

Orientation in leaf-cutting ants (Formicidae: Attini)

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Abstract. The cues used for orientation on chemical trails by laboratory colonies of *Atta cephalotes*, *Atta laevigata* and *Acromyrmex octospinosus* were studied. The ants used cues other than the odour trail itself when homing. When cues gave contradictory information, *Atta* workers used them in a certain hierarchy: presence of an odour trail, visual cues or spatial layout of the trail, 'odour cues' on the trail and gravitational cues. *Acromyrmex octospinosus* seemed to have a different hierarchy, giving more importance to odour cues on the trail. The possible nature of the 'odour cues' on the trail is discussed.

Leaf-cutting ants explore large areas and return to the nest without difficulty by day and night. They use trunk trails, leading outwards from the nest, and smaller recruitment trails, leading from the trunk trails to the cutting sites (Cherrett 1968; Lewis et al. 1974).

The most important positional cue is the trail pheromone; for discussion of those already identified, see Parry & Morgan (1979) and Cross et al. (1979, 1982).

Although trails made with a species-specific pheromone help the ants to orientate to the nest or to food sources already discovered, many trails from a normal attine nest cross or bifurcate, forming a complex network. In spite of this, foraging ants travel to the same cutting site along the same trail and regularly use the same nest entrances (Lewis et al. 1974). The pathways inside the nest are also very complex with numerous galleries and chambers forming an intricate labyrinth as described by Autuori (1942), Jacoby (1952, 1955), Gonçalves (1945) and Mariconi (1970). According to Jaffé et al. (1979) exploring leaf-cutting ants take tortuous routes when leaving the nest, but, when returning they sometimes take a relatively straight path. The ants never explore new territory alone, but recruit nestmates in the process. These observations suggest that leaf-cutting ants probably use other orientation mechanisms, in addition to trail-following, when orientating along the trails and through the gallery systems. These may include visual cues, olfactory cues other than the trail pheromone, gravitational cues, the earth's magnetic field, kinaesthesia and tactile information.

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The use of visual signals, such as landmarks, is probably common to most ant species and has been described, for example, in *Lasius niger* by Carthy (1951), in *Formica fusca* by Möglich & Hölldobler (1975) and in other ants of the genus *Formica* by Rosengren (1971). Hölldobler (1971, 1976) studied various species of *Pogonomyrmex* and concluded that visual landmarks may play a significant role in accurate orientation along the trunk trails in conjunction with sun-compass orientation. Ants may use trees, rocks, physically defined trails and even colours (Plekhanov & Kaul 1977) as visual landmarks. Gravitational cues are used in orientation by *Formica polyctena* (Markl 1962), *Lasius niger* and *Myrmica ruginodis* (Vowles 1954). Markl (1962) showed that the gravity receptors were similar to those of honey bees. The existence of a kinaesthetic sense in ants has been postulated by Pieron (1904), but experimental evidence for this is lacking (Carthy 1951; Wilson 1971). It has been postulated that electromagnetic radiation in the far infrared region of the spectrum is used for orientation by ants and other insects (Callahan 1977), but, again, no direct experimental evidence exists for this. The use of tactile information in orientation has been demonstrated in the army ant *Neivamyrmex nigrescens* (Topoff & Lawson 1979) which uses tactile stimuli to complement chemical signals in orientation.

Cues such as those described above may be used as back-up systems that aid the ants in following a chemical trail, particularly when they are foraging in three-dimensional space. The following experiments investigate the role of various cues in the orientation of workers of laboratory colonies of the leaf-cutting ants *Atta cephalotes*, *Atta laevigata* and

Acromyrmex octospinosus, especially in their ability to navigate between the nest and an external food source.

GENERAL METHODS

The leaf-cutting ants used for this study were one large colony of *A. cephalotes*, two small colonies of *Acromyrmex octospinosus* and one medium-sized colony of *A. laevigata*. These colonies were cultured in the laboratory at $27 \pm 2^\circ\text{C}$ and 75–85% r.h. They were supplied daily with water and a wide range of leaves, mainly cabbage, *Brassica* spp., bramble, *Rubus fruticosus*, and privet, *Ligustrum vulgare*.

The diurnal experiments were performed between 1100 and 1230 hours and the nocturnal experiments between 1700 and 1830 hours (lights off at 1530 hours) using red light for the measurements and observations. The ants were submitted to a continuous 12 h:12 h L:D cycle.

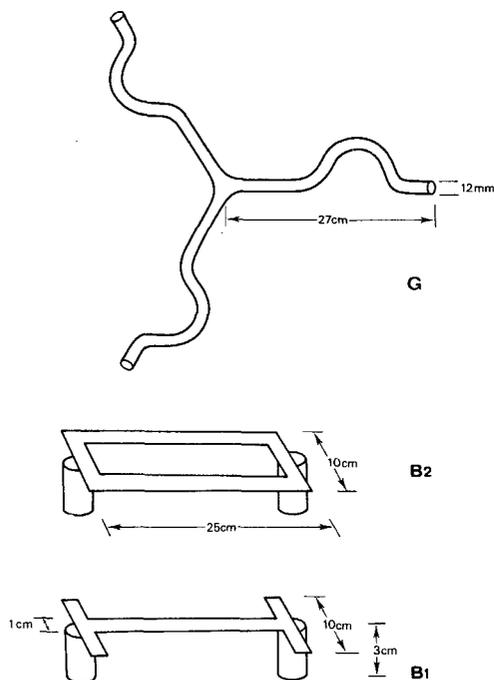


Figure 1. Mazes used in the experiments: G = glass tube maze with 120° between arms; B1 and B2 are cardboard bridges.

Four different types of maze were used (Fig. 1). Two of them were cardboard bridges (B1 and B2) and the other two were both three-armed glass tubes (GO and GC). B1 and B2 were both 25 cm long. B1 had a single runway, 1 cm wide, whereas B2 had two such runways. They were supported on corks 3 cm high, one at each end of the bridge. The glass mazes consisted of three arms 27 cm long, made of glass tubes with an internal diameter of 12 mm, each at an angle of 120° to the other and in the same plane. Each arm had an oxbow bend in the middle.

Table 1. Time (s; $\bar{X} \pm \text{SD}$) for the first leaf-carrying ant to cross a bridge, following a trail, when the bridge was turned through either 360° (control) or 180°

	Bridge B1 (one runway)		Bridge B2 (two runways)	
	Light	Dark	Light	Dark
<i>Atta cephalotes</i>				
Control	21 ± 5	29 ± 7	29 ± 9	45 ± 20
180°	25 ± 11	34 ± 17	28 ± 13	65 ± 29
<i>P</i> *	NS	NS	NS	< 0.01
<i>Atta laevigata</i>				
Control	16 ± 4	28 ± 8	20 ± 5	36 ± 10
180°	18 ± 9	38 ± 23	24 ± 11	48 ± 19
<i>P</i> *	NS	NS	NS	< 0.05
<i>Acromyrmex octospinosus</i>				
Control	10 ± 3	18 ± 5	16 ± 4	33 ± 17
180°	11 ± 2	43 ± 27	28 ± 12	68 ± 30
<i>P</i> *	NS	< 0.001	< 0.001	< 0.001

*Mann-Whitney *U*-test, $N = 24$.

The glass mazes were classified according to their termini and their transparency to light. The GC type had one end open and the other two ends sealed by conical plastic cups (4 cm in diameter and 3 cm deep). The open end led to a nest table, and the others to the plastic cups which served for capturing the ants. The GO type of maze had all three ends open and could be connected simultaneously to three different tables. The glass mazes were either transparent to light (L; GO-L and GC-L) or were covered with aluminium paper and black tape to prevent any light penetrating (D; GO-D and GC-D). The oxbow bend in each arm prevented transmission of light from the ends of the glass tubes. Tests with a maze broken in the centre, similar to one arm of the dark mazes, showed that practically no light was detectable by the human

eye or by a photometer at the centre of the maze. Only a very intense light (4300 lx or more) with the beam closer than 30° to the central axis of the arm produced a dim illumination of the glass walls on the other side of the oxbow. The light intensity in the laboratory was much lower (190 lx by day and 43 lx with red light) than the minimum required to produce a dim light in the centre of the maze. Thus in the L mazes the ants would be able to see a changing visual pattern as they traversed the maze, while in the D mazes they would detect dim light only when nearing the end of one of the arms.

Table II. Percentages of the first leaf-carrying ants that went to the nest after a 180° turn of the bridge when the ant was in the centre of it following a trail

	Bridge B1 (one runway)		Bridge B2 (two runways)	
	Light	Dark	Light	Dark
<i>Atta cephalotes</i>	100**	50	100**	67
<i>Atta laevigata</i>	75*	67	75*	58
<i>Acromyrmex octospinosus</i>	100**	25*	92*	50

* $P < 0.05$; ** $P < 0.001$; binomial test with an expected partition of 50% (Siegel 1956); $N = 24$.

The nest-end of the GC mazes and all ends of the GO mazes had a plastic collar coated with Fluon (ICI, England) to prevent ants from climbing on to the outside of the tubes. The glass mazes were cleaned with water, acetone and ether before each bioassay.

METHODS AND RESULTS

Polarity of Trails

Bridges B1 (one runway) and B2 (two runways) were used to connect the nest-table with the foraging table where food was offered to the colony. With bridge B2, the ants have the possibility of relying upon memory of turns for orientation, in addition to other cues. The ants laid a trail over the bridge leading to the food source and the leaf-carrying ants returning to the nest also laid trails by dipping the gaster on the ground. Thirty min after the food was offered to the colony, and then every 24 h, the bridge was turned through

Table III. Time (s, $\bar{X} \pm SD$) for the first food-carrying ant to emerge from the end of an illuminated maze leading to the nest table

	360° turn (control)	120° turn	P^*
<i>Atta cephalotes</i>			
GO-L maze	195 ± 68	173 ± 67	NS
GO-D maze	203 ± 74	261 ± 76	< 0.01
P^*	NS	< 0.001	
<i>Acromyrmex octospinosus</i>			
GO-L maze	103 ± 44	215 ± 129	< 0.001
GO-D maze	120 ± 48	313 ± 136	< 0.001
P^*	NS	< 0.05	

GO mazes had three arms, all of which were open. GO-L mazes were transparent, GO-D mazes were opaque.

Kruskal-Wallis one-way analysis of variance; $H = 17.5$, $P < 0.001$ for *A. cephalotes* and $H = 42.7$, $P < 0.001$ for *A. octospinosus*; $N = 24$.

* Mann-Whitney U -test.

either 360° (control) or 180° and placed again between the tables as close as possible to the previous position. After the rotation, the time taken by the first leaf-carrying ant to walk across the bridge was measured. In order to avoid a large number of leaf-carrying ants returning across the bridge simultaneously, the food offered was first cut into small pieces so that it could be picked up and transported immediately by the first ant to arrive; thus there was no time for any new recruitment process to come into play indicating a new food source and attracting a new group of ants. The same bridge was used every day in order to avoid exploratory behaviour induced by the ants encountering new territories or new objects (Jaffé et al. 1979).

In another set of experiments, the bridges B1 and B2 were turned through 180° when the first leaf-carrying ant returning to the nest was in the middle of the bridge, and the new direction taken by the ant was noted. Observations were confined to leaf-carrying ants because they are easily recognized and their motivation is probably similar. There were 24 replicates for each experimental situation.

The results (Table I) showed no difference between the control (360° turn) and the experimental groups (bridge turned through 180°) either by day or by night when B1 (one runway bridge) was used for *A. cephalotes* and *A. laevigata*. Results with *A. octospinosus* showed a significant difference between the control and the experimental group

Table IV. Number of times out of 24 replicates that the first ant, *Atta cephalotes*, of an arrested group emerged from the nest end of a maze, and the time taken to emerge at any end (s; $\bar{X} \pm \text{SD}$) of the illuminated maze

Maze	Ants released at ‡		
	(O-O)	(O-X)	(O-X', 120°)
No. of times			
GC-L	20**	20**	20**
GC-D	22**	28*	14
Time taken			
GC-L	52 ± 20	70 ± 38	86 ± 52†
GC-D	58 ± 28	104 ± 54††	91 ± 36††

GC mazes had three arms, one open and two closed. GC-L mazes were transparent; GC-D mazes were opaque.

‡ The ants were released into the same arm in which they were captured (O-O), into the alternative arm (O-X), or into the arm previously leading to the nest after it was turned through 120° (O-X', 120°).

* $P < 0.05$; ** $P < 0.001$; binomial test with an expected partition of 50%, † $P < 0.01$; †† $P < 0.001$; Student's *t*-test (Bailey 1981) comparing all with GC-L (O-O).

using bridge B1 (one runway bridge) at night. Thus, *A. cephalotes* and *A. laevigata* do not seem to recognize any polarity on the trail but the same cannot be said for *A. octospinosus* orientating in the dark.

When bridge B2 (two runways) was employed the ants tended to use one of the runways more than the other during the recruitment period. Experiments using these B2 bridges showed a significant difference between the control (360° turn) and the experimental group (180° turn) only at night for all species studied, except *A. octospinosus* which also showed a significant difference during the day. It is interesting that the bridge was crossed more rapidly in the light than the dark, even in the controls, suggesting that the ants are able to use memory of visual cues to supplement information gained from other cues.

Experiments (Table II) showed that ants were able to find their way to the nest in day-time even when the ants were turned with the bridge 180°. At night the same 180° turn seemed to confuse the ants and no clear orientation to the nest could be observed.

Table V. Number of times out of 24 replicates and time taken (s, \pm SD) for a single *Atta cephalotes* ant to emerge from the nest end of a GC-L maze

	Ant released at ‡	
	(O-O)	(O-X)
No. of times	24*	16
Time taken	57 ± 19	101 ± 31†

The GC-L maze had one open and two closed ends, and was transparent.

‡ The ants were released into the arm in which they were captured (O-O) or into the alternative arm (O-X).

* $P < 0.001$; binomial test; † $P < 0.001$; compared with (O-O) using Student's *t*-test.

Table VI. Number of times out of 24 replicates and time taken (s, \pm SD) for the first *Atta cephalotes* ant to emerge at any end of an old or new GC-L maze maintained at the same spatial position

	First ant of a group	Single ant	<i>P</i> †
No. of times			
Old	20**	24**	NS
New	18*	20**	NS
<i>P</i> †	NS	NS	
Time taken			
Old	52 ± 20	57 ± 19	NS
New	132 ± 91	289 ± 190	< 0.01
<i>P</i> ‡	< 0.001	< 0.001	

The GC-L maze had one open and two closed ends and was transparent.

† Chi-squared test.

‡ Mann-Whitney *U*-test.

* $P < 0.05$; ** $P < 0.001$; binomial test with an expected partition of 50%. Kruskal-Wallis one-way analysis of variance for time taken: $H = 65.8$, $P < 0.001$.

These results suggest that visual cues are also used in orientation along an odour trail. *A. octospinosus* seems to be more sensitive in detecting differences in the orientation of the trail. A supposed polarity of the trail could also explain the behaviour of *A. octospinosus* and of the two *Atta*

spp. in the night-time experiments: 75% of *A. octospinosus* followed the pheromone trail on B1 in the dark. The following experiments tested for trail polarity.

Table VII. Number of times out of 24 replicates for the first *Acromyrmex octospinosus* ant to emerge from the nest end, and time taken (s, \pm SD) to emerge from any end of a GC-L maze.

	Ants released at †	
	(O-O)	(O-X)
No. of times	20*	10
Time taken	156 \pm 69	154 \pm 70

The GC-L maze had one open and two closed ends and was transparent.

† The ants were released in the arm in which they were captured (O-O) or in the alternative arm (O-X).

* $P < 0.001$; binomial test, expected partition of 50%. Comparison between the values for time taken: Student's *t*-test, NS.

Homing in Glass Mazes

One end of a GO (open three-armed glass) maze was placed on a nest-table, and the other two open ends were placed on two additional separate tables with identical food on each. The ants found the food source and laid trails through the maze back to the nest-table. Every 24 h the maze was removed and the ants inside the maze were blown out with carbon dioxide with air from a vacuum pump. The maze was then rotated either through 360° (control), or 120° in a clockwise direction, and placed again connecting the tables in such a way that the ends of the maze coincided, after the rotation, with the previous points of connection. The whole process of cleaning and rotating the maze lasted less than 5 min. Then, the ants were allowed to enter the maze from the respective tables. The time for the first food-carrying ant to emerge from the end of the maze leading to the nest-table was measured with 24 replicates per experimental situation. There were at least four food-carrying ants waiting for a trail every time that the maze was replaced between the tables. Timing began when one of these ants touched the open end of the maze.

All experiments were performed during the day with *A. cephalotes* and *A. octospinosus*. Again, the food-carrying ants were selected because they are easily recognized. The total time taken for the first ant to reach the nest-end after the rotation was taken to be representative of the difficulties the leaf-carrying ants had in finding their way through the maze to the nest.

For *A. cephalotes* (Table III), with one exception, there were no significant differences between the mean times that workers spent in finding their way back to the nest. In spite of the apparent symmetry of the maze being maintained, and the fact that all the arms of the maze had been marked with trail pheromone, the ants were hindered in finding their way back to the nest when the GO-D maze was rotated through 120°. Thus, there seems to be an asymmetry of information inside the maze and the change when the maze is rotated interferes with the ability of the ants to orientate when other cues are also absent. *A. octospinosus* showed difficulties in returning to the nest after the rotation in both situations, but these were even greater without illumination. Thus it seems that the *Acromyrmex* workers depend more upon the trail itself than upon other cues when orientating in the maze. The experiments below were designed to establish more precisely the nature of the other cues.

Table VIII. Comparison between the frequencies at which the first *Atta cephalotes* ant emerged from the nest-end of a GC-L maze (data from Tables IV and V)

	First ant of a group	Single ant	<i>P</i> *
GC-L (O-O)	20	24	NS
GC-L (O-X)	20	16	NS

See Tables IV and V for further details. Chi-squared test; $N = 24$.

Odour Differences on the Trail

The open end of freshly washed GC-type maze was placed on an *A. cephalotes* nest-table and the two remaining ends sealed with plastic capsules. The ants exploring the maze eventually entered the capsules. They then laid a trail to the nest and recruited nest-mates. A likely explanation for this is

that the interior of the capsules was unmarked territory, which will normally stimulate exploratory behaviour, recruitment and marking with pheromone (Jaffé et al. 1979). The first group of more than 10 ants remaining in each capsule was trapped by removing the capsule and closing the entrance with cotton wool. The ants in the maze were then blown out with currents of carbon dioxide and air as described previously, and the maze was replaced in its previous position (360° turn, as the control). Contact with the nest-table was avoided to stop the ants in the nest from penetrating the maze. The group of ants trapped was then released either into the same arm in which it was captured (indicated by O-O in Table IV), into the alternative arm (indicated by O-X) or into the arm previously leading to the nest (indicated by O-X', 120°) such that the X end was now on the nest-table, by connecting the capsule to the corresponding arm. A few ants entered the labyrinth soon after the capsule was connected to the maze and they walked to and fro, each ant dipping its abdomen onto the lower part of the glass tube and finally emerging from one end of the remaining arms.

Table IX. Number of times out of 24 replicates the first *Atta cephalotes* ant emerged from the nest-end of a maze, and time (s, \pm SD) taken by this ant to emerge at any end of an illuminated GC-D maze with its nest-end downwards

	Ants released at ‡	
	(O-O)	(O-X)
No. of times	22*	20*
Time taken	70 \pm 33	94 \pm 41 †

The GC-D maze had one open and two closed ends and was opaque.

‡ The ants were released into the same arm in which they were captured (O-O) or into the alternative arm (O-X).

* $P < 0.001$; binomial test, expected partition 50%.

† $P < 0.05$; compared with (O-O) using Student's *t*-test.

The time taken for the first ant to emerge from one end of the maze leading to the nest or from the alternative end (X- or O- end) was measured and the arms they used were noted. The number of times they emerged from each end was also noted. The 'first ant' means not necessarily the first ant to leave the capsule, but the first to emerge from the end of one of the arms. Sometimes the ants returned to the capsule but their times of arrival were not recorded.

All experiments were performed during the day with 24 replicates for each experimental situation. Subsequently, these experiments were repeated in the dark to test for the possible influence of light at the end of the maze arm.

When ants were reintroduced into the arm of the maze from which they were taken (O-O situation) no difference was observed between their orientation in light and dark mazes (Table IV). Ants released into a different arm in a dark maze, however, took significantly longer to reach the nest. When the ants were released into the end of the maze previously adjacent to the nest (O-X', 120°) the number of ants taking the 'correct' route became highly variable ($P = 0.5$) in the darkened maze. This did not occur under illumination, but the ants then took longer to go to the nest, although a high percentage went there. Thus, *A. cephalotes* shows a homing pattern significantly different under illumination from that in the dark.

As shown in Table V, single ants cross a maze that they have previously exploited, and always make the 'correct' choice when they are released into the same arm in which they were captured. When released in a different arm from where captured, 16 out of 24 were able to find home, but took much longer to do so. This indicates that the choices of single ants are not reinforced by sister foragers. Single ants seem to be more sensitive to changes in the maze. When clean mazes were tested (after washing with water, acetone and then ether, after the ants were captured in the capsules but before they were released again into the maze) a single ant took significantly longer to explore a new maze alone than ants in a group (Table VI) as Jaffé et al. (1979) also concluded. In old mazes, ants in groups seem to home with the same ease as isolated individuals. When a new maze was used after trapping the ants they then took much longer to reach the nest and individuals took longer than groups. These results may suggest that, without an odour trail, the motivation for homing changes to

motivation for exploration (Jaffé & Howse 1979) and thus they take longer to reach the nest. It may also be that the altered chemical trail produces confusion.

whether this species orientates equally well by day or night, and whether, for example, night-time orientation may be influenced by the moon or other cues.

Table X. Number of times out of 24 replicates the first *A. cephalotes* ant emerged from the nest-end of a maze, and time (in s, \pm SD) taken by this ant to emerge at any end of a GC-D maze

	Ants released at ‡		
	(O-O)	(O-X)	(O-X', 120°)
Light			
No. of times	22**	18*	14†
Time taken	58 \pm 28	104 \pm 54†	91 \pm 36†
Dark			
No. of times	24**	18*†	10†
Time taken	68 \pm 32	114 \pm 62†	126 \pm 51†

‡ The ants were released where captured (O-O), in a different arm of the maze from where they were captured (O-X), or in the arm previously leading to the nest after the maze was turned through 120° (O-X', 120°).

* $P < 0.05$; ** $P < 0.001$; binomial test, expected partition 50%.

† $P < 0.05$; †† $P < 0.001$; for comparison with (O-O); χ^2 , 2 \times 2 contingency test for No. of times emerged; Student's *t*-test for Time taken.

Homing in *A. octospinosus* (Table VII) seems to be different from that in *A. cephalotes*. The former are more sensitive to changes in the maze and visual cues seem to play a less important role in orientation than in *A. cephalotes*. Even in the light and in groups they chose arms randomly when released in a different arm to that in which they were captured (O-X), although those ants that went to the nest did so in a time equal to that of ants released in the same position (O-O).

From these experiments we may conclude that these ants are capable of detecting differences in the maze. *A. octospinosus* seems to have a greater capability than *A. cephalotes* for detecting these differences, confirming the experimental results using bridges B1 and B2. Single *A. cephalotes* workers also seem able to find home even if released at a different arm (O-X), though they spent much more time finding home in this situation (Tables V and VIII). In spite of the fact that *A. cephalotes* uses light as an orientational cue, they can forage nocturnally. Field studies are required to find out

Gravity and Homing

Mazes of the GC-D type (two encapsulated ends with dark arms) were used as described above, except that the maze was inclined at 20° to the horizontal. The lowest point of the maze was the end of the arm leading to the nest-table. When the ants were released for the orientation test, the labyrinth was first emptied by blowing through carbon dioxide and air and was placed either in the same position as before (nest-end downwards) or with a change in the plane of inclination (O-end downwards and nest-end upwards). The number of times the first ant of a group of 10 appeared first in the end leading to the nest or at the alternative end was noted. Also the time taken by the first ant to get to the nest-end was measured. All experiments were performed during the day with 24 replicates for each experiment.

When ants were released at the end of the maze at which they were captured (O-O situation) the change in the inclination of the maze did not influence their ability to find the nest (Table IX). But if the inclination of the maze was changed when they were released at an end different from where captured (O-X situation), the ants did not emerge first from the end leading to the nest: they chose the arm leading downwards and a minority went to the nest taking longer to do so.

These results suggest that gravitational cues are used for orientation and that for an ant on a trail previously used by it (O-O), other cues are used in preference to the gravity cues, but for an ant following a trail not previously used by it (O-X), the decision about which way to go is determined more by remembering gravitational cues than by remembering other cues.

Orientation in Darkened Conditions

Although no light could penetrate the GC-D maze (the arms were enveloped in aluminium foil and black tape) the ants in the above experiments had free visibility when they were captured and released. Also, they could walk round an oxbow perceiving the incoming light and then turn around and return to make the choice again. In order to

reduce still further the possibility of the ants using light as a cue, maze GC-D was used at night. The experimental procedure was identical to that described for homing in a glass maze.

Orientation in the GC-D maze at night was the same as in day-time (Table X). Thus, the ants do not seem to be able to use any visual cue in the GC-D maze.

The fact that the ants turned the correct way even if released in a different arm (O-X, day or night-time) would indicate that they recognize the trail leading to the nest, although they took significantly much longer to reach home. An hypothesis to explain this is that the trail leading to the nest may be wider than the one going to the food. It is also possible that a double trail is formed because the traffic of ants is twice that to either of the other arms. A different concentration of the pheromone on the trails or even the use of more subtle orientation cues by the ants could also explain these percentages being different from the random choice (50%). In any case, the recognition of the arm leading to the nest seems to be based on odour, although tactile and magnetic cues cannot be excluded. The fact that orientation is hindered if the ants are released in the arm formerly leading to the nest indicates some odour differences in the O and X arms which confuse the ants. Thus, if the ants are released into an O or X arm when in the centre of the maze they will recognize the arm formerly leading to the nest but if released into the 'nest-arm' they seem unable to choose between the X or O arm.

DISCUSSION

The mazes used in these experiments can be classified into three distinct categories: those with direct visual cues available for orientation (bridges and light glass mazes); those with visual cues reduced (GC-D maze); and mazes that also had gravitational cues.

Ants may thus orientate in the presence of a trail with the additional aid of direct visual cues. In the absence of a trail, however, orientation to the nest can still be achieved by *Atta*. Nevertheless, they spent a significantly longer time homing than when they followed a trail, possibly because in the absence of a trail they would normally start exploratory behaviour. A group is necessary for exploration but a single ant is able to home without

any difficulty. A new area, for example a maze, induces behaviour different from that induced by an area previously explored by worker ants. In the presence of a trail, but in the absence of direct visual cues, additional cues may be used by the ants for orientation. No evidence for polarization of trails could be found in *A. cephalotes* and *A. laevigata*. An ant following a reversed trail with spatial cues but no visual cues (two-runways bridge, B2, with 180° turn, at night, for instance) took more time to cross the bridges than the control. If, however, the 'one-runway bridge' (B1) was reversed with no other cues available, no differences could be found between the experimental and control situations. The ants therefore seem unable to distinguish between a trail leading to the nest and one leading away from it on the basis of odour signals alone (Table I). This confirms previous observations of other ant species (Chauvin 1948; Sudd 1967). Thus, *Atta* workers find their way back home when following a trail by a kind of maze learning. They are able to detect any differences in the spatial layout of a trail even if no visual cues are present. However, in the dark, orientation is less accurate, indicating the importance of supplementary visual cues (Tables I, III and VI).

Acromyrmex workers seem to be more dependent on the trail (Tables I and II). The experiments suggest a kind of polarity which may be based on odour differences or very small spatial differences of the trail as the experiments with *Atta* suggest. In any case, *Acromyrmex* colonies used were very much smaller than the *Atta* colonies, thus fewer ants were responsible for laying the trail. It is also possible that individual ants can recognize trails they have laid themselves.

The use of different inclinations of the dark maze (GC-D), shows that leaf-cutting ants also use a gravitational sense in orientation. The use of gravity cues appears to override memory of the turns in a horizontal plane (O-X, nest-end downwards) except when using the same odour trail as before their capture (O-O, downwards).

Experiments in the dark (Tables III-IX) show that ants, if deprived of any visual and gravitational cues, follow the trails leading to the nest, probably recognizing them by means of odour differences. Such odour differences may explain the results using GO-D mazes (Table III). The presumed odour differences in the various arms of the maze could be due to one of several factors: (1) differences in the distribution of odour, producing

a kind of 'odour landscape'; (2) the use of special markers, placed at strategic points on the trail; (3) differences in the proportion of the minor components of the multicomponent trail pheromone of different individuals; (4) the use of another pheromone in addition to the trail pheromone, which would allow for individual differences of the trails; (5) an odour gradient which would polarize the trail; (6) different concentrations of pheromone or different trails or different width of the trail.

Alternative 3 depends on the possibility that each individual ant may have a slightly different trail pheromone complex. Inter-caste and inter-individual differences in alarm pheromone complexes have been found in ants such as *Oecophylla longinoda* (Bradshaw et al. 1979) and *Myrmicaria eumenoides* (Howse & Bradshaw 1980). Different groups or populations of ants may prefer to follow trails with a pheromone composition similar to the one they themselves produce. This difference in the composition of the trail pheromone complex could be due to minor components such as those present in the poison gland secretion of *Atta texana* (Tumlinson et al. 1972) and *A. cephalotes* (Riley et al. 1974). The end result would be the existence of different trails with slightly different proportions of the pheromone components, which would be used by different groups of ants from the same colony. Rosengren (1977) concluded that the trail fidelity of individual *Formica polyctena* workers depends only on visual memory during the day, but probably depends on odour differences on trails at night.

Alternative 4 would imply the existence of a pheromone from a different glandular source conferring individual differences on the trails laid by the ants. Ants encountering a junction of a trail leading to the nest and another leading to the place in which they were captured would be able to distinguish both trails. One would have a 'specific odour' only, whereas the other would have the 'specific odour' plus another odour from ants using the second arm of the maze.

Our experiments do not allow us to decide between these six possibilities, although they strongly suggest that such odour differences exist. The possible use of tactile cues or orientation using the earth's magnetic field cannot be excluded, but there have been no reports of magnetic field orientation in ants. The use of tactile differences in the glass mazes is very improbable.

Under the experimental conditions the results

suggest a hierarchy of cues used for orientation in leaf-cutting ants: (1) presence of an odour trail; (2) visual cues; (3) spatial layout of the trail; (4) odour differences on the trail; and (5) presence of gravity cues.

It seems that the ants are better prepared for exploration during the day than during the night, but that with the help of trunk trails (permanent trails), the spatial layout of which has been learned by the ants, and with recruitment trails with odour differences on them, orientation at night is possible. The apparent difficulty in orientating without trails at night is probably counteracted by the exploratory behaviour of these ants. Leaf-cutting ants never explore a new territory alone, but recruit nestmates with odour trails in order to do so (Jaffé et al. 1979).

The importance of specific odour cues in orientation could explain why ants are able to return repeatedly to certain foraging sites, always using the same routes in a complex of trails (Lewis et al. 1974). Different methodology could explain the apparent contradiction between these results and those of Robinson et al. (1974). They reported that the workers of attines are unable to distinguish between trails of different colonies and in some cases even of different species. This negative result may be due to the experimental method used; no allowances were made for effects due to visual orientation, gravity cues and so on.

According to Jander (1963) orientation to odour alone is as rare among ants as it is among other animals, and integrated navigation seems to be the more general pattern (Sudd 1967). Different ant species will probably differ in the hierarchy of cues used for orientation. *Formica polyctena* for instance, uses odour trails as a complement to visual navigation (Horstmann 1976). Orientation in *Atta* species seems to be achieved similarly in the different species studied so far, but *Acromyrmex* in these studies proved to be more dependent on the trail and possible odour differences on it for orientation.

In studying the mechanisms used for orientation of ants to and from their nest, the assumption has commonly been made that odour trails and menotaxis (the maintenance of a visual axis during locomotion) are the most important, and that either may dominate according to the species. This view now has to be re-examined as it is becoming increasingly clear that many different cues may be used, as the present study shows, and that ants may

either integrate several sources of information or select between them in a hierarchical fashion which reduces ambiguity. This places great stress on the critical design of experiments on orientation and navigation. There are interesting parallels here in the study of bird navigation (reviewed, for example, by Walcott & Lednor 1983), where factors such as visual memory, the earth's magnetic field, the sun and odour of the home may be used separately or in combination in certain species. Although many experiments here show that pigeons navigate using the sun as a compass, Keeton (1969) was the first to design an experiment capable of demonstrating that they are able to navigate in overcast conditions by switching to alternative cues, including the earth's magnetic field.

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