

MANDIBULAR GLAND CHEMISTRY OF GRASS-CUTTING ANTS: SPECIES, CASTE, AND COLONY VARIATION

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Abstract—The compositions of the alarm pheromones of two species of grass-cutting ant, *Atta bisphaerica* and *A. capiguara*, were examined, and caste and colony variations quantified. The pheromones of *A. bisphaerica* and *A. capiguara* were remarkably similar and were composed of a complex mixture of volatiles in which 4-methyl-3-heptanone and 2-heptanone were the most abundant compounds. Small but consistent intraspecific differences were found between the worker castes and between individual colonies. The results support the view that alarm pheromones are rarely species specific. The possible importance of intercolony variation is discussed.

Key Words—Leaf-cutting ants, alarm, pheromone, caste, colony, *Atta bisphaerica*, *Atta capiguara*, mandibular gland, Formicidae.

INTRODUCTION

Alarm behavior is one of the most obvious behaviors of ants, but it is also one of the hardest to define because of the wide range of responses that it can involve. The chemistry of ant alarm pheromones is equally diverse, but compounds typically have a molecular weight of between 100 and 200 and have 5–10 carbon atoms (Wilson and Bossert, 1963). The range of possible molecular weights is related to the function of alarm pheromones. An alarm response is a rapid reaction and so requires compounds of high volatility and low molecular weight in order that they evaporate rapidly (Bossert and Wilson, 1963; Wilson and Bossert, 1963). Furthermore, there is less need for the specificity shown by other pheromones, such as trail or sex pheromones, and so large, complex

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molecules, which are energetically more expensive to produce, are unnecessary (Blum, 1969; Hölldobler and Wilson, 1990; Vander Meer and Alonso, 1998).

Leaf-cutting ants (Hymenoptera: Formicidae: Attini: *Atta* and *Acromyrmex*) are dominant herbivores in the neotropics (Hölldobler and Wilson, 1990). They can account for as much as 50% of the total herbivory in some areas (Blanton and Ewel, 1985) and are one of the most destructive pests in many of the regions where they occur (Weber, 1972; Cherrett, 1986). *Atta* colonies, in particular, can be extremely large, with worker populations that are highly polymorphic and polyethic. When alarmed, leaf-cutting ants exhibit an aggressive response (Wilson and Regnier, 1971) and release an alarm pheromone from their mandibular glands (Butenandt et al., 1969; Blum et al., 1968; Moser et al., 1968; Riley et al., 1974; Knapp, 1995).

A number of studies have examined the chemistry of this mandibular gland secretion. In the earliest work, Butenandt et al. (1959) identified citral as the main component in *Atta sexdens rubropilosa*. The presence of this compound gives the crushed heads of *A. sexdens* workers a characteristic lemon smell. Later, Moser et al. (1968) found 4-methyl-3-heptanone and 2-heptanone at a ratio of 4 : 1 to be the principal components in major workers of *A. texana*. These ketones were also identified at species-specific ratios in the alarm pheromones of six other species of *Atta*, including *A. bisphaerica* and *A. capiguara* (Blum et al., 1968). However, subsequent research by Schildknecht (1976) and Nascimento et al. (1993) has revealed the chemistry of the mandibular gland secretion to be far more complicated than these early studies suggested, and some 56 compounds have been identified to date in *Atta* alone. However, for only very few has any behavioral activity been demonstrated. Although Butenandt et al. (1959) described citral as being the alarm-stimulating compound in *A. sexdens rubropilosa*, all other studies have concluded that 4-methyl-3-heptanone is the most important releaser of alarm behavior in *Atta* (Blum et al., 1968; Moser et al., 1968; Riley et al., 1974; Knapp, 1995; Pow, 1996).

The composition of alarm pheromones can vary between castes of ants. This was first found to be the case in the African weaver ant, *Oecophylla longinoda*, in which the glands of minor workers lack three of the main compounds found in major workers (Bradshaw et al., 1979). Similar variation between castes has been found in *Atta sexdens rubropilosa*. Nascimento et al. (1993) analyzed the mandibular gland secretion of workers of this species and found that the extracts of large workers consisted of a complex mixture of compounds dominated by citral. The secretion of small workers was 89.5% 4-methyl-3-heptanone and neither of the isomers of citral were present. A similar pattern has been found in *Atta laevigata* with 4-methyl-3-heptanone again dominating the secretion of the smaller workers (Hernández et al., 1999).

Alarm pheromones also can vary between colonies. Bradshaw et al. (1979) found that the mandibular gland chemistry of colonies of *Oecophylla longinoda*

differed significantly between geographical localities and even between colonies from the same location. Cherix (1983) also found that the alarm pheromone of *Formica lugubris* differed both qualitatively and quantitatively between colonies at the same location. This aspect has not been examined in leaf-cutting ants. Most studies (e.g., Butenandt et al., 1959; Moser et al., 1968; Schildknecht, 1976; Nascimento et al., 1993; Hernández et al., 1999) have examined ants only from a single nest. However, Whitehouse and Jaffé (1995) found that workers of *A. laevigata* respond more aggressively to the crushed heads of nonnestmates than nestmates. This suggests not only that there are intercolony differences in the alarm pheromone, but also that these differences are detectable by the ants.

The alarm pheromone chemistry of *A. bisphaerica* and *A. capiguara* has not been examined since the early study by Blum et al. (1968). Furthermore, our knowledge of caste variation in the alarm pheromone of leaf-cutting ants remains limited and that of colony variation is nonexistent. Identification of the chemistry and variation in the alarm pheromone is essential to further studies on the alarm behavior of these species. The compositions, therefore, of the alarm pheromones of *A. bisphaerica* (Forel) and *A. capiguara* (Gonçalves) were examined. Compositions for the four worker castes of each species also were compared, and intercolony differences were quantified in *A. capiguara*. The behavioral activity elicited by the compounds will be examined elsewhere (Hughes et al., 2001).

METHODS AND MATERIALS

Sample Collection. Samples of the heads of both *Atta bisphaerica* and *A. capiguara* workers were collected between March and June 1998. For both species, 10 samples of each of the four worker castes were collected, based on the estimated head width of the ants. Minors were less than 1.4 mm head width, medias 1.5–2.0 mm, foragers 2.0–3.0 mm, and soldiers had head widths >3.0 mm. The minor and media worker samples contained the heads of 20 individuals, samples of foragers comprised 10 heads, while the soldier samples contained only a single head. The *A. bisphaerica* samples were collected from a single nest near Viçosa, Minas Gerais, Brazil. The nest from which the *A. capiguara* samples were collected was located near Capinópolis, Minas Gerais. In addition, 10 samples of the heads of *A. capiguara* foragers were collected from each of four nests located on the UNESP campus in Botucatu, São Paulo, Brazil.

Nascimento et al. (1993) found that the chemical composition of the mandibular glands of *A. sexdens rubropilosa* does not differ between ants on the foraging trails and those within the nest. To reduce the disturbance to the ants, therefore, they were collected from the trails. Ants were gently removed from the trails and immediately cooled, which prevented them from becoming alarmed. They were then transported to the laboratory where their heads were

removed and placed in glass vials containing 500 μl of dichloromethane solvent. The mandibular glands are probably the only source of volatile compounds in the heads of ants (Howse and Bradshaw, 1980), and both Nascimento et al. (1993) and Knapp (1995) have found that there is no difference in the volatiles contained in crushed heads and mandibular glands of *A. sexdens rubropilosa*. Only crushed heads were examined here. Once samples contained the required number of heads, the heads were crushed thoroughly with a glass rod. After 24 hr the extract was removed and stored between -20°C and -60°C until analysis.

Chemical Analysis. The sample extracts were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). The gas chromatograph used was a Hewlett Packard 5890A with a flame ionization detector. The column was DB5, 30 m \times 0.25 mm, with a film thickness of 0.25 μm . Nitrogen was used as the carrier gas. The temperature program was 40°C for 2 min, then increasing at $10^{\circ}\text{C}/\text{min}$, with a final temperature of 280°C for 30 min. Compounds were quantified with 5-methyl-3-heptanone (97%, Aldrich) as an internal standard.

To identify the compounds, a VG-Analytical 70-250SE mass spectrometer was used, coupled to a Hewlett-Packard 5790 gas chromatograph. The column was BP1, 25 m \times 0.22 mm, with a film thickness of 0.25 μm . Helium was used as the carrier gas and the temperature program was 40°C for 3 min, increasing at $10^{\circ}\text{C}/\text{min}$ to 75°C , then increasing at $20^{\circ}\text{C}/\text{min}$, and ending with 5 min at 300°C . The conditions were 70 eV electron impact (EI) ionization, and an ion chamber temperature of 200°C . Compounds were provisionally identified by examination of the fragmentation pattern of their mass spectrograms. This was then confirmed by comparing the results with library mass spectrograms and with the mass spectrograms of synthetic versions.

Statistical Analysis. The GC integrator produces peak areas for each compound that are dependent upon the amount of material in the sample. To avoid this, the areas of each peak are divided by the sum of areas of all the peaks in the sample. This results in proportional data that adds up to one. However, as the data are compositional, the peak proportions cannot be used for multivariate analysis. The data, therefore, were standardized by the formula proposed by Aitchison (1986):

$$Z_{ij} = \ln [Y_{ij}/g(Y_j)]$$

where Z_{ij} is the standardized area of peak i for ant j , Y_{ij} is the observed area of the peak, and $g(Y_j)$ is the geometric mean of the areas of all the peaks of ant j included in the analysis.

The standardized data were then examined by multivariate analysis of variance (MANOVA) to determine whether the groups differed in the abundances

of compounds present. Differences between the groups were examined further by a canonical discriminant analysis. In addition, a one-way univariate analysis of variance (ANOVA) also was carried out for each peak in each analysis. Only peaks that were abundant enough to be quantified in all of the samples being examined in a particular analysis (5–10 peaks) were included. This avoided the problem of large numbers of independent variables relative to the sample size resulting in significant discriminations being found where no actual groupings exist (Panel on Discriminant Analysis, Classification, and Clustering, 1989). As a small number of samples had evaporated during storage, the analysis was weighted for the number of replicates. Only the first two discriminant functions were considered to be important, because together these always accounted for at least 90% of the variance between groups. The discriminant scores for the functions were also used to predict group membership and thus to confirm the effectiveness of the functions in discriminating between the groups.

To quantify the similarity between the groups (castes, nests, etc.), Nei's distances were calculated (Nei, 1972; Ferguson, 1980). This technique provides an index of similarity between two groups from 1 (identical) to 0 (totally different). The formula used is:

$$I = \frac{\sum x_i y_i}{\sqrt{(\sum x_i^2 \sum y_i^2)}}$$

where I is Nei's coefficient of identity, x_i is the quantity of peak i for sample x , and y_i is the quantity of peak i in sample y . After converting the data into binary values (1 = present, 0 = absent), Nei's distances were calculated with Ochiai's resemblance coefficient. This takes the same value as the cosine coefficient (Nei's distance) when binary attribute data are used (Romesburg, 1989) and allowed the trace compounds to be included in the analysis.

RESULTS

A total of 41 peaks of high volatility occurred consistently in one or more groups. Of these, nine were identified with a high degree of confidence, including almost all of the most abundant peaks. Seven of these were ketones in the C_7 to C_9 range, and the others were nonanal and the monoterpene, limonene. A provisional identification was made for a further seven peaks.

Caste Differences in Composition. In both species, there was very little differences between the castes in the composition of volatiles in their mandibular glands, as represented by the extremely high Nei's values (Figure 1). Medias

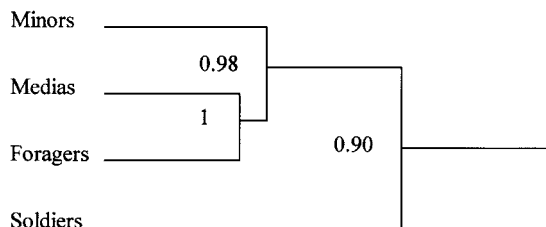
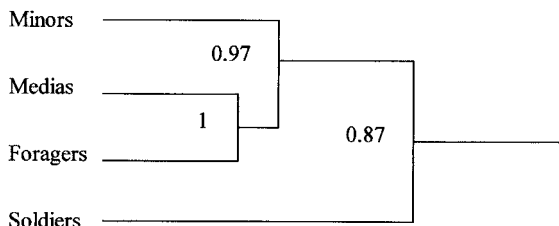
(A) *A. bisphaerica*(B) *A. capiguara*

FIG. 1. Dendrograms based on Nei's distances as a measure of the degree of similarity between the composition of volatiles (presence/absence) in the extracts of the four castes (1 = identical, 0 = totally different).

and foragers contained the same compounds, and the composition of the minor worker samples was also very similar. Soldiers differed to a greater degree, and the castes differed in the proportions of the most abundant compounds (*A. bisphaerica*: $F_{40,100} = 4.66$, $P < 0.001$; *A. capiguara*; $F_{28,124} = 7.20$, $P < 0.001$) (Tables 1 and 2).

The discriminant analysis of the *A. bisphaerica* castes was based upon 10 peaks. Soldiers, and to a lesser extent minors, separated out from the other castes on function 1 (64% of the variance) (Figure 2). The function structure coefficients indicated that 4-methyl-3-heptanone contributed most to this variance. The ANOVA showed that the proportions of this ketone differed significantly between the castes ($F_{3,31} = 13.0$, $P < 0.001$), with the soldiers containing a lower proportion than the other castes (Table 1). Soldiers also had a higher proportion of 2-heptanone, although this was not significant ($F_{3,31} = 1.31$, $P = 0.29$), and they were the only caste to contain 2-nonanone and peak 29. Minor workers were distinguished from the other castes by function 2 of the discriminant anal-

TABLE 1. VOLATILES IN MANDIBULAR GLANDS OF FOUR CASTES OF *A. bisphaerica*^a

Peak	Volatiles (% , mean \pm SE)			
	Minors (N = 9)	Medias (N = 8)	Foragers (N = 10)	Soldiers (N = 8)
1	0.7 \pm 0.2	trace	trace	trace
2	0	0	0	0
3	5.5 \pm 2.0	1.8 \pm 0.1	1.6 \pm 0.1	2.2 \pm 0.3
4	trace	0.3 \pm 0.1	trace	trace
5	trace	trace	trace	trace
6	1.5 \pm 0.3	1.0 \pm 0.1	0.7 \pm 0.1	1.4 \pm 0.3
7 (3-hexanone)	trace	trace	trace	trace
8 (3-hexanol)	0.9 \pm 0.3	0.9 \pm 0.1	1.7 \pm 0.2	trace
9	0	0	0	0
10	trace	trace	trace	0
11	0	0	0	0
12	0	0	0	0
13 (2-hexanol)	0	0	0	0
14	0	0	0	0
15 4-methyl-3-hexanone	7.1 \pm 0.7	5.6 \pm 0.4	6.8 \pm 0.7	5.9 \pm 0.8
16 4-methyl-2-hexanone	4.8 \pm 0.6	3.2 \pm 0.3	3.5 \pm 0.5	4.6 \pm 0.9
17 (2-pentenoate)	3.7 \pm 0.7	2.4 \pm 0.6	2.2 \pm 0.3	3.4 \pm 0.6
18	0.4 \pm 0.1	0.6 \pm 0.1	0.2 \pm 0.1	0
19	0.4 \pm 0.2	0.3 \pm 0.1	1.0 \pm 0.6	1.1 \pm 0.5
20	2.0 \pm 0.5	1.8 \pm 0.5	1.8 \pm 0.4	2.0 \pm 0.4
21 2-heptanone	22.9 \pm 2.1	27.6 \pm 1.8	23.4 \pm 1.2	31.6 \pm 3.9
22	0	0	0	0
23	0	0	0	0
24 (2-heptanol)	1.9 \pm 0.2	2.5 \pm 0.2	1.9 \pm 0.2	2.6 \pm 0.6
25	0	0	0	0
26	0	0	0	0
27 4-methyl-3-heptanone	29.7 \pm 1.5	34.9 \pm 1.1	38.4 \pm 1.1	21.7 \pm 2.0
28	0	0	0	0
29	0	0	0	0.5 \pm 0.2
30 (4-methyl-3-heptanol)	0.7 \pm 0.2	2.3 \pm 0.4	2.5 \pm 0.2	trace
31 3-octanone	6.9 \pm 0.6	6.8 \pm 0.5	6.5 \pm 0.3	5.5 \pm 0.8
32 2-octanone	3.4 \pm 0.8	2.4 \pm 0.7	1.9 \pm 0.5	5.2 \pm 0.8
33	0.8 \pm 0.2	0.9 \pm 0.1	0.8 \pm 0.1	trace
34	0	0	0	0
35 (pinene)	0	0	0	0
36 limonene	3.7 \pm 1.0	2.4 \pm 1.3	2.5 \pm 0.6	3.3 \pm 1.0
37	0	0	0	0
38	0	0	0	0
39 2-nonanone	0	0	0	7.2 \pm 1.9
40 nonanal	1.3 \pm 0.3	1.8 \pm 0.1	2.1 \pm 0.1	1.2 \pm 0.2
41	0	0	0	0
Total amount per ant (μ g)	0.41 \pm 0.03	0.89 \pm 0.07	2.51 \pm 0.29	23.8 \pm 2.03

^aCompound names in parentheses represent tentative identification only.

TABLE 2. VOLATILES IN MANDIBULAR GLANDS OF FOUR CASTES OF *A. capiguara*^a

Peak	Volatiles (% , mean \pm SE)			
	Minors (N = 10)	Medias (N = 10)	Foragers (N = 10)	Soldiers (N = 8)
1	1.2 \pm 0.2	1.6 \pm 0.2	1.5 \pm 0.4	1.7 \pm 0.3
2	0	\pm	0	trace
3	7.1 \pm 0.9	5.8 \pm 0.3	5.6 \pm 0.4	7.3 \pm 1.0
4	trace	trace	trace	trace
5	0	0	0	0
6	1.2 \pm 0.5	trace	0.7 \pm 0.2	1.1 \pm 0.3
7 (3-hexanone)	1.3 \pm 0.4	trace	1.0 \pm 0.2	1.1 \pm 0.3
8 (3-hexanol)	trace	trace	trace	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13 (2-hexanol)	0	0	0	0
14	0	0	0	0
15 4-methyl-3-hexanone	10.0 \pm 0.8	13.3 \pm 0.6	17.8 \pm 1.7	11.0 \pm 2.1
16 4-methyl-2-hexanone	6.6 \pm 0.4	6.7 \pm 0.4	6.9 \pm 0.8	6.4 \pm 0.7
17 (2-pentenoate)	2.4 \pm 0.7	1.1 \pm 0.4	1.4 \pm 0.3	2.3 \pm 0.9
18	0	0	0	0
19	0.6 \pm 0.2	trace	0.6 \pm 0.1	0.6 \pm 0.1
20	1.2 \pm 0.4	trace	1.0 \pm 0.3	1.4 \pm 0.7
21 2-heptanone	21.0 \pm 2.1	23.6 \pm 1.2	20.3 \pm 1.4	21.6 \pm 2.7
22	0	0	0	0
23	trace	trace	trace	0.5 \pm 0.1
24 (2-heptanol)	0.9 \pm 0.1	trace	trace	trace
25	0	0	0	0
26	0	0	0	0
27 4-methyl-3-heptanone	29.2 \pm 1.2	29.4 \pm 0.9	25.9 \pm 0.8	20.9 \pm 1.4
28	trace	trace	0	0
29	0	0	0	trace
30 (4-methyl-3-heptanol)	1.4 \pm 0.3	0.9 \pm 0.2	0.6 \pm 0.1	1.1 \pm 0.1
31 3-octanone	12.4 \pm 0.8	14.1 \pm 0.6	13.4 \pm 0.4	7.5 \pm 0.7
32 2-octanone	0	0	0	2.7 \pm 0.5
33	trace	trace	trace	trace
34	0	0	0	0
35 (pinene)	0	0	0	0
36 limonene	2.7 \pm 1.2	1.1 \pm 0.4	2.1 \pm 0.7	2.4 \pm 0.9
37	0	0	0	0
38	trace	trace	trace	trace
39 2-nonanone	0	0	0	9.2 \pm 3.5
40 nonanal	0	0	0	0
41	0	0	0	0
Total amount per ant (μ g)	0.46 \pm 0.04	0.51 \pm 0.04	0.96 \pm 0.09	8.9 \pm 0.08

^aCompound names in parentheses represent tentative identification only.

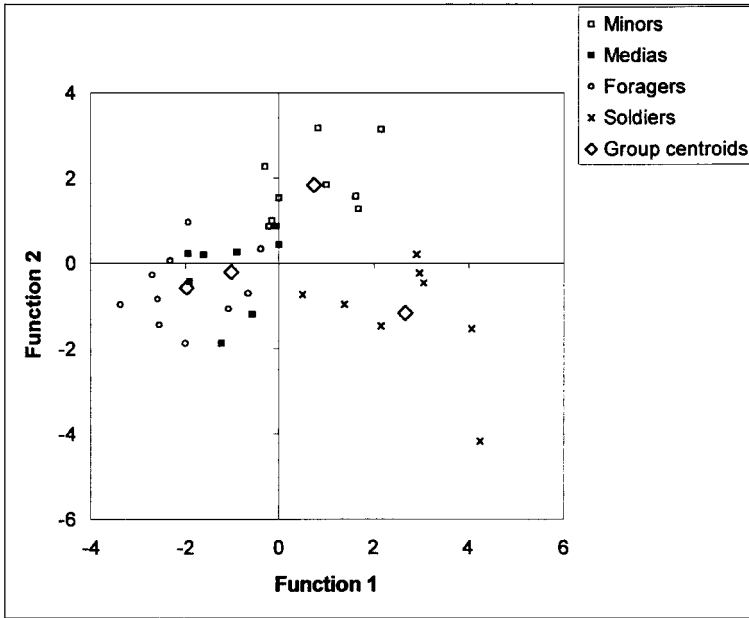


FIG. 2. Discriminant analysis of the four castes of *A. bisphaerica* [minors ($N = 9$), medias ($N = 8$), foragers ($N = 10$), and soldiers ($N = 8$)]. The proportions of 10 peaks were compared after standardization. Data points are the scores for each sample on functions 1 and 2 of the discriminant analysis, and the group centroids for each caste.

ysis (27% of variance) (Figure 2), which was mainly due to peak 3. This was higher in minors than in the other castes ($F_{3,31} = 5.30$, $P = 0.005$) (Table 1). Medias and foragers were not differentiated by either function (Figure 2), and their chemical profiles were virtually identical. The predicted classification based on the discriminant scores misclassified only 14% of samples (0/9 minors, 3/8 medias, 1/10 foragers, and 1/8 soldiers).

Separation of the castes of *A. capiguara* by the discriminant analysis followed a similar pattern, with the analysis being based upon seven peaks. Both function 1 (55% of variance), and function 2 (36% of variance) separated soldiers and minors from the other two castes, with medias and foragers being very similar (Figure 3). Four peaks contributed to function 1. Peak 3 was higher ($F_{3,34} = 6.16$, $P = 0.002$), and 4-methyl-3-hexanone lower ($F_{3,34} = 3.99$, $P = 0.015$), in minors and soldiers compared with the other two castes (Table 2). There was also less 3-octanone in the soldiers samples ($F_{3,34} = 3.14$, $P = 0.038$), and the proportion of 4-methyl-3-heptanone decreased from minors to soldiers, although this was not significant ($F_{3,34} = 2.32$, $P = 0.093$). Function 2 was due

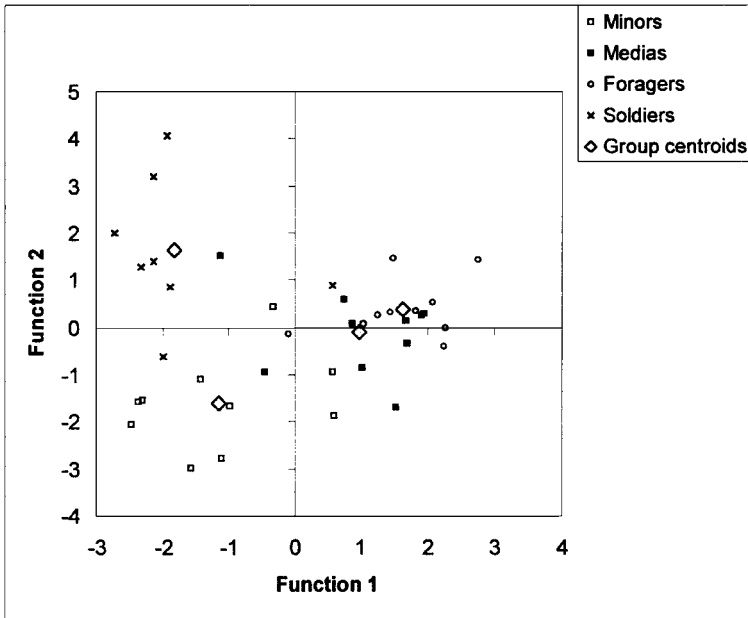


FIG. 3. Discriminant analysis of the four castes of *A. capiguara* [minors ($N = 10$), medias ($N = 10$), foragers ($N = 10$), and soldiers ($N = 8$)]. The proportions of seven peaks were compared after standardization. Data points are the scores for each sample on functions 1 and 2 of the discriminant analysis, and the group centroids for each caste.

mainly to 2-heptanone, although this did not differ between the castes ($F_{3,34} = 0.84$, $P = 0.481$). In addition, 2-octanone and 2-nonanone were found only in the soldiers (Table 2). Misclassification based on the discriminant scores was greater than with *A. bisphaerica*, with 26% being misclassified (2/10 minors, 4/10 medias, 2/10 foragers, 2/8 soldiers).

Species Differences in Composition. There was surprisingly little difference between *A. bisphaerica* and *A. capiguara* in the compounds present in their profiles (Nei's distance of 0.86), although they did differ in the proportions of the most abundant compounds ($F_{12,134} = 49.24$, $P < 0.001$). They were separated with a single function in the discriminant analysis, which used six peaks. This was contributed to mainly by 4-methyl-3-heptanone and 3-octanone. There was more 4-methyl-3-heptanone ($F_{1,71} = 28.44$, $P < 0.001$), and less 3-octanone ($F_{1,71} = 5.95$, $P = 0.017$) in *A. bisphaerica* (Tables 1 and 2). Overall, the proportion of 2-heptanone was also greater in *A. bisphaerica* ($F_{1,71} = 24.9$, $P < 0.001$), while there was more 4-methyl-3-hexanone ($F_{1,71} = 9.03$, $P = 0.004$) and peak 3 ($F_{1,71} = 44.5$, $P < 0.001$) in *A. capiguara*. Nonanal, peak 19, and peak 10

were found only in *A. bisphaerica*, and while 2-octanone was present in all the *A. bisphaerica* castes, it was found only in soldiers of *A. capiguara*. Prediction from the discriminant scores misclassified only 4.1% of the samples (3/35 *A. bisphaerica*, 0/38 *A. capiguara*).

Nest Differences in Composition. The nests at the Botucatu site differed from one another in the compounds present in their profiles (average Nei's distance of 0.76), and the proportions of the most abundant compounds also differed between the nests ($F_{12,124} = 9.85$, $P < 0.001$) (Table 3). In the discriminant analysis, with six peaks, nest B was clearly separated from the others on function 2 (26% of variance) (Figure 4). This was due mainly to 4-methyl-3-heptanone, of which there was more in nest B ($F_{3,33} = 19.84$, $P < 0.001$), and to 4-methyl-3-hexanone, of which there was less ($F_{3,33} = 12.33$, $P < 0.001$) (Table 3). 2-Heptanone was also more abundant in nests A, B, and C, than in nest D. All nests were separated by function 1 (73% of variance) (Figure 4). This was due to peak 7, which varied between an average of $18.1 \pm 0.8\%$ in nest D and only $1 \pm 0.1\%$ in nest A, with nests B and C being intermediate. The nests also differed considerably in the minor peaks that were present in their profiles (Table 3). Only 8.1% of samples were misclassified by predictions based on the discriminant scores (0/10 nest A, 2/9 nest B, 1/9 nest C, 0/9 nest D).

DISCUSSION

The volatile compounds identified in *A. bisphaerica* and *A. capiguara* were all within the 100–200 molecular weight range that is characteristic of alarm pheromone compounds (Wilson and Bossert, 1963). The main compounds were mostly methyl and ethyl ketones, which are the groups that dominate the alarm pheromones of myrmicine ants (Blum and Brand, 1972; Parry and Morgan, 1979). 4-Methyl-3-heptanone, 2-heptanone, 3-octanone, and 4-methyl-3-hexanone have previously been identified in the alarm pheromones of *Atta* (Schildknecht, 1976; Blum et al., 1968; Moser et al., 1968; Riley et al., 1974; Nascimento et al., 1993; Knapp, 1995; Hernández et al., 1999). In fact, 4-methyl-3-heptanone and 2-heptanone are extremely common alarm compounds and are found in a number of other ant species (Hölldobler and Wilson, 1990). The small quantities present of minor compounds prevented accurate mass spectrometry, and so their identity remains uncertain. However, at least some appeared to be low molecular weight aliphatic ketones and alcohols of the types commonly seen in the alarm pheromones of other leaf-cutting ants.

There were small but consistent differences between the castes in both species, although the profiles of medias and foragers were virtually indistinguishable. The similarity contrasts with studies on *A. sexdens rubropilosa* (Nascimento et al., 1993; Knapp, 1995) and *A. laevigata* (Hernández et al., 1999),

TABLE 3. VOLATILES IN MANDIBULAR GLANDS OF FORAGERS FROM FOUR
A. capiguara NESTS^a

Peak	Volatiles (%; mean \pm SE)			
	Nest A (N = 10)	Nest B (N = 9)	Nest C (N = 9)	Nest D (N = 9)
1	1.7 \pm 0.3	1.1 \pm 0.3	1.5 \pm 0.3	2.9 \pm 0.2
2	0	trace	0	0
3	7.6 \pm 0.7	4.6 \pm 0.4	6.8 \pm 0.9	3.1 \pm 0.3
4	0	0	0	0
5	0	0	0	0
6	0.6 \pm 0.2	0	0	0
7 (3-hexanone)	1.0 \pm 0.1	1.8 \pm 0.3	6.1 \pm 1.7	18.1 \pm 0.8
8 (3-hexanol)	trace	2.2 \pm 0.4	trace	0.6 \pm 0.2
9	trace	0	trace	0
10	0	trace	0	trace
11	0	0.5 \pm 0.2	0	0.7 \pm 0.2
12	0	0	0	trace
13 (2-hexanol)	0	trace	0	0.7 \pm 0.1
14	0	0.8 \pm 0.2	0	0
15 4-methyl-3-hexanone	15.3 \pm 0.8	5.9 \pm 1.9	18.0 \pm 1.3	11.0 \pm 0.6
16 4-methyl-2-hexanone	3.6 \pm 0.4	2.8 \pm 0.6	2.2 \pm 0.6	2.3 \pm 0.5
17 (2-pentenoate)	1.6 \pm 0.8	0.1 \pm 0.1	0	0
18	1.6 \pm 0.2	2.0 \pm 0.2	2.9 \pm 0.5	trace
19	trace	0.8 \pm 0.2	trace	8.7 \pm 0.5
20	1.5 \pm 0.5	0	0	0
21 2-heptanone	19.7 \pm 1.3	20.2 \pm 0.9	17.2 \pm 1.2	11.0 \pm 1.1
22	0	trace	0	0
23	1.0 \pm 0.2	trace	trace	trace
24 (2-heptanol)	trace	2.0 \pm 0.3	trace	trace
25	trace	trace	0	0
26	trace	0	0	0
27 4-methyl-3-heptanone	18.2 \pm 0.7	34.6 \pm 1.9	18.5 \pm 1.2	13.8 \pm 1.4
28	0	0	0	1.1 \pm 0.2
29	0	0	0	0.9 \pm 0.2
30 (4-methyl-3-heptanol)	1.1 \pm 0.2	4.4 \pm 0.8	2.0 \pm 0.5	5.4 \pm 0.3
31 3-octanone	17.2 \pm 0.7	9.9 \pm 1.6	18.3 \pm 1.1	10.9 \pm 0.3
32 2-octanone	0	0	0	0
33	1.8 \pm 0.2	0.8 \pm 0.2	trace	1.6 \pm 0.3
34	0	0	0	trace
35 (pinene)	0	0	trace	2.4 \pm 0.4
36 limonene	2.0 \pm 1.0	trace	trace	0
37	trace	trace	0	0
38	0	0	0.2 \pm 0.2	1.4 \pm 0.2
39 2-nonanone	trace	trace	trace	0
40 nonanal	1.6 \pm 0.1	3.4 \pm 0.5	1.2 \pm 0.3	2.2 \pm 0.1
41	trace	trace	0	0
Total amount per ant (μ g)	0.74 \pm 0.06	0.98 \pm 0.07	0.65 \pm 0.07	0.94 \pm 0.05

^aCompound names in parentheses represent tentative identification only.

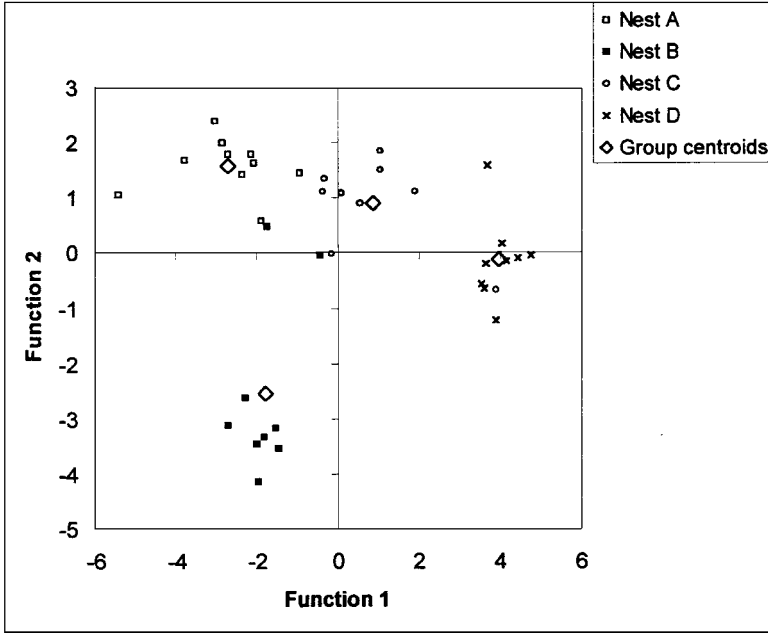


FIG. 4. Discriminant analysis of foragers from the four Botucatu nests of *A. capiguara* [nest A ($N = 10$), nest B ($N = 9$), nest C ($N = 9$), and nest D ($N = 9$)]. The proportions of six peaks were compared after standardization. Data points are the scores for each sample on functions 1 and 2 of the discriminant analysis, and the group centroids for each nest.

in which the alarm pheromone of the larger ants was a complex mixture of compounds while that of the smallest workers was almost pure 4-methyl-3-heptanone. Although the soldiers of both, *A. bisphaerica* and *A. capiguara* did have proportionally less 4-methyl-3-heptanone than the other castes, such a strong division between small and large workers was clearly not present in these species. In part, this may be ascribed to the size of the ants in the samples. Nascimento et al. (1993) and Hernández et al. (1999) reported that the alarm pheromones of ants <1.8 mm and <2.5 mm in head width, respectively, did not differ in composition. However, Knapp (1995) found that it was only the pheromones of workers with a head width of <1 mm that were dominated by 4-methyl-3-heptanone. The minors of *A. bisphaerica* and *A. capiguara* analyzed here had a head width of up to 1.4 mm, and so included individuals that were larger than the size range which Knapp (1995) found to be dominated by 4-methyl-3-heptanone. It would be interesting to examine in more detail the mandibular gland chemistry of the minor workers of *A. bisphaerica*,

A. capiguara, and indeed other species, to see if the caste differences described in *A. sexdens rubropilosa* and *A. laevigata* are a widespread phenomenon. The reason for caste variation is another area that deserves further research. Nascimento et al. (1993) and Bradshaw et al. (1979) have suggested that differences in chemical composition between castes may be due to living in different environments. However, individuals from every leaf-cutting ant worker caste may be found both inside and outside the nest, and no direct evidence for this theory has been found. The reason for caste variation, therefore, is still unclear.

There was also very little difference between *A. bisphaerica* and *A. capiguara* in the composition of the mandibular glands, although they could be consistently distinguished by the proportions of their major components. They were both very different from *A. sexdens rubropilosa*, and none of the larger molecular weight compounds, such as citral, were found. The most behavioral active compound in the alarm pheromone of *A. sexdens rubropilosa*, as well as *A. laevigata*, *A. cephalotes*, and *A. texana*, is 4-methyl-3-heptanone (Blum et al., 1968; Moser et al., 1968; Riley et al., 1974; Knapp, 1995; Pow, 1996). This was the most abundant compound in all the worker castes of both *A. bisphaerica* and *A. capiguara*, apart from the soldiers. It also has been identified in the mandibular glands of *A. colombica* and *A. robusta* (Blum et al., 1968). The lack of species specificity of alarm pheromones has been recognized by a number of authors (Wilson, 1965; Blum, 1969; Parry and Morgan, 1979; Vander Meer and Alonso, 1998). Unlike many other pheromones, discrimination between species is not essential to the function of alarm pheromones (Law and Regnier, 1971; Hölldobler and Wilson, 1990). Indeed, there could even be benefits to being able to recognize and respond to alarm pheromones produced by other species (Blum, 1969; Vander Meer and Alonso, 1998).

One explanation for the discrepancies between studies on the same species is colony-specific differences. Both the presence of minor compounds and the proportions of major compounds differ significantly among the nests of *A. capiguara*. In fact, the relatively high number of trace compounds that varied between colonies caused average Nei's distances between colonies to be lower than that between the two species. Similar intercolony differences in both the major and trace components have been found in weaver ants, *Oecophylla longinoda* (Bradshaw et al., 1975, 1979). Why colonies should differ to such a degree is unclear. Possibly the subtle differences in the composition of the alarm pheromone allow the recognition of nestmates (Howse, 1979; Bradshaw and Howse, 1984). Whitehouse and Jaffé (1995) have shown that the ability of leaf-cutting ants to distinguish between nestmates and nonnestmates is dependent upon a cue present in the head of the target ant. We present here the first evidence that the chemistry of leaf-cutting ant heads differs among colonies, supporting the hypothesis that alarm pheromones play an important role in nestmate recognition.

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