

## CHEMICAL COMMUNICATION SYSTEMS

### IN THE ANT *ATTA CEPHALOTES*

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#### SUMMARY

Leaf-cutting ants have elaborate chemical communication systems. They communicate alarm with a pheromone from the mandibular gland ; use a trail pheromone to orientate nestmates to the food source and to inform them about the quality of the food they are recruited to ; mark their territory with the secretions of the valve glands ; and have many exocrine glands with a possible role in chemical communication. *Atta cephalotes* recognise workers from another species (*Acromyrmex octospinosus* and *Atta sexdens*) and from other colonies of its own species as aliens. Species-specific recognition appears to be based on volatile substances on the cuticle, probably produced by the mandibular glands. There is some evidence that colony-specific recognition rests on detection of colony differences in the composition of the cephalic alarm pheromone. These ants dispose of waste products from the nest in a way different from that in which they dispose of wastes of other origins. The process of dumping refuse seems to involve a different programme of behaviour from that used for collection of food, although no caste-specificity in this behaviour could be detected. Chemical signals are used to recognise the wastes originating from the nest.

#### RESUMEN

##### Los sistemas de comunicación química en la hormiga *Atta cephalotes*

Las hormigas del género *Atta* tienen varios sistemas de comunicación química. Ellas comunican alarma con una feromona proveniente de la glándula mandibular ; usan una

feromona de camino para orientar a las compañeras a la fuente de alimento y para informarlas acerca de la calidad del alimento ; marcan su territorio con la secreción de las glándulas de los palpos del aguijón ; y tienen varias glándulas exocrinas más con una posible función en comunicación química. *Atta cephalotes* reconoce a obreras de otras especies (*Acromyrmex octospinosus* y *Atta sexdens*) y de otras colonias de su misma especie como extrañas. El reconocimiento especie-específico parece posible gracias a sustancias volátiles en la cutícula, probablemente provenientes de la glándula mandibular. Hay evidencias de que el reconocimiento específico para cada colonia de *A. cephalotes* se logra gracias a la capacidad de estas hormigas de detectar diferencias en las proporciones relativas de los componentes de la feromona de alarma. Estas hormigas disponen de los desechos del nido de forma diferente a como disponen de desechos de otro origen. Parece existir un programa comportamental específico para botar los desechos del nido en el sitio específico que tiene la colonia para ello. No se pudo observar una especificidad de una de las castas por ese comportamiento. Señales químicas le sirven a estas hormigas para reconocer los desechos del nido.

## INTRODUCTION

The communication systems of the leaf-cutting ants from the genus *Atta* are complex and could be classified among the most evolved in the Hymenoptera. Various different exocrine glands have been described in workers of these ants (Fig. 1). For some of them a function in communication is known, but for others not. It is to expect that eventually a function in communication for most of these glands are found. Thus, the mandibular gland secretes a multiple function alarm pheromone, which is used to attract nestmates and to direct the attack to the source of disruption (Crew and Blum, 1972). The same pheromone seems to be used to recognize the individuals from the same colony as we shall see later, and possibly is also used as the colony specific component in territorial recognition (Jaffé et al., 1979). The valve glands (Bazire-B and Zylberberg, 1979) are used for marking the territory at least with the species-specific component (Jaffé et al., 1979). The poison gland is used by these ants for chemical mass recruitment to the food source (Moser and Blum, 1963). It orientates the workers to the food source, giving spatial information, and at the same time it gives information about the quality of the food (Jaffé and Howse, 1979, and Jaffé, 1980). The mayor component of this pheromone, the methyl-4-methylpyrrole-2-carboxylate has two different effects on the ants. If applied as a trail at concentrations down to  $10^{-14}$  gr/cm it produces its known orientation effect. At higher concentrations (about  $5 \times 10^{-11}$  gr/cm or more) it works as an attractant. This double effect is used by the individual ant to modulate the amount of

recruitment required to exploit the food source. The function in communication of the other exocrine glands is still unknown. These ants also use pheromones or chemical substances for recognizing the waste products from the nest and thus take them to the refuse deposit, as will be described below. They recognize their brood with the help of an unknown chemical signal (Robinson and Cherrett, 1974) and probably also use pheromone for sex attraction. Inside the nest, communication for the coordinate building of the galleries, the recognition of castes, of the fungus, etc., are very probably also due to the help of chemical signals.

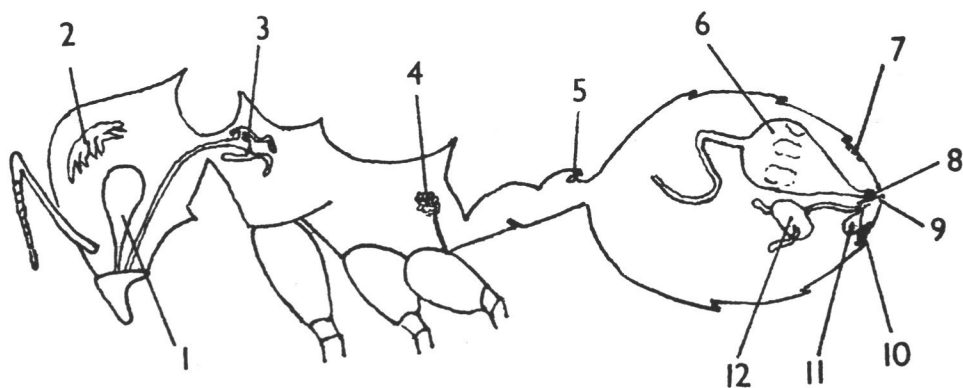


Fig. 1 — Exocrine glands of *Atta cephalotes* and its known function. 1 — Mandibular gland (alarm, individual recognition and probably also territorial marking) ; 2 — Postpharyngeal gland ; 3 — Salivary gland ; 4 — Metapleural gland (produces antibiotics) ; 5 — Stridulatory apparatus (ultrasound production) ; 6 — Rectal sac ; 7 — Tergal gland ; 8 — Valve glands (territorial marking) ; 9 — Sting ; 10 — Sternal gland (defence secretions) ; 11 — Dufour's gland ; 12 — Poison gland (orientation along a trail, information about the quality of the food).

Fig. 1 — Glándulas exócrina de *Atta cephalotes* y sus funciones conocidas. 1 — Glándula mandibular (alarma, reconocimiento individual y probablemente también marcaje territorial) ; 2 — Glándula post-faríngea ; 3 — Glándula salivar ; 4 — Glándula metapleural (produce antibióticos) ; 5 — Aparato estridulador (produce ultrasonido) ; 6 — Saco rectal ; 7 — Glándula tergal ; 8 — Glándulas de los palpos del aguijón (marcaje territorial) ; 9 — Aguijón ; 10 — Glándula esternal (secreción de defensa) ; 11 — Glándula de Dufour ; 12 — Glándula de veneno (orientación en caminos, información sobre la calidad del alimento).

## RECOGNITION OF NESTMATES

### Materials and methods

Tests were carried out in the laboratory on nest tables of *Atta cephalotes* colonies at Southampton University, England. Ants were killed by plunging them into finely crushed dry-ice. Dead ants were used in experiments after the body temperature equilibrated with the room temperature. Freeze-drying was carried out after killing the ants as just

described, using a vacuum pump and a refrigerated supporting plate. Pressures of about  $0.5 \times 10^{-3}$  mm Hg at  $-40^{\circ}\text{C}$  during 12 hours were applied to the ants during the process. Impregnated freeze-dried ants were those freeze-dried ants which were placed in a small closed plastic cup together with two crushed heads, thoraxes or gasters, for 5 minutes. Gas chromatography on 5 % Carbowax columns together with the solid sample technique (Morgan and Wadhams, 1972) was used for studying the relative amounts of the alarm pheromone components of *A. cephalotes*. 4-Methyl-3-heptanone was identified by assuming it to be the major component of *A. cephalotes* head volatiles (Riley et al., 1974a). 2-Heptanone was identified by co-injection with the synthetic compound using the Carbowax columns.

## RESULTS

### General observations

Test ants were placed on an *Atta cephalotes* nest table and the reaction of the workers of the host colony was observed. An ant from the same colony was examined briefly with the antennae of the workers after being placed on the table, and then entered the nest. An ant of the same species, but from a different colony was attacked. The foreign ant, as soon as it was placed on the table showed alarm behaviour, probably due to the presence of a colony-specific territorial pheromone. The workers attacked the alien ant until it jumped from the table or until it was killed. The same was observed when an ant from a different species (*A. sexdens* or *Acromyrmex octospinosus*) was placed on the nest table; in this case, the attack was more violent and the workers of the host colony seemed to detect and find the alien ant much faster. In all three cases, alarm behaviour was elicited in the host colony. If the same kind of ant were anaesthetised with carbon dioxide and then placed on an *A. cephalotes* nest table, different responses from the host colony were observed. The workers attacked only the ant of a different species, which was sometimes dropped over the edge of the table before it recovered. Both kinds of *A. cephalotes* ants were examined extensively with the antennae, and the workers attacked the ant from a different colony only when it recovered.

When the three type of ants, killed with dry-ice, were presented on the *A. cephalotes* nest table, behaviour similar to that with anaesthetised ants could be observed. All three types of ants were eventually dropped over the edge of the table, but only the ant from a different species (*Acromyrmex octospinosus*) elicited aggression from the workers. *A. cephalotes* workers were not attacked, even if they came from a different colony. The time the



dead ants remained on the nest table before they were picked up and dropped off the table (Table I) was less for dead *Acromyrmex* than for dead *A. cephalotes* workers. Freeze-dried ants, when presented to an *A. cephalotes* colony were very soon picked up and dropped off the edge of the table (Table I). No attack or extensive examination by the workers could be observed with any of the freeze-dried ants.

**Table I** — Mean and standard deviation of the time (min) an ant remains on the nest table of an *A. cephalotes* nest before it is picked up and dropped off the edge of the table ( $n = 8$ ).

**Tabla I** — Media y desviación estandar del tiempo (min) que una hormiga permanece sobre el nido de una colonia de *A. cephalotes* antes de ser recogida y botada fuera del mismo ( $n = 8$ ).

Form of presentation	Ants from same colony	Ants from different colony	Ants from different species ( <i>Acromyrmex octospinosus</i> )
Dead	25.3 $\pm$ 12.1 a	24.1 $\pm$ 10.7 a	5.2 $\pm$ 2.8 b
Freeze-dried	0.6 $\pm$ 0.5 c	0.7 $\pm$ 0.3 c	0.8 $\pm$ 0.3 c

a, b, and c indicate statistically different means ( $p < 0.05$ ) by Hartley's test (ANOVA,  $F = 25.4$ ,  $p < 0.001$ )

These observations show that ants from different species are recognised even if dead ; but ants from different colonies of the same species are recognised as such only if they are alive and active. The experiment with freeze-dried ants suggests that the species recognition signal is a volatile chemical.

### The odour source for species recognition

Body parts of freshly-killed *A. cephalotes* and *Acromyrmex octospinosus* workers were presented to an *A. cephalotes* colony. The body parts, head, thorax and gaster, were examined by the ants of the host colony for different lengths of time (Table II) before they were picked up and dropped off the edge of the table. All the body parts of *A. cephalotes* were examined for a longer period than those of *Acromyrmex octospinosus*, although all came from a colony different to the host colony, which suggests the presence of a chemical cue all over the body, probably on the cuticle, which is used in species recognition. When body parts of freeze-dried *A. cephalotes* ants were presented to an *A. cephalotes* colony, none were examined for long but were dropped off the table immediately, which again suggests a volatile chemical cue, acting as the species recognition signal. All body parts were

examined for a much shorter period if freeze-dried, with the exception of the gaster, in comparison with the body part of a freshly-killed ant (Table II).

Table II — Mean time and standard deviation (min) for which different body parts of freshly-killed ants from two different species remained on the nest table of an *A. cephalotes* nest before they were picked up and dropped off the table (n = 6).

Tabla II — Media y desviación estandard del tiempo (min) que diferentes partes del cuerpo de una hormiga recién muerta de dos especies diferentes permanece sobre el nido de una colonia de *A. cephalotes* antes de ser recogida y botada fuera del mismo (n = 6).

Species	Body part examined :		
	Head	Thorax	Gaster
<i>A. cephalotes</i>	11.4 ± 3.3 bd	12.6 ± 11.6 bd	6.1 ± 7.1 cd
<i>Acromyrmex octospinosus</i>	2.7 ± 1.6 c	4.3 ± 3.5 c	1.0 ± 0.6 a
Freeze-dried <i>A. cephalotes</i>	0.6 ± 0.2 a	0.8 ± 0.3 a	1.3 ± 0.7 ac

a, b, c, and d indicate statistically different means ( $p < 0.05$ ) by Students t-test (ANOVA,  $F = 5.77$ ,  $p < 0.001$ )

When freeze-dried *A. cephalotes* workers were impregnated with the odours from crushed body parts of *A. cephalotes* from a different colony and of *Acromyrmex octospinosus* workers, and were then presented to an *A. cephalotes* colony, different «pick-up» times were observed (Table III). Freeze-dried ants, impregnated with odours from the heads of either *A. cephalotes* or *Acromyrmex octospinosus* were examined for a longer period than those impregnated with odours from the thorax and gaster. No difference between either species could be detected in this experiment (Table III).

Table III — Mean time and standard deviation (min) a freeze-dried *A. cephalotes* ant, impregnated with vapors from crushed body parts from two different species, remained on an *A. cephalotes* nest table before it was picked up and dumped off the table (n = 6).

Tabla III — Media y desviación estandard del tiempo (min) que una hormiga liofilizada de *A. cephalotes*, impregnada con vapores de diferentes partes del cuerpo de hormigas de dos especies diferentes, permanece sobre el nido de una colonia de *A. cephalotes* antes de ser recogida y botada fuera del mismo (n = 6).

Species	Body part examined :		
	Head	Thorax	Gaster
<i>A. cephalotes</i>	4.2 ± 2.3 b	0.8 ± 0.6 a	0.8 ± 0.3 a
<i>Acromyrmex octospinosus</i>	4.5 ± 1.2 b	1.3 ± 1.2 a	1.6 ± 1.2 a

a and b indicate the means which are statistically different ( $p < 0.05$ ) using the Hartley's test (ANOVA,  $F = 6.83$ ,  $p < 0.001$ )

These experiments suggest that the species-specific recognition signal is a volatile compound or mixture of compounds over all the cuticle of the ant. The source of this volatile seems to be the head.

### The colony-specific recognition of individuals

Colony-specific recognition is achieved only if the test ant presented is alive and active. Therefore, the different body parts : head, gaster and thorax ; were cut off from live *A. cephalotes* workers from different colonies and were presented to an *A. cephalotes* colony (Table IV). No significant difference could be observed between body parts from ants of the same colony and from different colonies. In this experiment, thoraces with their legs removed were used for the test. The reason is that the legs can easily be grasped by the workers and a thorax with legs is therefore sometimes picked up by more than one ant, which results in pulling and competing for the same body part, which then will give unrealistic pick-up times for the thorax. If, instead of small body parts, more complete parts of an ant were presented to the colony, different results were obtained (Table IV). Ants with their legs and gaster removed were picked up and dropped off the table much faster if they came from different colonies of the same species than if they were from the same colony. Headless ants with or without legs were not examined extensively or attacked, in spite of the fact that they were showing movements similar to those of ants with head, but they remained on the nest table for several hours, whichever colony they originated from. Ants with only their feet removed, were attacked and dropped off the table much faster if they came from a different colony (Table IV).

Table IV — Mean time and standard deviation (min) for which a body part of a live *A. cephalotes* ant remains on the nest table of a colony of the same species before it is picked up and dropped off the table (n = 6).

Tabla IV — Media y desviación estandar del tiempo (min) que partes de una hormiga viva de *A. cephalotes* permanece sobre un nido de una colonia de su misma especie antes de ser recogida y botada fuera del mismo (n = 6).

Body part tested	Ant from same colony	Ant from different colony	Probability (t-test)
Head	12.9 ± 5.6	11.0 ± 8.5	> 0.1
Thorax without legs	7.8 ± 7.2	3.2 ± 4.5	> 0.1
Gaster	11.4 ± 10.5	12.9 ± 8.7	> 0.1
Head and thorax without legs	9.5 ± 3.6	2.4 ± 2.0	< 0.05
Head, thorax and gaster without legs	14.6 ± 7.9	4.9 ± 4.2	< 0.05
Thorax and gaster without legs	11.2 ± 6.0	10.5 ± 3.7	> 0.1
Thorax with legs and gaster	> 60	> 60	—

The results suggest that the colony-specific recognition of *A. cephalotes* workers is due to some signals from the head which are not present in isolated heads. These signals are probably volatile compounds from the head together with some kind of movement which is absent in isolated heads.

### Colony differences in head volatiles

Gas chromatograms of heads of workers from different colonies show the same compounds but in different proportions (Fig. 2). Analysis of the amount of the major components of head volatiles from young media workers showed that at least the relative amounts of 4-methyl-3-heptanone and 2-heptanone are more or less constant between of the same colony but different between colonies of *A. cephalotes* (Table V). The four colonies examined were fed and cared for in an identical way for over 3 years.

Fig. 2 —Solid-sample gas chromatograms of the head of one *Atta cephalotes* media worker, on a 5 % Carbowax column. Trace reads from right to left; temperature program : 4° per min increase from 70° to 190°C. A and C indicate the colony of origin. 1 is 4-methyl-3-heptanone, 2 is 2-heptanone, 3 and 4 are probably 3-octanone and 3-octanol respectively.



Fig. 2 — Cromatogramas de muestras solidas gasificadas de cabezas individuales de obreras medias de *Atta cephalotes*, usando columnas de Carbowax al 5 %. El gráfico se lee de derecha a izquierda; programa de temperatura : incremento de 4° por min de 70° a 190°C. A y C indican la colonia de origen. 1 es 4-metilo-3-heptanona, 2 es 2-heptanona, 3 y 4 son probablemente 3-octanona y 3-octanol respectivamente.

Table V — Ratio between peak areas of 4-methyl-3-heptanone and 2-heptanone in gas chromatograms of *A. cephalotes* media workers heads.

Tabla V — Relación entre las áreas de los picos de 4-metilo-3-heptanona y de 2-heptanona en cromatogramas de cabezas de obreras medianas de *A. cephalotes*.

A : Mean and standard deviation

Colony	Ratio of peak-area
A	$18.2 \pm 1.6$
B	$11.8 \pm 2.0$
C	$8.6 \pm 2.2$
D	$6.6 \pm 1.7$

a  
b  
c  
c

a, b, and c indicate means which are different ( $p < 0.05$ ) by Hartley's test.

B : ANOVA

Source of variability	Sum of squares	Degrees of freedom	Mean squares	F
Treatments	278.0	3	92.7	23.0 ***
Error	52.3	13	4.0	
Total	330.4	16		

\*\*\* indicate  $p < 0.001$

## DISCUSSION

From these experiments we may conclude that *A. cephalotes* workers recognise ants of their species by chemical cues spread on the cuticle which probably originate in the head. Colony-specific recognition seems to be achieved by perception of cephalic volatile substances, but it seems that ants react to these only if the intruder shows movement. That is, dead nestmates are not distinguished from those of other colonies of the same species. It is also possible that the way the volatiles are released from the head is different in an anaesthetised ant or an isolated head from a live ant or a live ant with the legs and gaster removed. There could be different rates of release of the alarm pheromone from the mandibular glands. The biological assays and the chemical analysis of the alarm pheromones from workers of different *A. cephalotes* colonies suggest that the ants use the alarm pheromone complex for recognising their nestmates or alien ants. Major differences in the alarm pheromone such as exist between *A. cephalotes* and *Acromyrmex octospinosus*,

can thus be detected easily, even in trace amounts of the pheromone on the cuticle, whereas minor inter-colony differences can presumably be detected by the workers only if sufficient pheromone is released. The alarm pheromones of different species of leaf-cutting ants vary in their components (see Crew and Blum, 1972 ; Riley et al., 1974b ; Parry and Morgan, 1979 ; Blum and Hermann, 1978). The alarm pheromone of *A. cephalotes* and *Acromyrmex octospinosus* differ in that *A. cephalotes* has 4-methyl-3-heptanone as the major component and smaller amounts of 3-octanone, 3-octanol, 4-methyl-3-heptanol and 2-heptanone (Riley et al., 1974b), whereas *Acromyrmex octospinosus* has 3-octanone and 3-octanol as major components (Crew and Blum, 1972).

Inter-colony differences in the alarm pheromone have been reported in other ant species (Cavill and Hintenberger, 1960 ; Bradshaw et al., 1979). *Oecophylla longinoda*, for example, shows differences in the composition of cephalic chemicals between colonies of the same species and between castes and ages of workers in the same colony (Bradshaw et al., 1979). Thus, the use of the alarm pheromone as colony-specific recognition signal or even caste-specific recognition signal could be common in ants. Bradshaw (unpublished observations) showed with high speed films that the recognition signal for the detection of *A. cephalotes* ants by *Acromyrmex octospinosus* colonies was mainly the cephalic volatiles of *A. cephalotes*. Thus, at least in leaf-cutting ants, species- and colony-specific individual recognition seems to be achieved using the alarm pheromone.

Ants marking their territory have their mandibles open (Jaffé et al., 1979) which could suggest that they mark their territory not only with the valves gland secretion, but also with their alarm pheromone, which could be used for colony specific territorial marking. Experiments to investigate this possibility require that the relative proportions of the different components must be accurately formulated if synthetic chemical are used.

## NEST HYGIENE

### Materials and methods

The laboratory colonies of *A. cephalotes* and *Acromyrmex octospinosus* were used for this study. Fifty observations on individual ants of different *Acromyrmex octospinosus* colonies and twenty on *A. cephalotes* workers were carried out. In each observation, an object (piece of spent fungus, freshly-cut leaf, dry leaf, etc.) was placed on a specific area of the nest (food place, waste dumping place or nest), and the activity displayed and the route walked by the ant which picked up the object, were recorded on a scale map of the

corresponding nest table. The time spent in each activity was noted separately. Each colony had a different distribution of nest area on the tables. Despite this, the following areas could be clearly identified in each colony : nest, food place, waste dumping place (waste pile), and the trail leading to the food place. All observations were performed in day-time between 11:00 am and 2:00 pm (lights off at 4:00 pm). Experiments in the dark were performed using red safety lights.

## Results

The waste dumped by the ant colonies in the laboratory cultures consisted mainly of spent fungus and dead nestmates. Occasionally, pieces of paper or other materials used in experiments were found in the rubbish pile. The dumping place was generally on the nearest edge of the table from the nest. When the nest was shifted in position on the table, ants began to dump their waste materials at a site, parallel in position from the former one. This suggests that the ants do not use marks or permanent chemical trails to find the position of the waste dumping place, but they probably find it by orientating from the nest with the help of general spatial cues.

If a pile of spent fungus is laid on the nest table next to the nest, some ants will begin to dump more waste materials on it. At the same time, other ants will carry the waste from this artificial pile to the one normally in use. Sometimes an equilibrium is established, which allows the artificial pile to exist for various weeks. If the spent fungus is spread over a larger area around the nest, the waste will be transported to the nest and then to the dumping place, without being reinforced with more waste. This suggests that the waste products of the nest, if present in a certain quantity and concentration, will stimulate workers to identify it as a dumping place and therefore use it as such. A piece of spent fungus placed inside the nest, or very close to it, is picked up by workers and carried directly to the dumping place. If a similar piece of spent fungus is placed on a different part of the nest table, it will be picked up in the same way, but it will be taken to the nest. Once in the nest or near the nest entrance, the ant carrying the waste will dump it there and another worker will then take it to the dumping place. Even if the piece of spent fungus is placed near the dumping place, it will be carried to the nest. The ant picking it up in this position, normally comes from having dumped its piece of rubbish and is on the way back to the nest. Only on very few occasions (4 %), could an ant be seen carrying a piece of spent fungus, picked up at a distance from the nest, directly to the edge of the table or to the dumping place. If a dry leaf, or a filter paper impregnated with the defence secretion is placed distant from the nest on the table, it is carried directly to the edge and dumped there. This is true for both ant species studied.

**Table VI** — Mean time and standard deviation (s) taken by an *Acromyrmex octospinosus* worker carrying a freshly-cut leaf (F) or a piece of spent fungus (R), from either the food site (FP) or the rubbish dumping site (RP) to the nest.

**Tabla VI** — Media y desviación estandard del tiempo (s) que tarda una obrera de *Acromyrmex octospinosus* en cargar hacia el nido un pedazo de hoja recién cortada (F) o un pedazo de hongo muerto (R), desde la fuente de alimento (FP) o del área del basurero de la colonia (RP).

	FP	RP
F	31 ± 12 (n = 11) a	72 ± 42 (n = 8) b
R	156 ± 98 (n = 18) c	24 ± 12 (n = 11) a

a, b, and c indicate the means which differ significantly ( $p < 0.05$ ) using the Student's t-test.

**Table VII** — Mean time and standard deviation (s) taken by an *Acromyrmex octospinosus* worker in carrying a piece of spent fungus (R) or a freshly-cut leaf (F) from the food site to the nest.

**Tabla VII** — Media y desviación estandard del tiempo (s) que tarda una obrera de *Acromyrmex octospinosus* en cargar un pedazo de hongo muerto (R) o una hoja recién cortada (F) del sitio del alimento al nido de día o de noche.

	Day-time (n = 12)	Dark, with red lights (n = 9)	Probability (t-test)
R	150 ± 114	162 ± 48	> 0.3
F	29 ± 10	56 ± 20	< 0.01

A piece of spent fungus, placed near the food source, is carried to the nest over a longer time period than a piece of freshly-cut leaf (Table VI). The inverse is true on or next to the dumping site; a piece of spent fungus is carried to the nest much faster than a piece of freshly-cut leaf. This slowness to reach the nest does not seem to be caused by a deficiency in orientational cues. If the lights are turned off, and red safe light is used instead, no differences in the time taken to transport the piece of spent fungus to the nest can be observed (Table VII), although an ant transporting a freshly-cut leaf will take a longer time before finding the trail to return to the nest. If the increased time taken by the ant in finding the nest after picking up the spent fungus on the food place is due to difficulties in orientation (lack of a «rubbish trail» for example), the absence of visual cues should increase it even more, as it does in leaf transport behaviour. Thus, the probable reason for the increased time taken to arrive at the nest and for the tortuous route taken by an ant after finding an «unexpected object» (Fig. 3) is not part of the orientation mechanism.



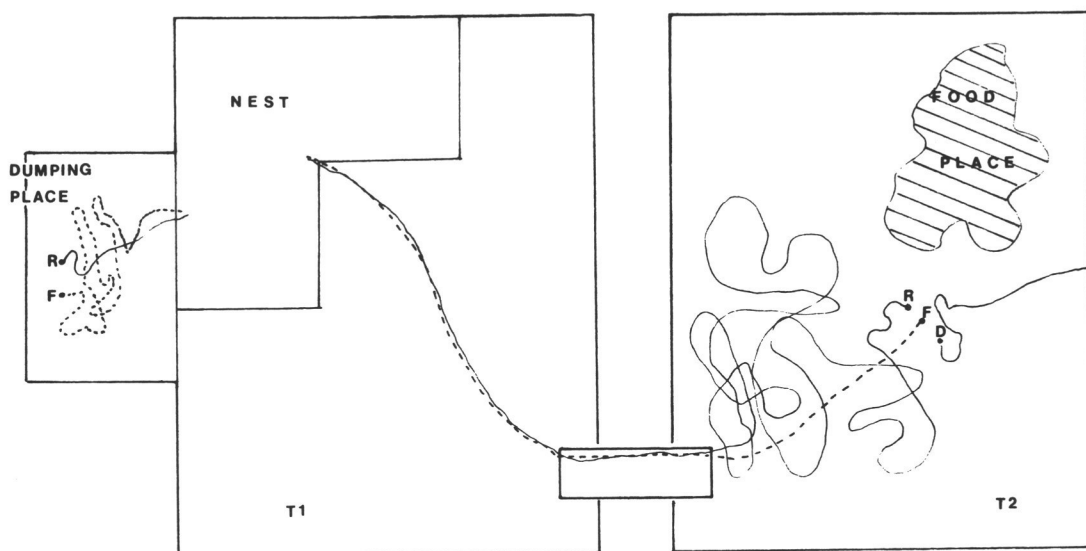


Fig. 3 — Routes taken by *Acromyrmex octospinosus* workers after picking up a piece of spent fungus (R), a freshly-cut leaf (F), or a dry leaf (D).

Fig. 3 — Rutas tomadas por obreras de *Acromyrmex octospinosus* después de recoger un pedazo de hongo muerto (R), una hoja recién cortada (F), o una hoja seca (D).

Solvent extracts of spent fungus, if applied to filter papers, will elicit the same behavioural patterns as a piece of spent fungus. Water is the best solvent, followed by methanol, ether and cyclohexane respectively (Table VIII). All the results have been confirmed in both ant species studied (*A. cephalotes* and *Acromyrmex octospinosus*). Both species have a similar behaviour in regard to nest hygiene. No differences between them could be observed. No species- or colony-specific recognition of the waste products seem to exist in either *A. cephalotes*, *A. sexdens* or *Acromyrmex octospinosus*. Wastes from the nest are treated in the same way, independently of the colony they originated from.

### Discussion

The ability of the ants to find the waste dumping site seems to be based on learning of the route from the nest, although a waste pile in itself may be used as a signal for identification of the waste dumping site of the colony.

Table VIII — Mean time with standard deviation (s) taken by *Atta cephalotes* workers picking up a filter paper impregnated with spent fungus extracts in different solvents. The papers dropped at the rubbish dumping site is indicated in brackets as a percentage of the total number of papers of the same kind presented to the colony.

Tabla VIII — Media y desviación estandar del tiempo (s) que tarda una obrera de *Atta cephalotes* en recoger un papel de filtro, impregnado con extractos de hongo muerto usando diferentes solventes. El porcentaje de los papeles botados en el basurero esta indicado en parentesis en función del total de papeles del mismo tipo ensayados.

A —

Solvent	(n for mean)	Filter paper with extract		Filter paper with pure solvent	
None (Spent fungus)	10	42 ± 55	(100 %)	—	
Water	6	71 ± 61	(100 %)	> 1500	(6 %)
		a		c	
Ethanol	5	180 ± 112	(100 %)	> 1500	(10 %)
		ab		c	
Methylene chloride	6	250 ± 198	(100 %)	> 1500	(20 %)
		ab		c	
Cyclohexane	5	484 ± 244	(80 %)	> 1500	(30 %)
		b		c	

a, b, and c indicate statistically different means ( $p < 0.05$ ) using Student's t-test.

B — ANOVA for solvent extracts

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F
Treatments	299 462.8	3	99 820.9	3 480 *
Error	516 251.2	18	28 680.6	
Total	815 714.0	21		

\* :  $p < 0.05$

The fact that a piece of spent fungus, if found in an unexpected place, will induce the ant to transport it to the nest, even if it is found near the dumping site, suggests that an object is first recognized as belonging to the nest and then as a waste product. This suggests that it is the context of the nest which classifies an object as waste or otherwise. The different treatment received by the spent fungus, according to whether it is found in- or outside the nest, does not seem to be related to caste-differences, as can be deduced from the fact that even ants which have just transported waste to the dumping site will behave in the same way as ants which were carrying leaves just beforehand.

The increased length of time taken in transporting a familiar object (spent fungus or freshly-cut leaf) to the nest if found in the «wrong» place suggests a coordination mechanism in the behaviour of ants, which is programmed for certain tasks in specific situations. If an unfamiliar or contradictory situation is encountered, the ant needs time to switch from one activity programme to the other.

The experiments show clearly the existence of two different types of rubbish in an ant colony. One type is the waste from the nest (spent fungus mainly) and the other is probably related to the cleaning of trunk trails (Dry leaves, etc.). These two types of rejected materials are treated differently. One is first transported to the nest, whereas the other is disposed of, without being first transported to the nest. The first type of rejected material is dumped in a specific place, whereas the second type is dumped off the table in any place.

The signal used for recognition of the waste from the nest appears to be chemical, as can be deduced from the experiments using spent fungus extract. Pure solvents on filter papers are not treated as spent fungus, but in a similar way to dry leaves.

These findings suggest that ants treat their waste products from the nest in a careful and special way. A specific programme of behaviour exists, which prevents potentially hazardous materials, capable of breeding diseases which could affect the fungus or the ants, from being disposed of in a random way.

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