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A comparison of volatiles in mandibular glands from three *Crematogaster* ant symbionts of the whistling thorn acacia

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Abstract

GC–MS analyses of dichloromethane extracts of the mandibular glands from three coexisting *Crematogaster* species, *C. mimosae*, *C. nigriceps*, and *C. sjostedti*, showed distinct differences in the 28 volatile compounds that were identified. The variations of gland components in these ant species may facilitate species identification and lead to species-specific alarm and defence responses that influence their competitive interactions. 3-Hexanol, 3-methylbutanoic acid, 2-methylbutanoic acid, 3-octanone, 3-octanol, phenylacetaldehyde, 2-phenylpropenal, and 3-decanone were found in all three species. The mandibular glands of *C. nigriceps* contain 7 compounds not detected in the two other species; 3-methyl-2-pentanone, 3-methyl-2-pentanol, 3-methyl-2-hexanol, 3-methyl-2-heptanone, 3-methyl-2-heptanol, 2-phenylethanol and 2-methylheptanoic acid. *C. sjostedti's* mandibular gland secretion also contains 7 compounds not detected in the other two species; butanoic acid, 3-heptanol, 2-methylpentanoic acid, glycerol, 6-methyl-3-octanol, 2-pyrrolidinone and 3-nonanol. Nonanal was the only compound detected solely in extracts of *C. mimosae*.

Keywords: *Crematogaster mimosae*; *C. nigriceps*; *C. sjostedti*; *Hymenoptera*; Formicidae; *Acacia drepanolobium*; Whistling thorn acacia; Mandibular gland; Alarm pheromone

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1. Introduction

The whistling thorn acacia, *Acacia drepanolobium*, is the dominant tree species on many black cotton soils in east Africa. A striking feature of this tree is the hollow thorn swellings after which it is named. The average tree is about 2 m in height and tree densities can be up to 400 per acre, with an average of 200 (Hocking, 1970). Three species of *Crematogaster* ants live symbiotically with this tree. *C. mimosae*, and *C. nigriceps* use the hollow thorn swellings as brood chambers and shelter while *C. sjostedti* nests in hollow spaces within dead or dying branches. The complex symbiotic relationship between the whistling thorn acacia and these ants has been the subject of several studies (Hocking, 1970; Stanton et al., 1999; Young et al., 1997). Competition for host trees leads to fierce conflicts between these species. The red-and-black cocktail ant, *C. mimosae*, and the black cocktail ant, *C. sjostedti*, forcibly displace the black-and-yellow cocktail ant, *C. nigriceps*, if branches on neighbouring trees come in contact.

These *Crematogaster* ants produce a potent alarm pheromone in their mandibular glands: ants disturbed on one tree will cause ants of the same or different species on neighbouring trees to display alarm behaviour (Stanton, unpublished; Wood and Chong, 1975). We found that the chemical composition of the mandibular gland secretion from each species is distinct and that all are more complex than has previously been reported for any *Crematogaster* species.

2. Materials and methods

Worker ants of *C. mimosae*, *C. nigriceps*, and *C. sjostedti* were collected at the Mpala Research Centre in Kenya (36°50′E, 0°15′N). For *C. mimosae* and *C. nigriceps*, thorn swellings were collected from 10 different whistling thorn acacias and within 1 h placed in a freezer to kill the ants. *C. sjostedti* workers were collected directly from 10 different tree trunks, placed in conventional Ziploc® storage bags, which were transferred within an hour to a freezer. After 1–4 h in the freezer, the heads of 50 individual ants of each species were placed in glass vials with Teflon-lined stoppers containing 2.0 ml of dichloromethane. A second sample of 50 heads for each species was likewise collected for comparison. To prevent cross contamination between species, all equipment used in the collection of the ant heads was thoroughly cleaned before use.

Gas chromatography–mass spectrometry (GC–MS) was performed on the dichloromethane extracts of the ant heads in a splitless mode, using a Hewlett–Packard GCD Plus fitted with a 30 m×0.25 mm cross-linked phenyl methyl silicone capillary column (HP–5MS). The gas chromatograph was programmed so the oven temperature was kept at 40°C for 4 min, then increased to a final temperature of 280°C at a rate of 30°/min. Mass spectral fragments below m/z=39 were not recorded. The

relative amount of each component was calculated from the total ion current. The amounts reported are an average (and standard deviation) of the two 50 ant head samples. Impurities identified in control samples of dichloromethane were not reported as were minor components less than 0.2%.

All compounds were initially identified by comparison of mass spectra in the NIST 1998 computerised mass spectral library, except for 6-methyl-3-octanone and 6-methyl-3-octanol. These two compounds were identified by comparison with spectra published in a study by Bjostad et al. (1996). All identifications were confirmed by comparison of spectra and retention times to those of authentic standards, except for 6-methyl-3-octanone, 6-methyl-3-octanol, 2-phenylpropenal and 2-methylheptanoic acid. Authentic samples were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Pfaltz & Bauer (Waterbury, CT), except as follows. 3-Methyl-2-pentanol, 3-methyl-2-hexanol, and 3-decanol were prepared from the corresponding ketone by reduction by sodium borohydride in methanol. 3-Methyl-2-heptanone was prepared by oxidation of 3-methyl-2-heptanol.

3. Results and discussion

Decapitation and storing ant heads in solvent is a standard method for extraction of the mandibular gland secretions of *Crematogaster* ants when they cannot be analysed immediately (Blum et al., 1969; Brand and Pretorius, 1986; Crewe et al. 1970, 1972; Schlunegger and Leuthdold, 1972; Wood and Chong, 1975). The GC–MS analyses of the dichloromethane extracts showed a composite of 28 different volatile compounds (Table 1) of which only 3-octanone, 3-octanol, 6-methyl-3-octanone, 6-methyl-3-octanol, and 3-nonanone have been previously identified from *Crematogaster* mandibular gland extracts (Wood and Chong, 1975; Blum, 1981; Scheffrahn and Rust, 1989).

Of the 28 compounds (Table 1), the following eight were common to all three species, 3-hexanol, 3-methylbutanoic acid, 2-methylbutanoic acid, 3-octanone, 3-octanol, phenylacetaldehyde, 2-phenylpropenal, and 3-decanone. *C. nigriceps* had seven compounds not detected in the two other species; 3-methyl-2-pentanone, 3-methyl-2-pentanol, 3-methyl-2-hexanol, 3-methyl-2-heptanone, 3-methyl-2-heptanol, 2-phenylethanol and 2-methylheptanoic acid. *C. sjostedti* also had seven compounds not detected in the other two species; butanoic acid, 3-heptanol, 2-methylpentanoic acid, glycerol, 6-methyl-3-octanol, 2-pyrrolidinone, and 3-nonanol. The only compound detected solely in extracts of *C. mimosae* was nonanal. In addition, *C. mimosae* and *C. nigriceps* had one compound in common that is not found in *C. sjostedti*; 3-(methylthio)-propanal. *C. sjostedti* and *C. nigriceps* had three compounds in common that were not found in *C. mimosae*; 2-methylhexanoic acid, 6-methyl-3-octanone, and 3-nonanone. No compounds were found in *C. sjostedti* and *C. mimosae* that were not in *C. nigriceps*.

Surprisingly, extracts from *C. nigriceps* contain a series of related alcohols and ketones that were not found in the other two species of acacia ants. These compounds are 3-methyl-2-pentanone, 3-methyl-2-pentanol, 3-methyl-2-hexanol, 3-methyl-2-

Table 1
Volatile compounds from the mandibular glands of the three species of *Crematogaster* ants

Compound name	*C. sjostedti* (%)	*C. nigriceps* (%)	*C. mimosae* (%)
3-Methyl-2-pentanone[a]		3.3±0.2	
Butanoic acid[a]	0.9±0.2		
3-Hexanane[a]		1.0±0.2	
3-Methyl-2-pentanol[a]		14.2±0.2	
3-Hexanol[a]	0.3±0.0	2.7±0.7	2.6±0.3
3-Methylbutanoic acid[a]	0.5±0.0	17.7±3.1	25.6±0.9
2-Methylbutanoic acid[a]	14.9±0.6	2.3±0.5	2.0±0.1
3-Methyl-2-hexanol[a]		2.2±0.0	
3-Heptanol[a]	0.4±0.0		
3-(Methylthio)-propanal[a]		1.6±0.4	3.6±0.5
2-Methylpentanoic acid[a]	0.5±0.0		
3-Methyl-2-heptanone[a]		9.2±0.6	
Glycerol[a]	0.7±0.2		
3-Methyl-2-heptanol[a]		2.7±0.3	
3-Octanone[a]	38.1±0.4	21.3±0.4	10.8±3.4
3-Octanol[a]	34.6±0.2	5.6±2.4	37.3±4.9
2-Methylhexanoic acid[a]	1.6±0.1	1.1±0.3	
Phenylacetaldehyde[a]	0.5±0.1	4.7±0.7	7.7±0.6
6-Methyl-3-octanone[b]	2.7±0.1	0.9±0.0	
6-Methyl-3-octanol[b]	1.1±0.0		
2-Pyrrolidinone[a]	0.9±0.1		
3-Nonanone[a]	0.9±0.0	0.8±0.1	
3-Nonanol[a]	0.5±0.2		
Nonanal[a]			2.1±0.1
2-Phenylethanol[a]		4.1±0.4	
2-Phenylpropenal[b]	0.4±0.0	2.4±0.2	5.5±1.5
3-Decanone[a]	0.5±0.0	0.7±0.1	2.9±0.3
2-Methylheptanoic acid[b]		1.5±0.3	

[a] Identified by comparison with an authentic sample.
[b] Identified from published spectra.

heptanone, and 3-methyl-2-heptanol and make up 30% of the volatiles in the analysis. To our knowledge none of these compounds have previously been reported from any ant species, however, a related compound, 3-methyl-2-hexanone has been reported as a trace component in the mandibular glands of queen leaf cutler ants, *Atta laevigata* (Hernandez et al., 1999).

The chirality of the mandibular gland compounds was not investigated since samples of enantiomericly pure mandibular gland compounds were not readily available. Ten of these 28 compounds have one stereogenic centre and four have two stereogenic centres. Each of the reference samples of 3-methyl-2-pentanol, 3-methyl-2-hexanol and 3-methyl-2-heptanol was a mixture of four stereoisomers as each has two stereocenters. The diastereomers for each of these reference compounds were well resolved into two GC peaks with different retention times. A single GC peak was observed in the ant samples, so the ant compounds must be a pure stereoisomer

or a racemic mixture of enantiomers. It is likely that all 14 chiral ant compounds exist as a single stereoisomer, since previous researchers found *C. castanea* and *C. liengmei* to produce only *S*-(+)-3-octanol (Brand, 1985).

The different compounds in the mandibular glands of these three species may facilitate species identification and lead to species-specific alarm and defence responses that influence their competitive interactions. 3-Octanone and 3-octanol have been reported as alarm pheromones of *C. mimosae* and *C. nigriceps* (Wood and Chong, 1975) so the difference in concentration between these compounds may be important in species discrimination. For *C. sjostedti*, the ratio of 3-octanone to 3-octanol was 1:1, for *C. nigriceps*, 4:1 and for *C. mimosae*, 1:3.5. Also of note are the different concentrations of 2- and 3-methylbutanoic acid. For *C. sjostedti*, the ratio of 2-methylbutanoic acid to 3-methylbutanoic acid was 30:1, for *C. nigriceps*, 1:8 and for *C. mimosae*, 1:13.

The previous analyses of the mandibular gland secretion of *C. mimosae* and *C. nigriceps* using a chromatographic column packed with a liquid phase covering a solid support, showed 3-octanone and 3-octanol to make up 95% of the volatiles detected (Wood and Chong, 1975). In the present study using a bonded phase glass capillary column, these compounds comprised 48.1% and 26.9% of the volatiles in extracts of *C. mimosae* and *C. nigriceps*, respectively (Table 1). While it may be possible that the difference is due to geographic variation, it is more likely due to differences in analyses between packed columns and capillary columns. In packed columns, highly volatile compounds such as 3-methyl-2-pentanone are likely to elute with the extraction solvent. Highly polar compounds like 3-methylbutanoic acid may bond to the solid phase or have extended retention times, and minor components such as phenylacetaldehyde may blend in with the baseline and not be seen.

An examination of published reports indicates there may be other compounds in addition to 3-octanone and 3-octanol that contribute to the alarm pheromones of *Crematogaster* ants. A detailed study by Leuthold and Schlunegger (1973) showed the alarm response to ant head extracts by *C. scutellaris* was greater than that to a mixture of 3-octanone and 3-octanol. Similarly, *C. californica*'s attraction to a synthetic mixture of mandibular gland secretion containing, 3-octanone, 3-octanol, 6-methyl-3-octanone, and 3-nonanone was not the same as to crushed heads of this species (Scheffrahn and Rust, 1989). Finally, published gas chromatograms used to illustrate 3-octanone, 3-octanol, 6-methyl-3-octanone, 6-methyl-3-octanol and 3-nonanone as components in mandibular secretions, also show unidentified compounds in mandibular gland extracts of *C. clariventris*, *C. gabonensis*, *C. stadelmanni*, *C. minutissima*, and *C. buchneri* (Crewe et al., 1972).

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