

AN ANALYSIS OF BUILDING BEHAVIOUR OF THE TERMITE MACROTERMES SUBHYALINUS (RAMBUR)

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Thou seekest hard and findest not
Seek not and thou wilt find.

(Alchemists Rosarium, in A. Koestler, 1969)

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AN ANALYSIS OF BUILDING BEHAVIOUR OF THE TERMITE MACROTERMES SUBHYALINUS (RAMBUR)

Proefschrift ter verkrijging van de graad
van doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 31 oktober 1979
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

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ONTV. TIJDSCHR. ADM

STELLINGEN

I

Vrijwel alle aspecten van het bouwgedrag van Macrotermes subhyalinus (Rambur) kunnen afdoende worden verklaard uit het feit dat feromonen een dominante rol spelen in dit sociale gedrag.

Dit proefschrift.

II

Het zogenaamde vetlichaam van een termietenkoningin is een bron van feromonen, welke door de stigmata aan de buitenwereld worden afgegeven.

Dit proefschrift.

III

De door Grassé onderscheiden eerste fase in het bouwgedrag van termieten, waarin de dieren gronddeeltjes op 'willekeurige' plaatsen deponeren, de 'ongecoördineerde' fase, berust op een gedeeltelijk onjuiste interpretatie van zijn waarnemingen.

Grassé, P.P. (1959) Ins. Soc. 6, 41-83.

Grassé, P.P. (1967) Ins. Soc. 14, 73-102.

IV

Het bestaan van twee typen termietennesten die van elkaar verschillen in vorm en structuur, wordt ten onrechte alleen toegeschreven aan Macrotermes subhyalinus (Rambur)

V

Het tuten van bijenkoninginnen kan niet worden beschouwd als overspronggedrag zoals gesteld door Fletcher.

Fletcher, D.J.C. (1978) J. Apic. Res. 17
(1), 14-26.

VI

De berekening gemaakt door Botterweg betreffende de gemiddelde grootte van het gebied waarin een willekeurige verdeling van paringen van de dennespanner Bupalus piniarius (L.) optreedt, is aanvechtbaar.

Botterweg, P.F. (1978) Neth. J. Zool. 28
(3-4), 341-464.

VII

Bij de beantwoording van de vraag, waardoor de mensheid er toe gebracht is voor het eerst landbouw te gaan bedrijven, dient meer aandacht te worden besteed aan het feit, dat de mogelijkheid tot zaadopslag een voorwaarde vormt voor het ontstaan van landbouw.

Allen, T.H.F. (1977), J. Theor. Biol. 66,
169-180.

VIII

Ratten, waarvan de voorouders gedurende 20 generaties werden gevoed met een calorie- en eiwit-deficient dieet, die daarna enkele generaties een optimale voeding kregen, vertoonden een geringer leervermogen vergeleken met dieren waarvan de voorouders steeds een optimaal dieet kregen. In het licht van de schatting, dat één op de drie mensen in de wereld zich voedt met een dergelijk deficient dieet, geeft het bovenstaande te denken.

Galler, J. (1979), MIT Reports on Research.

IX

De vrees, dat het invoeren van de 'chip' technologie tot grote werkloosheid zal leiden, is in belangrijke mate het gevolg van het onvermogen van de industrie nieuwe toepassingsmogelijkheden te creëren.

O.H. Bruinsma : An analysis of building behaviour of the termite
Macrotermes subhyalinus. Wageningen, 31 oktober 1979.

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CHAPTER I GENERAL INTRODUCTION

PHEROMONES INVOLVED IN THE BUILDING BEHAVIOUR OF *MACROTERMES SUBHYALINUS* (RAMBUR)*

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Nest architecture is one of the most fascinating manifestations in termite societies. Nevertheless relatively little information exists about morphogenesis of nest structures. The large and elaborate structures are built from tiny soil particles which are cemented together by numerous workers. The coordination involved in the construction has been investigated for several termite species by Grassé (1959, 1967), but little experimental evidence is available on the behavioural mechanisms involved. Which stimulatory situation releases a determined pattern of building behaviour? Which structural signals or communicative pheromones modulate building behaviour, and how far do intrinsic elements of behaviour determine the spatial outcome of the structure?

In order to investigate these questions it was necessary to select a reproducible experimental situation in which the building of defined structural elements could be tested. Such a situation was found in the roofing of an open laid physogastric queen. The queen which normally lives together with the king in the royal cell elicits, when laid open, construction behaviour of the workers after a certain time lapse. This leads finally to the construction of a preliminary shelter capsule. Even in a relatively small isolated group workers start grasping soil particles in the vicinity of the queen and deposit them at 2-5 cm distance around the queen's abdomen. Within an hour building concentrates on several agglomerations of soil particles. This results in the erection of single pillars. When such pillars have reached the height of 1.5-2 cm,

*Preliminary report. The detailed data will be published separately.

soil particles are added in a more or less horizontal plane forming lamellae. This frame work is gradually extended and connected to form an earthen vault that covers the queen completely except for a few holes on the sides for in- and outgoing workers. Construction thereupon ceases. It was possible in a standardised test situation to focus observation on either the release and disposition of building or on the development of single pillars.

In a first experimental series it was demonstrated that chemical factors from the queen (pheromones) are involved in the release of characteristic building behaviour. A dead queen still induces shelter building whereas a wax dummy does not. A piece of abdominal cuticle initiates building as does an intact queen. A standard bioassay was designed to test semi-quantitatively extracts of various body parts applied in a drop of paraffine oil hanging centrally from the cover in a petridish testing chamber. High building releasing activity was found in the so called 'royal fat bodies' of the queen. It seems that in the intact queen the active components are given off via the stigmata. They appear to be volatile. From preliminary investigations C.D. Prestwich (unpublished) showed that a mixture of fatty acids may constitute part of the building stimulus. However, neither the fatty acid mixture nor the 'royal extract' nor the isolated tissue release completion of the shelter structure as does a live or dead intact queen. Additional cues responsible for the full scope of behaviour have to be postulated. When a testing queen was placed in a one-directional slow air stream (5-8 cm/sec) it was observed that a biased shelter was built. The soil particles were placed significantly nearer in the direction of the wind source than on the other side of the queen. This suggests that the local concentration within the gradient of pheromone may be a parameter involved in the determination of the building distance.

In a second set of experiments analysis was focussed on factors arising from already built structures that modulate building behaviour. The shaping of columns and arches was the ideal assaying situation. In accordance with Grassé's (1959, 1967) principles of 'stigmergy' it was confirmed that physical and chemical properties of particles once deposited influence the proceeding of further vertical deposition. It was also found that the thereby erected pillars after reaching a certain height (1.5-2 cm) provoke building towards the horizontal plane. Beyond Grassé's findings, it was possible to obtain more precise information on the chemical factors involved in this feedback system. Short range attraction (1-2 cm) was demonstrated for a worker intestinal secretion that is added to the soil particles while these are being shaped with the mandibles and wetted. Further work is in process that suggests the use of trail

pheromone as possible communicative cue in shape building behaviour.

The aim of this series of investigations is to work out a valid model to explain building of a specific structure as the consequence of releaser and modificatory signals interacting in a spatial-temporal sequence with the behavioural repertory of the worker termites.

References

Grassé, P.P., 1959. La reconstruction du nid et les coordinations inter-individuelles chez *Bellicositermes natalensis* et *Cubitermes* sp. La théorie de la stigmergie: essai d'interprétation du comportement des termites constructeurs. *Insectes Sociaux* 6: 41-83.

Grassé, P.P., 1967. Nouvelles expériences sur le termite de Müller (*Macrotermes müller*) et considérations sur la théorie de la stigmergie. *Insectes Sociaux* 14: 73-102.

CHAPTER II

A BUILDING PHEROMONE FROM THE QUEEN OF MACROTERMES SUBHYALINUS (RAMBUR).

INTRODUCTION

Construction behaviour in social insects is a complex form of social interaction with a high degree of co-ordination between the activities of individual workers. This results in nest structures of diverse architectural design (Noirot, 1969; Wilson, 1971; Jeanne, 1975). Termites of the subfamily Macrotermitinae are known to construct large mounds, dominating landscapes in tropical Africa. The mound nests of the East African subterranean grass-feeding, fungus-growing termite Macrotermes subhyalinus (Rambur) in the study area consist of an epigeous mound, internally penetrated by ramifying air passages and with a large hypogeous central area (the hive), containing special structures such as fungus combs, the nursery and the copularium (the royal cell). Such nests may contain up to four million individuals (J. Darlington, personal communication).

The means of co-ordination involved in the building of such elaborate and intricate structures have been investigated for several termite species by Grassé (1959, 1967). According to Grassé, previously built structures act as cues for further work; he names this principle 'stigmergy'. Basic shortcomings in this concept are that it gives neither an explanation as to the variety of structural elements within the nests (Chauvin, 1968; Harris and Sands, 1965), nor as to the stimulus which stops construction activity (Stuart, 1967 and 1969). Through a different approach it was experimentally shown that in Nasutitermes corniger, Zootermopsis angusticollis and Z. nevadensis the use of odour trails is involved in spatial co-ordination of building behaviour (Stuart, 1967). Furthermore, the importance of air movements for the positioning of building sites is emphasized in Z. angusticollis (Howse, 1966).

None of the proposed mechanisms fully explains details of the formation of internal structural elements composing a nest. In an attempt to fill this gap, we investigated the basic behavioural principles involved in the construction of defined structural elements present in the mound nests of M. subhyalinus, like the royal cell, a pillar or a gallery.

In this paper the royal cell is the subject of investigation. The royal cell or copularium is a unique structure, a chamber enclosing the royal pair. It has an ovoid shape and is generally situated near the base of the hive. When a physogastric queen is laid open, the nearby workers are stimulated shortly afterwards to building behaviour. Building workers will cover the queen with an earthen vault of cemented soil granules, a replacement royal cell.

In M. bellicosus a similar phenomenon of royal cell reconstruction is reported (Escherich, 1909). In this species workers, prior to construction, rapidly circulate for a few minutes around their exposed queen, displaying a 'caroussel'. Lüscher (1970) has confirmed this observation, and assumes that the workers lay a trail pheromone zone and that they only accumulate soil granules at the periphery of this area. He has observed similar behaviour in *Zootermopsis*.

The fact that in M. subhyalinus no 'caroussel' of workers is normally observed around the queen, but rather a group of more or less stationary workers engaged in grooming her, suggests that other cues exist additionally to the possible use of trail pheromone for initiating and orienting the building behaviour of workers engaged in cell construction. The results of a laboratory study of the construction of a replacement royal cell by workers of this species is reported here. These findings reveal the existence of a volatile agent, produced by the physogastric queen: a building pheromone, which initiates and orients building behaviour of workers involved in replacement cell construction.

Although this building pheromone transmits crucial information for cell construction, building workers are influenced by additional cues of chemical and mechanical nature for their co-ordination. These additional cues will be dealt with in a subsequent paper.

MATERIALS AND METHODS

Experimental animals

Mound nests of M. subhyalinus were opened near Kajiado, Kenya. Parts of the nests, including the royal cells and adjoining nest structure containing numerous workers were transported to the laboratory. Workers had to be used within 48 hrs to obtain building response. In order to obtain highly active workers for use in the bioassays, workers were collected (always from the same mound, located near the laboratory in Nairobi) from repairing an induced damage to their nest, immediately before use. Termite queens could be kept alive for 5 - 7 days on moistened filter paper in Petri dishes containing about 100 minor workers, which were changed every day.

Observation and bioassay procedures

Worker's responses to live and/or experimentally treated queens were assessed by observation, and quantified in bioassays. All experiments were done under laboratory conditions of 23 - 25°C and 70 - 80% RH, unless otherwise stated.

1. Observation of building behaviour

Observations were carried out in observation boxes of 25 x 20 x 5 cm in perspex. They were filled with a layer of 0.5 cm sieved sterilized (3 hrs, 110°C) friable red volcanic soil with a pellet size not exceeding 1 mm, and sprayed with 10 - 15 ml distilled water (prepared soil). In two opposite corners of the box wetted balls (\emptyset : 2 cm) of cotton wool served as water supplies. A physogastric queen was placed centrally in the arena. To start the experiment, a standard number of 640 major workers* was introduced here. The observation boxes were covered with a plexiglass lid, leaving an air gap, since virtually no cell building behaviour could be observed in airtight boxes within 60 min. The activities of the workers gathering around the queen were observed by eye, through a conventional stereomicroscope, or a pair of magnifying glasses (1.5 x). Data were collected during the first 60 min following worker introduction.

Recorded were:

- 1) The time which elapsed between the start of the experiment (worker introduction) and the first observed grasping of a soil granule within a 0.5 cm wide zone around the queen (the grasping zone): the building latency time.
- 2) The total number of soil pellets removed from the grasping zone.
- 3) The total number of soil pellets placed in a 3 cm wide zone, located at between 2 and 5 cm distance around the queen (the deposition zone).

In order to record the behaviour of single workers during a pre-determined period of time, workers were marked with a dot of nitro-cellulose paint on the abdomen. These were newly added to the arena 50 min after the start of the experiment and continuously observed during a 10 min period. Concerning them 1) the number of soil granules removed by these workers from the grasping zone and placed in the deposition zone or elsewhere in the arena, and 2) the time required to transport a pellet from the grasping site to the deposition site (the transport time) were recorded. The behavioural sequence of grasping, transporting and depositing of a soil pellet is hereafter referred to as a 'run'.

2. Bioassay of queen's body parts and tissues

In order to locate the source of the building stimulus in the queen, the abdomen, head and thorax, and different tissues from the abdomen separately

*Structures removed from a nest within 1 hr after the completion of mound digging hardly contained any minor workers. Members of this caste were observed to return to the building sites beyond this period of time.

were tested in the following bioassay (Fig. 1a): The bottom halves of two 11 cm petri dishes were charged with a 0.3 cm layer of prepared soil. In both dishes, a solid glass rod (L: 5 cm, \emptyset : 0.6 cm) wrapped in filter paper moistened with insect Ringer was positioned centrally. In one dish this dummy was used as blank to act as control. In the second dish a queen substitute was attached in one of the following forms:

- The cuticle of the abdomen, or segments of it with or without fat body patches was draped over the wrapped rod.
- The ovaries, fat body or intestines were fixed on the rod underneath a metal netting (mesh size: 1 per mm^2), while haemolymph was tested with filter paper soaked in haemolymph instead of Ringer solution.

The head, head plus thorax, or the abdomen as well as intact live queens with varnished and non-varnished abdomens were tested without the rod.

Fifty freshly collected workers were then placed into each of the two dishes; a lid covering the dish with an air gap. After a 10 min 'exploration' period records were made of workers depositing soil pellets in a 1 cm wide zone located at 1 cm distance on either side of and parallel to the rod or queen fragment during a maximum period of 20 min. A dummy was considered active if the test dish contained a minimum of five times more deposited soil granules than the control dish, or when the control dish scored zero, a threshold value of five soil pellets was deposited. Because of possible 'stigmergic' effects (Grassé, 1959) of the deposited soil granules, the experiments were stopped when either of the dishes contained 20 depositions.

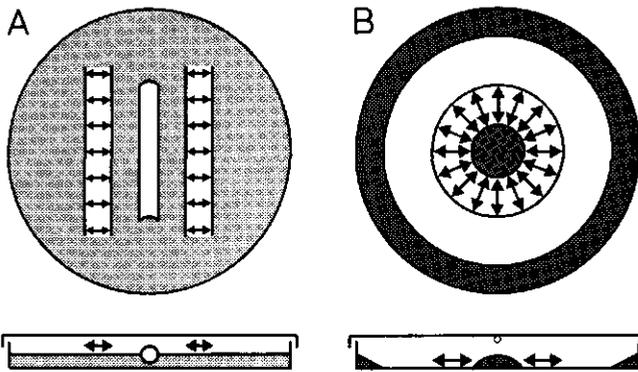


Fig. 1a and b. Body fraction and tissue bioassays arrangements (a).
 Extract bioassay arrangement (b).
 The scoring area's are indicated with arrows (see text).

3. Extract bioassay

A second bioassay was devised to screen for cell building activity induced by extracts of the various body parts and tissues of queens. In addition, extracts of major and minor workers, larvae, nymphs and soldiers were tested. The intact animals, body parts or homogenized tissues were extracted in di-ethyl ether during 24 hrs at -16°C .

Lots of fifty freshly collected major workers were introduced into two bottom halves of 11 cm petri dishes, each loaded with a ring (at the periphery of the dish) and a central mound (\varnothing : 2 cm) of prepared soil on filter paper ground (Fig. 1b). After a 10 min 'exploration' period, 10 μl of the ether tissue extract was applied to a droplet of mineral oil (Merck), hanging on the lid of the dish, 1.5 cm above the bottom. Ether alone was applied to the oil droplet in the control dish. The lids were placed with an air gap. The number of soil pellets deposited in a 1.5 cm wide zone around the central mound was scored during a 20 min period. An extract was considered active or inactive, according to the scoring system as described above.

4. Windtunnel, a test for spatial information of the building pheromone

A small low speed windtunnel (Fig. 2) was designed in order to investigate

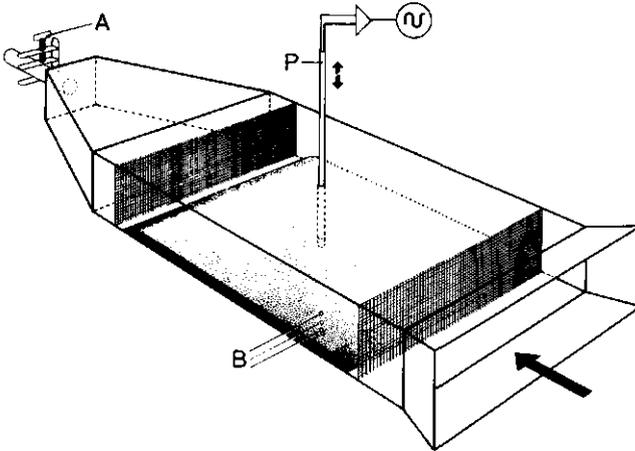


Fig. 2. Low speed windtunnel. A: valve for the regulation of air speed.
B: Holes for the injection of smoke puffs.
P: Hot wire micro-probe for air speed measurements at specific heights above soil level (shaded area). For further explanation see text.
The fat arrow indicates the direction of the air movement.

the effect of artificial changes of the pheromone concentration on the deposition distance from the queen maintained by the building workers. A water-jet pump sucked air through the experimental chamber. This chamber was a rectangular perspex box (20 x 10 x 5 cm), of which two sides consisted of wire gauze. The wire gauze of fine mesh (9 per mm²) located at the air inlet side decreased the air turbulence in the experimental chamber. The entering air passed first through an inlet funnel covered with water soaked filter paper to provide a relative humidity level in the experimental chamber of 60 - 75% (measured with Telemax probe RH 1). The experimental chamber was charged with a 1.5 cm thick layer of 'prepared' soil. The physogastric queen was positioned centrally in the test area, longitudinally with respect to the direction of the laminar air flow, and immobilized with a small plastic clip fitting over the thorax and fastened in the soil.

One hundred major workers were then introduced, and after an 'exploration' period of 5 min, the air flow was turned on to a standard value of 5 cm/sec, at 1.5 cm above soil level. The deposition sites of the first 20 soil pellets on both sides of the queen were recorded on the transparent lid of the experimental chamber, and their horizontal distances from the queen were measured. The air flow velocities in the chamber were measured with a sensitive anemometer with a miniature temperature compensated hot-wire probe in combination with an oscilloscope. These measurements were performed at 20°C at defined distances above soil level, on either side of the queen at a horizontal distance of 2 cm from the queen (Fig. 3).

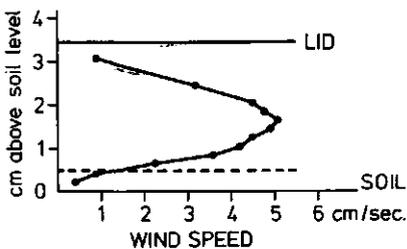


Fig. 3. Windspeed at specific heights in the windtunnel. Each point represents the average of 10 measurements. The broken line indicates the maximum average height (including antennae) of soil pellet transporting major workers.

In order to calibrate the anemometer and to obtain visual information on the degree of turbulence in the moving air, tiny puffs of smoke were injected in the test chamber with a syringe via tiny holes at 0.5, 1.0 and 1.5 cm above soil level, and their travelling times measured with a stopwatch.

RESULTS

Description of the building behaviour around live queens

In the following an average feature of behaviour is described as extracted from a total of 10 observations, each lasting 6-8 hrs, the time required by 640 major workers to complete a replacement royal cell. A physogastric queen of M. subhyalinus aroused considerable interest when present among workers. She was quickly surrounded by a group of between 30 to 50 workers, most of which displayed prolonged periods (up to 6 min) of arrested locomotion while engaged in inspecting the abdomen with their antennae, and grooming the cuticle of the distended intersegmental membranes with their mandibles (Fig. 4). After a latency time of a few minutes, a first worker grasped a soil pellet very near to (within 0.5 cm) or from underneath the queen's abdomen. This worker then turned around, and walked along the queen while kneading the pellet with its mandibles, often changing it into a more or less fluid paste with a secretion released from the buccal cavity: the cement (Noirot, 1969; personal observations).



Fig. 4. Major workers surrounding their exposed physogastric queen, are engaged in grooming it, prior to cell construction.

During this excursion the worker performed a more or less serpentine path around the queen, in which it sometimes made brief antennal contacts with the abdomen of the queen, after which it resumed its journey (Fig. 5). After an excursion time (the transport time) of 10 - 15 sec, it usually deposited the pellet somewhere within the above 3 cm wide zone (in 7 out of 10 cases). Sometimes (in 3 out of 10 cases) the granules were deposited beyond this zone, against the arena walls. The soil bits were easily cemented by the worker with characteristic 'rocking' movements of the head, and the often regurgitated soil material would harden within a few minutes. The first worker depositing its load in the deposition zone returned in all experiments to the queen, and resumed grooming.

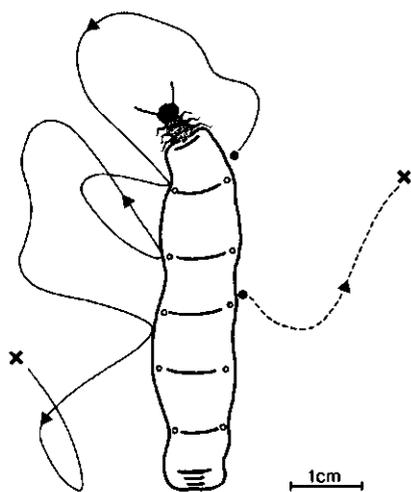


Fig. 5. Characteristic walking patterns of two soil transporting workers around their queen. Solid line: path of first observed transporting worker. Broken line: path of a transporting worker 30 min after the start of the experiment. Solid dot: grasping site. X: deposition site.

The second observed grasping of a soil pellet occurred within 0.5 - 1.5 min (\bar{x} : 1.1 min, $n = 10$) after the first, and was executed in 9 out of 10 times by another worker. Deposition occurred after a similar transport time at another place within the deposition zone (in 8 out of 10 cases). The time interval between successive runs then rapidly decreased: more workers started grasping soil pellets near the queen and placed them anywhere but mostly in the deposition zone. The involvement of pheromones released by the worker during soil transport and the process of cementing the pellet, acting as a social stimulus, will be discussed in another paper. During the first 5 min following the building latency time an average of 15 depositions was observed of which 50 - 60% were in the deposition zone. The average number of depositions recorded per 10 min increased to 470, during the interval of min 20 - 30 after worker introduction, after

which time building activity gradually decreased to a level of about 350 depositions per 10 min, and remained on that level until the end of the 2 hrs recording period (Fig. 6). The percentage of depositions placed in the deposition zone increased with time and reached its maximum during min 40 - 50 with 90 - 95% of the total number of depositions occurring in that time interval and thereafter remained at that level. The serpentine walking patterns of the transporting workers began to stop after about 25 min after the start of the experiment, and were gradually replaced by rather straight paths from the queen to the deposition site (Fig. 5). This led to a reduction of the transport time to 3 - 5 sec.

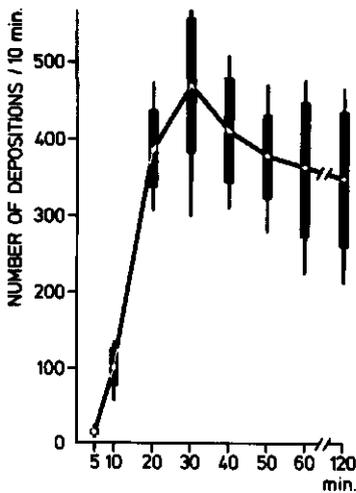


Fig. 6. The total number of depositions, scored per 10 min, displayed by 640 major workers around their physogastric queen. The figure shows the mean (open dot), standard deviation (vertical bar) and range (vertical lines); $n = 10$.

In all assays, the deposition sites in the initial building stage, the first 40 - 60 min, were distributed at random in the deposition zone; beyond this period, the deposition activities of the workers began to concentrate at several loci within that zone at distances of 1.9 - 2.6 cm (\bar{x} : 2.2 ± 0.3 cm) from the queen. At these loci soil pellets were placed against or on top of previously deposited ones, resulting in the formation of incipient (0.2 cm high, 0.2 - 0.4 cm wide) soil columns. Within minutes thereafter these tiny pillars received in comparison to their surrounding area (\emptyset : 2 cm), a disproportionate number of pellets; this leading to their enlargement. When such a structure reached a certain critical height (0.5 - 0.8 cm), workers began adding the pellets to the top of the column in a more lateral direction, resulting in the formation of curved lammellae originating at the pillar apex and pointing towards the queen. Four to eight of these growing pillars with developing lamellae on both sides



Fig. 7. A physogastric queen surrounded by several pillars with developing lamellae under construction which are gradually extended laterally to form the covering arch.

of the queen (Fig. 7), were gradually extended and connected against each other until an earthen vault completely spanned the queen (Fig. 8). A few holes in the cell wall at soil level, sometimes connected with covered runways, were left open for in and outgoing workers as in the original cell. After completion of such a replacement cell construction activity around the queen was reduced. The average free space between the queen and the lateral cell wall at the most distant point was 2.41 ± 0.19 cm and the average clearance between the dorsal side of the queen and the roof at the most distant point was 0.57 ± 0.8 cm. The corresponding measurements of 10 royal cells found in natural circumstances were respectively, 3.66 ± 0.24 cm and 0.72 ± 0.1 cm.

The results obtained with single marked workers ($n = 40$) introduced into the building arena 50 min after the start of the experiment and observed for

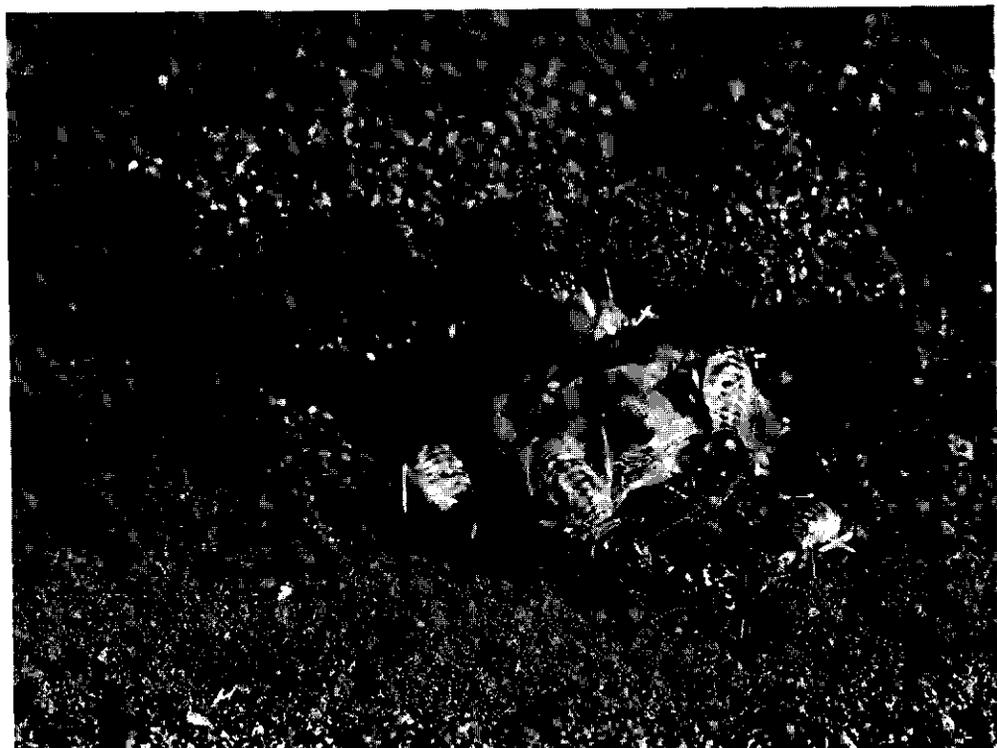


Fig. 8. An earthen vault, the 'replacement royal cell' spanning the queen.

10 min showed, that workers are capable of completing several runs (\bar{x} : 6; range 3 - 8) before they stop building. When they stop, the workers invariably moved away from the queen and the construction sites and displayed various activities such as trophallaxis, drinking water, allo- or self grooming etc. Some of these marked workers (9 out of 40) after visiting the water supply again joined (with distended abdomen) the building group around the queen.

Construction around killed, varnished and caged queens

The building latency time of workers around freshly killed queens (10 min at -16°C) was similar to those obtained with workers around live queens, and the replacement cells were constructed in periods ranging from 6 - 9 hrs. The average distance of the cell wall to the dead queen ($n = 4$) was 0.6 - 0.9 cm smaller than the corresponding distance to a live queen (Table 1). The possible cause of this difference will be discussed in a later section.

Table 1. Distance (cm) of the interior of the cell wall to the queen measured on either side opposite spiracles 2 and 5. The difference between live queen and killed queen is significant (t-test, $p < 0.01$).

Spiracle nr.	Live queen		Spiracle nr.	Killed queen	
	Left	Right		Left	Right
2	1.7	1.8	2	1.3	1.5
5	2.0	1.8	5	1.2	1.3
		$\bar{x}=1.8$			$\bar{x}=1.3$
2	2.2	1.9	2	1.3	1.2
5	2.4	2.2	5	1.4	1.0
		$\bar{x}=2.2$			$\bar{x}=1.2$
2	1.9	1.6	2	1.3	1.1
5	2.3	2.0	5	1.2	1.1
		$\bar{x}=1.9$			$\bar{x}=1.2$
2	1.8	2.3	2	1.1	1.4
5	2.2	1.8	5	1.3	1.3
		$\bar{x}=2.0$			$\bar{x}=1.3$

Live and killed queens with completely varnished abdomen were not able to arouse the workers to start building, although the unvarnished head, thorax and anus received some attention from a greatly reduced group of encircling workers (4 - 8, rather than 30 - 50, around intact control queens; measured every 30 min during 6 hrs). It was thus apparent that neither the shape and/or movements of the queen, nor sounds produced by her provided the essential initiating stimulus for the workers. In order to decide whether chemical and/or mechanical stimuli provided by the queen were involved in the initiation of cell building behaviour, live and killed queens were placed inside cages which fitted her closely. These cages were made of two layers of copper wire gauze of fine mesh (4 per mm²), which prevented the workers from making antennal contact with the queen. A glass rod (L: 5 cm, Ø: 0.6 cm) wrapped in filter paper soaked with Ringer solution placed in a similar cage served as control in a second arena. The caged queens (n = 5) were immediately surrounded by 30 - 55 workers, probing the netting with their antennae, and often observed to be trying to bite it with their mandibles. After a building latency of 2 - 6 min workers started constructing and completed the earthen vault in 7 - 9 hrs. These results led to the conclusion that a volatile stimulus, which we named a building pheromone,

was released by and evaporated from the queen, and that it plays a role in the initiation of building behaviour of workers around their queen. This volatile stimulus was found only in physogastric queens (see below) and seemed to be continuously released since live queens, when replaced in a new arena directly after replacement cell completion, were covered again, twice a day on four consecutive days.

Construction around parts of queens and their extracts

An intact abdomen, with its severed side sealed with beeswax, was treated like an intact live queen, i.e. it was groomed by surrounding workers and covered with an arching vault in 6 - 8 hrs. No building response was obtained with the head or the thorax.

Queen abdomens were then dissected in order to locate the stimulus source(s). From freshly killed queens the haemolymph was collected via a small incision near the anus. The queen was then positioned on her dorsal side and the abdominal cuticle was opened by a longitudinal cut from anus to thorax. The ovaries and the digestive tract could easily be removed. The remaining organ was the 'royal fat body', a characteristic secondary tissue, present in physogastric Macrotermes queens (Grassé, 1949). It consists of six pairs of dark brown, lobate but compact patches arranged in two lateral rows and attached to the interior of the tergal region of the cuticle. Each of the patches is clustered around a spiracle and penetrated by a bundle of ramifying trachea arising thereof. These patches were easily removed and collected by flushing them with small jets of Ringer solution, leaving the cuticle with the tracheal system largely intact. The results of a comparative study of the effects of various parts and their di-ethyl ether extracts, tested in the body part/tissue and extract bioassay are given in Table 2 and 3. They indicate that the 'royal fat body' is the source of a volatile stimulus, which initiates a type of building behaviour similar to that around intact queens: the grasping of a soil granule near the stimulus source and its deposition further away.

The significance of the spiracles in stimulus release

Although an extirpated fat body released a construction response, the presence of this tissue on a dummy (glass rod) in an arena with 640 major workers did not lead to the construction of an arched vault as occurs with intact queens. The building activity of the workers (20 - 40% of the number of runs displayed around intact control queens during the first 60 min) gradually declined with time and ceased 1.5 - 2 hrs after the start of the experiment. Thereafter 3

Table 2. Body fraction and tissue bioassay results.

	Score range	Score average
Queens:		
Intact live queen	++++ ^c	++++
Intact dead queen (fresh)	++++ ^c	++++
Intact dead queen (5 days at 4°C)	+ / ++	++
Live queens with varnished abdomen	-	
Live queens with varnished abdomen but with unvarnished spiracles	++++	++++
Queen substitutes: ^a		
Head	-	
Thorax	-	
Abdomen	++++ ^c	++++
Ovaries	-	
Haemolymph	-	
Digestive tract	-	
Abdominal cuticle plus attached 'fat body' (rinsed for 1 min in Ringer at 4°C)	++++ ^c	++++
Abdominal cuticle (rinsed for 1 min in Ringer)	++++	++++
Abdominal cuticle plus attached 'fat body' (rinsed for 30 min in Ringer at 4°C)	++++ ^c	++++
Abdominal cuticle (rinsed for 30 min in Ringer at 4°C)	- / ++	+
Royal 'fat body'	++++	++++

^a Details of bioassay to be found in the text. Number of repetitions for each experiment: 6 - 8.

^b Controls scored 0 or 1 soil pellet. The various ranges of number of depositions in the test dishes were scored as follows: 0-4, (-); 5-9, (+), 10-14, (++); 15-20, (+++); 20 soil pellets before 20 min completed: (++++).

^c Subsequent transfer of the queens or dummies into a building arena with 640 major workers resulted in replacement cell construction.

Table 3. Extract bioassay results.

Extract of: ^a (in di-ethyl ether)	Score ^b range	Score average
Intact queen	++/++++	+++
Abdomen	++/++++	+++
Head	-	
Thorax	-	
Head plus thorax	-	
Abdominal cuticle (Ringer, 1 min)	+ /+++	++
Abdominal cuticle (Ringer, 1 hr)	- /+	-
'Fat body' (Ringer, 1 min)	++/++++	+++
'Fat body' (Ringer, 1 hr)	++/++++	+++
Haemolymph	+ /++	++
Ovaries	-	
Digestive tract	-	
300 major workers	-	
200 minor workers	-	
200 minor/major soldiers	-	
400 larvae (various stages)	-	
150 last stage nymphs	-	

a Details of bioassay in text. Number of repetitions for each experiment 5-8.

b Scored as in Table 2.

trials were conducted with 1000 major workers under conditions of 29.5°C and 90% RH (comparable to the nest condition), while moistening the fat body every 10 min with a few drops of Ringer solution. Again, the building activity with respect to the dummy stopped after 2 - 2.5 hrs, and no pillar construction was observed. In contrast, an abdominal cuticle with attached fat body patches draped over the glass rod caused initiation and continuation of building behaviour for at least 12 hrs, sufficient for the 640 workers to complete a replacement cell. This difference was interpreted to be due to the fact that the

release of the building pheromone from the fat body was hampered when the gland was extirpated and placed on the rod. Extracts made of fat body patches which appeared inactive on the dummy (after 3 hrs) showed to be active when tested in the bioassay. Since each fat body patch is clustered around the permanently open spiracles (Bordereau, 1971) a series of experiments was conducted with varnished live queens to assess the role of the spiracles in the release of the pheromone. Queens received the following treatments:

- a) Varnishing of the abdomen but not of the spiracles.
- b) Varnishing of the abdomen and the spiracles, but not of patches of 0.5 cm^2 each of abdominal cuticle in between the ipsilateral spiracles.
- c) Not varnishing of the abdomen but sealing of the spiracles.
- d) Not varnishing of the abdomen but varnishing patches of 0.5 cm^2 of the cuticle between the ipsilateral spiracles.

In all four series of tests the number of workers was 320.

During the first 30 min, construction activity could be observed in all series, reduced however in series a) and b). In series b) and c) building activity ceased completely after 30 - 50 min; in series a) the construction activity led in four out of five trials to the erection of 1 - 3 pillars each time, at distances of 1.6 - 2.3 cm from the queen. However, no cell completion was observed. Workers in series d) constructed complete replacement cells in a time interval of 8 - 10 hrs. The results of building activity during the first 60 min are summarized in Fig. 9.

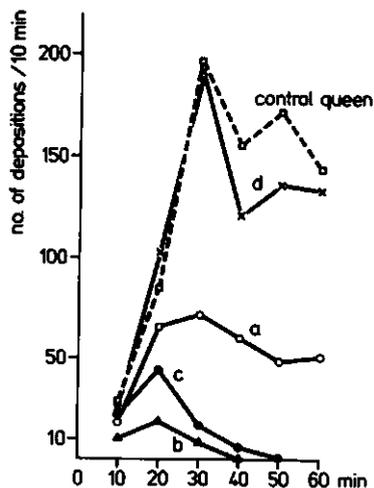


Fig. 9. Number of runs displayed by 320 major workers around queens, scored per 10 min. Intact queen (control queen); queens ($n = 5$) with varnished abdomens but unvarnished spiracles (a); queens ($n = 5$) with varnished abdomens and spiracles, but unvarnished patches of abdomen (b); queens ($n = 5$) with unvarnished abdomen, but varnished spiracles (c); queens ($n = 4$) with patches of varnish on the abdomen (d).

In a final investigation, the abdomens of live queens ($n = 10$) were rinsed in pentane (22°C) for 15 sec; the queens were then placed after a 2 min interval in an arena, where subsequently 640 major workers were introduced. Five of

these queens had their spiracles sealed prior to the pentane treatment. Initially none of the queens aroused any interest of the workers that is to say neither grooming nor construction activity was observed. After 10 - 13 min however, workers were attracted to the control queens and started grooming and antennating the abdomen. The first run was observed between 13 - 16 min after the queen's introduction and the earthen vault was completed in a period of time of 5 - 8 hrs. The workers around queens with sealed spiracles remained inactive with respect to the queen until the end of the observation period of 12 hrs. From the above findings, it is concluded that the building pheromone is released from the spiracles. The spiracles no's 2 to 7 consist of elongated depressions (approximately 2 mm long) in which tracheal orifices are visible (Bordereau, 1971; personal observations). Since, as stated before, each of the fat body patches is clustered around a rosette of trachea arising of a spiracle, it seems possible that the tracheal system is involved in the transport of the pheromone to the spiracles.

Therefore, cuticles ($n = 5$) with attached fat bodies were draped over the rod. Prior to this the 12 tracheal bundles arising from the respective spiracles were severed as close as possible to the spiracle. These cuticles released a building response during a period of 55 - 100 min (320 major workers), compared to 8 hrs in the controls with intact tracheal system. It thus seems possible that the tracheal system is involved in the transport of the building pheromone from the fat body to the spiracle.

The results obtained in the aforementioned experimental series a: only pillar construction around queens with varnished abdomen but with open spiracles, and the fact that queens with sealed spiracles elicit a building response during a limited period of time following sealing (Fig. 9), called for an investigation of the possible role of the abdominal cuticle in the release of a complete building response leading to cell construction. First, 2 live queens with varnished spiracles were each placed in a container filled with broken nest material and numerous workers. The queens were observed at regular intervals during 2 hrs in order to confirm their inability to induce building behaviour. These queens were placed next in observation boxes, each containing one live queen, and positioned centrally in the arena parallel and adjacent to the live queen. The queens were separated from each other by a 9 cm long, 1 cm high and 0.1 cm wide wall in perspex. A standard lot of 640 workers were then allowed to enter the box. After a period of 8 hrs, workers had completed the earthen vault over the not treated queen, and the cell wall at the control side of this queen was located at an average distance of 1.9 ± 0.22 cm (measured each cm

along the abdomen). On the other side of the untreated queen the cell wall had been constructed for a part on the dorsal side of the treated queen and for a part along her, however with no free space between the cuticle and the wall. In a second series, 2 similarly treated queens were also placed parallel and adjacent to live queens, but this time touching each other. After a period of 8 hrs, the workers had completed the earthen vault but now over the two queens. The average distance from the untreated respectively the treated queen to the cell wall was 2.1 ± 0.20 cm and 0.8 ± 0.17 cm.

These results suggest that part of the building pheromone after being released from the spiracles spreads over the abdominal cuticle by surface diffusion. Although the building pheromone is as yet unidentified, palmitoleic acid assumingly constitutes part of the pheromone (G.D. Prestwich and O. Bruinsma, unpublished). The cuticle of insects is permeable to oil-soluble molecules, which can disperse rapidly over the surface (Lewis, 1962). According to Locke (1965) the rapid diffusion of e.g. lipids in the surface wax layer of the cuticle is explained if this wax layer is in a liquid-crystalline phase. It will therefore be of interest to study the spread of a radioactive preparation of a lipid over the queen's abdomen by an autoradiographic technique.

Does the building pheromone transmit spatial information?

By changing the position of freshly killed queens it was clearly demonstrated that the cell walls are constructed at a fairly constant distance from the queen (Fig. 10). The question then arises how do workers perceive this distance? An odour evaporates in an equable way from an odour source while temperature and air conditions remain constant. Under such conditions, an expanding sphere of diffusing odour will remain centered on the source, if one assumes that the molecules are free to diffuse infinitely in all directions. If the odour source

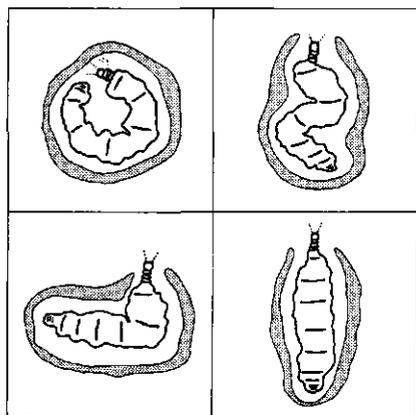


Fig. 10. Freshly killed queens, placed in four different postures. The shaded area's indicate the 'foundation' of cells constructed around them in 8 hrs.

is located on an impervious plane, the diffusion plane can be described as a hemisphere.

After a certain evaporation time, within the diffusing sphere a gradient of concentrations is established due to the odour dilution in space.

The building pheromone is continuously evaporating from the queen under constant temperature and calm air conditions. It is thus plausible that a diffusing sphere of pheromone exists around the queen. Since worker's building responses are elicited by olfactory perception, the hypothesis presents itself that the distribution of the building pheromone influences workers' responses. That is to say can the observed worker behaviour be theoretically explained as mechanistic responses to changes in the quality and quantity of the pheromone emanating from the queen?

In an attempt to investigate this complicated matter, three experiments were performed.

The first experiment focussed on the effect of a laminar air flow of low velocity on the distance between the queen and the deposition site. The queens were positioned centrally in the test chamber of the windtunnel with their longitudinal axis parallel to the direction of the air flow. The maximum air velocity measured at 0.5 cm above soil level was 0.9 - 1.1 cm/sec. The mean of maximum heights including antennae of transporting major workers is 0.45 ± 0.06 cm, $n = 35$). A reduction was observed in the mean distance at which soil pellets (the first 20 on either side of the queen) were deposited (Table 5).

Table 5. The average distance from the first 20 depositions to the queen on either side, in the windtunnel with an air velocity of 0.9 - 1.1 cm/sec 0.5 cm above soil level. The results of subsequent control experiments (still air) using the same queens are given in brackets. The difference between experiment and control is significant (t-test, $p < 0.01$).

	Left side		Right side	
	cm		cm	
Exp 1	1.65	(2.38)	1.48	(2.27)
Exp 2	1.37	(1.93)	1.31	(1.95)
Exp 3	1.52	(2.12)	1.45	(2.28)

In a second experiment intended to manipulate the pheromone distribution, one live queen was positioned as usual in the arena. A second one, freshly killed, was hung in a metal netting of coarse mesh (0.5 per mm^2) 1.5 cm above one depo-

sition zone generally used building workers around a live queen (Fig. 11). The results of 4 trials show that workers did not deposit their pellets within the expected range of sites, but 1.8 - 2.0 cm further from the live queen compared to the distance range of the deposition sites on the control side. The data obtained in these two experiments strongly suggest that workers employ the pheromone distribution in order to locate the deposition zone. In other words, they may respond to changes in pheromone concentration as the distance between workers and queen increases. The perception of a threshold concentration (or ratio of threshold concentrations if the pheromone is multi-component) may facilitate deposition behaviour.

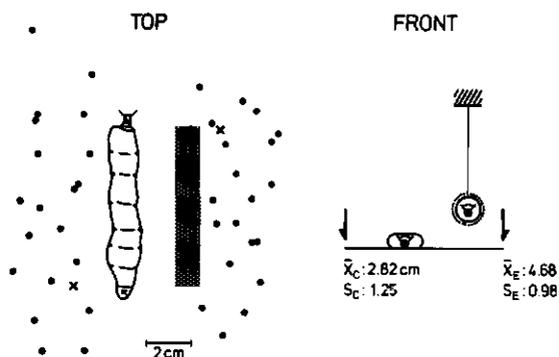


Fig. 11. The distribution of the first 20 deposition sites on either side of a live intact queen (black dots). The shaded area represents the position of a freshly killed queen hanging in a netting parallel to the live queen. The average distance of both groups of sites with reference to the live queen (arrows) together with their standard deviation (C: control side, E: experimental side) of one experiment are given. X: position of first observed pillar on each side of the queen.

In order to demonstrate the feasibility of such a mechanism of orientation the following experiment was performed. A live but immobilized queen (fastened to the soil with a plastic clip over her head and thorax) was present in the observation box 6 hrs before 640 major workers were introduced. During that period the box was closed. The rationale behind this experiment is that under these conditions the building-up of a further extended pheromone zone, compared to the usual procedure, may be expected. Following the 6 hrs time interval workers entered the arena via a sliding door in one of the walls. After replacement cell completion the distances were measured between the interior of the cell wall and the queen's abdomen. The means of these distances were longer by 0.3 - 0.7

cm than those of the control cells (Table 6). Although this result is consistent with the hypothesis, the extension of the pheromone zone may very well be due to another factor than diffusion: measurements with the anemometer showed the existence of puffs of air, emitted from the spiracles. Air velocities up to 4 cm/sec were recorded within a few mm from the spiracles. These air puffs from single spiracles occurred in frequencies of 3 - 4 per min, and often coincided with an outwardly directed movement of that side of the abdominal segment at which the spiracle was located. Although no detectable air movements existed at 1 cm from the spiracles, fluctuations in relative humidity of 5 - 10%, coinciding with the occurrence of air puffs could be recorded up to 1 cm from the spiracles. The air emanating from the spiracles serves as a transporting medium so that the diffusion constants are properties of the moving air rather than of the pheromone itself. However, this phenomenon may account for the difference in size observed between cells constructed over live queens and freshly killed queens (no air puffs) as shown in Table 1.

Table 6. Distance to the interior of the cell wall of the queen, measured in cm on either side of the queen, opposite spiracles nr 2 and 5. Each queen was used twice, first in B, then in A. The difference between A and B is significant (t-test, $p < 0.01$).

Spiracle nr.	A Workers introduced simultaneously with queen		B Workers introduced after 6 hrs	
	L	R	L	R
Queen 1	2	2.2	3.2	2.9
	5	2.5	2.9	2.7
		$\bar{x} = 2.2$		$\bar{x} = 2.9$
Queen 2	2	2.2	3.2	2.7
	5	2.3	3.1	2.9
		$\bar{x} = 2.3$		$\bar{x} = 3.0$
Queen 3	2	2.6	2.6	2.9
	5	2.2	2.8	1.8
		$\bar{x} = 2.4$		$\bar{x} = 2.8$
Queen 4	2	1.8	2.2	2.6
	5	2.0	2.5	2.3
		$\bar{x} = 1.9$		$\bar{x} = 2.4$

In a final experiment it is intended to show that the distribution of the building pheromone around the queen is influencing worker building responses, and that consequently this stimulus is involved in the formation of structures built around the queen. In this trial, 3 live queens were each placed centrally 2 cm above a building arena on a copper netting of coarse mesh (0.5 per mm²). In the arena were 640 workers. Over the queen at a distance of 0.2 cm above her, a second netting was installed parallel to the first one, of finer mesh (2 per mm²) carrying prepared soil arranged in an ovally shaped ring of 5 cm width around the queen. Also here 640 workers were present. Underneath the queen workers constructed in 8 hrs 6 - 9 slender, outwardly inclined and often curved pillars connecting the soil surface with the netting. The distance between the pillars on either side of the queen was minimal at their base (0.8 - 1.1 cm), and reached a maximum of 4.3 - 5.6 cm at about 0.5 cm underneath the netting. The arrangement of the pillars underneath the queen was reminiscent of the frames of a ship. The frontal view is schematically depicted in cross section (Fig.12). Above the queen, pillars were also constructed on either side, 2 - 2.6 cm apart. These pillars after reaching a height of 0.3 - 0.4 cm were usually extended in a horizontal direction. The growing lamellae joined over the underlying queen, and formed a roof with a maximum distance to the netting of 0.45 cm.

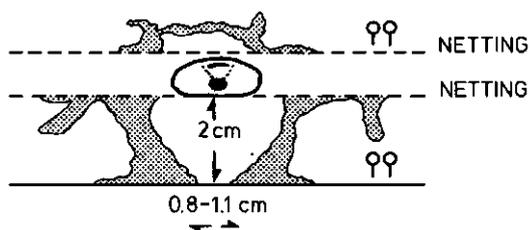


Fig. 12. Live queen on a netting over an arena, and underneath a second one. The shaded areas schematically represent a cross section through the structures built around her.

In conclusion it can be stated that the building pheromone produced and released by the queen is the primary stimulus accounting for queen-oriented building behaviour, and that this pheromone has a likely function in the formation of the royal cell.

DISCUSSION

This study demonstrates that building behaviour of workers of Macrotermes subhyalinus around their exposed physogastric queen, leading to the construction of an earthen vault covering her (replacement royal cell), is initiated and maintained by a volatile stimulus produced by the queen. This stimulus, which was named a building pheromone, originates from the 'royal fat body' (Table 2 and 3). The 'royal fat body' is formed during the transition of a female de-alate into a physogastric queen (Bordereau, 1971). The precise physiological function of this organ is unknown; its oxygen consumption is however extremely high (Gabe and Noirot, 1960; Wyss and Lüscher, 1975; for a review see Han, 1978). Recent investigations into the metabolic processes of this tissue have shown a high activity in the oxydative metabolism of fatty acids (Abo-Khatwa, personal communication), and preliminary investigations have demonstrated that unsaturated fatty acids with a chain length of 16 carbon atoms released a building response of workers in the extract bioassay (G.D.Prestwich and O. Bruinsma, unpublished). Holmgren (1909); Mukerji and Raychauduri (1942) suggested that the tracheal system might serve as its secretory ducts. Bordereau (1971) contested this hypothesis because according to him the trachea are air filled. Our results demonstrate that the fat body could well function as an exocrine gland, and that the spiracles are involved in the release of the pheromone. The specific function of the tracheal system in this respect has not yet been investigated in detail, however based on the fact that a dummy consisting of an abdominal cuticle with attached fat body but with tracheal rosettes disconnected of the corresponding spiracles yielded a short lived building response compared to the control, suggests that the tracheal system collects the pheromone and leads it to the spiracles. The morphological arrangement of fat body patches, each clustered around a rosette of trachea arising of a spiracle and ramifying into the patches, is not inconsistent with the possible function of the tracheal rosette as a pheromone conducting system. Furthermore, Han (1978) concludes that the 'royal fat body' originates from larval adipocytes, part of which may transform to tracheal epithelium.

Arriving at the spiracles, the building pheromone may evaporate directly into the air, as indicated in an experiment employing queens with varnished abdomen and open spiracles, as well as spread over the abdominal cuticle by surface diffusion and evaporated from there. The surface monolayer of wax of insect cuticles, according to Locke (1965) is suited for rapid diffusion of lipids if lipid-water liquid crystals are continuous with the monolayer at the surface. Lewis (1962) discovered that a radio-active preparation of di-iodo octadecane

applied on the tarsi of adult *Phormia* spreads within minutes over the whole of the integument, provided that the integument was part of a living, active insect. This finding is significant since the abdomen of the queen is continuously making pulsating movements. Queens with sealed spiracles continued to make these movements up to approximately 5 hrs after sealing.

The disparity in results obtained with caged queens (cell completion) and queens with varnished abdomens, but with intact spiracles (some pillar construction) indicates the importance of the abdominal cuticle in the release of full building behaviour leading to cell completion. Workers in between their runs frequently groomed and antennated the abdomen of the queen. The number of workers displaying this behaviour was reduced around queens with varnished abdomens and intact spiracles: 4 - 10 workers compared to 20 - 40 workers around intact queens (measured every 10 min during a 2 hrs interval). Furthermore, the average number of runs per worker per 10 min (workers introduced between min 20 - 30, $n = 34$) was 3.5 (range 1 - 5) around varnished queens and 6 (range 3 - 8, $n = 28$) around intact control queens.

Whether these differences in response are due to additional close range chemical information provided by the abdominal cuticle or to a decrease in building pheromone concentration and/or a change in its composition around the varnished queens is not known.

Spatial information is received by building workers, in view of the fact that these workers placed their load on the average at a certain distance from the queen. The results of the present investigation suggest that workers employ the distribution of the building pheromone as a cue for distance orientation. The precise mechanism by which they detect the proper distance is unknown. However, it is interesting to speculate that workers may possibly be searching for pheromone threshold concentrations as indicated by a typical serpentine walking pattern depicted in Fig. 5 (solid line), facilitating deposition behaviour.

The conclusion that a volatile agent, a building pheromone, provides a building releasing stimulus and may be involved as a parameter for distance orientation (= Elasis, Jander, 1970) for workers engaged in replacement royal cell construction around the termite queen, adds a fascinating dimension to the still poor understanding of the formation of structural elements occurring in termite nests.

SUMMARY

1. Workers of the termite Macrotermes subhyalinus (Rambur)(Termitidae, Macrotermitinae) construct a replacement royal cell around an open-laid physogastric queen with soil pellets.
2. A specific sequence of behavioural activities is initiated around the queen, after a 2 - 5 min time lapse following worker introduction: (a) grasping of soil pellets near the queen; (b) transport of the pellet to the site of deposition, a zone around the queen located approximately at 2 - 5 cm distance from her; (c) deposition and cementing of the soil granules somewhere in that zone. After about 40 - 60 min of building activity, workers start to concentrate their depositions in one or more specific areas in the deposition zone. This leads to the construction of incipient pillars or columns, at 1.9 - 2.6 cm from the queen. These pillars are lengthened until they reach a certain height of 0.5 - 0.8 cm. Building workers then change the direction of building at the pillar apex in a lateral sense: the formation of lamellae. The growing lamellae are extended and connected to one another, to form a roof over the queen, while pillars are connected to form a wall.
3. The sequence of behavioural activity is initiated by a volatile stimulus, a building pheromone, which is found to be produced in the queen's 'royal fat body' (Table 2 and 3), and which is released via the spiracles. Part of the pheromone evaporates directly from the spiracles, and another part very likely spreads over the cuticle of the queen's abdomen by surface diffusion, and probably evaporates from there.
4. The results of experiments intended to manipulate the distribution of the building pheromone around the queen suggest that building workers employ this distribution as a cue for deposition distance orientation.

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CHAPTER III

BUILDING BEHAVIOUR OF WORKERS OF MACROTERMES SUBHYALINUS AROUND THEIR QUEEN

INTRODUCTION

The social behaviour of termite workers involved in the (re)construction of their often-very elaborate nests is a fascinating but rather poorly understood phenomenon (Wilson, 1971). Most of these nests result from the building activity of countless workers. The question arises how do building workers communicate and how are their individual activities co-ordinated in space and time? In the previous chapter the construction is described of an arched roof (a replacement royal cell) made of cemented soil pellets over an exposed physogastric queen of Macrotermes subhyalinus (Rambur) by workers of the colony. In general, workers grasp soil granules near the queen and place them in a deposition zone on either side approximately between 2 and 5 cm removed from her. The behavioural sequence of grasping, transporting and depositing/cementing a pellet is shown to be initiated by a pheromone produced by and emanating from the queen. The hypothesis is advanced that workers employ the distribution of this volatile building pheromone in their deposition distance determination. Such a mechanism is sufficient to explain the initial random distribution of the depositions in the zone around the queen, but it does not elucidate the succeeding concentration of part of the depositions at several specific sites in that zone. Such a concentration is a requirement for the construction of pillars, which are essential in cell building. Furthermore, the rapid increase of the number of workers participating in construction and the general decrease in transport time between the sites of soil removal and of deposition are not satisfactorily explained.

It has been well established that termite workers use odour trails in their orientation between important sites in- and outside of the nest (Stuart, 1969; Wilson, 1971; Leuthold, 1975). It is therefore of interest to investigate the role of the trail pheromone and other factors possibly involved in the construction of an earthen vault over the queen. In this chapter the results are reported of a study in which the workers around an exposed physogastric queen were exposed experimentally to extracts of the glandular sources of several pheromones and to extracts made of soil cemented by the workers. The consequences of the presence of the pheromones evaporating from these extracts, often combined with mechanical cues, on the building performance are discussed.

MATERIALS AND METHODS

Experimental animals

The termites were obtained, kept and observed by the same technique and procedures as described in the preceding chapter.

Extracts

Trail-active extracts were prepared from a variable but known number of extirpated sternal glands, immersed in a calibrated volume of redistilled hexane, and extracted during 24 hrs at -16°C . Their biological activity was measured in 'trail-units' (TU) according to the method of Leuthold and Lüscher (1974). Extracts of salivary glands were made of 40 excised glands, homogenized in 0.5 ml of distilled water (0°C), extracted for 1 hr at 4°C and subsequently filtered. In order to collect the undiluted salivary gland content, glands after extirpation and rinsing in a Ringer solution (0°C) for a few seconds, were punctured with the tip of a capillary tube. Extracts of fore- and midgut were made of 40 intestine sections extirpated, homogenized in 0.5 ml of distilled water (0°C), extracted for 1 hr at 4°C and filtered. Extracts of soil from nest mound repair sites (soil was collected between 12 and 24 hrs after nest damaging from those areas where workers were still observed to construct) were prepared from a variable but unknown amount of soil, immediately immersed in a calibrated volume of distilled water, extracted during 24 hrs at 4°C , and finally filtered. Extracts acting as controls were prepared in a similar fashion from sterilized (6 hrs, 110°C), sieved friable red volcanic soil (referred to as 'prepared' soil) collected near the laboratory. The behavioural sequence of grasping, transporting and deposition/cementing a soil pellet is referred to as a 'run'. Additional methodological details will be given with the description of the individual experiments.

RESULTS

The influence of group size on building activity around the queen

Since the building behaviour of workers of M. subhyalinus is a mass phenomenon, in a first approach the influence of group size on several aspects of construction behaviour was studied. These aspects were 1) the building latency time (the time which elapsed between the introduction of the workers in the arena with the queen and the first observed grasping of a soil pellet within 0.5 cm from the queen); 2) the total number of depositions in the deposition zone during the first 60 min after worker introduction; and 3) the number of soil granules placed in the deposition zone by single workers (marked with a

dot of non-toxic nitro-cellulose paint on the abdomen) introduced 50 min after the start of the experiment, and observed during a 10 min interval. The tested groups consisted of 20, 40, 80, 160 or 320 major workers respectively. These groups were observed during a 60 min interval. The results of observations focussing on the first two aspects are depicted in Fig. 1. They reveal that the larger the group of workers, the shorter its building latency time. The relation between the two was established to be exponential (Jonckheere-Terpstra test, $P < 0.001$). Furthermore, the total number of depositions in 60 min shows a more than proportionate increase with increasing group size up to and including the group of 80 workers. In the larger groups, a largely proportionate increase with the number of workers was observed (Fig. 1). The data recorded in the third

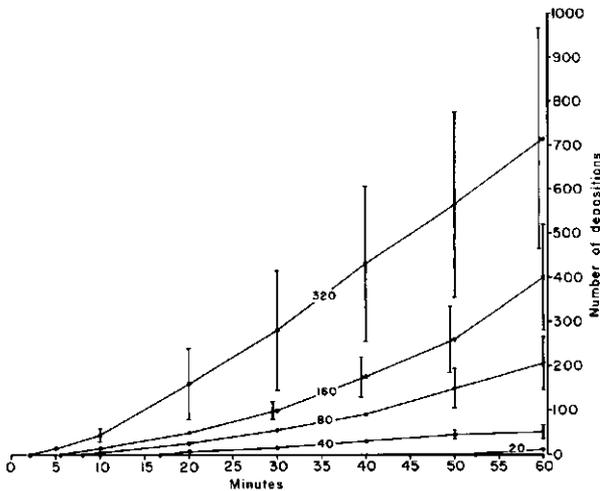


Fig. 1. The mean number of depositions observed in 60 min after worker introduction in differently sized groups around the queen, presented in a cumulative way. The vertical lines represent the standard deviations (no. of replicates = sample size: 6-8).

experiment, the introduction at min 50 and subsequent observation of marked workers, show an increase in individual building activity of a sigmoidal form, with an increase in the size of the group into which these workers were introduced (Fig. 2). In summary, these results clearly show that group performance as well as single worker performance with respect to building activity is influenced by the size of the group. Although these observations are in accordance with the phenomenon of mutually reinforcing stimulation of activity, typical for social insects (Wilson, 1971), the underlying causal mechanism is unknown.

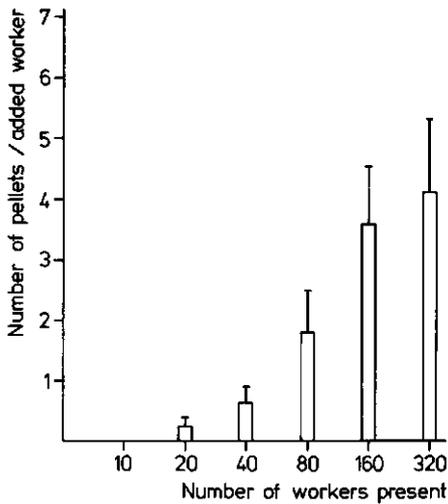


Fig. 2. The mean number of depositions per worker introduced at min 50 after the start of the experiment in differently sized groups, measured in a 10 min interval following introduction. The vertical lines represent the standard deviations. Data are based on 30 replicates of 10 min tests.

The influence of trail pheromone on building behaviour around the queen

In termites, the information required for orientation in space is frequently mediated by pheromones, e.g. chemical trail orientation. From this observation the hypothesis was deduced that workers may lay odour trails around the queen while engaged in building or while encircling the queen. In order to investigate whether building workers lay pheromone trails, a thin layer of top soil in a zone around the queen was collected 45 min after 320 workers and a queen were introduced in the observation box. From the 3 cm wide zone located between 1 and 4 cm distance, 5 g of soil was collected and immediately immersed in 5 ml of redistilled hexane and extracted during 24 hrs at -16°C . Soil collected in a similar zone around queens 45 min after their introduction in arenas without workers served as control. In all three cases trail activity was detected in extracts obtained from boxes with workers, employing a method described by Leuthold and Lüscher (1974). The extracts contained between 10 - 30 TU/2.5 μl ; no biological was found in control extracts. These results suggest that workers may lay pheromone trails around the queen. In order to assess whether building workers around the queen were able to follow pheromone trails connecting the grasping and the deposition zone the following experiment was executed. A group of 75 major workers with varnished 4th and 5th sternite, preventing the sternal gland from releasing its trail pheromone, were introduced around the queen together with 5 untreated but marked (dot of nitro-cellulose paint on the abdomen) major workers. After building activity had started, 7 - 13 min

after worker introduction the pathways were recorded of the marked workers during soil transport. Out of 60 pathways (recorded on the transparent lid of the observation box), 4 were followed for at least 5 cm by transporting varnished workers. The four followed trails were between 2 and 8 sec "old". In another trial, out of 35 recorded pathways, 6 were followed by varnished transporting workers. The observation period was confined to 30 sec following the record of the pathway. The marked workers were removed from the arena for a period of 5 min in case trail following had been recorded, after which period they were replaced by another lot. None of the control pathways (n = 95), laid by varnished workers, was followed for at least 5 cm. These results indicate that transporting workers under these experimental conditions are capable of following individual pheromone trails.

The possible function of the trail pheromone was then studied using major workers with varnished sternites. These workers were introduced around the queen in the already mentioned differently sized groups, and observed during a 60 min interval. The following aspects were recorded: 1) the building latency time; 2) the number of workers crowding around the queen, i.e. present in a 0.5 cm wide zone bordering the queen; and 3) the total number of depositions in the deposition zone.

It appears from the results presented in Table 1, that the building latency times of the respective varnished groups were similar to those with correspondingly sized groups of untreated workers. Furthermore, no significant difference could be observed with respect to the total number of depositions between corresponding groups of treated and untreated workers, e.g. 160 varnished workers: average 540 depositions (range 403 - 721); 320 varnished workers: average 985 depositions (range 788 - 1216); data were in each case obtained from 5 trials. In treated groups, the number of workers crowding around the queen was higher than this number in not treated groups up to and including groups of 40 workers. In bigger sized treated groups of 160 and 320 workers this number did not differ to an appreciable extent from control groups (Table 2). These results suggest that the building latency time is affected by the number of workers crowding around the queen, regardless whether these workers are varnished or not.

In conclusion, the absence* of trail pheromone did not seem to influence the general building activity in a negative way. Unexpectedly however, the building activity of even the largest group of varnished workers did not lead to the

*Extracts of top soil collected in two tests employing 320 varnished workers did not contain any detectable trail activity.

Table 1. Building latency times (min) of differently sized groups of varnished and not varnished major workers. Details in text.

Varnished						
	Experiment no.	1	2	3	4	5
Group size	10	57	59	> 60	> 60	> 60
	20	18	24	38	42	49
	40	7	8	13	18	
	80	5	5	8		
	160	2	3	7		
	320	2	4	6		
Not varnished						
	Experiment no.	1	2	3	4	5
Group size	10	> 60	> 60	> 60	> 60	> 60
	20	45	50	52	58	> 60
	40	13	15	18	22	
	80	6	9	10		
	160	4	6	7		
	320	2	3	3		

construction of a replacement royal cell, nor to its prerequisite the pillar. Instead, after 12 hrs two oblong flattened ridges of deposited soil granules had been constructed parallel to and on either side of the queen (four observations). The ridges were 0.3 - 0.4 cm above soil level, 0.9 - 1.5 cm wide and located at distances ranging from 0.8 - 1.7 cm from the queen (Fig. 3). Extension of the experimental period up to 24 hrs, and the introduction of an additional 400 varnished workers did not alter this situation to a large extent (3 replicates), although the queen was slowly 'sinking' below the original soil level due to the continuing removal of soil pellets near and underneath her. Three control experiments were then conducted, employing 320 workers each, of which the first two sternites were varnished. In all three trails cell completion was observed in 8 - 10 hrs. These results prove that trail pheromone plays a role in the co-ordination of building activity leading to pillar construction. In order to investigate the role of trail pheromone more closely, highly trail-active extract in hexane (500 TU/2.5 μ l) was used in the following way: extract

Table 2. Summary of data from varnished and unvarnished workers crowding around the queen (present in a 0.5 cm wide zone around the queen). Data are means, based on the lumped replicates of observations made every 10 min during 60 min after worker introduction.

Varnished						
Experiment no.		1	2	3	4	5
		No. of workers crowding				
Group size	10	10	10	10	10	9
	20	17	18	18	20	20
	40	36	36	38	38	38
	80	35	45	51	53	57
	160	46	50	51	54	58
	320	45	50	53	55	60

Not varnished						
Experiment no.		1	2	3	4	5
		No. of workers crowding				
Group size	10	3	3	5	5	7
	20	9	10	10	11	12
	40	17	17	20	24	25
	80	30	35	35	38	39
	160	36	40	40	44	45
	320	42	45	48	50	52

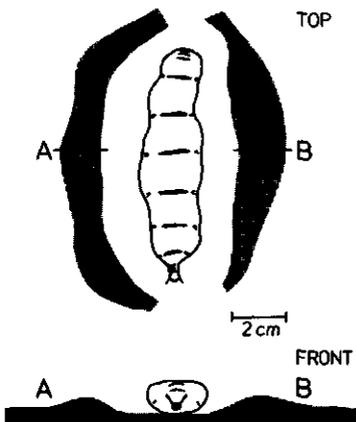


Fig. 3. A schematic representation of the results, after 24 hrs, of building activity of workers with varnished sternal glands around their queen.

was applied as an artificial trail with the aid of a cotton thread (L: 7 cm, \varnothing : 0.3 mm) soaked in the extract and placed at right angles to the queen's longitudinal axis; the extract was reapplied every 10 min. After the introduction of the trail a group of 80 varnished workers was placed in the arena, and observed for a 60 min interval. Workers grasping soil bits near the queen and within 1.0 - 1.4 cm from the artificial trail were frequently observed to follow that trail while transporting the soil pellet. Records were made in three trials during the first 30 min after worker introduction: of a total of 78 observations of a worker grasping a pellet in the above area, 55 followed the trail whilst transporting the pellet. They deposited their load within 1.5 - 5 cm from the queen along the trail (Fig. 4) after an average transport time of 4.3 ± 0.9 sec ($n = 45$); after deposition, only 16 of these workers followed the trail again while returning to the queen. The respective period of time of

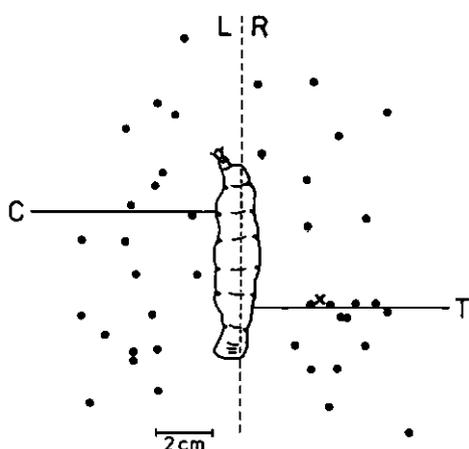


Fig. 4. A characteristic example of the distribution of the first 20 deposition sites (black dots) on either side of the queen, made by 80 major workers with varnished sternal gland.

C: cotton thread soaked in hexane, acting as control.

T: cotton thread soaked in trail-active extract.

X: location of the first observed pillar, constructed after the introduction of an additional 80 varnished workers (see text for further explanation).

45 workers neither grasping a soil bit near the trail nor following the trail during transport was 8.9 ± 2.2 sec. In order to test whether the observed concentration of depositions along the trail could lead to pillar construction, the above experiment was continued beyond 60 min with the introduction of an additional lot of 80 varnished workers under these conditions, in three out of four replicates pillar construction was observed, along the trail at distances of 1.6 - 2.3 cm from the queen. In five additional tests using 160 varnished workers the results proved to be positive in four out of five trials. At min 74, 96, 110 and 83 after the introduction of the workers, the first incipient pillars (structures of 0.2 cm high) were constructed at 1.7, 2.0, 2.4 respec-

tively 2.4 from the queen and all within 0.5 cm from the trail. It is apparent from these results that directional information is mediated by extract trails, leading to a biased distribution of soil transporting workers and consequently of depositions. Although the absence of trail pheromone around the queen may prevent for some period the required bias in deposition distribution, it remains unclear whether this factor is the only one involved. One could expect that through the employment of a large number of varnished workers, as shown in a previous experiment, by chance a few soil pellets would be cemented together forming an incipient pillar. However, whether a concentration of depositions will lead to pillar construction is also dependent on two other cues (see following sections).

The influence of the cement on building behaviour around the queen

Workers cement the soil pellets with a secretion emerging from their buccal cavity. Although according to Noirot (1969) a considerable amount of saliva is present in the cement, its nature as well its precise origin is still unknown. A first indication that this secretion influences the behaviour of nearby workers was found in a series of observations on building workers in petri dishes (\emptyset : 11 cm) charged with a 0.5 cm thick layer of 'prepared' soil. Quite often a worker was cementing its granule against another one already placed against the lid. No precise estimates were made of the 'age' of the first pellet when the second one was cemented, although it was clear that this time period did not exceed a few minutes.

Additional tests ($n = 40$) were done in similar dishes in which 50 workers were confined with soil this time with a freshly cemented pellet, applied 10 min after worker introduction, centrally on the lid which covered the dish with an air gap. The distance between the soil level and the lid was 1.0 cm. In each test, observations were made during 3 min after pellet introduction on workers passing in an area of 1 cm radius located directly underneath the pellet. Out of 27 soil transporting workers passing the scoring area, 14 stopped to inspect the pellet with the antennae, while 6 of the inspecting workers cemented, or attempted to cement the granule against the one already on the lid. In control tests, the application underneath the lid of granules of prepared soil moistened with distilled water, or of cemented pellets 2 - 3 hrs after deposition did not seem to attract the interest of the workers passing by. Employing the same experimental approach, it appeared that freshly cemented pellets exerted such influence on workers passing nearby for a period of 1 - 3 min. It is assumed that the cement during a relatively short period of time releases a volatile

agent, attracting nearby workers in search for a deposition site.

While conducting the above series of tests the impression was obtained that this pheromone influences the behaviour of non-building workers as well. Non transporting workers passing nearby the pellet were sometimes observed to grasp a pellet in the scoring area and to cement it against the lid or near the one already present.

The following bioassay was employed to locate the source of the volatile agent, hereafter referred to as the 'cement' pheromone, and to examine more closely its influence on the behaviour of non-building workers. Two bottom halves of petri dishes (\emptyset : 11 cm) were charged with a 0.3 cm layer of 'prepared' soil. Into each of the dishes 50 freshly collected major workers (see previous chapter) were introduced. After an 'exploration' period of 10 min, a capillary tube (\emptyset : 0.1 cm) containing 2 μ l of an extract of soil, or pure salivary gland content, or an extract of fore- and midgut sections, was suspended from the plexiglass plate which covered both dishes with an air gap (Fig. 5). The tip of the capillary tube was at 1.5 cm above soil level in the centre of the dish. In controls the tubes contained distilled water. Starting from the moment of tube introduction the time was recorded (maximum time allowed: 3 min) within which the first worker grasped or deposited a soil bit within a circle of radius 1 cm centered underneath the tip of the capillary tube or attempted to cement its load against the tube. The results of the bioassay are shown in Table 3. It is clear from the data that the salivary gland is the

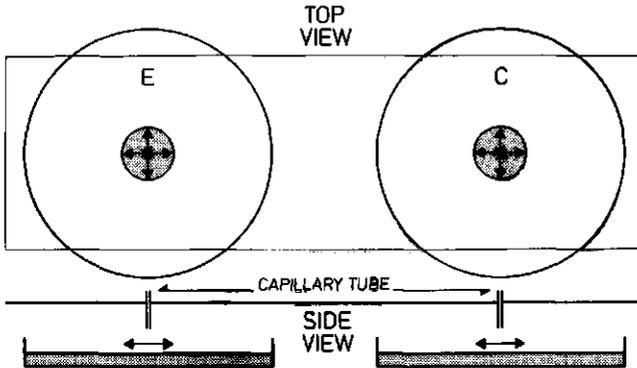


Fig. 5. The 'cement' pheromone bioassay arrangement. The shaded circle with arrows (top view) represents the scoring area for soil pellet grasping or deposition. E: with capillary containing the various extracts or salivary gland content. C: Dish with capillary containing distilled water (control). For further explanation see text.

Table 3. Results of the 'cement' pheromone bioassay.

The figures refer to the period of time (sec) elapsed between capillary introduction and the first worker displaying a building response. Maximum time allowed 180 sec.

(-) : no building response within 180 sec.

\bar{x} : mean of data only referring to periods \leq 180 sec.

Workers and soil were replaced every 3rd experiment.

Capillary content	Latency time (sec)	Fraction of response within 180 sec
Salivary gland contents	3 x (-), 43, 55, 74, 62, 10, 9, 18, 27, 36, 50, 31, 56, 68, 49, 138, 122, 37, 41, 43, 14, 50, 71, 110, 143, 151, 160, 90.	27/30
	\bar{x} : 65.1 \pm 44.5 sec.	
Extract of fore- and midgut	24 x (-), 88, 91, 123, 149, 169.	6/30
Extract of freshly cemented soil	8 x (-), 62, 39, 73, 103, 116, 136, 32, 39, 63, 69, 78, 81, 133, 121, 158, 50, 61, 40, 36, 112, 139, 16.	22/30
	\bar{x} : 79.9 \pm 41.0 sec.	
Extract of cemented soil, 7 days old	5 x (-), 13, 21, 56, 36, 88, 126, 45, 73, 113, 93, 135, 60, 56, 43, 25, 87, 165, 119, 85, 40, 56, 71, 33, 19, 114.	25/30
	\bar{x} : 70.9 \pm 40.7 sec.	
Extract of 'pre-prepared' soil	26 x (-), 98, 153, 140, 168.	4/30
Distilled water	27 x (-), 116, 148, 155.	3/30

source of the 'cement' pheromone. The response of non-building workers can be summarized as follows: when a worker was approaching the tube containing 2 μ l of salivary gland content, it stopped (71 out of 90 workers) at a horizontally measured distance of 0.8 - 1.5 cm from the capillary tip while making probing movements with its antennae, mostly in the vertical plane. After 1 - 3 sec it turned towards the tip and while standing on his four posterior legs it inspected the tube with its antennae (64 out of 71 workers; the remaining lot continued their path). It was observed frequently that when the capillary tip was low enough above the soil (1 cm) workers drank from the tube content. Out of the 64 inspecting workers, of which 31 drank from the tube, 41 grasped soil pellets in the vicinity (circle of 1 cm radius centered around, underneath the tube) of the capillary, and tried to cement them against the tube (15 out of 41 workers) or walked away from the tube while kneading the soil granule with the mandibles. Soil transporting workers passing within 1 cm from the capillary tube gave similar responses. Out of 36 workers, 24 deposited their load within the scoring area around the tube or tried to cement it against the tube. In control experiments with capillary tubes containing 2 μ l of distilled water, out of 40 transporting workers passing within 1 cm from the tube, 3 deposited their pellets in the scoring area; out of 40 non-transporting workers, 1 worker was observed to grasp a granule in that area.

The data clearly reveal that the 'cement' pheromone is involved in several behavioural responses. It orients workers from distances of 1.0 - 1.5 cm to the site of pheromone release (the capillary tip). Moreover, it induces in this area grasping behaviour in non-building workers and a deposition response in soil carrying workers. The 'cement' pheromone could then be considered as a volatile marker for a building site, and hence might be directly involved in cementing together of soil pellets.

In addition to saliva, compounds originating from the fore- and midgut may constitute part of the cement. In the absence of suitable radio-active markers the following procedure was employed to examine this assumption. Major workers from nests kept in the insectary were allowed to forage green grass. After 2 - 3 hrs a lot of 100 of these workers were introduced around an open-laid queen. Samples of water extracts of the freshly built structures examined under the microscope contained chloroplasts and plant debris particles, which were also present in the fore- and midgut preparations of simultaneously dissected nestmates. It is concluded that part of the secretory originates from the fore- and midgut.

An interesting phenomenon is the limited period of time a freshly cemented pellet is influencing the behaviour of nearby workers. Since extracts made in distilled water of dry and hardened structures (e.g. 1 week old replacement royal cells, or parts of royal cells obtained in the field), tested in the bio-assay appeared to elicit a response similar to the response induced by extracts made of freshly built structures (Table 3), the possibility is considered that the evaporation period of the 'cement' pheromone is influenced by the degree of moisture contained in the pellet. Very often soil pellets are transformed during transport and emerge from the buccal cavity as a fluid paste. In a pilot experiment in which cemented pellets ($n = 40$; each pellet was positioned centrally underneath a lid covering a petri dish containing 50 major workers and soil) immediately after application on the lid were touched with a strip of filter paper, draining the liquid cement, it was shown that such treated pellets did not elicit the above response. Subsequent wetting of these pellets with distilled water demonstrated that 15 out of 40 of the pellets induced again a building response. That is to say it has been observed 12 times that a worker grasped a pellet in the scoring area, and 3 times that a worker cemented or attempted to cement its load against the pellet on the lid. Regnier and Goodwin (1977) concluded from experimental results that the evaporation of polar odorants is affected by humidity changes. At high humidity the evaporation of such odorants substantially increases. The humidity level recorded at a few mm above soil level of deposition sites used by varnished workers (700; 3 replicates) 4 hrs after their introduction around the queen ranged between 75 - 90% at 23°C; records obtained within a few mm from 15 incipient pillars under construction ranged between 93 - 96% at 23°C. Further experimentation is required for a precise evaluation of this phenomenon.

In order to investigate the consequences of the behavioural responses elicited by the 'cement' pheromone during the initial building stage around the queen, the following experiment was conducted. On either side of the queen a wetted soil patch (\emptyset : 1 cm) was introduced at 2.5 cm removed from her. One patch was soaked with 0.5 ml of distilled water, the other with 50 μ l of salivary gland extract dissolved in 0.5 ml of distilled water. Both patches were wetted with 50 μ l of distilled water every 3 min, and received 0.5 ml of distilled water respectively diluted extract every 15 min, in order to maintain a high relative humidity level of at least 90 - 95% (measured with Telemax probe 1) within a few mm above the patches. The building latency time and the site of the first 20 depositions on either side of the queen were recorded while employing 80 major workers with varnished sternal gland. The result of one of

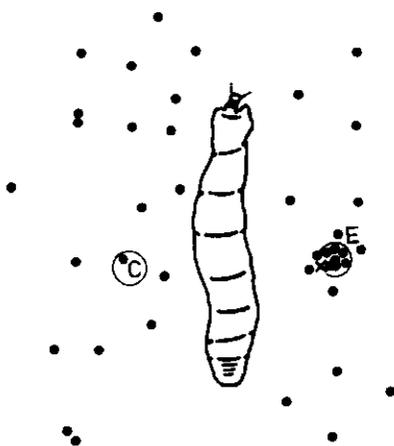


Fig. 6. A characteristic example of the distribution of the first 20 depositions on either side of the queen, made by 80 varnished major workers. The circles (\emptyset : 1 cm) indicate the presence of a water soaked patch (C) and a patch soaked in water extract of salivary glands (E). X: location of the first pillar constructed after the introduction of an additional 80 major workers.

the experiments is depicted in Fig. 6. The outcome of four replications showed that the initial deposition distribution was biased on one side of the queen towards the extract treated patch. Moreover, the latency time was reduced compared to that obtained in controls (queens with water soaked patches on both sides) from an average of 8 min (range 5 - 12 min), to an average of 2.5 min (range 1 - 4 min). In three tests a lot of 80 varnished workers were additionally added 60 min after worker introduction of the first 80 varnished workers. Incipient pillar construction was observed on or near the extract treated patch 30 - 50 min after the introduction of the additional workers (Fig. 6). In five similar trials using 160 major workers with varnished 4th and 5th sternite, on or near the extract patch the first incipient pillar (a structure of at least 0.2 cm high) was constructed. This occurred at min 53, 65, 88 and 92 after worker introduction. In one experiment no pillar was constructed. These data confirm the role of the 'cement' pheromone as a building initiating and building co-ordinating agent.

The cumulative release of 'cement' pheromone during the building process around the queen may, for a part, be responsible for the rapid increase of the number of depositions (Fig. 1). This is indicated by the results of an experiment in which salivary gland extract (0.5 ml) was introduced together with 0.5 ml of distilled water in an area (4 x 1 cm) located in the deposition zone at a distance of 2 cm from and parallel to the queen. The number of depositions was recorded during a 10 min interval following the building latency time of a

group of 80 varnished workers. In three replicates, these workers deposited 58, 65 and 94 pellets, while in controls, the area wetted with 1.0 ml of distilled water, workers scored 12, 16 respectively 9 pellets.

The influence of tactile stimuli on building behaviour around the queen

The observation that workers in arenas without a queen invariably start constructing along and against the walls, indicates the importance of tactile stimuli for workers in search for a deposition site. In order to investigate this influence on building behaviour around the queen, four steel globes (\emptyset : 0.2 cm) were placed on the soil in the observation box, 2 on either side of the queen at a distance of 2 cm opposite spiracles no. 2 and 5. These tiny globes were held in place with small magnets* underneath the box. In this experiment 160 workers with varnished sternal gland were employed. Within 15 min after the start of the experiment the tiny spheres became foci of deposition activity, and after a period with a mean length of 33 min (range: 28 - 40 min; n = 5) they were already transformed into the first pillars (0.4 cm high) around the queen. After reaching a height of 0.6 - 0.8 cm the workers started adding soil pellets in a more lateral direction marking the beginning of the construction of lamellae on the pillar apex. The observations lasted 60 min. These results prove that workers are sensitive to mechanical stimuli, and that workers may employ this tactile information in the precise location of a deposition site. In order to assess whether mechanical stimuli only elicited a deposition response, the termites were presented with spheres around the queen placed in the above arrangement, however this time within a few mm from the queen. During the first 30 min after worker introduction (160 varnished major workers) it was observed 27 times that a termite attempted to grasp a sphere (mandibles wide open), while a deposition response with respect to the sphere was not observed. This observation will be treated in detail in the following section.

The influence of stimulus combination on building behaviour around the queen

Since under more 'natural' conditions workers are engaged in replacement cell construction in the presence of three pheromones: the building pheromone of the queen, the trail and the 'cement' pheromone, together with tactile stimuli (the influence of worker crowding is not considered here), it is of

*Magnets alone did not influence the distribution of deposited soil pellets in the deposition zone.

interest to study the influence of several combinations of chemical and mechanical signals on the building performance around the queen. The time required to construct a first pillar around the queen proved a useful criterion to assess the building performance. The first pillar is defined as a structure of at least 0.2 cm high without a globe respectively 0.4 cm high with the globe. The experimental approach is outlined below. Major workers (160) with varnished sternal glands were introduced around a queen in arenas with different arrangements:

- 1) An artificial trail (described above) was placed perpendicular to the queen's longitudinal axis, opposite spiracle no. 5. The trail-active extract was re-applied every 10 min (Arrangement T, Fig. 7).
- 2) In addition to 1), a soil patch (\emptyset : 1.0 cm) soaked in salivary gland extract (50 μ l) and distilled water (0.5 ml) was introduced at 2.5 cm from the queen and at 1 cm from the extract trail. The mixture of water and extract was re-applied every 10 min (Arrangement S + T, Fig. 7).
- 3) In addition to 2), a steel sphere (\emptyset : 0.2 cm) was introduced and held in place with a magnet in the centre of the extract patch. The extracts were regularly reapplied as described above (Arrangement G + S + T, Fig. 7).
- 4) The fourth arrangement (G, Fig. 7) with the four spheres placed at 2 cm distance from the queen, has been described already.

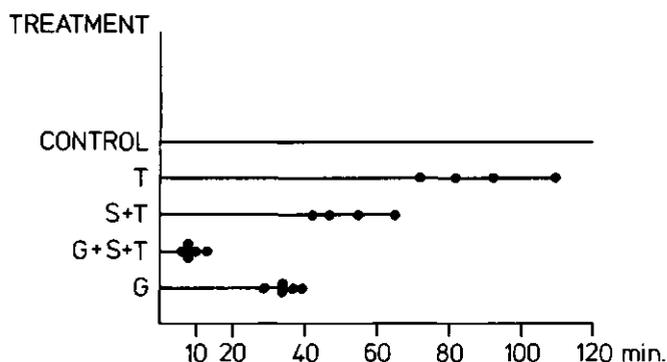


Fig. 7. The time of appearance (black dots) of the first pillar (0.4 cm high when constructed on a globe; 0.2 cm high without the globe) constructed in tests using 160 major workers with varnished 4th and 5th sternites. Each black dot represents one test.

CONTROL: Queen is present (a total of 8 tests).

T: In addition to the queen, an extract trail is introduced.

S+T: In addition to T, a patch soaked in salivary gland extract is present.

G+S+T: In addition to S+T, a metal sphere is placed centrally in the extract treated patch.

G: Four spheres are positioned around the queen.

For details see text. The horizontal lines have no significance and are only drawn for the sake of clarity.

Acting as control, hexane trails, water soaked patches and holes (\emptyset : 0.2 cm) in the centre of these patches were offered simultaneously on the other side of the queen. The results are summarized in Fig. 7. They clearly demonstrate a reduction of the time required to construct a small pillar when the various cues were presented in combination. The presence of only the mechanical stimulus (Arrangement G) immediately released a deposition and cementing response in soil carrying workers when arriving at the sphere. This fact explains very well the relatively short period of time required to construct a pillar under these conditions. The difference in transport time of workers following the extract trail in situation G+S+T: mean 3.4 ± 0.8 sec ($n = 35$) and in situation S+T: mean 6.1 ± 1.9 sec ($n = 35$) was significant (t-test, $P < 0.01$). These effects may result from the fact that workers in situation S+T arriving at the extract patch were standing on, or walking on and around the treated area prior to deposition, while corresponding workers in situation G+S+T in general deposited their load when they encountered the globe.

In view of the complex stimulus environment of workers building around their exposed queen, it seems necessary to re-investigate building behaviour of an individual worker. What induced the worker around the queen to grasp or to deposit a soil granule? The experimental procedure simply consisted of the introduction at specific distances between 0 and 2.5 cm from the queen, of steel spheres (\emptyset : 0.2 cm). The globes were placed opposite spiracle no. 3 on the soil and held in place by small magnets underneath the arena. The number of graspings, respectively depositions with respect to the sphere was recorded between min 20 and 40 after the introduction of 160 workers. When a soil pellet had been cemented on or against the sphere, it was replaced by another one. The results of a combination of 2 trials are depicted in Fig. 8. They show that the ratio grasping/depositing is 1 near the queen and rapidly decreases when the spheres are at distances exceeding 1.0 cm. It is assumed that this effect is specifically due to the influence of the building pheromone of the queen. This assumption is based on the fact that the addition of neither the trail pheromone nor the 'cement' pheromone changed the above results in an appreciable way. This was demonstrated in an experiment in which the spheres were positioned at some of the above mentioned distances from the queen, each globe located at 0.3 cm distance along an artificial trail (L: 4 cm, trail-active extract re-applied every 10 min) or placed in the centre of a patch with radius 0.5 cm wetted with 10 μ l of salivary gland extract and 10 μ l of distilled water, or both. The results are given in Table 4: data depicted in Fig. 8 serve as control.

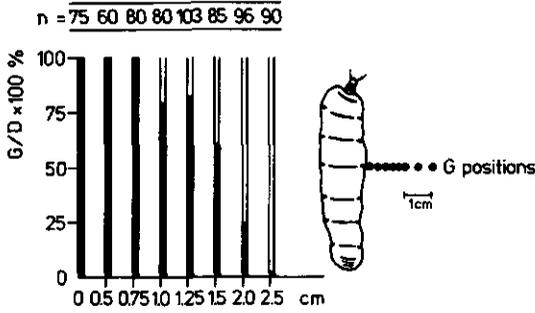


Fig. 8. The ratio between grasping (G) and deposition (D) behaviour with respect to metal spheres (\emptyset : 0.2 cm) placed at defined distances from the queen. n: the number of workers displaying a building response. Data are based on a combination of two tests, each employing 160 major workers.

Table 4. The ratio (%) between grasping and deposition with respect to metal spheres placed between 0.5 and 1.5 cm from the queen opposite spiracle no. 3.

n: number of workers displaying a building response.

T: arrangement with trail-active extract.

S: arrangement with salivary gland extract.

S+T: arrangement with trail-active and salivary gland extract. For details see. text. Data from Fig. 8 serve as control.

T			
n:	50	50	50
Grasp/deposit ratio (%)	100	82	48
Distance from the queen	0.5 cm	1.0 cm	1.5 cm
S			
n:	50	50	50
Grasp/deposit ratio (%)	92	66	22
Distance from the queen	0.5 cm	1.0 cm	1.5 cm
S+T			
n:	50	50	50
Grasp/deposit ratio (%)	90	78	30
Distance from the queen	0.5 cm	1.0 cm	1.5 cm

DISCUSSION

Three different chemical signals are influencing building behaviour of workers of Macrotermes subhyalinus engaged in replacement royal cell construction: 1) The building pheromone emanating from the queen; discussed in the previous chapter; 2) The trail pheromone originating from the worker sternal gland; and 3) A newly described pheromone, named the 'cement' pheromone, originating from the worker salivary gland.

Two of these pheromones, the building pheromone of the queen and the 'cement' pheromone constitute agents for the initiation of building behaviour. Although no effort was made to determine the effect of trail pheromone in this respect, it is possible that the presence of this pheromone around the queen may initiate or facilitate a building response. In addition to the chemical stimuli, unknown crowding effects are also involved in the initiation of building behaviour. This has been clearly demonstrated by the recorded building latency time of the differently sized groups; the latency time decreased with an increase in group size. The observed accelerating effect of time and group size in the building activity (measured as the number of depositions per time unit) during the first 60 min around the queen, may therefore well be due to the above three pheromones in concerted action with unknown effects initiating building and generated by workers crowding.

A very important second aspect of the discussed pheromones is that they contain an orientational component: distance orientation is mediated by the queen building pheromone (see previous chapter) while direction orientation provided by the trail and the 'cement' pheromone is demonstrated in this chapter. The question implicated in the introduction of this chapter is whether building workers do employ cues additional to the building pheromone of the queen in order to construct pillars. In order to build a pillar in the deposition zone around the queen (and elsewhere), workers have to place some pellets against or on top of each other. In other words, to increase the probability that a few soil pellets are cemented together, the distribution of soil carrying workers and of depositions has to be biased temporarily. The results of experiments employing extract trails and the observation that building workers lay pheromone trails around the queen, which may be followed during soil transport, are interpreted to indicate that trail pheromone is involved in the orientation of soil carrying workers. Such orientation may lead to a concentration of transporting workers at certain sites in the deposition zone and hence to a concentration of depositions (Fig. 4), which may be responsible for a local concentration increase in cement pheromone. As has been shown experimentally,

workers preferentially deposit their pellet in areas treated with salivary gland extract (Fig. 6). This volatile agent apparently is involved in the short range (1 - 1.5 cm) orientation of transporting workers to a building site. In this respect, somewhat unexpected was the finding that even large groups of varnished workers (700) were unable to construct pillars. A possible explanation for this failure to construct soil columns may be that due to the random distribution of pellets in the deposition zone the moisture content of the pellet was reduced to such a level which impaired the evaporation of the 'cement' pheromone. The absence of a building response of workers nearby freshly cemented pellets of which the liquid cement had been drained, may suggest such a mechanism. Regnier and Goodwin (1977) demonstrated that the evaporation of an odorant could be modified by the humidity, that is to say, at high humidity the evaporation of the odorant (polar compounds) substantially increases.

In conclusion it is suggested that both trail pheromone and 'cement' pheromone may serve the purpose of concentrating the distribution of soil transporting workers, respectively their depositions, around the queen, a prerequisite for the construction of pillars.

Once an incipient pillar has been constructed, tactile information by such a structure alone as simulated with small globes, is already sufficient to release further deposition behaviour. In spite of the presence of the complex of chemical and mechanical cues around the queen, the ratio between grasping and deposition at a particular site seems to be only dependent on the distance of that site to the queen. This suggests a qualitatively determining influence of the queen's building pheromone on worker response with respect to building. An important implication of the existence of the proposed mechanism involved in the co-ordination of workers engaged in replacement royal cell construction is that workers are capable of simultaneously perceiving and evaluating various different cues.

SUMMARY

- 1) Building behaviour of Macrotermes subhyalinus (Rambur) around their exposed physogastric queen was studied in the laboratory. Experiments were performed to investigate factors influencing building behaviour additional to the building pheromone of the queen, and to assess their possible role in the co-ordination of building workers.
- 2) Workers transporting soil pellets between the site of grasping near the queen and deposition, the latter in a zone of approximately 3 cm wide, 1 - 2 cm from the queen, may lay chemical trail marks. In the deposition zone soil pellets are cemented with a liquid secretion originating from the fore- and midgut and the salivary gland. A volatile agent, a newly described pheromone, named 'cement' pheromone, is evaporating during a few minutes from the drying cement, and may during that period induce a building response in workers nearby the site of pheromone release.
- 3) Both the trail pheromone and the 'cement' pheromone contain an orientation component. Soil transporting workers may follow chemical trails leading to the deposition zone. Some workers will converge upon sites of 'cement' pheromone release and may deposit their load at that site. Attraction is effective up to a radius of 1,5 cm. The resulting bias in deposition distribution is a prerequisite for pillar construction.
- 4) Tactile perception of agglomerations of granules stimulated to contributive deposition behaviour, leading to the construction of pillars.

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CHAPTER IV

FACTORS INVOLVED IN THE FORMATION OF SOME BASIC BUILDING ELEMENTS IN
MACROTERMES SUBHYALINUS (RAMBUR).

INTRODUCTION

The nest mounds of *Macrotermes subhyalinus* (Rambur) are composed of a variety of different architectural elements which, with the exception of fungus combs, are all constructed of soil pellets. These elements can be roughly grouped into several categories: 1) The thick and massive structure of the outer nest wall, with air passages ramifying in the epigeous part of the nest mound; 2) The laminar structure composing the central nest area (the hive), consisting of layers of thinly constructed horizontal lamellae supported by pillars; 3) The royal cell, situated at different levels in the hive, but most frequently located near the base of the hive; 4) The layer of fungus combs 'draped' over the hive, separating the hive from the outer nest wall; 5) The fungus chambers, which are cavities enclosed between modified lamellae, connecting the laminar structure with the dome shaped roof over the hive formed by the outer wall structure; and finally 6) A peripheral system of galleries constructed under- as well as overground (covered runways), connecting the nest mound with e.g. foraging areas.

From the experimental studies presented in the previous chapter, which yielded insight into the predominantly chemical mediation of building behaviour around an exposed physogastric queen, it appears that building workers release and employ two pheromones to initiate and to co-ordinate their building response. These two pheromones are, the trail- and the 'cement' pheromone. In this chapter the rôle of these pheromones is examined in the formation of pillars and galleries. In addition, an attempt is made to answer the question which factors lead to the construction of lamellae in repair of damage done to the nest mound.

RESULTS

The rôle of trail pheromone in pillar construction

Groups of 320 major workers when confined in observation boxes with soil (for details see chapter 2) may construct spontaneously between 30 and 50 soil pillars in a few hrs. Termites constructing at the pillar apex started adding pellets in a more lateral direction, after the column had attained a height of 0.5 - 1.6 cm: the onset of lamella construction (Fig. 1). Most of these growing lamellae are then connected with those constructed on neighbouring pillars, forming arches (Grassé, 1959). Arches are subsequently connected with others until after 6 - 8 hrs a nearly completed roof over the greater part of the arena floor is constructed (Fig. 2). However, groups of 320 workers with varnished sternal gland when placed on the soil covered floor of the arenas were not observed to build pillars at all. Nevertheless, no appreciable difference could



Fig. 1. Pillar with lamella under construction by major workers of M. subhyalinus. Bar indicates 1 cm.

be found between control (not treated and treated groups in building latency time and in the total number of depositions recorded during 60 min after worker introduction. Data are presented in Table 1. In three additional experiments

Table 1. The building latency time (min), and the total number of depositions recorded in 60 min, of 320 workers with varnished respectively not varnished sternal glands (5 replicates).

	Varnished	Not varnished
Building latency time	3; 3.5; 3.5; 4; 5.	3.5; 4.5; 6; 6.5; 7.
Total no of depositions	948; 1057; 1115; 1183; 1236.	1121; 1195; 1251; 1388; 1413.

an increase in the number of varnished workers to approximately 500 did not alter the above situation for a period of 36 hrs. In all controls as well as treated groups, the workers invariably started building in the corners of the arena, grasping soil granules within a few mm from the arena wall, either cementing them against the walls near the site of grasping or transporting the pellets and depositing them in zones of approximately 1 to 2 cm from and parallel to the wall. In this respect a difference between the two groups in the distribution of depositions was observed: control groups cemented a considerable number (\bar{x} : 61 out of the first 100 depositions, $n = 5$) of pellets against the walls at variable heights up to 1.1 cm above soil level. This behaviour was unlike that of varnished workers who cemented soil material at the walls exclusively at soil level (\bar{x} : 28 out of the first 100 depositions). The remainder of the first 100 pellets was placed by both groups in the indicated zones. With control groups, incipient structures of at least 0.2 cm high appeared in respectively 25, 34, 38, 44 and 49 min after worker introduction, all located between 1.3 and 3.5 cm from a corner of the arena. Varnished workers continued beyond such a period of time to deposit granules in a random fashion in the zone along the walls. Due to the continuous soil transport, the deposition zones in this case were transformed into flattened accumulations of granules, while depressions occurred in the area's where grasping activity predominated (Fig. 2).

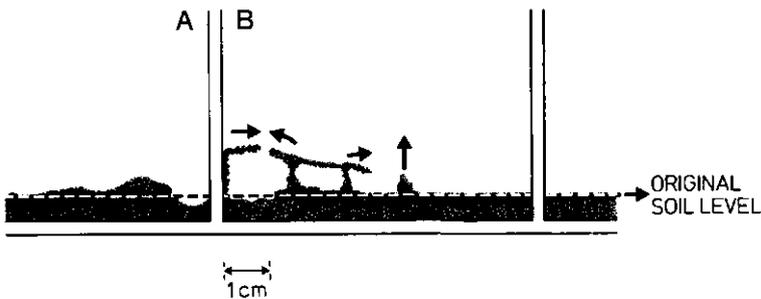


Fig. 2. Schematic representation of a cross section of the results after 4 hrs of building activity of 320 workers with varnished sternal glands (arena A), respectively untreated workers (arena B). Arrows indicate the direction of building. S = original soil level. For further explanation see text.

In an attempt to investigate the cause of this difference in construction behaviour, a first experiment was designed to determine whether tactile stimuli could substitute for the presence of trail pheromone, by directing deposition behaviour and subsequently could initiate pillar formation. For this purpose small metal spheres were placed in two of the corners of the arena, at a dis-

tance of 1.5 cm from either wall. The spheres were held in place as usual with small magnets underneath the box. Thereafter 320 varnished workers were confined in each of these boxes. Control workers (not varnished) immediately made use of the spheres as focus sites for deposition; within respectively 22, 28, 31, 34 and 35 min after worker introduction the first pillar was erected on or against the globule. Treated workers also concentrated soil material on and around the spheres, however instead of adding part of the pellets on top of each other, or on top of the small globe, they cemented their load mostly on soil level against the sphere or other pellets. Such a response led to the formation of flattened ridges of between 0.3 and 0.4 cm high and 2 - 3 cm long, more or less parallel to the arena walls. No pillar construction was recorded during the 6 hr observation period. These results suggest that workers in the absence of trail pheromone are unable to build in the vertical plane. An additional observation was in line with this suggestion. When 320 varnished workers were confronted with an arena in which 24 hrs earlier they had built themselves (when they were still untreated) during 4 hrs pillars and arches in various stages of development (workers were removed from the arena after 4 hrs and kept in petri dishes with wetted filter paper), these workers, unlike control workers, hardly climbed the pillars. Varnished workers did not display any building behaviour on lamellae but concentrated their efforts around the base of pillars, while untreated workers in this second observation period of 4 hrs connected lamellae and continued to cover the arena with a soil sheeting. The next experiment was performed to confront varnished workers with trail-active extracts, in order to assess whether the presence of extract trails fulfilled the condition required to construct pillars. Arenas were prepared according to routine. On the arena floor a cotton thread (L: 15 cm, \emptyset : 0.3 mm) was placed parallel to and 1.5 cm removed from a wall. The thread was soaked in trail-active extract containing 300 TU/2.5 μ l. Extract was applied every 20 min. In three out of four replicates between 2 and 4 pillars were constructed at distances of between 0.3 - 0.8 cm from the trail. Lamellae construction was observed twice in two replicates at a height of 0.6 - 0.9 cm. The lamellae were all directed towards the trail. Varnished control workers in arenas with hexane trails did not construct pillars during the experimental period of 3 hrs. In a final test, a modification of the preceding one, varnished workers (the usual lot of 320) were confronted with 5 pairs of small spheres positioned at 3 cm from another along the trail. The spheres belonging to one pair were placed on either side of the trail, one cm from each other. Within 5 - 12 min after worker introduction in four replicates 1 - 5 spheres had been plastered completely

with soil pellets. Within 60 min, on top or against 7 - 10 globes a pillar was constructed.

After a period of 6 hrs, the lamellae of 1 - 3 pairs were connected over the extract trail to form an arch. Varnished workers building in arenas with spheres placed in an arrangement similar to the one above, this time along hexane trails did not construct a single pillar but erected ridges of cemented soil material more or less parallel to the arena wall, connecting most spheres. The mean width and height of these ridges in four replicates ranged respectively between 0.3 - 0.6 cm and 0.2 - 0.4 cm. From these results it is concluded that trail pheromone plays a role in the co-ordination of construction behaviour i.e. it influences the distribution of depositions and thus is conditional in pillar construction. Moreover, at least under these experimental conditions it appears to be a necessary stimulus for the workers to build in the vertical plane.

An important feature is the height of a pillar. As mentioned earlier, above a certain height the pillars are extended by the termites into a more lateral direction. The conditions for this specific performance to occur were investigated in the following way. Hand made pillars of 'prepared' soil, 3 cm long and approximately 0.5 cm wide were positioned in a row parallel to, and at a distance of 2 cm from the arena wall, spaced 2 cm from another. A volume of 10 μ l of trail-active extract (a total of 1200 TU) was applied around (0.5 cm radius) the base of one pillar out of the row of 6, every 10 min. An equal amount of hexane was used around a control pillar. One hr after the introduction of 320 unvarnished major workers, the height was measured of the site on the pillar at which pellets were cemented in a direction deflecting at least 20⁰ from the vertical plane, hereafter referred to as 'pillar height'. The results of 6 replicates are summarized in Table 2. Data show that the 'pillar height' is positively influenced by the extract introduction. It could also be shown that it is

Table 2. The height (cm) of the site on a hand made pillar at which pellets were cemented in a direction deflecting at least 20⁰ from the vertical plane. Extract: trail-active extract around pillar base. Hexane: hexane around pillar base. Control: neither extract nor hexane around pillar base (6 replicates).

Test no.	1	2	3	4	5	6	\bar{x}
Treatment: Extract	1.2	1.4	1.3	2.2	1.7	1.6	1.6
Hexane	0.5	0.8	0.7	1.1	1.2	0.8	0.8
Control	0.7	0.6	0.7	0.9	1.3	0.7	0.8

by the number of workers passing along the respective pillar. This could be demonstrated in the following test. Workers were confined in observation boxes of 20 x 10 x 5 cm in perspex. The soil covered floor carried 28 hand made pillar of the above dimensions placed in a grid pattern 2 cm away from each other. The total number of workers passing in between certain pairs of pillars was recorded during 60 min following the introduction of 300 workers, and then the respective 'pillar heights' were measured. The results are presented in Table 3.

Table 3. The 'pillar height' of hand made pillars along which base workers passed in defined frequencies.

no of workers passing/min	'pillar height' range (cm)	mean 'pillar height' (cm)	no of pillars
0 - 10	0.5 - 0.7	0.5	12
11 - 30	0.5 - 0.9	0.7	9
> 30	0.8 - 1.6	1.2	10

In order to confirm that the positive correlation between 'pillar height' and the number of termites passing underneath is the result of changes in trail-pheromone distribution, which is assumed to be determined by the number of termites passing, the above experimental approach was also used, employing varnished workers. This time, 75% of a group of 300 major workers was varnished prior to the introduction into the arena. Recorded were during 60 min the total number of workers passing in between certain pairs of pillars, and after that the 'pillar heights' were measured. The results are given in Table 4. Data confirm that trail pheromone is involved in the determination of the height of the site on the pillar at which workers begin to construct a lamella.

Table 4. The 'pillar height' of hand made pillars along which base workers passed in defined frequencies. 75% of the worker population in the arena had varnished sternal glands.

no of workers passing/min	'pillar height' range (cm)	mean 'pillar height' (cm)	no of pillars
0 - 10	0.4 - 0.7	0.6	7
11 - 30	0.4 - 0.7	0.6	11
> 30	0.6 - 1.0	0.8	8

The role of the 'cement' pheromone in pillar construction

Grassé (1959) described the construction of pillars or columns by workers of M.bellicosus. Like in M.subhyalinus, when these pillars had attained a certain height, direction of building at the pillar apex started to deflect mostly towards neighbouring pillars; subsequently two were then connected to form an arch. Grassé (1959) and Wilson (1971) have suggested that the sense by which the termites detect the proximity of another column is olfaction. The following two experiments were designed to test the hypothesis that spatial information required to join lamellae from neighbouring pillars is mediated by the 'cement' pheromone, which as has been shown in the previous chapter is originating from the worker salivary gland. For this purpose observation boxes (25 x 20 x 5 cm) were supplied with a 1.5 cm thick layer of soil. In a first attempt, 3 hand made soil pillars, 4 cm long and 0.3 - 0.4 cm wide were placed in a row 2 cm from another, with their apex 3 cm above soil level. After the introduction of 320 major workers, records were made of the height above soil level of the junction of two lamellae built from neighbouring pillars (Fig. 3a). This height ranged between 0.6 and 1.1 cm (mean, 0.78 cm; number of replicates: 15). Manipulation of the height of a lamella by carefully pulling the pillar in an upward direction to 0.6 - 0.8 cm above its original position, provided evidence that the direction of the growth of a lamella is influenced by the presence of a nearby construction site (Fig. 3a). In order to demonstrate that this influence is exerted by the 'cement' pheromone, assays were made of the effect of salivary gland extract under experimental conditions essentially identical to those just described for the 'pillar pull' test. The experimental design is depicted in Fig. 3b. Salivary gland extract was applied to a cotton ball (\emptyset : 0.2 cm), which position could be adjusted continuously to maintain a distance of approximately 1.0 cm to the tip of a lamella under construction, to prevent physical contact with the termites. Between 1 and 5 μ l of extract or distilled water was added every 20 min during a 90 min period. After this period of time, the height was recorded of the lamella tip opposite the extract and control ball. The results of 12 replicates are given in Table 5, and they demonstrate that the direction of the growth of a lamella is adjusted (here in an upward direction) according to the position of the extract ball. The lamellae were invariably connected with the extract ball when the position of the ball was not changed. No influence of the salivary gland extract was observed in case the ball was more remote than 1.0 - 1.5 cm from the lamella tip. In conclusion it can be stated that the 'cement' pheromone constitutes a directional cue (effective radius < 1.5 cm) for the workers engaged in building (a conclusion already arrived at in the

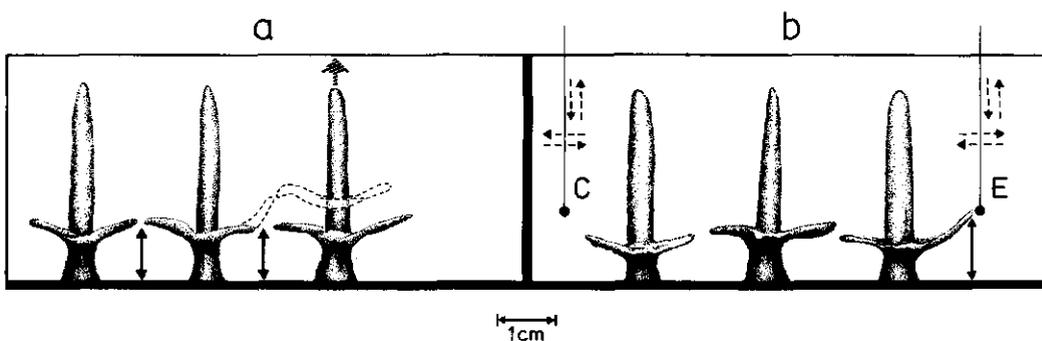


Fig. 3a and b. The construction of lamellae on hand made pillars. In (a) it is demonstrated that a change in the position of a nearby lamella (lamella position is gradually changed in an upward direction) influences the direction of building of a second one. In (b) it is shown that extracts of salivary glands (E) exert a similar influence; (C) cotton ball wetted with distilled water. The solid arrows indicate the sites of lamella height measurement.

Table 5. Height of the tips of lamellae above soil level, in the presence of salivary extract (E), respectively water (C). Data in cm. For further explanation see text.

Test no	1	2	3	4	5	6	7	8	9	10	11	12	\bar{x}
E	1.5	1.6	1.6	1.8	1.9	1.9	1.9	1.9	2.0	2.0	2.1	2.1	1.8
C	0.7	0.7	1.1	1.1	1.0	0.9	0.8	0.9	0.9	0.9	1.1	0.8	0.9

previous chapter, and that it is involved in the junction of lamellae and hence in the formation of arches.

The construction of a gallery

Many termite species are known to construct tunnel-like galleries through which they can pass protected from the outside air. In *M.subhyalinus* a system of galleries for a part constructed underground and for a part built on the soil surface (covered runways) connects the nest mound with e.g. foraging areas. Since the construction of covered runways follows odour trails (Stuart, 1969) it is of interest to examine the role of trail pheromone in the formation of this building element.

In this section the possibility is examined that soil transporting workers following a scent trail employ the distribution of the diffusing trail substance

as a cue to dispose the soil pellets along the trail. A consequence of such a mechanism would be that a change in the distribution of the diffusing trail substance should result in the construction of a gallery with a width and height different when compared to a control gallery.

In a preliminary test it is intended to demonstrate the effect of introduction of trail-active extract on the width of a gallery. For this purpose, workers were allowed to construct a gallery over a 30 cm long plexi-glass bridge connecting two parts of a nest, kept in the laboratory. In the middle of the bridge a 5 cm long paper covered section of the floor could be removed in order to interrupt the trail or gallery. Workers of this species constructed a runway cover over the bridge with a mean width of 1.2 ± 0.2 cm, and a mean height of 0.6 ± 0.08 cm. (Data refer to internal dimensions of four galleries, measured every 1 cm along the bridge). Following gallery completion, it was interrupted by sliding the floor of the central section. Thereafter, a trail was applied with a capillary containing 10 μ l (1200 TU) of trail-active extract or an equal amount of hexane (control). The trail was drawn parallel to and on either side of the runway midline, 0.5 cm apart, and connected both broken ends. Workers started to deposit soil pellets along the edge of the runway 15 - 35 min after extract/hexane introduction, in a zone of about 0.2 cm wide. Building activity in the open section was not clearly concentrated at particular sites, and hence two more or less regularly shaped walls were erected which after attaining a height of 0.3 to 0.5 cm became deflected to the runway centre: the onset of roof construction over this section. In general the gallery was closed again, nearly always centrally over the runway, 2 - 3 hrs after extract/hexane introduction. Over the central section a wider gallery, as compared to the control was constructed. The results are presented in Table 6. These results demonstrate that the edge of a trail constitutes a zone co-ordinating the deposition of soil pellets. The resulting narrow deposition areas are the foundations for the construction of a gallery.

In order to obtain a better resolution of the effect of the trail pheromone distribution on the shape of a gallery (especially its height) the following experimental approach was used. The experimental design is depicted in Fig. 4. One thousand major and minor workers were introduced in two arenas, charged with a 0.5 cm thick layer of moistened soil and each containing a water soaked cotton ball (\emptyset : 2 cm) as water supply. The two arenas were connected by a 27.5 cm long runway which was enclosed between two plexi-glass walls each 5 cm high. The distance between the walls could be varied between 0.4 and 3.5 cm. In a standard control situation, the passing of workers through the 3.5 cm wide plexi-glass

Table 6. The width (internal) of four 30 cm long galleries. Measurements (cm) were taken every 5 cm along the gallery (control). The width of a 5 cm long central section of the gallery, the floor of which is supplied with a trail of trail-active extract (10 μ l, a total of 1200 TU) every 15 min during 60 min following the removal of part of the gallery covering this section (n = 4). Measurements (cm) were taken every 1 cm along the section (extract).

CONTROL				
Test	1	2	3	4
	1.4	1.4	1.3	1.2
	1.2	1.1	1.1	1.5
	1.3	1.5	1.2	1.4
	1.6	1.3	1.3	1.3
	1.0	1.4	1.4	1.2
\bar{x} : 1.3 \pm 0.1 cm				
EXTRACT				
Test	1	2	3	4
	1.8	1.9	2.1	1.9
	1.7	1.7	1.9	1.7
	1.6	1.6	1.6	1.8
	1.8	1.6	1.6	1.8
\bar{x} : 1.8 \pm 0.1 cm				

channel was initiated by the introduction of an extract trail drawn centrally in the channel using a capillary containing 15 μ l of trail-active extract (a total of 60 TU). The number of passing workers was manipulated by two sliding doors on either channel ending, to obtain 40 - 80 passing workers per min. Although construction activity in the arenas started within 5 min after worker introduction, it required between 31 - 49 min (n = 6) before the first workers deposited a pellet in the channel along the trail. In the channel most of the soil granules were placed in two zones, each about 0.2 cm wide. The zones were located at the edge of the trail and the distance between them was 1.1 - 1.5 cm. In 6 replicates the gallery walls became deflected to the centre after they attained a height of 0.3 - 0.5 cm. The gallery was completed 4 - 6 hrs after worker introduction. The internal dimensions were: mean width 1.34 \pm 0.14 cm;

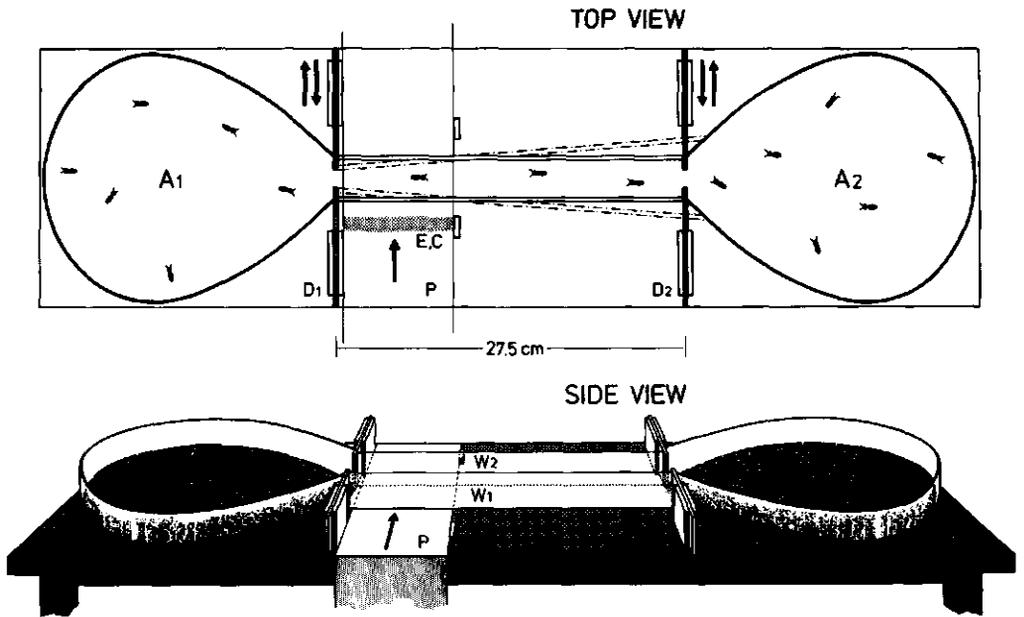


Fig. 4. Arrangement for the study of the construction of covered runways. Two arena's A1 and A2 are connected by a runway enclosed between two plexi-glass walls W1 and W2. The nr of workers passing the runway is regulated with sliding doors D1 and D2. P: paper sheet forming the sliding floor in a 7.5 cm long section of the runway. Arrow indicates direction for travel. Shaded band indicates the position of trail-active extract (E) and hexane (C) introduction. The distance between the plexi-glass walls could be changed, e.g. in a non-parallel arrangement (broken lines).

mean height 0.62 ± 0.04 cm (measured every 1 cm along the runway). Corresponding measurements of runway covers collected in the field, over a total of 2.5 m, yielded averages of 1.56 ± 0.26 cm and 0.58 ± 0.06 cm respectively.

In a further application of this assay worker building responses to a change in space available around the trail were tested for evidence of the effect of the trail pheromone distribution. It is assumed that due to the impervious walls the pheromone distribution is different from that around control trails. In order to attain this, the plexi-glass walls are placed in a wedge-shaped arrangement, 0.4 cm apart on one ending of the runway (here, the workers could only pass one by one) and 3.5 cm apart on the other ending (Fig. 4, broken lines). The results of 6 replicates demonstrate that a decrease in width of the gallery is accompanied by an increase in gallery height (Fig. 5, solid dots). In the wider section of the channel (walls > 1.5 cm apart) workers constructed a normally shaped gallery, not connected with the channel walls and with a mean height and width of 1.2 ± 0.15 cm and 0.6 ± 0.12 cm respectively. In the more narrow section of the channel (walls < 1.5 cm apart) workers began to plaster pellets against the

enclosing walls. This occurred at ground level in the area where the walls were 1.4 - 1.7 cm apart. Beyond this area, going towards the narrow channel ending, the walls of the gallery were entirely plastered against the plexi-glass walls. The height at which the gallery walls became deflected to form a cover, gradually increased from 0.3 - 0.5 cm to about 4 cm, the latter at the narrow channel ending. A runway cover was formed in this way after 4 - 7 hrs.

In order to confirm that the distribution of the diffusing trail pheromone is involved in this phenomenon the experiment was repeated employing a population of workers (1000) of which 50% had varnished sternal glands. The number of workers passing the trail were regulated to be of the same order of magnitude as in the controls and varied between 30 - 75 workers per min. Data concerning the cover heights are depicted in Fig. 5 (open dots; data represented by solid dots are controls). The observed reduction in cover height is interpreted to show that trail pheromone distribution is involved in cover height determination. However, this pheromone is probably not the only cue involved in this respect, since the dimensions of the gallery built in the wider section of the channel did not differ from the dimensions of galleries built in this section by untreated groups.

In a final attempt to demonstrate that the active space of the diffusing trail pheromone (a zone around the point of pheromone emission within which the pheromone concentration is at or above a threshold concentration required for a behavioural response) is involved in gallery formation the following experiment was performed. The floor covering of a 7.5 cm long section of the above

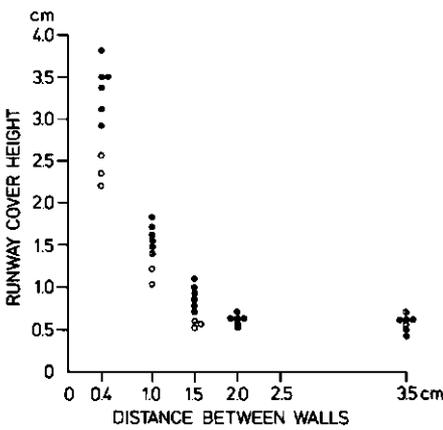


Fig. 5. The relation between the height at which the gallery wall became deflected and the distance between the plexi-glass walls enclosing the runway in an arrangement depicted by broken lines in Fig. 4. The mean width of the gallery (internal dimensions), constructed along the midline in the section where the distance between the plexi-glass walls exceeded 1.5 cm, is 1.2 ± 0.2 cm.

runway connecting the two arenas, could be shifted continuously. The floor of this section was covered with paper which was pulled with the aid of an electrical motor at variable but defined speeds underneath the walls enclosing the runway, in a direction perpendicular to the longitudinal axis of the runway (Fig. 4). In this manner the trail pheromone laid on the floor covering could be constantly removed in this section. The trail enclosing walls were placed parallel to the runway 1 cm from each other. The number of workers passing ranged between 20 - 60 individuals per min. The mean heights of four control galleries (the paper speed was 0 cm/min) were 1.5, 1.4, 1.5 and 1.6 cm. Measurements were taken every 1 cm along the runway, except the two sections of 2 cm length, bordering the arenas. In four assays with the paper speed initially set at 2 cm/min, and increased after 3.5 hrs to 3 cm/min, the mean heights of the gallery over the 5.5 cm long sections were 0.9, 0.9, 1.0 and 1.1 cm, while the remaining part of the galleries reached mean heights of 1.5, 1.5, 1.6 and 1.6 cm. A typical example of the height reduction is depicted in Fig. 6. In a last series of experiments trail-active extract* (100 TU/2.5 μ l) was

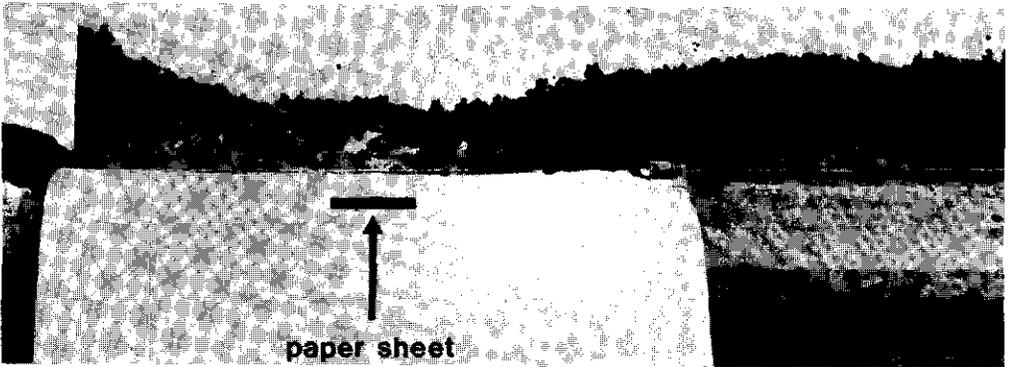


Fig. 6. A characteristic example of a gallery constructed over a runway section in which the trail pheromone is continuously removed by a sliding floor (paper sheet). The paper speed was set at 2.8 cm per min. Bar indicates 1 cm. The difference in height between the gallery over this section and the rest of the gallery is between 0.5 and 0.65 cm (internal dimensions).

* Extracts were made of a variable but known number of workers immersed in a calibrated volume of hexane, and extracted during 24 hrs at - 16°C. Their biological activity was determined with a method described in detail by Leuthold and Lüscher (1974).

continuously applied on a 1 cm wide zone over the total width of the moving paper, prior to its introduction in the section; the zone was placed parallel to and at a distance of 1 - 1.5 cm from the plexi-glass wall to allow for solvent evaporation. Hexane was used as a control.

The data show no reduction in the gallery height over the moving floor; on the contrary, in two of the four replicates a slight increase in cover height was observed, 1.6 cm (section) versus 1.5 cm (rest) and 1.5 cm versus 1.4 cm.

On the basis of the results presented in this section, it can be stated that trail pheromone is involved in the formation of a gallery. It is tempting to conclude that the three dimensional volatile zone of diffusing pheromone molecules along the scented line provides the required directional information for the building workers, however other cues like the cement pheromone may play a role as well.

The repair of small damages to the nest mound

The ability of termites to detect and repair damages in the outer wall structure of the nest is well known (Howse, 1970), and M.subhyalinus proves no exception. Due to the well regulated micro-climate in the nest (Lüscher, 1961), the mean temperature in the nests in the study area ranged between 23.5 and 29.5°C. Since these temperatures were in general 5 and 12°C higher than the mean maximum and the mean minimum temperature of the ambient air respectively, the creation of a small opening in the outer wall structure of the mound resulted in an outwardly directed air current of warm humid nest air. The first termite became visible in the opening within minutes (mean: 1.8 min, range 0.8 - 5.0 min; 58 holes with a maximum diameter of 4 cm). In 54 cases this was a minor soldier and in 4 cases a minor worker. Invariably more workers rapidly appeared in the openings and between 2.5 and 10 min (mean: 4.1 min) after making the hole, a worker brought a soil pellet, which it often deposited outside the actual hole on the oblique section of the surrounding damaged wall. Additional soil carrying workers joined in, nearly all depositing their load in similar positions in a zone of 0.5 - 3.0 cm wide around the hole. Although the workers seemed to act independently, they concentrated the soil material in narrow, approximately concentric areas 1 - 3 pellets wide. These lines of cemented material provided a basis for lamella construction (Fig. 7). The lamellae were built in a direction more or less perpendicular to the surface they were built on. Further inside the hole other lamellae were erected and building activity declined rapidly on the outer construction sites. The workers closed the hole, in a way when viewed from the outside, reminiscent to the closing of a camera



Fig. 7. A schematic representation of a section through an opened air passage (A) and the mound mantle. The position of the growing lamellae closing the hole are indicated.

diaphragma, in 60 - 90 min after the gap was made.

In this section the question is considered what factors are initiating workers to deposit soil granules in zones around the hole. In this respect, workers initially are searching for a deposition site on the outside of the mound. There they encounter environmental conditions which differ in many respects (temperature, relative humidity, CO_2 content, air turbulence, etc) from the nest environment. In contrast to all the experimental situations discussed in this study, where concentration gradients of volatile pheromones may play a role in the location of a deposition site, it is assumed that in this situation where air flow velocities in the hole of 40 - 80 cm/sec were recorded, a different mechanism occurs. In a first approach to analyse such a mechanism observations were made of the closing of holes ($n = 60$). These holes (one per mound) were made at a defined height 30 cm underneath the mound apex. Half of the holes was positioned on the windward side of the mound, the other half on the leeward side. Record was made of the position of the closing lamella with reference to the edge of the hole. Windward holes were closed from inside the edge (1 - 6 cm), while leeward holes were repaired with lamellae placed 0.5 - 4 cm outside the edge. Building activity around the latter holes often resulted in protrusions of cemented material, covering part of the mound mantle. Prior to and during the hole closing activity measurements were taken of the relative humidity (Telemechanics, RH 1 micro-probe), centrally in the hole at various but defined distances from the edge. The ambient temperature was $19.5^\circ - 21.5^\circ\text{C}$ (RH: 41 - 33% respectively) in the period between 09.00 - 11.00 a.m., while the air emerging from the hole, hereafter referred to as 'nest air', measured $23.4^\circ - 24^\circ\text{C}$ (RH: 82 - 90%). Rapid fluctuations in RH between the above extremes were recorded in the area where nest air mixed with ambient air. Workers in search of a deposition site preferred the areas in which the RH did not fall

below a mean value of 80%, and retreated from areas where RH fluctuations occurred down to 70%. The most outwardly located line of depositions coincided with the occurrence of RH fluctuations between 75 and 85%. On the basis of these observations it is assumed that the workers preferentially deposit their load in the area where nest air begins to mix with ambient air. In order to test this hypothesis the following experimental approach was employed. A PVC tube with a diameter of 4 cm, and with a length of 5, 10 or 20 cm was placed over the hole immediately after it was created. Workers were recruited to the hole in the expected time interval of 3 - 6 min, and within a few minutes started cementing the first soil pellets against the lower end of the tube. Several workers climbed the interior of the tube but retreated after a few cm. More workers were recruited and began to plaster the inside of the tube with a layer of soil. Most of the 5 and 10 cm tubes (6 out of 8) had their interior completely covered in 4 - 6 hrs. Between 1 and 3 cm from the outer end of the tube (outside wind speed less than 1 m/sec), the direction of building suddenly changed to a direction perpendicular to the tube wall - the beginning of the formation of closing lamellae. RH measurements showed the occurrence of rapid fluctuations between 70 and 83%, measured with the probe in the centre of the tube opposite the respective sites. In a similar fashion, the tubes with a length of 20 cm were covered and closed at 8 - 5 cm from their outer end. Also here, the area where RH fluctuations within the indicated limits occurred, coincided with the zone where the building direction changed. In a further experiment tubes were employed which had a transverse slit of 5 x 0.3 cm, located halfway the tube. In 5 out of 7 replicates, workers did not continue with the plastering of the interior of the tube beyond this slit, but constructed closing lamellae at the very site. RH measurements showed fluctuations between 75 and 85% also here. The conclusion is drawn from these results, that workers perceive differences in 'nest air' and ambient air and are able to employ these differences to locate a deposition site.

DISCUSSION

The variety of architectural elements, and their complex arrangement in nest mounds of M. subhyalinus is impressive. Until recently (Bruinsma and Leuthold, 1977) it was difficult to envision how such arrangements or even individual elements were formed (Wilson, 1971). On the basis of experimental evidence presented in the preceding chapters, it was possible to show that termite workers are induced by stimuli of chemical and tactile nature to initiate and co-ordinate building behaviour around an exposed physogastric queen. In this study it is

examined how of the chemical stimuli, the trail and the 'cement' pheromone are involved in the construction of several building elements other than a replacement royal cell.

The basis of starting any structure on a surface was found to be the mechanism to concentrate soil pellets in specific areas. This increases the probability of the development of agglomerations of pellets, which will eventually result in the construction of a building element, generally a pillar. The presence of trail pheromone appeared to be an essential condition for the construction of pillars. It was shown that workers were not able to concentrate pellets in particular areas in the absence of this pheromone. When scenting lines of trail-active extract were offered to varnished workers, two zones of deposited material were constructed parallel to an on either side of the line. Such a concentration of depositions was often sufficient to induce pillar construction. As expected from evidence presented in the previous chapter, the presence of spheres providing tactile information to the workers reduced the time required to construct a pillar. Nevertheless, in the absence of trail-active extract, varnished workers failed to construct pillars although they were concentrating part of their depositions on and against the spheres. Apparently the trail pheromone has a second function, it initiates or facilitates building behaviour in the vertical plane. Preliminary experiments, confronting varnished workers with patches of soil treated with salivary gland extract did not result in pillar formation (Bruinsma, unpublished data).

The direction of building at a particular site can be influenced, as demonstrated with the construction of a lamella on the pillar apex, by the position relative to that site of the point of emission of 'cement' pheromone, provided that the distance between the two does not exceed 1.5 cm. According to theoretical considerations concerning the diffusion of a volatile substance in space, made by Neuhaus (1964), in undisturbed air, osmotropotaxis can contribute to the orientation towards the place of origin of an odour from distances of 0.5 - 2 cm. Such a mechanism might therefore be of importance to co-ordinate building behaviour at distinct sites, since as pointed out by Lindauer and Martin (1963), it exceeds several times the reach of tactile perception.

Stuart (1969) observed that the construction of a gallery follows pheromone trails. Bossert and Wilson (1963) formulated a hypothesis according to which the active space of a pheromone trail is assumed to be a semiellipsoidal volatile zone along the scented line, with a steeply decreasing gradient of concentration in width and height. According to these authors, in order to achieve greater accuracy in trail following it is importance that the gradient of con-

centrations near the effective boundary of the diffusing trail substance be so large to correct erring followers. It has been demonstrated in this chapter that termites employ the edge of a trail as a co-ordination zone for the deposition of soil pellets, prior to the construction of a gallery over that trail. The stimulus to deposit is unknown, however with respect to the cited hypothesis, it is feasible that zones of iso-concentration parallel to the line of travel may constitute such a stimulus. Manipulation of the distribution of the trail pheromone by the addition of trail-active extract (Table 6) suggests such a mechanism. The most clear indication of the involvement of trail pheromone in the determination of the height of a gallery was obtained in a test employing a sliding floor (Figs. 4 and 6). Since the observed reduction in gallery height was compensated by the introduction of trail-active extract, a significant contribution of the mechanical disturbance caused by the sliding floor had to be excluded. Again, with respect to the postulated 3 dimensional tunnel-like active space of the diffusing trail substance, it is assumed that workers engaged in building the wall and the roof of the gallery over a trail may employ zones of iso-concentration near the effective boundary of the trail, to determine the building direction. A mechanism similar to the just proposed mechanism may be involved in the determination of the 'pillar height'. Pillars are constructed by workers which have established a network of diffusing trails on the soil surface of the arena. Although the distribution of locations on which a pillar will be constructed is influenced by surface irregularities, it is uncertain whether the zones co-ordinating deposition behaviour along individual runways are important in this respect.

In contrast to all previously discussed situations the building of lamellae, closing holes in the nest mound, occurred under conditions where pheromone concentration gradients are unlikely to exist for longer periods of time in view of the fact that the making of such a hole created an outwardly flowing air current of about 0.5 m/sec. Despite this, the workers concentrated soil granules in narrow zones around the hole. On the basis of preliminary tests it is suggested that workers perceive the differences between the air emanating from the hole and the ambient air, and initially use these to locate a deposition area. The nature of this complex set of differences has not yet been investigated.

SUMMARY

1. The factors influencing building behaviour leading to the formation of pillar and galleries (covered runways), and the construction of lamellae closing small induced damages to the mound are investigated.
2. Two parameters providing directional orientation are employed by the termites: trail pheromone and 'cement' pheromone.
3. The edge of a chemical trail constitutes a zone for the co-ordination of depositions, forming the gallery foundation. The 'cement' pheromone emanating from drying cement at active deposition sites attract nearby workers to these particular sites.
4. The width and height of a gallery and the height of a pillar are influenced by the trail pheromone distribution.
5. The 'cement' pheromone is responsible for the junction of lamellae growing from neighbouring pillars.
6. In addition to directional orientation provided by chemical cues, it has been demonstrated that termites employ differences between 'nest' air and ambient air to locate deposition zones for the purpose of the construction of lamellae closing a gap in the mound.

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CHAPTER V GENERAL DISCUSSION

Although the work presented in this study is little more than a first survey of the causal factors involved in construction behaviour of Macrotermes subhyalinus, it clearly indicates a fertile field of research which has scarcely been explored. Past studies have concentrated on the release of building behaviour and have done little (with the exception of Grassé, 1959) to account for the formation of the various types of structural elements present in termite nests. The present study gives a general account of building behaviour and of the formation of several structural elements: volatile chemical cues in concerted action with tactile stimuli provided by physical objects, cause workers to grasp soil pellets, transport them, and subsequently deposit them at a building site. The ability of termite workers to employ pheromones in a versatile way in their orientation is the underlying condition which gives rise to the formation of a variety of structural elements.

Building behaviour in this species is predominantly released by chemical stimuli like the building pheromone of the queen, the 'cement' pheromone and the trail pheromone (Bruinsma, unpublished). In view of the fact that the active space of the latter two pheromones directly depends on the number and distribution of workers, it would appear, then, that the building latency time is inversely related to the workers group size. Grassé (1959) has described such a density dependent effect on building activity in *Cubitermes*.

The orientation required by building workers is mediated by trail- and 'cement' pheromone (directional orientation) and in the appropriate situation also by the building pheromone emanating from the queen (providing distance orientation). Other authors have documented examples of chemical orientation among termites. Leuthold (1975) has reviewed a body of evidence which shows that termites to a great extent rely on odour trail orientation. Stuart (1967) discovered that *Zootermopsis* is recruited to building sites by chemical trails. Grassé (1959), Wilson (1971) and Deneubourg (1977) indicated the possibility of odours emanating from construction sites, orienting nearby motivated workers to such sites. This study furnishes convincing authentication of a pheromone emanating from a building site and confirms its rôle in worker orientation. Soil transporting workers arri-

ving at a building site, e.g. located along a trail or in a deposition zone around the queen, attain a stable orientation with respect to that site prior to and during cementing their load. This response is probably to be due to the perception of a local stimulus, or stimulus complex. Such a mechanism of orientation is named telotaxis (Schöne, 1973), and implies as already emphasized by Fraenkel and Gunn (1961), simultaneous perception and evaluation of several stimuli.

The results obtained with physical objects (small spheres) are in accordance with those of Stuart (1967) who demonstrated that surface irregularities release building behaviour in *Nasutitermes*. In respect of the ability of *M. subhyalinus* to perceive spatial relations between the discussed stimuli, it is of interest to mention a mechanism of orientation which has been described by Forel as: "By topochemical I mean a sense of smell which informs the ant as to the topography of the places surrounding it by means of chemical emanations which give an odour to objects" (Forel, 1928; cit. by Wilson, 1971, p252). To date such a mechanism integrating olfactory and mechanical information has been only demonstrated in honey bees (Martin, 1965) and the termites referred to in this study.

Grassé (1959, 1967) described in detail building behaviour in *M. bellicosus*, *M. mülleri* and *Cubitermes* sp. According to this author, it is the product of work previously accomplished, which constitutes the social stimulus for the workers to perform additional work (the concept of 'stigmergy'). In summary, two stages are distinguished: 1) A phase of 'unco-ordination' during which the workers first explore the container they are placed in, and after a certain time lapse start depositing soil pellets anywhere in the arena. When at some place the deposited material reaches a 'critical density' that is when several soil pellets are stuck together, this incipient structure proves to be very attractive to the workers compared to single pellets. 2) Subsequently the phase of 'co-ordination' is started during which workers transform the incipient structures into pillars, which, when a neighbouring one is within a critical distance, are combined to form arches. The concept of 'stigmergy' can be very well viewed in terms of the mechanism treated in the present study, except the observation that, initially, building is random. This observation is at variance with that of Stuart (1969), and the finding in the current study that workers employ the edge of a pheromone trail as a zone to co-ordinate soil depositions.

Stuart (1967, 1969, and 1972) concludes that the behavioural basis of building is a response to a 'low level excitatory' stimulus, like air movement, odour, light, temperature etc., deviating from the normal nest environment. The subse-

quent building activity gradually eliminates the causal stimulus, and when no more environmental stimuli are forthcoming, building will stop. In conclusion, the immediate function of building is a homeostatic one. Since termites like most other social insects control the environment within the nest by actively maintaining several steady states, based on behavioural and physiological regulation (this phenomenon has been named 'social homeostasis' by Emerson, 1956), it is important to know whether the building mechanism proposed in this study is compatible with homeostatic regulation. At present it is only possible to indicate that there are several negative feedback mechanisms operating during building activity. At first, the ephemeral activity of both the trail- and the 'cement' pheromone is likely to modify the number of workers recruited to building sites. Secondly, it could be shown that major and minor workers during subsequent building runs loose between 20 - 40% of their fresh weight. In case workers are not allowed to compensate this loss by drinking at the water supply, they invariably stop building (Bruinsma, unpublished).

The fact that pheromones, together with structural signals, are involved in the initiation and co-ordination of building behaviour in termites constitutes a plausible mechanism which accounts for most aspects of nest building behaviour in M. subhyalinus. The findings presented in this study may serve a useful purpose, increasing the understanding of the way in which termites perform such remarkable engineering feats as the construction of their impressive nest mounds.

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SAMENVATTING

Bij termieten speelt de reukzin een overheersende rol in de communicatie tussen individuen van een kolonie. Het onderzoek beschreven in dit proefschrift, heeft betrekking op het bouwgedrag van Macrotermes subhyalinus (Rambur), een schimmelkwekende en een heuvelbouwende termietensoort. Een nest gebouwd door deze soort bestaat uit een aantal verschillende structurele elementen zoals een koninginnekamer, een schimmeltuyn, pilaren en galerijen.

De factoren die ten grondslag liggen aan het bouwgedrag werden bestudeerd. Aan de hand van experimenten die grotendeels in het laboratorium zijn uitgevoerd, kon worden vastgesteld dat olfactorische oriëntatie de basis vormt van het bouwgedrag, dat leidt tot de constructie van bovengenoemde structurele elementen (hoofdstuk 1).

Drie deelonderwerpen zijn onderzocht, namelijk a) de constructie van een vervangende koninginnekamer, b) de constructie van pilaren en galerijen, en c) de constructie van lamellen die kleine gaten dichtten, die tijdens het experiment werden gemaakt in de nestwand.

Een koningin, die uit haar kamer of cel is gehaald, wordt door leden van de werkerkaste opnieuw overdekt met een aarden overkapping. De directe aanleiding tot dit bouwgedrag, alsmede de bepaling van de bouwafstand tot de koningin kon worden teruggevoerd op de aanwezigheid van een pheromoon. Dit pheromoon wordt geproduceerd in het vetlichaam van de koningin, en wordt afgegeven via haar stigmata (hoofdstuk 2).

De aanwezigheid van dit pheromoon alleen is niet voldoende voor het tot standkomen van de overkapping. Werksters en werkers leggen geursporen rond de koningin. Deze sporen werken coördinerend op het deponeren van gronddeeltjes, zodat er ophopingen van gronddeeltjes kunnen ontstaan waarop vaak pilaren worden geconstrueerd. Termieten die in contact komen met deze pilaren worden door tactiele prikkels gestimuleerd tot verdere bouwactiviteit.

Gedurende het transport van het gronddeeltje wordt het vermengd met een pheromoon dat afkomstig is uit de speekselklier. Dit pheromoon trekt gemotiveerde termieten aan over een afstand van maximaal ongeveer 2 cm van de plaats van verdamping en stimuleert de dieren tot additionele bouwactiviteit (hoofdstuk 3).

In afwezigheid van een koningin bouwen de termieten galerijen en pilaren. Nabij de rand van een geurspoor ligt een zone waarin termieten bij voorkeur gronddeeltjes deponeren. Aangezien het aanbrengen van veranderingen in de vorm van de ruimte waarin de geurstof diffundeert, tot resultaat heeft dat galerijen met afwijkende afmetingen (breedte en hoogte) worden geconstrueerd, wordt geconcludeerd dat termieten bepaalde concentratie-zones in de geurwolk waarnemen en ervaren als signaal tot het deponeren van gronddeeltjes. Pilaren bereiken een bepaalde hoogte, waarna de bouwrichting in een zijdelingse richting wordt afgebogen. Dit resulteert in de constructie van een horizontale lamel. De hoogte van de pilaar kan worden beïnvloed door veranderingen aan te brengen in de hoeveelheid spoorpheromoon rond de pilaarvoet. Uit de positieve correlatie die tussen beide parameters wordt gevonden, kan worden geconcludeerd dat termieten ook bij de constructie van pilaren van de ruimtelijke verdeling van het spoorpheromoon gebruik maken.

Het pheromoon afkomstig uit de speekselklier dat van een bepaalde constructieplaats verdampst, met name van een lamel, beïnvloedt de bouwrichting van een tweede lamel onder constructie op een nabij gelegen pilaar. De beide lamellen worden op deze wijze met elkaar verbonden tot een boog.

In het laatste gedeelte van deze studie wordt de constructie van lamellen bestudeerd waarmee kunstmatig gemaakte openingen in de nestwand worden gedicht. Dergelijke openingen veroorzaken het ontstaan van een relatief sterke, naar buiten gerichte luchtstroom. Onder deze omstandigheden zijn bouwende termieten in staat, in zodanige richting te bouwen, dat de opening zo snel mogelijk gedicht wordt, door zich te oriënteren met behulp van de optredende verschillen tussen 'nestlucht' en buitenlucht (hoofdstuk 4).

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Oebele Herman Bruinsma werd op 25 augustus 1946 geboren te Wolvega. Hij behaalde het eindexamen hogereburgerschool B aan de Rijks-H.B.S. te Tiel, en begon in hetzelfde jaar zijn studie aan de Landbouwhogeschool te Wageningen. Juni 1974 behaalde hij aan deze hogeschool het doctoraalexamen richting Planteziektenkunde met als hoofdvak Entomologie en als verzwaard bijvak Dierkunde.

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