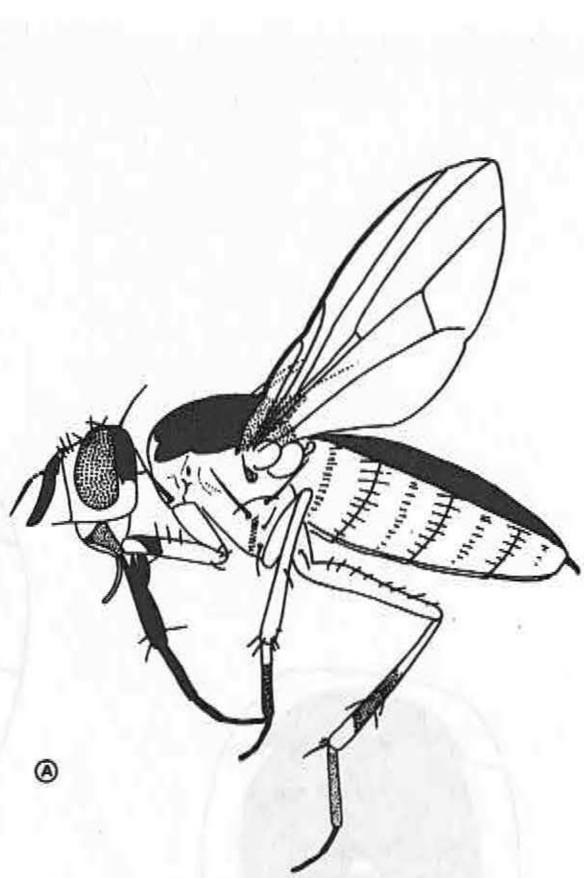


Fig. 4. *Atherigona laevigata.*
(a) Female. Note long thin black antennae, small black patch on foreleg, thin yellow antennae and yellow meta—and Meso—pleurae. The shiny black dorsal surface contrasts sharply with the bright yellow sides.

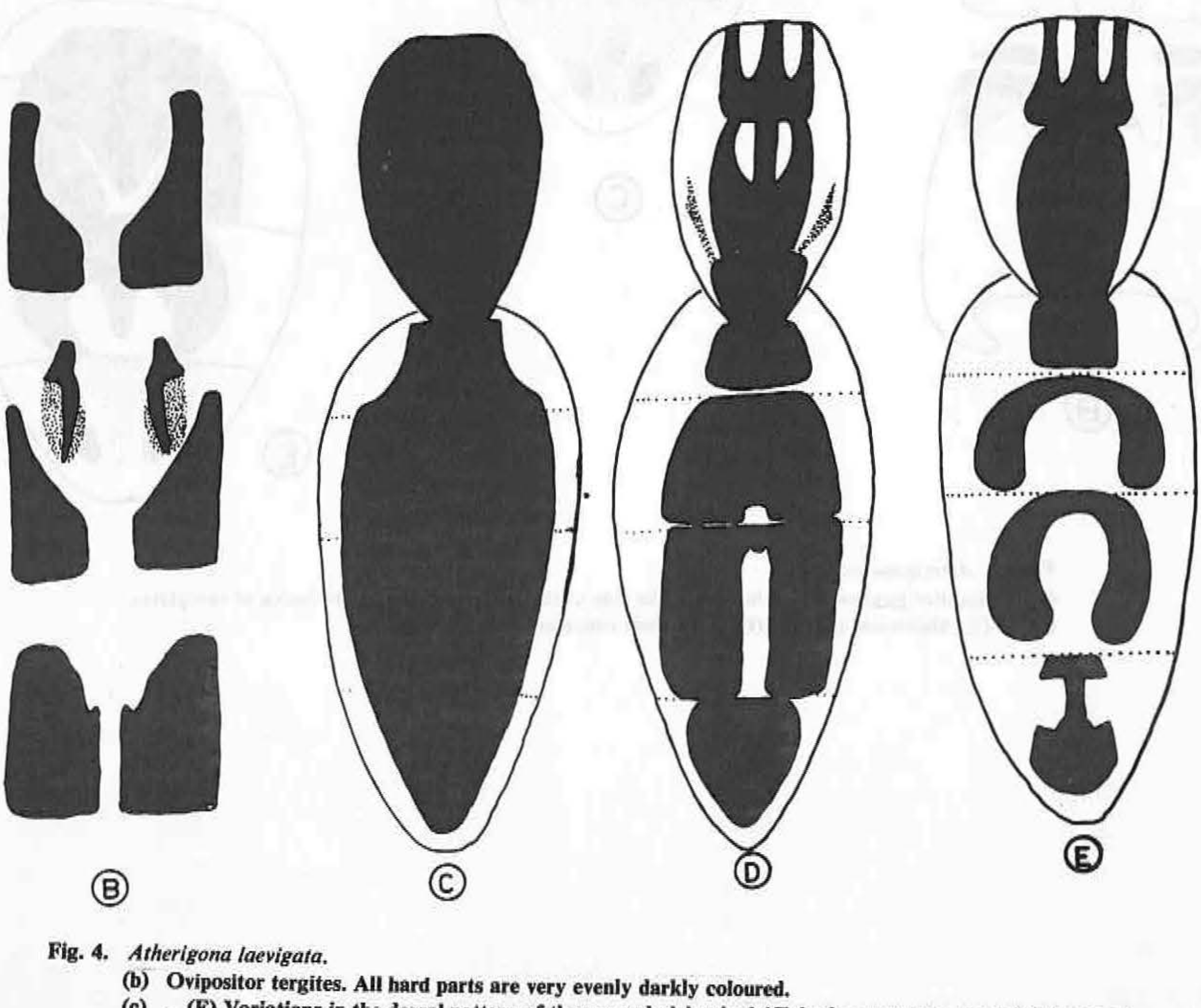


Fig. 4. *Atherigona laevigata.*
(b) Ovipositor tergites. All hard parts are very evenly darkly coloured.
(c) —(E) Variations in the dorsal pattern of thorax and abdominal (C) is the most common (E) is the least.

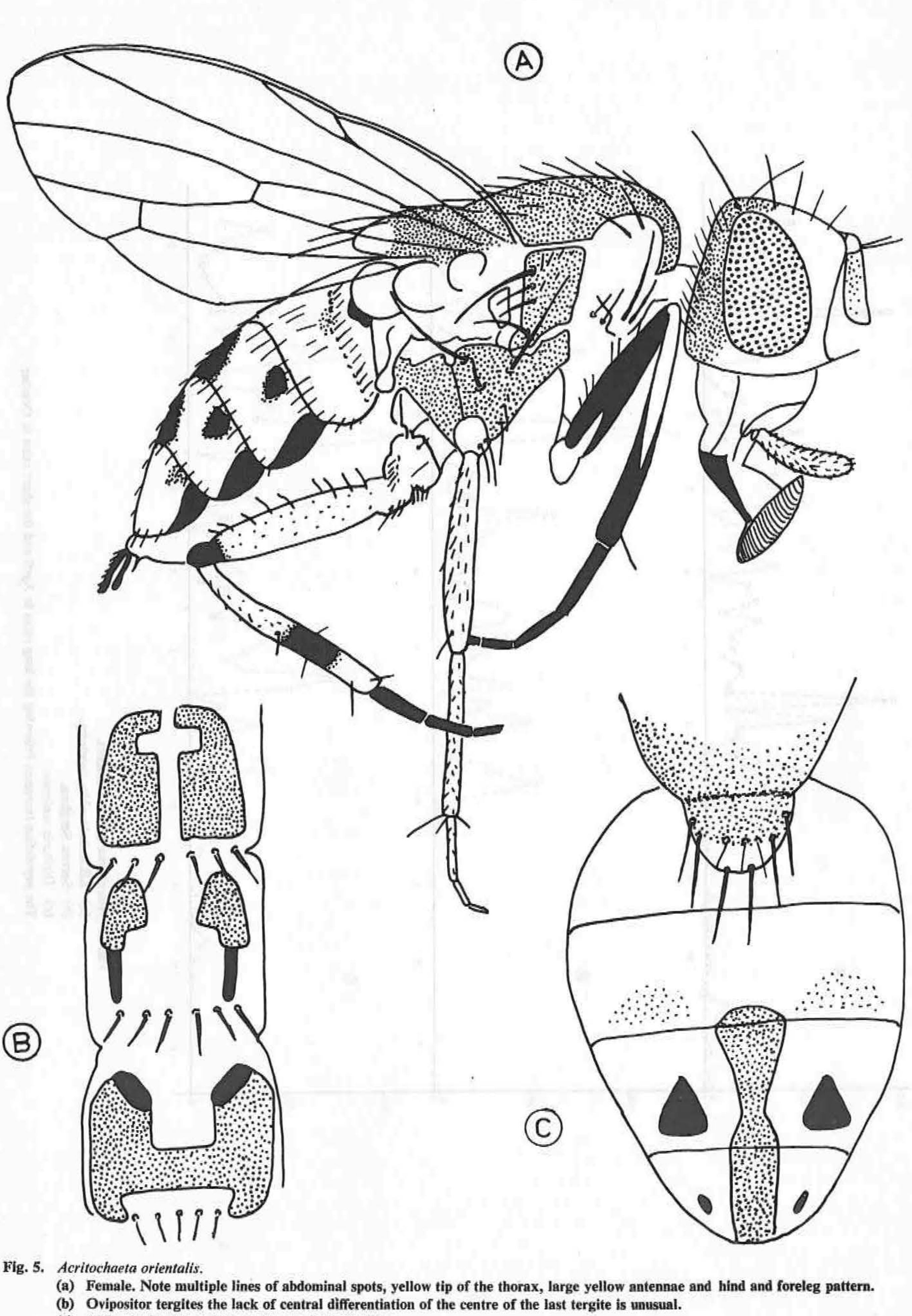


Fig. 5. *Acritochaeta orientalis.*

(a) Female. Note multiple lines of abdominal spots, yellow tip of the thorax, large yellow antennae and hind and foreleg pattern.

(b) Ovipositor tergites the lack of central differentiation of the centre of the last tergite is unusual.

(c) Abdominal pattern. The spots vary in distinctions.

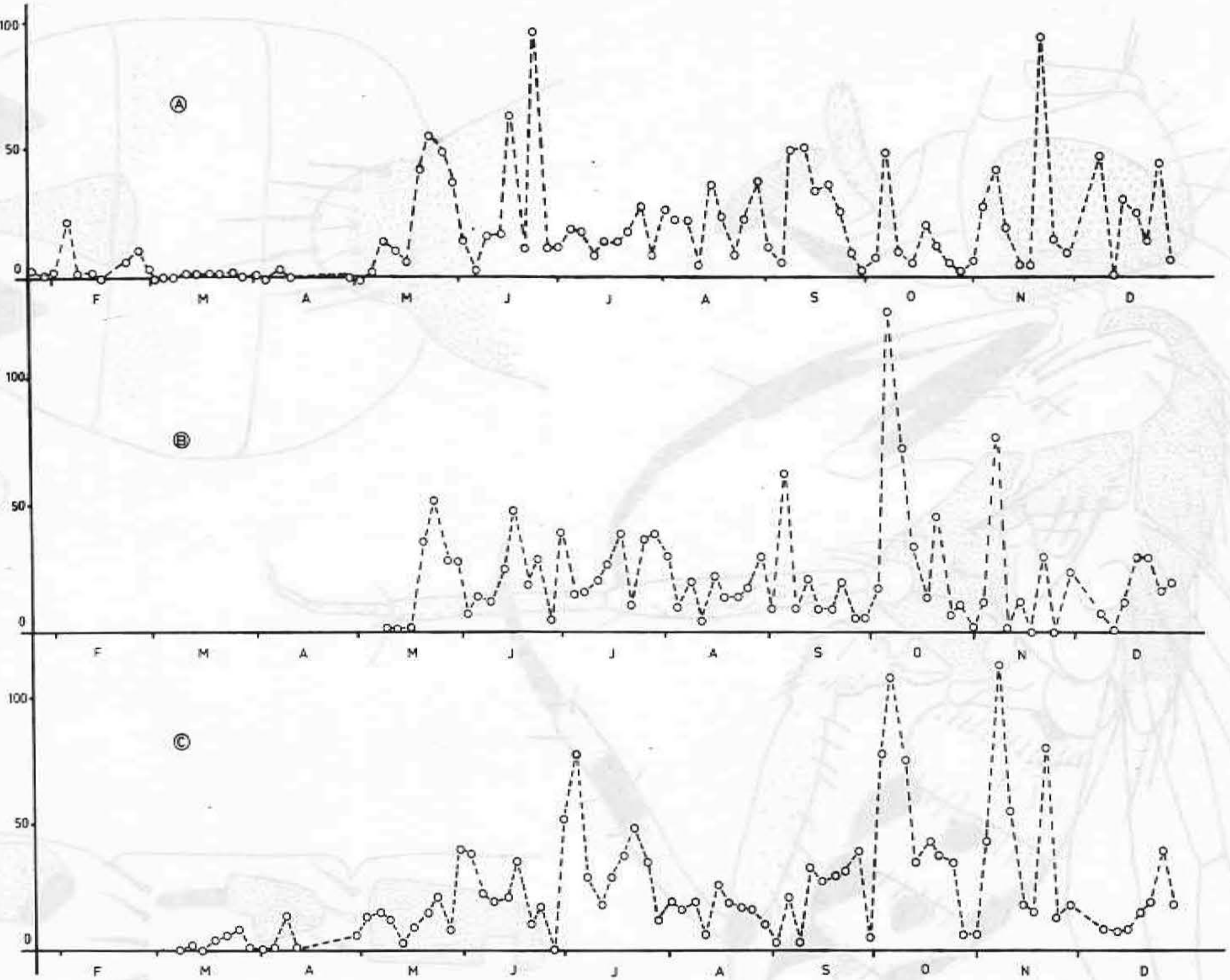


Fig. 6. ***Atherigona sp*** **Total catches.**
(a) Kikambala white sorghum.
(b) Serena Sorghum.
(c) ***Digitaria scalarum***
The population increases following the long rains in April and the short rains in October.

contained 13 preadults out of 89 plants, and 20 out of 100 plants—substantial decreases. On the morning after this shower five live third instar larvae were found in the waterfilled trays at the base of the sorghum plants. (These trays were baited with fishmeal for capturing adults). The decaying core of the plant was dry in the dry season sample and very moist in the long rain samples. These preliminary results suggest that the larvae aestivate in the attacked plant till the beginning of the long rains. The larvae then migrate out of the stems into the soil for pupation.

There is a significant difference in the ability of different varieties of sorghum to maintain *A. soccata*. *Sorghum bicolor* Var Serena (a high yielding dwarf) receives fewer eggs, and is unable to sustain numbers of larvae or adults, in comparison with *S. bicolor* (indeterminate variety) a tall white grained variety from the coastal plains of Kenya (Fig. 10). The population of adults on "Serena" decreases sharply from August onwards perhaps due to a decrease in recruitment of newly emerged adults (Fig. 7). This is in turn due to a decrease in the number of larvae (Fig. 11). Antibiosis may be involved but the drop in larvai numbers may be more simply explained in terms of decrease in numbers of sorghum shoots in the susceptible size classes in "Serena". The "Serena" crop was noticeably uniform in size, while the local variety contained numerous small shoots right throughout the growth season. The different numbers of eggs laid may be due to different degrees of attractiveness to the ovipositing female, rather than a reflection of the different growth strategies of the two sorghum varieties.

Atherigona laevigata Loew

The population of *Atherigona laevigata* like *A. soccata* builds up locally. *A. laevigata* is found almost exclusively on *Digitaria scalarum* and is captured in greatest numbers by a sweep net. Few are caught in the baited traps.

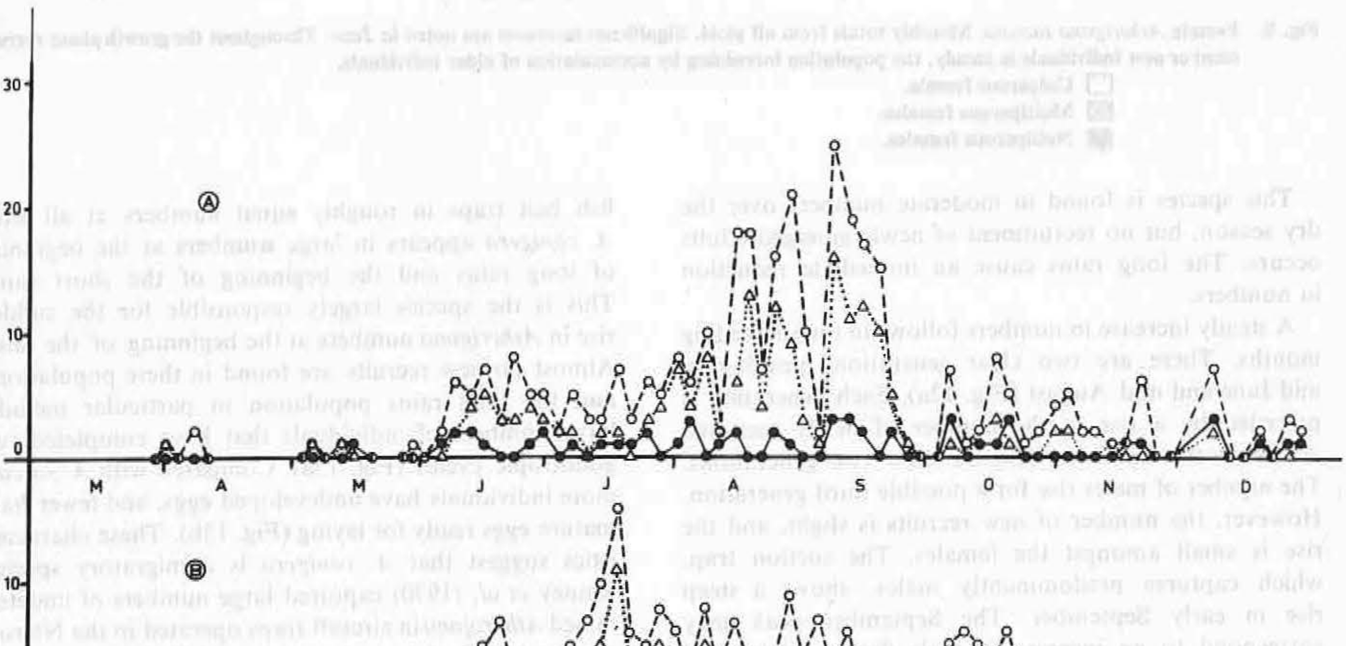


Fig. 7. ***Atherigona soccata***
(a) Kikambala white. A steady recruitment of newly emerged adults results in an increasing population of shoot flies.
(b) Serena. Significant recruitment only occurs in June and October. This is a declining population.
General
= No. of insects (total)
= No. of insects that have completed two or more gonotropic cycles.
= No. of newly emerged insects with pupal fat balls in the haemocoele.
X axis = Months or the year in 1976.

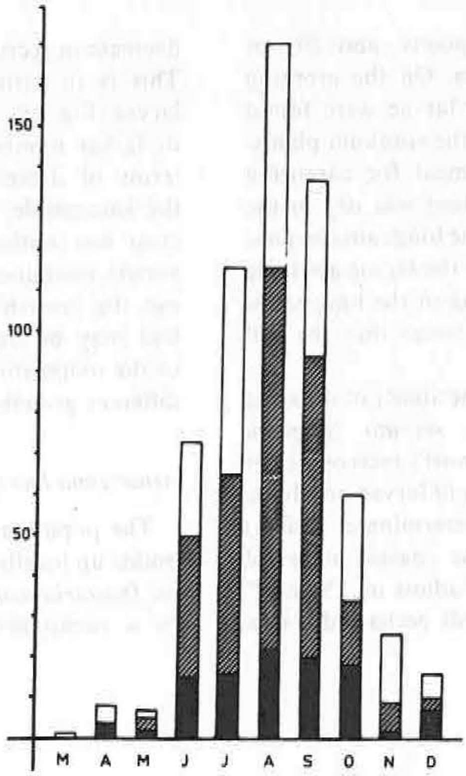


Fig. 8. Female *Atherigona soccata*. Monthly totals from all plots. Significant increases are noted in June. Throughout the growth phase recruitment or new individuals is steady, the population increasing by accumulation of older individuals.
☐ Uniparous female.
▨ Multiparous females.
■ Nulliparous females.

This species is found in moderate numbers over the dry season, but no recruitment of newly emerged adults occurs. The long rains cause an immediate reduction in numbers.

A steady increase in numbers follows in the succeeding months. There are two clear generations peaking in mid June and mid August (Fig. 12a). Each generation is preceded by a rise in the number of newly recruited adults. Both males and females show two generations. The number of males rise for a possible third generation. However, the number of new recruits is slight, and the rise is small amongst the females. The suction trap, which captures predominantly males, shows a steep rise in early September. The September peak may correspond to an increase in male flight activity and movement rather than a third generation.

The number of actively egg laying females is higher than in *A. soccata*. This may partially reflect different method of capture (sweep net *VS* baited trap). The number of active ovarioles per ovary is lower in *A. soccata*, which may balance the apparent greater frequency of egg production *in A. laevigata*. (Fig. 12b).

Atherigona conigera van Emden

Atherigona conigera is the most commonly encountered species of *Atherigona* in Nairobi. Unlike the first two species discussed which are captured near one species of host plant, *A. conigera* was captured with fish bait traps in roughly equal numbers at all sites. *A. conigera* appears in large numbers at the beginning of long rains and the beginning of the short rains. This is the species largely responsible for the sudden rise in *Atherigona* numbers at the beginning of the rains. Almost no new recruits are found in these populations, and the long rains population in particular includes large numbers of individuals that have completed two gonotropic cycles (Fig. 13a). Compared with *A. soccata* more individuals have undeveloped eggs, and fewer have mature eggs ready for laying (Fig. 13b). These characteristics suggest that *A. conigera* is a migratory species. Rainey *et al*, (1970) captured large numbers of undetermined *Atherigona* in aircraft traps operated in the Nairobi area, in May and June. I would consider that *A. conigera* is the most likely identity of these air trapped individuals.

Atherigona trapezia van Emden

Atherigona trapezia is comparable with *A. conigera* appearing in the fishmeal baited traps at all sites at the beginning of the long rains and short rains. The long rains population of *A. trapezia* appears slightly before the comparable population of *A. conigera* and unlike *A. conigera* contains significant numbers of new recruits (Fig. 14). Though likely to be a migratory species, it is possible that there is also a local population. The host plant has not been identified.

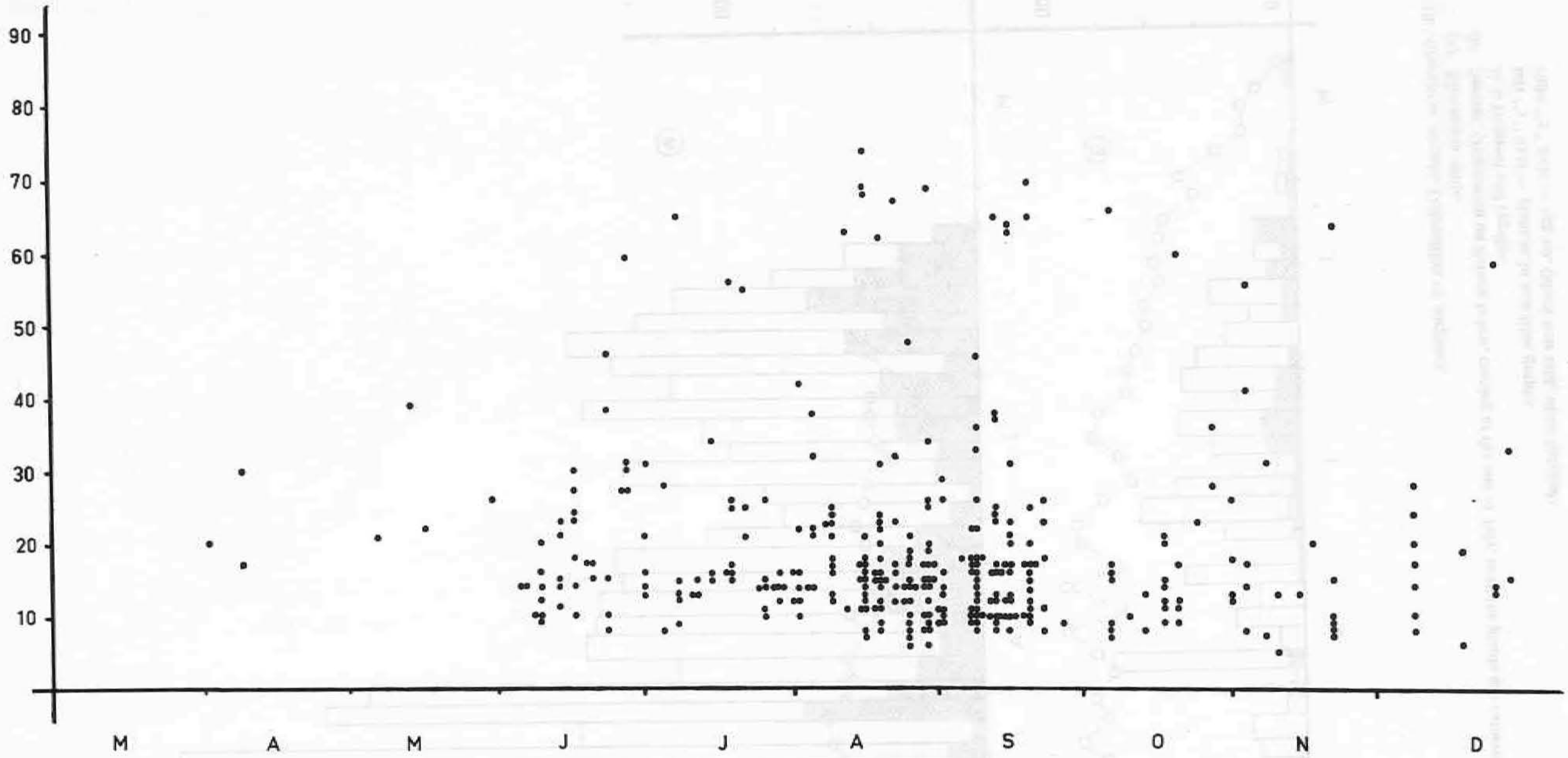


Fig. 9. *Atherigona soccata.* **Egg development with time. Mature eggs are found from June to December. The bulk of the population is in the early stages of development. (Baited trap sample). Y axis = arbitrary Micrometer units.**

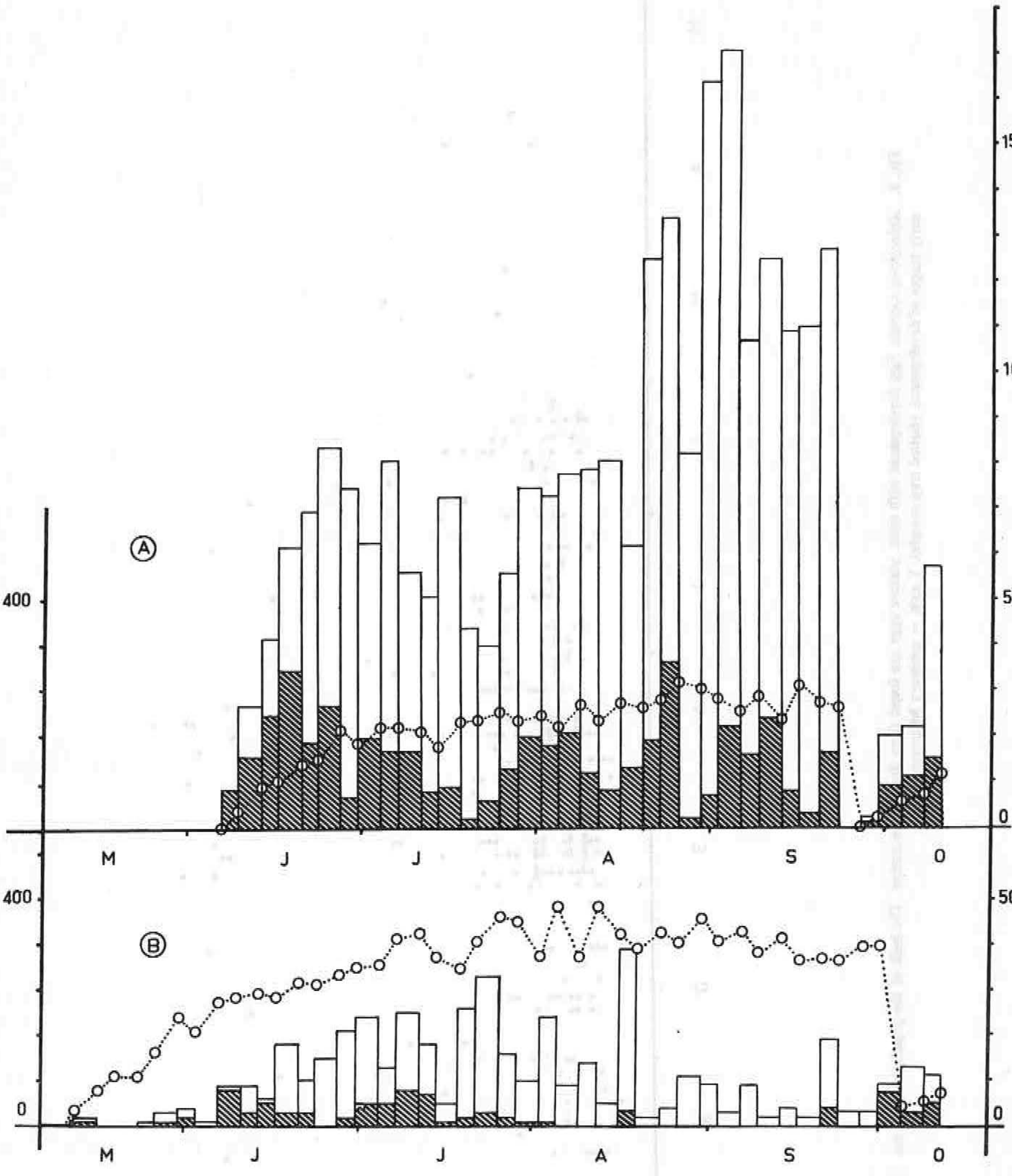


Fig. 10. ***Atherigona soccata.*** **Oviposition on sorghum.**

(a) Kikambala white.

(b) Serena. Oviposition on Serena is low, ceasing at the end of July, until the plants are ratooned in October. Oviposition on Kikambala is frequent and regular.

left "y" axis = plant ht in mm (line graph).

right "y" axis = egg no. (black new laid, white hatched).

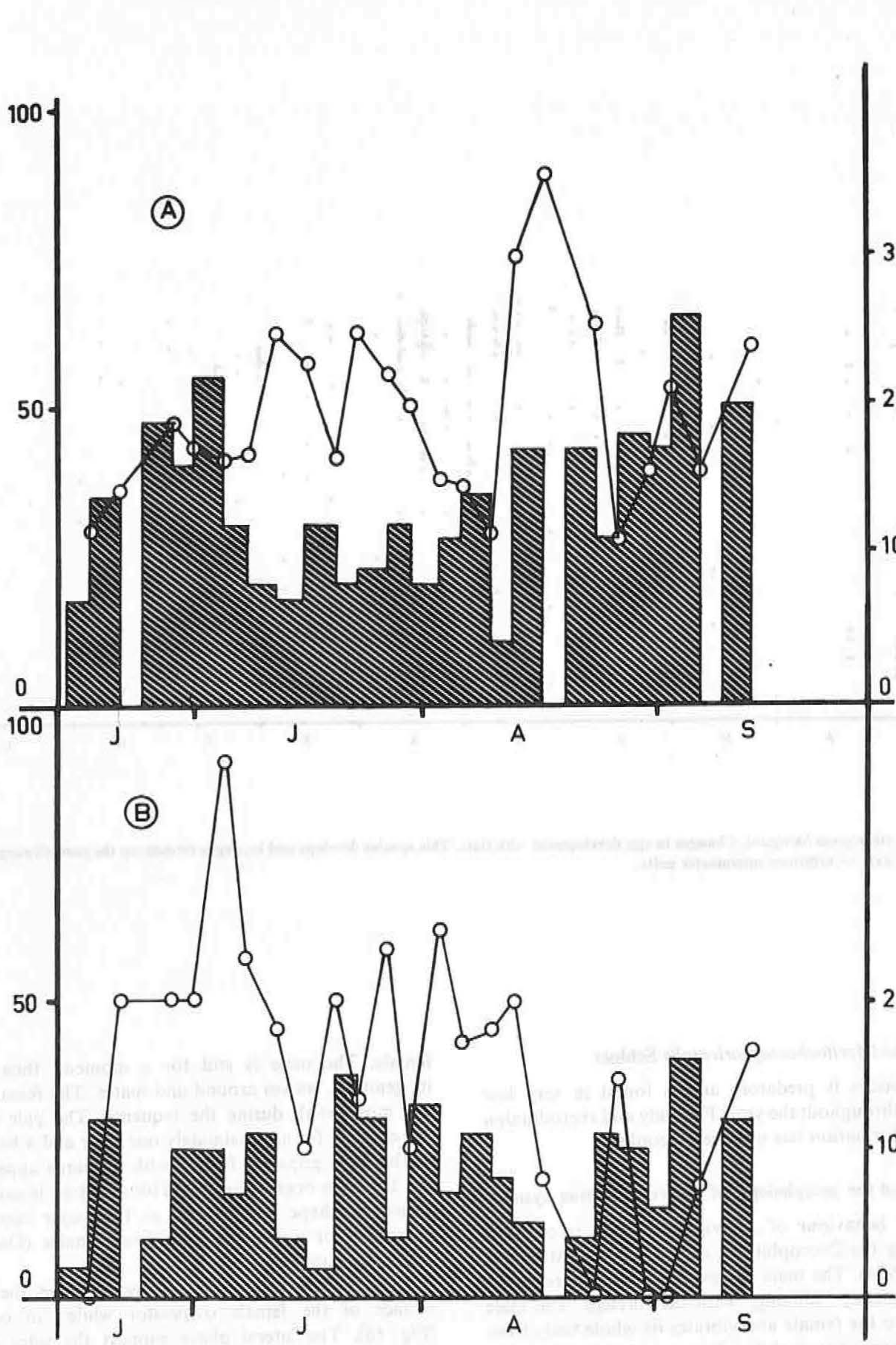


Fig. 11. ***Atherigona soccata*, Larvae on different varieties of sorghum.**

(a) Kikambala white.

(b) Serena. Significantly more Kikambala plants are damaged compared with Serena. Throughout the four month period, an average of 50% of the damaged Kikambala are occupied by larvae. Fewer of the damaged Serena plants are occupied in August and September.

"y" axis = % damaged plants occupied by larvae.

right "y" axis = No. of damaged plants/100.

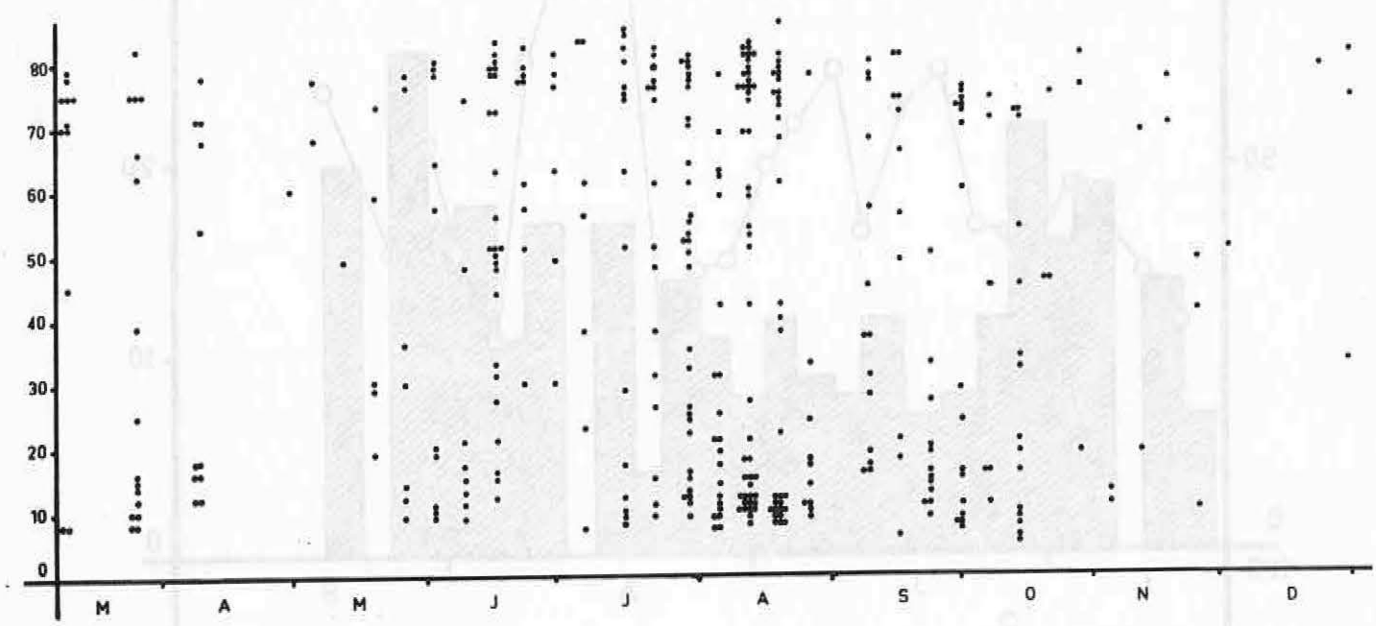


Fig. 12b. ***Atherigona laevigata*. Changes in egg development with time. This species develops and lays eggs throughout the year. (Sweep Sample). y axis = arbitrary micrometer units.**

Atherigona (*Acritochaeta*) *orientalis* **Schiner**

This species is predatory and is found in very low numbers throughout the year. Fat body and reproduction system information has not been recorded.

Mating and the morphology of the reproduction system

Mating behaviour of *Atherigona soccata* is complex resembling the Drosophilidae rather than the Muscidae (Spieth, 1974). The male chases the initially unreceptive female typically "fanning" with the forelegs. The male slides up to the female and vibrates its whole body from side to side very rapidly. Two or three approaches are necessary. Finally the female does not move away at the male's approach. The male comes close, suddenly flicks open its wings, curves its abdomen and brushes the tip against the head of the female. The male slowly shakes its body and touches the abdominal tip of the female. The male is still for a moment, then everts its genitalia, moves around and mates. The female does not move at all during the sequence. The pair remain "in copula" for approximately one hour and a half.

The male grips the female with a unique appendage, the trifoliate organ (Fig. 15). This structure is extremely varied in shape and is used as the major taxonomic character for distinguishing between males (Deeming, 1922 van Emden 1940).

The trifoliate organ of *A. laevigata* grips the lower surface of the female ovipositor while "in copula" (Fig. 16). The lateral plates support the sides of the ovipositor, while the sensilla bearing tip of the median piece tucks into the inter-segmentai membrane caudal to the seventh sternite of the ovipositor. In some species e.g. *A. conigera*, the flanges possess inner ridges that appear to clip the trifoliate process onto the seventh sternite.

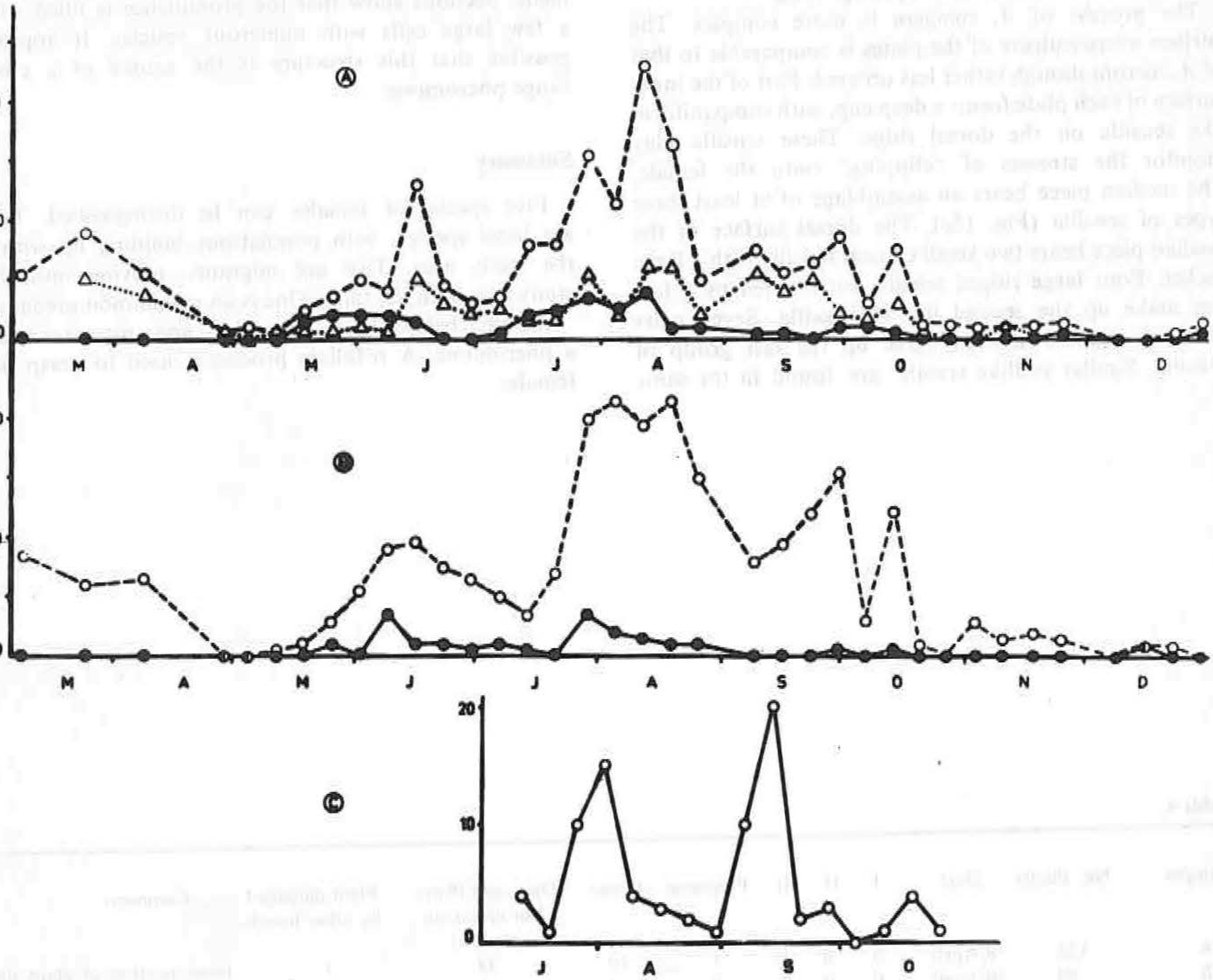


Fig. 12a; ***Atherigona laevigata***

(a) Females (Sweep Sample).

(b) Males (Sweep Sample).

(c) Males (Suction Trap Sample).

This population shows three phases—low numbers during the rains (April—May, November—December); high numbers with newly emerged adults being recruited (June,—August); and moderate numbers, many of which have completed at least two gonotropic cycles (March—April, September—October). These insects were captured on ***Digitaria Scalarum.***

The trifoliate process of *A. soccata* and *A. conigera* are examples of a simple and a complex form respectively. The process of *A. soccata* consists of two elliptical lateral plates and a deeply curved median piece which join a long extension of the cercal plate at the same point (Fig. 15a). The outer surface of the lateral plates is covered by a microsculptured carpet of fine rods, the inner surface by cross-linked ridges. Careful examination failed to reveal any sensilla on the plates. The median place is triangular in section and bear three paired and one unpaired sensilla on the tip (Fig. 15b).

The process of *A. conigera* is more complex. The surface microsculture of the plates is comparable to that of *A. soccata* though rather less ordered. Part of the inner surface of each plate forms a deep cup, with campaniform like sensilla on the dorsal ridge. These sensilla may monitor the stresses of "clipping" onto the female. The median piece bears an assemblage of at least three types of sensilla (Fig. 15c). The dorsal surface of the median piece bears two small conical sensilla with a large socket. Four large ridged sensilla surrounded by a fine rim make up the second line of sensilla. Seven pairs of short rod-like sensilla make up the last group of sensilla. Similar rodlike sensila are found in the same position in *A. laevigata*.

Another structure (also used taxonomically) that appears to be involved in reproduction is the median prominence of tergite 8 (hypogial prominence). This dorsal protrusion consists of single or multiple knobs, and appears to be the structure that the male brushes against the head of the female in courtship. A group of fine hairs surround a central pore or depression on the single lobed hypogial prominence of *A. laevigata*. The hairs seem to hold a droplet of fluid in airdried specimens. Sections show that the prominence is filled with a few large cells with numerous vesicles. It appears possible that this structure is the source of a close range pheromone.

Summary

Five species of females can be distinguished. Two are local species, with populations building up within the study area. Two are migrants, moving into the study area with the rains. One is an uncommon predator.

Mating behaviour is complex and may involved a pheromone. A trifoliate process is used to grasp the female.

Table 4

Sample	No. Plants	Date	I	II	III	Prepupae	Pupae	Damaged Plants But no larvae present)	Plant damaged by other insects	Comment
A	125	8/April	0	0	59	1	10	48	7	Inner portion of plant dry
B	89	10/April	0	0	9	0	4	76	0	Inner portion of plant wet
C	100	10/April	1	5	14	0	0	80	0	Inner portion of plant wet

Rainfall 1–8April = Omm, 9/April = 7mm, 10/April, = Omm, 11/April = 18mm, (Reading at 8'oclock in the morning)

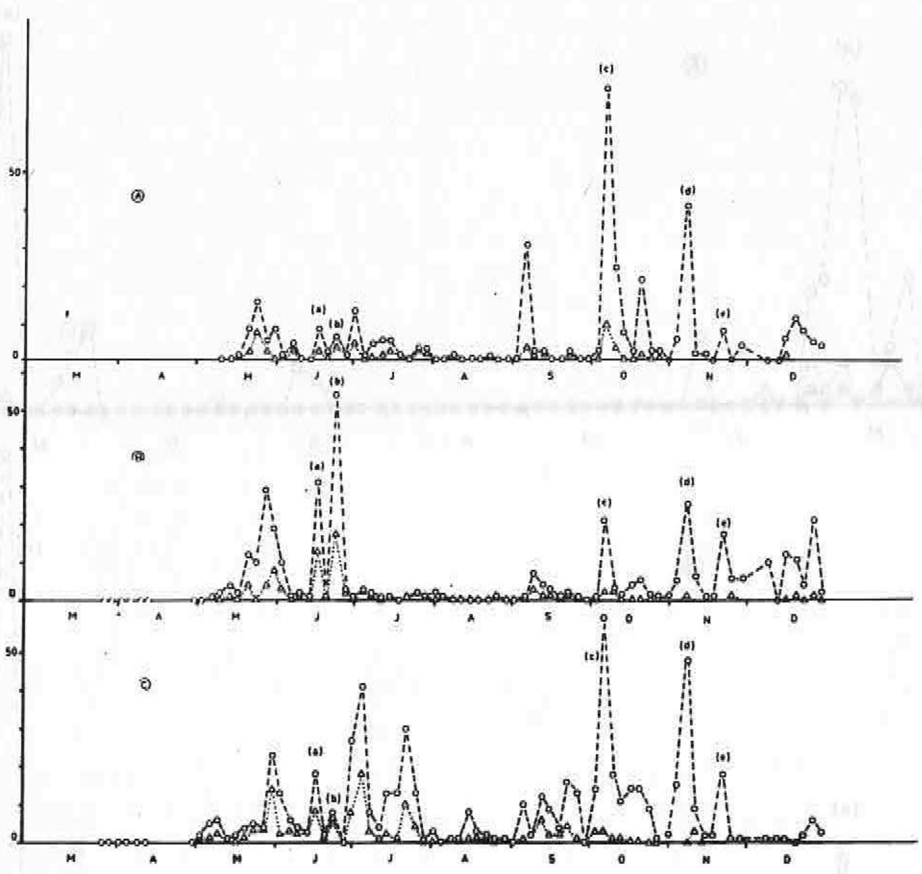


Fig. 13a. ***Atherigona conigera***
(a) Serena Sorghum.
(b) Kikambala White Sorghum.
(c) ***Digitaria scalarum.***

This species arrives in large numbers after the long (April) and short (October) rains. Large numbers arrive in all areas on the same day.

(a) 17/June
(b) 24/June
(c) 7/October
(d) 8/November
(e) 22/November

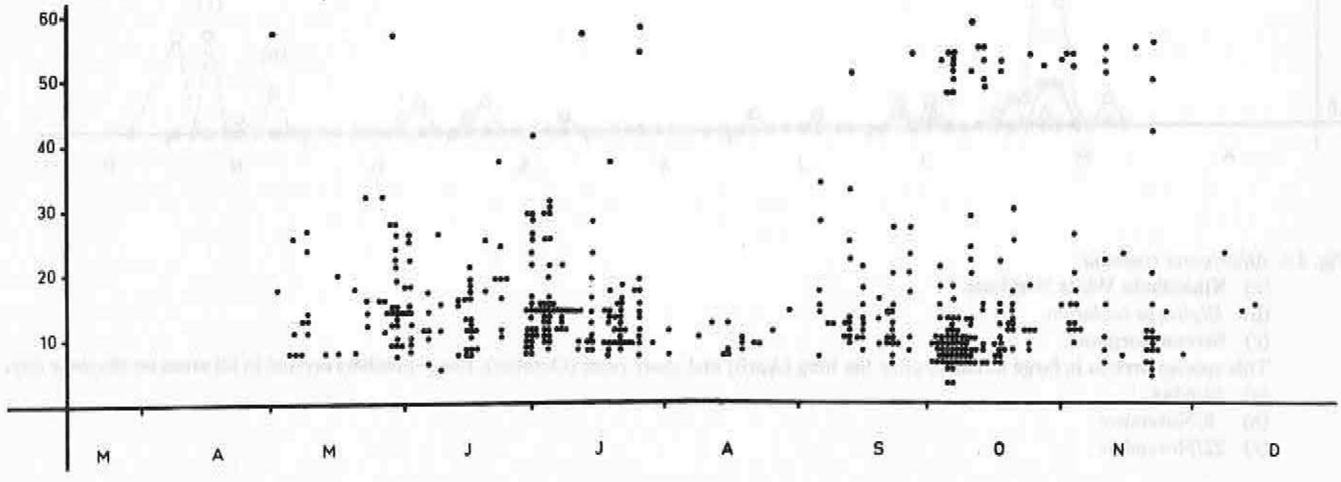


Fig. 13b. ***Atherigona conigera***

Egg development with time. More mature eggs are found during the short rains. Many individuals contain small eggs that do not appear to be developing.

y axis = arbitrary micrometer units.

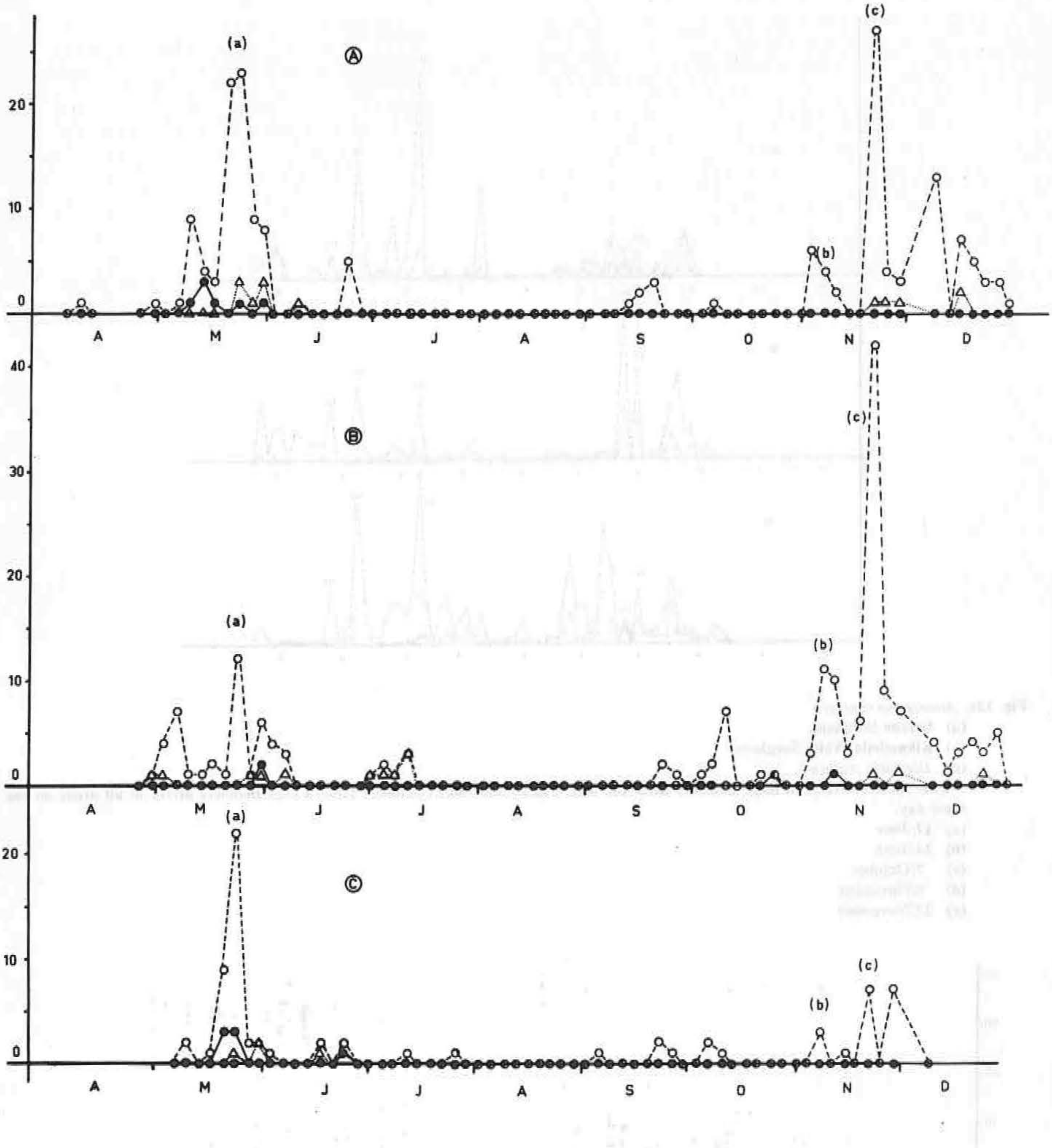
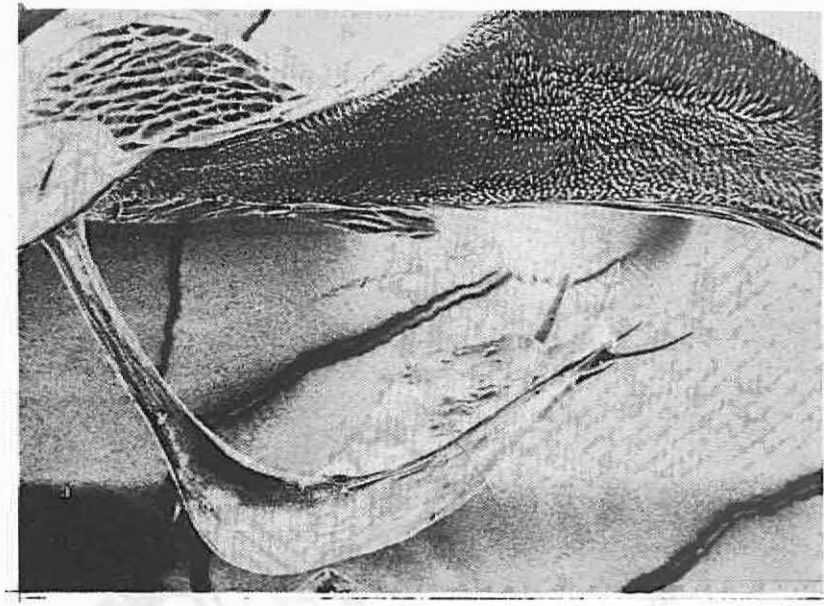


Fig. 14. ***Atherigona trapezia***

(a) Kikambala White Sorghum.
(b) ***Digitaria scalarum.***
(c) Serena Sorghum.

This species arrives in large numbers after the long (April) and short rains (October). Large numbers arrived in all areas on the same day.

(a) 24/May
(b) 8/November
(c) 22/November



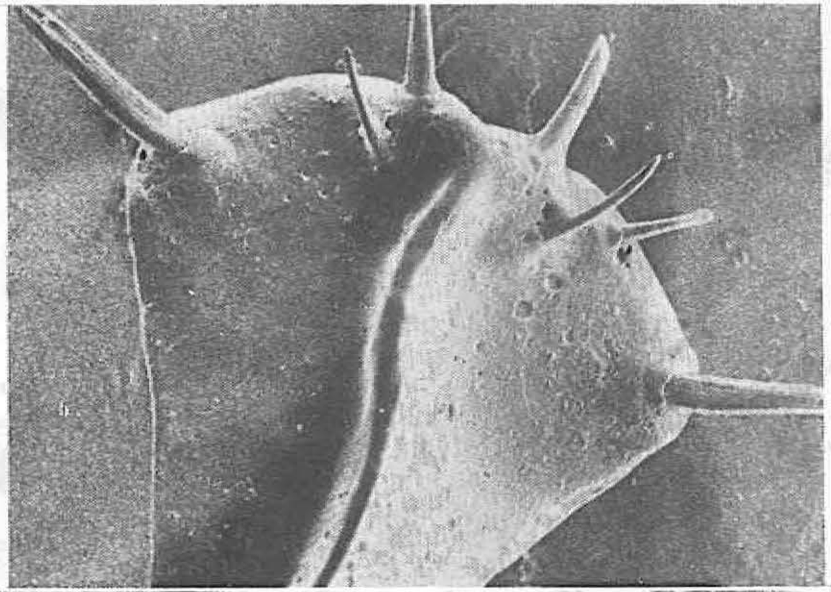


Fig. 15. *Atherigona soccata*

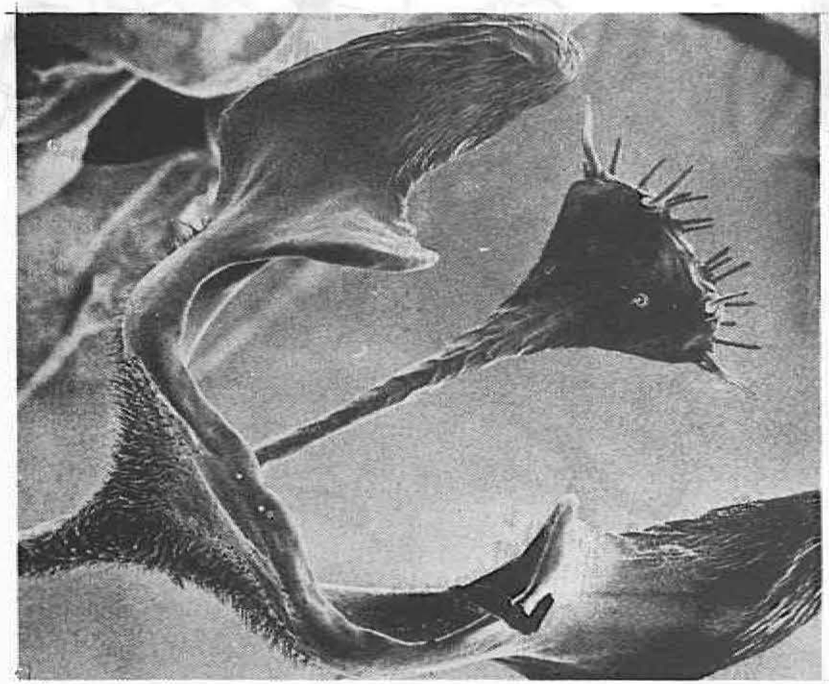
(a) The trifoliate organ.

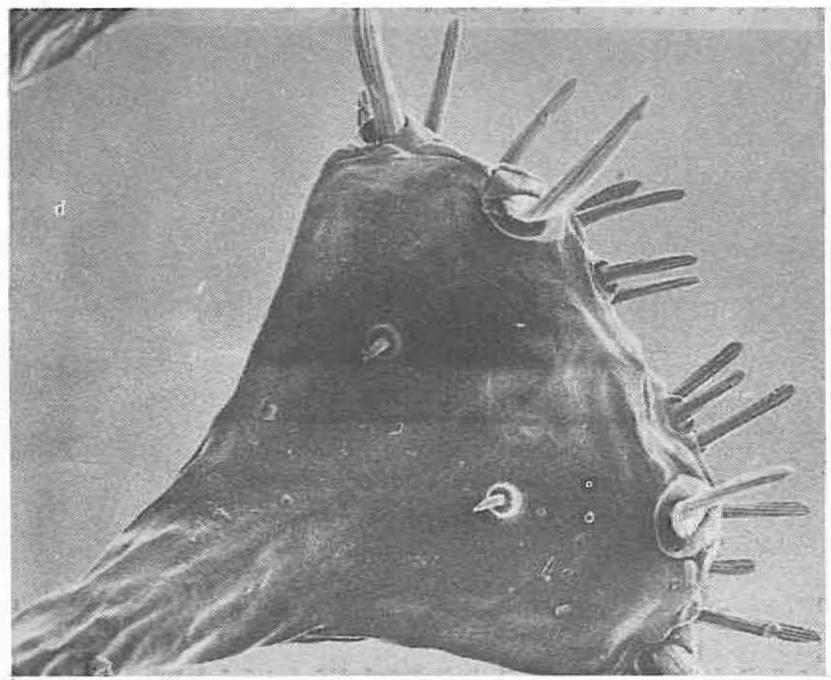
(b) The underside of the median piece, showing the sensilla.

Atherigona conigera.

(c) The trifoliate organ.

(d) The upper surface of the median piece showing the three types of sensilla.





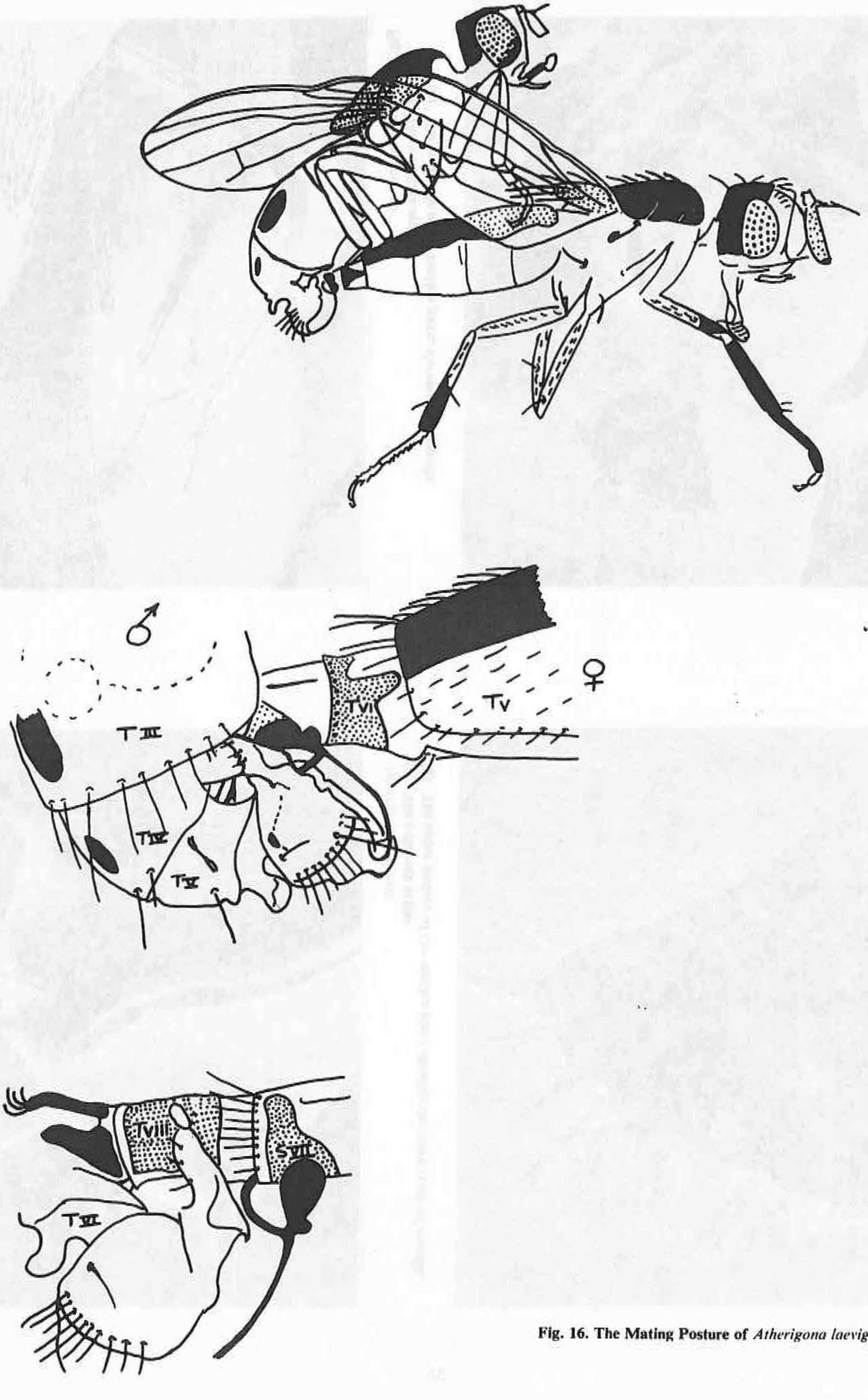


Fig. 16. The Mating Posture of *Atherigona laevigata*.

The immature stages of sorghum shoot fly, *Atherigona soccata* Rondani (Diptera, Anthonyiidae)

Kenuel Ogwaro

Introduction

Deeming's (1971) description of the immature stages of *A. soccata* included that of the full grown larva and the puparia, but identifying younger stages remained very difficult. Determining species even in the adult stage is still difficult due to their small sizes and rather similar morphologies and colour patterns. Inconspicuous but consistent differences can, however, be seen in reared material and these, along with distinctive characters in the male genitalia, are being used in taxonomic studies. Other kinds of supporting data such as the life history information and descriptions of the immature stages are badly needed.

The immature stages (Figs.17–22)

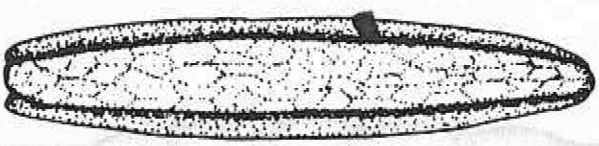
The eggs are dull white in colour, 1.3mm in length and 0.2m wide; finely sculptured on the outside and possessing a raised, flattened keel running along the mid length. The anterior end is narrower than the posterior end, with the keel ending just behind the tip of the median body. Posteriorly, the keel extends beyond the median egg body. The median area shows a reticulate, somewhat regular pattern of ridges enclosing seemingly sunken hexagonal cells (Fig. 17a). The keel area is also in a hexagonal pattern but the cells are smaller and appear as round circles (Fig. 17b).

The first larval instar has a semi-transparent integument and is pale white in colour. Its length is 2.2mm and greatest width 0.3mm. The head capsule bears characteristic rows of gill-like "cephalic brushes" covering the whole area below the frontal palps. The cephalopharyngeal skeleton (Fig. 20) has one pair of simple, slender mouth hooks (mh) without accessory teeth; a short hypostomal sclerite (hys), and an elongated pharyngeal sclerite (phs). Each hook is curved ventrally and has a wide dorsal process (dp).

The second larval instar is 4mm long and 0.5mm at its greatest width. The integument shows deposition of the fat body beginning about the third and fourth segments, thus giving characteristic shades of dull white and yellow colour. The cephalopharyngeal skeleton has two pairs of mouth hooks which vary in size and shape with the age of the larva.

The third instar larva is creamy white in colour with an opaque, granular integument. Its length is 8mm and its greatest width 1.4mm. The head capsule is distinctly bi-lobed along the cephalic region with short but thickened papilla-like antennae anterodorsally. The frontal palps are more conspicuous and are raised on two circular rings. The cephalopharyngeal skeleton (Fig. 22) is heavily sclerotised. The mouth hooks are a single pair, the hook parts heavy and sharply pointed, with 5–8 accessory teeth. Each hook has a window and a narrow dorsal toothlike projection.

The puparium, which is barrel shaped, is 5mm long and 1.2mm at its greatest width (Fig. 19). It is reddish brown but becomes dark brown towards adult emergence. The anterior end is round with a circular disc bearing the two anterior spiracles. There are 9 segments visible; the head and the first two thoracic segments being drawn in. The cephalopharyngeal skeleton remains visible through the ventral pupal skin.



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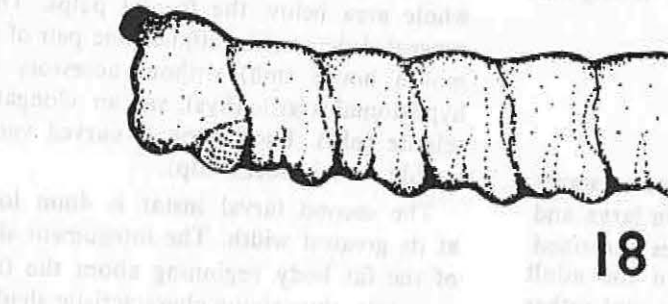
17a

17b



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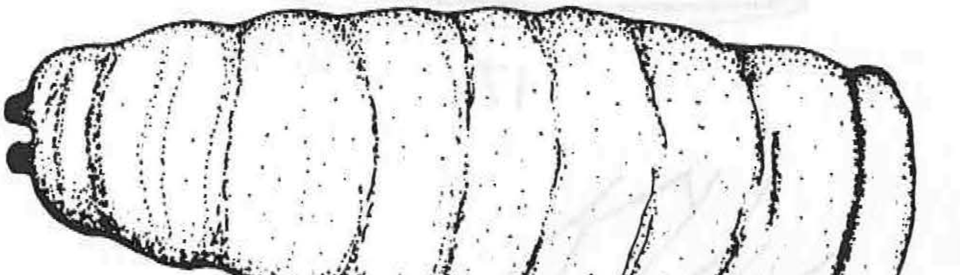
18a

18b



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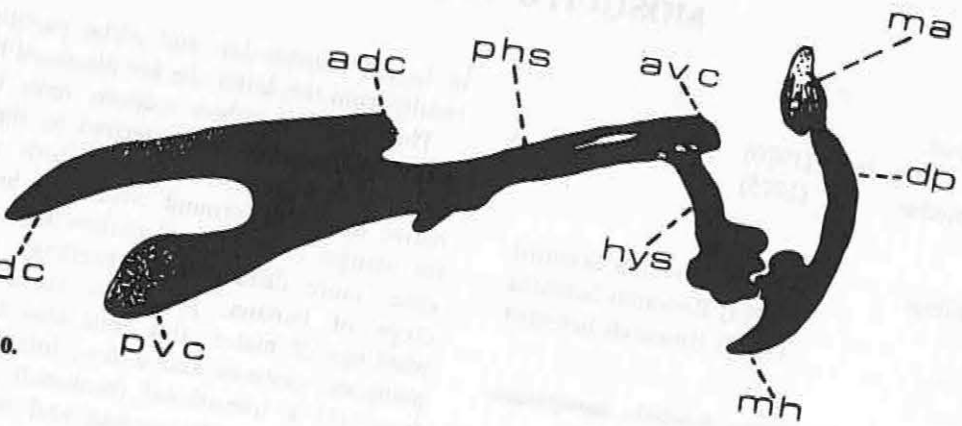


Fig. 20.

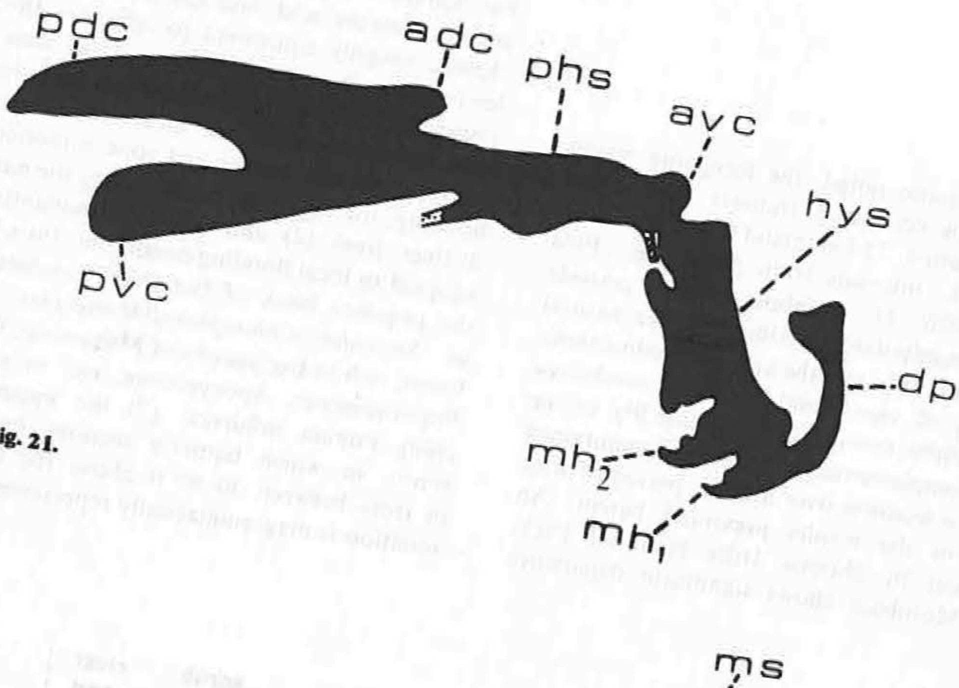


Fig. 21.

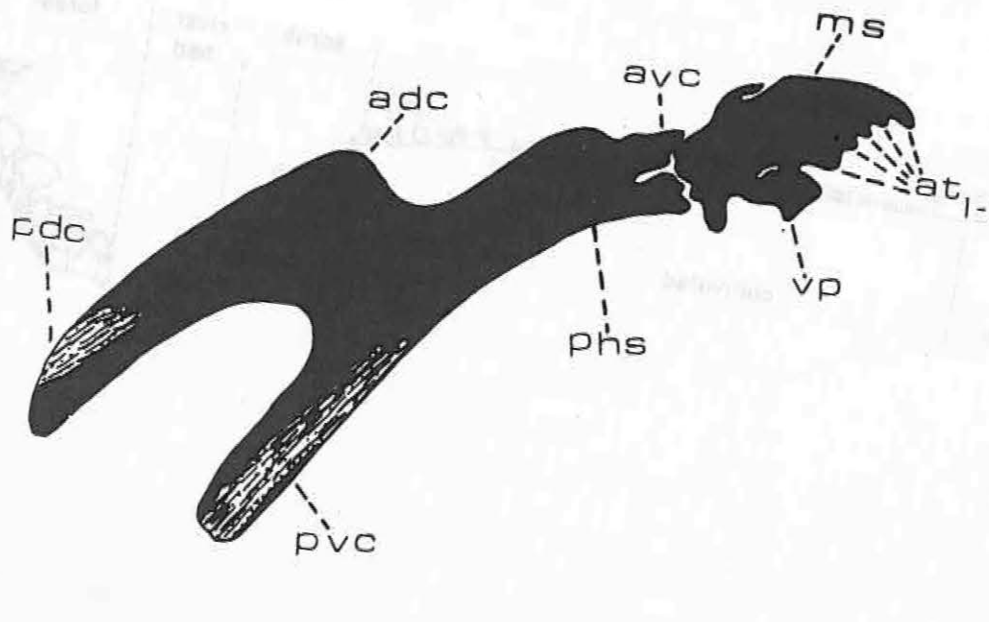


Fig. 22.

MOSQUITO RESEARCH

Directors of Research:
Professor G. B. Craig, Jr. (1970)
Professor J. Mouchet (1975)

Scientists:
Dr. L. P. Lounibos (1974) Research Scientist
Dr. F. Ogah (1973) Research Scientist
Dr. R. Subra (1975) Research Scientist

Aspects of niche utilization among treehole mosquitoes of the Kenya coast

L. P. Lounibos

Introduction

Except where otherwise noted, the foregoing results are derived from an ecological transect of bamboo sections in Rabai location, 25 km inland from Mombasa, sampled at 5–14 day intervals from November, 1974 through August, 1976. The bamboo sections provide an experimentally-manipulable facsimile for the natural treehole environment, although the artificial maintenance by water addition of year-round breeding conditions represents a departure from natural seasonality of the habitat. Two complementary transects maintained elsewhere in Rabai location over a lesser period of time essentially confirm the results presented herein. An additional transect in Shimba Hills National Park, 40 km SW of Mombasa shows significant departures in faunal constitution and niche partitioning but the results from this latter site are discussed here.

The twenty bamboo sections have been placed in seven environments characterized by their predominant vegetation: (1) a periodomestic zone within 50 m of native huts; the ground cover has been cleared save for mango, coconut, and cashew trees. (2) a cultivated zone, more distant from dwellings, characterized by crops of banana, pineapple, cassava, and seasonal plantings of maize; this zone also contains coconuts, mangoes, cashews and a few, interspersed indigenous trees. (3) a transitional (ecotonal) zone subjected to human influence by cutting and occasional planting, but dominated by savannah (grasses), introduced weeds such as *Lantana* and interspersed secondary trees and shrubs, roughly equivalent to *Afzelia—Albizia/Panicam* lowland mosit savannah. (4) a scrub zone descending toward the Kombeni River; the vegetation is predominantly indigenous, but most trees have been felled for firewood. (5) a river bed zone subjected to seasonal flooding; the vegetation surrounding the embankments is distinct from (4) and (6), predominanting in species adapted to local flooding conditions. (6) a forest zone on the opposite bank of the river, commonly recognized as *Sterculia—Chlorophoral/Memecylon* lowland rain forest, rich in tree species of Moraceace, Combrectaceae, Euphorbiaceae, Apocynaceae, etc. in spite of encroaching human influence. (7) the upper story of this forest, in which bamboo sections have been placed in trees between 30–60 ft above the forest floor. The zonation is diagrammatically represented in Fig. 1.

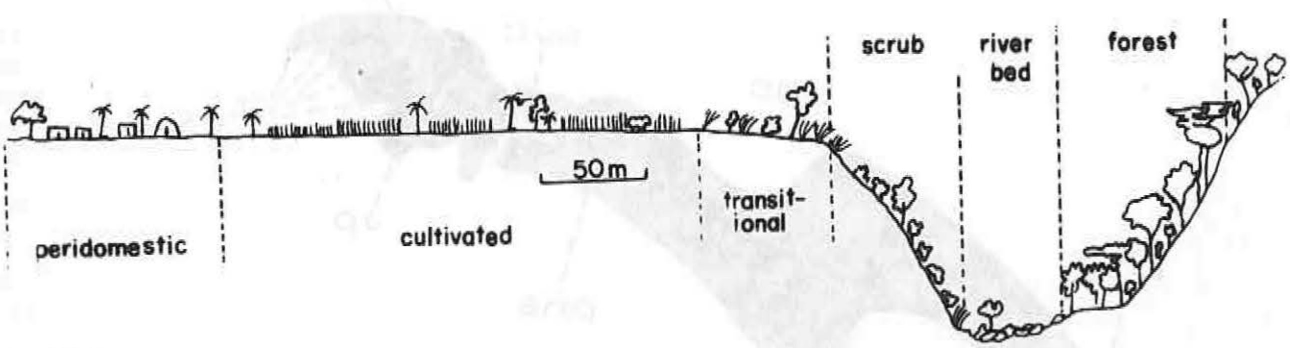


Fig. 1.

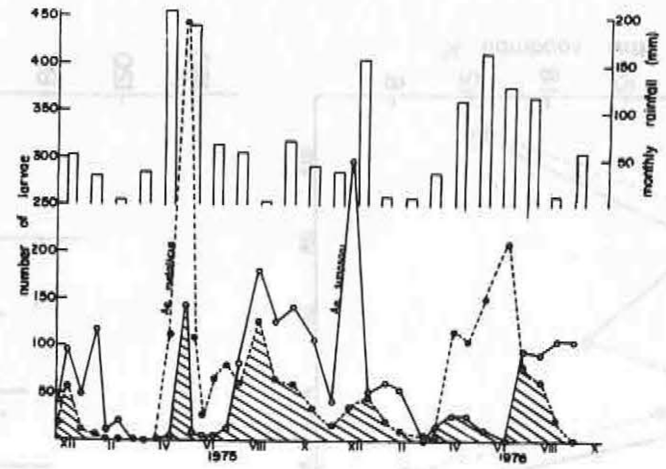


Fig. 2. The numbers of *Ae. simpsoni* and *Ae. metallicus* larvae collected weekly from 25 blackened, ½ litre tins suspended from trees in the peri-domestic habitat of a Rabai village. Each point represents the mean value of three collections. Rainfall data is from a separate village of 2 km away. Today no. of *Ae. simpsoni* collected between Dec. 1974–Sept. 1976 is 211; *Ae. metallicus* = 2153.

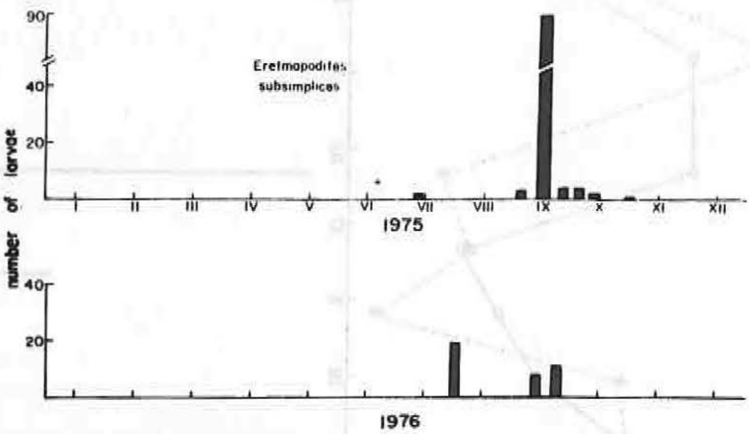


Fig. 3. The seasonal occurrence of *Eretmapodites subsimplices* from bamboo sections on the ecological transect.

Seasonal partitioning

Separation of species by season is less pronounced since all treehole mosquitoes necessarily rely on the periodic rains to increase population size. Nonetheless, certain species such as *Ae. metallicus* are more common during the long rains of April-June while others such as *Ae. simpsoni* predominate around the short rains of October-November (Fig. 2). The "generalist" container breeder *Eretmapodites subsimplices* has only been collected from bamboo sections between July and October (Fig. 3) although adults of this species have been caught in sweep net samples much earlier in the year; the tendency of this species to oviposit in bamboos during the drying months may indicate a population of gravid females lacking more suitable oviposition sites in this season.

The occurrence of the predatory mosquito *Toxorhynchites brevipalpis* on the transect is related to suitable rainfall and prey abundance (Fig. 4). Peaks in predator abundance lag prey increases by an interval determined, in part, by the severity of the intervening dry season. When the preceding dry season is severe as early in 1975, the interval between prey and predator peaks may be as much as two months.

Predation by *T. brevipalpis*

An effort to estimate the intensity of predation by *T. brevipalpis* in bamboos has been made by comparing prey densities in the presence and absence of the predator during six collection periods corresponding to times of high *T. brevipalpis* abundance (Fig. 5). The mean prey density was. 03 prey/ml collection fluid in the presence of the predator and .09 prey/ml in its absence. The prey distribution patterns are highly significantly different in the two samples ($p < .0005$, Wilcoxon $t = 3.64$). Eighteen transect collections in which *T. brevipalpis* fourth instars occurred at least twice were further compared. In predator-free bamboos the mean larval (prey) density was between 75–80 prey/bamboo; in the presence of *T. brevipalpis* fourths this value was 15–20 prey/bamboo (Fig. 6).

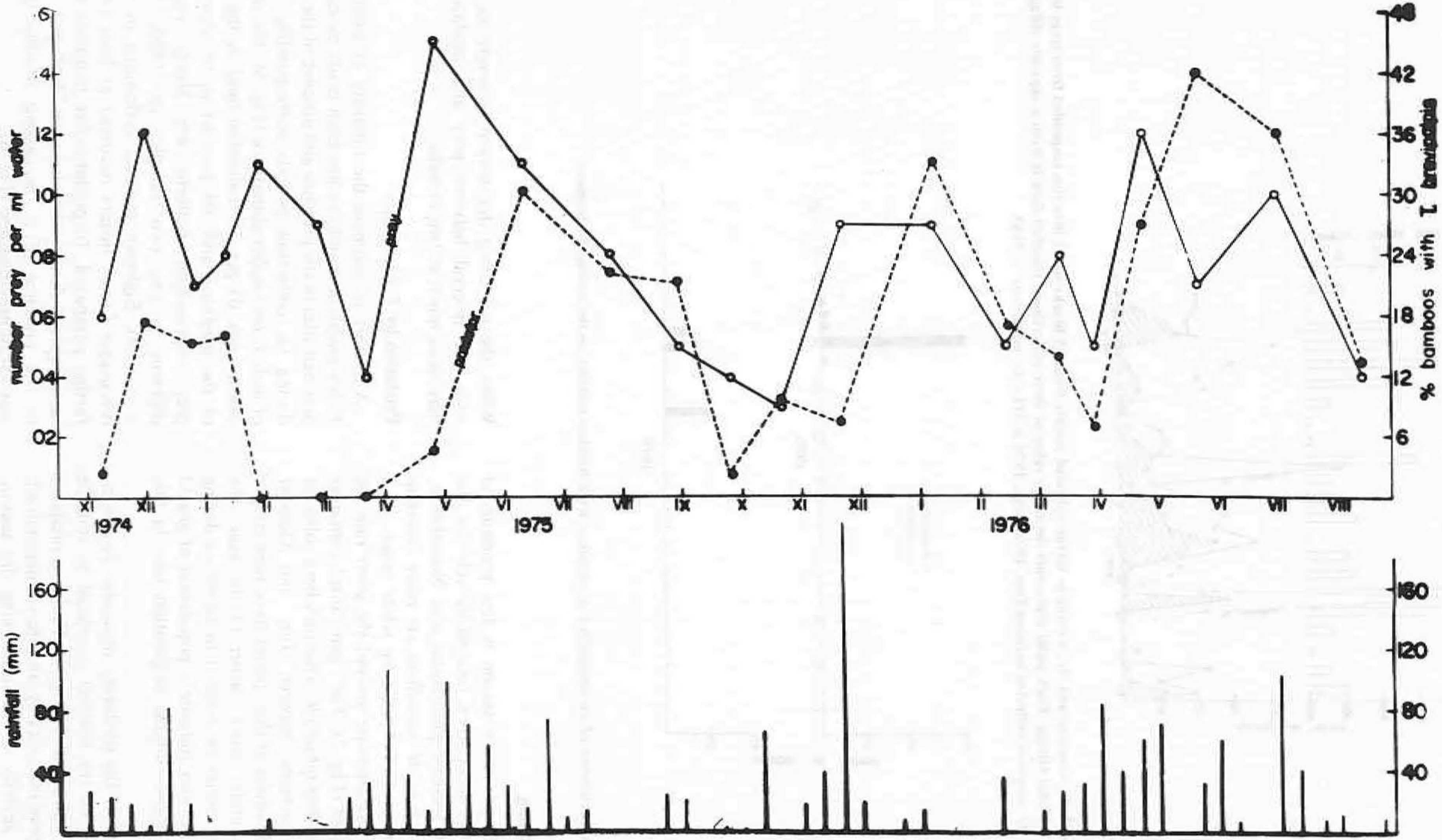


Fig. 4. The seasonal abundance of *Toxorhynchites brevipalpis* (predator) and its prey (all other mosquito species) on the transect between Nov. 1974. 1974–Aug., 1976. Each point represents a mean value from three collections of all transect bamboo sections. Rainfall data is from the predomestic zone.

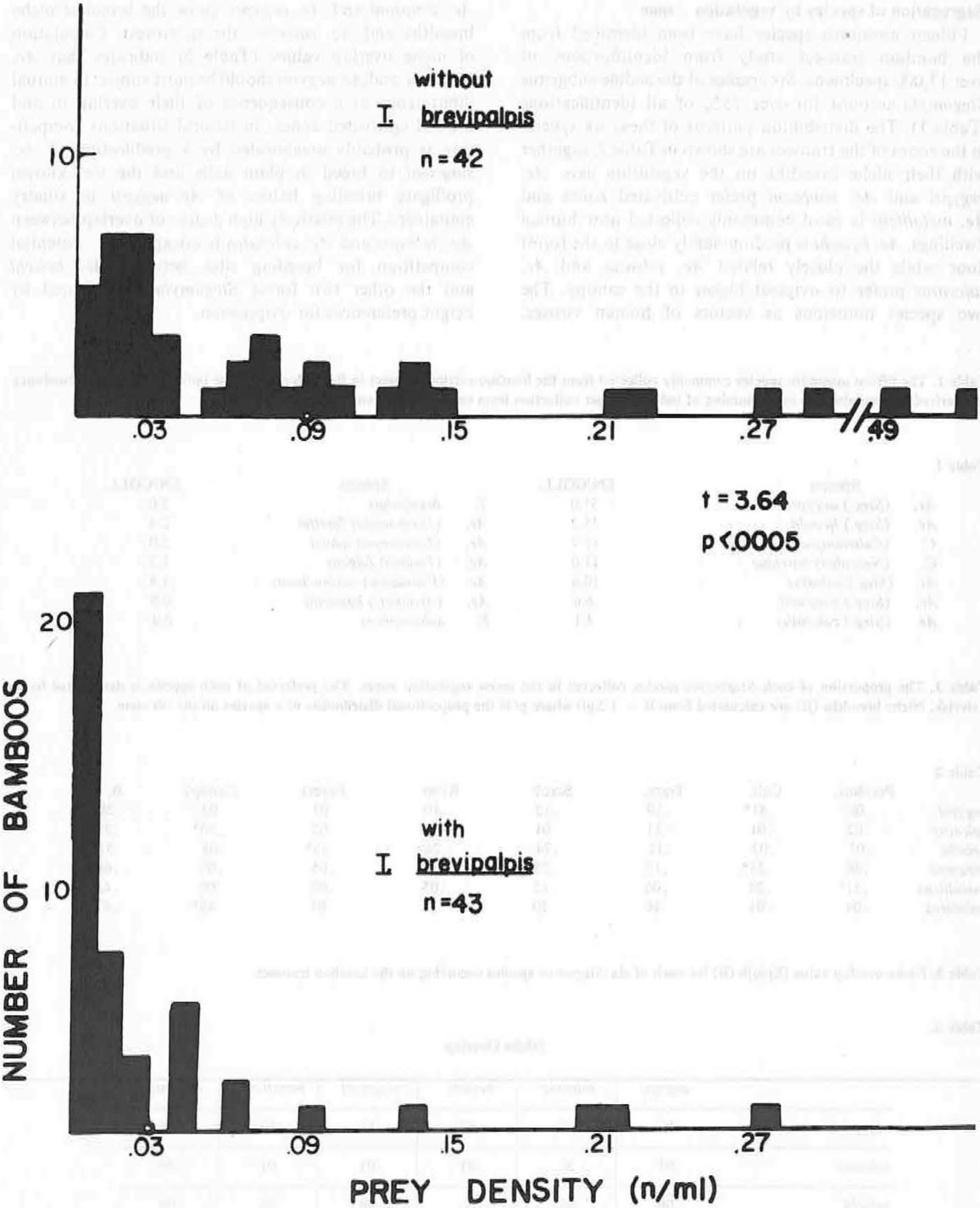


Fig. 5. The distribution in prey density in presence and absence of *T. brevipalpis* from six transect collections made during periods of peak predator abundance. Open circles represents geometric means of prey density of the two samples, calculated from Wilcoxon two-sample test. Prey density average from Nov. 74–Aug. 76 in both of the two compared samples is .08 prey/ml.

Segregation of species by vegetation zone

Fifteen mosquito species have been identified from the bamboo transect study from identifications of over 17,000 specimens. Six species of the aedine subgenus *Stegomyia* account for over 75% of all identifications (Table 1). The distribution patterns of these six species in the zones of the transect are shown in Table 2, together with their niche breadths on the vegetation axis. *Ae. aegypti* and *Ae. simpsoni* prefer cultivated zones and *Ae. metallicus* is most commonly collected near human dwellings. *Ae. heischi* is predominantly close to the forest floor while the closely related *Ae. soleatus* and *Ae. calceatus* prefer to oviposit higher in the canopy. The two species notorious as vectors of human viruses, *Ae. simpsoni* and *Ae. aegypti*, show the broadest niche breadths and *Ae. soleatus*, the narrowest. Calculation of niche overlap values (Table 3) indicates that *Ae. simpsoni* and *Ae. aegypti* should be most subject to mutual interference as a consequence of their overlap in and around cultivated zones; in natural situations competition is probably ameliorated by a predilection of *Ae. simpsoni* to breed in plant axils and the well-known profligate breeding habits of *Ae. aegypti* in sundry containers. The relatively high degree of overlap between *Ae. soleatus* and *Ae. calceatus* is unexplained. Potential competition for breeding sites between *Ae. heischi* and the other two forest *Stegomyia* is mitigated by height preferences for oviposition.

Table 1. The fifteen mosquito species commonly collected from the bamboo-section transect in Rabai location. The indices of relative abundance are derived by summing the mean number of individuals per collection from each of the seven vegetation zones.

Table 1

	Species	ΣN/COLL.		Species	ΣN/COLL.
Ae.	*(Steg.) aegypti*	51.0	*T.*	*brevipalpis*	3.0
Ae.	*(Steg.) heischi*	35.2	*Ae.*	*(Diceromyia) furcifer*	2.4
C.	*(Culiciomyia) nebulosis*	11.7	*Ae.*	*(Diceronyia) adersi*	2.0
C.	*(Neoculex) horridus*	11.0	*Ae.*	*(Finlaya) fulgens*	1.7
Ae.	*(Steg.) soleatus*	10.6	*Ae.*	*(Pseudarm.) michaelimati*	1.4
Ae.	*(Steg.) simpsoni*	6.6	*Ae.*	*(Aedimor.) haworthi*	0.6
Ae.	*(Steg.) calceatus*	4.1	*E.*	*subsimplices*	0.4

Table 2. The proportion of each *Stegomyia* species collected in the seven vegetation zones. The preferred of each species is designated by an asterisk. Niche breadths (B) are calculated from B = 1/Σpi) where p*i* is the proportional distribution of a species on the *i*th zone.

Table 2

	Peridon.	Cult.	Trans.	Scrub	River	Forest	Canopy	B
aegypti	.09	.41*	.19	.12	.10	.07	.03	.59
soleatns	.02	.01	.11	.01	.02	.02	.80*	.22
heischi	.03	.03	.11	.24	.26	.33*	.03	.57
simpsoni	.08	.33*	.12	.25	.12	.05	.05	.68
metallicus	.51*	.24	.06	.12	.05	.02	.00	.42
calceatus	.08	.01	.16	.20	.04	.03	.48*	.47

Table 3. Niche overlap value (Σpipjh (B) for each of six *Stegomya* species occurring on the bamboo transect.

Table 3.

Niche Overlap

	aegypti	*soleatus*	*heischi*	*simpsoni*	*metallicus*	*calceatus*
aegypti	X	.03	.07	.13	.10	.05
soleatus	.01	X	.01	.01	.01	.09
heischi	.06	.02	X	.08	.04	.04
simpsoni	.14	.05	.09	X	.11	.07
metallicus	.07	.01	.03	.07	X	.03
calceatus	.04	.19	.04	.05	.04	X

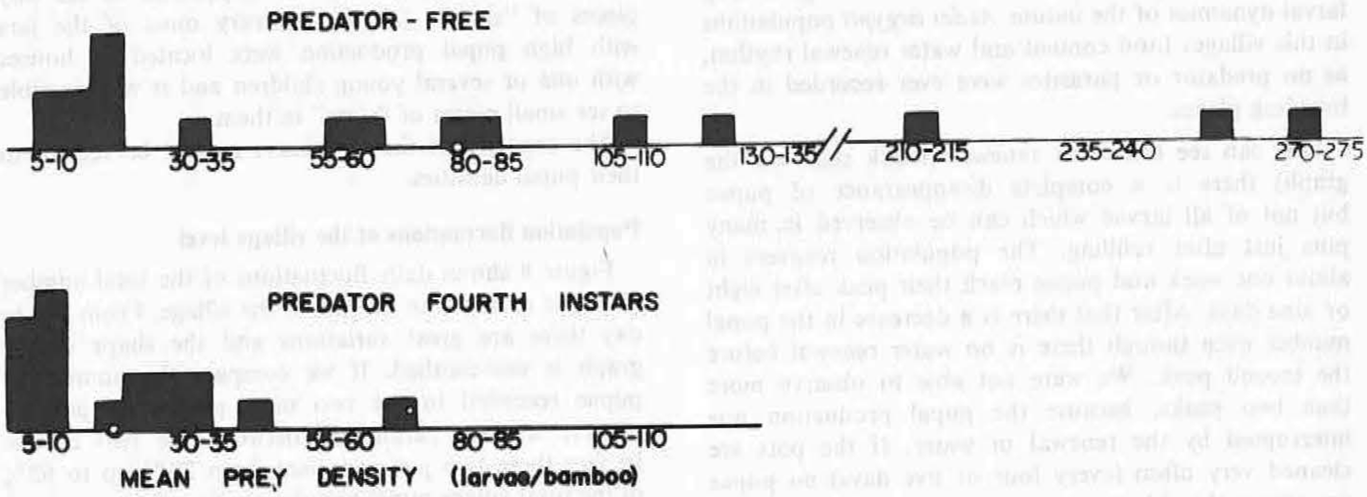


Fig. 6. Prey density compared from 18 transect samples in which *T. brevipalpis* fourth instars occurred two or three times. Each histogram point represents a mean of 3.0 ± 1.6 bamboo sections with predator fourths and 6.0 ± 2.8 bamboos without predator.

The regulation of preimaginal populations of *Aedes aegypti* L. "type form" on the Kenya Coast

R. Subra

During 1976 we studied the preimaginal dynamics of an indoor breeding *Aedes aegypti* population belonging to the type form subspecies. The only breeding containers were jars used for domestic purposes (drinking and cooking), and our observations were carried out during the dry season when no peridomestic breeding occurs. The selected village was Kwa Bendegwa (Rabai location) twenty kilometers inland from the Kenya Coast, inhabited by a very homogenous population of about one hundred people. The source of water is a tap in a neighbouring village and also a small permanent pool. Women bring water three times a day. The small size of the village (only 36 houses) made possible the daily checking of all the containers. Water is stored mainly in clayjars, rarely in drums. The capacity of these containers varies from 18 to 80 litres.

We chose to check pupae because they are more easily counted and they represent the final stage of development and give a good figure of adult population.

Daily observations dealt with the four following points:

- number of pupae in each container every morning. After counting the pupae were replaced in the container.
- noting the presence, but not counting of larvae at different stages.
- origin of water; whether tap or pool.
- whether the water in each container has been completely renewed.

Dynamics of preimaginal populations at container level

Three different types of situations can be observed in Kwa Bendegwa (Fig. 7).

- first situation, containers in which the number of pupae shows very pronounced variations, from zero to sometimes more than one hundred in a few days. The graph has one or two peaks depending on whether the jar has been cleaned or not. In some cases it is only possible to observe the increasing part of the first peak, after which the population drops nearly to zero, due to the removing and refilling of the port water.
- second situation; containers in which pupae are found irregularly, always in small numbers, less than ten, and during short period (three or four days).

● third situation is intermediate; graphs can show peaks but not very high ones. The pupal production might last several days and sometimes more than one week. Of course, these three categories represent the commonest classes of water pot along a continuum.

It clearly appears that two factors are governing larval dynamics of the indoor *Aedes aegypti* populations in this village: food content and water renewal rhythm, as no predator or parasites were ever recorded in the breeding places.

One can see that after renewal (Black spots on the graph) there is a complete disappearance of pupae but not of all larvae which can be observed in many pots just after refilling. The population recovers in about one week and pupae reach their peak after eight or nine days. After that there is a decrease in the pupal number even though there is no water renewal before the second peak. We were not able to observe more than two peaks, because the pupal production was interrupted by the renewal of water. If the pots are cleaned very often (every four or five days) no pupae are observed inside.

On the other hand, in some jars where the water is never renewed the pupal number stays nevertheless very low. It is obvious that in this case, the limiting factor for preimaginal population is food, as water renewal cannot be indicated. The source of food in jars is mainly the same, a maize gruel called "sima", the basic diet in the Rabai area. This "sima" is introduced into jars by young children who drink water from a big coconut spoon which they dip in the containers thus introducing it into the jar. We observed that no children were present in those houses where pupal population was very low in spite of a lack of water renewal. When looking into these jars it was impossible to see any pieces of "sima". On the contrary most of the jars with high pupal production were located in houses with one or several young children and it was possible to see small pieces of "sima" in them.

The capacity of the containers cannot be related to their pupal densities.

Population fluctuations at the village level

Figure 8 shows daily fluctuations of the total number of pupae recorded in the jars of the village. From day to day there are great variations and the shape of the graph is saw-toothed. If we compare the number of pupae recorded in the two most productive jars we observe a clear parallelism between the two curves. In fact these two jars represent from 20% up to 63% of the total village pupal population. So a few containers having a high pupae population are responsible for the most mosquito production in the village. The most productive jars are not the same every day. But, in fact, in 63 days of continuous observations we found that the same jars have the highest pupal numbers in 35 of the 63 days. Finally, only 17 out of 53 jars played a significant role in pupal production during the period of the study.

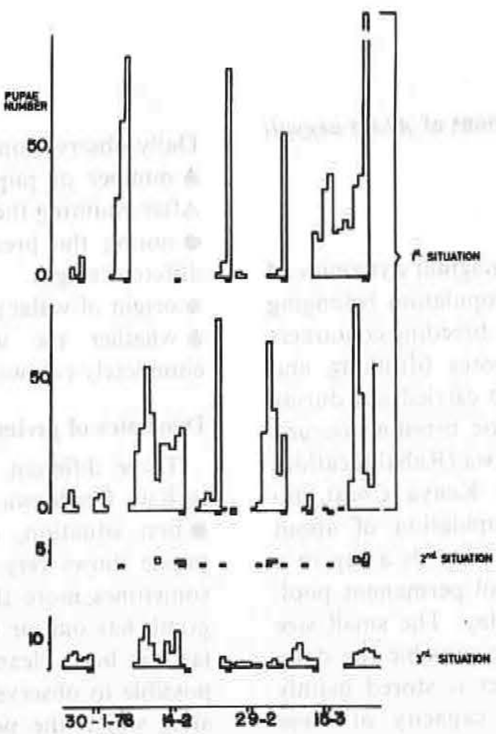


Fig. 7. Different types of situations observed at container level.

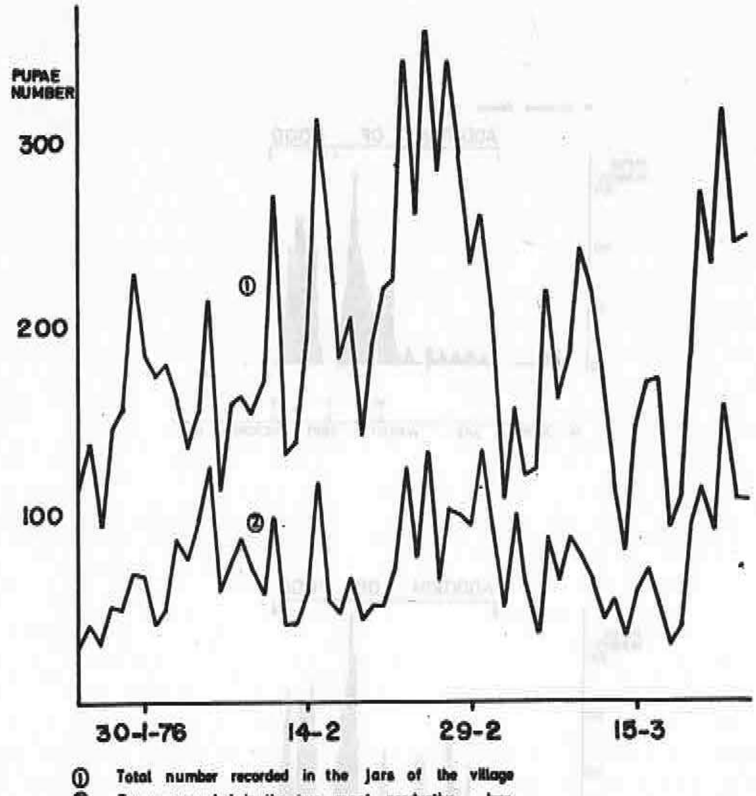


Fig. 8. Population fluctuations at the village level.

Importance of food as a regulatory factor

This study was carried out from May 1976 up to October 1976. We attempted to check the hypothesis of the importance of sima, by adding food into type 1 and type 2 jars. 8 jars were selected, 4 belonging to type 1 and 4 to type 2. At this period of the year (end of the long rains, "cold" season) some inhabitants take drinking water from pools whose water is occasionally muddy. Under such circumstances making correct scientific observations might be difficult. So these people were asked to use tap water and were refunded the price of it when we started to add "sima".

Preliminary observations ranging over several weeks were done on variations in pupal numbers under normal conditions. In early July we started to add food daily to each of the 8 jars up to the end of September. During October we continued our observations to check if in the absence of food there was a decrease in pupal densities. Thus our whole observations covered about five months:

- stage I: preliminary observations, 1 month
- stage 2: addition of food, 3 months
- stage 3: follow-up, 1 month

All observations were made on a daily basis, according to the method previously described. The number of pupae in each container was counted every morning and replaced in the container. Complete water renewal, when occuring was also noted. We were unable to do our observations on days of funerals when people were mourning. This happened 6 times during the course of our observations.

The different jars in which food was added were numbered from 1 to 8.

Type 1 Jars

- Jar 1 (Fig. 9, low). During stage 1, the maximum density observed was 51 pupae. During stage 2 the peaks of the graph showed daily pupal variations but the numbers always exceeded 100 and even 200 pupae in one case. When food addition was stopped the maximum pupal densities declined below one hundred.

- Jar 2. This jar was initially supplied with pool water, so stage 1 observations were short. The maximum density during stage 2 was 192 pupae. It dropped to 75 during stage 3.

- Jar 3. The maximum density observed during stage 1 was 50 pupae. It rose above 100 at the beginning of stage 2. In spite of food addition pupal densities stayed low and irregular in September, during Ramadhan; Muslim jar owners were not using water in the day time, but they were frequently almost emptying the jar at night. During stage 3 maximum densities dropped below 100.

- Jar 4. During the preliminary observations maximum pupal density reached 77. It reached 197 when sima was added. As in the above case and for the same reason September densities were low and irregular. Stage 3 densities were lower than those for stage 2.

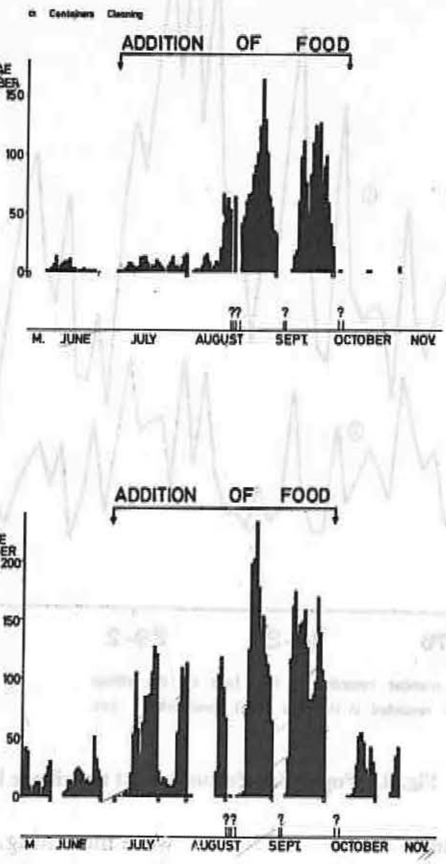


Fig. 9. Influence of food on the regulation of *Aedes aegypti* preimaginal populations.

● Jar 5. Before adding food, the maximum pupal density was only 18 pupae. During stage 2 it reached 141 in August and 129 (twice) in September. As soon as food was stopped those densities decreased markedly to 23.
● Jar 6. Low densities observed during stage 2 (maximum density: 14) increased with the addition of food, but less markedly than in other jars. For the same reasons cited for jars 3 and 4 we did not maintain observations during Ramadhan time. Stage 3 densities pupae were very low.
● Jar 7 (Fig. 9, up). 14 pupae was the maximum density during stage 1. In the first weeks of stage 2, in spite of food addition, densities stayed at the stage 1 level. Then they rose slowly reaching a maximum of 164. They were very low during stage 3.
● Jar 8. Stage 1 maximum densities were low (11). They increased slightly in the first two weeks of stage 2, then peaked at values between 56 and 67. As in the above cases, stage 3 densities were low.

The water temperature in jars during stage 2 was lower than in stages 1 and 3, thereby increasing the pupal life span. If one wants to compare pupal densities during these different stages one has to take into account that stage 2 densities have been slighlty overevaluated.

In all cases food addition promotes an increase in pupal densities. In type 1 and some type 2 jars, this increase is instantaneous. The food addition allows a greater number of larvae to develop and there is an increase in the pupal densities. In this case food is a limiting factor of preimaginal populations. In other jars of type 2 the increase in pupae is not instantaneous. The number of available eggs might be limited. Such a hypothesis suggests there is a difference in the attractiveness of gravid females towards the types of jars. Type 1 jars might be more attractive than some type 2 jars because of the presence of a greater number of old larvae and pupae (this has been demonstrated for other species of mosquitoes). The food addition in these type 2 jars will slowly increase preimaginal numbers. As a consequence of the addition of food the jars attractiveness will also increase and after several weeks densities in the two types of jars will have similar values.

In the last part of our investigations we will study the attractiveness of gravid females towards jars of both types in order to check the aforementioned hypothesis.

Isoenzyme genetic polymorphism in *Aedes aegypti* of the Kenya coast

Fidelis Ogah

Introduction

The objectives of isoenzyme studies on *Aedes aegypti* at the Kenya Coast are three fold: to look for isoenzymes which will characterise the population in various habitats, to use these markers to study the population dynamics of each population and also to use the markers to study the degree of interbreeding between domestic populations and ferral populations. In late 1974, and part of 1975 the population dynamics was studied by monthly survey using esterase 6–isoenzyme (ICIPE 3rd Annual Report 1975). This did not yield any analysable data because of the tremendous variation shown by esterase–6 locus. The ideal isoenzyme system should be a two allele system and with clear cut differences in allele frequencies between the forest and domestic populations.

Search for suitable isoenzyme systems

Throughout the greater part of 1976 surveys were carried out on other isoenzymes. Polyacrylamide gel electrophoresis was used in all the surveys. Table 4 below is a summary of the results of the survey. The table shows the enzymes stained for, the number of

Table 4. List of isoenymes of *Aedes aegypti* surveyed

Enzyme	No. of mosquitoes screened	Nature of the enzymes
Glyrophosphate dehydrogenase (2Gpdh)	140	monomorphic
Glucose-6-phosphate dehydrogenase (G6–pd)	327	monomorphic
Leucine amino peptidase (lap)	415	monomorphic
Tetraxolium oxidase (To)	670	monomorphic
Xanthine dehydrogenase (xdk)	250	monomorphic
Malate dehydrogenase (mdh)	300	Highly polymorphic

mosquitoes used and the nature of the enzymes. As can be seen from the table most of the enzymes are monomorphic. Apart from the monomorphic enzymes systems in both forest and domestic populations, polymorphic enzyme systems were also present. These were PGM, Alkaline phosphatase, Acid phosphatase, and Aldehyde oxidase. Aldehyde oxidase is weakly polymorphic. The highly polymorphic systems were either to hard to score or staining results were not consistent.

So far the only reliable and very ideal system found is that of isocitric dehydrogenase (Idh). This is one locus two allele system. The forest populations are homozygous for the slow allele shile the domestic populations are highly polymorphic. A survey for Idh was carried out with samples from Majengo, Ererwani and Shauri Moyo villages, and also from Kombeni Forest and Shimba Hills Forest. The data proved to be extremely interesting. The staining result was consistent. As shown in Table 5 all samples from Shimba Hills and Kombeni Forest were homoxygous for the slow allele while the samples from the villages were polymorphic.

Table 5. Frequencies of Idh genotypes of *Aedes aegypti* from domestic and forest habitats at the Kenya Coast

Habitat	Genotypes SS	SF	FF
Majengo	44	49	34
Shauri Moyo	302	92	64
Kombeni Forest	324	—	—
Simba Hills	398	—	—

The data on Idh is interesting for two reasons. It is a good system to use to carry out a long term survey of domestic and forest populations of *Aedes aegypti*. It could become a marker for typical forest *Aedes aegypti* from the Kenya Coast depending on the outcome of what collections from other villages and forests will be. This aspect of the work will be followed up later.

Reference

OGAH, F. (1975). Frequencies of Esterase-6 genotypes from Shauri Moyo village and Kombeni Forest (ICIPE Annual Report 1976).

TERMITE RESEARCH

Directors of Research:

Professor M. Lüscher	(1970)
Dr. W. A. Sands	(1973)

Scientists:

Dr. N. Abo Khatwa	(1974)	Research Scientist
Ing. O. H. Bruinsma	(1974)	Research Scientist
Dr. G. Buhlmann	(1975)	Research Scientist
J. P. E. C. Darlington	(1975)	Research Scientist
Dr. M. Lepage	(1975)	Research Scientist
Dr. G. W. Oloo	(1974)	Research Scientist

Collaborators:

Dr. G. D. Prestwich (1974–1976) Chemistry

Consultant:

Professor C. Noirot

ECOLOGY

Introduction

The year ending 31st December 1976 is the first in which both reseatch scientists have been fully active throughout.

Their studies have advanced to a point where the results are beginning to converge towards a predictive potential.

The mound sampling (Dr. Darlington) has followed two annual cycles and is detecting seasonal activity and population changes. The technique has been refined to produce estimates of un-predented accuracy.

The studies of foraging and feeding (Dr. Lepage) are evaluating consumption by natural populations in the field.

The final step will be to combine population estimates with those of foraging activity to quantity the role of Macrotermes sp as primary consumers.

Ecology of *Macrotermes subhyalinus* Nests

J. P. E. C. Darlington

Seasonal sampling of nests

The series of monthly nest samples started in September 1975 is being continued. Complete interpretation is not attempted at this stage, partly because of the difficulty of standardising the samples, and partly because of the drought which has persisted through a large part of the sampling period, but some preliminary conclusions may be drawn.

It appears that the caste composition of the nest population does not fluctuate greatly with the season. Healthy nests seemed able to cope with the drought, possibly through the buffering action of the fungus combs on a fluctuating food supply. The intensity of foraging activity towards the end of the drought did suggest that the nests were then short of food. Since the beginning of the Short Rains there has been extensive regrowth of grass and herbs, and within the nests many new fungus combs are being constructed, the old ones being chewed up and removed.

The development of the 1976 brood of reproductives has been followed since April when they were first found in the nests. Fresh and dry weights of individuals in samples taken at intervals will allow the growth rates of reproductives to be calculated. It was noticed that although there was broad synchrony between nests, the state of development of the brood of reproductives could be out of step by about one month between different nests. However, all the reproductives had completed their final moult to the alate well before the Short Rains began.

Observations on alate flights

Observations on the dates, times and associated weather conditions for *Macrotermes* alate flights have now been made through two flight seasons, in 1975 and 1976. In 1975 the Short Rains were meagre, and although flight holes were built and regularly opened over a long period, only a small proportion of the alates flew. The rest remained in the nests until the beginning of the Long Rains in April 1976 when they again attempted to fly. By this time they had lost 5–10% of their fresh weight, and were weak and in poor condition, so that it seems doubtful if they will have been successful in establishing new colonies. The loss of weight suggests that the alates were not fed by the parent colony during the waiting period. In 1975, adequate rain fell in the Short Rains, and most of the alates flew in November-December—early January.

Comparisons can be made of the distribution of flight holes on mounds in these two years. Almost all mounds known to have had flight holes in 1975 also had them in 1976. Of those mounds which had many flight holes (over 200) in 1975, about half had similarly large numbers in 1976. The remainder had noticeably fewer flight holes in 1976, but an approximately equal number of mounds which had few flight holes in 1975 had many in 1976, so that the total number of mounds with many flight holes remained about the same.

Mounds with many flight holes constituted 15% of the total, while another 14% of nests thought to be alive had no flight holes at all. It is, however, very difficult to be certain whether some mounds are alive or dead. The presence of flight holes on a degenerate mound was taken as proof that the mound was still alive, and mounds in similar condition but without flight holes were also counted as being alive. On this basis, 70% of all visible mounds were considered to be alive. This compares with an earlier survey of the same area based solely on the external condition of the mound, in which only 44% of the mounds were thought to be alive. The difference may represent nests which are in decline or dying, perhaps with the royal pair already dead.

Foraging and feeding of *Macrotermes subhyalinus*

M. G. Lepage

Introduction

This study concerns the foraging activities of *Macrotermes subhyalinus* and the estimate of the food intake. *M. subhyalinus* is mainly a grass-feeder and forages using underground galleries branching out and opening on the outside through foraging holes. The workers come out mostly at night to collect plant materials.

The work is carried out near Kajiado (80 km south of Nairobi), in a semi-arid grassland ecosystem, where one hectare has been protected from large mammals.

Two types of methods are used in relation with the two phases of the study.

a. Foraging activities. The methods are based on the measure of the foraging parameters, either inside plots or along line-transects: the number of foraging holes per unit area, their level of utilisation (by masking them with plaster) and the number of foraging workers on the ground or from the nest.

b. Food intake. Several methods are employed together, to give parallel data: comparison between protected and unprotected plots in vegetation, consumption in litter-bags, sampling of foraging workers and the food they collect.

A. Relationships between foraging parameters

The foraging parameters are intercorrelated. The following correlations are drawn from 28 foraging cycles:

a–maximum population of foraging workers per hour (W_M) and total number of holes open (H_M) per foraging cycle:
Log $W_M = 3.97 + 0.00789 H_M$; r + 0.8989; $p < 0.01$.

b–Accumulated number of foraging workers per hour (w.h) and total number of holes open (H_M) during a foraging cycle: Log w.h $= 5.46 + 0.00822\ H_M$; $r = 0.9088$; $P < 0.01$

c–w.h and foraging workers during the first hour of a foraging cycle (w_1). Log w.h $= 4.78 + 0.726\ w_1$; $r = 0.6893$; $P < 0.01$. These relationships are used to express the level of the foraging activity by measuring only a parameter easier to obtain. For instance, it was a theoretical number of foraging workers (w.h) was estimated for the period February-September 1976, from the total holes open (HM), which estimation differs from the real figures by only 3.7%.

B–Territory—area foraged

Each colony moves its foraging area throughout the season, even from one cycle to another. The size of the area foraged during a day-night cycle varies from 133 m² in April to 552 m² in May. This pattern enables a full exploitation of the area. The holes become more densely spaced and there is a negative correlation between the number of holes per unit area in March 1976 and the new holes dug up to August 1976 ($P < 0.05$).

The coefficient of variation (standard deviation divided by mean) of the number of holes per 100 m² shows also the even distribution of the foraging: $V = 149$ in March, 57 in August.

The accumulated areas foraged give the picture of territory belonging to each nest. Depending on the season the activity is related to the distance from the mound, as shown on Figure 1. From these curves was calculated the total of foraging holes per "theoretical" colony: from 22000 in November 1975 to 51000 in August 1976.

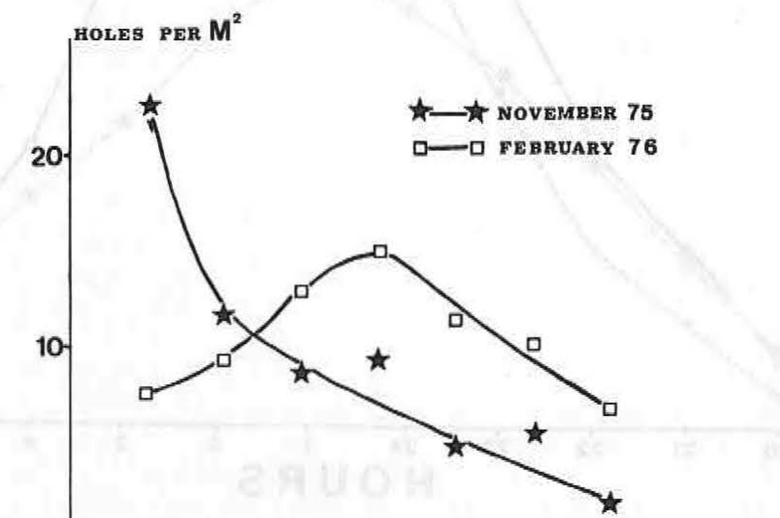


Fig. 1. Foraging holes per square meter correlated to the distance from the nearest mound.

C—Periodic variation in activity

a—Day-night variation

The workers start collecting food above the ground soon after dark and stop in the early morning. These foraging workers are checked at intervals (1–2h) during the cycle and the experience is repeated several times a month.

Figure 2 gives the example of two monthly foraging cycles and the average for the period February-September 1976.

b—Seasonal variation

This variation is based on the level of utilisation of the foraging holes. This evolution is irregular (Figure 3 quotes the results per 10 days), but we are able to draw a clear picture of the general pattern. Before describing this pattern it is necessary to point out that the period January to October 1976 was particularly dry. Broadly, the activity shows a peak before the rains (February—March), decreases during the rainy season (April) and goes up again during the dry season (June-July).

Table 1 also summarises the seasonal activity, quoting the number of holes per m^2 and their level of utilisation per foraging cycle.

c—Determination of foraging activities

The explanation of the foraging activities is still in progress and only preliminary conclusions can be drawn.

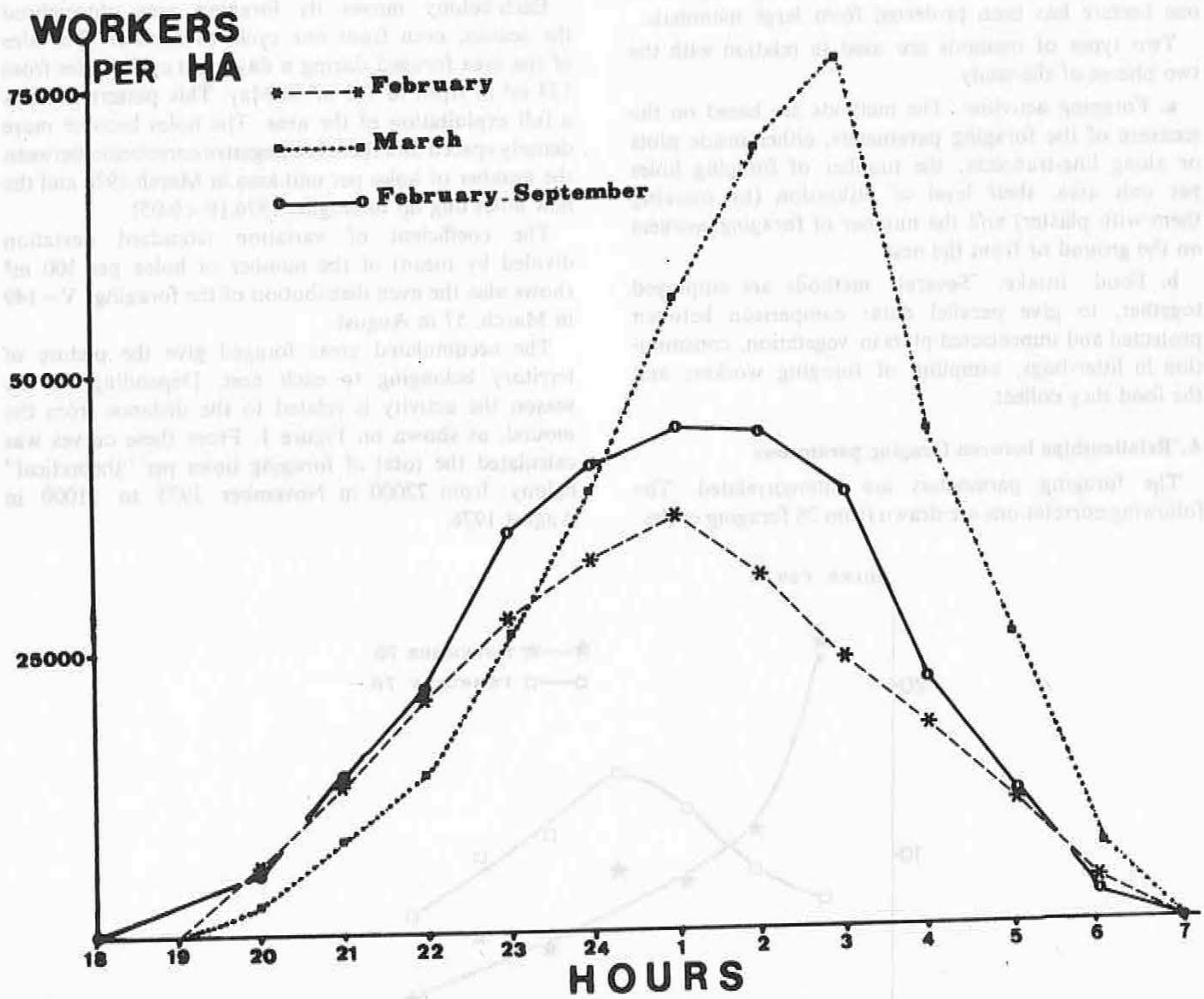


Fig. 2.

Temperatures. The foraging activity is correlated with the temperature on the ground level and especially with the difference between the maximum and the minimum (M-m), at least for the period January-May. The data from June-July cannot be fitted with this relation. The relationship between the percentage of activity and (M-m) per 10 days is significant ($p < 0.05$) and the correlation is even better with (M-m) of the 10 previous days. The analysis of 27 foraging cycles gives the following correlation between the foraging workers (w.h) and the difference $(M\text{-}m)d_{-1}$ during the previous day:

$\text{Log w.h} = 3.73 + 0.253\ (M\text{-}m)d_{-1};\quad r = +0.5335;\ P < 0.01$

Rainfall. The influence of rain is complex. Probably small rains increase the foraging activity, whereas too much rain stops or decreases the activity. The correlation per month between rainfall (0.0 to 20.5 mm) and percentage of holes open is significant and positive ($r = +0.8290$; $P < 0.05$), while the correlation per 10 days between rainfall (0.8 to 95.8 mm) and that percentage of utilisation is significant and negative ($r = 0.9163$; $P < 0.01$).

Also, we point out a negative correlation between the number of foraging workers ($w.h_d$) during a cycle and their number on the previous cyle ($w.h_{d-1}$):

$\text{Log w.h}_d = 8.61 - 0.000114\ w.h_{d-1};\ r = -0.7025;\ P < 0.05$

We set up the hypothesis of the division of the foraging activities between two phases in *M. subhylinus:* first the plant materials are collected from the surface and secondly this food is brought back to the nest. This hypothesis implies storage structures below the ground, still to be proved. But the observations of the foraging populations as quoted below, brought some evidence about this hypothesis.

D—Foraging populations

The foraging populations are studied under two aspects:

- The populations foraging above the ground, checked along a line-transect.
- The populations coming in and out of a nest surrounded with a trench, checked through clear pipes (24) which castes are obliged to use.

Table 1. Foraging holes, level of utilisation, foraging workers and food collected per square meter throughout the year

Month	Foraging Holes Number	Foraging Holes Used	Foraging Workers	Food Collected (g.) minimum	Food Collected (g.) maximum
October	6.75	0.50	4.27	2.17	2.55
November	8.02	0.19	—	0.82	0.97
December	8.65	0.39	0.53	1.69	1.99
January	9.03	0.58	6.99	2.51	2.95
February	10.84	1.91	52.24	8.28	9.73
March	13.00	2.69	78.78	11.65	13.70
April	13.58	0.23	—	1.00	1.17
May	13.66	1.64	43.06	7.11	8.35
June	13.75	3.64	111.01	15.77	18.54
July	13.83	2.39	68.57	10.36	12.17
August	12.86	1.16	26.73	5.03	5.91
September	11.75	1.53	39.32	6.63	7.79

Vegetation. The foraging holes are distributed differently according to the vegetation and their number per unit area increases from a tall grass to a short grass cover (P—0.05). The number of holes follows proportionally the decrease of standing crop and litter per m².

Internal factors. In some cases, we cannot explain the foraging pattern only from environmental parameters: particularly the peaks established in June-July. During these months, probably the foraging activity is related to the maturation of the sexual castes.

a. Foraging population above the ground

Table 1 summarises the numbers of workers per m² facing the number of holes open.

b. Foraging population from a colony

This foraging population outside the nest is calculated by difference between the out- and the in-going populations. We focused the following points:

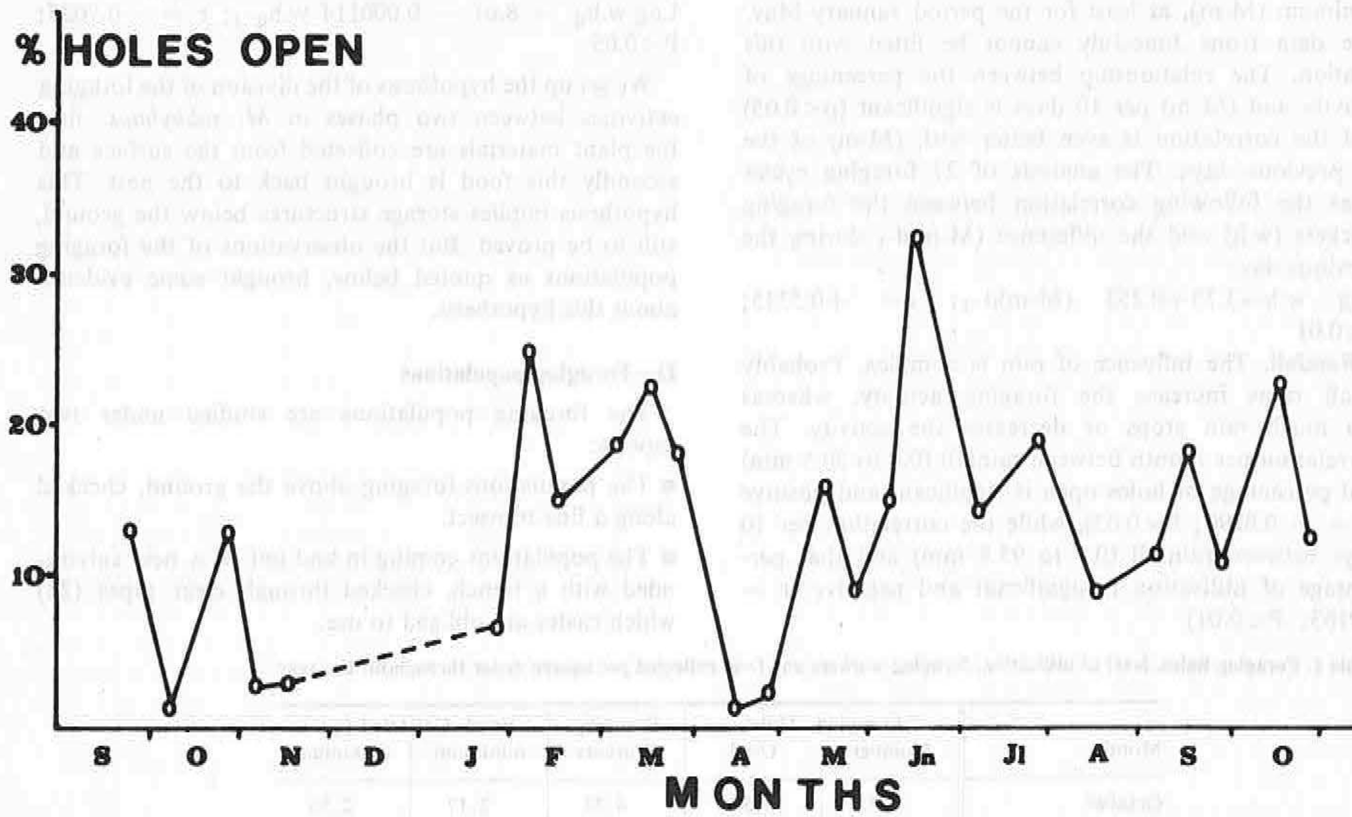


Fig. 3. Percentage of foraging holes utilized throughout the year (10 days averages).

The maximum number of out-going workers occurs during the night and the peak is slightly before the peak seen for the above ground activity.

The activity decreases in the morning (low temperatures) and around 5–6 p.m. (high temperatures), but, more or less, this activity neverstops and maintains quite a high level throughout the day (often 500–1000 out-and ingoing workers per minute at midday). This continuous activity when the above ground activity has ceased, corroborates the hypothesis of two phases in the food collection.

c. Comparison between the above ground activity and the foraging population from a single nest

We compared the difference in/out-going workers per minute from the nest at the peak of activity and the workers found on the transect. The relationship, logarithimically, is significant and positive. As our study nest is far enough from the transect, this relationship set up the interesting problems of the synchronism between colonies within the same area.

E—Food collected

This study is still in its preliminary phase and the figures given should be considered as provisional.

a. Comparison between protected and unprotected plots.

We settled a triangular experiment for measuring the standing crop, litter and roots biomass in the vegetation: (1) expressed to all the grass-consumers, (2) protected (fence) from large mammals and (3) protected (fence + trench) from both large mammals and termites. The main results of this study are plotted in Fig. 4

We analysed the figures concerning the termites food in-take. This in-take is as follows: for the period May-September (4 months), the standing-crop in-take reaches 192 Kg and the litter, 201 Kg/ha. On the assumption of a yearly consumption at the same level (probably not entirely true, because this period corresponds to a high foraging level), we come to a total of 1179 Kg. ha^{-1}. year^{-1} (standing crop + litter). From the comparison between the peak (standing crop + litter) in May (2060 Kg/ha) and the minimum in September (1498 Kg/ha), we find that 68 % of the missing quantity is the intake by *Macrotermes subhyalinus*.

b. Food collected by the foraging workers

The workers in the foraging area collect their food in two ways: they can ingest food in their digestive lobe or they can carry back to their holes the pieces of grasses.

We found in comparing samples of in/out-going workers from the foraging holes, a quantity of food ingested around 6–8 %, of the large workers dry weight (3.18 to 3.75. 10^{-4}g). At the same time, the workers lose water by evaporation: 21.6 to 37.4. 10^{-4}g per worker. By integration of the number of foraging workers (w.h), we come to a total of 39–52 Kg.ha^{-1}. $year^{-1}$ of material ingested and to a loss of 283–490 Kg of water. ha^{-1} $year^{-1}$. But these figures need to be corrected according to the exact time spent outside by each worker.

The food collected in the workers mandibles is estimated by picking workers coming back to the nest. Few such experiments have been made in this very promising way. Figure 5 shows the example of the food collected during a 43h period (22–24 September 1976) when about 450 g were collected, or 250 g per 24h.

c. Litter–bags method

Sixteen litter-bags are disposed on the ground and fitted with litter. After 15 to 30 days, they are removed and weighed. We relate the food taken to the number of days and to the number of active foraging holes. The differences between eaten and uneaten bags ($P < 0.01$) is, from the first experiment, 0.15 to 0.17 g.$m2^{-1}$. $hole^{-1}$. We estimated the total consumption throughout the year (to be corrected when more figures are available) using monthly levels of utilisation of foraging holes. The results are given in Table 1. From this first attempt, the food collected is estimated to be 730 to 850 Kg.ha^{-1}. $year^{-1}$.

d. Other possibilities

Some other methods are still not yet employed for measuring the food intake: Determination of the fungus-combs turnover and food consumption of colonies. For this purpose we are rearing young colonies of *Macrotermes* from dealates. The populations of these colonies—one year old—already pass one thousand.

Conclusion

These preliminary studies already draw the influence of *Macrotermes subhyalinus* in this semi-arid ecosystem and point out the termite impact on the pasture. This importance would certainly be emphasized if we take into consideration the action of termites on soils and organic matter. For instance, some of our experiments (not reported here) show that more than one ton of soil is brought to the surface per hectare per year in the foraging activites.

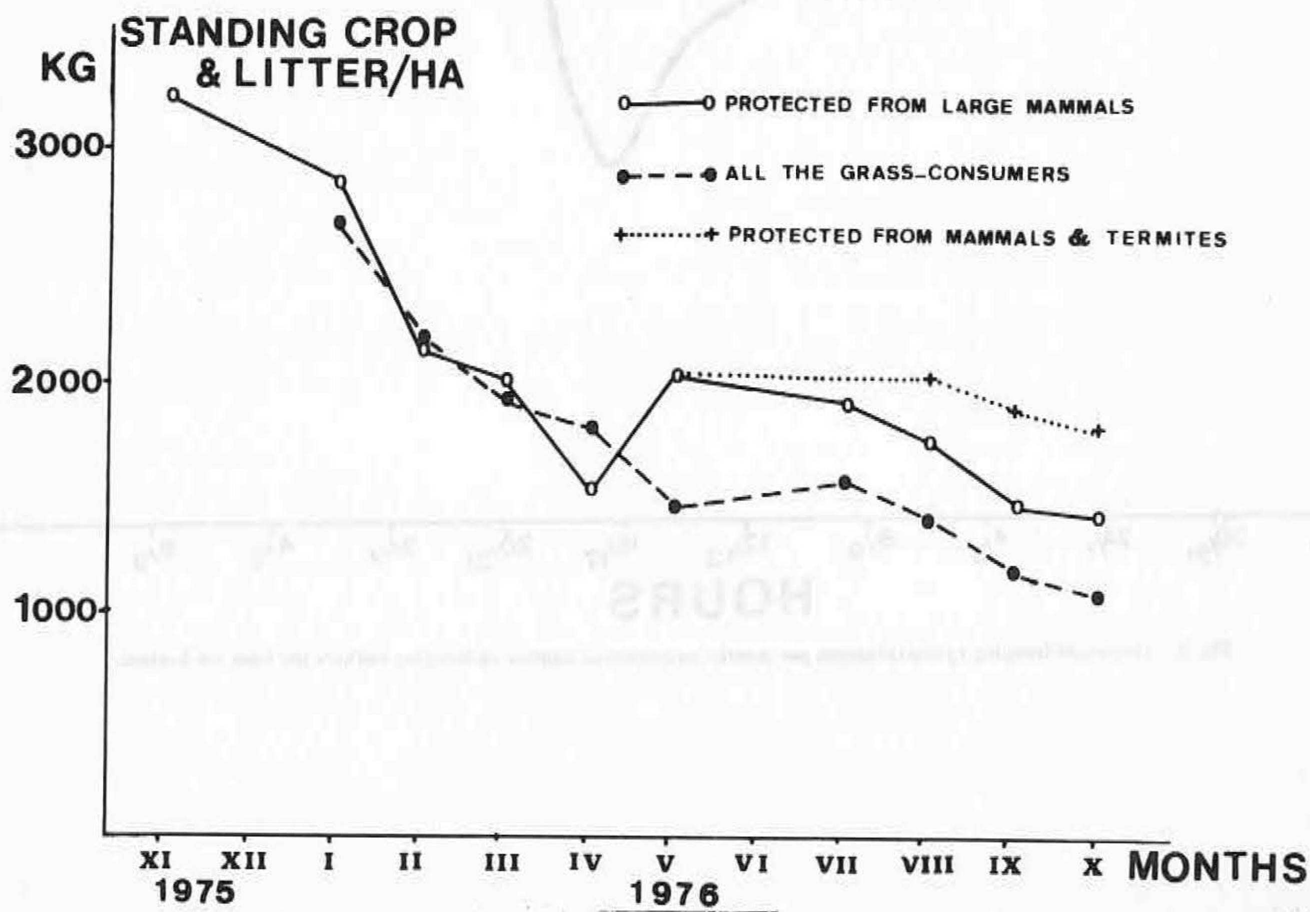


Fig. 5. Food collected to the *Macrotermes subhyalinus* nest in g. per hour (22–24 September 1976).

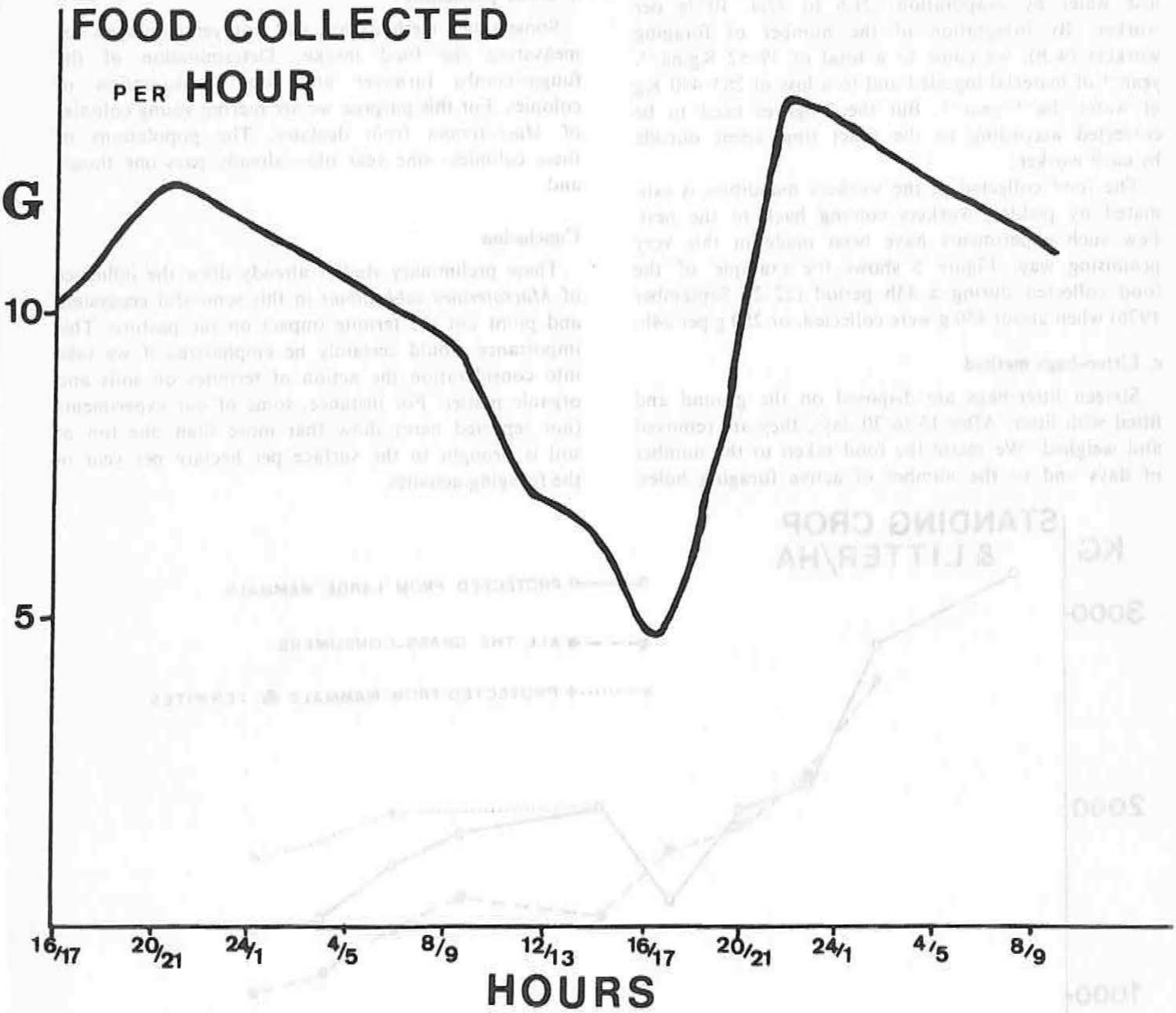


Fig. 2. Day-night foraging cycles (averages per month) expressed as number of foraging workers per hour per hectare.

PHYSIOLOGY

Termite physiology and caste differentiation

Georges Buhlmann

Nitrogen determinations

A Kjeldahl set up was established in order to carry out quantitative determinations of organic nitrogen in a semi-micro scale at ICIPE. The technique was standardized and our technical personnel instructed. Nitrogen determinations are now being performed with great accuracy and are open for a broad field of applications.

The investigation of soil from termite mounds shows a considerable difference in nitrogen content according to the location from which the sample was obtained. The samples were provided from a *Macrotermes subhyalinus* mound in Kajiado.

It is evident that the activity of termites is responsible for the reshuttling of nitrogeneous compounds in and around the mound.

An analysis was carried out about the termitomyces which is cultured in the mounds of *Macrotermes*. No significant difference could be detected in the nitrogen : dry weight ratio of fungus comb material of different quality (newly deposited, overgrown by white mycelia, grasped by termites). However, if the white conidia of termitomyces were analyzed separately, a seven times higher nitrogen : dry weight ratio was found. Termitomyces therefore very likely plays an active role in reorganizing nitrogenous material within the fungus comb.

Macrotermes caste formation

Eggs, larvae, nymphs and the adult castes of *Macrotermes subhyalinus* were collected from a mound near Kajiado and analyzed for head width, dry weight and total nitrogen content. The results are summarized in Table 2. One of the most interesting facts is the low nitrogen : dry weight ratio found in higher instar nymphs. It reflects a higher rate of incorporation of non-nitrogeneous material, i.e. fats and carbohydrates in the future reproductives, in anticipation of the long period of time they will have to live on their reserves after the flight. It is possible that nymphs receive diet different from that of larvae from the neuter line, but at present it can not be ruled out that nymphs simply make other use of their diet.

Alates collected after the flights of December, January and March showed little difference in total nitrogen per animal. Dry weight, however, was highest in December and declined afterwards. This suggests that the alates, which probably all hatch around November, are only poorly provided with food by their worker nest mates during the waiting time. They have to live on their reserves and consume mainly fats and carbohydrates. The highest loss of dry material was marked

Table 2. ***Macrotermes subhyalinus*** **averages of at least 3 measurements**

	Head width mm	Wing pad length mm	Total nitrogen µg	Dry weight µg	Relative nitrogen % dry weight
Eggs			2.3	31	7.4
Larvae	0.74	0	17.0	410	4.1
	1.17	0	53.0	1075	4.9
	1.80	0	148.5	2620	5.7
Small workers	1.79	0	149.0	3350	4.5
Large workers	2.85	0	430.0	7070	6.1
Small soldiers	2.78	0	1360.0	17725	7.7
Large soldiers	4.86	0	2860.0	36300	7.9
Nymphs	0.59	0.06	10.5	233	4.5
	1.17	0.39	143.0	2700	5.3
	1.72	1.45	440.0	18200	2.4
	2.31	3.29	805.0	35200	2.3
	—	—	4855.0	105970	4.6
Imagines	—	—	6695.0	104395	6.4

in the abdomens. No difference could be observed between the two sexes.

A pronounced increase of dry material and nitrogen could be observed in all castes during the time when the newly ecdysed insects undergo pigmentation. This is connected with sclerotisation and the development of functional mandibles, musculature, exocrine glands etc. Since unpigmented animals are not able to take food up actively, this means that the termite society has to provide a considerable amount of nutrition for the formation of the new workers and soldiers.

We have failed so far to detect any difference between workers found in the queens chamber and workers from the nursery in respect of head width, dry weight and nitrogen content. It is possible that the two locations are occupied by the same groups of workers, but it still can not be ruled out that workers involved in taking care of the royal pair and nursing termites happen to have the same head widths, dry weights and nitrogen contents.

In a similar investigation, major workers carrying out repair work on the surface of the mound and average workers from the inside were compared. In all characteristics measured the repair workers were significantly heavier. It is not known if each worker changes his activities according to age or if the largest are straightaway determined to become repair workers.

Macrotermes colony foundation

After flight the dealates increase their body weight during the first days of colony foundation. This is due to water uptake and feeding on soil. Later on, especially after onset of egg laying and raising of offspring, they shrink because of depletion of their reserves. In healthy young colonies, 200 days after the flight, both male and female showed a loss of about 70% of their dry weight and 50% of their total nitrogen. In colonies where eggs failed to hatch for some reasons, the reproductives gained weight after the same period because of feeding on soil and oophagie. It is interesting that such males showed a slight increase in nitrogen, while the females showed a slight nitrogen loss.

Our incipient colonies started at the laboratory suffered from high initial mortality. Losses of 60% after three weeks seem to be quite the rule and it is very likely that conditions in the field are even worse. Another critical phase is the time when the workers start foraging and caring for the reproductives. This is also the time when the colony has to be provided with the symbiotic termitomyces. If the nutritional pathways fail to be established, the reproductives have no chance to recover any more. It was never possible to observe a dealate female developing signs of physogastry.

Macrotermes Yolk Protein

The major constituent of *Macrotermes* eggs is a yolk protein. It was possible to precipitate this protein by lowering the ionic strength of homogenates of eggs or ovaries. By means of a twice precipitated yolk protein preparation antiserum was gained from rabbits with high titre and high specificity. Preliminary studies have shown that the yolk antigen is not only found in eggs and ovaries but also in the haemolymph of female dealates and physogastric queens. It could never be traced in males. Similarities to female specific proteins or vitellogenins in cockroaches or other insects are obvious.

Breeding of *Macrotermes* eggs

It was possible to implant fertile eggs into pairs of dealates which had failed to hatch larvae. The eggs were accepted, groomed, they hatched and some of the larvae were even brought up to worker stage. Other suitable adoptive parents were pairs of *Macrotermes* males or females and even pairs of *Odontotermes* males or females. Heterosexual pairs of *Odontotermes* somehow recognize the foreign eggs and refuse. Adoptive parents from the other Genus seem to be not adequate for the care of the larvae, whereas hatching was commonly observed.

In all combinations there was generally a minor soldier ecdysing with the first workers, irrespective of whether the eggs came from an incipient colony or if they were collected from mature field colonies. Both, field collected and laboratory eggs. from incipient colonies gave rise to minuscule workers and soldiers as do the eggs of dealates cared for by their own parents. Somehow the soldiers development, the number of soldiers and the size of workers and soldiers is dependent on the environment of the eggs and larvae.

Changes in corpora allatta volume in *Macrotermes subhyalinus* in relation to caste differentiation

B. M. Okot-Kotber

Corpora allata (CA) are endocrine glands which play an important role in insect development and behaviour. They exert their action through juvenile hormones (JH). There is now conclusive evidence that JHs play an important role in caste differentiation in lower termites.

However, there is not much in literature about the mechanisms that may be involved in caste differentiation in higher termites. The aims of this work have been to investigate:

a. The relationship between the CA volume (activity) and the development of castes.

b. The changes in CA volume in dealates reared in the laboratory as related to the age of the colony.

Materials used were termite kings, queens, workers, soldiers and presoliders, collected from mounds in the field. Alates were also collected from the same area during flights. Materials were kept in the laboratory under moist conditions at about 30°C and fed with grass at appropriate time intervals. Random samples

Table 3. Corpora Allata Volume and Head Width of Presoldiers and Adults of *M. subhyalinus* Castes

Caste or Larval Stage	Sample size (n)	Corpora Allata Volume (μ^3) × 10^6 ± S.D.	Head width (mm) ± S.D.
Queens (19,5059 gm) ± 1,59	6	582,48 ± 296,78	—
Kings (0,2130 ± 0.01 gm)	6	19,02 ± 14,84	—
Alates	5	3,41 ± 0,85	—
Alates	5	2,78 ± 0,52	—
Major Presoldiers	57	0,283 ± 0,148	3,39 ± 0,65
Minor Presoldiers	36	0,147 ± 0,074	1,91 ± 0,31
Major Soldiers	34	0,065 ± 0,023	4,49 ± 0,33
Minor Soldiers	4	0,018 ± 0,007	—
Major Workers	30	0,04 ± 0,029	2,48 ± 0,23

were sacrificed at intervals. The head capsules were dissected, CA dimentions measured *in situ* and the volume calculated.

Corpora allata of soldiers, and presoldiers were elongated and rather ellipsoid in shape. Those of alates and workers were oval shaped. The queens had CA of special interest in that these glands are lobular in structure. Each of the glands consists of several fairly large lobes and some of them are pushed posterially to the prothoracic segment of the thorax. On the other hand, kings had CA smaller in size and without lobes.

The volumes of CA from different castes and presoldiers are shown in Table 3.

The data in Table 3 clearly show that queens have the largest CA volume which is about 30 times that of the kings and about 150 times those of the alates.

Male and female alates statistically have the same size of CA ($P<0.1$) as determined soon after flight. These glands are much larger than those of soldiers ($P<0.001$). Also major presoldiers have larger CA ($P<0.05$) than major soldiers. Major workers have some of the smallest CA among all the castes studies.

The frequency distribution of CA volumes of major workers and soldiers are illustrated in Figs. 6 and 7, respectively.

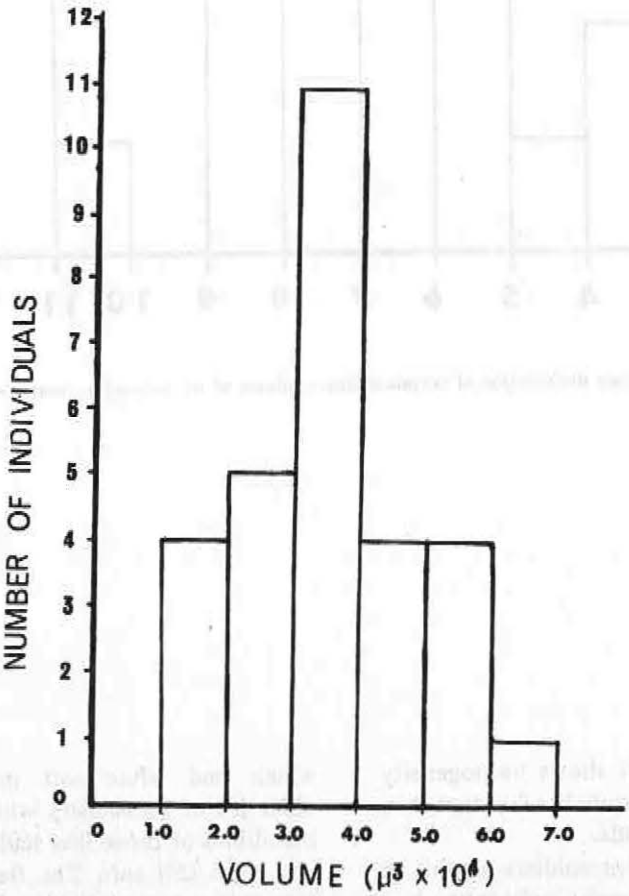


Fig. 6. Frequency distribution of corpus allatum volume of *M. subyalinus* major workers.

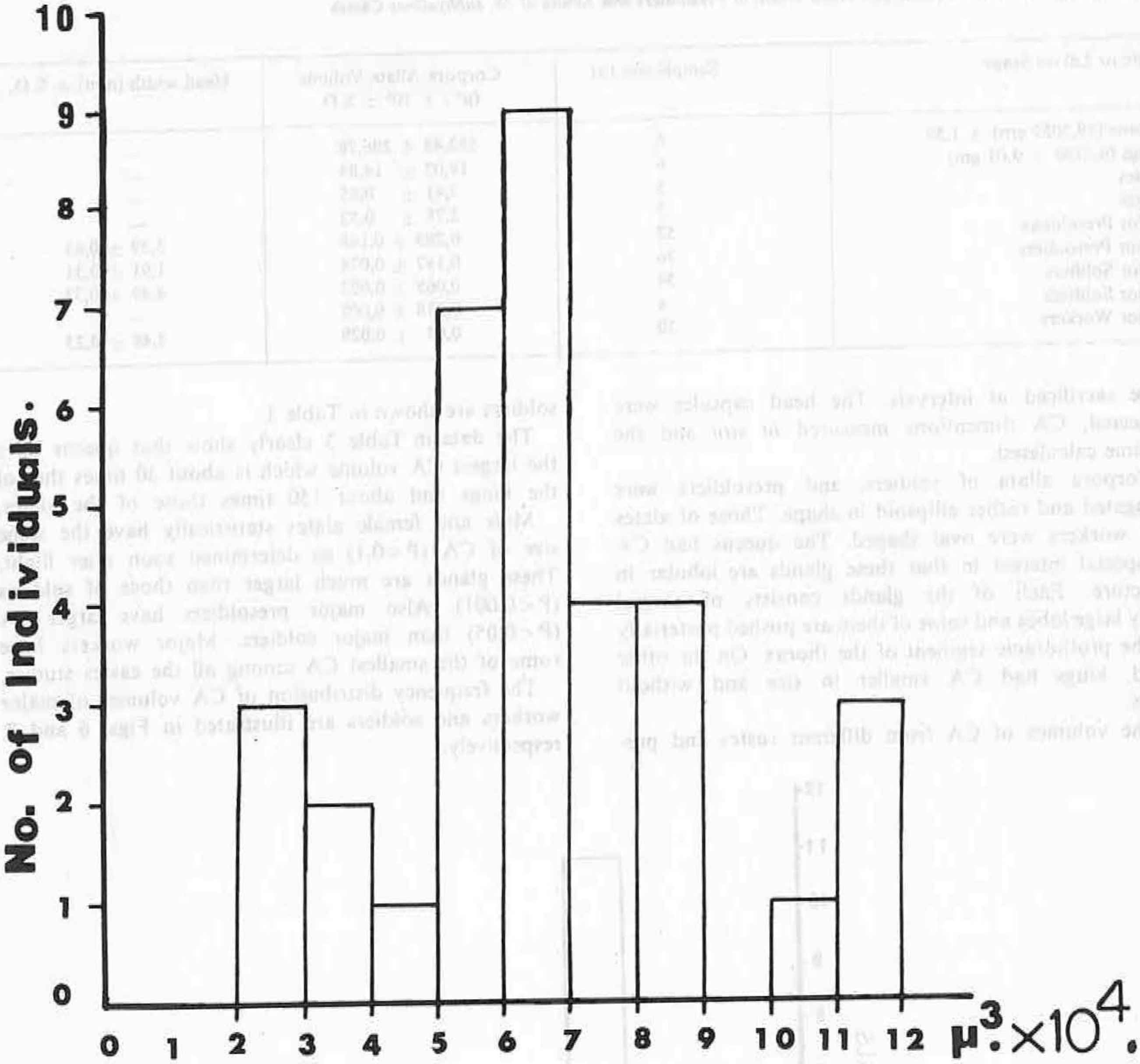


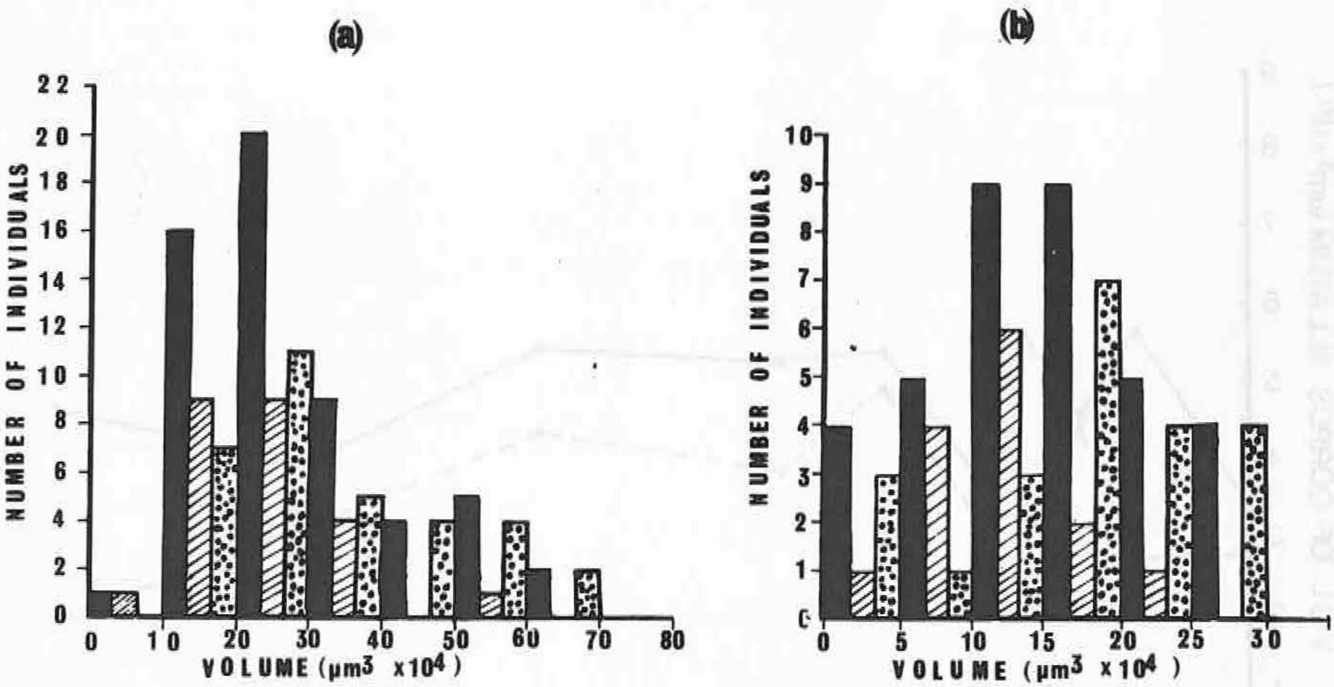
Fig. 7. Frequency distribution of corpus allatum volume of *M. subyalinus* major workers.

The distribution is normal and shows homogeneity in the samples used in the study despite the fact that they were collected from different mounds.

Groups of minor and major presoldiers could be subdivided into two subgroups each: subgroup A—this subgroup made up of relatively young presoldiers which had white soft mandibles. Subgroup B—an older lot of presoldiers which had soft but, sclerotised mandibles or those that had newly moulted into soldiers and were still soft. The frequency distributions of the CA volumes are presented in Figs. 8 and 9, respectively.

Figs. 8 and 9 show that the distributions of CA volumes of subgroup A presoldiers lie basically on the right of the curves whereas those of subgroup B are on the left.

Figure 10 shows CA volume changes in dealates following colony founding in the laboratory for a period of 152 days. Throughout the follow up study, the volume of the glands of female dealates were larger than those



ig. 8. Corpus allatum distribution of *M. subhyalinus* (a) Major presoldiers; (b) minor presoldiers: Total number of individuals split into Subgroups—A and B where A is a subgroup of presoldiers consisting of relatively young individuals and B consisting of old ones.

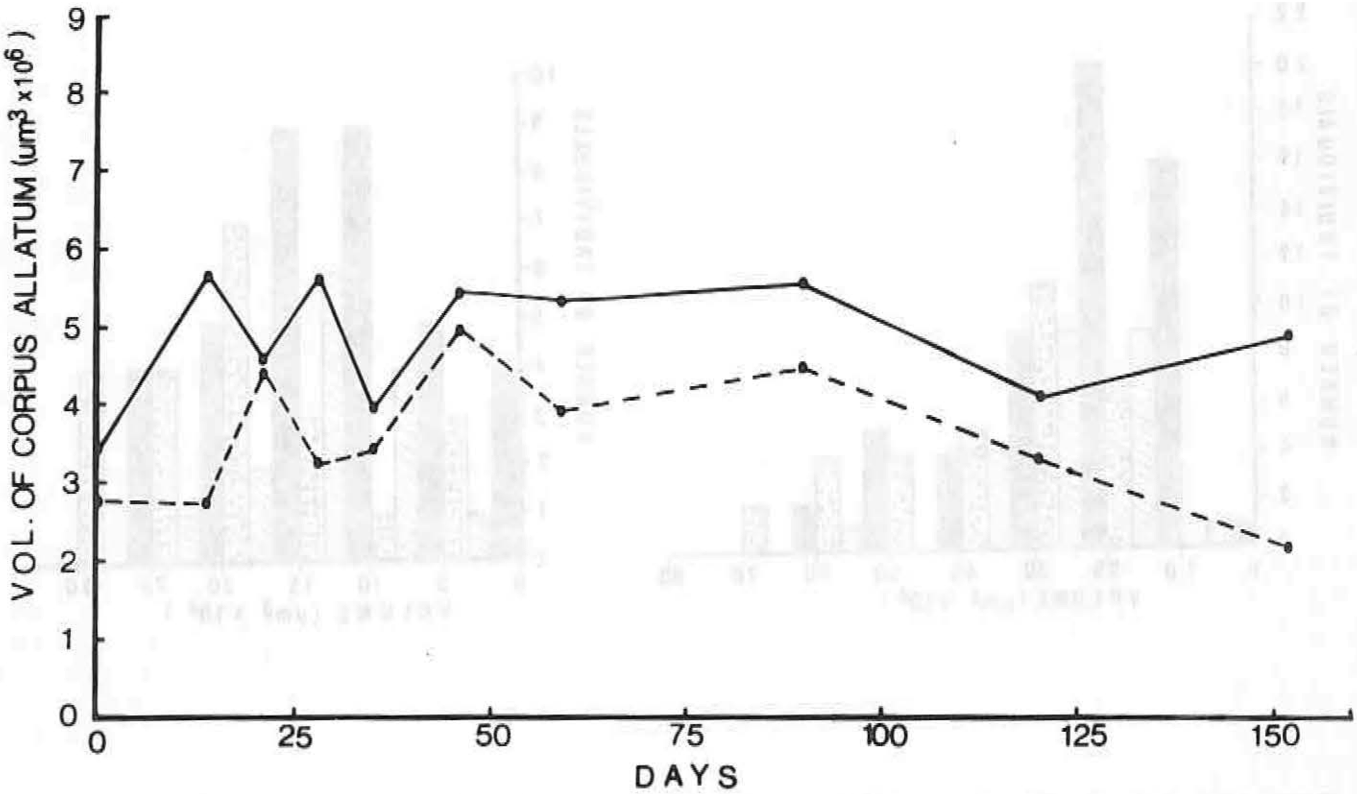


Fig. 9. Changes in the volume of corpora allata of colony founding females (—•— —) and males (—•—) of *M. subyalinus dealates*.

of males. The volume increased rapidly in both sexes during the first two weeks following colony founding and in females a peak was reached at week 2 and in males at week 3. There were no appreciable changes in the volume of the glands after the peaks were achieved in both sexes until after 90 days when the CA volume of males started dropping and continued to do so for the rest of the study period. However, those of the females remained at high plateau.

Throughout the follow up study, the volume of the glands of female dealates were larger than those of males. The volume increased rapidly in both sexes during the first two weeks following colony founding and in females a peak was reached at week 2 and in males at week 3. There were no appreciable changes in the volume of the glands after the peaks were achieved in both sexes until after 90 days when the CA volume of males started dropping and continued to do so for the rest of the study period. However, those of the females remained at high plateau.

Results have shown that *M. subhyalinus* queens have the largest CA of all castes studied. Next in magnitude were those of the kings then alates, presoldiers, soldiers and finally workers. These findings indicate that CA may be playing an important role in caste differentiation in *M. subhyalinus* as has been shown to be the case in lower termites. Since queens lay an enormous number of eggs during their life span, it is reasonable to assume that they require large CA to synthesise adequate JH necessary for pysiological processes of egg production e.g. vitellogenesis. Bordereau (1975) has recently demonstrated that the royal pair of *M. bellicosus* exerts inhibition on the development of nymphs. This mechanism may be through pheromones and/or JH. If this is true then this could be an additional reason for the large CA volume of the queens to control development of individual castes in their large colonies. Corpora allata of dealates may be playing the same role as those of the royal pairs as it has been partly evidenced here that the glands become larger at about the time when the first eggs are laid.

It has been demonstrated in lower termites (1958) that implantation of CA from reproductives could induce soldier development and that JHA may as well induce soldier development (Luscher 1969). Kaiser (1956) also demonstrated that CA of *Neocapritermes* and *Nasutitermes* increase in volume during presoldier development. Results reported here show that CA of *M. subhyalinus* presoldiers are not the exception. This seems therefore to add to the evidence that CA play a role in caste differentiation in higher termites as well.

Further studies are being conducted to investigate the changes that may occur in the CA during larval development as well as in the adult stages at histological, cellular and subcellular levels. Also the direct influence of JH and or JHA on differentiation of castes will be investigated.

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BEHAVIOUR

1. Orientation and recruitment to food source in *T. bettanianus*

G. W. Oloo

Part of this work was reported in the 1975 Annual Report. In this report, a summary of the overall conclusions from the study is given. During the year, investigations on the orientation and recruitment in *Trinervitermes bettonianus* were concluded and a short field study on the foraging behaviour of *T. geminatus* was carried out at Mokwa in Nigeria.

Laboratory and field studies showed that *T. bettonianus* locates food by random exploration and that food odour plays a minor role as an orientation cue during foraging. However, as soon as food is detected, its location is communicated to nestmates and recruitment is subsequently directed to it. A single food-finder (worker) is capable of alerting several nestmates, but for mass recruitment to occur, a sustained source of stimulus (food) is essential (Fig. 11). The stimulating factor in food is being investigated.

Chemical trails are involved throughout the foraging activity (Table 4) and are maintained by re-inforcement at all stages of foraging. It was further shown that food-finders (workers) re-inforce trails more intensively and that the trail of two such workers only was sufficient to divert recruitment from a well-established trail (leading to a depleted food source) to a new food supply (Table 5). Thus, pheromonal trails appear to give directional information about a food-find. It also implies that a "recruitment" trail (a trail laid by the first food-finders) differs from the regular trail either in quantity or quality. Faecal trails were often followed by scouting termites but, attempts to elicit trail-following response in a bioassay with hexane extract of faecal deposits were unsuccessful. It is therefore likely that the effect of faecal deposits is tactile rather than olfactory, but further investigation with different solvents would be necessary to establish their relative importance in orientation.

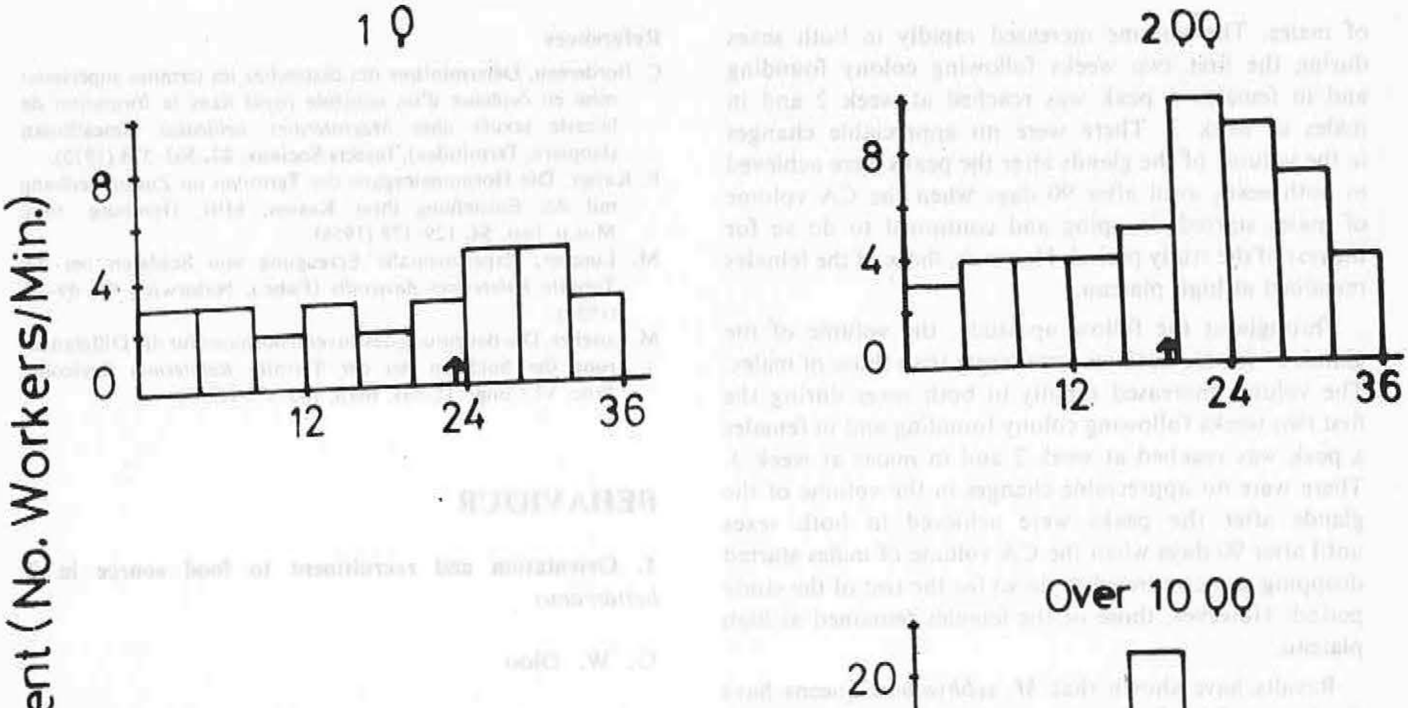

Fig. 11. Recruitment of one or more food finders (workers) in *T. bettonianus*. The arrow indicates time the first food-finder returns to the nest.

Table 4. Involvement and properties of pheromonal Trails in *T. Bettonianus* (N.—nest; FS—food source;—destination of trail-layer; in all cases trail laid and tested by worker castes)

Trail Tested	No. Runs	Total No. Choices	+VE Choices
N → FS	10	17	17(p=0.01)
N → FS	13	26	26(p=0.01)
FS → N	20	40	40(p=0.01)
Outgoing trail tested by homing worker	10	20	20(p=0.01)
Trail from FS tested by outgoing worker	7	14	14(p=0.01)

2. Field study on *T. geminatus* at Mokwa

The short field survey (jointly carried out with Dr. Leuthold of the Zoological Institute, University of Bern) included general observations on foraging behaviour; trail deviation experiments; trail polarity; and alarm behaviour.

a. Foraging behaviour general observations

In the period of study (March) foraging activity commonly occurred from 6 a.m. to 8.30 a.m. They forage in the open and form foraging columns which may extend up to 1½ m from the foraging hole. A single nest may have several foraging holes situated within about ½ m from the mound, rarely opening up directly on the mound itself. At the beginning of foraging, workers open up holes of about 6 mm in diameter; foraging columns consisting of workers and soldiers are formed—the columns eventually branching up to form a network of trails; and workers forage while soldiers guard foraging routes. Foraging ends by workers withdrawing back to the nest first, then followed by soldiers, and finally the foraging holes are closed. Although foraging activity occurred daily throughout this period, individual colonies made only occasional foraging expeditions and foraging intensity varied from one day to the next. The species is a true harvester and stores large quantities of food in special chambers in the nest. The termite exhibits more or less similar features of foraging behaviour to that of *T. bettonianus* except that the latter takes back to the nest partially-chewed food rather than large pieces of grass and has no special food stores. Furthermore, *T. bettonianus* usually forages in the evening (6 p.m.–9 p.m.) rather than morning hours.

Table 5.

I

Situation	Ratio A	Ratio B
Equal food at A & B	1	1
Food added to B	1	5
Trail test*	1	9
Trail test after 2 food-finders return from A	4	1

II

Situation	Ratio A	Ratio B
Equal food at A & B	1	1
Food added to A	5	1
Trail test	10	0
Trail test after 2 food-finders return from B	1	9

III

Situation	Ratio A	Ratio B
Food at A only	71	1
Trail test	10	0
Trail test after 2 food-finders return from B	0	10
Counts after trail test	1	51

***Trail activity tests carried out with the first 10 workers either directly on the feeding bridge as in (A) or by using the "y" assay as in (B) A and B, feeding chambers; N, Nest; T, Test termite (Worker); NT, natural trail on paper. The sketches above not drawn to scale.**

b. Trail deviation experiments

In these experiments, trail pheromone extracts from *T. gratiosus* and *T. bettonianus* and a synthetic analogue (Dodecenol) were used. The materials had shown trail activity in the interspecific trail-following tests in the laboratory at ICIPE. At Mokwa the analogue showed no activity, while the extracts were followed in laboratory tests. However, several attempts to divert foraging traffic in the field with the extracts at various concentrations were unsuccessful—the termites preferred their own natural trails.

c. Trail polarity at junctions

The relative strength of trails was studied by directing termites through prepared "Y" channels on paper and bioassaying for trail activity on both arms of "Y". In addition, counts of foraging termites were recorded from selected "Y" trail junctions in the natural trail work. It was found, as expected from laboratory experiments, that trail strength was proportional to the number of workers laying the trail, i.e. the base of "Y" in a trail network was always stronger than either of the two branches. From this, it could be speculated that a homing worker recognizes the way home by activity differences or polarity at trail junctions. However, in one case workers were observed proceeding home correctly even in a reversed situation where the branch trails of the "Y" were leading into rather than away from the nest as is usually the case, i.e. they were moving from a theoretically higher to a lower trail pheromone gradient. Furthermore, occasionally a few "homing" workers (carrying food) went the wrong direction, although they eventually turned homewards. Thus, the question of how workers determine the correct way home in a network of trails remains unresolved.

d. Alarm behaviour

The alarm behaviour appeared to be characterized by a sequence of distinct stages—the spread of alarm by soldiers; the haphazard escape of workers in all directions in panic; the "streaming" of workers back into the nest; and finally the withdrawal of soldiers from the foraging arena.

An attempt to film alarm and foraging activity was unsuccessful because light conditions were still inadequate at the time optimum activity occurred and the termites withdrew back to the nest as soon as sunlight was becoming strong.

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Analysis of building behaviour in termites

O. H. Bruinsma

The cooperative construction of nests is one of the forms of group behaviour on which our analysis can be undertaken in order to reveal the intricacies of social interactions prevailing in insect societies.

The construction behaviour in termites is discussed together with the information necessary for the workers to complete various structures.

It is important to choose several situation which are both reproduceable in the laboratory and in the field, since workers, encountering environmental conditions different from the normal nest environment will display building behaviour.

1. The reconstruction of the royal cell around an exposed physogastric queen

The queen of *Macrotermes subhyalinus* (Rambur) lives in an individualized oval shaped structure—the royal cell. The opening of this cell creates an emergency situation among the retinue (workers attending the queen). This results in a rebuilding process, and the retinue will eventually (within a few hours) cover the queen again under a "roof" of soil. In short, workers—both major and minor—run towards the queen; and grasp with their mandible soil particlcs. They thereupon turn around and walk a certain distance and deposit their load at various sites at about 2–3 cm from the queen. After an hour two ridges of deposited soil particles lie on either side of the queen parallel to her longitudinal axis. On these elevation pillars are erected which are extended in both the horizontal and ventral planes, gradually forming two walls. When these walls reach a certain height (1–2 cm) they start inclining towards the queen and will eventually cover her when the arches touch and thus completing an earthen roof upon which the construction ceases. The main stimulus releasing and orienting the building activities emerges from the queen and is of a votatile nature.

The pheromone consists of a mixture of free fatty acids of which palmitoleic acid is an essential component.

However, this pheromone by itself is not enough to stimulate workers to complete a cell; other stimuli are therefore necessary.

2. Foraging gallery reconstruction

The repair of galleries is studied in the laboratory with two dampwood termites, *Schedorhinotermes lamainiamus* (*sjostedt*) and *Nasutermes vempoe* (Harns).

The technique used is the removal of a section of an intact gallery, leaving no information (chemical or tactile) for the termites.

In summary, the reconstruction is preceded by "traffic" of scouting soldiers, later on followed by workers until a more or less continuous stream of workers and soldiers connect the two gallery ends. A chemical trail is laid by the trespasses. After about 1–2 hours an additional behavioural faecal pellet deposition can be observed. These depositions provide a more permanent orientation cue than the rapidly evaporating trail. The faecal droplets near the trail are used as foundations for the following steps: soil particle deposition and cementing.

Here a difference exists between the two species. *S. lamaniams* only extends both gallery ends by adding soil particles onto the intact structure, while *N. vampae* also builds in the inter section.

By manipulating the chemical information in terms of changing the trail pheromone distributors, in either the horizontal and the vertical plane, we collected evidence that a part of the gallery is built around a trail pheromone gradient. However, in competition with tactile information (intact galleries) the intact structure acts as a mixture for future work. The interactions between chemical and tactile information is under investigation.

The additional situations are chosen around pillar and fungus comb building which are constructed with a complex of chemical and tactile information now under investigation.

In the field the closing of artificial holes (damages) in the nest was studied. Preliminary data suggest that air streams of both "nest" air and ambient air are of prime importance for the orientation of the workers.

TERMITE BEHAVIOUR (CHEMISTRY)

Termite soldier frontal gland secretions

G. D. Prestwich

1. Rhinotermitinae

As reported last year (ICIPE Annual Report, 1975), soldiers of the termite *Schedorhinotermes lamanianus* produce a mixture of aliphatic ketones. This work has now been published (Prestwhich *et al.*, 1975),

2. Termitinae

No further progress can be reported in the secretions of *Noditermes* or *Amitermes* soldiers (ICIPE Annual Report, 1975; Prestwich, 1975). However, further studies into the diterpene hydrocarbons isolated from six different species of *Cubitermes* species indicate the presence of an artermisencetype carbon skeleton (Prestwich, Wiemer, Meinwald, unpublished results). This new monocyclic diterpene hydrocarbon has been named "cubiterpene." The distribution of the cubiterpenes among *Cubitermes* soldiers is species-specific and of potontial value in chemical taxonomy of this genus.

3. Macrotermitinae

Soldiers of the East African fungus growing termite *Macrotermes subhyalinus* employ both mandibular and chemical defenses. Soldiers release a chemical secretion composed of long-chain saturated and monounsaturated hydrocarbons into wounds inflicted by their powerful mandibles. Chemical analysis of the secretion shows the paraffin fraction to consist primarily of n–tricosane, n–pentacosane, 3– and 5–methylpentacosanes, and 5–methylheptacosane. The major olefins were identified as (Z)–9–heptacosene and (Z)–9–nonacosene. The secretion originates from the frontal glands of both major and minor soldiers; however, the hypertrophied gland of the major soldiers contains 500–fold more secretion than that of the minor soldiers (Prestwich *et al.*, 1976e)

4. Nasutitermitinae

The sticky secretions ejected from the frontal glands of the soldiers of *Trinervitermes* species have been totally analyzed. The major component is a novel diterpene, which we call TG2, possesses the newly-named "trinervitene" carbon skeleton. This novel cembrane-derived substance possesses a dome-shaped 6.5.11 skeleton with a bridge head olefin. Other trinervitene congeners were also structurally elucidated, and finally a description of the overall secretion was formulated (Prestwich *et al*, 1976b; Prestwich *et al*, 1976c; Prestwich, 1976d).

Trail pheromones of termites

Evidence has been collected with several East African species from different subfamilies to show that termite trails are multi-component blends possessing genus and/or species specificity but lacking colony-specificity. However, all species tested would follow the same trail in an interspecific fashion when the alternative choice was an (inactive) solvent trail (Bruinsma *et al*, 1976).

The chemical orientation of *Hodotermes mossambicus* in the absence of light has been under continuing study for five years. The latest results show the presence of a nonpolar, high molecular weight component (*ca.* 250 amu) and a more polar, lighter component in an activity ratio of 100:1. No structural assignments can be made until further material can be collected and purified.

Building peromones of termites

It has been found that workers of the termite *Macrotermes subhyalinus* will build a replacement queen cell around an unsheltered queen in response to a volatile stimulus emerging from the royal fat body. Chemical identification of this volatile stimulus indicates the major activity to reside in the fatty acid, palmitoleic (Z–9–hexadeconoic) acid. Other isomers and homologs are inactive in eliciting the desired behaviour. Further studies, both behavioural and chemical are in progress (Bruinsma and Prestwich, 1976).

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BIOCHEMISTRY

Biochemical and physiological studies on *Macrotermes subhyalinus*

Nabil Abo-Khatwa

1. Termite hormones

Recently Meyer *et al.*, (1976), in collaboration with the termite research team at ICIPE, identified juvenile hormone III (JH–III) (methyl (2E, 6E(–10, 11–epoxy 3, 7, 11–trimethyldodeca–2, 6–dienoate) as the major JH in the haemolymph of the physogastric queen of *M. subhyalinus*. JH activity seems to be related with the enormous egg production of the queen, although the mechanism of vitellogenin production and its incorporation into the growing oocytes is not fully understood (Lüshcer, 1976).

JH titer of the queen haemolymph, based on the Galleria wax test conducted at ICIPE, varies significantly throughout the year, ranging from 10,000–51,000 G.U./ml haemolymph in August—November, 1975 to a titer of 192,000 G.U/ml in January 1976 (1 G.U. = 2–5 picograms of the natural JH1). More data on JH titer has to be collected during 1976–1977 season to be able to draw a conclusion whether or not juvenile hormones, in the subfamily of Macrotermitinae, are involved in caste regulation as it is the case in lower termites. JH titer of alates from incipient colonies of *M. subhyalinus* was also determined. As shown in Fig. 12, there is a sudden and rapid JH activity after colony foundation particularly at the time when the first eggs were laid (about 7–days). This finding suggests that JH might be involved in promoting oocyte maturation during the early phase of colony foundation and probably thereafter.

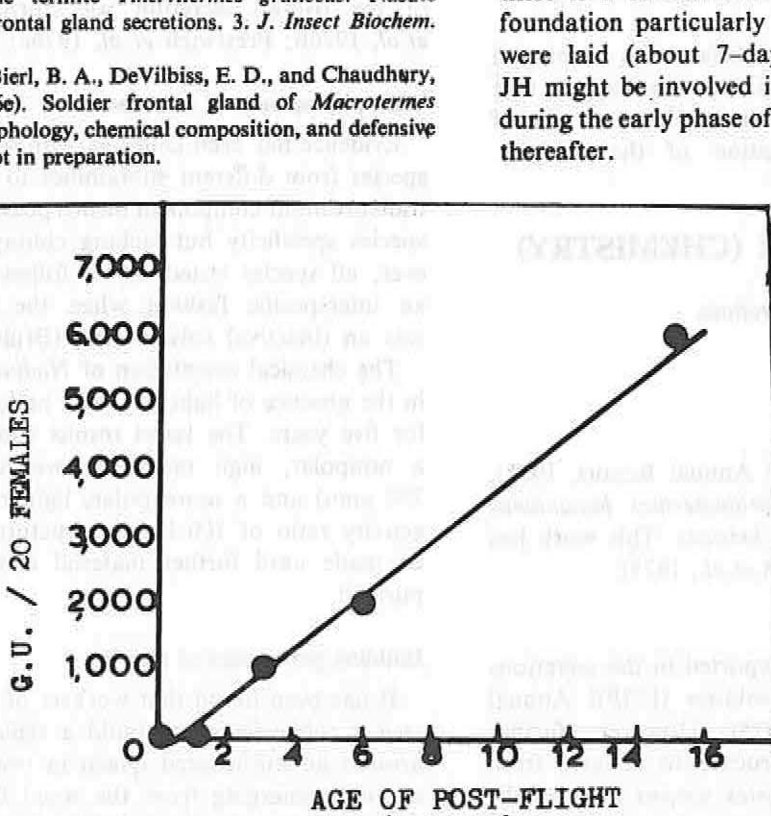

Fig. 12. JH titer of dealate females of *M. subhyalinus* after colony foundation. Date of collection: December 9, 1975. Place: Kajiado District.

Last year (ICIPE Annual Report 1975), I reported on the influence of physiological concentrations of ʙ-ecdysone on the induction of basal and oxidative metabolism of the ovarian tissue of the termite queen. Accordingly, I proposed, at the time, that ecdysones might be present in the ovaries and probably function in a manner similar to that of juvenile hormone in promoting oocytes maturation. The possibility of the presence of ecdysones in the ovaries was investigated on aqueous methanolic extracts. I would like here to confirm my previous results (ICIPE Annual Report 1975) on the absence of ʙ-ecdysone as well as α-ecdysone from the ovarian tissue of the termite queen. This finding is based on the results of various chromatographic analysis (TLC and high pressure liquid chromatography) and mass-fragmentory analysis (Nakanishi, personal communication). However, I was able to recover a significant amount of a vanillin-positive and a U.V. active material, from the ovaries and the haemolymph having a polarity higher than that of ʙ-ecdysone (Table 7).

Subjecting this vanillin and U.V. positive fraction to a poragel-PN column, on a high pressure liquid chromatograph, revealed that this fraction is eluted faster than ʙ-ecdysone (Fig. 13) indicating its greater polarity.

Table 7

Steroid	R_fX100[a]	color produced[b]
Cholesterol	86	Purple
Ponasterone A	42	Dark green
Cyasterone	35	Yellow grey
Makisterone A	22	Light brown
Inokosterone	16	Orange
β-ecdysone	16	Yellow green
Unknown steriod (From the ovaries)	10	Yellow orange

[a]On thin layers of silica gel (0.25mm), developed with chloroform—96% ethanol (80:20 v/v).

[b]Color produced with the vanillin—sulfuric acid spray reagent.

The recovered steroidal substance was also found to co-chromatograph with a standard material of 26-hydroxyecdsyone that was recently reported to be present in the eggs of the tobacco hornworm *Manduca sexta* (Kaplanis *et al.* 1973). Further confirmation on the chemical structure and on its moutling activity will be carried soon. Whether ecdysones of the termite queen are directly or indirectly involved in regulating the protein synthetic activity of the ovarian tissue or in supporting development of the new cuticle that is produced during the process of physogastry remains to be investigated.

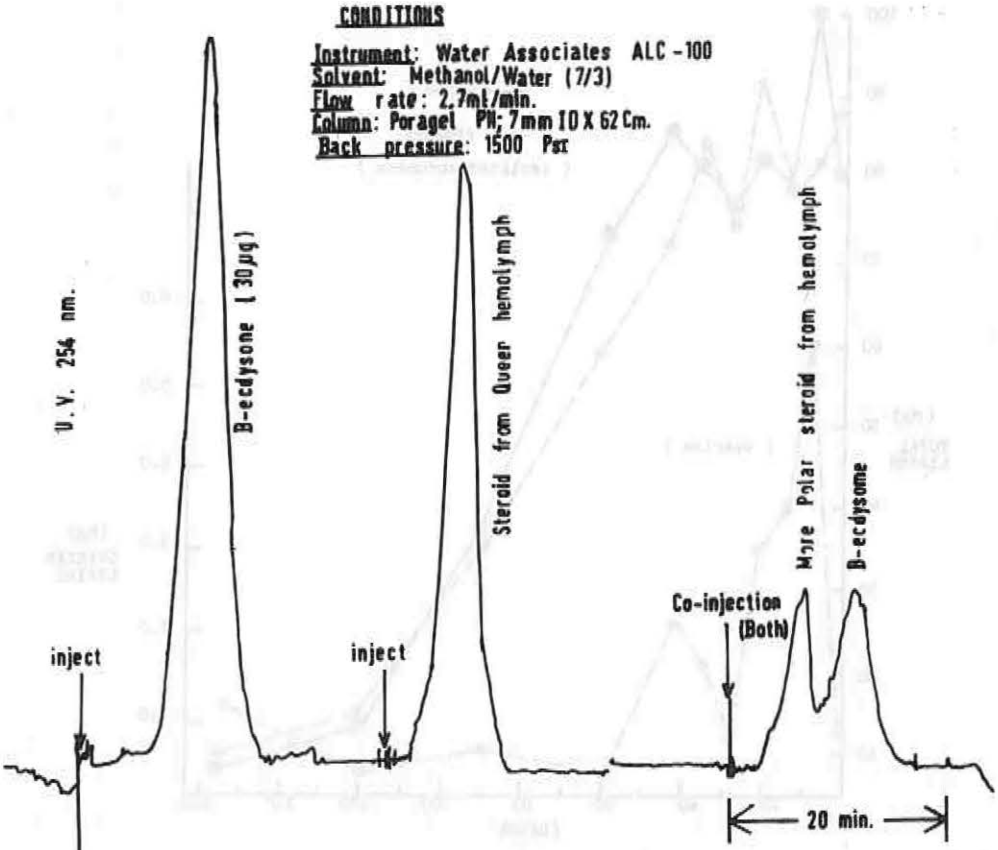

Fig. 13. Elution pattern of B—ecdysone and the unknown steroid recovered from the queen hemolymph. Conditions are indicated in the figure.

2. Lipids and lipid composition of incipient colonies and of the queen termite

Lipids are recognized as an ideal and a major source of energy for an embryonic insect. It contains more energy per unit weight and yields more metabolic water per unit energy than any other catabolic fuel. However, as a stored energy reserve, the quantity and composition of lipids are subject to considerable variability induced by environmental factors particularly important being the abundance or lack of food. Post flight termites cease to feed until the time when the first patch of workers appear. This is an ideal situation to perform biochemical studies of lipids where the effects of nutrition are greatly minimized since parental growth depends on their fat reserves. We followed the lipid content of incipient colonies of *M. subhyalinus* after colony foundation together with those of the physogastric queens that were brought from their natural habitat.

a. Liquid content of incipient colonies, after colony foundation

Fig. 14 shows the results of total liquid content from incipient colonies kept under laboratory conditions for a period of nearly 6 months.

The quantity of abdominal lipids (isolated abdomens without the digestive system) of males and females showed a cyclic pattern of activity until the time of appearance of the first larvae (i.e. about 42 days). A drastic decline in abdominal lipids of both sexes was evident thereafter. Thus males and females of 154-days old lost 88–90% of their abdominal lipids. One must not however, underestimate the physiological stress that these animals are facing by keeping them under unnatural conditions for extended periods.

The ovaries, on the other hand, contained the highest amount of lipids at a time just before or during the first batch of eggs were laid (i.e. about 7 days). A second peak of high lipid content reached the maximum at 42 days i.e. the time when the first larvae appeared.

It is interesting to note that during the first week after flight, total abdominal lipids decreased by 5–4 mg while the gain of ovarian lipids was 4.8 mg. This finding suggests that the increase in ovarian lipids must have originated from the fat body (which is present in the abdomen) and probably lipids of the flight muscle which is assumed to degenerate after colony foundation, contributes little. Currently we are in the process of analyzing the lipid composition of alates during development in relation to vitellogenesis. Thereby, understanding the physiological mechanisms operating in this vital process

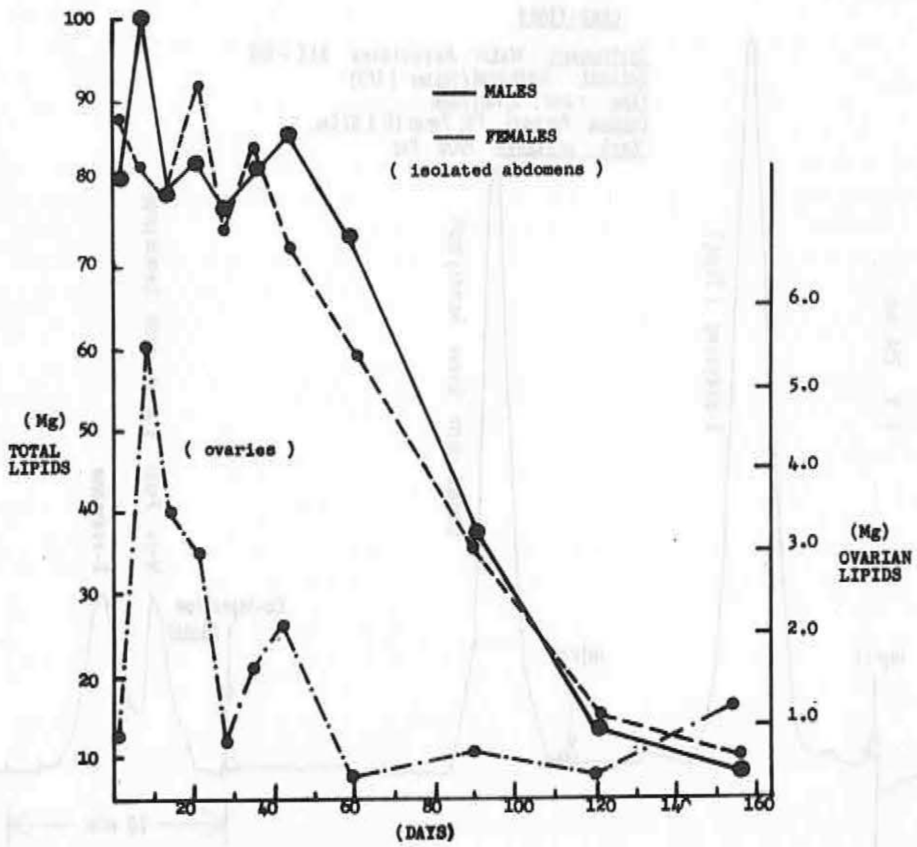

Fig. 14. Total lipid content of isolated abdomens and ovaries of incipient colonies of *M. subhyalinus* kept for about 6 months.

(b) Lipid composition of the queen termite

Lipids from various organs of the physogastric queen were extracted, quantitated, and analyzed by means of a Florisil column chromatography (Carrol, 1961) to determine their relationships to the queen's diet. Since the queen termite cannot feed itself and must depend on the worker caste, an investigation on the chemical composition of saliva from workers would shed some light on the very complex problem of termite feeding. The last problem is currently under investigation.

Table 8 shows the lipid content and composition of various organs from the termite queen. Relatively, a large amount of lipid was recovered from the reproductive organs (12.4%) as compared to the fat body (6.5%). Most of the lipids of the reproductive organs are undoubtedly located in the eggs (mature and immature) which comprise the bulk of the weight of the ovaries. More than 80% of the ovarian lipids are neutral lipids particularly triglycerides and sterol esters. The sterol esters fraction seemed to include also several other highly pigmented non-polar lipids which could not be identified conclusively. In contrast, the lipids of the fat body were mainly phospolipids (46.2%) and neutral lipids (48.3%). Triglycerides were the most abundant neutral lipids of the fat body while free fatty acids comprised the least (3.2%).

Although haemolymph lipids were similar to that of the reproductive organs in terms of the total content of neutral and phospholipids, higher amounts of mono-and diglycerides, free fatty acids, and hydrocarbons were recovered from the haemolymph. The high concentration of hydrocarbons is rather striking. This fraction was highly pigmentated which indicates that probably the characteristic pale yellow colour of the haemolymph is a hydrocarbon in nature. These lipids of the digestive system were mainly phospholipids (64.1%). Most of the neutral lipids were mono-and diglycerides which might suggest that these metabolites are probably intermediates of lipolytic and lipogenetic pathways brought by active digestive lipases. Since the lipid composition depends on the diet intake and metabolism no conclusion can be drawn from these data until further examinations on the composition of saliva lipids are carried out.

3. Biochemical studies on "fungus–gardens" in relation to termites

The true fungus-termite relationships have been a subject of controversy since Konig discovered the fungus in the eighteenth century. There is no agreement in literature as to whether the fungus combs provide termites with nutritional ingredients (and vitamins) or is primarily concerned with humidity control combined with heat production inside the nest.

Chemical analysis was performed on "fungus-gardens" and on various castes of the termite *M. subhyalinus* with the ultimate objective of studying the importance of "fungus-gardens" to the nutrition of termites. A sugar alcohol was isolated from "fungus-gardens" and was identified as D-mannitol by means of spectroscopic and chromatographic methods. Metabolic and feeding studies showed that termites do not utilize mannitol but the latter was found to be essential in supporting the growth of monocultures of *Termitomyces*. When *Termitomyces* conidia were allowed to grow on synthetic agar media (fungus inoculations were done *in locu* under sterilized conditions using a flame and ethyl alcohol) in the presence of various sugars serving as carbon sources, only agar media containing D-mannitol (3%) were able to support the growth of monocultures of *Termitomyces*. The inclusion of other sugars (sucrose, glucose, mannose and sorbitol) in the agar media resulted in the growth of a variety of other foreign fungi some of which were tentatively identified as *Aspergillus* and *Fusarium sp.*, The source of these fungi is likely to result from their association with *Termitomyces* conidia which were not able to proliferate into the "garden" substrate but were allowed to grow when favourable conditions existed (Abo-Khatwa, 1976).

Table 8 Composition of Various Classes of Lipids in Various Organs of the Termite Queen of *M. subhyalinus*[a]

Lipid Composition	Ovaries	Fat body	Organ[b] Hemolymph	Intenstine
1. Total Lipids	12.4	6.5	2.5	C
% Neutral lipids	82.6	48.3	83.2	33.3
% Phospholipids	9.6	46.2	10.7	64.1
2. Neutral Lipids:				
% Mono—and diglycerides	2.3	8.9	13.0	14.3
% Triglycerides	25.0	11.4	1.6	3.6
% Free fatty acids	1.4	3.2	16.8	1.0
% Free sterols	2.3	9.4	6.9	1.6
% Sterol esters	51.5	8.9	11.6	3.9
% Hydrocarbons	0.1	6.5	33.3	8.9

[a]Values are average of two independent determinations using 2 animals collected during May 1976 from Kajiado district, Kenya.
[b]% of total lipids based on fresh weight of the organ.
[c]Two intestine; were collected from two animals weighing 39.6 gm produced a total of 116 mg lipids.

An additional function, of mannitol, seems to be concerned with temperature regulation inside the mound throughout the year. It is known that dissolving mannitol in water at lower concentrations (0.1–0.2 mole/Kg H_20) produces positive (endothermic) enthalpies (i.e. absorption of heat) while at higher concentrations (0.3–1.0 mole Kg/H_20) it produces negative (exothermic) enthalpies) i.e. production of heat. By simple calculations as to the amount of mannitol and water in fungus combs of *M. subhyalinus* one reaches a value of about 0.7 mole/Kg water which corresponds to exothermic reactions being carried out in these combs. An additional advantage to the presence of mannitol in the fungus combs is that unlike sorbitol (a stereoisomer analog), mannitol possesses a dual effect on either heat production or absorption depending upon its final concentration in the combs. This reversible effect could be an important factor in regulating the heat content of fungus combs thereby regulating the temperature inside the mound.

Another nutritionally important group of substances found in fungus combs were the nitrogenous constituents. Our results showed no significant difference in the nitrogen content of various parts of fungus combs of varying stages of maturity. These findings suggest that fungus combs nitrogen is derived essentially from plant material while fungus tissues contributed very little. This suggestion is also supported by the fact that the nitrogen content of fungus combs was significantly much lower (1.2%) than that of the fungal conidia (7.9%). These facts together indicate that the comb material (without the conidia) contribute very little to the termite requirements for nitrogen.

Part of the nitrogen compounds found in fungus combs was uric acid. The amount of uric acid present in the combs depends upon its stage of maturity; being at high concentration in the newly deposited material (0.66 mg/g dry weight) and at low concentration (0.33 mg/g) in the older combs. It is assumed that the reduction of uric acid concentration during comb maturation is probably a result of fungal growth. Furthermore, the presence of uric acid in the combs of *M. subhyalinus* suggests that the origin of the building material for these combs is faecel material which was previously shown to contain uric acid (Hungate, 1941).

The importance of lipids in terms of metabolic and structural function in insects is well documented. Moreover, the diet on which insects feed has a strong influence not only on the total lipid content but also on the relative proportion of lipid classes. On the assumption that termite workers are responsible for feeding other castes (larvae, soldiers and reproductives) the quantity and quality of lipid classes were examined together with those of fungus combs and conidia. Considerable differences of lipid composition were found between fungus combs, conidia and various termite castes (Fig. 15).

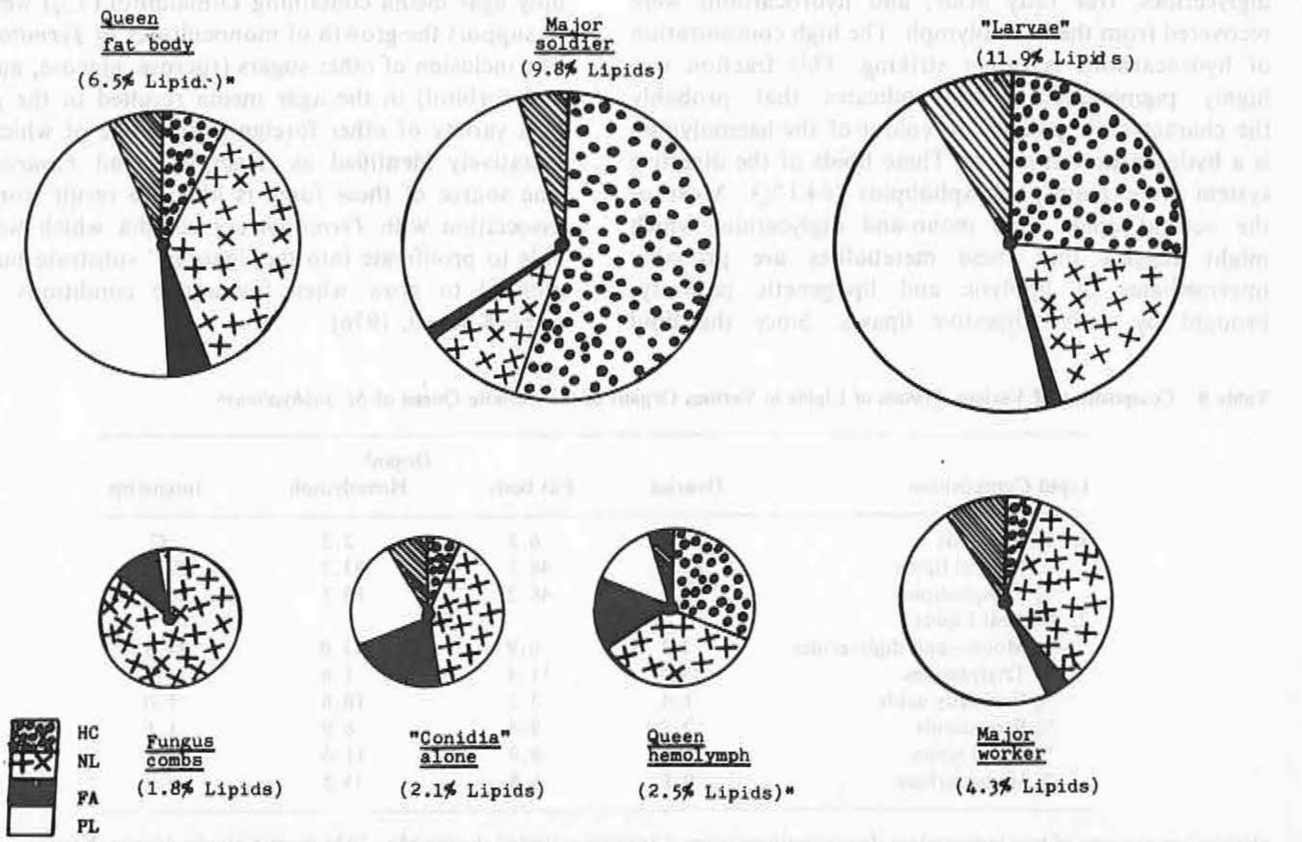

Fig. 15. Lipid composition of "fungus gardens" in relation to termite castes.

Very little similarities between the composition of lipids of fungus combs or conidia and that of various termite castes did exist. These findings indicate that most of the termite castes are themselves capable of modifying the lipid composition of their diets according to their needs. Only in one case, was there a striking similarity between the lipid composition of the queen fat body and that of major workers, which might suggest that lipids present in the saliva of workers when they are received by the queen will be channeled directly to the fat body without major metabolic alterations.

Based on our findings, we concluded therefore, that "fungus-gardens" seem to contribute little to the nutrition of termites and probably only provide them with essential ingredients such as vitamins (vitamins A and C were detected in fungus combs) and nitrogen. The latter requirement should be obtained mainly from the white nodules (conidia) which are rich in nitrogen content. The main function of "fungus-gardens" associated with macrotermitines however, seems to be concerned with the heat and water regulation (the amount of water present in a mound containing 10 Kg of fungus combs is estimated to be about 5 Kg) within the mound and annual fluctuations of mannitol content could be a factor in temperature control. We are continuing this study by looking into the cellulose and lignin break-down enzymes that might be associated with fungus combs and termite

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TICK RESEARCH

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Professor R. Galun (1970) Physiology

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Mrs. C. K. A. Mango (1971) Experimental Officer
Dr. R. M. Newson (1974) Senior Research Scientist
Dr. F. D. Obenchain (1976) Research Scientist
Mr. D. K. Punyua (1973) Experimental Officer (on study leave)

Collaborators:

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Mr. T. S. Dhadialla (1973) Reproductive Physiology
Dr. A. Maradufu (1974) Chemistry
Dr. S. J. Mchinja (1975) Head, Division of Protozoology, EAVRO
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ECOLOGY

Introduction

During the year under review Dr. F. D. Obenchain has joined the tick project as a Research Scientist. He brings with him a fine background in endocrinology and physiology and will continue these aspects of the work on hard ticks. Mr. J. W. Chiera also joined the project Research Assistant to assist with the ecological work. He takes Mr. D. K. Punyua's place while the latter is engaged on studies for his Master of Science degree at the University of Nairobi. Mr. A. Mongi has been seconded to the project from Tanzania, and is also studying for his Master of Science degree.

The main lines of study, as in the previous report, concern aspects of the physiology of hard ticks, in particular *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, the causative organism of East Coast fever of cattle. Work on the pheromones of this and other ticks continues. Studies on the reaction of hard ticks to environmental factors likely to be experienced by the ticks while they are on the ground, has also continued.

A new, long-term experiment designed mainly to determine the influence of the density of hosts on the chances of survival and reproduction of *R. appendiculatus* has been under way for more than a year. Studies on the longevity of ticks in pasture have given results that throw light on certain tick-control practices. These studies have been made possible by the provision of land and other assistance by the Director of the East African Veterinary Research Organization at Muguga.

The physiology of the soft tick *Ornithodoros moubata*, especially the influence of insect hormones on growth and development, and the role of pheromones in aggregation behaviour, is also continuing.

A summary of the main findings during the year follows.

Studies on experimental *Rhipicephalus appendiculatus* populations and East Coast fever transmission

R. M. Newson and D. K. Punyua

In 1973 an experimental *R. appendiculatus* population infected with *Theileria parva*, the causative organism of East Coast fever (ECF) in cattle, was set up on a double-fenced paddock of 2 ha in cooperation with the East African Veterinary Research Organization (EAVRO) at Muguga. Its use has been described in the 1974 and 1975 ICIPE Annual Reports, The paddock was then divided in the ratio of 1(A): 3(B). Weekly collections of ticks were made as before from the vegetation in paddocks A and B, and an adjacent unstocked, uninfected control paddock, to monitor the changes in the larval and nymphal tick populations. A number of studies have been completed or are in progress.

(a) Effect on ECF infectivity of allowing ticks to feed only on immune hosts

The object was to simulate an ECF vaccination campaign in order to examine the changes in infectivity that would occur in the infected vector population when they could only feed on immune hosts.

Six cattle immune to *T. parva* were kept in paddock B from November, 1975—September, 1976, under intensive veterinary supervision. The ticks on them were counted *in situ* once per week. Six pairs of susceptible control cattle were exposed in the paddock during the course of the experiment; they were always removed to quarantine after 6 days for observation. The ticks on them were killed by spraying on day 6 in the first three cases, but allowed to feed to repletion (and thus increase the disease chellenge) in the fourth and fifth pairs, whilst in the last case they were sprayed 5 days after removal from the paddock to avoid direct harmful effects from the ticks themselves.

In the smaller paddock A, eight pairs of susceptible controls were introduced at intervals and remained there until they died of acute ECF, in all cases but two (see below), thus confirming the continued ECF transmission cycle, and also maintaining the tick population. The results of the experiment are shown in Table 1.

Table 1. History of disease and tick infestation whilst using immune cattle as hosts for the ticks in paddock B, compared with susceptible controls in paddocks A and B

	Susceptible controls		Immunes		
	A	A	B	B	B
Month	ECF deaths	Mean tick[1] load	ECF deaths	Mean tick[1] load	Mean tick load[2]
1	2/2	446	2/2	645	320 (6)
2	2/2	250	—	—	65 (37)
3	1/2[3]	87	—	—	74 (70)
4	2/2	95	0/2	72	321 (91)
5	2/2	306	0/2	293	390 (126)
6	1/2[4]	487	1/2	253	461 (161)
8	2/2	827	1/2	616	444 (224)
9	2/2	1454	0/2	1719	362 (301)

1. Mean tick count for both ears together on day 6 of exposure.
2. Mean tick count for both ears together, (with days of continuous exposure).
3. The surviving animal was known to be *T. parva* immune, and had been used as a contro in error.
4. The second animal died of acute anaplasmosis on day 11 of exposure.

The first pair of controls was introduced into B at the same time as the six immunes at the start of the experiment. They both died in quarantine of acute ECF. Meanwhile the immunes, as expected, showed mild ECF reactions and quickly recovered. The next pair of controls (month 4) showed only mild ECF reactions and recovered. This result was not expected so early in the work, so the third pair of controls was immediately introduced to confirm it, and even with a much heavier tick load gave the same result. The longer and more nearly natural exposure of the fourth and fifth pairs of controls resulted in severe reactions with two deaths, but even the very heavy challenge to the last pair of controls gave only mild reactions with recovery.

Though they remained healthy, *Theileria*-like parasites were occasionally seen in blood smears of the immunes, and these were shown in further experiments to be serologically distinct from *T. parva* and *T. mutans* (a normally benign parasite) and to confer no cross-immunity to *T. parva* infection when transferred to other cattle. It appeared that in the susceptible controls exposed from the sixth month onwards it was this organism, and not *T. parva*, that was causing the ECF-like reactions. Thus, although the use of immune cattle appeared to have markedly decreased *T. parva* infection in the tick population in three months, helped perhaps by the low tick population in the early stages, another, and so far unknown, *Theileria*-like organism manifested itself.

The level of tick numbers on the immune cattle appeared to have reached some sort of equilibrum by the fourth month, although the tick populations in both paddocks as assessed by the numbers attaching to naive cattle were rising steadily.

This study was made in close collaboration with Dr. D. E. Radley and Dr. M. P. Cunningham.

(b) Daily collection of ticks from isolated quadrats

R. M. Newson and J. W. Chiera

A set of 3 × 3 1m² quadrats, separated from each other by 1 m of bare earth and surrounded by a fence to exclude cattle, was set up in each paddock (A, B) in January, 1976. Daily collections of ticks were made throughout the year. Adults were collected by searching by eye and picking the ticks off the grass with forceps; larvae and nymphs by standardised brushing of a small cloth flag over the tips of the grass. The object was to attempt an estimation of absolute numbers and also to study seasonal variation in activity, without the complications of migration, recruitment and depletion by removal on the host. In April, 1976 an additional set of nine quadrats identical with the others was started in paddock B and ticks were collected as before on five plots, but collecting for immatures on the remaining four plots was repeated five times per day.

Table 2. Ticks collected on two sets of quadrats each consisting of 3 × 3 1 m² plots

Date (1976)	A adults	A nymphs	A larvae	B adults	B nymphs	B larvae
Jan. 14—Mar. 31	25	543	1631	40	259	722
Apr. 1—June 30	209	343	5284	200	210	4783
July 1—Sep. 30	121	336	8274	64	256	7702
Oct. 1—Dec. 31	21	603	4216	11	541	4138
Total yield/m²	41.7	202.8	2156.1	35.0	140.7	1927.2

The results to date are summarised in Table 2. Only the adults behaved as might have been predicted, with increased activity in the second quarter (during the rainy season) and a decline thereafter. The nymphs showed relatively little change until the fourth quarter and then the numbers collected increased. The larvae increased to maximum numbers in the third quarter and now appear to be decreasing. The collecting technique for the immatures is known to be very inefficient—the repeated daily collections produce a yield directly proportional to effort—and because of this we have been able to follow the larval and nymphal populations over a long period. The results demonstrate how long they can survive under natural conditions in the field, and also that the proportion active at any time must be only a small part of the total population.

(c) Reproduction and survival of *R. appendiculatus* at different host stocking densities

R. M. Newson and J. W. Chiera

At a high rate of stocking, ticks should have a good chance of acquiring a host, but at the same time heavy grazing pressure is likely to produce conditions on the ground that are less favourable for survival of the ticks when they are not feeding, than if the host stocking density were less.

Seven double-fenced paddocks were prepared at EAVRO, Muguga, with areas of 300 m² (1), 1000 m² (3) 4,000 m² (2) and 12,000 m² (1). In June, 1976 each plot was seeded with disease-free ticks at the rate of 4 nymphs/m² and 9 larvae/m², except one plot of 1,000 m², which is an unstocked, non-infested control. Ten days later one steer was introduced into each of five plots, leaving the 300 m² plot as an unstocked, but tick-infested, control. At this time the grass cover was dense as there had been no grazing for a year, and the area was known to contain only stray *R. appendiculatus* and a population of *R. hurti*, with many adults awaiting their final bovine host, having fed as larvae and nymphs on rodents.

Once per week all the ticks are counted *in situ* on each animal, basic haematological data are collected, and the ticks on the vegetation are sampled. Once per month all the adults on one side of the body of each animal are collected for identification, and the vegetation is measured in detail along two transects in each paddock.

The changes that have taken place in the tick populations are summarised in Table 3. Adult tick counts are given for the ears as well as the whole body, because three-quarters of the adult *R. appendiculatus* are normally found there. *R. hurti* adults are generally rare on the ears and feed elsewhere on the body. Adults began to be picked up immediately by the cattle and comprised 95% *R. hurti* but this figure had dropped to 11% by December. There was an increase in adult *R. appendiculatus* in mid-August when the seeded nymphs began to emerge after feeding and moulting. On the two plots with the densest stocking rate, the number of adults then decreased, but they remained at a high level on the others, where the reduction in grass cover was less marked.

Table 3. Mean monthly total body counts (*in situ*) of adult and nymphal ticks on cattle living on plots of different sizes, larval abundance I= 1—10; II= 10—100 etc.

Plot size (m²) and host	Ticks	July	Aug.	Sep.	Oct.	Nov.	Dec.
	Adults (ears)	2	45	87	53	17	9
1000	Adults	34	88	169	77	29	13
(K124)	Nymphs	180	47	31	7	8	27
	Larvae	0-II	0-I	0	0	0-III	II-III
	Adults (ears)	4	82	219	127	98	57
1000	Adults	48	199	388	226	128	69
(K143)	Nymphs	145	61	22	11	10	196
	Larvae	0-II	0	0	0	0-V	IV
	Adults (ears)	11	36	146	82	79	98
	Adults	171	221	423	230	164	172
4000	Nymphs	245	211	57	33	12	14
(L535)	Larvae	II	0-II	0	0	0-III	IV
	Adults (ears)	4	11	90	141	127	163
4000	Adults	92	121	276	301	228	302
(L536)	Nymphs	332	175	67	41	18	16
	Larvae	II	0-I	0	0	0-III	III-IV
	Adults (ears)	5	19	99	148	137	214
12000	Adults	93	83	226	299	248	329
(L49)	Nymphs	32	179	63	35	24	8
	Larvae	II-III	0-II	0	0	0-II	III

Relatively few of the seeded larvae appear to have been picked up by the cattle and larvae disappeared entirely from the collections for 3–4 months. The first larval offspring of females which fed on the cattle appeared in November and remained most numerous on the plots with lower grazing pressure. The introduced nymphs persisted much longer than the larvae, and their numbers on the hosts declined gradually, and must have been augmented by the emergence of some others which had fed as larvae on the study area. By late December there were signs of increase in the nymphs as members of the big larval cohort, hatched and fed during the course of the study, began to join the nymphal population.

The changes in the vegetation are most marked on the two most heavily stocked plots. Already however, the tick population of each host/paddock combination seems to be developing differently.

Ecological study of *R. appendiculatus* and associated species and two tick-borne cattle diseases

R. M. Newson and D. K. Punyua

A site was chosen at Lolngoswa in Kajiado District in which the conditions are almost too dry for *R. appendiculatus* and in fact the principal ticks infesting the cattle are *R. pulchellus*, *R. evertsi*, *R. pravus* and *Amblyomma gemma*. Nevertheless there was recent clinical and serological evidence of East Coast fever, and in conditions like these the disease situation and the vector (*R. appendiculatus*) might be expected to show considerable, and instructive, variation. *Theileria mutans* which is widespread and generally believed to be benign is also present.

Monthly collections of ticks, serum samples and blood smears were made from the same group of marked adults and calves from July, 1975 to August, 1976, and will be resumed in 1977. Unfed ticks from the vegetation were also sampled intensively at each visit. During 1975–1976 the climate was drier than usual.

The results so far indicate that the *R. appendiculatus* population is very low and may temporarily become extinct. Although some of the cattle showed serologically positive reactions for *T. parva* many of the calves, in particular, are probably now susceptible and on the return of wetter weather and the re-introduction of *R. appendiculatus*, conditions could be conducive to an epizootic outbreak of ECF. *T. mutans* infection has been found in all the cattle from serological and histological evidence. The vector appears likely to be *A. gemma* but this requires confirmation as vectoring has not been demonstrated before in this species.

The most abundant, but little known, species is *R. pulchellus*. Our results show that it has a life cycle resembling that of *R. appendiculatus* in the drier parts of the range of that species. Maximum adult activity occurs in the main rainy season, followed by the appearance of the larvae whilst the habitat on the ground is still fairly humid. These, in turn, produce nymphs in the dry season. All stages are, however, active to some extent the whole time. At this site all that can be said

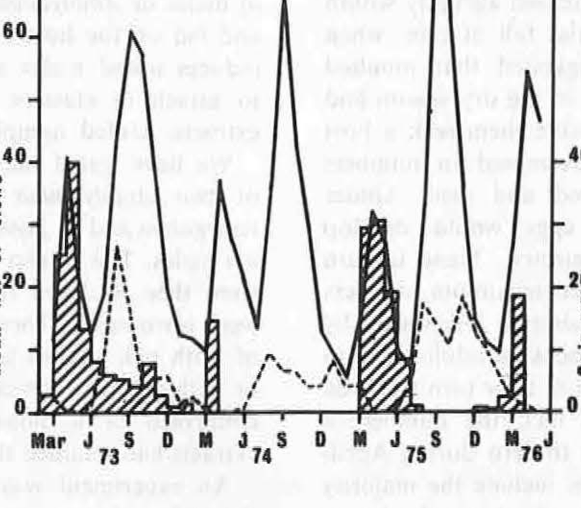

Figure 1.—Mean number of *R. appendiculatus* per host per week, during each month. Total rainfall for each month is also shown (no records May 1974—March, 1975 and September—December, 1975).

so far about *R. appendiculatus* is that the adults were at their most numerous during June–August and that they disappeared altogether from the collections for December-February.

We are grateful to Dr. A. S. Young for carrying out the serological tests and slide examinations.

Population study of *R. appendiculatus* on the Kenyan coast

R. M. Newson

Five marked indigenous cattle at Ukunda in Kwale District were used from March 1973 to May 1976. All ticks were collected from the ears and anus of each animal every week, with samples from the rest of the body at intervals of 1–3 months. Daily records of rainfall were made on the study area and a thermohygrograph placed at ground level was operated for the first 15 months.

The mean annual rainfall is approximately 1,200 mm of which half falls in April-June, with most of the remainder fairly evenly distributed from July—December, and very little from January until late March-early April when the rainy season starts. Both the daily and seasonal temperature ranges are small. We found an overall mean of 25°C. In the rainy season relative humidity was never less than 60% and was always above 90% from dusk to dawn. In the driest part of the year the RH was below 60% for 4–6h/day and above 90% for 0–6h/night. These records were made in a well-vegetated site. Undoubtedly, as the year progressed and the grass cover dried out and was grazed back, the conditions on the ground experienced by the ticks were hotter and drier than shown above.

The most numerous species was *R. appendiculatus*. The changes in the numbers collected on the ears and anus are summarised in Figure 1. The adults were fewest in the dry weather and increased abruptly within a few days of the first substantial fall of rain when the wet season began. This suggested that moulted adults accumulate on the ground in the dry season and require the stimulus of rain to make them seek a host and start feeding. The adults decreased in numbers again quickly as they fed, bred and died. Under the prevailing conditions the eggs would develop rapidly and larvae were soon detected. These in turn gave rise to nymphs which reached maximum numbers in August-September, followed shortly afterwards by a more numerous and sustained peak of adults than in April–May. These adults were not in their turn followed by more larvae or nymphs. In fact, the number of nymphs on the cattle fell almost to zero during April-June each year. Whilst ear counts include the majority of the adult *R. appendiculatus* on the host, the larvae and nymphs are widely distributed elsewhere on the head, neck and legs, and only a small proportion appear in the ear collections. It appears that the reproductive effort of those adults feeding in September-October is entirely lost, due to the death either of the engorged females before they oviposit, or the eggs and larvae.

These results are not typical of *R. appendiculatus* but illustrate a situation in which climatic conditions may be critical. Aridity increases northwards up the coast and *R. appendiculatus* gives way to *R. pulchellus*, a species typical of drier conditions. A point must be reached at which successful reproduction in one rainy season is insufficient to sustain the population until the next, despite the production of a large second generation later in the year. At Ukunda, however, small numbers of nymphs descended from the first generation of adults maintained the population by feeding during the dry season, after most of their contemporaries had already reached the ill-fated second adult generation.

Five other tick species appeared regularly in the samples. None showed a bi-modal pattern. *R. evertsi* maintained a uniformly low population with only a doubling in numbers of adults during August-December. Three little-known species which only feed on cattle as adults showed single annual peaks; *R. kochi* and *R. muehlensi* in July, *R. maculatus* in September. *Amblyomma variegatum*, a cattle-feeding species in all stages, was present as adults and nymphs on the body throughout the year and showed no marked seasonality.

PHYSIOLOGY

Attraction of unfed females to attached males in *Amblyomma* ticks

F. D. Obenchain, R. M. Newson and J. Chiera

The presence of an extractable pheromone has been demonstrated (Y. Rechav, personal communication) in males of *Amblyomma hebraeum* which have attached and fed on the host for 7 to 10 days. The pheromone induces unfed males and females of the same species to attach in clusters around the fed males or their extracts. Unfed nymphs are similarly attracted.

We have tested the responses of males and females of two *Amblyomma* species common in Kenya, *A. variegatum* and *A. gemma*, to ether extracts of *A. hebraeum* males. The extract was applied to bull scrota which were then enclosed in cloth bags to which the ticks were introduced. There was no response by either sex of both tick species to the extract within a 3h period, or within 24h in the case of *A. variegatum*. It was later confirmed by a bioassay with *A. hebraeum* that the extracts had retained their activity.

An experiment was then performed to see whether fed males of *A. variegatum* could attract unfed females of the species and induce them to attach around the males. Single males were fed for 10 days in bags on the scrota of three bulls, and 30 unfed females were then introduced into each bag. Thirty females, in the absence of males, were also placed in scrotal bags on two control bulls. After 3h, 7% of the control females had attached (scattered) whereas 47% of the experimental group females had attached in tight clusters around the fed males. Only two experimental females had attached away from the males. At the end of 24 h the proportion of control females which had attached in the absence of males was 14%, still without clustering. For the experimental females, 61% were attached in tight clusters around the males and an additional 6% were attached elsewhere on the scrotum.

A similar experiment was performed with *A. gemma*, except that both unfed males and females were tested for their response to fed males. After 24h, 71% of the females had attached near the fed male, 4% had attached elsewhere and 25% were unattached. Among the unfed males, 17% had attached near the fed male, but 79% had attached elsewhere.

It is clear that the males of both Kenyan species of *Amblyomma* produce an aggregation-attachment pheromone, similar to that of *A. hebraeum*. Still, there are differences in the responses of males of *A. variegatum* and *A. gemma*. In the latter species the males appear to be largely indifferent to the pheromone produced by other males of their species. Further behaviour studies and studies on the sites of pheromone synthesis and release, mechanisms involved in the control of production and the chemical nature of the pheromone(s) are planned.

The effects of ecdysone, juvenile hormone and their analogues on *Ornithodoros moubata*

C. K. A. Mango

Work started and reported in the ICIPE Annual Report (1975) on the effects of ecdysone, juvenile hormone and their analogues on the soft tick *Ornithodoros moubata* was continued. The effects of beta-ecdysone and ponasterone A on nymphal stages when given at different concentrations in the bloodmeal and when topically applied to fed nymphs at different times were investigated.

O. moubata 2nd, 3rd and 4th stage nymphs were fed on defibrinated porcine blood treated with either beta-ecdysone or ponasterone A in 95% ethanol at doses of 1, 2, 4, 8, 16, and 32 μg/ml of blood.

Second, 3rd, and 4th nymphal stages were fed on untreated porcine blood and then they were treated either on the same day of the meal or four days later at doses 1, 2, 4 and 8μ/μl/nymph.

Nymphs were fed on bloodmeal containing cercropia oil or ZR512 at doses of 1,2,4 and 8μg/ml of blood juvenile hormone. Topical application trials were also carried out at doses of 0.25, 0.50, 1 and 2μg/nymph.

In the beta-ecdysone and ponasterone A tests, accelerated moulting was observed (Fig. 2 and Table 4), compared to normal controls. This was more evident in the advanced nymphal stages particularly among the 4th

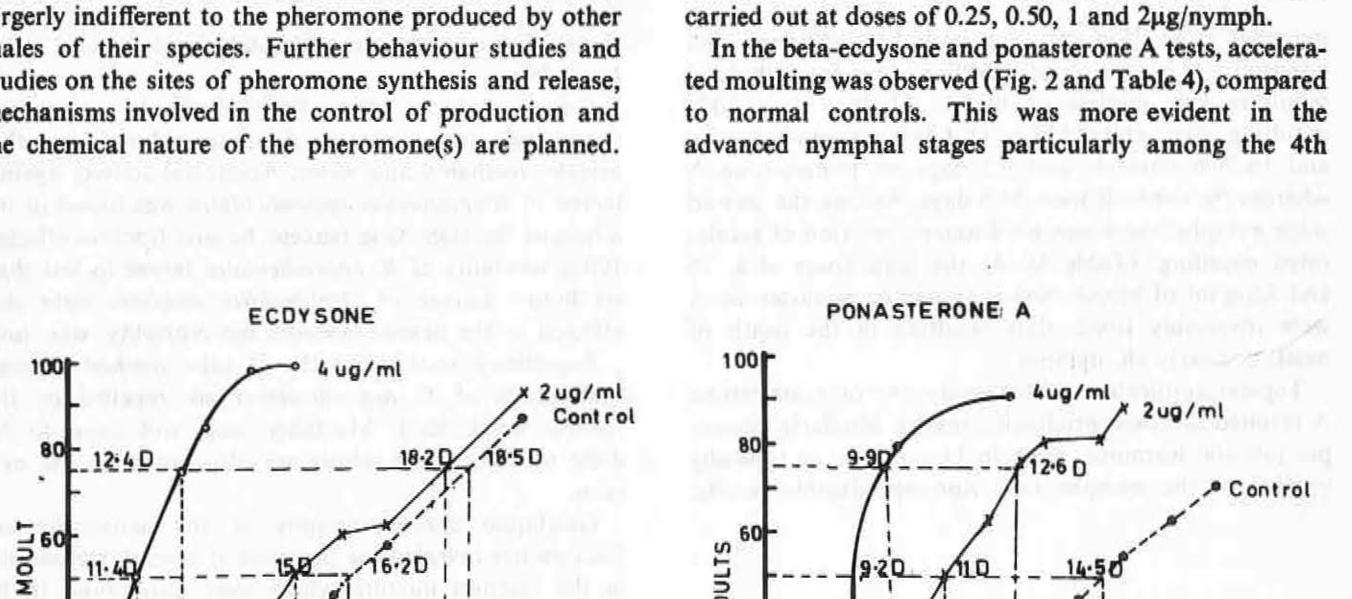

Fig. 2. Effects of ecdysone and Ponasterone A on 4th Stage *O. moubata* nymphs when given bloodmeal.

Table 4. Effects of beta-ecdysone and ponasterone A on 2nd, 3rd and 4th nymphal stages of *Ornithodoros moubata* when given in bloodmeal

Dosage (μg/ml blood)	2		3		4	
beta-ecdysone	Days within which 50% moulting was achieved	Percent mortality	Days within which 50% moulting was achieved	Percent mortality	Days within which 50% moulting was achieved	Percent mortality
0 -Control	8.2	1.8	12.3	2.6	16.2	6.5
1	7.0	2.0	8.0	0	15.3	0
2	6.0	2.0	7.3	0	15.0	2.0
4	7.3	2.0	8.6	0	11.4	0
8	8.9	27.0	0	55.0	11.0	6.0
16	—	100.0	0	100.0		
32	—	100.0	0	100.0		
ponasterone A						
0–Control	8.2	16.0	16.0	0.7	14.5	10.5
1	7.4	0	10.7	6.0	16.2	4.0
2	6.0	2.0	7.7	4.0	11.0	10.0
4	7.5	5.0	8.6	5.0	9.2	4.0
8	—	62.0	—	41.0	0	84.0
16	—	100.0	—	100.0		
32	—	100.0	—	100.0		

nymphal stage (Fig. 2). For both beta-ecdysone and ponasterone A, the most effective dose for enhanced moulting was 4μg/ml of blood. At that dose 50% moulting was achieved after 11.4 days on beta-ecdysone and 16.2 in controls and 9.2 days on ponasterone A whereas the controls took 14.5 days. Among the second stage nymphs, there was no dramatic reaction of accelerated moulting, (Table 4). At the high doses of 8, 16 and 32μg/ml of blood, beta-ecdysone or ponasterone A were invariably toxic, thus resulting in the death of most, or nearly all, nymphs.

Topical application of beta-ecdysóne or ponasterone A resulted in non-reproducible results. Similarly cecropia juvenile hormone given in bloodmeals or topically applied to the nymphs gave non-reproducible results.

Chemical research on natural acaricides

A. Maradufu

In Northern Kenya the local people use extracts of plants to reduce the tick population on their livestock. The extracts are quite stable and are sold in the local shops.

Crude extracts were fractionated by extraction successively into *n*-hexane, benzene, chloroform, ethyl acetate, methanol and water. Acaricidal activity against larvae of *Rhipicephalus appendiculatus* was found in the *n*-hexane fraction. One percent hexane fraction effected 100% mortality of *R. appendiculatus* larvae in less than six hours. Larvae of *Ornithodoros moubata* were also affected by the hexane fraction, but mortality was low.

Repellancy tests using the Y tube method showed that adults of *R. appendiculatus* are repelled by the natural acaricide(s). Mortality tests will have to be done to ascertain if adults are also killed by the extracts.

Gas-liquid chromatography of the active hexane fraction has revealed the presence of several compounds in the fraction mixture which were also found to be U.V. active. Plans are now at hand to separate the mixtures on the high pressure liquid chromatograph and carry out bioassay tests of the pure components. Chemical identification of the active principle(s) will then be done, and field tests carried out using live animals.

TSETSE RESEARCH

REPRODUCTIVE PHYSIOLOGY (*Glossina morsitans*)

Directors of Research:

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

Scientists:

Dr. M. F. B. Chaudhury (1974) Research Scientist
Mr. T. S. Dhadialla (1973) Experimental Officer
Mr. J. Kawooya (1973) Experimental Officer (on study leave)
Mrs. J. Kongoro (1974) Research Assistant
Mrs. R. Kunyiha (1975) Research Assistant

Collaborators:

Mr. J. Owor (1973) Experimental Officer
Dr. E. D. Kokwaro (1975) Experimental Officer

SALIVARY GLAND PHYSIOLOGY

(*G. morsitans, G. pallidipes* and *G. brevipalpis*)

Director of Research

Professor Thomas R. Odhiambo (1970)

Scientists:

Miss N. Darji (1974) Research Assistant
Mrs. N. Patel (1975) Experimental Officer
Dr. L. H. Otieno (1973) Research Scientist

ISOLATION MECHANISMS

(*Glossina pallidips*)

Director of Research

Professor Wim Helle (1971)

Scientist:

Dr. J. van Etten (1974) Research Scientist

ACCOUSTIC COMPONENT OF TSETSE FLY COMMUNICATION

Directors of Research:

Professor Thomas R. Odhiambo (1970)
Professor A. R. Møller (1973)

Scientists:

Mr. R. K. Saini (1975) (Experimental Officer (on study leave)

Technical Staff:

Miss E. I. Carina Erickson
Consultation Technician (from May 1974)

REPRODUCTIVE PHYSIOLOGY

M. F. B. Chaudhury, T. S. Dhadialla and R. W. Kunyiha

Introduction

Studies on various aspects of the cyclical events of vitellogenesis, ovulation, functioning of milk gland, *in utero* larval development, parturition and abnormalities of pregnancy are being conducted. Possible endocrine involvement in the regulation of these events are particularly being considered.

Regulation of ovulation

To determine the factor(s) which regulates the release of ovulation-stimulating hormone (OSH) (Chaudhury and Dhadialla 1976, ICIPE Annual Report 1975) in the haemolymph, effect of age and stages of ovarian development of donor females on the ovulation of mature (12–day-old) virgin female recipients were investigated. Results showed that most of the females receiving haemolymph from mated donors (mated when 3-day-old and haemolymph obtained when 9-day-old) with at least one mature follicle ovulated, whereas those receiving haemolymph from mated but physiologically young (absence of a mature egg) donors did not. Haemolymph obtained 24–48 h after mating from physiologically mature females (mated when 10-12-day-old and having at least one mature egg) induced ovulation in about 60% mature virgin recipients. Haemolymph from physiologically mature *virgin* females did not have any effect on ovulation of recipients.

Haemolymph from a pregnant female failed to induce any ovulation in the virgin recipients. However, haemolymph obtained from parturient or from a female which has recently larviposited (within 4 h post parturition) induced ovulation in a large number of virgin recipients (50–70%), indicating the presence of the factor (or titer thereof) to result in the second ovulation.

The effect of donor's brain implant or brain homogenate injection on the ovulation of mature virgin recipients was observed. Implants from younger females (5–10 day, mated or virgin) failed to induce ovulation in recipients but those from the older virgin females (20–21 old) induced ovulation in 20% recipients. Brain homogenates from younger (mated or virgin) females did not induce ovulation, but those from older virgin females (21–day old) induced ovulation in 50% recipients.

The above findings suggest that the appearance of OSH in donor haemolymph is not only dependent on donor's mating status but also on the stage of ovarian development. Results also indicate that sufficient OSH to induce ovulation appears in circulation within 24 h after mating of a physiologically mature female. It appears that OSH disappears from the circulation after ovulation has occurred and reappears in the circulation before the time of larviposition to induce the next ovulation. If it is suggested that the act of parturation is responsible for triggering the release of OSH in the circulation to induce the second (and subsequent) ovulation, it seems from the evidence that the actual parturition activity may roughly coincide with the maturity of the next follicle and starts well before the actual larviposition.

It is certain that the OSH is not released spontaneously when the follicle is fully matured but a prolonged and successful mating is required to trigger the release. Alternatively, it is possible that in addition to a factor from the brain, a second ovulation stimulating factor is released from a mature follicle into the haemolymph. Whether such a factor originates from the follicle remains to be investigated.

Results of the brain implant and brain homogenate injection suggest that the donor females which remain virgin for 3 weeks or so may accumulate relatively large amount of brain neurosecretory material (the proposed source of OSH) which causes ovulation. Detailed histological studies on the neurosecretory cells are being pursued to fully understand these findings.

Studies on corpus allatum (CA) and corpus cardiacum (CC)

Although the surgical removal of CA or CACC complex from the tsetse fly is a difficult procedure, this surgery can now be performed at the ICIPE with success. However, the accumulation of results is a slow process, particularly because of routine histological examination of the operated fly at the end of the experiment.

While allatectomy within 12 h after emergence and before the first blood meal did not seem to affect vitellogenesis, mating, blood-meal size, ovulation or parturition it always prolonged the normal 9-day interlarval period, sometimes extending it to 15 days. In addition, the allatectomized females produced smaller offsprings (pupae weighing 15–21 mg as opposed to 28–31 mg by sham operated females) for first 3–4 cycles and abortion of larval stages for 1–2 cycles thereafter. Replacement therapy on the allatectomized females with juvenile hormone analogue, ZR-515 (Zoecon Corporation, Palo Alto, California, U.S.A.) administered topically on ventral abdominal segments at the rate of 0.1–0.2µg per fly on alternate days throughout the pregnancy cycle resulted in viable offspring of subnormal size (pupae weighing from 24–27 mg). Explanation of these results cannot yet be given, but it is fairly clear that the developmental processes of the larva is under maternal endocrine control.

The question of the role of CA in the egg maturation remained unsolved at this point. While most females allatectomized within 12 h of emergence but before having taken blood meal larviposited for at least the first five cycles, about 10% did not produce any progeny but revealed an under-developed ovary with very little yolk deposition in the developing follicle. Because yolk deposition commences during the late pupal stage in this species of tsetse fly, it is possible that the gonadotropic effect of the CA hormone, if any, becomes functional before eclosion.

Investigation on the histological changes of the CA and CC during two pregnancy cycles has been undertaken. Changes of CA and CC in virgin females for the same duration are also being studied. This work is still in progress and no results can be given at present.

SALIVARY GLANDS PHYSIOLOGY

L. H. Otieno and N. Darji

1. Infective development o^f^ *Trypanosoma brucei* in *Glossina morsitans*

Using batwing membrance technique developed in our laboratory, it has been possible to show that *Gossina morsitans* harbouring *Trypanosoma brucei* do extrude trypanosomes in their saliva as early as day six after infective bloodmeal.

The midgut trypanosomes are extruded during the early stages of infection but later other forms (proventricular and metacyclics) appear. Infected flies do not always extrude trypanosomes when they salivate.

During salivation, the salivary drops are produced in sequence; from the first to the third, sometimes up to the fourth, and the first usually being the largest. Emuneration of the number of trypanosomes in each drop has shown that the first drop usually contains the highest count. This observation indicates that a hungry fly may transmit trypanosomes even if it does not get an opportunity to be fully engorged.

Dissection of flies which have individually been shown to salivate infective trypanosomes has indicated that some infective organisms may be present among the proventricular forms since no salivary glands infection could be demostrated and yet the flies were highly infective to mice.

2. Tsetse trypanosomes interactions

It was reported in the ICIPE Annual Report 1974 that partly digested blood meal from old *G. morsitans* when incubated with blood form *T. brucei*, the trypansomes tended to stick together in a definite (rosette) pattern. This study has been extended, noting particularly the effect of partly digested normal blood meal, from a rabbit on the blood form *T. brucei in vitro*.

a. Effect of partly digested blood meal on *T. brucei*

A comparison of the effects of freshly ingested blood meal from young and old *G. morsitans* on blood form *T. brucei in vitro* has confirmed that the blood meal from old flies. causes trypanosomes to clump together in a rosette form. The effect is also seen in young flies but after the blood meal has been digested for 10 or more hours.

b. Effect of the incubation temperature on trypanosome rosette formation

In order to see whether the incubation temperature had any effect on the clump (trypanosome) formation, digested blood was extracted from 8 days old *G. morsitans* and incubated with infected (trypanosome) mouse blood at 28°C and 4°C. It was found that incubation at 4° did not alter the ability of trypanosomes to form rosettes.

c. Effect of inactivating partly digested blood on rosette formation

Blood meal from 8 days old *G. morsitans* was inactivated at 56°C for 30 minutes then incubated with infected mouse blood as described above. The results showed that the use of inactivated sample did not inhibit the formation of rosette.

d. Effect of partly digested blood meal on tryapanosome infectivity to mice

Having confirmed that partly digested blood meal from old *G. morsitans* caused trypanosomes to form rosettes more easily than those from young flies, it was of interest to find out whether blood meal from similar flies affected the infectivity of trypanosomes to mammalian hosts. Using Lumsden's (ID_{63}) infective dose tests, it was found that significantly higher numbers of trypanosomes incubated with samples from old flies lost their infectivity to mice than those incubated with samples from young flies (Table 1).

e. Is the rosette formation due to an immunological phenomenon

In order to see whether the clumping of trypanosomes as a result of incubation with partly digested blood from old flies was due to an immunological phenomenon, infected mouse blood was mixed with:

i. Normal mouse blood
ii. Antimouse globulin (Ig)

Incubating trypanosomes with normal mouse blood resulted in heavy haemolysis of the red blood cells (RBC). The parasites became heavily granulated with marked vacuolation around the posterior end. Many parasites appeared damaged. There was, however, no agglutination of trypanosomes.

Incubating trypanosomes with —antimouse globulin resulted in agglutination of RBC and also of trypanosomes, but not in the rosette shape observed after the use of partly digested blood.

f. Electrophoretic analysis of partly digested blood from young and old *G. morsitans*

Disc acrylamide electrophoresis was used to study the protein patterns of the partly digested blood from young and old *G. morsitans* in order to see whether there was some differences in the ingested blood meal from two groups of flies, and if so, could this be associated with the observed clumping of trypanosomes. Midgut extracts were obtained from young and old *G. morsitans* and analysed three hours after feeding. The results of these analyses showed that in old flies there was a dinstinct band of haematin while in young flies the haematin band was very faint and sometines not seen at all.

Experiments are in progress with the aim of trying to isolate the factors which contribute to the rosette formation.

Table 1. The effect of incubating blood form *T. drucei* with partly digested blood meal from young and old *G. morsitans*. The partly digested blood meal was tested 4 hours after the flies had fed

	Young *G. morsitans*						Old *G. morsitans*					
	Fly No. 1	2	3	4	5	6	1	2	3	4	5	6
Test Sample	5.5±0.3	5.3±0.2	4.8±0.5	5.1±0.3	5a8±0.5	6.1±0.5	3.4±0.3	3.6±0.3	3.4±0.5	5.4±0.3	5.1±0.3	4.3±0.5
		5.6±0.3						5.6±0.3				
Test Sample	6.8	6.0	6.0	5.9	6.5	6.3	5.6	5.0	6.5	6.7	6.7	6.6
Control		6.7				6.7						

Biochemical composition of tsetse fly saliva

N. Patel

Introduction

Preliminary results on the nature of the extruded tsetse saliva were mentioned in previous report (ICIPE Annual Report 1975). The histochemical results obtained from that study indicated the presence of proteins, polysaccharide containing glycogen, lipids and phospholipids.

In the later study use of electrophoretic and chromatographic techniques was made for the identification of various proteins, amino acids, sugars and lipids present in the tsetse saliva.

Collection of tsetse fly saliva

About 15–20 salivary probes from *G. morsitans* (reared at ICIPE) were collected on marked microscope slides and 30 slides were used for each analysis. The saliva collection was according to the technique developed by Youdeowei (1975).

Disc electrophoresis

The standard canalco equipment for disc electrophoresis was modified at the ICIPE Workshop for the use of glass tubes with an internal diameter of 2.5 mm instead of the standard tubes with an internal diameter of 7 mm. Gels containing 7.5% Cyanogum "41" were prepared using ammonium persulphate for polymerization (T. S. Dhadialla, personal communication).

Samples containing saliva from 6 slides C dissolved in 1μl of Calliphora meditm) were applied directly on the surface of the gel and the electrophoresis was run in a Boric acid buffer of pH 8.6 at 1.5 mA/gel for 35 minutes. The gels were fixed in 12.5% TCA for 10 minutes and stained in 0.25% Coomassie brilliant blue

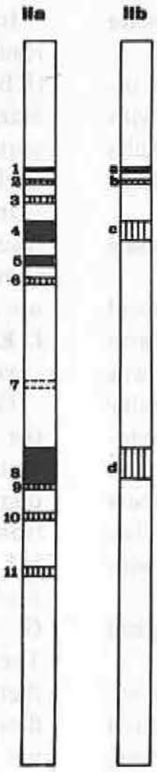

Fig. 1.

G for proteins (Diezel *et al* 1972). Color reactions for glyco-proteins were followed with Periodic-Acid.-Schiff's (PAS) reagent (Olembo 1972). Following electrophoresis, gels were also stained with Sudan Black B (Prat *et al* 1971) for lipids and Fast Blue RR (Whitmore *et al* 1974) for non-specific esterases.

Chromatographic analyses

Amino acids analysis

The proteins from saliva were hydrolysed in sealed capillarly tubes in 6N HcL at 110° for 24 hours. Paper chromatography was performed by the method of Smith (1960) and the spots were developed with 0.2% ninhydrin in acetone.

Sugar analysis

Saliva from 30 slides was hydrolysed in 20 μl ofl 0.5N H_2SO_4 at 100°C for 24 hours followed by remova of the acid with $Ba(OH)_2$ solution (Partridge 1948). Sugar analysis was done on silica gel G plates impregnated with 0.2 M sodium acetate using n-propanol: ethyl acetate ÷ water (3:2:1 v/v) solvent and the spots located by alkaline $AgNO_3$ solution (Trevelyan *et al* 1950).

Lipid and phospholipid analysis

Lipids were extracted by chloroform: methanol (2:1 v/v) and evaporated by rotary vacuum evaporator to dryness and redissolved in 50μl of 2:1 chloroform: methanol. The solvent used was petroleum ether: diethyl ether: acetic acid (60:40:1 v/v) for the separation of neutral lipids and chloroform: methanol: acetic acid: water (60:30:10:3.5 v/v) for the phospholipids. The spots were detected by exposing the plates to iodine vapours.

Disc electrophoresis run on 2.5 mm tubes showed 10 proteins bands (Fig. 1) from saliva on 6 slides as compared to only 5 bands using 7 mm tubes and using saliva from 30 slides. On staining the gels with PAS reagent, 4 PAS positive bands appeared. The most intensely PAS positive bands, a, b and d of I (Fig. 1) corresponded to bands 1, 2 and 8 of II. These bands (a, b and d) probably formed the major carbohydrate-bound protein fractions in the saliva.

When the gels were stained for lipids, no bands were located on gels indicating absence of lipo-proteins. Similarly absence of non-specific esterases was demonstrated when the gels were stained with Fast Blue RR.

However when a capilary tube of 7 mm external diameter with an internal diameter of 1 mm was used, saliva from only 3 slides was required to obtain as good a separation as that obtained by 2.5 mm tubes.

Methanolic extract of saliva on paper chromatograms gave a faint reaction at the original spot site only, suggesting absence of free amino acids. However, when the hydrolysed saliva was spotted, 16 ninhydrin reactive spots appeared.

Similarly study of hydrolysed saliva for sugar content showed negative results indicating absence of free sugars. Hydrolysed saliva gave only one spot after spraying with alkaline $AgNo_3$ reagent.

TLC of tsetse saliva lipids revealed phospholipids, sterols, free fatty acids, triglycerides and sterol esters.

Phospholipid analyses showed three faint spots when exposed to iodine vapours.

Attempts are also being made to run electrophoresis in a 20 µl capillary with a diameter of 0.6 mm using saliva droplets from one single fly. This would help in analysing saliva from individual infected and non-infected flies.

Determination of moleculer weights and fractionation and isolation of saliva proteins by gel filteration with sephadex is in progress.

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ISOLATION MECHANISMS

Jaap Van Etten

Introduction

The comparative studies of several characteristics of *G. pallidipes* populations in the two selected areas Nkruman and Mwalewa forest has been continued (ICIPE Annual Report 1975). The additional data we have obtained in the past year do not change the conclusions with respect to the efficiency of traps and the differences in the ratios in the different trapping systems used, which means that the overall results found last year are constant and are more or less independent of the seasons. The same conclusion can be applied to the diurnal activity patterns which also remain the same (ICIPE Annual Report 1975).

In future attention will be paid to the possibility of the existence of interpopulation diversity and if it is found, an effort will be made to determine to what extent this diversity influences the conclusions already made on the matter.

Field studies

Some preliminary results were obtained in the studies on breeding sites in the two areas of Mwalewa Forest and Nkruman. In both areas breeding sites are typical "floorsites"—breeding places. No correlation between preference site and vegetation could be found except that all sites are shadowish, dark places. This agrees with the experimental results obtained with *G. palpalis* in the laboratory in which it was found that females use dark places for larvi-position.

A remarkably higher percentage of the pupae in the coastal area were parasitized. While only 2.3% of the pupae from Nkruman were parasitized by Thyridanthrax, 36.2% of the pupae collected in Mwalewa forest were parasitized. These results stress the need for further research on the role of parasites and predators in tsetse fly population dynamics.

Nutritional studies

The nutritional studies have been restricted to studies on the fat reserves of flies caught in traps and in a stationery car in both areas and to studies on blood-meal size and frequency of feeding of flies from both areas under laboratory conditions.

The total amount of lipids extracted by chlorophorm, which in accordance with literature is called fat reserve, is different in flies from the two areas. Females from Mwalewa have about 40% more fat than females of Nkruman, while males from Mwalewa have about twice as much fat as the males from Nkruman. There was no indication of influence from the trapping systems used or the date on which the flies were sampled. Neither did differences in size or age composition have a bearing on the differences that were found. However, the following possible reasons may explain these differences:

- Flies from Mwalewa take bigger meals
- Flies from Mwalewa take meals more often
- Flies from the two areas feed on different hosts
- Flies from the two areas have a different behaviour towards the used catching systems.

To come two some understanding to experiments were initiated under constant laboratory conditions, using rabbits as a host:

- Determination of the size of bloodmeals and the number of meals taken in a fixed period
- Determination of the fat reserve after a fixed period of daily feeding. In both experiments the fixed periods were 20 days for males and 30 days for females

From the second experiment there is yet no available data but the feeding experiment has given some interesting results.

Males from both areas take, on the average, meals of the same size. The same is true of females although these take 1.5 times as much blood as the males do per meal.

Males from both areas take during twenty days the same number of meals. However, females from Mwalewa take about 30% more often a meal than females from

Nkruman do in the same period. This results in about a 30% higher intake of blood by females from Mwalewa. This might explain the higher fat reserves found in females from this area. The difference in fat reserves of the males cannot be explained on the same basis because no differences are found in the blood intake. The most likely explanation is that the trapping systems in the two areas catch different samples. A recent experiment in which males from Nkruman caught in a moving car had fat reserves equal to the fat reserves found in males from Mwalewa lends more weight to this point.

The fact that females from Mwalewa feed more often means they have to fly more often. This behaviour has to be taken into account in any population estimation since it will influence the apparent density on which relative population estimations are based.

The more frequent feeding of females from the coast has other implications. It increases the chances of transmitting trypanosomes, which might be a part of the explanation for the high infection rate found in the coastal area (Gazi-farm and near Lamu, J. Liroux, personal Communication).

In Summary then, it can be stated that further studies in the past year have provided additional information on the existing diversity between the two populations of *G. pallidipes* under investigation. The confirmation that this aspect of the difference in the feeding frequency in females is genetically determined since flies which are used in the experiments are maintained for at least two generations under constant laboratory conditions, supports the idea that at least some aspects of the diversity may have a genetic base.

CHEMISTRY AND BIOCHEMISTY RESEARCH UNIT

Directors of Research:

Professor Jerrold Meinwald (1970)
Professor Koji Nakanishi (1970)

Scientists:

Dr. N. Abo-Khatwa (1974) Research Scientist
Dr. A. Maradufu (1974) Research Scientist
Dr. G. D. Prestwich (1974–76) Research Scientist

The Chemistry and Biochemisty Research Unit is by nature a fluid group and interacts with other programmes of the Centre depending on the development of projects. The details of the investigations carried out by the staff in this unit appear under each appropriate project in this report. But in summary, the investigations are as outlined below:

Dr. N. Abo-Khatwa looked into termite hormones; lipids and lipid composition of incipient colonies and of the queen termite; biochemical studies on the "Fungus Gardens" in relation to termites.

Dr. A. Maradufu worked on natural acaricides in local plants, finding acaricide activity against larvae of *Rhipicephalus appendiculatus* in the extract from one of the plants.

Dr. G. D. Prestwich who left towards the end of the year, worked on hemipteran scents, termite frontal gland secretions; trail and building pheronones of termites.

HISTOLOGY AND FINE STRUCTURE UNIT (HFSRU)

Directors of Research:

Professor Thomas R. Odhiambo (1970)
Professor David S. Smith (1970)

Scientists:

Mrs. E. D. Kokwaro (1975) Experimental Officer
Mr. J. R. Owor (1972) Experimental Officer

There was a considerable increase in the use of FSHRU facilities by ICIPE Scientists as compared to last year and an overall increase in the output of work by the unit inspite of periods of technical problems with both the Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Transmission Electron Microscopy played quite a singificant frole in the work of both ICIPE Scientists and non-ICIPE collaborators as detailed below.

In spite of a few intermittent failures of the microscope, over 3,000 micrographs were taken during the year ending December 1976. Some of these micrographs are shown.

Scanning electron microscopy

The SEM worked without any major breakdown. Scanning electron microscopy was boosted by the acquisition of the critical point drier, which solved most of the problems we had with soft non-cuticular tissues. The demand for the SEM was quite high. Over 3,000 micrographs for about 14 scientists within and outside ICIPE were taken in the course of the year.

Photographic Section

This continued to play a vital role in the production of high quality macro-and micro-photographs and other reproductions for both staff and university staff members.

Training and visits

The Unit had the honour of extending its facilities to Professor E. L. Beneditti of the Institute of Molecular Biology, Paris, who conducted a UNESCO Course on Cellular Organization for postgraduate African students from 4th to 16th April 1976.

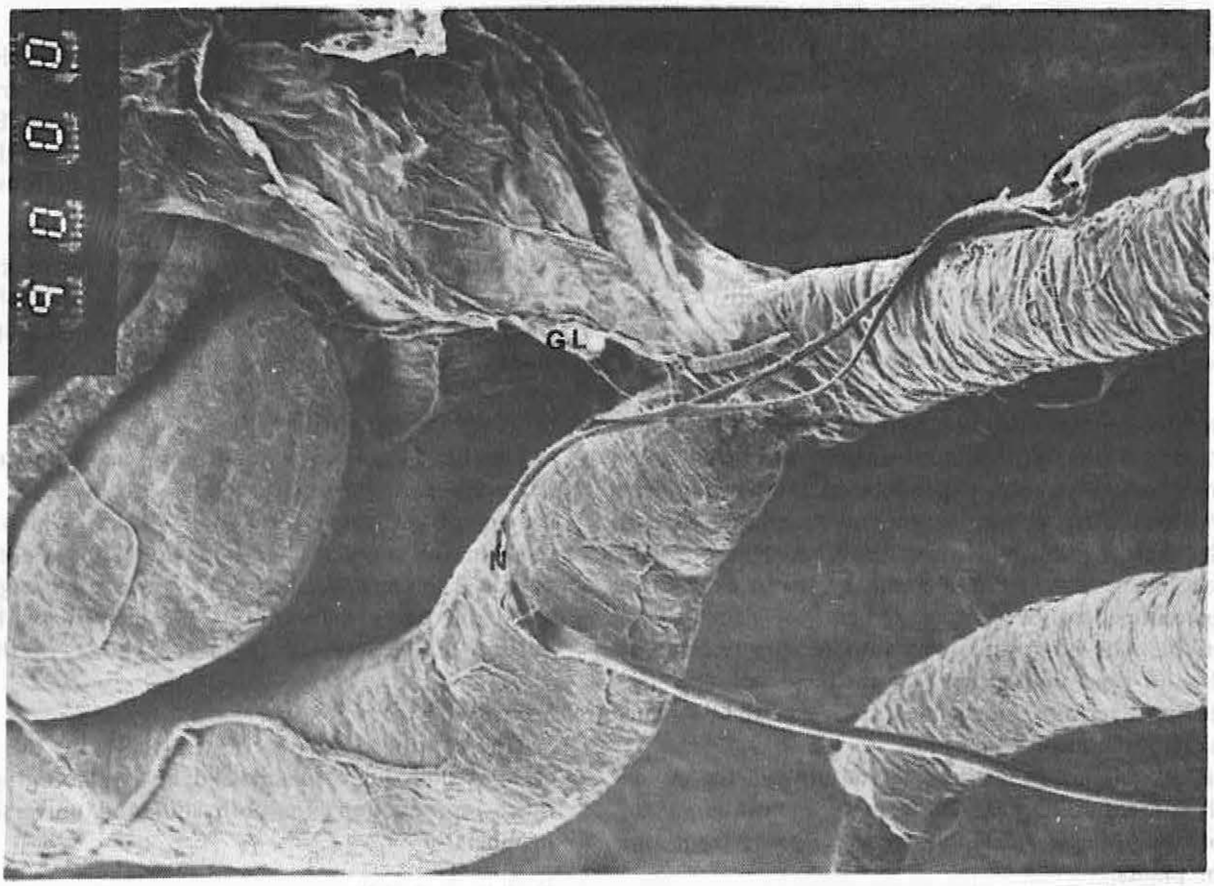
Dr. A. M. Glauert, Strangeways Research Laboratory, Cambridge, England made use of Fine Structure facilities, while she was at Wellcome Nairobi.

Mr. S. D. Ajayi, Senior Technician, University of Ibadan, was a trainee in EM techniques, operation and routine maintenance for 3 months, till 31st May 1976. By the end of this period, he became competent in both TEM and SEM techniques.

Mr. John Kaddu, a Research Officer at EATRO, and Mrs. Jedida Kongoro continued their training in EM techniques.

ICIPE Scientist	Project	Subject
Dr. L. H. Otieno	Salivary Gland	Salivary gland of *T. brucei*
Dr. N. Abo-Khatwa	Termite	Fat-body, Mitochondria of *Macrotermes*
Dr. M. F. B. Chaudhury	Reproductive physiology	Meidian Neurosecretory cells of *Glossina*
Ir. P. Sheltes	Stemborers	1. Diapause and non-diapause brain of *Chilo* 2. Corpora allata caridaca complex of *Chilo*
Dr. J. Clearwater	Sorghum shootfly	1. Trifoliate Organ of *A. laevigata* 2. Hypogial prominence *A. laevigata*
Mr. Okot-Kotber M. B.	Termite	Corpus allata caridaca complex *Macrotermes sub-hyalium*

Non-ICIPE Scientist	Institution	Subject
Professor Tucker	University of Nairobi	Waterbuck glomerilli
Dr. Kimeto	University of Nairobi	East Coast Fever *Theileria parva*
Dr. Mathai	University of Nairobi	Tick Salivary Gland
Dr. Oduor	University of Nairobi	Hyrax placenta
Mr. Mutanda	East African Virus Research Institute	Rotavirus



ig. 1. Scanning Electron Microscope (SEM) micrograph of the the innervation of the *G. morsitans* testis. Note the nerves (N) and the 'ganglion-like' structures (GL) 900.

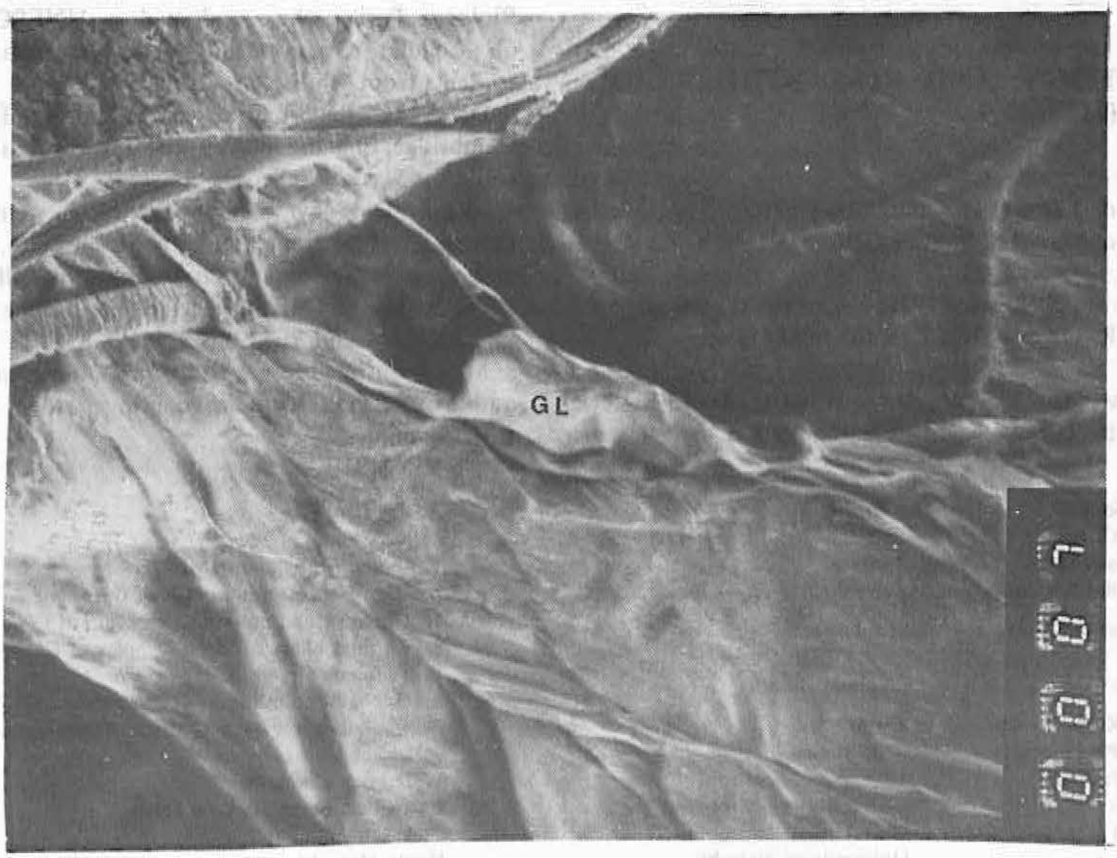


Fig. 2. Higher magnification of the GL region × 3000.

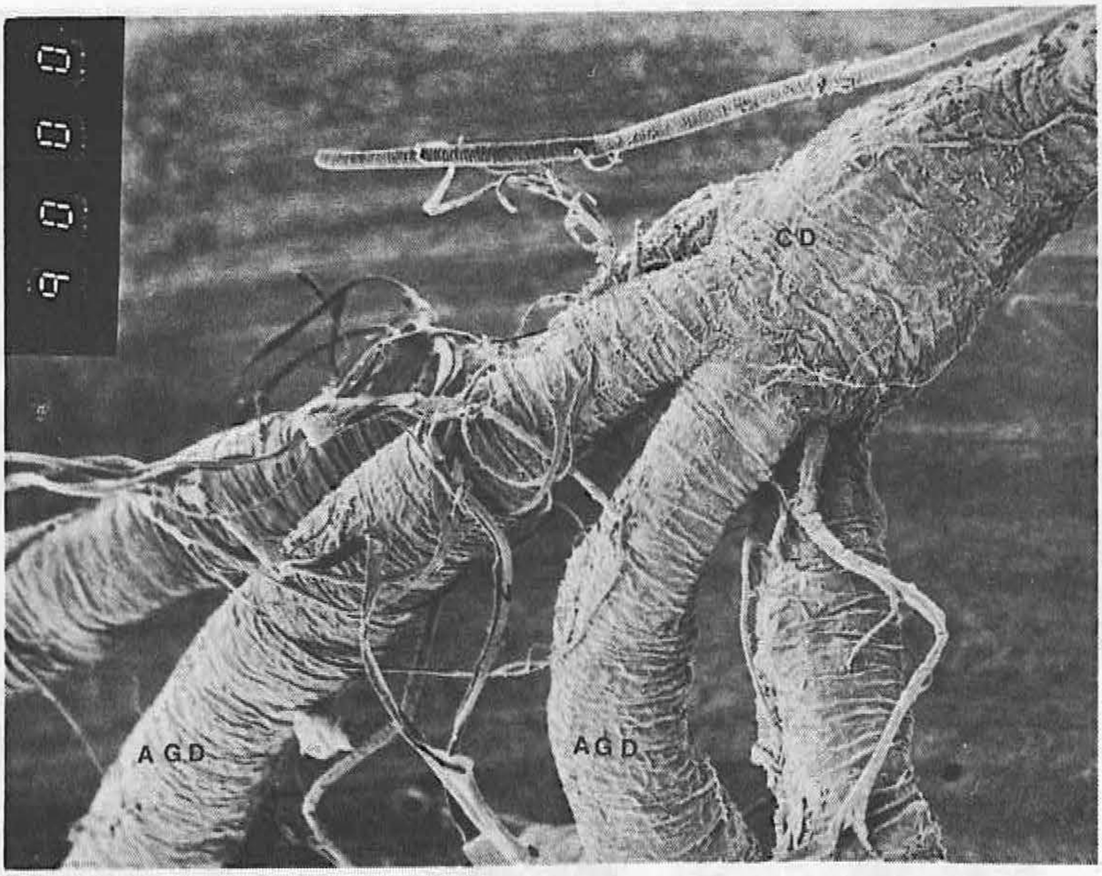


Fig. 3. Part of the nerves (n) to the accessory gland duct (AGD). CD is the common duct ×900.

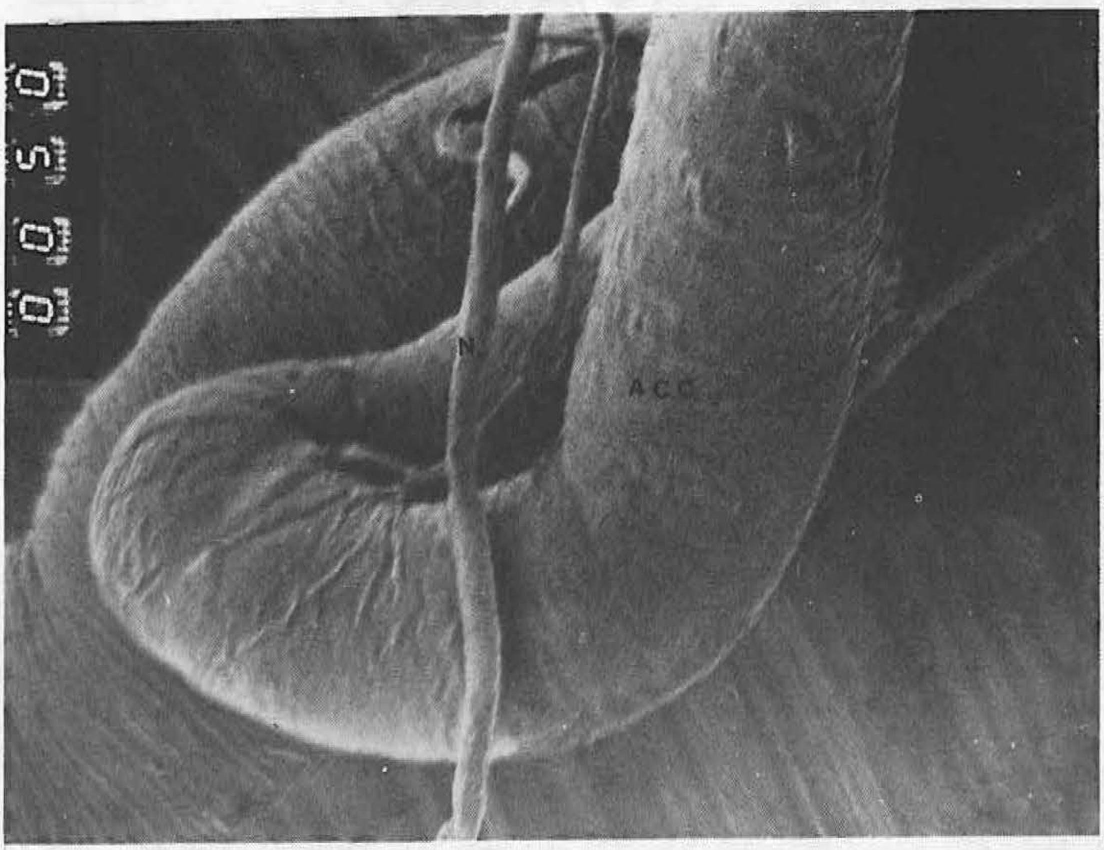


Fig. 4. Innervation to the accessory gland (ACC). ×900.

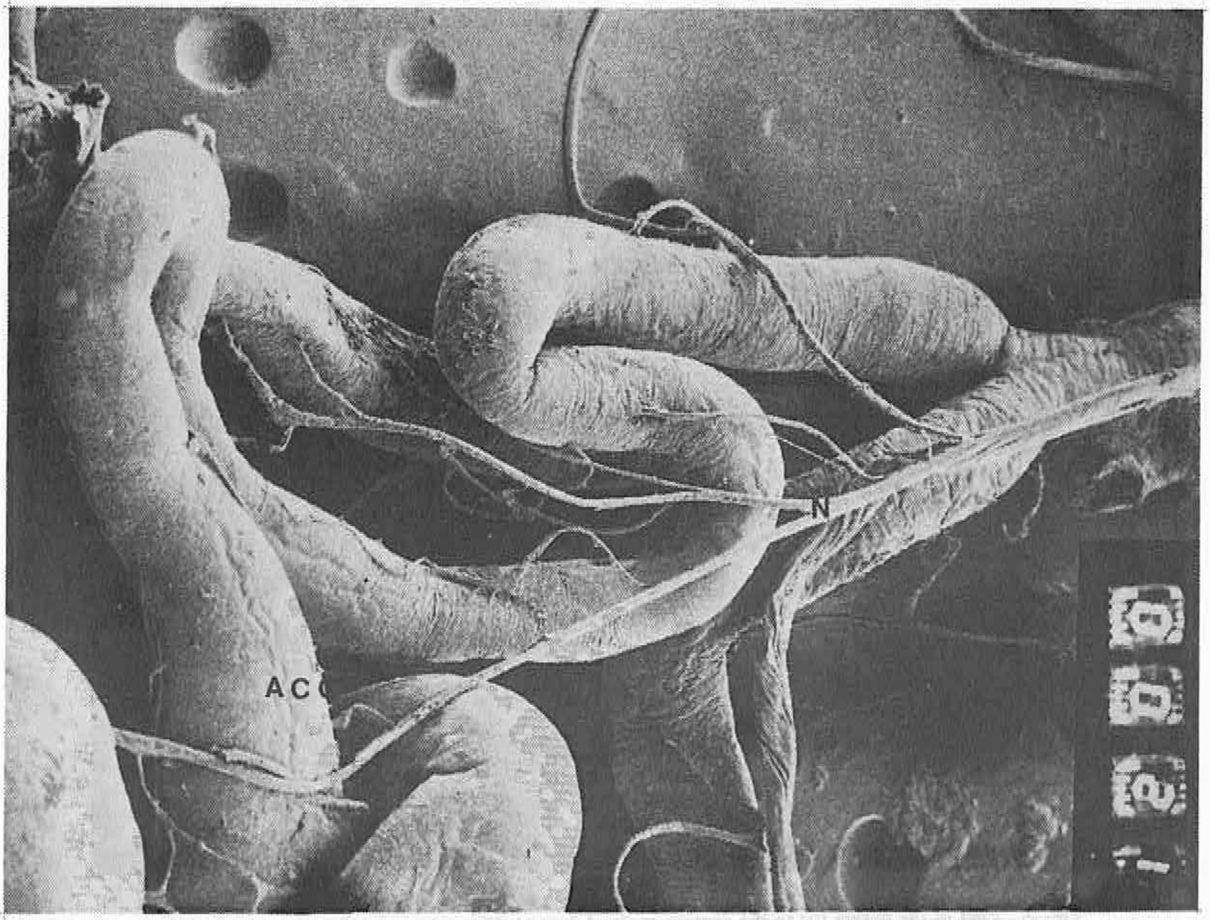


Fig. 5. A low power SEM micrograph, showing the innervation of the accessory gland (ACC) × 600.

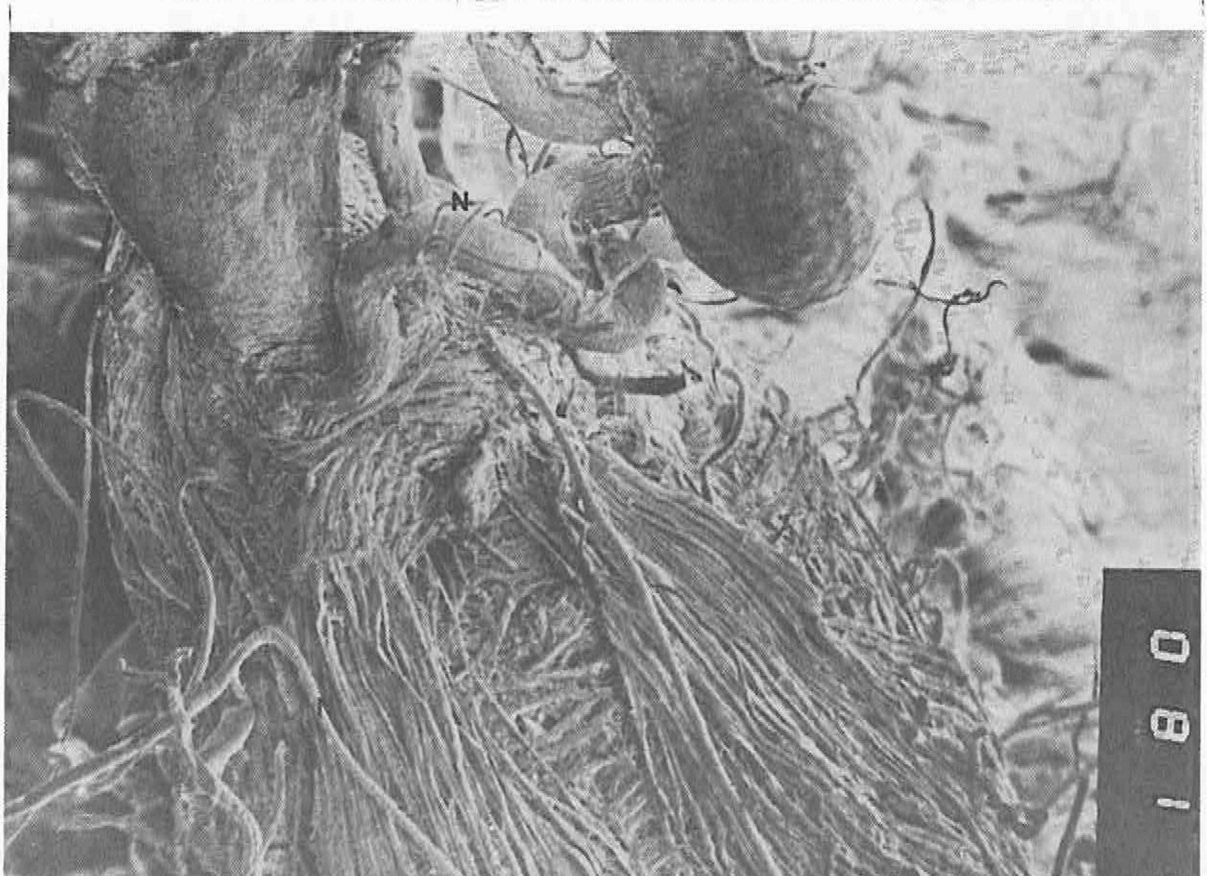


Fig. 6. Nerves to the anterior region of the uterus of *G. morsitans* × 600.

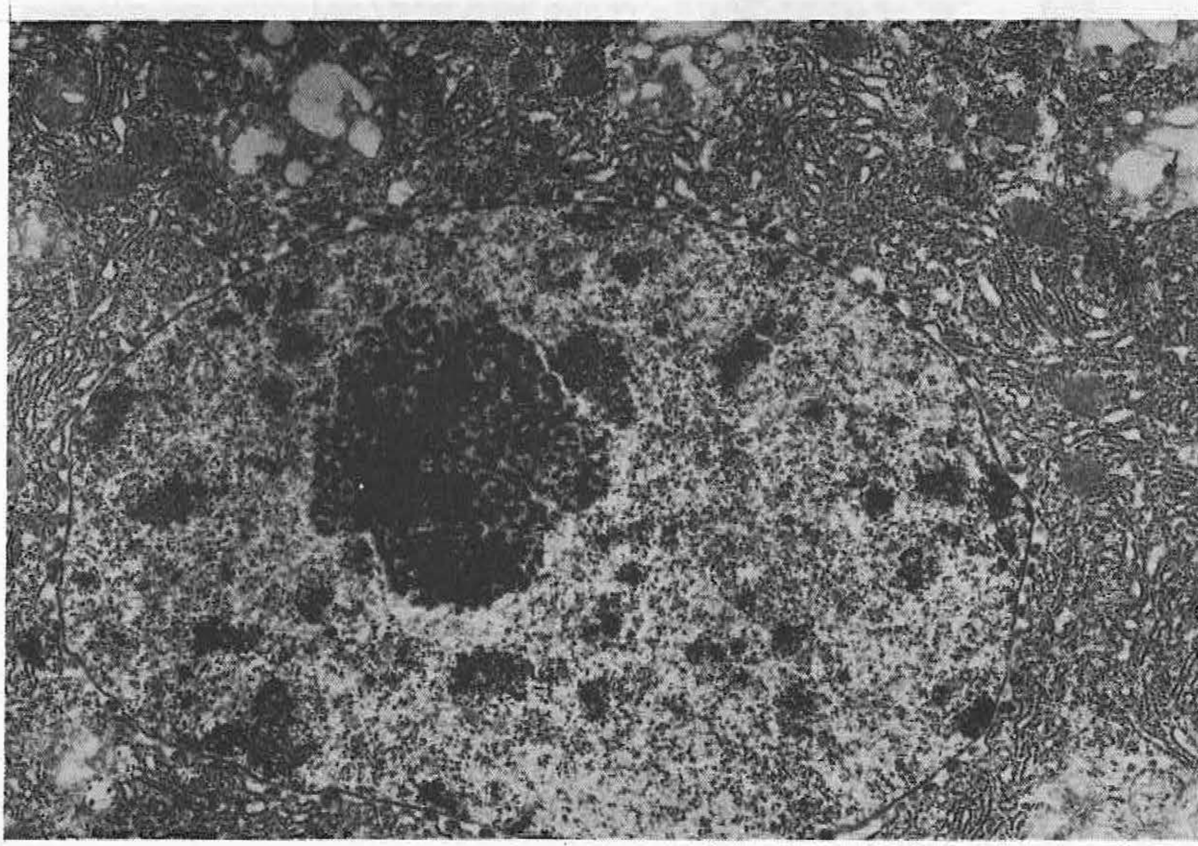


Fig. 7. Normal salivary gland cells of the distal region × 12,000.

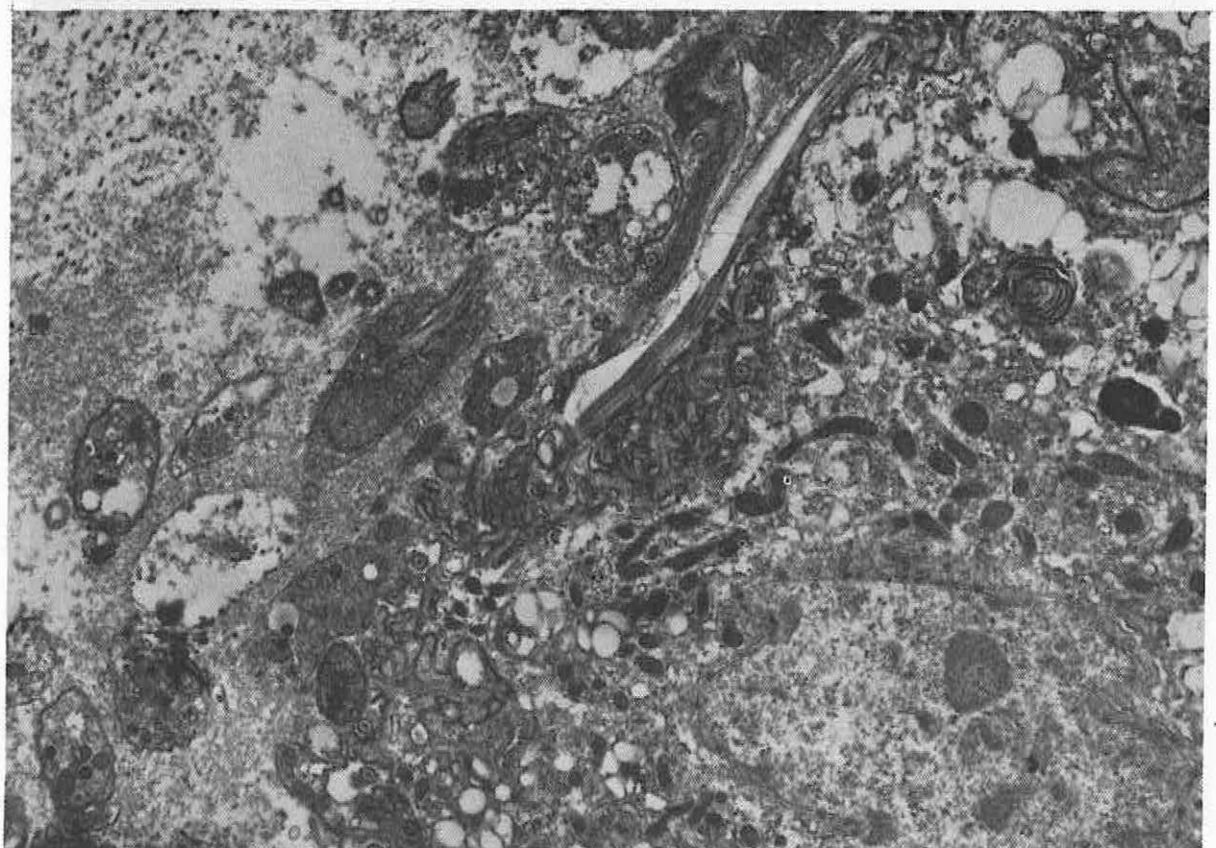


Fig. 8. *T. brucei* infected salivary gland × 13,500.

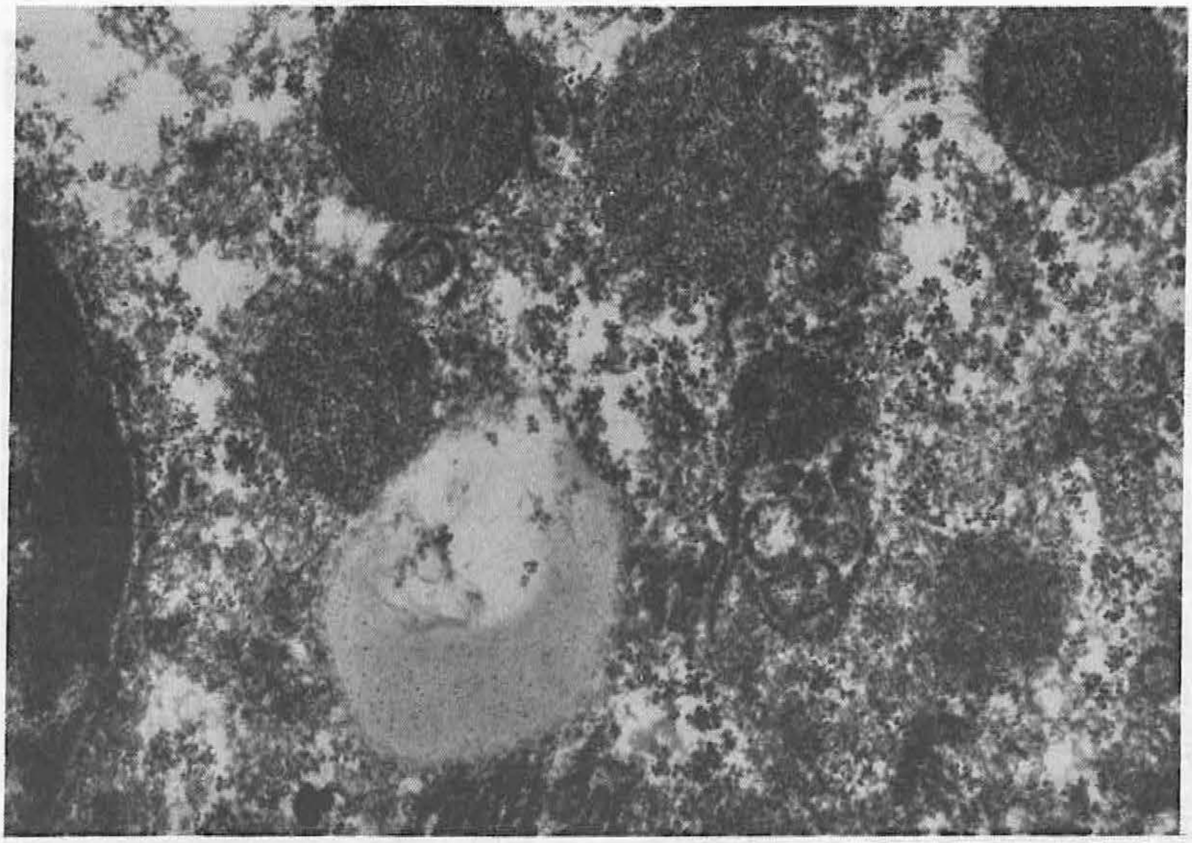


Fig. 9. Liver biopsy of a suspected cancer patient × 60,000.

Fig. 10. Rotavirus × 120,000.

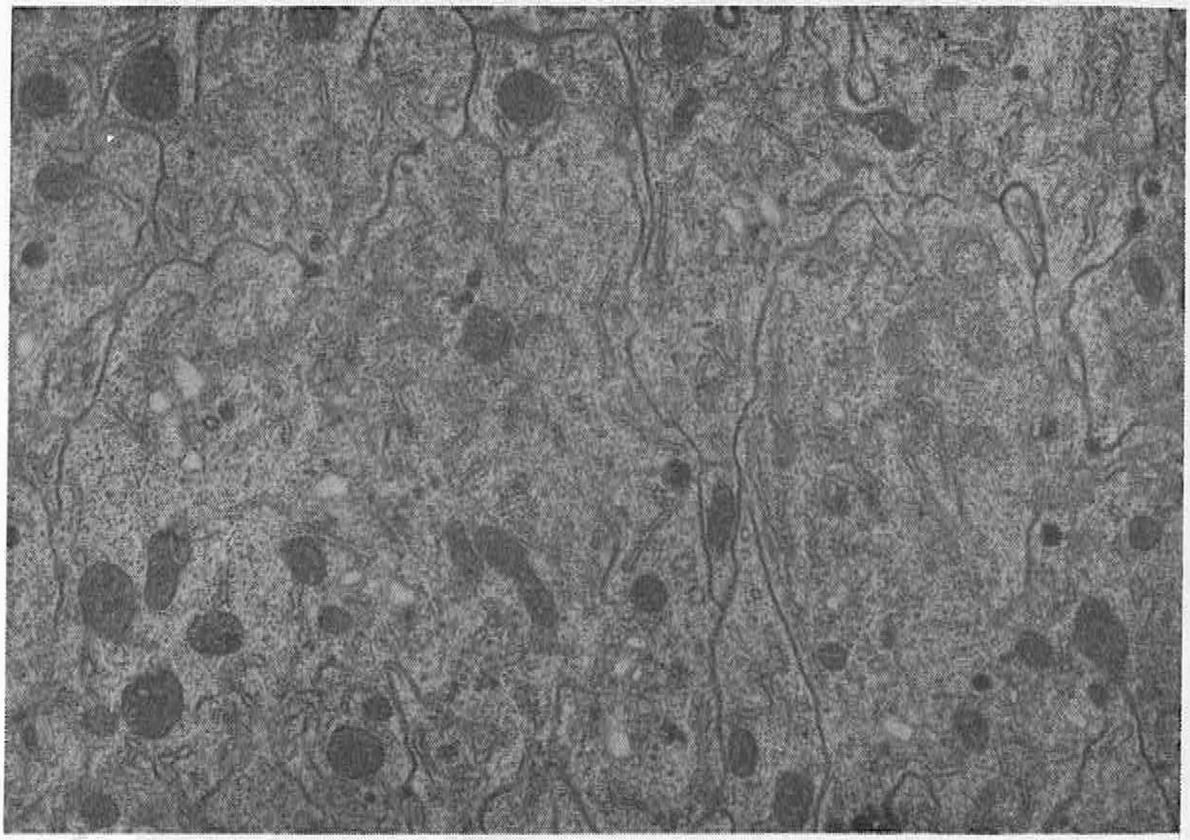


Fig. 11. Cell-type III of the *G. morsitans* proventriculus × 24,000.

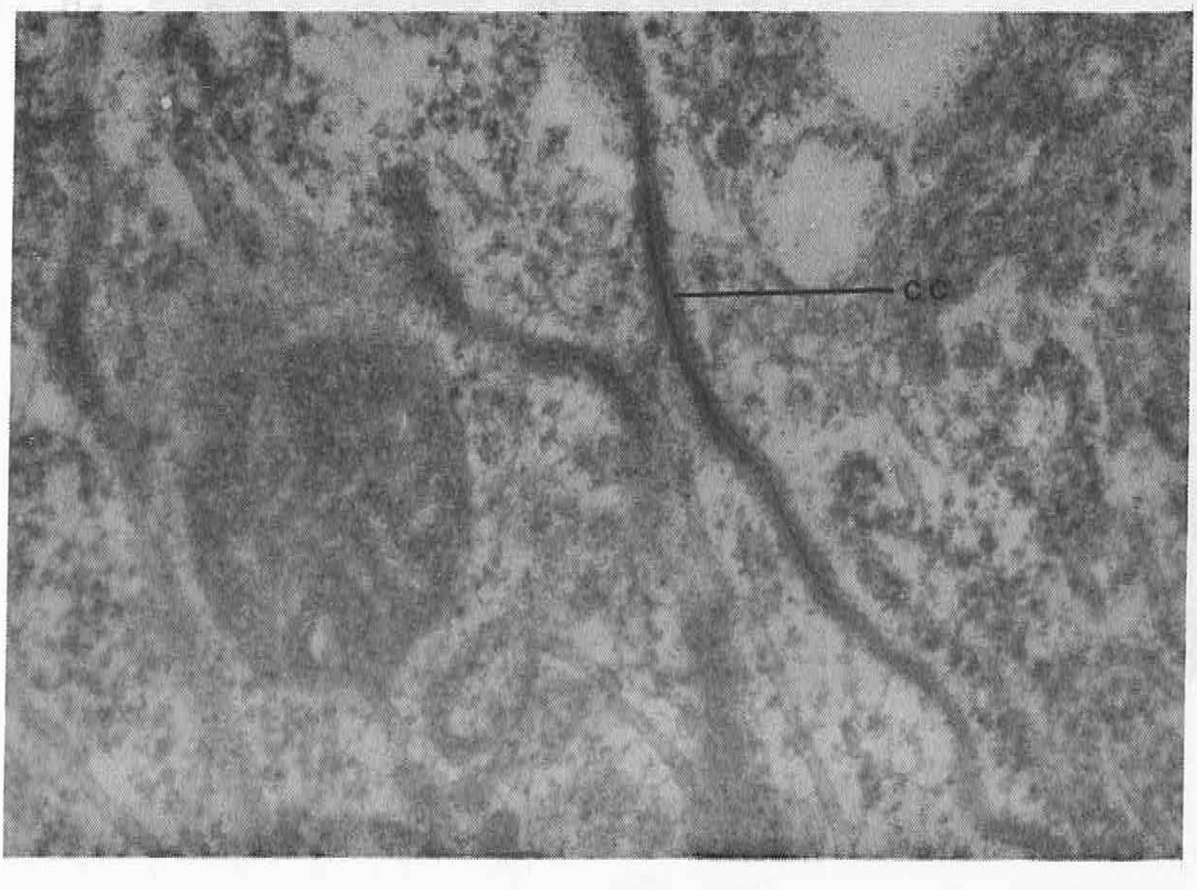
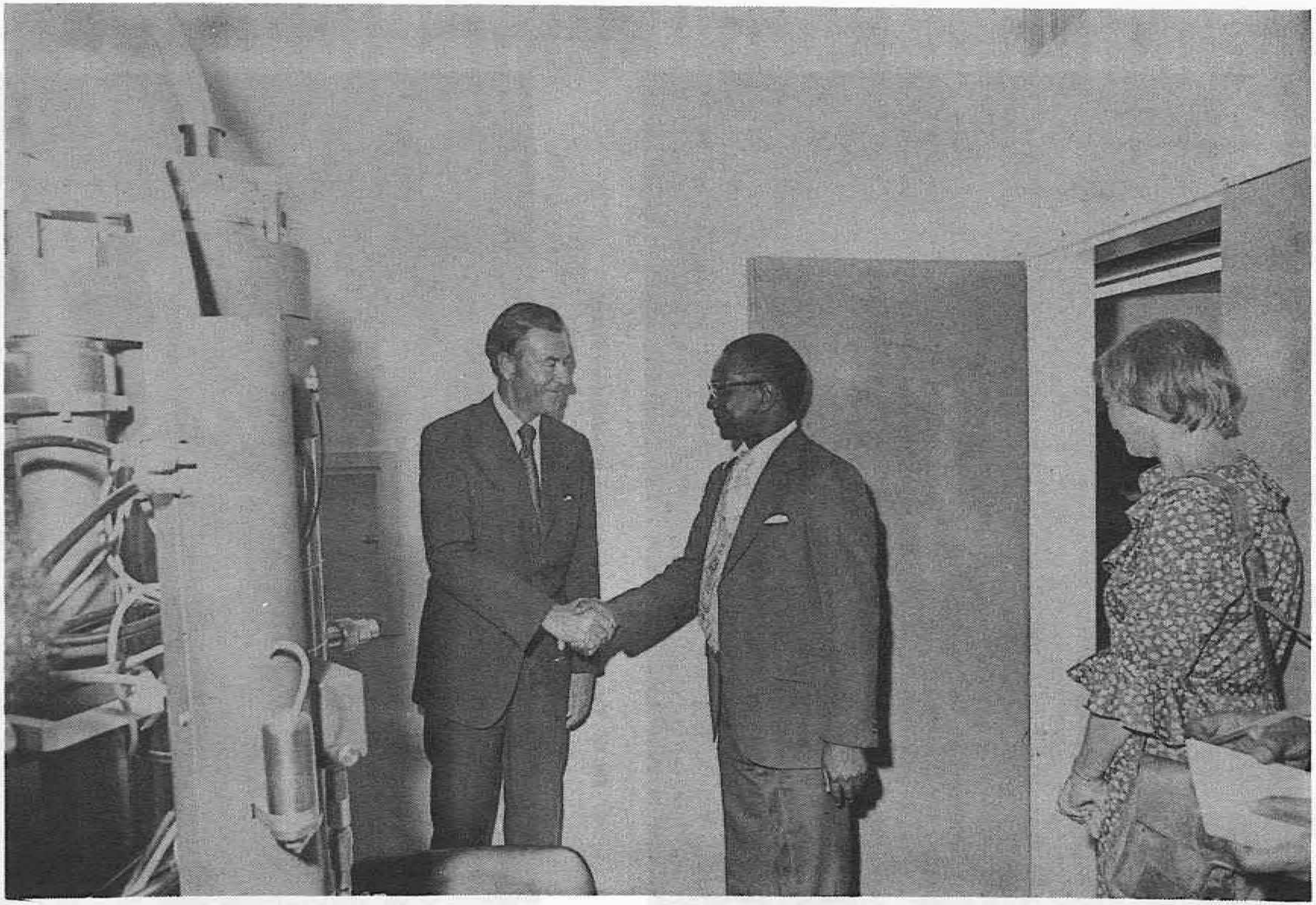


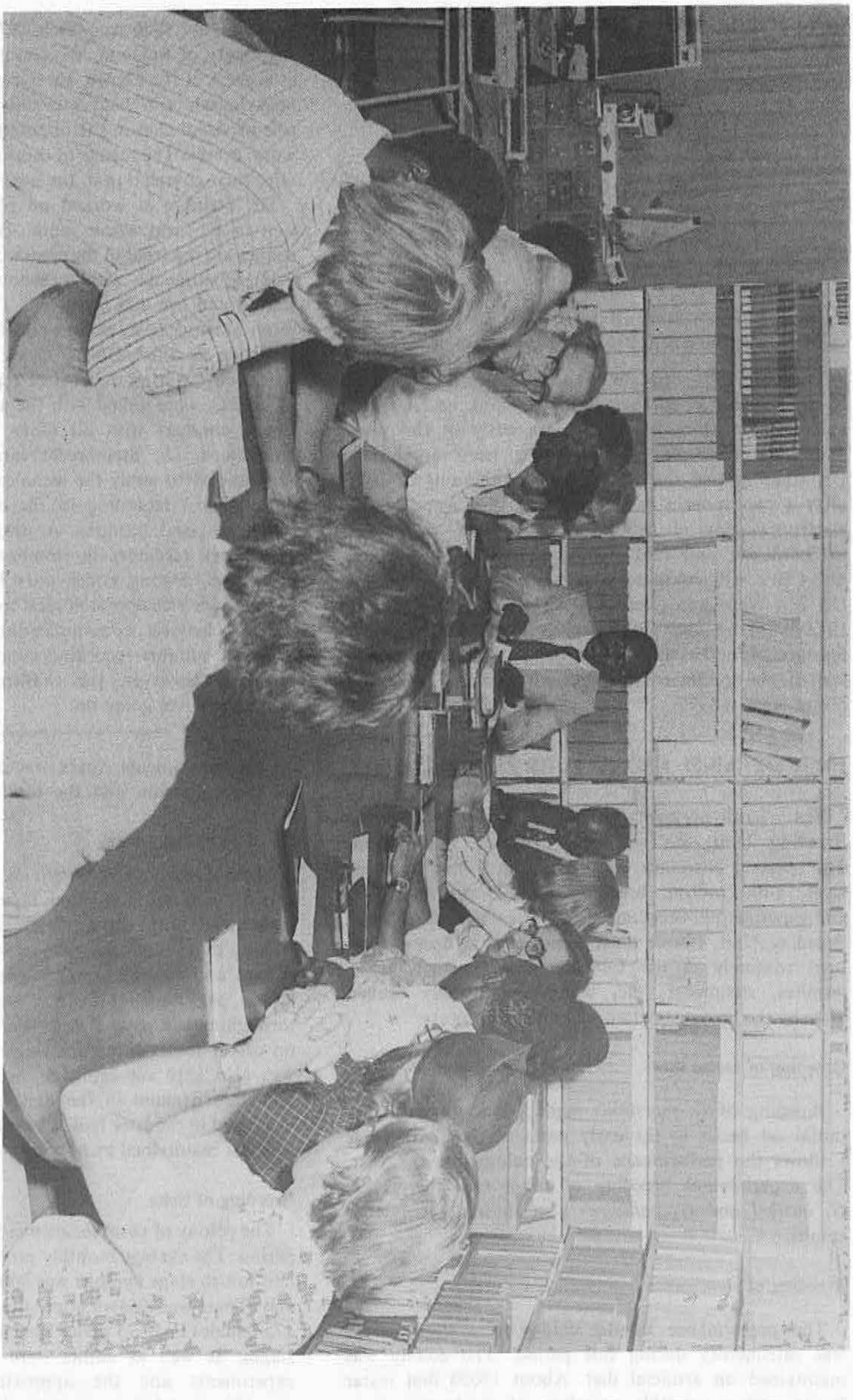
Fig. 12. Colloidal carbon marker (CC) in the intercellular space of cell-type III.



The Danish Ambassador to Kenya H. E. Mr. H. Kühne shaking hands with Professor Thomas R. Odhiambo at the handing over of the Transmission Electron Microscope to the ICIPE.

Dr. William Gamble, Director-General of the International Institute of Tropical Agriculture (I.I.T.A.) is introduced to Dr. (M.B.) E. D. Kokwaro of the ICIPE by Prof. Thomas R. Odhiambo.

Prof. Koji Nakanishi performing one of his conjuring tricks ata party to bid him and Prof. T. O. Browning farewell at the end of their terms as visiting Directors of Research at ICIPE.



A joint meeting of ICIPE and ILRAD Staff at the ICIPE to dicuss possible areas of collaboration.

SENSORY PHYSIOLOGY RESEARCH UNIT

Director of Research:
Professor D. Schneider (1970)

Scientists:
Dr. R. A. Steinbrecht (1975) Senior Research Scientist
Dr. M. Kaib (1973–1976) Research Associate
Dr. G. Karuhize (1972–1976) Research Scientist
Professor H. Langer (1976–1977) Visiting Research Associate
Dr. J. V. Clark (1976) Research Scientist
Dr. J. MacFarlane (1976) Research Scientist

The year 1976 brought major changes in personnel to the Sensory Physiology Research Unit, as Dr. Kaib and Dr. Karuhize left the centre early in the year. Two new research scientists have been appointed. Dr. Clark joined the Centre at the beginning of 1977 after a two months special training in Europe at the electrophysiological laboratories of Prof. Schneider, in Seewiesen, and Prof. Schoonhoven in Wageningen, Dr. Clark will continue some aspects of the work of Dr. Ma Wei-Chun (Research Associate at the Centre 1972–1975) on the sensory basis for acceptance and rejection of food in the armyworm larva. Dr. MacFarlane started his special training abroad and will join the centre later in 1977.

From July 1976 to March 1977 Prof. Langer of the University of Bochum, W. Germany, joined the SPRU as a guest of the Centre. He started electrophysiological, behavioural, and field experiments to investigate the role of visual cues in the orientation of migrating armyworm moths. The results of these studies will be reported after their completion in the next Annual Report.

Dr. Steinbrecht worked on pheromone communication in the armyworm moth. His electrophysiological studies are reported in the armyworm section. Additional field experiments with pheromone traps have been started and are still going on. The morphology of the moth antenna is studied in collaboration with the Fine Structure Research Unit.

Although during the year of report the main activities of SPRU were linked with the armyworm programme, strong contacts with all other target programs were maintained. Dr. Steinbrecht carried out various pilot experiments to study the technical feasibility of electrophysiological recording in the antennae of tsetse fly, stemborer, and termites. A project to study the role of sensory receptors in stemborer diapause has been postponed. Strong endeavours have been undertaken to introduce eletrophysiological techniques in the field of termite chemical communication, but to obtain and maintain suitable recording conditions is still a major problem. However, the challenge is recognized and experiments are going on.

INSECT AND ANIMAL BREEDING UNIT

The overall performance of the Insect and Animal Breeding Unit was satisfactory during this period. The present Insectary consists of the Tsetse Breeding Unit, with routine breeding of *Glossina morsitans* and experimental breeding of *G. austeni* and the Animal Breeding Unit. Beside these some more arthropods are bred routinely at the Centre e.g. armyworm, ticks, termites, stemborers etc, which are usually looked after by the Scientists attached to these projects.

Breeding of tsetse flies

Breeding of *G. morsitans* made good progress after initial set backs in the early parts of the year. Table I shows the performance of the colony for the year. The experimental breeding of other two species i.e. *G. austeni* and *G. pallidipes* also showed satisfactory results.

Breeding of stemborers

The performance of the colony of *Chilo zonellus* was satisfactory during this period. The colony was maintained on artificial diet. About 15000 first instar larvae with a monthly variation of production from 200 to 2700, were produced during the year. As most of the larvae of the 5th-6th instar (15 days old) were used up for experiments, there was a general reduction in pupae production and the total came to about 3050.

Breeding of armyworm

Three species of *Spodoptera* i.e. *S. exampta*, *S. littoralis* and *S. triturata* were bred regularly. *S. exampta* was maintained both on artificial diet and maize plants. Among these three *S. exampta* was the most demanded species and had an average production of 4000–5000 larvae per week. This rate was maintained almost throughout the year. *S. littoralis* was maintained mostly on castor leaves though a small portion of the colony was also kept on artificial diet. This species had a moderate demand in the first half of the year but it decreased in the later half. The other species, *S. triturata*, was just maintained as nobody worked on it.

Breeding of ticks

The colony of *Ornithodors moubata* did well during this period. The average monthly production of second, third and fourth stage nymphs was 860, 730 and 720 respectively. There was also an approximate production of about 175 females and 195 males per month. All the nymphal stages, as well as adults were supplied regularly for experiments and the approximate monthly supply was 620 second stage nymphs, 430 third stage nymphs, 430 fourth stage nymphs and 130 each of females and males.

Animal breeding unit

Rabbits, guineapigs, rats and mice are kept and bred at the Centre to be used in feeding of arthropods and other experimental work.

Rabbits: About 280 rabbits were produced during the year. These were used in feeding tsetse flies and ticks. Due to shortage of space it was not possible to produce as many rabbits as required and a good amount of money was spent in purchasing rabbits from outside sources. There was also a fall in supply of rabbits from EAVRO, Muguga. It is expected that some additional space will be provided this year for expanding the rabbitry so that the Centre can become self sufficient in the requirement of rabbits.

Guineapigs: Though very few guineapigs were used for experiments, a good colony was maintained during the year. There were about 300 animals at the beginning of the year. About 200 were also produced during the year. As these animals were not used much it was decided to reduce the number. After regular culling the number was reduced to 120. When there was a shortage of space to keep them they were reduced to 10 only.

Rats: There was also very little demand for rats. About 250 rats were produced during the year and only 150 were used. To reduce the number it was decided to distribute them to different schools in Nairobi. Accordingly, the number was reduced to 20 only.

Mice: The Animal House produced about 4500 mice during the year. A good number of these were used by The ICIPE Coastal Research Station, Mombasa and others by Scientists at the Centre.

Outside support

Beside the internal supplies the department also provides support to a number of outside organisations in various ways. The Departments of Entomology and Biochemistry, University of Nairobi, were provided with tsetse flies and pupae and army worms from larval stages.

The National Council of Scientific Research, Zambia got a regular supply of large numbers of *G. morsitans* pupae for establishing their own colony.

Secondary schools around Nairobi got mice, rats and rabbits. Egerton College and Kenya Science Teachers College were supplied with armyworm from larval stages.

Pupal stages of armyworm were also sent to West Germany. ILRAD was supplied with adults and pupae of *G. morsitans*. The Centre also received a regular supply of pupae of *G. morsitans* and *G. austeni* from University of Bristol, U.K. and Institut D'élerage et de Médecine Veterinaire des Rays Tropicaux, France.

Table 1:

Months	New Emergence		Adult Females Death		Old Females removed	Mated Females added	Used in experiments		Pupae	
	Females	Males	Virgin	Mated			Female	Male	Total collected	Used in experiments
January	1227	1112	17	365	55	927	—	—	3595	—
February	1584	1584	—	378	214	1107	—	—	4027	—
March	2100	2041	—	606	400	1406	105	221	4808	—
April	2258	2058	—	539	219	1400	185	304	5997	—
May	2708	2492	45	974	404	1810	10	10	6369	—
June	3033	2964	—	1726	408	1765	351	62	5648	—
July	2649	2742	—	1112	—	1940	61	73	6508	—
August	2510	2350	—	1160	—	1386	524	381	7826	—
September	3570	3484	—	987	—	2296	321	314	7317	—
October	3528	3496	—	1635	—	1443	538	638	9677	—
November	4029	4188	—	1345	49	2200	470	675	9738	30
December	4181	4328	—	1499	—	2540	837	402	11438	30
TOTAL	33377	32839	62	12326	1749	20220	3402	3080	82948	60

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A group photograph of the participants in the ICIPE Study Workshop on Tick Ecology and Behaviour held at the Centre in October, 1976.

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