

## MANDIBULAR GLAND SECRETION IN DIFFERENT CASTES OF THE LEAF-CUTTER ANT *Atta laevigata*

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**Abstract**—Gas chromatography analyses and behavioral assays showed that *Atta laevigata*, a highly polymorphic ant species, has a mandibular gland secretion that varies with castes. All castes contain 4-methyl-3-heptanone as the main component and its concentration is proportional to head size. Small workers and soldiers, but not medium size workers, also contain 4-methyl-3-heptanol. Queens show variations in their chemical composition after mating, as virgin males contain a secretion dominated by 4-methyl-3-heptanol, and, in a lesser proportion, 4-methyl-3-heptanone. In mated males these proportions are inverted. The compounds 4-methyl-6-hepten-3-one, 4-methyl-4-hepten-3-one, 6-methyl-tetradecene, and 2,6-dimethyl-2-dodecene are found only in queens. The behavioral response elicited by the secretion is mainly alarm, which is elicited more strongly by glands of larger workers. The results suggest that chemical castes, behavioral castes, and morphological castes overlap in this species.

**Key Words**—4-Methyl-3-heptanone, 4-methyl-3-hexanone, 4-methyl-3-heptanol, castes, *Atta laevigata*, Formicidae, Attini, leaf-cutting ant, mandibular gland, behavior.

### INTRODUCTION

The mandibular glands in the Formicidae are a source of volatile organic compounds. These compounds have strong effects on ant behavior (Cammaerts et al., 1983; Hölldobler and Wilson, 1990). Alcohols and their respective ketones are the chemical compounds most frequently found in the mandibular glands of

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Myrmicinae ants; many of these secretions have been analyzed and the results have been discussed and reviewed. The main function of the secretion from the mandibular glands is in signaling alarm behavior (Blum and Hermann, 1978; Parry and Morgan, 1979; Attygalle and Morgan, 1984; Hölldobler and Wilson, 1990).

Leaf-cutting ants are a serious problem, because they are widely distributed throughout the neotropics and are considered important pests in agricultural and arboricultural systems (Mayhé-Nunes, 1995; Cherrett and Peregrine, 1976; Hernández and Jaffe, 1995). These ants have a sophisticated communication system that is their main tool for the maintenance of the structure of their complex societies. Their colonies are strongly territorial (Jaffe et al., 1979; Vilela, 1983; Salzemann and Jaffe, 1990a,b) and the nestmate recognition system is based on odors present mainly in the head (Whitehouse and Jaffe, 1995), possibly from volatiles produced by the mandibular glands (Jaffe et al., 1979; Salzemann and Jaffe, 1991; Hernández and Jaffe, unpublished data). *Atta* colonies show a marked polyethism and polymorphism between members. Wilson (1980) divided *Atta sexdens rubropilosa* into four castes according to the tasks performed: gardener–nurses, generalists, forager–excavator, and defenders. These categories have raised questions regarding the chemical composition of the mandibular gland secretion in the different castes and the particular responses of the castes to this secretion (Do Nascimento et al., 1993).

The chemical composition of the mandibular gland secretion of other *Atta* species has been reported by several authors (Butenandt, 1959; Moser et al., 1968; Blum et al., 1968; Riley et al., 1974; Schildknecht, 1976), and almost all agree that the ketone 4-methyl-3-heptanone is the alarm pheromone in *Atta* species. Nonetheless, all these studies have been undertaken with massive extracts of all worker castes, without taking into account the strong polyethism and polymorphism present in the genus. Recently, Do Nascimento et al. (1993) reported the existence of variation between castes in the composition of the mandibular gland secretion of *Atta sexdens rubropilosa* and established that in the smaller worker caste the mandibular gland secretion is dominated by 4-methyl-3-heptanone, while in the larger worker caste this secretion is dominated by a mixture of neral and geranial. Virgin and mated queens contain mainly 4-methyl-3-heptanone, which increases in quantity after mating. Virgin males contain 4-methyl-3-heptanone and 4-methyl-3-heptanol in equal proportions, but in mated males the alcohol is absent.

Using the technique of individual gland analysis combined with gas chromatography–mass spectrometry (see Do Nascimento et al., 1993), we undertook chemical analysis of the mandibular gland secretion of the different castes of the ant *A. laevigata*. Additional experiments were performed in order to determine the possible behavioral relevance of the differences in chemical composition between castes.

## METHODS AND MATERIALS

*Ant Collection*

*Workers.* Workers with different head widths were collected individually with a forceps from a colony located on the campus of the Universidad Simón Bolívar and were immediately introduced into 2- × 7-cm vials and placed in a container with Dry Ice. They were transported to the laboratory and stored at -20°C until analysis. Prior to the analysis the head width of each worker was measured.

*Virgin Queens and Males.* During the nuptial flights in June 1994, virgin females and males were collected as they left the ant nest at the pine tree plantations of CVG-PROFORCA, El Merey, Estado Anzoátegui, Venezuela. Each individual was treated in the same way as previously described for the collection of the workers.

*Mated Females.* Incipient colonies located in the pine tree plantations of CVG-PROFORCA were excavated and brought to the laboratory. Nests were kept at: 80–90% relative humidity, 24°C, 12L:12D. The queens were kept with their respective workers until the analyses. Prior to the analyses, queen activity was slowed by cooling the insect to -20°C for 5 min.

*Mated Males.* Males were collected at the pine plantations as they landed from the nuptial flights in June 1995. The males were transported and stored until analysis in the same way as described for the workers.

*Extracts*

For all castes, each cephalic capsule was placed in a clean porcelain dish with 100 µl of hexane (pesticide grade, Fisher Scientific) and was crushed with a clean glass rod. One microliter was taken for analysis (see Do Nascimento et al., 1993). This method was carried out, because preliminary experiments showed that there were no differences between results from dissected mandibular glands extracts.

*GC and GC-MS Analyses*

Volatiles were analyzed by gas chromatography (GC). GC was performed on a Hewlett Packard 5890 Series II chromatograph attached to an HP 3396A integrator. The GC was equipped with a FID, a splitless injector, and a fused silica DB-5 capillary column (Quadrex, 25 m × 0.18 mm ID). The carrier gas was helium (flow rate 1 ml/min) and the oven was programmed with two temperature ramps, the first starting at 50°C, maintained for 4 min, and then increased to 150°C at 6°C/min. The second ramp started at 150°C, increased at 20°C/min to 280°C, and was then maintained at that temperature for 20 min. Compounds were identified by means of their mass spectra, with a Perkin-Elmer GC (Autosystem) coupled to a Perkin-Elmer MS (QMass-910). GC condi-

tions and column were the same as those used above. Identification of the compounds was achieved either by comparing their mass spectra with those from the NIST library and/or with synthetic standards synthesized in the laboratory or purchased commercially, or by comparison of the GC retention times of the standards. The purchased compounds were 4-methyl-3-hexanone, 3-heptanone, 2-heptanone, and 4-methyl-3-heptanol. 4-Methyl-3-heptanone was prepared from its alcohol by oxidation with pyridinium chlorochromate (PCC). Quantification was performed with 4-methyl-3-penten-2-one as an external standard. This compound was chosen as standard because it has a relative FID response factor similar to all the other identified compounds. For each caste, five replicates were undertaken, and all data were analyzed by means of one-way ANOVA (Siegel and Castellan, 1988) with SPSSPC.

#### *Behavioral Bioassays*

Behavioral tests were performed on colonies kept in the laboratory. Tests were undertaken on a foraging area, which consisted of a plastic container (18 cm high, 60 and 40 cm upper and lower diameter). In the bottom of the container we placed fine cardboard (white) with concentric circles drawn on it in order to aid in estimating the distance between the ant showing a particular behavior and the odor source. Over the cardboard a glass sheet (40 cm diam.) was placed to facilitate cleaning the substrate of the arena for each bioassay. As foraging substrate, leaves of *Hura crepitans* were placed daily on the foraging area so that ants could recognize the foraging arena as a colony territory (Salzemann and Jaffe, 1991). We then presented different odors to workers foraging on this arena. The odors were body parts taken from live queens and workers of *A. laevigata*, crushed on a glass sheet (40 cm diam.) with a clean glass rod. Each test was filmed for 10 min with a video camera (SONY Handycam CCD-F360). Films were used to quantify the behavior of workers elicited during the first 5, 15, 30, 45, and 60 sec after presenting the odor and then during 15 sec for each minute up to minute 10. In each observation we determined the percentage of ants exhibiting a specific behavior. Controls were lyophilized worker and queen bodies, submitted to high vacuum at  $-20^{\circ}\text{C}$  for 26 hr (Freeze Dryer 4.5, Labconco). Castes were classified according to their head width.

## RESULTS

#### *Chemical Analyses*

Cephalic capsules of 35 ants belonging to different castes of *A. laevigata* were analyzed. The compounds identified and the average compositions nanograms per head for each caste are given in Table 1. The different castes showed differences in the chemical composition and the relative proportions

TABLE 1. VARIATION OF COMPOSITION AND ABSOLUTE QUANTITY OF MANDIBULAR GLAND SECRETION IN DIFFERENT CASTES OF *A. laevigata*<sup>a</sup>

Compounds	Virgin queen	Mated queen	Virgin male	Mated male	Soldier	Worker 2.5-4 mm	Worker <2.5 mm	$\chi^2$	P
1) 4-methyl-3-hexanone	14 ± 13	3.5 ± 5	1.5 ± 1.4	1.7 ± 1.5	0.3 ± 0.4	0.02 ± 0.1	15.8	0.002	
2) 3-methyl-2-hexanone	t	t							
3) 4-methyl-3-hexanol	t	t							
4) 3-heptanone	t	t					t		
5) 2-heptanone	t						t		
6) 4-methyl-6-hepten-3-one	0.6 ± 0.6	0.1 ± 0.2					t	15.2	
7) 4-methyl-3-heptanone	1013 ± 162	167 ± 120	3.4 ± 2.8	51 ± 47	16 ± 12	4.5 ± 2.6	1.9 ± 0.5	27	
8) 4-methyl-3-heptanol	9 ± 3	1.8 ± 1.3	48 ± 36	2.7 ± 1.5	0.4 ± 0.4	t	1.1 ± 1.5	23.8	
9) 3-octanone	0.4 ± 0.4	1.1 ± 2.6				t		29.4	
10) 4-methyl-4-hepten-3-one	1.2 ± 0.2	0.2 ± 0.5						4.4	
11) 6-methyl-tetradecene	3.0 ± 3.1	1.3 ± 2.3						15.4	
12) 2,6-dimethyl-2-dodecene	26 ± 8							35	

<sup>a</sup>Kruskal-Wallis ANOVA if  $P < 0.05$ . The values are the mean of the relative abundance of compounds (ng/head ± SD).  $N = 5$  for all replicates; t = trace compounds.

of compounds present in their mandibular gland secretion. In all castes, except virgin males, 4-methyl-3-heptanone was the main component of the secretion. Queens had more compounds than other castes, with their secretion dominated by the 4-methyl-3-heptanone, 4-methyl-3-hexanone, and 4-methyl-3-heptanol. In addition queen mandibular glands contained 3-methyl-2-hexanone, 4-methyl-3-hexanol, 3-octanone, 4-methyl-4-hepten-3-one, 3-methyl undecene, and 6-methyl tridecene, which were absent in all other castes, or if present in other castes, in quantities we were unable to detect. The relative abundance of the compounds in mandibular glands from virgin queens differed markedly from that of mated queens (Table 1). Mandibular glands of virgin males contained only 4-methyl-3-heptanone and 4-methyl-3-heptanol, the latter compound being the most abundant in the secretion. After mating, the relative proportion of both compounds was inverted, and in addition 4-methyl-3-hexanone was detected. We observed a decreasing concentration of all the compounds identified linked to decreasing head width in the castes. In virgin males (head width >2.36 mm), however, the amount of 4-methyl-3-heptanone contained in the secretion was greater than that of both workers and soldiers. Mandibular glands of soldiers contained 4-methyl-3-hexanone, 3-heptanone, 4-methyl-3-heptanone, and 4-methyl-3-heptanol. Workers, independent of head width, also produced 4-methyl-3-hexanone and 4-methyl-3-heptanone (Table 1). 4-Methyl-3-heptanol was not, however, observed and the 3-octanone was only detected in trace amounts. We also identified with GC-MS analysis the compounds 3-hexanone, 2-hexanone, 3-hexanol, 2-hexanol, hexane-3-hydroxyperoxy, and hexane-2-hydroxyperoxy in the extracts of the mandibular glands of all castes; however, these compounds are artifacts produced by the reaction of the samples with the hexane (W. Francke, personal communication). The results presented here are statistically significant, as an ANOVA of the analyzed samples showed statistically significant differences in the composition and relative abundance of the volatile compounds found in the mandibular glands between the castes analyzed (Table 1).

#### *Behavioral Bioassays*

Tests presenting the different body parts to ants colonies allowed us to characterize the following behaviors:

*Attraction.* Ants orient toward the source (body part presented) and walk (move more than half their body length per second) or run (move more than half their body length per sec) toward it.

*Alarm.* Ants detecting the source open their mandibles, bend their gaster ventrally, and run in irregular circles around the source.

*Antennation.* Ants touch the source with the tip of their antennae, pausing for more than 1 sec at the source.

*Attack.* Ants stand in front of the source with their bodies tilted upwards ante-

riorly and with their mandibles open. Then they quickly rock forward towards the source pivoting on their back pair of legs and quickly return to their original position. Occasionally they close their mandibles at the most anterior point of the cycle, opening them again as they rock back to their original position.

*Biting.* Ants grasp the source with their mandibles and pull backwards, some times dragging the source.

*Trembling.* Ants vibrate rapidly (i.e., shiver) and sometimes press their bodies against the ground.

*Grooming.* Ants groom appendages by either passing them through their mouthparts or by repeatedly rubbing a leg against it.

*Alert.* Ants stop and lift their head, thorax, and antennae.

*Pause.* Ants stand with their legs more or less straight and stiff so that their bodies are held high above the substrate. The thorax of each ant is slightly tilted upward anteriorly while the head is strongly tilted upwards. The gaping mandibles are almost perpendicular to the substrate.

*Transport.* Ants pick up the source with their mandibles and move it off the trail (in the field) or carry it to the waste (in the laboratory).

In tests presenting the different body parts to ants in colonies, only a few of the behaviors detected were analyzed because of the low frequency of occurrence of most of them. Tests revealed that during the first minute of presentation, the head of the queen was the body segment that elicited the most behaviors and the strongest response in workers, followed by the head of workers. Among those behaviors, only alarm behavior showed statistically significant differences among castes (Figure 1A and B). The differences in alarm elicited by the head of queens compared with that elicited by the thorax and abdomen was highly significant (chi-square test,  $P < 0.0001$ ). Among body segments of workers, head odors elicited the strongest alarm response in workers followed by the gaster. The thorax did not elicit significant behavioral responses (Figure 1B). Behavior decreased during the 10-min observation period in all worker castes (Figure 2). During the first 2 min of evaluation, alarm was the preponderant behavior but attack behavior was also observed in frequencies that varied between 2 and 8% of workers.

#### DISCUSSION

Wilson (1980) reported the existence of four morphological and behavioral castes in workers of the ant *Atta sexdens rubropilosa*. Our observations suggest a similar polymorphism in *A. laevigata*. Chemical composition of the mandibular glands of the castes differ. However, our results are very different from those reported for *A. sexdens rubropilosa* (Do Nascimento et al., 1993). In that ant, only the smallest workers (head width < 1 mm) contain the compound 4-methyl-3-heptanone as the main component of the mandibular gland secretion,

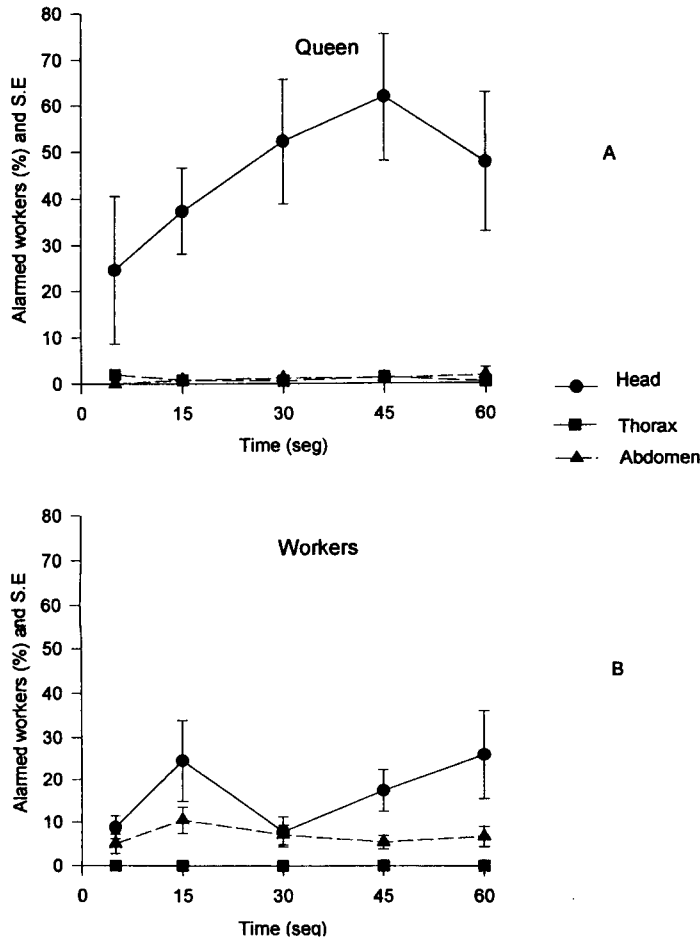


FIG. 1. (A) Alarm behavior produced in forager workers of *Atta laevigata* by odors from crushed queen's body parts. (B) Alarm behavior produced in forager workers of *Atta laevigata* by odors from crushed worker's body parts.

while the dominant compound in larger workers was citral. In *A. laevigata*, all castes, except virgin males, contain 4-methyl-3-heptanone as the main component. The fact that in workers the relative proportions of 4-methyl-3-hexanone and 4-methyl-3-heptanone decreased with head width is congruent with morphological studies of the mandibular glands of *Atta laevigata* (Hernández and Caetano, 1995). These authors reported that the size of the glands is proportional to the size of the cephalic capsule. It is known that the same compound

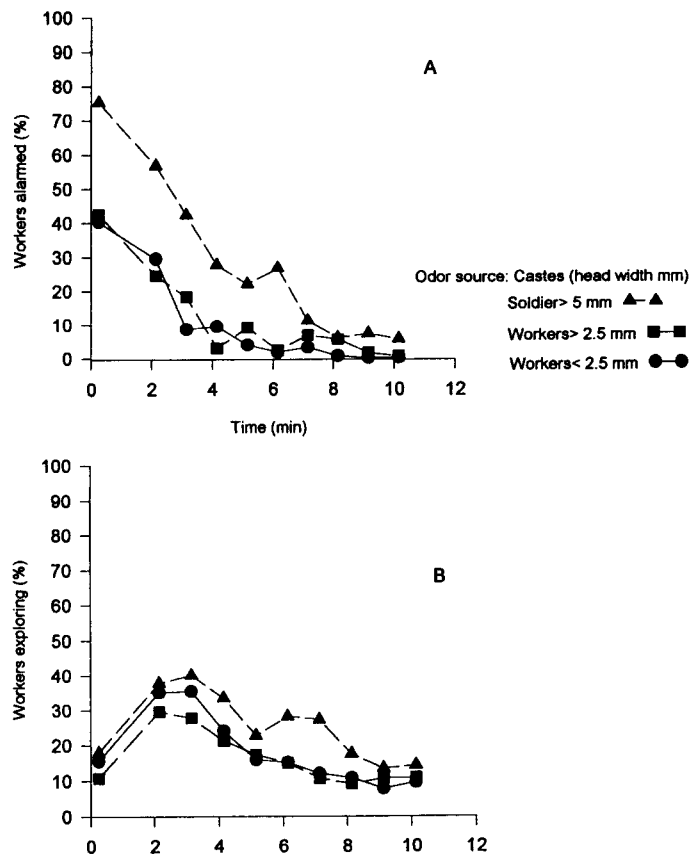


FIG. 2. (A) Alarm behavior produced in forager workers of *Atta laevigata* by odors from crushed head from workers of different castes. (B) Exploring behavior produced in forager workers of *Atta laevigata* by odors from crushed head from workers of different castes.

can release different behaviors in ants depending on its concentration (Moser et al., 1968; Blum, 1996). In leaf-cutting ants, each worker caste is involved in different activities within the colony (Wilson, 1980). A single compound may also release different behaviors depending on the context of the signal. For example 4-methyl-3-heptanone could be used by workers as an alarm signal, and the same compound may be used by females and males as mating cues.

The chemical composition of the mandibular gland secretion in males and queens of *A. laevigata* is different from results reported for *A. sexdens rubropilosa* (Do Nascimento et al., 1993). The facts that in *A. laevigata* the amount of

secretion in males and queens decreases after the nuptial flight and that males show a very different composition compared to queens suggest that these compounds may be used in the communication between the sexes. Do Nascimento et al. (1993) suggested that *A. sexdens rubropilosa* uses the alcohol as an attractant for mating, which could also be the case in *A. laevigata*. Nonetheless, these authors do not explain the high quantities of 4-methyl-3-heptanone found in mated males. The reason for high amounts of 4-methyl-3-heptanone in males after the nuptial flight is not clear, given the short time they survive after mating. It is possible that 4-methyl-3-heptanol can be converted to the ketone by means of oxidation. Thus, males may synthesize the ketone from the alcohol as a precursor during the nuptial flight, and use this ketone for communication, resulting in a change in the mandibular gland chemistry between virgin and mated males.

Wilson (1980) proposed that the smaller worker castes in *Atta sexdens rubropilosa* are more primitive than higher castes. He based this hypothesis on the fact that they are morphologically more similar to the less modified monomorphic members in the same genus and that they carry out fewer activities within the ant society, acting only as nurses and gardeners within the nest. Do Nascimento et al. (1993) supported this hypothesis based on the fact that workers in the smaller castes contain far fewer chemical compounds in the mandibular gland than workers in larger castes. We found that the chemical composition of the mandibular gland is more or less similar in all worker castes in *Atta laevigata*, except for the medium size caste, which according to the primitive caste hypothesis would be primitive caste. Our results suggest the existence of chemical castes that seem to overlap with the caste system proposed by Wilson (1980) based on morphological and behavioral characteristics.

Jaffe (1982, 1987) suggested that nestmate recognition is achieved by the odors produced by the mandibular glands, with respect to the nestmate recognition system in *Atta* ants. Our results suggest the possibility that the mandibular gland secretions could be used by workers to differentiate the castes. These compounds are released continuously in low quantities and are adsorbed by cuticular hydrocarbons over the ant's body.

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