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OBSERVATIONS ON TRAIL PHEROMONE, TRAIL LAYING AND LONGEVITY OF NATURAL TRAILS IN THE TERMITE, *MACROTERMES MICHAELSENI*

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Abstract—The sternal gland in *Macrotermes michaelseni* is located in the anterior half of the fifth abdominal sternite between the third and fourth abdominal ganglia and overlapped by the fourth sternite. It is found in all castes including newly-swarmed de-alates. The gland is largest in major workers followed by major soldiers, minor soldiers and minor workers and is the only source of trail pheromone. Major workers have the highest potential trail pheromone activity followed by minor workers, male and female de-alates, minor soldiers and major soldiers.

There is a change in trail laying behavior with time and direction of travel, from the culture or from the arena and it can be correlated with the deposition of a light or heavy trail, or no trail at all.

The trail of one major worker leaving the culture is attractive to a significant number of workers for 1 hr 30 min and that of five major workers for 1 hr 45 min. The trail of workers leaving the arena is not as strong, one worker trail is attractive for 1 hr and five worker trails for 1 hr 15 min. The well-established trail is attractive to a significant number of workers for 9 hr. All trails lose attractiveness at a constant rate.

Key Words: Sternal gland, trail pheromone, trail laying, natural trail, major worker, termite

INTRODUCTION

TERMITES lay chemical trails to orient to the mound and food sources. The time that these natural trails remain effective has been investigated only in *Trinervitermes trinervoides* and *Hodotermes mossambicus* (Hewitt *et al.*, 1969). The manner in which the trails are established has received only casual observation in *T. trinervoides*, *H. mossambicus*, *Zootermopsis nevadensis* (Stuart, 1963a,b; Hewitt *et al.*, 1969; Tschinkel and Close, 1973).

The sternal gland, found in all castes of all termites examined so far, has been shown to be the source of the chemical trail pheromone (Stuart, 1963a; Tschinkel and Close, 1973). A size polymorphism among the various castes has been correlated with potential trail pheromone activity in *T. bettonianus* (Leuthold and Lüscher, 1974).

The paper presents the results of a study carried out on the sternal gland, trail pheromone and the natural trails of the termite *Macrotermes michaelseni*.

MATERIALS AND METHODS

Experimental animal

Macrotermes michaelseni mounds were dug at weekly intervals near Kajiado town, Rift Valley Province and workers, soldiers, larvae, nest material, fungus and a royal cell containing a king and queen were removed to the laboratory and stored in plastic basins. The termites were used in the various experiments within 3 days of collection. Any reference to the age of the termites refers to the time since mound was dug.

Termite culture and foraging arena

A simulated foraging situation was set up in the laboratory where the termites were allowed to move freely between a termite culture and foraging arena (Fig. 1). The foraging arena was a 25 × 25 × 5 cm plexiglass chamber containing small pieces of dry grass collected in the area where the mounds were dug. The termites were kept in a plastic chamber, 18 × 12 × 8 cm, painted black. The chamber or culture contained 100–150 termites; the majority of which were major workers but also included a few minor workers, major and minor soldiers, larvae, nest material, fungus, moist mound soil and a piece of wet cotton to maintain high humidity.

The foraging arena and termite culture were placed on a plexiglass platform and connected by a plexiglass gallery 1 cm wide and 25 cm long. An 8 cm section of this foraging gallery could be removed for insertion of the apparatus for the figure-8 and diamond bioassays, cinematography and sooted slide technique (Figs 3, 4, 5 and 6). The number of termites entering and leaving the centre section from either culture or arena was controlled by a double gate system.

Location of the sternal gland

Thirty specimens of each caste from different mounds were fixed in acetic-formalin for 24 hr and

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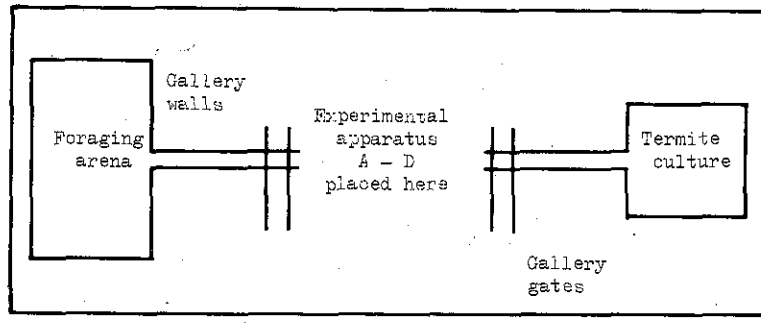

Fig. 1. Diagram of culture and foraging arena used in the laboratory experiments.

then transferred to 70% alcohol. The abdominal sternum was removed by cutting along the pleuron, anterior of the metathoracic legs and posteriorly in the last abdominal segment, then all adhering tissue was removed; stained with haematoxylin for 10 ± 2 min, dehydrated in alcohol, cleared in xylene and mounted on slides in DPX for measurement of length and width of the sternal gland. A few specimens were cleared in KOH and the ventral cuticle mounted on slides for more careful examination.

Source of trail pheromone

The sternal cuticle containing the sternal gland was dissected from three groups of major workers, 50 from each of three different mounds, placed in 2 ml of hexane at 16°C and extraction continued for 18 ± 2 hr. After removal of the tissue each extract was bioassayed twice in the figure-8 apparatus. The remainder of the workers were placed in an equivalent amount of hexane, extracted and bioassayed.

The figure-8 bioassay used the simple maze described by Leuthold and Lüscher (1974) (Fig. 2) and inserted into the centre section of the foraging gallery (Fig. 1). The extract to be tested was spread on a strip of lightweight typing paper, 5 × 30 cm, (Fig. 2, paper) placed under the maze. Five microliters of the test-extract and hexane were spread on the two S-shaped pencil lines previously drawn on the paper strip to conform to the figure-8 maze. Each pattern was tested once by a single worker which had to decide twice between trails *h* and *e*. In a complete test, this procedure was repeated 10 times with systematic alternation of the trails to yield a total of 20 decisions between *h* and *e*. If the trails were chosen an equal number of times there was no preference, that is, no difference in the strength of the trails. However, if one trail was chosen 15 or more times (15 or more positive choices) it was significantly stronger ($P = 0.05$) than the other. In the bioassays to determine the source of trail pheromone, extracts of sternal glands alone and whole workers minus sternal glands were tested against each other and against hexane.

Potential trail activity in various castes

Crude trail pheromone extracts were prepared of the various termite castes by placing a known number of each caste (from 100 to 1000 termites) in a container and then adding hexane (16°C) to completely cover them. The extraction procedure was the same as described above. The termites were removed after extraction, the extract measured and the equivalent number of termites per volume calculated. Cold hexane provided the most active extracts because the termites were killed immediately upon contact with the cold hexane and consequently there was little or no regurgitation of material from the crop. The extracts were bioassayed in the figure-8 apparatus where 5 μl of crude extract was tested against 5 μl of hexane both spread over a distance of 5 cm on the S-shaped pencil lines previously drawn on the paper strip (see previous section). If the crude extract was chosen more than 15 times it was serially diluted until

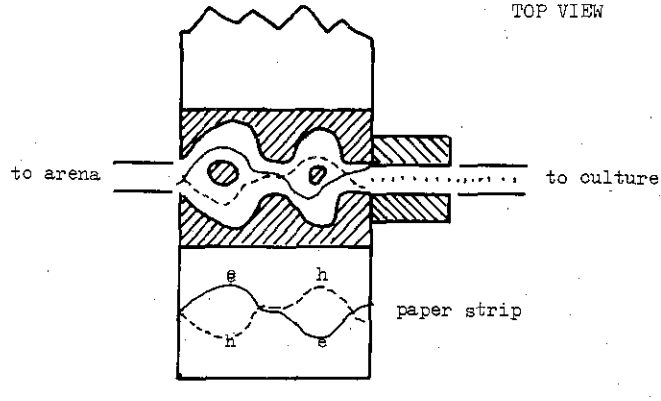

Fig. 2. Diagram of figure-8 bioassay apparatus for hexane extracts. *e* = extract, *h* = hexane.

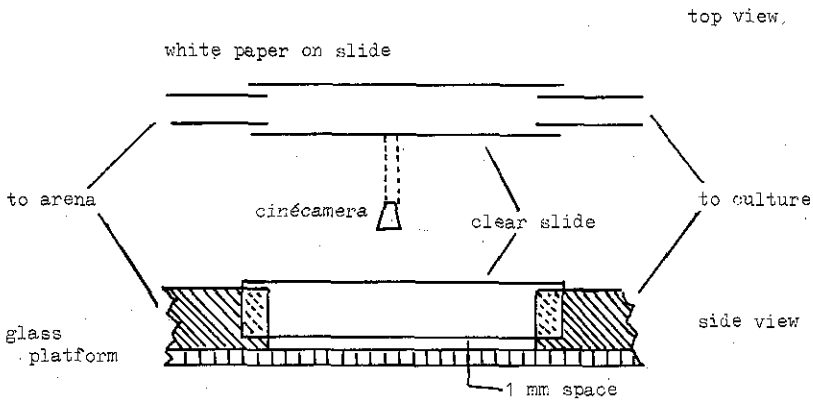

Fig. 3. Diagram of cinématography apparatus for trail laying behavior.

it was chosen exactly 15 times out of possible 20 decisions (5% level of significance). Therefore, the definition of one trail unit (TU) is that amount of pheromone in 5 μl of diluted crude extract which, when spread over a distance of 5 cm, is chosen 15 times out of a total of 20 decisions. The number of trail units per caste could be calculated when the dilution factor, the amount of extract and the number of termites in the extract are known.

Trail laying behavior

Major workers were allowed to establish trails between the culture and foraging arena. The culture and arena were set on a clear glass plate 15 cm above the laboratory bench. A mirror set at 45° angle was placed under the centre of the foraging gallery which allowed the termites to be photographed from below and also provided more light for photography. A viewing chamber, consisting of a pair of microscope slides (Fig. 3), was placed in the centre section of the foraging gallery (Fig. 1). The slide away from the viewer was covered with white paper as a background for cinématography. The slide towards the viewer was clear and placed with the bottom edge 1 mm above the glass platform. This space provided a clear view of the lower half of the termite abdomen and the edge of the slide served as a reference point when the films were studied. The termites were photographed as they passed a 3-cm-wide section of the viewing chamber by a Beaulieu R16 semi-automatic ciné camera with a 75 mm Macro lens at 64 frames per sec. Kodak 2484 B & W high speed negative film permitted filming in the laboratory without artificial lighting. The films were viewed on an editor and the various types of trail laying behavior and their frequencies recorded. Examples of the various types of trail-laying behavior were sketched from projections of the films.

As a supplement to the filming, the workers were allowed to walk over sooted-slides to determine which parts of the body touched the substrate during trail laying, a modification of the technique used by Hangartner (1969) to study trail laying behavior in *Solenopsis geminata*. The apparatus for the sooted slide (Fig. 4) was inserted in the foraging gallery (Fig. 1). The centre section of the foraging gallery, centre-section-depression, was modified to place a microscope slide at the same level as the rest of the foraging path. The workers were confined to the sooted-slide by a plexiglass cover, 8 × 2 cm and 3 mm deep, which is the same size as a microscope slide. It was found in some preliminary experiments that workers hesitated to move over the sooted-slide in the absence of a trail, the deposition of soot destroyed any previous trail, especially when there was a well-established trail in the foraging path at both ends of the slide. Preliminary observations showed that major workers could detect and follow a trail without hesitation placed above them, that is, a trail placed a few mm above their path of travel even when no previous trail had been established on the path. This behavioral fact was used to obtain a continuous trail over the sooted slide. The plexiglass cover (Fig. 4, cover) when turned over (inverted-cover) can be placed in the centre-section-depression so that the ceiling of the cover, instead of a sooted-slide, is on the same level

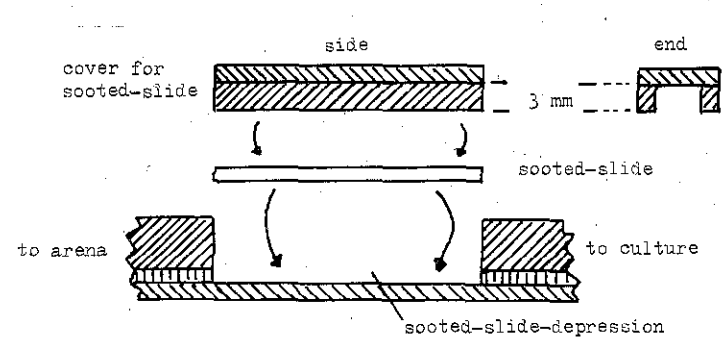

Fig. 4. Diagram of sooted-slide apparatus for trail laying behavior.

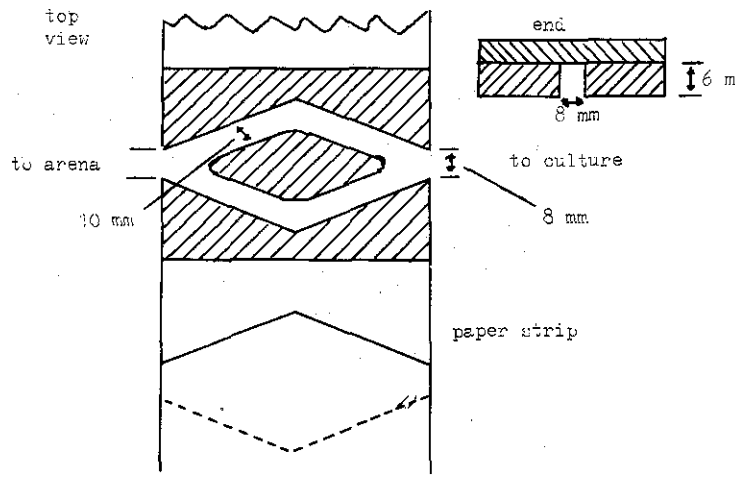

Fig. 5. Diagram of diamond bioassay apparatus for natural trails.

as the other sections of the foraging path and forms a continuous uninterrupted path between culture and arena. To record which part/parts of the termite abdomen touches the substrate during trail laying, the inverted-cover is removed from the centre-section-depression and a sooted-slide is inserted in this space. The cover is then placed over the slide which puts the trail previously laid on the ceiling a few mm above the sooted-slide which can be detected and easily followed by the termites. The strength of the trail established on the ceiling of the inverted-cover depended on the time after the culture and arena were set-up. The records were taken, from zero time to 1440 min (24 hr). The slides were coated with soot from a candle about 5 min before an experiment since it was found that workers could not make clear impressions in the soot more than 30 min after its deposition. The slides were photographed immediately for a permanent record making note of direction, to or from culture and the time since culture and arena were set-up. The prints of the sooted slides were similarly correlated with the sketches from the 16-mm films.

Longevity of major worker natural trails

Natural trails of major workers were collected and bioassayed in the laboratory using the diamond-apparatus (Fig. 5) placed in the centre section of the foraging gallery (Fig. 1). The diamond-apparatus was constructed of plexiglass 8 cm long, 6 cm wide and 10 mm deep. The diamond paths, 1 cm side and 6 mm deep, at the widest point were 5 cm apart and converged to 8 mm wide openings at each end of the apparatus. In some preliminary experiments major workers from either culture or arena were allowed to walk over the paper strip placed under the apparatus for about 10 min of continuous use. The apparatus was removed and the path of workers marked in pencil. This paper was bioassayed in the same apparatus to determine the presence or absence of a trail and by reversing the paper strip detect any directional information in the trails.

In other bioassays one or more workers from either culture or arena were allowed to walk over a strip of light-weight typing paper, 8 × 30 cm and marked with pencil to conform to the diamond-apparatus. If trails were bioassayed immediately, the paper strip was left in the apparatus, the first worker through the apparatus establishing the trail and the second worker was the bioassay worker. If trails were to be bioassayed later, five individual trails to conform to the pencil marks were collected on the same paper strip which was then stored in a closed container in the laboratory to be bioassayed in the same apparatus at various times after collection. Workers used to establish the trails and in the bioassays were actively foraging and moving freely between culture and arena, thus simulating as nearly as possible in the laboratory a natural foraging situation. The apparatus was frequently changed and cleaned to prevent contamination and bias due to nest odor and other compounds.

Trails laid by 1, 5, and very many major workers (well-established) were collected and bioassayed in the diamond-apparatus. Well-established trails were collected by using three separate diamond-apparatus in series so that three complete diamond trails or six half-diamond trails could be collected at the same time. They were obtained by allowing major workers at a traffic density of 15 per min from both culture and arena to walk over the paper for 2 hr. The papers containing the trails were stored at room temperature until bioassayed. Multiple worker trails were verified as having an active trail at time of collection by allowing the final worker to walk over the paper after removal of the diamond-apparatus. If the worker followed the same diamond path completely and without hesitation the trail was retained for later bioassay.

RESULTS

Location of the sternal gland

Examination of the abdomen of freshly killed specimens and KOH cleared specimens with low power microscope showed no morphological variations in the abdominal sternites. In fixed material, the sternal gland as well as the abdominal ganglia could be seen through the cuticle of major and minor

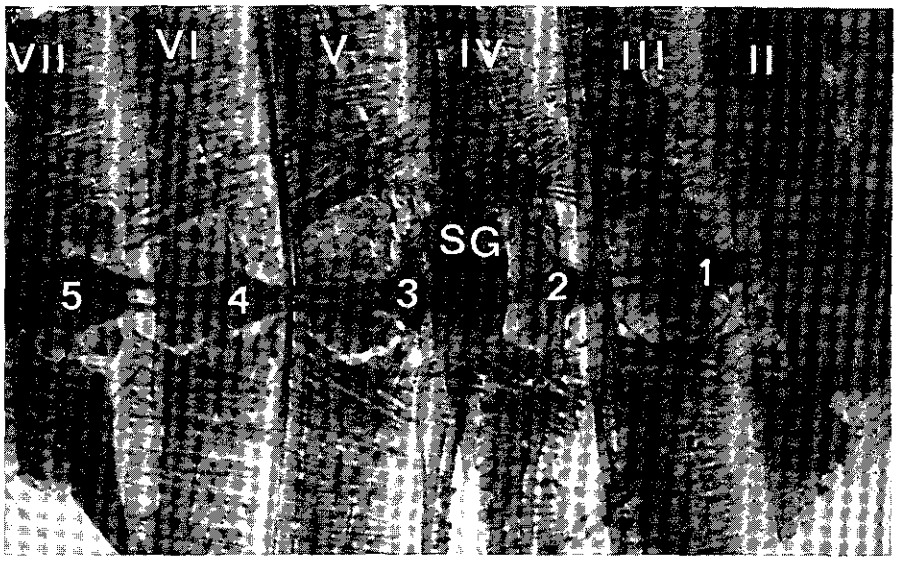

Fig. 6. Abdominal sternites (II–VII) of Macrotermes michaelseni showing the location of sternal gland (SG) and ganglia of ventral nerve cord (1–5).

workers but not in major and minor soldiers which have a darker and perhaps heavier cuticle. The sternal gland is a rectangular white mass of tissue located in the anterior part of the fifth abdominal sternite and between the third and fourth abdominal ganglia (Fig. 6). The anterior part of fifth abdominal sternite including the sternal gland is overlapped by the posterior part of the fourth sternite. The dimensions, length, width and area, of the sternal gland in the various castes are shown in Table 1. The length and width of the gland was measured at the widest points and then the area was calculated by multiplying length by width. A total of 30 specimens of each caste was measured. There was no relationship between gland size and potential trail activity in the various castes, for example, the gland in major and minor soldiers is much larger than would be expected based on their trail laying potential. The potential trail activity in the various castes will be discussed in more detail later.

Source of trail pheromone

The sternal gland is apparently the only source of trail pheromone in major workers (Table 2) since only the glandular extracts provided consistent trail following ($P = 0.005$) by major workers. The 5 μl of crude extract spread over 5 cm distance was equal to 0.125 worker-equivalents. The results of the three replicates and two tests of each replicate were pooled because there was no difference between them.

Table 1. Dimensions of the sternal gland in various castes of *Macrotermes michaelseni*

Caste	n	Dimensions (μm $\pm$ SE) Length	Width	Area
MW	30	172 $\pm$ 4	323 $\pm$ 6	55,556 $\pm$ 1851
mW	30	121 $\pm$ 4	250 $\pm$ 0	30,250 $\pm$ 1476
mS	30	87 $\pm$ 2	222 $\pm$ 3	19,314 $\pm$ 673
MS	30	135 $\pm$ 3	321 $\pm$ 7	43,335 $\pm$ 1490

MW, major worker; mW, minor worker; mS, minor soldier; MS, major soldier.

The behavior of the workers during the tests was an important part of the bioassay. Disturbed workers which travelled quickly through the maze with antennae very high were discarded. In most bioassays the workers moved slowly along the foraging path and paused a few seconds with a great amount of antennal waving before proceeding slowly along one or the other pathway. As the worker moved along the pathway, it frequently palpated the surface with its antennae and followed the line containing the crude trail pheromone extract with no deviation. Some workers proceeded a few mm in one direction before stopping, turning back, and then choosing the other pathway which usually contained the active extract. In the tests of extracts from whole worker minus sternal gland against hexane, the workers stopped before proceeding very slowly along either pathway. In many cases workers changed paths at the second choice, thus giving bioassays with opposite signs, this shows that there is no difference between the two choices.

Potential trail activity in various castes

The trail laying potential in the various castes (TU) is shown in Table 3. There was no correlation between gland size and trail pheromone potential.

Table 2. Number of positive responses of *Macrotermes michaelseni* major workers to extracts of sternal gland and whole worker minus sternal gland.

	Number of bioassays	Number positive	Level of significance
Sternal gland vs hexane	120	120	$P < 0.005$
Sternal gland vs whole worker minus gland	120	115	$P < 0.005$
Whole worker minus gland vs hexane	120	64	NS

Table 3. Number of trail units (TU) or potential trail pheromone activity in various castes of *Macrotermes michaelseni*

Caste	No. of extracts	TU
Major worker	10	186
Minor worker	8	100
Minor soldier	6	40
Major soldier	5	25
Male de-alate	1	80
Female de-alate	1	79

Crude extracts from major workers stored at 16°C for one month and frequently assayed did not lose activity. Crude extract stored in the laboratory for one week also retained its activity. In these tests the bioassay-worker came from one mound while the extract-worker came from a different mound. Sometimes this changed the behavior of the termites during the bioassay; workers which first contacted the figure-8 maze containing the extract very quickly retreated, then advanced slowly with much antennal motion along the path containing the extract.

Trail laying behavior

Trail laying behavior of major workers in the laboratory is conveniently divided into three categories related to the types of trails laid:

HT—head, thorax and abdomen low and parallel to substrate (Fig. 7a), occassionally a distention of the sternal gland area of the abdomen; numerous and heavy abdominal hair marks on sooted-slides (Fig. 7b).

LT—body at a slight angle with head and thorax elevated, and posterior abdomen lower than anterior; one-half to three-fourths of abdomen low and close to substrate (Fig. 7c); the abdomen of some workers was alternately raised and lowered, with some distention of the sternal gland area; light and/or intermittent abdominal hair marks on sooted slides (Fig. 7d).

NT—body raised at steep angle with head and thorax much higher than rest of body; abdomen not touching substrate (Fig. 7e); no abdominal hair marks on sooted-slides (Fig. 7f).

The first termites leaving the nest moved very slowly with the whole body low and close to the substrate. The head moved from side to side and the antennae rapidly palpated the substrate. Each worker advanced along the trail a bit further than its predecessor before it stopped and returned to the culture. This behavior continued until the termites reached the arena after which they began to move more quickly between culture and arena without hesitation even though the body of termites is still low and close to the substrate. It is assumed that these first termites were laying strong trails (behavior HT) to establish communication between culture and arena. Behavior HT was the most common in workers leaving the culture (Table 4), but the number slowly decreased and the number in behavior LT increased during the observations. Very few workers leaving the culture were observed in behavior type NT (no trails). Analysis of ciné films shows that disturbed workers which moved very fast, from culture or arena, held the abdomen very high and did not lay trails.

The majority of workers leaving the arena showed behavior type LT and were assumed to be laying light trails (Table 5) even during the establishment of the trail when workers leaving the culture were still laying strong trails. Very few workers leaving the arena were observed to lay strong strails. There is some evidence from the ciné films, but not supported on the sooted-slides that behavior LT of workers leaving the arena is different from the behavior of workers leaving the culture. Workers leaving the arena raise and lower the abdomen more frequently and may lay lighter trails than workers leaving the culture. The number of termites leaving the arena which lay no trails is very high. The presence or absence of food in the foraging arena seemed to have no influence on trail laying behavior and there is no evidence for recruitment trails under the experimental conditions.

When a termite loses contact with a trail, it moves quickly in circles with antennae rapidly palpating the substrate and then follows without hesitation in either direction when the trail is located again.

Longevity of major worker trails

The preliminary experiments showed that major workers lay trails when travelling from culture or from arena. When the trails were reversed they were followed without hesitation, which indicates that they are probably non-directional. In the longevity studies all trails, from culture or from arena, were bioassayed with workers leaving the culture.

Tables 6 and 7 show that the trails of a single major worker leaving the culture remained attractive to a significant number of workers for 1 hr 30 min but those laid by workers leaving the arena were attractive for only 1 hr. Accumulated trails of five major workers (Tables 6 and 7) remained attractive slightly longer, those from workers leaving the culture 1 hr 45 min and from workers leaving the arena only 1 hr 15 min. The trails of workers leaving the culture, whether one worker or five workers, were stronger than those of workers leaving the arena (Figs 8 and 9). Well-established trails (Table 6) remained attractive to a significant number of workers for 9 hr. All trails lost activity at a constant rate (Figs 8, 9 and 10).

DISCUSSION

The sternal gland is located in the fifth sternite in all castes including newly-swarmed de-alates of *M. michaelseni* as it is in all the higher termites so far reported (Noirot and Noirot-Timothée, 1965; Quennedey, 1977; Luethold and Lüscher, 1974). A size-polymorphism was likewise evident in the gland, i.e. *Reticulitermes lucifugus* (Mosconi-Bernardini and Vecchi, 1964), *R. santonensis* (Quennedey, 1977), but not to the extent found in *Trinervitermes bettonianus* where in newly-swarmed de-alate females, have an extremely large sternal gland (Leuthold and Lüscher, 1974).

In this species there is no relationship in gland size and potential trial pheromone activity. The area of the gland in minor workers is 1.4 times smaller than major soldiers but they have four times the potential trail activity (Table 3). In major workers, the area of the gland is 1.3 times that of major soldiers and yet they have 7.5 times the potential trail activity. Major

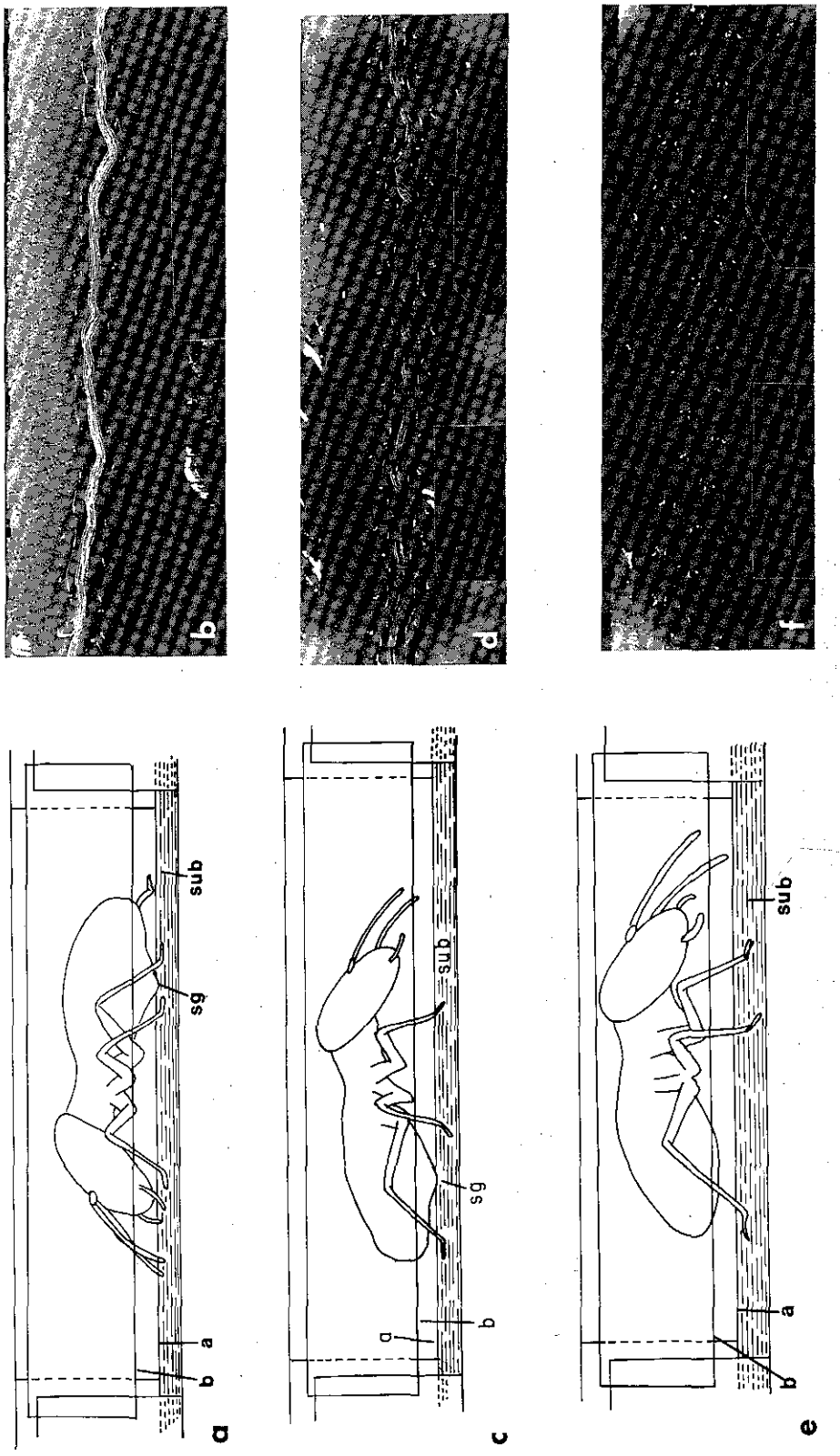

Fig. 7. Diagrams from 16 mm film of major workers during trail laying and their tracks on sooted-slides. Gallery wall *a* touching substrate (sub) and *b* 1 mm above substrate. SG = sternal gland. (a) Trail laying behavior HT, worker going from culture to arena within 3 min after start of foraging. (b) Tracks on sooted-slide of worker leaving culture 5 min after start of foraging. (c) Trail laying behavior LT, worker going from culture to arena 2 hr after start of foraging. (d) Tracks on sooted-slide of worker going from arena to culture 30 min after start of foraging. (e) Trail laying behavior NT, worker going from arena to culture 1 hr after start of foraging. (f) Tracks on sooted-slide of worker going from arena to culture 30 min after start of foraging.

Table 4. Per cent major workers in each trail laying behavior type at various times after start of foraging

Time (min)	Trail laying behavior types HT	LT	NT	Total workers
0	98.5	1.5	0	69
30	92.5	7.5	0	54
50	85.9	12.5	1.6	64
90	85.7	14.3	0	21
120	84.6	15.4	0	39
150	73.9	26.1	0	23
300	60.4	39.6	0	48
1440	69.7	30.3	0	33

Traffic from culture to foraging arena only. HT, heavy trail; LT, light trail; NT, no trail.

workers also have higher potential trail activity than newly-swarmed de-alates. In *T. bettonianus* the relationship between gland size and potential trail pheromone activity is striking. The volume of sternal gland in de-alated females is 65 times larger than in workers, but they have 1200 times the potential trail pheromone activity, while major workers have 40 times the potential of minor soldiers whose gland is much smaller (Leuthold and Lüscher, 1974; Quennedey and Leuthold, 1978).

Stuart (1963a) found that in *Zootermopsis nevadensis* the fourth sternite, which overlaps the sternal gland area of the fifth sternite, is much larger than the other sternites, and it acts as a reservoir for the trail pheromone. The fourth sternite in *M. michaelseni* is similar in all aspects including setal pattern to the other abdominal sternites.

The use of the sternal gland and potential trial pheromone activity in major soldiers is unclear since these termites are not actively involved in trail laying, foraging, or nest repair. It was observed that the majority of termites in mound repair details were major workers and minor soldiers, but as repairs continued a few major soldiers and minor workers appeared. Stuart (1963a, b) suggested that *Z. nevadensis* soldiers lay trails to and from repair sites to recruit workers for repair and soldiers for defense.

The assays with whole body and glandular extracts demonstrate that the sternal gland is apparently the only source of trail pheromone in *M. michaelseni* as it is assumed to be in all termites. However, it has been demonstrated only in *Z. nevadensis* (Stuart, 1963a), *T. trinervoides* (Tschinkel and Close, 1973) and *T. bettonianus* (Leuthold and Lüscher, 1974).

The crude trail pheromone extract of whole termites in this species is very stable, retains the same activity for one month when stored at 16°C and for 1 week when stored at room temperature. Very little is known about termite trail pheromone extracts but in *T. bettonianus* nearly 50% of the trail activity is lost from hexane extracts, in less than 1 week when stored at 16°C (Oloo, personal communication). The chemical nature of the trail pheromone of this species is unknown and has been partially characterized in very few species, *Nasutitermes exitosus* (Moore, 1966), *Z. nevadensis* (Hümmel and Karlson, 1968), *Reticulitermes virginicus* (Matsumura *et al.*, 1968, 1969; Tai *et al.*, 1969).

The first worker leaving the culture moves very slowly with abdomen close to the substrate, but traffic speeds up as the strength of the trail increases. Similar behavior has been observed in *T. trinervoides* and *Hodotermes mossambicus* (Hewitt *et al.*, 1969). In some workers the sternal gland is distended during trail laying, but it could not be determined even from the films if this occurs only when stronger trails are being laid or all the time. The distention of the sternal gland during trail laying has been observed in *H. mossambicus* (Hewitt *et al.*, 1969) and *Odontotermes* sp. (unpublished observations).

The presence or absence of food in the foraging arena in these experiments apparently did not stimulate the laying of recruitment trails. Recruitment

Table 5. Per cent major workers in each trail laying behavior type at various times after start of foraging

Time (min)	Trail laying behavior types HT	LT	NT	Total workers
0	7.0	61.4	31.6	57
30	6.8	56.8	36.4	44
60	12.5	55.0	32.5	40
90	19.2	73.1	7.7	26
120	7.4	74.1	18.5	27
150	11.8	64.7	23.5	34
300	15.2	54.5	30.3	33
1440	11.8	50.0	38.2	34

Traffic from foraging arena to culture only. HT, heavy trail; LT, light trail; NT, no trail.

Table 6. Longevity of trails from various number (1, 5, many) of major workers

Time	No. of trails	No. positive	Significance level
	1 worker trails		
60–75 min	56	40	$P < 0.01$
75–90 min	48	31	$0.01 < P > 0.05$
90–105 min	40	24	NS
105–120 min	50	31	NS
	5 worker trails		
75–90 min	40	29	$P < 0.01$
90–105 min	70	46	$P < 0.01$
105–120 min	45	27	NS
120–135 min	61	36	NS
	Well-established trails		
8 hr	45	37	$P < 0.01$
9 hr	36	27	$P < 0.01$
10 hr	23	10	NS
11 hr	24	12	NS

One- and five-worker trails by workers *travelling from culture to arena*, well-established trails by many workers *travelling in both directions*.

Table 7. Longevity of trails made by major workers (1 and 5) travelling from arena to culture in the laboratory

Time	No. of trails	No. positive	Significance level
	1 worker trails		
30–45 min	47	34	$P < 0.01$
45–60 min	41	30	$P < 0.01$
60–75 min	39	23	NS
75–90 min	49	30	NS
	5 worker trails		
45–60 min	48	37	$P < 0.01$
60–75 min	41	30	$P < 0.01$
75–90 min	39	23	NS
90–105 min	50	28	NS

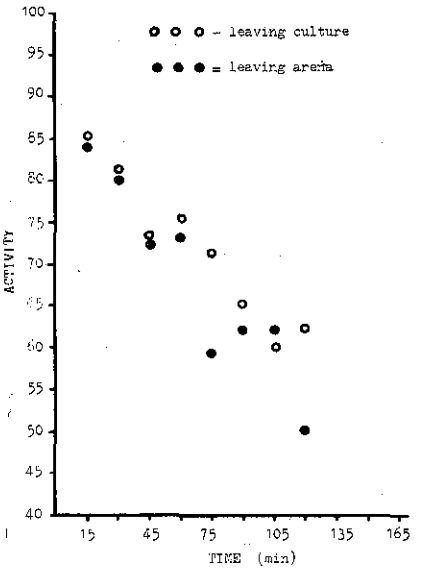

Fig. 8. Decay in attractiveness of trails of single workers travelling between culture and arena in the laboratory. Activity = percentage of workers following trails. Each point based on 1 bioassay of 40–70 individual trails.

trails have recently been demonstrated in termites for the first time by Oloo and Leuthold (1979) in *T. bettonianus*. The study on trail laying behavior of this termite showed that a high percentage of workers leaving the culture lay heavy trails for the first 30 min thereby establishing communication with the foraging arena. After this time a reducing percentage lay heavy trails for the duration of the observations and an increasing percentage lay light trails to maintain

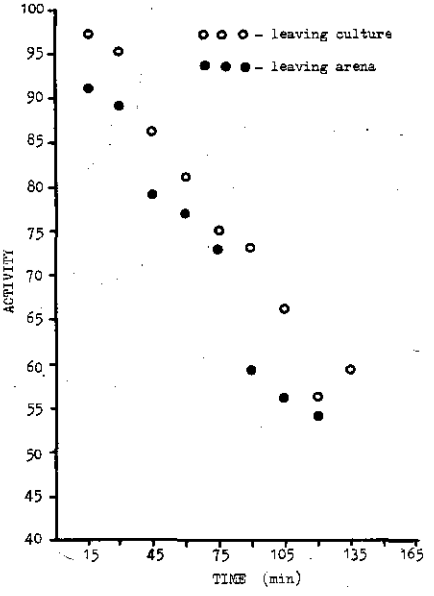

Fig. 9. Decay in attractiveness of trails of five major workers travelling between culture and arena in the laboratory. Activity = percentage of workers following trails. Each point based on one bioassay of 40–70 individual trails.

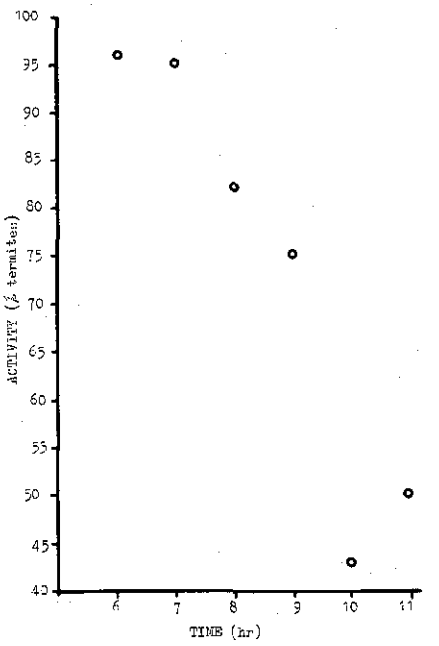

Fig. 10. Decay in attractiveness of well-established trail of major workers travelling between culture and arena in the laboratory. Well-established trails obtained by allowing 15 workers per min to walk over the paper for 2 hr. Activity = percentage of workers following trails. Each point based on one bioassay of 25–45 individual trails.

the trail at some steady state level. Tschinkel and Close (1973) suggested that *T. trinervoides* workers continuously reinforce the trails which never reach a steady state. Trails of *H. mossambicus* are also continuously reinforced by successive workers but to a lesser degree than those of *T. trinervoides* (Hewitt *et al.*, 1969).

The experiments also show that major workers lay trails which are followed by other workers as well as other castes. It has been assumed that all termites lay trails which are followed by their nest-mates but definite proof exists for only a few species: *Z. nevadensis* (Lüscher and Müller, 1960; Stuart, 1963a); *Nasutitermes corniger* (Stuart, 1963a); *T. trinervoides* (Hewitt *et al.*, 1969); *T. bettonianus* (Oloo and Leuthold, 1979). Lack of direction also has been shown for *T. trinervoides* (Tschinkel and Close, 1973).

Trails of major workers are very stable and can remain effective for up to 9 hr (well-established). Natural trails of *T. trinervoides* remain effective for 6 min when laid on glass, 15 min on damp soil and 25 min on paper, while those of *H. mossambicus* laid on paper remain effective for 90 min (Hewitt *et al.*, 1969). Trails of *T. bettonianus* laid on paper on the other hand remain effective for approx. 1 min (Oloo, personal communication).

The study presents some of the characteristics of trails laid by major workers of *M. michaelseni* in the laboratory as well as how these trails are established. Experiments are needed to determine the longevity of trails on different soil types at different moisture contents as well as in closed containers to simulate more closely conditions in the natural habitat of this termite.

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