Archives of Insect Biochemistry and Physiology 63:11–14 (2006)

Exocrine Chemistry of the Myrmicine Ant *Zacryptocerus pusillus* (Hymenoptera: Formicidae)

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Minor workers of the ant *Zacryptocerus pusillus* have unusual exocrine secretions in both their mandibular and Dufour glands. The mandibular glands contain a 3:1 mixture of 4-heptanone and 4-heptanol, a mixture found only in the related species *Z. varians*. The Dufour gland contains a mixture of 13 aldehydes from C_9 to C_{18}, not previously encountered in ant secretions. The venom glands gave variable results with only nonanal present consistently. Arch. Insect Biochem. Physiol. 63:11–14, 2006.

KEYWORDS: 4-Heptanone; 4-heptanol; Dufour gland; mandibular gland; venom gland; hexadecanal

INTRODUCTION

Myrmicine ants of the neotropical genus *Zacryptocerus* (tribe Cephalotini) are found widely in Brazil on the cerrado or savannah. *Zacryptocerus* is one of four myrmicine genera that includes species with distinct minor and major castes of workers (Wilson, 1976). These castes show a clear division of labour (Wheeler and Hölldobler, 1985). Soldiers are larger than workers. Their sole function is to guard the nest entrance, which they do by blocking it with their large, armoured heads. In *Z. pusillus* colonies, the proportion of major to minor workers is approximately 1:20–50. Possibly because of this low proportion of major workers, in this species, minor workers can also cooperate to form a shield to block the nest entrance, although these shields are rather ephemeral and do not last more than 10 to 15 sec (Delabie, 1994). Minor workers are distinguished morphologically from workers of other myrmicines by their ventrally flattened abdomens and extremely large Dufour glands. We report here on the chemical examination of the exocrine glands of the minor workers of this little-studied species.

MATERIALS AND METHODS

Minor workers of *Zacryptocerus pusillus* (Klug, 1824) were collected at Viçosa, Minas Gerais, Brazil. They were dissected there and samples of individual worker mandibular, Dufour, and venom glands were separately placed in soft glass capillary tubes (one gland per tube, pairs of glands or whole heads for mandibular glands) and sealed in a small flame for transport to the chemical labo-

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Contract grant sponsor: European Commission via the Training and Mobility of Researchers (TMR) Programme.

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Received 20 October 2005; Accepted 16 March 2006

DOI: 10.1002/arch.20136
Published online in Wiley InterScience (www.interscience.wiley.com)

ratory, as described by Morgan (1990). The dissection details were as follows: abdominal glands were removed under distilled water. The ant was held by the petiole with one pair of forceps, while the final tergites and sternites were parted and the posterior internal portion of the gaster was pulled out by the final sternite and sting lance. Other organs were carefully removed until only the poison apparatus remained. The required glands were then cut from the poison apparatus, placed on a fragment of glass as described in Morgan (1990), and sealed in a capillary. Mandibles were removed with the glands attached and similarly mounted on a fragment of glass and sealed in capillaries.

Analysis by linked gas chromatography and mass spectrometry (GC-MS) was carried out using a Hewlett Packard 5890 Gas Chromatograph and 5970B Mass Selective detector with HP59970C ChemStation. The column was a fused silica capillary column (12 m × 0.2 mm) coated with HP-1 (methyl silicone equivalent to OV-1 from Hewlett Packard, Wokingham, UK) with 0.33-μm film thickness. The column was linked to the mass spectrometer by a deactivated fused silica capillary (10 m × 0.2 mm). The carrier gas was helium at 1 ml min^{-1}. For mandibular and venom glands, the GC oven was held at 30°C for 10 min, then heated at 4°C min^{-1} to 200°C. For Dufour glands, the oven was held at 30°C for 2 min, then heated at 8°C min^{-1} to 200°C and held there 2 min. The mass selective detector was set to scan the ions from *m/z* 36 to 450 at about 1.7 scans sec^{-1} using 70 eV ionization. Samples in their glass capillaries were introduced into the GC with the solid sampling device described by Morgan (1990).

4-Heptanone, nonanal, decanal, dodecanal, tridecanal, and 11-hexadecenal were obtained from Lancaster Synthesis, Morecambe, UK. 4-Heptanol was made from the corresponding ketone by $NaBH_4$ reduction. A series of normal alkanes from C_{10} to C_{20} were available to check retention times.

RESULTS

Four samples of dissected mandibular glands and seven samples of whole heads were analysed.

TABLE 1. The Composition of the Contents of the Mandibular Glands of *Z. pusillus* ($n = 11$) and the Amount Per Worker

Compound	Proportion (%)	±SD
4-Heptanone	76.4	11.6
4-Heptanol	23.6	11.6
Total amount per individual (ng)	39.6	11.0

No differences were seen between dissected glands and whole heads. The mandibular glands contained only two substances, 4-heptanone and 4-heptanol, in a relatively small quantity for mandibular glands, and in the approximate ratio of 3:1 (Table 1). Compounds were identified by their mass spectra (Olubajo et al., 1980) and comparison with the NIST spectral library (4-heptanone no. 227072, 4-heptanol no. 231743).

The Dufour glands contain a large amount of secretion consisting entirely of aliphatic aldehydes (Fig. 1; Table 2). 2-Methylhexadecanal was identified by comparison with the spectrum (no. 36564) in the NIST spectral library. It showed a very weak molecular ion M^+ 254, another ion at *m/z* 196 for the alkene portion from a McLafferty rearrangement, and clusters of weak ions centred at *m/z* 168, 154, 140, 111, 97, and 83 (the strongest ion of the last group was at *m/z* 81). Other ions were at *m/z* 71 (15%), 58 (100%), 43 (30%), and 41 (25%). The base peak at *m/z* 58 arises from the ion $CH_3CH{=}CHOH^+$, the other ion that can arise from the McLafferty rearrangement. The straight chain

TABLE 2. The Composition of the Secretion of the Dufour Glands of *Z. pusillus* ($n = 8$) and the Amount Per Worker

Peak no. in Figure 1	Compound	Proportion (%)	±SD
—	Nonanal	0.9	0.4
—	Decanal	0.6	0.2
1	Dodecanal	0.8	0.2
2	Tridecanal	1.4	0.7
3	Tetradecenal	1.1	0.3
4	Tetradecanal	5.8	1.9
5	Hexadecenal	26.3	7.2
6	Hexadecanal	33.6	5.7
7	Heptadecadienal	3.8	3.0
8	2-Methylhexadecanal	9.2	2.5
9	Heptadecenal	3.0	2.0
10	Octadecenal	9.4	4.7
11	Octadecanal	3.1	1.1
	Total amount per individual (ng)	1,300	370

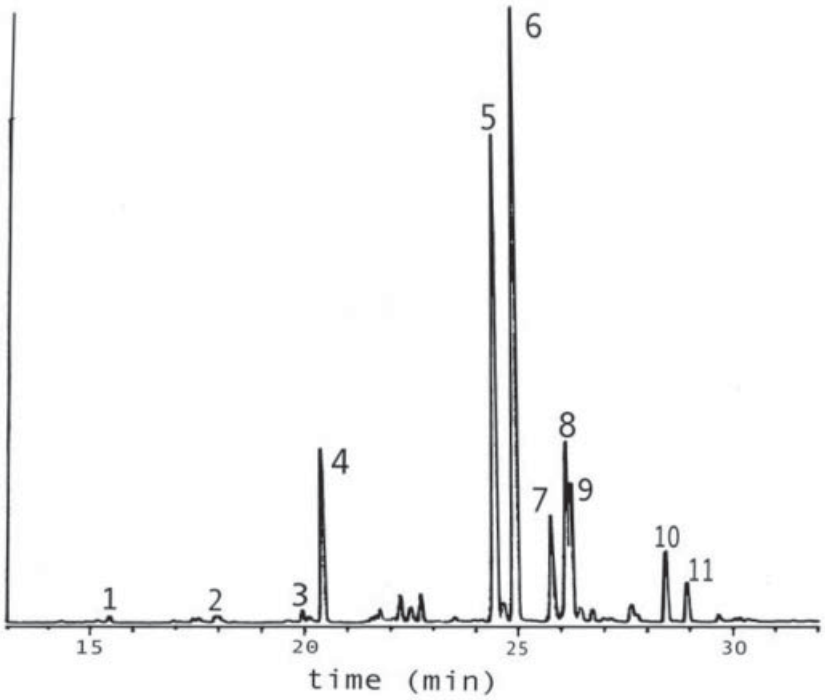

Fig. 1. A typical chromatogram of the contents of a Dufour gland of a worker of *Z. pusillus*. Numbers correspond to those in Table 2.

aldehydes were readily identified from their characteristic mass spectra. No more material was available to locate double bond positions in the unsaturated aldehydes.

The only compound found in all the venom glands analysed was nonanal. Otherwise the composition was variable. Three samples contained decanal as well, one contained 2-dodecanone, and one tetradecanal and hexadecanal (which may be contaminants from the Dufour gland during dissection). The pyrazines found in many myrmicine ant venom glands (Attygalle and Morgan, 1984) were not present.

DISCUSSION

The mandibular glands contain a very simple mixture of a ketone and its corresponding alcohol in the ratio of 3:1. It is interesting that the same pair of compounds were found in the mandibular glands of *Zacryptocerus varians* (ratio 2:1, Olubajo et al., 1980), a North American species that inhabits mangrove swamps. 4-Heptanone has been identified, along with other compounds, in the ant *Polyrhachis simplex* (Hefetz and Lloyd, 1982), and in the anal gland of *Tapinoma simrothi pheonicium* (Hefetz and Lloyd, 1983). Except for its presence in *Z. varians,* 4-heptanol has not been identified as a component of insect pheromones or secretions before. Olubajo et al. (1980) found that workers of *Z. varians* retreated from the odour of both ketone and alcohol. Delabie (1994) suggests the recruitment of minor workers to join together to form an entrance shield may be stimulated by an alarm or aggregation pheromone. The heptanone-heptanol mixture of *Z. pusillus* would, because of its high volatility, be a prime candidate for such a pheromone, but lack of live workers prevented us looking at this after the identifications had been made.

The Dufour gland of worker ants generally is smaller than their respective venom glands, but in *Z. pusillus* the volume of the Dufour gland of minor workers is much greater than that of the venom gland. The total volume of secretion was well over 1 μg per worker (Table 2). The Dufour gland is also unusual in containing a range of aliphatic aldehydes from C_9 to C_{18} (Fig. 1). Aldehydes of similar chain length are well known as part of lepidopteran sex pheromones and have been found in the Pavan gland of the ant *Dolichoderous thoracicus* (Allygalle et al., 1998), but to the best of our knowledge, a Dufour gland secretion consisting only of aldehydes

has not been encountered in ants before. It is known that workers of *Z. pusillus* follow trails (T. C. Della Lucia, personal communication). Wilson (1976) in his study of *Zacryptocerus varians* recorded that either the Dufour gland or the venom gland caused short-acting recruitment of workers from distances up to 25 cm but could not determine which of these glands was responsible.

ACKNOWLEDGMENTS

Funding for this work was obtained through the research network INSECTS of the Universities of Copenhagen, Firenze, Helsinki, Keele, Lausanne, Leuven, Oulu, Regensburg, Sheffield, Würzburg, and ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme. We thank Marcelo V.B. Quieroz for help in collecting and identifying the ants, and Eric Schoeters for helping with some of the analyses.

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