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## Mandibular Gland Contents of a Colony of the Queenless Ponerine Ant *Dinoponera australis*

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True sociality in insects (eusociality) has been defined as simultaneous possession of three traits: individuals cooperate in caring for the young; there is a reproductive division of labor, with more or less sterile individuals working on behalf of fertile individuals in life stages capable of contributing to colony labor, so that offspring assist parents during some period of their life [1].

In many species of the ant subfamily Ponerinae, a morphologically distinct female (queen) is completely absent, raising the question of whether these species could still be called eusocial. However, in all cases studied, reproduction is taken over by one or relatively few fertilized workers, called “gamergates” [2], confirming that there is a division of reproductive labor. Moreover, recent studies indicate that most queenless ponerine species are monogynous (only one mated worker at a time) and that physical dominance plays an important role in establishing reproductive dominance [3]. Although the references bring indirect evidence that pheromones can also play a role in determining reproductive division of labor, in only one case

(*Diacamma australe*) mutilation of a gland inside the thoracic appendages (gemmae) of newly emerged workers by the gamergates precludes their production of sexual attractants [4].

The genus *Dinoponera*, containing the largest ants in the world, is restricted to continental South America, where its six accepted species [5] live in very small colonies [6] occupying ground nests in wet and dry forests. *D. quadriceps*, from the Brazilian Northeast, has one or a few gamergates and there is evidence that its reproductive system is sustained by dominance, resulting from aggressive interactions [7].

*D. australis* colonies are even smaller than those of *D. quadriceps*, with only one gamergate per colony (worker number  $13 \pm 6$ ,  $n = 36$ , data from a population living in a tropical dry forest in Itirapina, São Paulo, Brazil). They forage individually at daylight, stinging a variety of medium-sized arthropod prey [8]. Individually marked ants, living in artificial gypsum nests, showed a structure composed of a least two age groups: older foragers and younger nurses. The gamergate, although relatively old (may

live up to 20 months), never leaves the nest chambers near the bottom of the nest and is constantly involved in aggressive interactions with nurses. This is particularly true after the emergence of callow nestmates (Paiva and Brandão, in preparation).

For the present paper two colonies of *D. australis* were collected at Itirapina in January 1990 and March 1991. They consisted respectively, of 13 workers, 5 eggs, 2 larvae and 10 pupae (Colony 1), and 14 workers and immatures (Colony 2). To determine the most common task performed by a given individual in Colony 2, we labeled them all with numbered marks [9], glued on the pronotal disk with cyanoacrylate.

On completion of the behavioral observations, Colony 2 was sacrificed. We determined relative age by the wear on the mandibles. The reproductive status was determined by the filling of spermathecae and by the stage of ovariole development. The colony had only one gamergate and the workers were divided into two groups, with three individuals acting as foragers, seven as nurses, and three with an unclear function. The glands were fixed for morphological studies (Billen et al., in preparation), with the exception of one mandibular gland from each individual, these were sealed individually in glass capillaries for GC-MS analysis, using the solventless technique in [10]. The low number of individuals composing each colony allowed us to study chemically, for the first time, all adult members of a society. Gas chromatography-mass spectrometry was carried out on a BP-1 fused silica capillary column of dimensions  $12 \text{ m} \times 0.2 \text{ mm}$  with a  $0.25 \text{ }\mu\text{m}$  film thickness (SGE Ltd.) in a Hewlett Packard 5890 gas chromatograph coupled to a 5970B Mass Selective Detector. The glass capil-

larvae were heated in the injection port at 200 °C for 2 min before crushing. The oven was held isothermal at 35 °C for 2 min before being raised to 250 °C at a rate of 7 °C min<sup>-1</sup>. Helium was used as the carrier gas with a flow rate of 1 ml min<sup>-1</sup>.

A large difference in the amount of secretion between the gamergate and a worker was first noted by analysis of the

glands of the only individual with fully developed ovaries and filled spermatheca in Colony 1 and a worker from the same colony [11]. A complete analysis was then carried out on Colony 2. The results of the chemical analyses are shown in Table 1. The numbers refer to the structures in Fig. 1. Identification was confirmed by comparison of GC retention time and mass spectra with

those of authentic samples, from the spectra listed in [12], and by further chemical syntheses [13]. Compounds 8, 9 and 11 are novel, and we have confirmed the identification of 7 and 10 for the first time.

Table 1 shows that 6 was the major component in the mandibular glands of all members of the colony, with the exception of the newly emerged worker, W12, where it was only present as 18% of the secretion. The gland of the newly emerged worker contained relatively more tetra-substituted pyrazines, with 8 and 10 as the major compounds (see Fig. 2A) when compared with an average worker (Fig. 2C). In 11 of the workers, compound 3 was the next major constituent, the exceptions being the gamergate (G) and W12. The mandibular gland of the gamergate was dominated by 6 (98%) with 2% of 5 and trace amounts of 1a and b. The major difference between the gamergate and the other workers was in the total amount of secretion; the gamergate had only 0.1 µg (see Fig. 2B), whereas the secretion of the others ranged from 5.3 to 48 µg. The worker numbered W11 in Colony 2, identified as the major forager, contained the largest amount of secretion in its mandibular glands (twice as much as any other worker, and approximately 500 times as much as the gamergate).

Several genera of ponerine ants have been shown to possess pyrazines in their mandibular glands [14, 15], and in each case they serve as alarm pheromones. We, however, have observed no such response when exposing workers of *D. australis* to a mandibular gland extract. Although the pheromonal function of the mandibular gland secretion of this species remains unknown, we suggest two possible roles. First, the presence of a relatively small amount of secretion in the gamergate indicates that the secretion may be linked to the reproductive division of labor and, secondly, the large amount of secretion in the major forager suggests a role related to foraging, such as a nest area or home-range marking pheromone. Further behavioral tests are required to confirm these suggestions.

The reason for the presence of relatively more tetra-substituted pyrazines in the newly emerged worker is unclear. A possible explanation is that they are biosynthetic precursors of the trisubstituted pyrazines, and are converted to these compounds over a period of time. It is

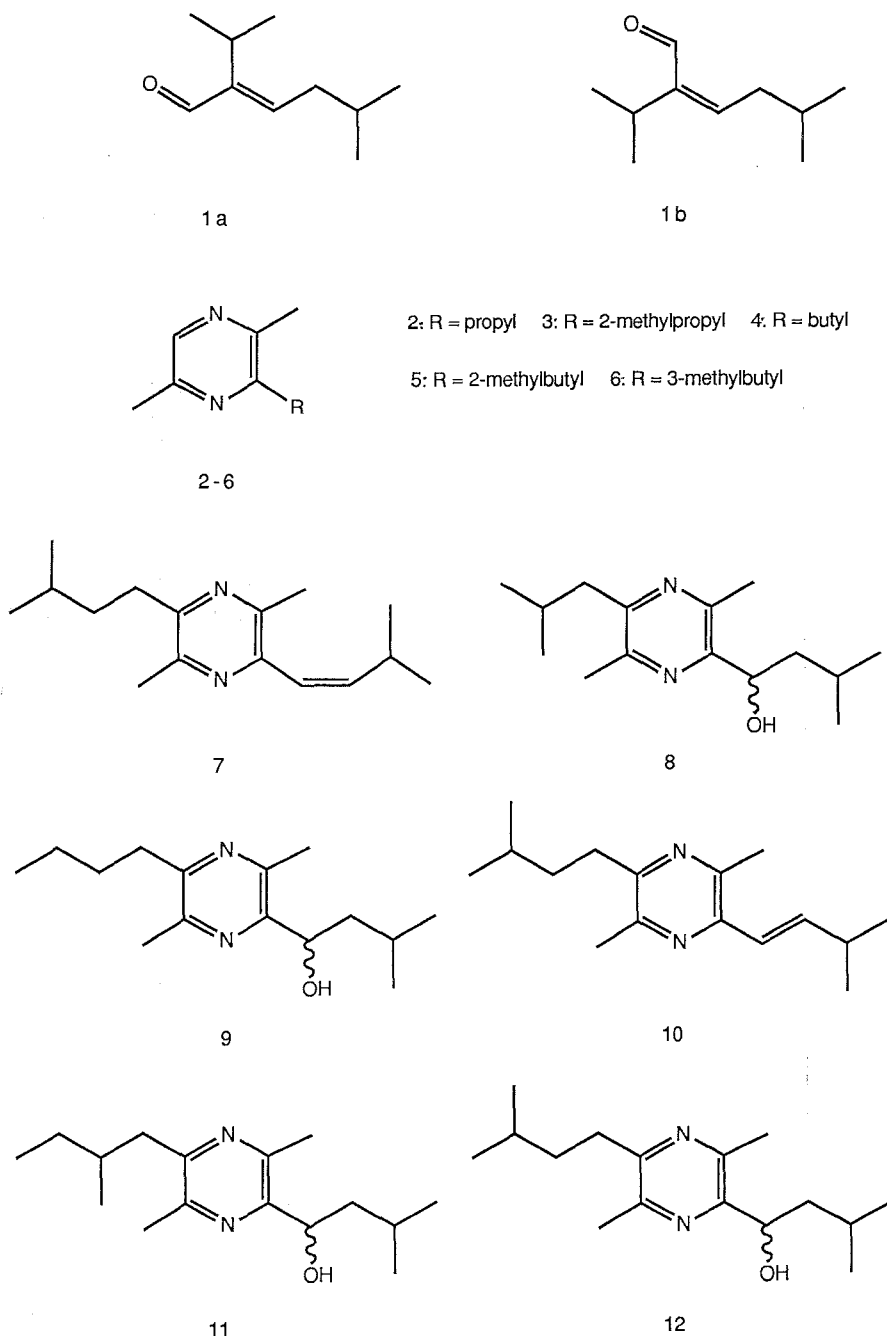


Fig. 1. The structures of the aldehydes and pyrazines found in *D. australis*

Table 1. Composition (%) of the mandibular gland secretion of Colony 2 of *Dinoponera australis* (t = trace component, G = gamergate, W = worker)

Structure	Compound	Number of individual												
		G	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
1a	(E)-2-Isopropyl-5-methyl-2-hexenal	t	t	-	-	-	-	-	t	t	-	-	t	-
1b	(Z)-2-Isopropyl-5-methyl-2-hexenal	t	t	-	-	-	-	-	t	t	-	-	t	-
2	2,5-Dimethyl-3-propylpyrazine	-	t	-	-	-	t	-	t	t	-	-	1.0	-
3	2,5-Dimethyl-3-(2'-methylpropyl)pyrazine	t	11	8.5	13	16	14	12	18	14	16	19	24	t
4	3-Butyl-2,5-dimethylpyrazine	-	t	-	0.8	t	0.7	t	1.7	1.0	t	0.9	1.8	-
5	2,5-Dimethyl-3-(2'-methylbutyl)pyrazine	2.0	7.3	5.3	5.3	5.0	3.0	5.6	6.5	5.9	6.8	8.4	5.8	t
6	2,5-Dimethyl-3-(3'-methylbutyl)pyrazine	98	73	71	69	66	66	65	62	61	61	59	58	18
7	(Z)-2,5-Dimethyl-3-(3'-methyl-1'-butenyl)-6-(3''-methylbutyl)pyrazine	-	t	t	t	-	t	t	t	0.5	t	t	-	t
8	3-(1'-Hydroxy-3'-methylbutyl)-2,5-dimethyl-6-(2'-methylpropyl)pyrazine	-	5.0	5.4	6.4	6.8	8.3	9.0	6.0	9.7	8.9	7.2	4.8	28
9	3-Butyl-6-(1'-hydroxy-3'-methylbutyl)-2,5-dimethylpyrazine	-	-	-	-	-	t	-	-	t	-	-	-	2.7
10	(E)-2,5-Dimethyl-3-(3'-methyl-1'-butenyl)-6-(3''-methylbutyl)pyrazine	t	4.2	9.4	5.8	5.7	6.0	7.8	5.8	6.6	7.6	5.0	2.0	30
11	3-(1'-Hydroxy-3'-methylbutyl)-2,5-dimethyl-6-(2'-methylbutyl)pyrazine	-	-	-	-	-	-	-	-	-	-	-	-	1.9
12	3-(1'-Hydroxy-3'-methylbutyl)-2,5-dimethyl-6-(3''-methylbutyl)pyrazine	-	t	t	t	-	1.1	t	t	0.9	-	-	1.1	18
	Total [ $\mu$ g]	0.1	24	11	15	14	24	5.3	22	22	8.7	9.8	48	27

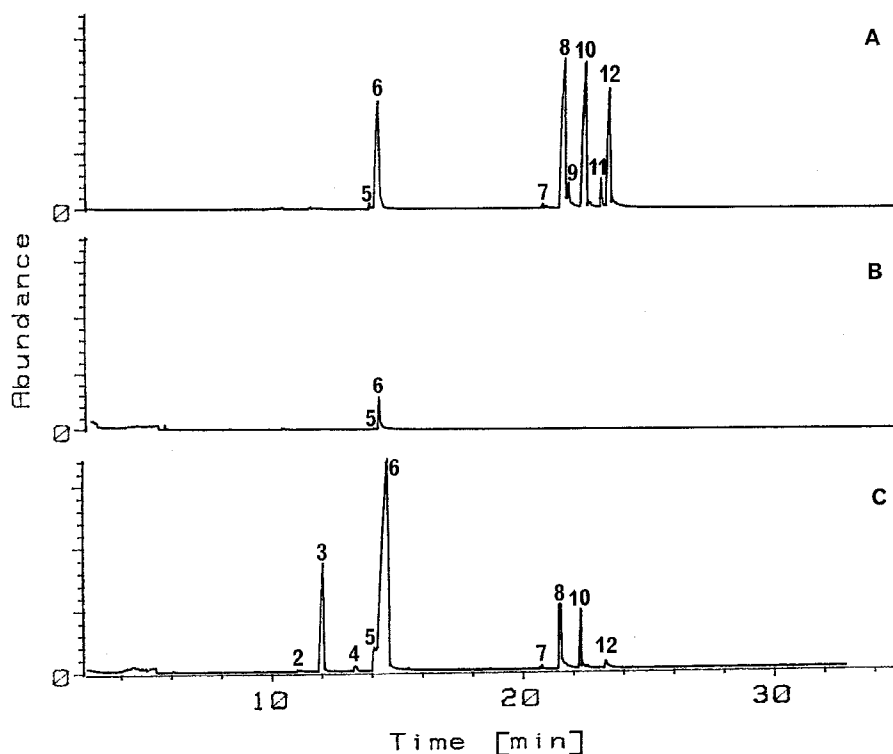


Fig. 2. Gas chromatograms of the contents of one mandibular gland of A) the newly emerged worker, W12, showing the large amounts of tetra-substituted pyrazines, B) the gamergate, G, and C) a "typical" worker, W5, all from Colony 2 and shown to the same scale. The numbering of peaks correspond to the compounds in Table 1 and Fig. 1

interesting that oxidative cleavage of the hydroxyisopentyl side chain of 8, 9, 11, and 12 would yield 3, 4, 5, and 6 and isovaleraldehyde. Although isovaleraldehyde itself was not detected in the secretion, its aldol self-condensation products 1a and 1b were.

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