

THE TRAIL PHEROMONE OF THE TERMITE, *TRINERVITERMES TRINERVOIDES*

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Abstract—By using a T-junction choice assay workers of *Trinervitermes trinervoides* have been shown to lay and follow pheromone trails. The trials are not polarized and there is a quantitative relation between the number of termites laying the trail and the fraction of test termites which follow correctly. Workers reinforce trails whenever they follow them, and the degree of this reinforcement seems to be independent of trail strength. The trail pheromone is volatile and need not be perceived by contact chemoreception. Activity loss from filter paper is approximately exponential with a half-life of about 2 hr. Extracts of papers over which workers had laid trails were used to show a linear relationship, on a log-probit scale, between dose and response. Soldier termites also follow trails about as well as do workers, but workers lay trails that are about six to seven times as strong as soldier trails. No preference of either caste for their own trails could be demonstrated. Thin-layer chromatography indicated that the trail pheromone(s) is a fairly polar substance.

INTRODUCTION

ALTHOUGH it has been known for some time that a number of termite species use trail pheromones for orientation toward food or sources of disturbance (LÜSCHER and MÜLLER, 1960; STUART, 1961, 1963a, b), the detailed properties and functioning of these pheromone systems have not been well worked out, despite reports of the isolation of several of the chemicals responsible (MOORE, 1966; HUMMEL and KARLSON, 1968; MATSUMURA *et al.*, 1968). The trail substance is produced, at least in *Kaloterms*, *Zootermopsis*, and *Nasutitermes*, in the sternal gland, and the ubiquity of this gland among termites has led to the postulate that most, if not all, termites secrete a trail pheromone in this gland (STUART, 1963a). Nevertheless, few termite species have actually been characterized with respect to trail-laying and following behaviour. In the present study, we have partially characterized the trail pheromone system of the termite *Trinervitermes trinervoides* with respect to trail-laying, trail-following, responsive castes, and physical properties of the pheromone. We have also demonstrated for the first time in a direct fashion that trail pheromone is actively deposited by the termites.

MATERIALS AND METHODS

Colonies of *T. trinervoides* were collected near Grahamstown, Cape Province, South Africa, and housed in circular glass nests constructed of 15 × 2 cm Petri

dishes with Perspex tops, similar in design to those used by WILSON (1962). Two such nests containing only adult workers were connected by plastic tubes to a Perspex experimental chamber ($15 \times 15 \times 3$ cm). (Hereafter the word termite refers only to adult workers unless otherwise stated.) Two plastic strips restricted the path of the termites through the experimental chamber to a straight line on a piece of chromatograph paper between the entrances. By placing clean paper in the chamber and allowing a certain number of termites to cross, trails laid by varying numbers of workers could be obtained. When experiments were not in progress, the paper substrates were replaced regularly and any organic substance deposited on them was extracted in diethyl ether. This extract was later used to lay artificial trails.

To minimize orientation to light, the only light source hung 30 cm over the centre of the experimental chamber and was kept permanently on. The nests and experimental chamber were kept on a flat surface on polyurethane foam to minimize vibrations. A fairly constant procession of termites was maintained by placing dry grass in one chamber and keeping the humidity of the other high. The termites then foraged in the drier chamber and formed their 'nest' in the moister one. All experiments were performed at 23°C.

EXPERIMENTS AND RESULTS

Preliminary experiments

Termites from both sides were allowed to cross the restricted path over the chromatograph paper strip. After 10 to 15 min of continuous use, the tubes to the chamber were closed and the chamber was aspirated clear of termites. The restricting plastic strips were removed, the trail was marked lightly in pencil, and the paper substrate cut and arranged into a 'T' junction. Junctions thus made, by placing paper strips together, did not interfere with trail following. Controls showed that pencil lines did not facilitate trail following in any way. The T junctions were made in the centre of the square experimental chamber in order to minimize orientation, if any, to its sides. Different termites were used to test such T trails for activity and were allowed to encounter the test trail one at a time. A termite was judged to have completed the *t* test successfully when it was able to follow the trail to the end of either one of the arms of the T.

Four such termite-crossed papers were tested for trail activity, three with 10 workers and one with 20. Of the 50 test termites 46 completed the trail correctly, 21 proceeding to one side of the T and 25 to the other. Thus, there is a great probability that termites will follow the same path that previous termites have taken.

Trail strength, reinforcement, and polarity

The tube entering the experimental chamber from the nest chamber was disconnected, and only termites from the foraging chamber were allowed to cross the restricted path over the clean chromatograph paper substrate. The number doing so was controlled and assumed to be the number that laid the trail.

The first few termites were reluctant to cross 15 cm of trailless substrate, and the paper strips were thus shortened to 8 cm. After a trail had been laid on them, these were cut in half and arranged in a T junction in the centre of the experimental chamber. Paper strips containing an active trail led from the chamber entrance to the T trail. Ten test termites from the 'nest' chamber were used to assess the strength of the experimental trail.

Trail activity or strength increases as a function of the number of termites laying the trail (Fig. 1) and Student's *t*-test (the large samples made the *t*-test reasonably valid) showed that the trails laid by successively increasing numbers of termites were significantly different ($P < 0.01$) in all cases except the trails laid by 2 and 3 termites (P between 0.1 and 0.05).

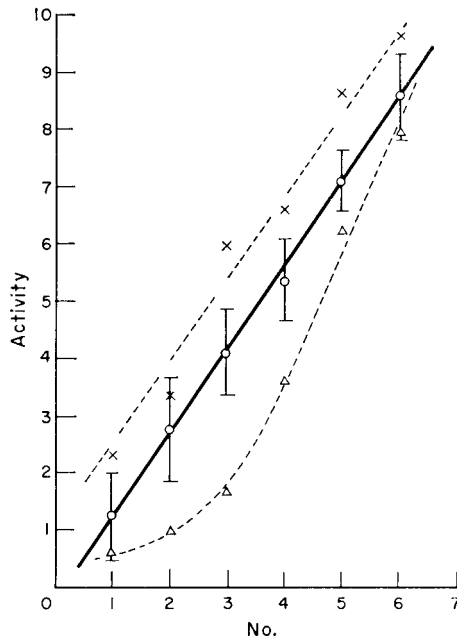


FIG. 1. Trail-following activity as a function of the number of termites laying the trail. Activity is given as the number of 10 test termites which followed the trail correctly. Open circles indicate the mean of 10 trials and the vertical bars ± 2 standard errors. The lower dashed line indicates correct following by the first 3 test termites, the upper dotted line by the last three.

The arm of the T taken by successful followers was noted (A or B). If the termites took side B, they would have proceeded in the same direction as the termites laying the trail, and therefore imply polarization of the trail. However, there is no significant difference between the choice of sides (Table 1) and it is unlikely that the trail is polarized.

The number of test termites within *each experiment* proceeding to the same side of the T junction as the first successful test termite is significantly higher than

those proceeding to the opposite side ($P < 0.005$ by χ^2 test) showing that trail-reinforcement by the test termites must be occurring. Similarly, in Fig. 1, the curve showing correct following by the first 3 termites in each test group of 10 is markedly lower than the curve for the last three. Again, trail-reinforcement is indicated.

TABLE 1—POLARITY AND REINFORCEMENT OF TERMITE TRAILS

Type and No. of trails tested	Choice with respect to first test termite		Choice of absolute direction	
	No. proceeding to same side	No. proceeding to opposite side	No. to side A	No. to side B
30 termite generated trails, each tested on 10 termites	218	75	150	149
	$P < 0.005$		N.S.	
100 extract generated trails, each tested on 10 termites	336	114	233	234
	$P < 0.005$		N.S.	

The test termites were presented with test trails in the form of T junctions, and of those successfully following the trail, the number proceeding to the same side as the first test termite or to the opposite side was scored, as well as the number proceeding to side A or B (i.e. right or left).

Trail extract experiments and the dose-response curve

The extract of trail-containing papers was concentrated to 8 ml and 8 cm trails were made using different volumes of extract, thus producing trails of different strengths. The trails were tested as in the previous experiments and the results plotted on a log-probit scale (Fig. 2). Again, the proportion that proceeded to the same side of the T junction as taken by the first test termite indicated that the test termites were reinforcing the trail. In addition, mean correct following by the first 3 termites in each test group is lower than the overall average. Again, no trail polarization was indicated.

The Student *t*-test indicated that the difference between the 10 and 15 μ l doses and the 20 and 25 μ l doses, respectively, were significant ($P < 0.05$). The others were not significant at the 0.1 to 0.05 level, but all differences between alternate doses are significant.

The control extract consisted of blank filter papers in ether and was concentrated to 2 ml after storage at -20°C for the same period as the trail extract. This control extract elicited no trail following and it is apparent that trail-following is due to a substance extracted from trail-bearing papers.

In Fig. 3, the number of termites laying a trail is plotted against the volume of extract required to yield equal activity in the assay (equipotent volume). When volumes larger than about 25 μ l are used, the extract becomes relatively less effective than the live termites and the equipotency curve deviated from the theoretical linear relationship. It seems likely that this is due to the difficulty of

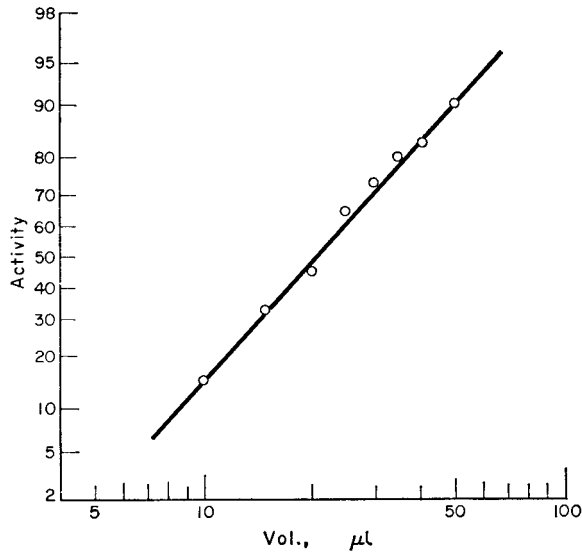


FIG. 2. Trail-following activity as a function of the volume of trail extract used to generate the trail. Activity is given as a percentage correct following, and the data are plotted on log-probit scales.

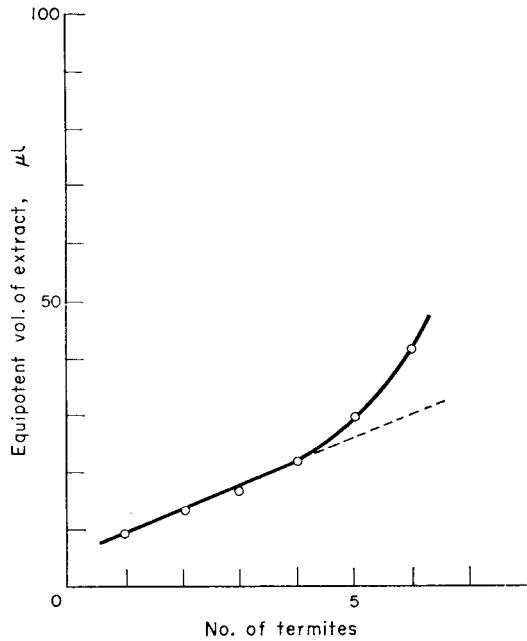


FIG. 3. Equipotency of termite- and extract-generated trails. The number of termites laying a trail is plotted against the volume (in μl) of extract causing the same level of correct following.

laying very narrow trails with large volumes of extract, so that the extract becomes less effective due to lateral spreading. This hypothesis was, however, not further tested.

Rate of trail-activity loss

Two millilitres of a strong trail extract (eliciting correct following by 44 out of 50 termites in a 10 μ l, 8 cm trail) was prepared. The strength of these trails was probably equivalent to about 50 μ l in the dose-response curve (Fig. 2). Five replicate trails were tested for each of ten elapsed-times. Thus, 10 sets of 5 separate trails were made, each set of 5 within a period of 5 min, and the time half-way through making each set was taken as the initial time. For example, if 5 trails were laid between 8.35 and 8.40 a.m., the initial time was 8.38 a.m. The activity of each set of 5 was tested during a 25 min period some time after the start of the experiment and the elapsed trail-life of a set was the difference between the initial time and the time half-way through the testing period. The means of each elapsed time are plotted on a semi-logarithmic scale in Fig. 4.

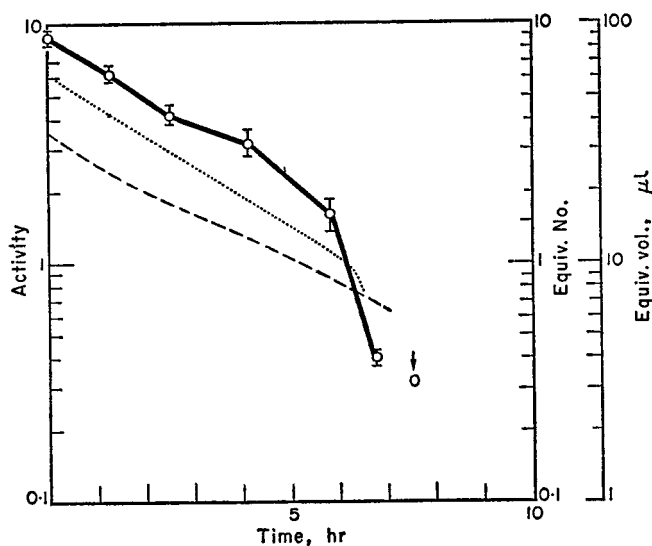


FIG. 4. The loss of trail-following activity from extract-generated trails (solid line and open circles). The vertical lines indicate ± 2 standard errors from the mean. The arrow and 0 indicate a response of zero. The dotted line gives the calculated number of termites required to lay an equipotent trail. The dashed line gives the calculated volume of trail-extract needed to make a trail of equal strength. Both lines are estimates of the mass of pheromone as a function of time. See text for explanation.

The trail retained detectable activity up to 7.5 hr, and the half-life of the trail appears to be approximately 2 to 2.5 hr.

Trail-laying and following by soldiers

It was difficult to induce soldiers to cross a clean experimental strip, and the apparatus used for the workers was not suitable. Thus, approximately 100 soldiers were transferred to a darkened circular container 3.5 cm in diameter. A small opening (0.5×0.5 cm) led into a narrow passage limited on either side by strips of plastic and with clean paper strips as a substrate. A drop gate assured that the number of soldiers proceeding along the passage could be controlled. The fact that soldiers in a dark container tend to move toward the light was used to induce them to leave their container and walk down the passage. The number doing so was postulated to be the number laying the trail and the strength of these trails was compared to worker trails as follows.

T junction trails were made with one arm of the T junction containing a trail by a number of soldiers while the other arm contained a trail laid by 6 workers. The relative strength of the trails on the two arms was then tested using 10 workers. Table 2 shows that trails laid by 40 soldiers are approximately equal to trails laid by

TABLE 2—RELATIVE STRENGTH OF WORKER AND SOLDIER TRAILS

Test No.	Trail choice tested	No. ($\pm$ S.E.) per 10 choosing	
		Worker trail	Soldier trail
1	6W : 6S	7.6 ± 0.51	0.0
2	6W : 16S	7.8 ± 0.52	0.20 ± 0.19
3	6W : 25S	6.4 ± 0.68	1.0 ± 0.45
4	6W : 40S	4.4 ± 1.1	3.2 ± 0.74

In each replicate, 10 workers were allowed individually to choose between a 6-worker trail on one arm of the T junction and a soldier trail of a particular strength on the other. Each experiment was replicated five times. W, Workers; S, soldiers.

6 workers. As will be shown below, these results do not reflect any preference by either workers or soldiers for their own trails, but show the relative strength of the trails laid by the two castes. The trails of soldiers and workers in tests 1 to 3 are all significantly different and show the extent to which the workers' trails were preferred, while the trails of test 4 are not significantly different and are of approximately the same strength. The large standard errors in test 4 are probably attributable to reinforcement, by subsequent test termites, of the random first choice made under conditions of approximate equality of trail strength. Thus a majority of termites proceed along one arm of the T test during any one experiment, while during the next experiment the opposite arm may be favoured.

Since soldiers' prime function is colony defence, it seemed possible that they need to be alarmed before laying a strong trail. Perhaps soldiers only lay trails for recruitment of a 'defence force'. STUART (1969) indicates that *Zootermopsis* lay

trails to the sites of disturbance. The experimental soldiers were thus alarmed by breathing into the entrance of their darkened container, and the soldiers proceeded hastily out, many of them walking the length of the experimental strip. The activity of such trails laid by alarmed soldiers was tested in 5 replicates (using 10 workers each) against trails by unalarmed soldiers, each trail again forming the arm of a T test. A mean of 3.6 ± 0.74 chose the trail laid by alarmed soldiers, while 2.8 ± 0.82 chose the unalarmed. The difference is not significant, indicating that alarmed soldiers do not lay stronger trails.

Possibly, soldiers may prefer their own trails to those of workers. Thus, soldier trails were tested with both soldiers and workers (Fig. 5). Although there was no significant difference between the effectiveness with which soldiers and workers follow soldier trails of each strength, the consistently higher values for workers in Fig. 5 nevertheless suggests that workers follow soldier trails better than soldiers do. It is likely that this is due to greater reinforcement by workers than by soldiers.

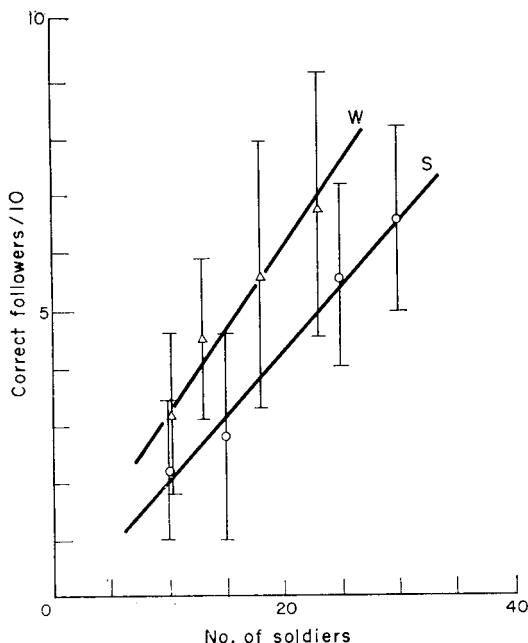


FIG. 5. The response of workers (Δ) and soldiers ($\circ$) to soldier-trails of various strengths. The means ± 2 standard errors of 5 replicates are indicated. W, Workers; S, soldiers.

The reverse experiment was also carried out to see if soldiers followed worker trails as well as workers do. The trails were produced with a diethyl ether extract of worker trail substance. The results (Table 3) indicate that workers follow worker trails significantly better than do soldiers at both the 10 and 20 μ l level. Most of

this difference probably resides in the fact that workers reinforce trails to a greater extent than soldiers do, for the differences between worker and soldier following are about equal to the difference between the first three followers in Fig. 1 and the last three. The test scores thus do not really reflect a difference in trail following ability, but only in tendency to add to the trail.

TABLE 3—THE RESPONSE OF SOLDIERS AND WORKERS TO WORKER-TRAILS OF TWO STRENGTHS

Correct followers per 10 ($\pm$ S.E.)					
10 μ l trails		20 μ l trails		40 μ l trails	
Workers	Soldiers	Workers	Soldiers	Workers	Soldiers
4.8 $\pm$ 0.33	3.2 $\pm$ 0.42	8.4 $\pm$ 0.50	7.0 $\pm$ 0.33	—	8.0 $\pm$ 0.48
$P < 0.001$		$P < 0.005$			

Trails were made using 10, 20, and 40 μ l of extract and tested both on workers and soldiers. Each experiment was replicated five times.

It is interesting that the standard errors obtained when assaying soldier trails are generally higher than those obtained with worker trails, irrespective of whether soldiers or workers are being used as test termites. This suggests that trails laid by soldiers are not only weaker, but more variable in strength as well.

Under natural conditions, soldiers periodically make brief forays from the edge of the foraging column and, as suggested by HEWITT *et al.* (1969), their trails may only serve to guide them back to the main worker trail. Furthermore, the weakness of soldier trails may ensure that workers are not side-tracked from the main foraging trail. Finally, it is possible that a strong trail-laying response may only be evoked in soldiers after adequate stimulus, and that such a stimulus may have been lacking during these experiments.

Degree of trail reinforcement

Reinforcement has been established above, but the following question is still open: Do termites continue reinforcing the trail indefinitely or do they decrease or stop reinforcement after the trail has reached a certain strength?

Some termites are known to produce trail substance in a sternal gland (STUART, 1963a; MOORE, 1966; NOIROT, 1969). Initially, visual observation was used to determine whether termites depositing trail substance pressed their abdomens against the substrate. The first 3 to 5 termites crossing a clean substrate did so slowly, and appeared to drag their abdomens, lifting them off the substrate at irregular intervals. But subsequent termites crossed rapidly and accurate observation of their abdomens was not possible.

It was thought that if termites did not reinforce trails above a certain strength, they would refrain from laying a trail on fine nylon gauze suspended just above a

very strong trail. It was first established that termites could, in fact, follow unhampered, a strong trail while suspended above it on nylon gauze, indicating that the trail is volatile and not detected only by contact chemoreception. When the gauze was more than 2.5 mm above the trail, the termites tried to get through it to the trail. Therefore, these experiments were carried out at a height of 1.5 mm above the trail. Six termites were allowed to cross the gauze following the trail, and then the trail beneath was removed and the gauze tested for trail activity. Ten worker termites were again used for each test and it was apparent that there was a significant difference between the strength of a 6-termite trail in Fig. 1 and a 6-termite trail laid on gauze while following a strong trail beneath. These results indicate that termites continually lay down trail substance, but apply less on a strong trail than on a weak one.

But it was possible that the termites on the gauze sensed they were not on the actual trail and may have responded by actively laying down trail substance, and that the reason for the lower activity was the perforated nature of the gauze. For this reason, the times required for trails laid by varying numbers of workers to completely lose all activity were determined. If termites cease reinforcing trails which have reached a certain strength, the time taken for a very strong trail to lose all activity should be independent of the number of workers laying that trail. Trails laid by 6, 12, 18, 50, 100, and approximately 500 termites were produced on chromatography paper and were tested for activity at various intervals. All activity was considered lost when only 1 out of 5 test termites could follow the trail for a distance of 4 cm. The results (Fig. 6) indicate that the fade-out time is dependent on the number of termites laying the trail up to 500 termites. Like the previous experiments, these results also indicate that the termites continue to reinforce even strong trails. But to draw the conclusion from Fig. 6 that the amount of reinforcement is or is not constant with increasing trail strength requires knowledge of the kinetics of pheromone loss from the filter paper substrate: (a) If trail pheromone is lost by desorption from the fibre surface, the decline would be exponential, as would the time required for trails of varying strengths to reach a given end point (as in this experiment). Under these conditions, the above result (Fig. 6) would indicate a constant increment of reinforcement independent of trail strength. (b) If evaporation from a free surface is assumed, or initial evaporation from a free surface followed by desorption, then Fig. 6 shows that the rate of reinforcement decreases as the trail strength increases.

In theory at least, the type of evaporative loss from paper should be accessible from the present experiments. We have established that for relatively weak, termite-generated trails (up to about 60 per cent following), each termite contributed about the same amount of material to the trail. This conclusion follows from the linear relation between the number of termites and an equipotent volume of extract (Fig. 3). It is also possible to calculate an equivalent volume of extract for any given response in an assay. Thus, for any point on the evaporation curve (Fig. 4), it is possible to calculate (a) the number of termites required to lay a trail of equal strength and (b) the volume of extract needed to lay a trail of equal

strength. Both (a) and (b) are directly related to the actual mass of pheromone in the trail and thus give an estimate of the mass of pheromone in the trail (the two lower lines in Fig. 4) at any time. Fig. 4 indicates that the loss of pheromone from the trail is approximately exponential, supporting the hypothesis that the increment of trail reinforcement stays constant, no matter what the strength of the trail. However, when the pheromone-loss data are plotted on a linear scale, the deviation from linearity is not very great. Considering the indirect nature of the derivation of the pheromone-loss curve and its resultant uncertainty, we draw the conclusion of constant-increment reinforcement very tentatively and the question is by no means closed.

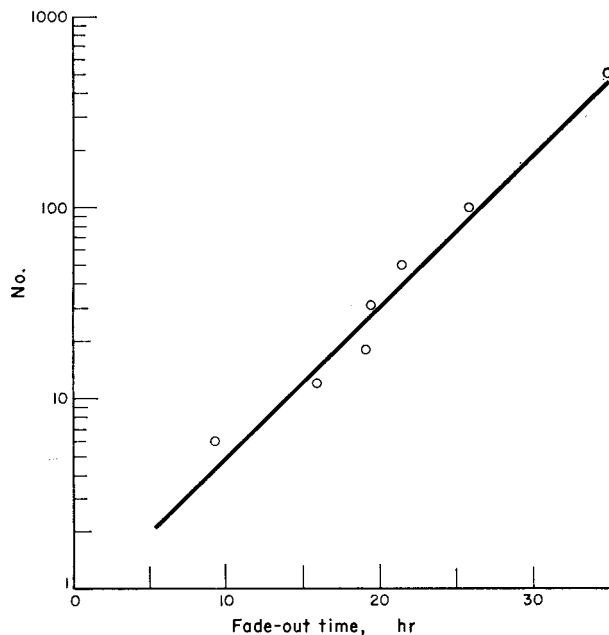


FIG. 6. Fade-out time of termite-generated trails. The time required for the trail to lose all activity (only 1 out of 5 able to follow) is plotted against the number of termites laying the trail.

Origin and detection of trail

The trail pheromone of the family Termitidae (which contains the Nasutitermitinae) is produced in a sternal gland near the anterior portion of the fifth abdominal sternite (STUART, 1961; NOIROT, 1969). Using the technique of HEWITT *et al.* (1969), termites were anaesthetized with carbon dioxide and clamped with their ventral surface uppermost. Under a dissecting microscope, nail varnish could be applied accurately to areas of the abdomen. It was found that control termites in which the tip of the abdomen was coated could still lay a trail while

those which had varnish applied to the fourth, fifth, and sixth abdominal sternites could not.

Since the trail pheromone acts through some distance, it was presumed that it was detected by means of the antennae. Amputation of these rendered the termites incapable of trail following. But, because of the traumatic nature of this operation and the obvious importance of antennae to the blind termites, this experiment is difficult to interpret. Experiments using the effect of crossing the antennae on trail following ability (as in HANGARTNER, 1967) were unsuccessful due to the shortness of the antennae of *T. trinervoides* and their insertion far apart on the head. However, termites which at least had their antennae stuck together, appeared confused and would not orientate to the trail, while sham-operated control termites followed trails normally. Thus, it seems very likely, from these experiments and those of others, that antennae are of primary importance in trail detection.

Thin-layer chromatography of the trail pheromone

Even strong trails over glass are not visible to the eye and exposure of such trails to hydrofluoric acid fumes did not prevent the glass from becoming etched. Thus, very small quantities of substance are applied during trail-laying.

The trail extract in diethyl ether was subjected to thin-layer chromatography on silica gel (Merck silica gel-G). Using a mixture of pentane and benzene (1 : 2) four spots (R_f 0.02, 0.30, 0.65, and 0.80) were observed under u.v. light or upon treatment with iodine vapours. Upon elution, only the spot of R_f 0.02 showed any trail activity and recombination of all fractions did not increase this activity. Only about one-quarter of the original activity was recovered from the plate, but since a trail of high activity could be generated by applying more of the eluted spot, it was tentatively concluded that this spot contained the trail pheromone(s). The relative polarity of the active fraction led us to try n-caproic acid, reported by KARLSON *et al.* (1968) to be the trail pheromone of *Zootermopsis nevadensis*. T-trails (8 cm long) were made using 10, 20, and 40 μ l of a 2 mM aqueous solution. Tests of these showed that they elicited, respectively, 0.6, 2.2, and 2.8 followers per 10 test termites. Thus, caproic acid has weak trail activity, but a strong trail could not be produced, for these alarmed the termites and had no trail activity at all. Termites have been reported to follow trails of many different substances and the demonstration of trail following in response to some pure chemical is far from proving that the natural pheromone has been found. Caproic acid is almost certainly not the trail pheromone of *T. trinervoides*.

DISCUSSION

These experiments provide the final step in the proof that termites not only contain substances eliciting trail following in conspecifics (as has been shown many times), but that they actually deposit this substance on the substrate. We have shown that the trail-following response is due (at least for moderate trail levels) to a substance extractable from trail-bearing papers, but not control papers. The deviation at high extract volumes of the linear relation between the number of

initial extract containing a small number of compounds of high specific activity. It has been well documented that termites will follow a wide variety of unrelated compounds, some of them non-natural (WATANABE and CASIDA, 1963; BECKER and PETROWITZ, 1967; KARLSON *et al.*, 1968; BIRCH *et al.*, 1970; TAI *et al.*, 1971), and it appears as if termites will follow trails of many of the compounds which attract them. Thus, extraction of whole termites (MOORE, 1966; KARLSON *et al.*, 1968; MATSUMURA *et al.*, 1968, 1969) is likely to result in solutions containing a variety of attractants, some of which may release trail-following as well (see also STUART, 1969; BLUM and BRAND, 1972). Since many substances release trail-following, high specific activity is, by itself, insufficient evidence of the isolation of the true trail pheromone, i.e. that substance actively deposited on the substrate by the termites.

Thus, for example, MATSUMURA *et al.* (1968, 1969) and TAI *et al.* (1969) isolated and identified an active trail substance both from *R. virginicus* and from the brown rot fungus of wood, *Lenzites trabea*, but their published data do not exclude the possibility that the substance isolated from the (whole) termites was actually due to the rotted wood in the gut. The fact that infected wood contained about twenty times the specific activity as the termites adds weight to this hypothesis, and it is possible that they are dealing with a very active attractant from the rotted wood in the termite gut which incidentally also releases trail-following, but is not the substance deposited by the termites during trail-laying. No indication is given whether the diet of the extracted termites may have contained *Lenzites*-rotted wood. MATSUMURA *et al.* (1972) also reported the lack of species specificity of the isolated trail substance, but give no data on whether the species specificity is also lacking in trails laid by the termites themselves. Still further, although SMYTHE *et al.* (1967) report on *Reticulitermes* sternal gland extracts, they actually extracted whole termites and give no evidence that the isolated compounds actually come from the sternal gland. In fact, as STUART (1969) points out, these authors have not shown, in their published work, that the sternal gland is the source of the trail pheromone, although this seems very likely from experiments on *Zootermopsis*, *Nasutitermes*, and *Trinervitermes*.

The work of KARLSON *et al.* (1968) is less subject to artifact since STUART (1963a) showed that no part of *Zootermopsis* other than the sternal gland region elicited trail-following, at least at the concentrations tested. However, hexanoic acid, which they report to be a component of the trail pheromone of *Zootermopsis*, has only about 2 per cent of the specific activity of even the high vacuum distillate: 2.5 to 3 units/ng as opposed to 0.05 units/ng for hexanoic acid (Tables 2 and 3 in KARLSON *et al.*, 1968), and it appears that the most potent pheromone(s) in this extract are not yet identified.

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