

ETHOLOGICAL AND CHEMICAL STUDIES OF THE ABDOMINAL GLAND EXTRACTS OF THE LEAF-CUTTING ANT *ATTA LAEVIGATA* (HYMENOPTERA: FORMICIDAE)

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INTRODUCTION

Of the many ethological or behavioral studies undertaken in ants, agonistic behavior has not been widely investigated although it comprises a host of important behaviors in intra-specific interactions, including territorial behavior, the defense of resources, recognition of intruders and of nest mates, and alarm behavior.

Abdominal secretions in ants emanate from several sites including the Dufour's and poison glands and the rectal sac. In the genus *Atta*, the Dufour's gland secretion has been associated with territorial behavior (Hölldobler and Wilson 1986; Salzemann and Jaffé 1990; Salzemann *et al* 1992). Methyl-4-methylpyrrole-2-carboxylate and 3-ethyl-2,5-dimethylpyrazine from the poison gland secretion has been shown to produce trail following behavior in several species of the same genus (Tumlinson *et al* 1971; Cross *et al* 1979; Oliveira *et al* 1990; Billen 1992) this secretion also functions as an attractant in *A. cephalotes* (Jaffé 1980; Hölldobler and Wilson 1986). Although the rectal sac is not recognized as a gland it is known that the secretion acts as a trail pheromone in some Formicinae, for example, *Laxius fuliginosus* (Attygalle and Morgan 1984). In the genus *Atta*, Hölldobler and Wilson (1986) reported that the contents of the hind gut contain slightly attractive substances and an arrestant. The function and chemical composition of the contents of the rectal sac in *Atta*, however, is unknown.

The studies just mentioned have only investigated one or a few of the compounds released from the glands and the complexity of the secretions implies that they may have functions other than those just described. Since agonistic behavior is so important in intra-specific ant interactions it is possible that secretions from these glands play a role in its mediation.

In this study we investigate the role of the secretions of the Dufour's and poison glands and the rectal sac in the agonistic behavior of the leaf-cutting ant *A. laevigata*.

MATERIALS AND METHODS

Nests of *A. laevigata* were collected in pine plantations (*Pinus caribaea*) managed by CVG-PROFORCA in Monagas state, Venezuela. Nests were transported in a thermic container to the Behavior Laboratory at the University Simón Bolívar. Nests were kept at 26-28°C, RH 80% with a 12 hour light-darkness cycle. Extracts of the contents of

each gland were prepared using 30 Dufour's glands, 36 poison glands and 31 rectal sacs respectively. Workers were removed from the nest and their activity reduced by cooling them to -5°C for 10 min. Glands were dissected in distilled water under a stereoscopic microscope. Finally each extract was submerged in an ultrasonic bath (Branson, mod. 5210) for 10 min.

Intra and intercolony bioassays

The behavior of workers when exposed to volatile compounds present in extracts of Dufour's gland, the poison gland, rectal sac and blends of these extracts was evaluated. Bioassays were undertaken to investigate the reactions of workers to gland extracts from workers from the same colony (intracolony bioassays) and from different colonies (intercolony bioassays).

The following bioassays were undertaken:

- a) A triangle of filter paper of 10 mm^2 (Whatman N° 10) impregnated with $1\text{ }\mu\text{l}$ of the extract of interest was placed in the foraging area of the colony.
- b) $0.5\text{ }\mu\text{l}$ of the extract of interest were injected on to the body of a foraging worker (between the propodeo and the abdomen).

Five replicates of each bioassay were undertaken for each extract. The behavior patterns of the ants in each experiment were observed directly. The behavior of the workers towards the filter paper or the ant (the source) was recorded using a tape recording Sony TCM-81. The ants were observed until they showed no reaction towards the source, or for a maximum of 10 min. Agonistic behaviors were measured as alarm behavior, attack the source and bites directed to the source.

Bioassay results were analyzed using the binomial test Siegel and Castellan (1988).

Chemical analysis of the extracts

Each extract was concentrated to $100\text{ }\mu\text{l}$ by blowing a low nitrogen stream on it and $1\text{ }\mu\text{l}$ of the extract was taken for analysis.

Gas chromatography-mass spectrometry (GC-MS) was performed on a Perkin Elmer Autosystem chromatograph coupled to a mass spectrometer QMass-910 Perkin Elmer, using 5% phenyl methyl silicone as the stationary phase (0.15 mm ID) in a 25 m . fused silica capillary column (0.18 mm ID). The carrier gas was helium (2 ml/min.). The injection port was set at 280°C . The oven was programmed at $40\times 4\times 6\times 280\times 30$. The split vent was set open 1 min after each injection.

The chemical substances separated were identified from their mass spectra and confirmed by comparison with the mass spectra in the Nist Library. Additionally comparisons of the retention times and the mass spectra of the compounds with synthetic standards were made.

RESULTS

Intra and Intercolony bioassays

The results of the behaviors with a significance of $P < 0.05$ released by ants at the foraging area when exposed to *n*-hexane extracts of abdominal glands (Dufour's gland, poison gland and rectal sac) and blends of these extracts are shown in Table 1.

Table 1. Behaviors with significance of $P < 0.05$ released by ants at the foraging area when exposed to *n*-hexane extracts of abdominal glands (Dufour's and poison glands and rectal sac) and blends of these extracts.

		Extracts												
		⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	
I N T R A C O L O N Y	Rectal Sac	+	+											
	Poison gland		+++	+++	+++		+++				+++	+++		
	Dufour's gland	+		+										
	Control	+												
	Rec Sac + Poison	+++	+++	+++	+++						+++	+++		
	Rec Sac + Duf	+												
	Poison + Duf	++		+++	+++							++		
	Pois + Duf + Rec. Sac		+		+++								+++	
	I N T E R C O L O N Y	Rectal Sac			+++	+++	+++	+++		+++		+++	+++	
Poison gland		+		+										
Dufour's gland		+												
Control		+												
Rec Sac + Poison		+									+++			
Rec Sac + Duf					+++	+++							+++	
Poison + Duf					+++	+++						+++	+++	
Pois + Duf + Rec. Sac		+	+						+		+++			

N = 5 replicates P < 0.05 Binomial Test
 ⊖ = Extract injected over filter paper unangle ⊖ = Extract injected over ant

Criteria used to categorize the behaviors observed (With the exception of "bite the source"):

+ = < 10 ants ++ = 10 - 20 ants +++ = > 20 ants

Criteria used to categorize "bite the source" behavior:

+ = Bite and release the source ++ = Continue biting the source between 1-2 min.
 +++ = Bite the source for a period of > 2 min. and frequent mutilating of the bitten area.

In the intracolony bioassays it can be observed that the poison gland extract produced exploration behavior (touching the source with the top of the antenna), orientation towards the source (directional movements towards the source) and vibratory movements and concentration of workers around the source. Agonistic behaviors such as attacking the source and biting the source were, however, not observed. In the intercolony bioassays exploration, orientation and vibratory movement and concentration of the ants around the source were again significant. Additionally, however, the ants

showed strong agonistic behavior (biting the source) in bioassays where the extract was applied onto the ant, but not where the source was the filter paper. In bioassays of the rectal sac + poison gland extract and rectal sac + poison gland + Dufour's gland extracts where the extract was applied onto the ant we observed mutilations of the test ant. Table 2 shows some of the compounds identified in each extract.

Table 2. Chemical compounds identified by GC-MS in abdominal glands extracts of foraging workers of *Atta laevigata*.

Rectal sac	Poison gland	Dufour's gland
1. Linoleic acid **	2-hexanone and **	Heptadecane **
2. Oleic acid **	Structural isomer **	Octadecene ***
3. Stearic acid **	Structural isomer **	Octadecane ***
4. Tricosane ***	Structural isomer **	Nonadecadiene **
5. Tetracosane ***	2-Heptanone dimethyl-branched **	Nonadecene *
6. Pentacosane **	Heptane 4-ethyl **	Hexadecanoic acid **
7. Hexacosane **	Octane 4-methyl **	Heptacosene **
8. Heptacosane *	1,6-heptadien-4-ol **	Docosene **
9. Octacosane **	Decane 5,6-dimethyl *	Tricosene *
10. Nonacosane **	Isomer *	Tricosane ***
11. Triacotane ***	Dodecane 2-methyl ***	Tetracosane ***
12. Hentriacontane ***	Methyl 4-methylpyrrol-2- carboxylate ***	Pentacosene **
13. Dotriacontane ***		Pentacosane ***
		Hexacosane ***
		Heptacosene ***
		Heptacosane **
		Octacosane ***
		Nonacosane ***

* Main
 ** Secondary
 *** Trace

DISCUSSION

The results show the existence of agonistic behavior in the leaf-cutting ant *Atta laevigata* and support the results of previous authors as regards the existence of colony specific odors and nestmate recognition (Jaffé *et al.* 1979; Hölldobler and Wilson 1986; Salzemann y Jaffé 1990; Salzemann *et al* 1992; Whitehouse y Jaffé 1995). Furthermore, they indicate possible mechanisms by which these defense systems are regulated in intraspecific encounters between members of different colonies.

Table 1 shows that none of the extracts release strong agonistic behavior where the extract was applied onto filter paper in neither intracolony nor intercolony bioassays. Where the extracts were applied onto an ant, in intracolony bioassays, no agonistic behavior occurred. In the intercolony bioassay, however, extracts of mixtures of the rectal sac + poison gland and of all three (rectal sac, Dufour's and poison gland) produced biting of the ant.

Ants marked with glandular extracts from nestmates are not targets for attack, which puts forward evidence for the existence of a colony specific nestmate recognition system. Furthermore, the fact that no agonistic behavior was observed, in neither the intra nor the intercolony bioassays when the source was the filter paper implies that other unknown factors, in addition to those in the glands studied, elicit agonistic behavior towards non nestmates. These compounds, we suggests could be colony specific cuticular hydrocarbons (Nowbahari *et al* 1990).

The results show that of the compounds identified in each extract. In the Dufour's gland we confirmed the three of the compounds previously reported as being used for territorial marking (Salzemann and Jaffé 1992) and in the poison gland one of the compound has been reported as being responsible for releasing trail following behavior, attractant and arrestant behaviors (Jaffé 1980; Hölldobler and Wilson 1986). The functions of the other compounds found in these glands and all of those from the rectal sac were previously unknown. In this study we have shown that they are important in stimulating agonistic behavior in *A. laevigata*. Further studies are needed to determine the precise role of each of these compounds in the agonistic system of this specie.

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