

## Nestmate recognition in the leaf-cutting ant *Atta laevigata*

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**Key words:** Ants, nestmate recognition, kin recognition, pheromones.

### Summary

We tested mature *Atta laevigata* colonies in the field to see if the ants used queen substances, environmental odours (in this case odours produced by the nest's fungi), an odour produced by each individual, or a gestalt odour (resulting from odours distributed between nestmates) as a discrimination signal for nestmate recognition. We found that nestmate recognition in *A. laevigata* appears to be largely based on an odour produced by each nestmate which appears to be concentrated in the head, although other odours may also be used. We found no evidence of genetic relatedness influencing the discrimination ability, nor did ants respond differently to neighbors in comparison to non-neighbors.

### Introduction

The ability of many species of ants to distinguish between nestmates and others is legendary (e.g. Fielde, 1904; Elton, 1932; Mabelis, 1979). However, there remains a lot of debate as to the means by which ants recognize nestmates. Different recognition cues have been postulated to be used by different ant species, but in no case has a clear relationship between specific cues and nestmate recognition been established. Four possible methods implicated in various species include: a) environmental odours such as food and/or nest material (with some experimental support from experiments with *Acromyrmex octospinosus*: Jutsum et al., 1979, for example); b) a discriminating substance produced by the queen which is distributed among workers (e.g. *Camponotus* sp: Carlin and Hölldobler 1986; 1987; but see Crosland, 1989a; 1990a); c) discriminators produced by each individual (e.g. *Atta cephalotes*: Jaffe, 1983; *Odontomachus bauri*: Jaffe and Marcuse, 1983; *Leptothorax curvispinosus*: Stuart, 1987; *Rhytidoponera* sp: Peeters, 1988); and d) odours which are distributed among all colony members to produce a collective discriminating substance or "gestalt" odour (e.g. *Rhytidoponera confusa*: Crosland, 1989c; *Pristomyrmex pungens*: Tsuji, 1990).

As regarding the anatomical origin of the individually produced recognition cue, two proposals have been put forward. The cues are thought to be odours produced in the head by some cephalic gland (Jaffe, 1983; 1987) or are chemical substances (mainly cuticular hydrocarbons) which are spread over the cuticle (Nowbahari et al., 1990) or both (Jaffe, 1987). This controversy seems to get more interlocked as recent evidence suggests that cuticular hydrocarbons are stored in the cephalic postpharyngeal gland (Hefetz et al., 1994). No other anatomical source for chemical cues has been proposed for nestmate recognition in ants, nor is there any compelling evidence of visual, tactile or auditory cues playing a role in nestmate recognition (Hölldobler and Wilson, 1990).

One reason why clear identification of nestmate recognition mechanisms has been difficult is that ants vary greatly in their tendency to respond to non-nestmates. Various factors may be responsible for this (Carlin and Hölldobler, 1987; Stuart, 1991). For example, not all workers are equally adept at recognizing non-nestmate (Crosland, 1990b) nor show the same recognition cues (Crosland, 1989b). In addition, ants may respond differently to different foreign nests. For example, ants may be more aggressive towards more distantly related non-nestmates, as has been shown in *Formica polyctena* (Gordon, 1989), or ants from some nests may produce particularly distinctive signals, making them more readily identifiable and therefore more prone to attack.

Leaf-cutting ants are known to indulge in large-scale wars where the ability to recognize nestmates is paramount (Salzemann and Jaffe, 1990). However the actual means by which these ants may recognize nestmates is currently unclear. The aim of this paper is to search for possible nestmate recognition mechanisms in the leaf-cutting ant, *Atta laevigata*. To do this we conducted a series of experiments to examine the methods by which *A. laevigata* recognize nestmates, and factors that may influence the ability of ants to recognize non-nestmates.

## Methods

These experiments were conducted in commercial pine plantations (*Pinus caribae*) in the State of Monagas, Venezuela, using mature nests of *A. laevigata*, a pest species in these plantations, which is present in high densities. In all these tests the same bioassay was used: two ants, (the "subjects") a nestmate and a foreigner, both of which had undergone the same treatment, were restrained using a pin (with care being taken not to touch the subjects with our hands, or to spread over their bodies their own defense secretion) and placed 2 cm apart on the foraging trail about 10 cm from the foraging nest entrance (where the "nestmate" had been collected). The restrained subjects were observed for 2 minutes, during which time we recorded the number of times each subject was "bitten" (i.e. mouth parts were applied to the subject) by foraging ants. All subjects were collected from foraging entrances and were about 7–9 mm in length. Tests were done "blind" in that the person recording the number of bites received by each subject did not know which was the nestmate and which the foreigner.

Three types of statistical analyses were used: Binomial, Mann-Whitney U test (M-W U), and the Kruskal-Wallis non parametrical one way ANOVA (K-W A).

For the binomial test we compared, for each nest, the number of times the foreign subject was bitten more with the number of tests the nestmate subject was bitten more. For the M-W U and K-W A, we calculated, for each test, the recognition index, which was the difference between the number of bites that the two subjects received. The recognition index was positive if the foreign subject in the test was bitten more often than the nestmate subject, and negative if the nestmate was bitten more often.

### *Methods of recognition*

The signals postulated to be used by ants to recognize nestmates are: a) environmental odours, b) a queen discriminator pheromone, c) individual discriminators, which may be a signature pheromone unique to each individual (but similar among workers of the same colony or from the same mother) or d) odours distributed between nestmates (gestalt odour). We conducted a series of tests to directly address a, c and d, but it was unfeasible to look directly for b (a queen discriminator pheromone), because *A. laevigata* colonies have only one queen, but may contain up to 3 million workers occupying a volume of 20 m<sup>3</sup> (Jaffe, 1993). Despite these constraints, the effect on nestmate recognition of the absence of a queen (i.e. measured on isolated workers) could be assessed.

Subjects underwent seven tests in the field, these were:

1. tested immediately
2. isolated for 20 minutes
3. isolated for 24 hours
4. tested immediately without head
5. isolated without head for 24 hours
6. isolated with nestmates and fungi from respective nests for 24 hours
7. isolated with nestmates only for 24 hours

The tests were conducted simultaneously throughout the year with tests 1 and 4 acting as controls. Subjects were isolated by placing them individually in glass vials (diameter 2 cm, height 7 cm) with a few pine needles in a cool dark place. Subjects isolated with nestmates only were placed in a large open-topped plastic jar (diameter 15 cm, height 22 cm) with about 40 nestmates of various sizes. Because fungi contains a contingency of omnipresent small ants, subjects isolated in the glass vials with their fungi were also isolated with numerous small nestmates.

### *Factors influencing recognition ability*

We examined two factors that may influence recognition abilities: reciprocity, and a neighbor/non-neighbor effect. A *neighbor effect* occurs when animals are able to distinguish between strangers which are neighbors and non-neighbors and consequently respond differently to the two groups. *Reciprocity* is when nests respond similarly towards each other. That is, if nest A responds strongly towards ants from nest B, then nest B will respond strongly to ants from nest A. If genetic relatedness

affects the ability of ants to discriminate nestmates, then one would expect the pattern of recognition to be reciprocal.

Thirty nests were tested in groups of 10. For each group active foraging entrances from different nests were chosen from 2 localities, El Meroy and Chaguaramas, which are about 20 km apart. At each locality 5 entrances (one for each nest) each about 100 m apart were chosen. We tested each nest with every other nest in the group so that each nest was tested with its neighboring nests and distant non-neighboring nests. In addition, because each nest was tested with each other nest in its group, we were able to see if nests responded similarly towards each other, that is, whether the response within the pair was reciprocal.

## Results

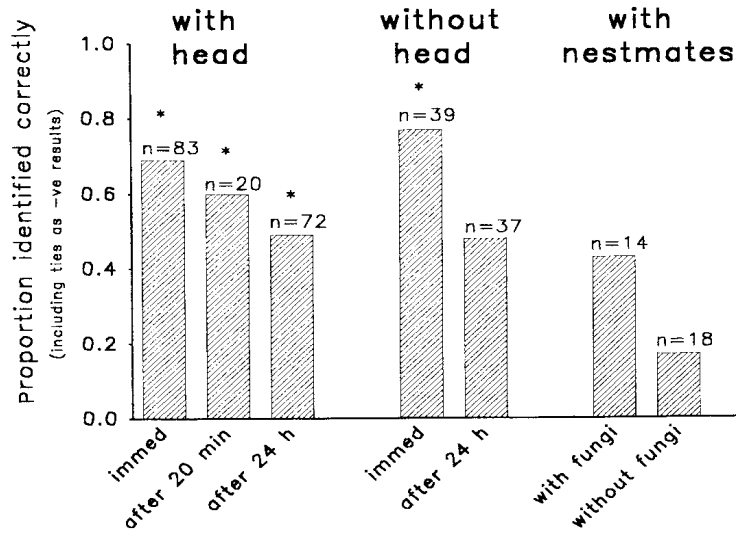
### *Methods of recognition*

Results from the field revealed that ants were able to distinguish between nestmates and non-nestmates which were taken from the nest and tested immediately both with and without their head (Fig. 1, binomial test  $p < 0.0001$ , ratio: 57:10, 16 ties;  $p < 0.0001$ , ratio 30:6, 3 ties; respectively) and which had been isolated for 20 min or 24 h (binomial test,  $p = 0.04$ ; ratio: 12:4, 4 ties;  $p = 0.0005$ , ratio: 35:15, 22 ties; respectively). Although ants recognized whole nestmates after 24 h, this ability appeared to have deteriorated, as there was a significant difference between subjects tested immediately, after 20 min, and after 24 h (K-W A:  $p = 0.002$ ,  $df = 2$ , mean ranks: immediate: 87.7; after 20 min: 74.5; after 24 h: 60). Subjects without a head were not recognized after 24 h (binomial test  $p = 0.24$ , ratio: 18:13, 3 ties) and although there was no difference between the ability of nestmates to recognize subjects with or without heads for 24 h (M-W U:  $p = 0.81$ ,  $n = 89$ ) ants were more likely to recognize headless subjects tested immediately than those headless after 24 h of isolation (M-W U:  $p = 0.01$ ,  $n = 76$ ).

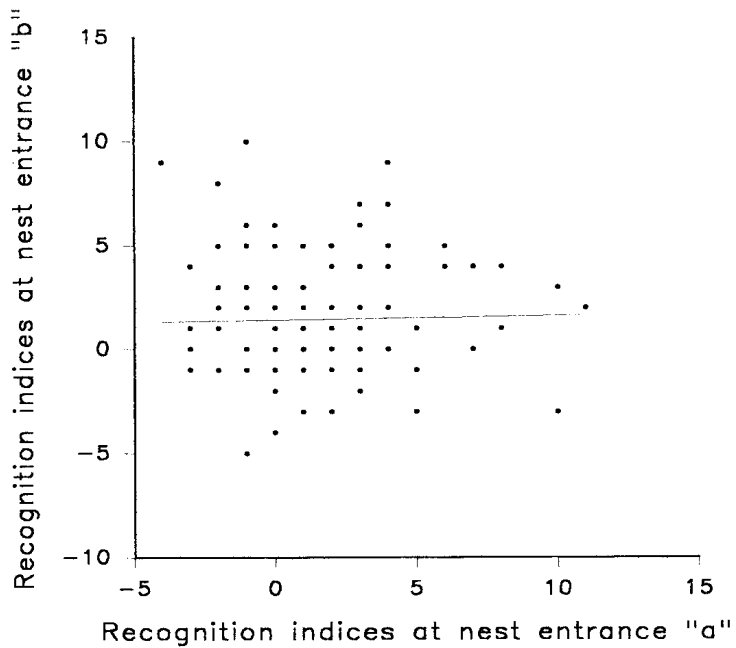
Ants were not able to distinguish between nestmates and foreigners maintained for 24 h with their respective fungi (binomial test,  $p = 0.38$ , ratio 6:4, 4 ties) nor with their respective nestmates (binomial test,  $p = 0.11$ , ratio 3:8, 7 ties). Even ants maintained with a foreign fungi elicited similar responses to those subjects maintained with their own fungus (M-W U,  $p > 0.1$ ,  $n = 32$ ).

### *Factors influencing recognition ability*

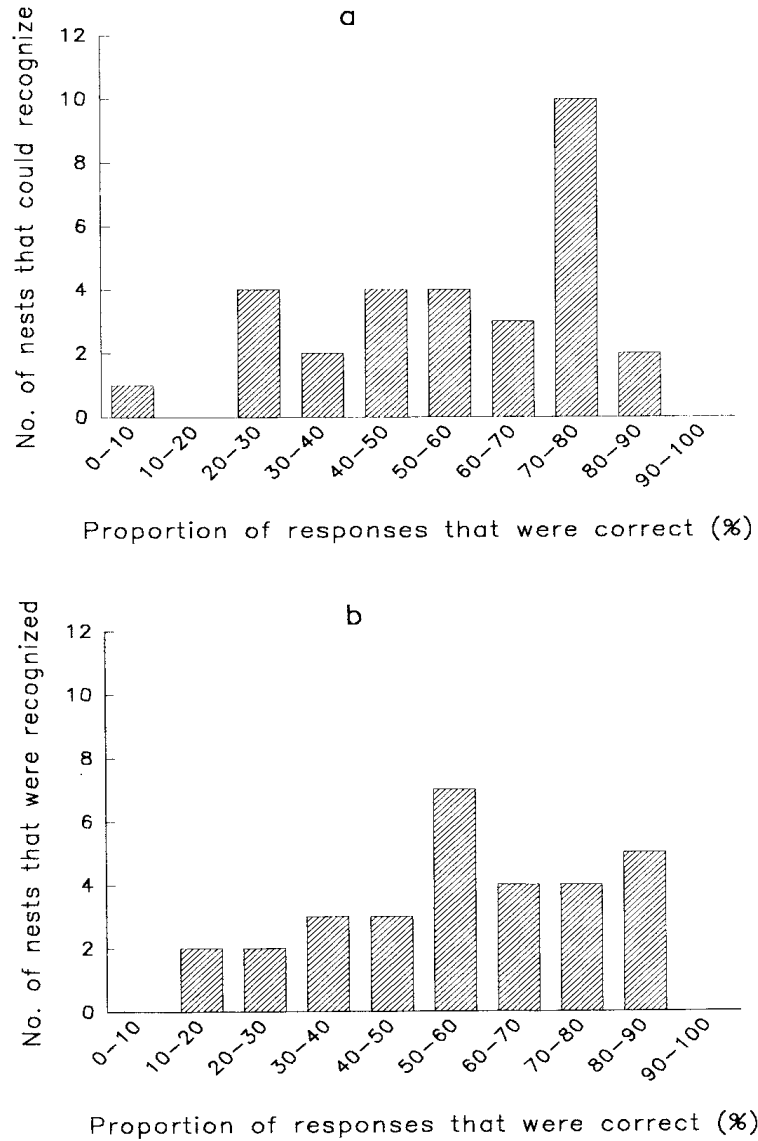
*Reciprocity:* A chi-square test of independence with 128 pairs revealed that there was no reciprocity. If one nest correctly identified an ant from a second nest as foreign, that nest would not necessarily identify an ant from the first nest as foreign ( $df = 4$ ,  $X^2 = 3.84$ ,  $p = 0.43$ ). This result was confirmed by a Spearman Rank Correlation which compared the recognition indices recorded for a pair of nests at each nest site (Fig. 2). The correlation coefficient was 0.06 ( $n = 128$ ) indicating that there was no reciprocity in responses within nest pairs. Thus it appears unlikely that ants respond more readily to more distantly related nests.



**Figure 1.** The proportion of nests that correctly identified the foreign subject. Tests in which the nest responded the same to both subjects are included as incorrect responses in this figure. The asterisks indicate the conditions under which ants were significantly more likely to attack the foreign subject



**Figure 2.** A comparison between the recognition indices of nest pairs (n = 128 pairs). There was no correlation between the recognition indices, as indicated by the regression line (correlation coefficient = 0.06)



**Figure 3. a:** The proportion of correct responses (expressed as a percentage) that ants from each nest made; and **b:** the proportion of correct responses (expressed as a percentage) that each nest elicited. For example, in Fig. 3a there were nine nests whose ants correctly recognized foreigners in 70 to 80% of the tests

Although we did not find reciprocity between nests, we found a few distinctive nests to which other nests reached strongly, but which did not necessarily reacted strongly towards other nests. Figure 3 shows the tendencies of the 30 nests tested to either correctly recognize other nests or to elicit correct responses (i.e. be recognized by other nests). Both of these characteristics varied considerably, indicating that there were some nests that were particularly astute at recognizing foreigners (K-W A:  $p = 0.003$ ,  $F = 51.6$ ,  $df = 29$ ), and also that there were particularly distinctive nests which elicited aggression (K-W A:  $p = 0.019$ ,  $F = 45.5$ ,  $df = 29$ ).

#### *Neighbor effect:*

To see if ants were more likely to recognize non-neighbors as foreigners than neighbors, we did a test of independence using the 258 interactions and found that in the interactions with neighbors, 63 correctly identified the foreign ant and 55 failed to identify the foreign ant, while in the interactions with non-neighbors, 85 correctly identified the foreign ant while 60 failed to. This difference was not significant ( $df = 1$ ,  $X^2 = 0.73$ ,  $p = 0.40$ ).

## Discussion

### *Methods of recognition*

Table 1 shows the results predicted by the various models of nestmate recognition, and the results we obtained. Our results did not match any of the models exactly, but were closest to the individual odour model. Predictions from the individual discriminator model fit the results well. However this model does not explain why ants were not recognized when they were kept with fungi or other nestmates. An explanation is that these conditions somehow masked the subject's odour. The results differ from those predicted by the queen discriminator model in that this model predicts that ants would be unable to discriminate between nestmates and non-nestmates after 20 min or 24 h of separation. We found that the ants could discriminate nestmates from non-nestmates after this period of time, but that this

**Table 1.** Results predicted from the four models of nestmate recognition and the results we obtained. A "+" indicates that the model predicts recognition, while a "-" indicates that the model predicts that the ants should not be able to distinguish between the subjects.

Test	Model				Results
	a (environ)	b (queen)	c (individ)	d (gestalt)	
1 (immed)	+	+	+	+	+
2 (20 mins)	-	-	+	-	+
3 (24 h)	-	-	+	-	+
4 (-h immed)	+	+	+	+	+
5 (-h 24 h)	-	-	-	-	-
6 (fungi + nm)	+	-	+	+	-
7 (nestmate)	-	-	+	+	-

ability was reduced. The reduction suggests that a queen discriminator could play a role in recognition, but as ants were still recognized after 24 h and the presence of the head improved long term recognition, a discriminator produced by the individuals may be more important.

Another explanation for the reduced recognition of subject ants after prolonged periods of isolation is that these ants were physiologically affected by being retained for 24 hours, which may have affected pheromonal production influencing recognition. If this is so, then it would strengthen the argument that the ant's inherent odour is predominately important for nestmate recognition in *A. laevigata*.

As the ability to distinguish between nestmates and non nestmates was not detected in the test with isolated headless ants, it is possible that the head may be an important site for nestmate recognition. This supports previous proposals that the mandibular gland may be important in nestmate recognition (Jaffe, 1984, 1987). Many workers have suggested that ants may use cuticular hydrocarbons for nestmate recognition (e.g. Mintzer and Vinson, 1985; Hölldobler and Wilson, 1990; Nowbahari et al., 1990). Recent evidence suggests that these hydrocarbons are stored in the head (Hefetz et al., 1994), thus the finding that recognition was reduced in headless ants also ties in well with this model. The fact that ants did recognize their nestmates when presented immediately after decapitation suggests that any odour discriminator produced in the head is spread all over the body of the insect.

#### *Factors influencing recognition ability*

We found no evidence that nests responded in the same way towards each other (reciprocity) indicating that genetic relatedness is unlikely to affect the response to non-nestmates. By using both close and widely separated nests we ensured that nests were tested with ants that would be both related and unrelated. If unrelated nests respond more strongly towards each other than related nests, we would expect reciprocal responses between pairs of nests. That is, if nest A responds strongly to ants from nest B, then nest B should respond strongly to ants from nest A. this was not evident from our tests.

However, we did find evidence of ants responding differently to particular nests. This suggests that particular nests may have distinctive characteristics to which other nests respond strongly. The strength of the response does not seem to be related to genetic relatedness although it may be due to some inherent characteristic of the ants or the colony as a whole. This last result confirms previous suggestions (Mintzer and Vinson, 1985; Jaisson, 1987; Stuart, 1988) in that genetic relatedness is not the primary factor determining nestmate recognition, suggesting further that nestmate-recognition, rather than kin-recognition is used by ants.

*A. laevigata* did not consistently treat neighbors differently from non-neighbors. Gordon (1989) argued that the harvester ant *Pogonomyrmex barbatus* which is able to distinguish between neighbors and strangers responds more strongly towards neighbors because a group of neighbors indicates a possible invasion, whereas a group of unrecognized ants are probably lost and not a threat to the nest. *A. laevigata* are known to engage in wars with neighboring nests, thus neighbors may pose

more of a threat to a nest than strange ants, but this was not translated into a different response towards neighbors compared to non-neighbors in this study.

In conclusion, nestmate recognition in *Atta laevigata* appears to be largely based on an odour produced by each nestmate, although other odours whose sources are external to the ant may also be used. Studies on other ant species has also indicated that they may use more than one method to recognize nestmates, (e.g. Carlin and Hölldobler, 1986; Crosland, 1990a; Jaffe, 1987) leading to the suggestion that ants in general may use more than one means by which to recognize nestmates. Our finding that recognition was not modulated by genetic relatedness, and was reduced, but not lost, over time, suggests that *A. laevigata* may use a blend of odour signals when recognizing nestmates.

### Acknowledgments

We thank El Centro de Investigaciones de Ciencias Forestales (CVG-Proforca) for allowing us to use their facilities and conduct the research on their land. In particular we would like to thank Catalina Ramos who generously provided us with equipment and backup support. We are also grateful to our field assistance, Antonio Acevedo, Jose Mora, Cruz Aray, Jose Aponte and especially Jesus Velasquez for their invaluable help in the field. This research was supported by grant QF-36-BID-Conicit and CVG-Proforca-Funindes.

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Received 15 September 1994;  
revised 19 December 1994;  
accepted 3 January 1995.