Insectes soc. 47 (2000) 383–389
0020-1812/00/040383-07 $ 1.50+0.20/0

Insectes Sociaux

Research article

Worker size polymorphism and ethological role of sting associated glands in the harvester ant *Messor barbarus*

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Received 20 June 2000; revised 7 September 2000; accepted 12 September 2000.

Summary. Though harvester ants are closely similar in ecology, species differ in their worker size polymorphism as well as in the glandular source of their trail pheromones and defensive compounds. In the harvester ant *Messor barbarus,* we find that the recruitment trail pheromone is located in the Dufour gland, while defence-alarm substances are produced in the poison gland. We also investigated how the glandular development and the ethological response to these abdominal glands are related to worker body size. For both glands, *M. barbarus* workers show monophasic and nonisometric growths with slopes of allometric regression lines lower than 1. The highest trail-following response is elicited by the Dufour gland secretion from media workers, responsible for most foraging activities in *M. barbarus*. Aggressive behaviour is more frequently observed in the presence of poison gland secretions from medium and large-sized workers. Differences between species and between worker size classes in the ethological role of sting associated glands are discussed in relation to the foraging ecology and defensive characteristics of harvester ants.

Key words: Size polymorphism, exocrine glands, defence, trail, harvester ants.

Introduction

Harvester ants, which feed essentially on seeds, are widespread in both Old and New World dry areas. As in most myrmicines, the two exocrine glands associated with the sting (Dufour and poison glands) are commonly involved in chemical communication during foraging or agonistic situations. The ethological role of these two abdominal glands is however highly variable amongst harvester ant species. In *Pogonomyrmex* species, the short-lived secretion of the poison gland serves as a recruitment pheromone and an orientation cue while the Dufour gland secretion produces additional orientation signals used on longer-lasting trails (Hölldobler and Wilson, 1970). On the other hand, in most but not all species of the genus *Messor* [*M. structor* (Hahn and Maschwitz, 1985); *M. bouvieri* (Jackson et al., 1991), *M. capitatus* (Grasso et al., 1998), *M. minor* and *M. wasmanni* (Grasso et al., 1999), but not *M. pergandei* (Blum, 1974)] it is the Dufour gland which is responsible for the production of the trail pheromone and which contains both recruitment and orientation substances, while the poison gland secretion only elicits a weak trail-following response.

Furthermore, harvester ants also differ in their degree of worker size polymorphism, which varies between species (Davidson, 1977) or even within the same species over its geographic range (Davidson, 1978). Worker size range has been considered as a specific adaptation to match the size distribution of available food resources (Davidson, 1977, 1978; Rissing and Pollock, 1984), to expand diet (Traniello and Beshers, 1991), to increase foraging efficiency (López and Haeger, 1999) or possibly to reduce competition (Davidson, 1977, 1978).

The adaptive significance of such size polymorphism has been widely discussed in literature, but little is known about 1) how differences in workers body size are related to differences in the development of exocrine glands involved in chemical communication and 2) how division of labour between worker size classes is coupled with a specialisation in the production of trail pheromones or defensive compounds. Our work on the highly polymorphic harvester ant *Messor barbarus* provides a first set of answers 1) by studying the relative development and the ethological function of abdominal glands associated with the sting (Dufour and poison glands) and 2) by comparing behavioural responses elicited by glandular extracts from the three worker size classes (minor, media and major).

Materials and methods

1. Rearing of colonies

The ant *Messor barbarus* is a common seed predator (Detrain and Pasteels, 2000) on Mediterranean grasslands of Southern Europe (Detrain et al., 2000). Nests can be easily detected in the field because of the clearly defined network of trunk trails. Colonies collected at Vidauban (Southeastern France) were reared in plaster nests in the laboratory. Each nest (20 × 30 × 0.3 cm) was subdivided in three interconnected sections (16 × 8 × 0.3 cm) covered by a red glass plate. Nests were kept at a room temperature of 22 ± 3 °C, a relative humidity of 60% and a constant photoperiod of 12h-light per day. Colonies were regularly fed on a diet of brown sugar solution (1 M) and mixed seeds (mainly canary grass and oats) and were supplemented twice a week with dead cockroaches (*Periplaneta americana*).

2. Morphometric measures

Sixty workers were taken from the foraging area of a *M. barbarus* colony and were killed by freezing (–12 °C) for a few minutes. Several morphological parameters were measured under a binocular microscope: head width across eyes; length of the alitrunk in profile (from the anterior edge of the pronotum to the posterior base of the propodeum); maximum length of gaster (from postpetiole insertion to gastral apex), maximum width and maximum height of gaster; maximum length and maximum width of abdominal glands associated with the sting (Dufour and poison glands). Volumes of gaster, Dufour gland and poison gland were calculated by assuming them to be ellipsoids.

3. Bioassays on Dufour and poison glands secretions

Three size classes – minor, media and major – are usually recognised among *M. barbarus* workers (Bernard, 1968; Cerdan et al., 1986; Chrétien, 1996; López and Haeger, 1999) which are arbitrarily defined according to workers body length: minors (< 5 mm); medias (5 > × < 10 mm); majors (> 10 mm).

Bioassays were carried out with glandular secretions from each of these three size classes. Two colonies of 1,500 and 2,000 workers (35% minors, 57% medias and 8% majors) were used from which 10 workers of each colony and of each size class were dissected in physiological saline (9‰). Dufour and poison glands were transferred separately to vials containing 100 µl of dichloromethane (stock solutions), were sonicated and stored at –30 °C. Three glandular extracts were tested: poison gland extract (PG), Dufour gland extract (DG) and a mixture of both glandular extracts (PG + DG). The extraction solvent (S) was used as control. Bioassays were carried out on nestmate workers and were video recorded. Media workers were chosen to test the ethological role of exocrine glands because 1) they are the numerically dominant size class in *M. barbarus* colonies and 2) they carry out most of the outside activities like foraging or defence against competitors (unpublished observations). Within each experimental series, successive tests were separated by at least 30 minutes. One day elapsed between experimental series carried out with workers coming from the same colony.

3.1 Aggressive responses

Twenty µl of the stock solution of each gland were diluted with solvent in order to reach a concentration of 2 glands/600 µl. Pieces of filter paper (5 × 5 mm) were impregnated with aliquots of 30 µl (0.1 gland equivalent) of each glandular extract (DG or PG) or with 60 µl (0.1 DG equivalent + 0.1 PG equivalent) of both glandular extracts. Tests were performed in the following order: S (30 µl), DG, PG and DG + PG extracts. For each test, the filter paper was placed in the centre of a 20 × 20 cm arena in which 20 medias had been previously introduced. A count was made of all workers approaching the paper at less than 2.5 cm during 5 min starting as soon as the paper was deposited. For those attracted workers, aggressive behaviours as mandible openings, bites to the paper and bites between nestmates were quantified. Percentages of bites between nestmates were calculated over the total number of encounters between workers. Six replications were performed with each tested glandular extract as well as with the solvent.

3.2 Trail following response

Sixty µl of the stock solution of each gland were diluted with solvent in order to reach a concentration of 6 glands/120 µl. A 10 cm diameter circle divided in 1 cm arcs was pencil-drawn on Bristol paper (350 g/m^2). Aliquots of 30 µl (1.5 gland equivalent) of each glandular secretion (PG or DG) or 60 µl (1.5 PG equivalent + 1.5 DG equivalent) of both glandular extracts were deposited with a metallic normograph pen along the circumference. Tests were performed in the following order: S (30 µl), PG, DG and PG + DG extracts. Each circular trail was presented in an experimental arena (20 × 30 cm) to 20 media workers, which had been isolated from the colony 3 h before testing. Three replications were carried out with each glandular extract as with the solvent. Within each replication, we observed 60 encounters of workers with the trail. A worker was considered as responding to the trail when following it over at least 1 cm. The number of cm followed by these responding workers was counted and averaged for each glandular extract tested.

3.3 Recruitment response inside the nest

Twenty µl of the stock solution of each gland were diluted with solvent in order to reach a concentration of 2 glands/40 µl. Filter papers (5 × 5 mm) were impregnated with aliquots of 2 µl (0.1 gland equivalent) of each glandular extract (PG or DG) or with 4 µl (0.1 PG equivalent + 0.1 DG equivalent) of both extracts. Solvent (2 µl) and glandular extracts were tested following the same order as in the previous bioassay. Test papers were introduced inside the nest through a hole (1.5 cm diameter) made in its glass cover at 4 cm from the entrance. We quantified differences between the number of workers leaving the nest during 1 minute prior to testing and the number of exiting ants during 1 min following the introduction of the test paper. Six replications were carried out with each glandular extract and with the solvent.

4. Data analysis

Normality of data was checked using the Kolmogorov-Smirnov test for goodness of fit. When data were normally distributed, the one-way ANOVA was used and, when a significant difference was found, was followed by the Tukey multiple comparison test. When data were not normally distributed, the Kruskal-Wallis test was used and followed by the Dunn's multiple comparison test. Chi-square test was used for comparison between proportions. All p-values above 0.05 were considered to be not statistically significant.

Results

1. Morphometric measures

The relation between head width and alitrunk length showed a monophasic allometric growth (Fig. 1 A). The slope of the regression line, which was greater than 1, indicated that large-sized workers had proportionally wider head capsules than smaller-sized individuals. Relations between alitrunk length and either poison or Dufour glands size (Fig. 1 B and 1 C) also showed monophasic allometric growths. Slopes of regression lines were not different (test of equality of slopes,

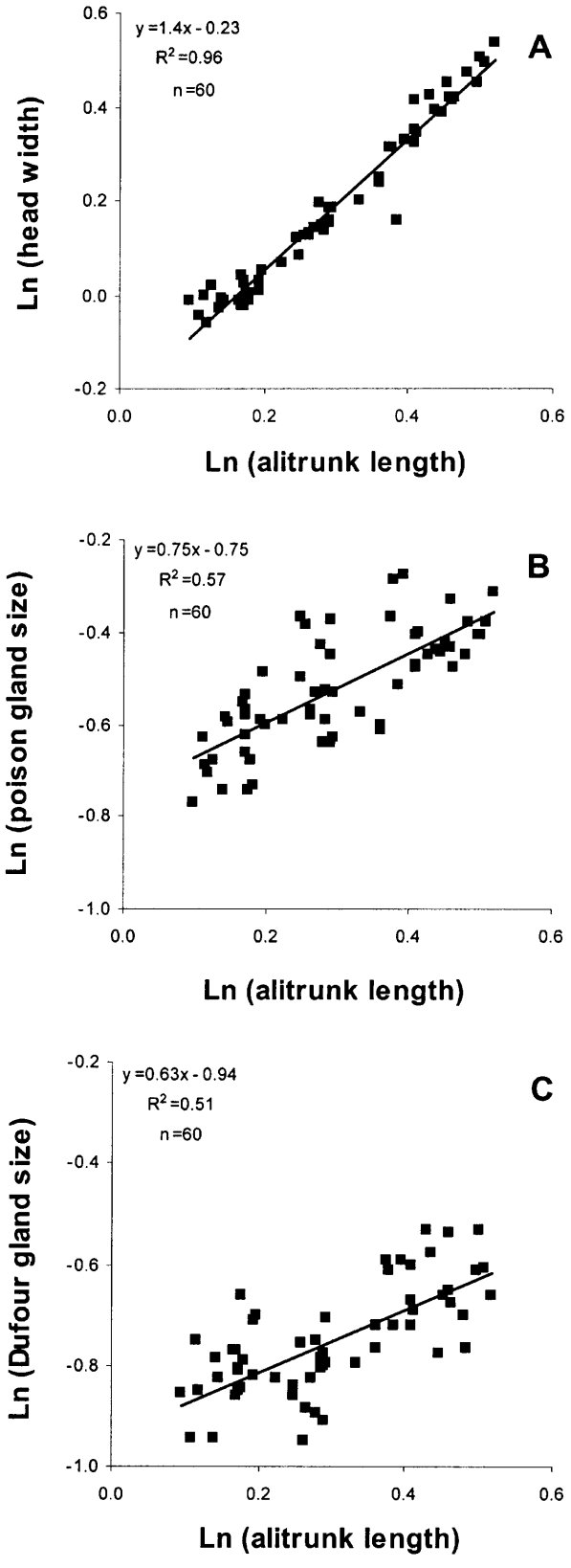

Figure 1. Relation between natural logarithms of alitrunk lengths (mm) and either head widths (mm) (A), poison glands sizes (mm) (B) or Dufour glands sizes (mm) (C). Sizes of exocrine glands are expressed as the cubic root of measured glandular volumes

$p > 0.05$) and were both lower than 1 (0.75 and 0.63, for poison and Dufour glands, respectively). Large-sized workers had thus proportionally smaller exocrine glands associated with the sting than smaller-sized individuals. This was confirmed by the relative development of abdominal glands in majors (Table 1: percentage of gasters volumes) which was similar to that observed in medias (Dunn's test, $p > 0.05$) and even significantly lower than in minors (Dunn's test, $p < 0.05$ and $p < 0.001$ for poison or Dufour glands, respectively). In terms of absolute volumes, poison and Dufour glands from majors were nevertheless larger than those of minors or medias (Table 1: Tukey test, $p < 0.001$). For a given worker size class, poison glands were on average 3.5 to 8 times larger than Dufour ones.

2. Bioassays on Dufour and poison glands secretions

2.1 Aggressive responses (Table 2)

Similar number of workers were attracted by pieces of paper impregnated with solvent (Kruskal-Wallis test, $p > 0.05$), indicating that ants showed the same activity level at the beginning of each experimental series. The Dufour gland secretion from majors as well as poison gland extracts from medias or majors attracted slightly more nestmates than the solvent (Kruskal-Wallis, $p > 0.05$). For a given glandular extract (Dufour or poison), the number of attracted workers seemed to increase with the worker size class from which this gland was extracted, probably due to the higher amount of available secretion. This trend was however not statistically significant (Kruskal-Wallis test, $p > 0.05$).

Poison gland secretions induced frequent aggressive behaviour among attracted workers. Indeed, when extracted from majors, poison glands induced mean percentages of mandible openings significantly higher than the solvent (Dunn's test, $p < 0.05$). Poison gland secretions from medias or majors also elicited significantly higher percentages of bites to paper or to encountered nestmates than the solvent (Dunn's test, $p < 0.05$). By contrast, Dufour gland secretions induced none or only slight aggressive behaviour, which were never statistically different from the solvent (Dunn's test, $p > 0.05$). No synergistic effect of the mixture of both glands was found since mean percentages of mandible openings or bites to paper were statistically similar to those induced by the poison gland alone (Dunn's test, $p > 0.05$). The mean number of bites to nestmates was even reduced, as for the mixture of both glands from media workers, which elicited a significantly lower aggressiveness than poison gland alone (Dunn's test, $p < 0.05$). The aggressive behaviour of workers in contact with the glandular mixture seemed thus essentially due to their perception of the poison gland compounds.

Aggressive responses increased with the worker size class from which glands were extracted. Around 5 times more bites were observed between nestmates when poison glands originated from medias (Dunn's test, NS, $p = 0.07$) or majors (Dunn's test, NS, $p = 0.06$) than from minor workers.

Table 1. Volumes of poison and Dufour glands from workers of each size class. The table gives absolute volumes (mean ± SD, n = 20) and percentages (mean ± SD, n = 20) of gaster volumes taken by glands. Volumes are compared using one-way ANOVA, followed by Tukey multiple comparison tests. Percentages are compared using Kruskal-Wallis tests, followed by Dunn's multiple comparison tests. Means sharing the same letter are not significantly different (level of significance $\alpha = 0.05$)

	Absolute volumes ($\times 10^{-3}$ mm^3)		% of gasters volumes	
	Poison	Dufour	Poison	Dufour
Minors glands	14 ± 8^{a}	4 ± 2^{a}	1.5 ± 0.7^{a}	0.5 ± 0.3^{a}
Medias glands	31 ± 32^{a}	4 ± 2^{a}	$1.6 \pm 1.3^{a,b}$	0.2 ± 0.1^{b}
Majors glands	69 ± 33^{b}	14 ± 6^{b}	1.1 ± 0.7^{b}	0.2 ± 0.1^{b}
Statistical tests	ANOVA $p < 0.001$	ANOVA $p < 0.001$	Kruskal-Wallis $p = 0.045$	Kruskal-Wallis $p < 0.001$

Table 2. Aggressive response of media workers to glandular extracts from each size class (0.1 gland) or to the solvent. The table gives percentages (mean ± SD, n = 6) of attracted workers performing mandible openings, bites to paper and bites to nestmates. Kruskal-Wallis tests are used and are followed, when statistical differences are found, by Dunn's multiple comparison tests. Means sharing the same letter are not significantly different (level of significance $\alpha = 0.05$)

		Number of attracted workers	% of mandible openings	% of bites to paper	% of bites to nestmates
Minors glands	Solvent	25.8 ± 8.9^{a}	5.2 ± 5.7^{a}	1.6 ± 2.8^{a}	0^{a}
	Dufour	25.5 ± 11.4^{a}	8.3 ± 6.6^{a}	3.7 ± 6^{a}	0^{a}
	Poison	23.7 ± 19.4^{a}	10.1 ± 7.3^{a}	4.1 ± 6.7^{a}	9.2 ± 17.2^{a}
	D + P	27.2 ± 8.6^{a}	18.8 ± 8^{a}	4.2 ± 5.9^{a}	19.5 ± 40^{a}
		NS $p = 0.77$	NS $p = 0.06$	NS $p = 0.91$	NS $p = 0.21$
Medias glands	Solvent	24.3 ± 11.3^{a}	3.1 ± 4.9^{a}	0.9 ± 2.2^{a}	0^{a}
	Dufour	27 ± 17.4^{a}	5.2 ± 4.1^{a}	$10.1 \pm 8.4^{a,b}$	0^{a}
	Poison	41.8 ± 12.1^{a}	7.6 ± 6^{a}	21.7 ± 8.4^{b}	49.9 ± 30.7^{b}
	D + P	29.2 ± 10.3^{a}	10.1 ± 6.5^{a}	18.5 ± 17.3^{b}	6.6 ± 10.7^{a}
		NS $p = 0.13$	NS $p = 0.19$	S $p = 0.003$	S $p < 0.001$
Majors glands	Solvent	28 ± 11.4^{a}	0^{a}	2.1 ± 4^{a}	0^{a}
	Dufour	40.8 ± 11.6^{a}	$5 \pm 7.2^{a,b}$	$9.5 \pm 5.1^{a,b}$	$18.5 \pm 40.2^{a,b}$
	Poison	45.2 ± 9.8^{a}	15.5 ± 12.4^{b}	13 ± 7.7^{b}	47.3 ± 14.1^{b}
	D + P	34.8 ± 12.4^{a}	15.1 ± 13.1^{b}	$4.8 \pm 4.3^{a,b}$	$19.8 \pm 24.7^{a,b}$
		NS $p = 0.06$	S $p = 0.01$	S $p = 0.01$	S $p = 0.01$

Similarly, while minors poison gland extract elicited 4.1% of bites to paper, 3 to 5 times more bites were observed in the presence of majors (Dunn's test, $p > 0.05$) or medias gland (Dunn's test, $p < 0.05$).

2.2 Trail following response (Table 3)

For all experimental series, workers that contacted trails laid with the solvent (36.7% to 47.8%), followed it over very short distances of 1.9 to 3.4 cm on average. A higher number of workers responded to trails drawn with poison gland secretions (χ^2 test, $p < 0.001$) but still followed them on rather short distances of around 5 cm. This weak trail-following response was often accompanied by typical alarm behaviour of workers, such as the opening of mandibles and excited running. Such alarm behaviour among workers was not due to a too high concentration of a possible component of the trail pheromone in the poison gland extracts as hypothesised by Grasso et al. (1998). Indeed, additional experiments showed that over less concentrated trails drawn with only 0.1 medias poison gland, *M. barbarus* workers followed similar short distances of 5.3 cm (± 4.7 cm, n = 71) on average. The highest response was observed for Dufour gland trails, which were followed by 76.1 to 83.9% of tested workers (χ^2 test, $p < 0.001$) over long distances (12.5 to 19.3 cm on average). This trail-following response to Dufour gland extracts was always significantly higher than those elicited by the solvent or other glandular secretions (Dunn's test,

Table 3. Trail following response of media workers to glandular extracts (1.5 glands) from each size class or to the solvent. The table gives percentages of workers responding to circular trails and numbers (mean ± SD) of cm followed. Percentages are compared using χ^2 tests. Means are compared using Kruskal-Wallis tests, followed by Dunn's multiple comparison tests. Means sharing the same letter are not significantly different (level of significance $\alpha = 0.05$)

		% of responding workers (n = 180)	Distance followed (cm)
Minors glands	Solvent	36.7[a]	2.1 ± 1.5[a] (n = 66)
	Poison	57.8[b]	5.7 ± 5.8[b] (n = 104)
	Dufour	76.1[c]	13.6 ± 17.7[c] (n = 137)
	P + D	63.3[b]	8.4 ± 11.8[b] (n = 114)
		χ^2 p = 0.0001	Kruskal-Wallis p < 0.001
Medias glands	Solvent	47.8[a]	3.4 ± 2.2[a] (n = 86)
	Poison	63.9[b]	5.3 ± 5.2[a,b] (n = 115)
	Dufour	83.9[c]	19.3 ± 20[c] (n = 151)
	P + D	67.8[b]	7.2 ± 6.6[b] (n = 122)
		χ^2 p = 0.0001	Kruskal-Wallis p < 0.001
Majors glands	Solvent	43.3[a]	1.9 ± 1.1[a] (n = 78)
	Poison	63.3[b]	5.2 ± 7.4[b] (n = 114)
	Dufour	81.7[c]	12.5 ± 17.8[c] (n = 147)
	P + D	57.2[b]	8.8 ± 13[b] (n = 103)
		χ^2 p = 0.0001	Kruskal-Wallis p < 0.001

p < 0.05). There was no synergistic effect of the mixture of both glands, since the addition of poison gland secretions even significantly weakened the trail response of workers to Dufour gland alone (Dunn's test, p < 0.05).

Trails drawn with Dufour gland extract from medias were followed over longer distances than those from minors and majors (Dunn's test, p < 0.001). We tested whether the weaker response to glandular extract from majors could be due to a repulsive effect of higher quantities of trail pheromone since majors glands were 3 times larger than those of medias. This seemed not to be the case since a 3 times diluted extract of majors Dufour glands (0.5 gland/trail) induced a similar trail-following response (11.3 ± 10.5 cm, n = 148) which was still weaker than that elicited by medias glands.

2.3 Recruitment response inside the nest (Table 4)

Dufour gland extracts from medias and majors elicited a significant recruitment of nestmates with up to 10 times more workers exiting the nest than for the solvent (Dunn's test, p < 0.05). On the other hand, poison gland secretions did not elicit nest exits significantly different from the solvent. Moreover, workers behaved quite differently: while workers in contact with Dufour gland secretions left the nest calmly, without signs of aggressiveness, ants remained near the test paper impregnated with poison gland extracts displaying opened mandibles, excited running and self-grooming. This latter behaviour could be a sign of a possible irritant effect of the poison gland secretions. The mixture of both glandular extracts had no additive effect, as it even reduced up to 5 times the recruitment response of workers to Dufour glands alone from medias or majors.

Although this trend was not statistically significant, Dufour glands extracted from minors seemed to elicit less exits from the nest than those extracted from medias or majors (Kruskal-Wallis test, p > 0.05).

Discussion

In the large subfamily Myrmicinae, the poison gland appears to be the primary source of odour trail pheromones while the Dufour gland elicits no response or simply produces additional orientation cues as in *Pogonomyrmex* harvester ants (Hölldobler and Wilson, 1970). Within the Pheidolini tribe to which belongs the genus *Messor*, the glandular source of the trail pheromone is nevertheless highly variable. While the poison gland contains the trail substance in most *Pheidole* (Hölldobler and Möglich, 1980; Ali et al., 1989) and all *Aphaenogaster* species (Hölldobler et al., 1978; Attygalle et al., 1998), the Dufour gland secretion releases trail following responses in all studied *Messor* species (Hahn and Maschwitz, 1985; Coll et al., 1987; Jackson et al., 1991; Grasso et al., 1998, 1999) excepting in *M. pergandei* (Blum, 1974). We here found that, as for *M. structor* (Hahn and

Table 4. Recruitment response to glandular extracts (0.1 gland) from workers of each size class or to the solvent. The table gives differences (mean ± SD, n = 6) between the number of workers exiting the nest before and after the introduction of the test paper in the nest. Kruskal-Wallis tests are used and are followed, when statistical differences are found, by Dunn's multiple comparison tests. Means sharing the same letter are not significantly different (level of significance $\alpha = 0.05$)

	Solvent	Poison	Dufour	P + D	Statistical tests
Minors glands	2 ± 3.9[a]	2.5 ± 1.8[a]	8.2 ± 5.5[a]	2.5 ± 3.9[a]	NS (p = 0.11)
Medias glands	1.3 ± 3.4[a]	2.7 ± 2[a,b]	13.5 ± 5.1[b]	3 ± 4.8[a]	S (p = 0.006)
Majors glands	2.2 ± 2.1[a]	2.3 ± 1.2[a]	15.3 ± 11.4[b]	3 ± 3.5[a,b]	S (p = 0.02)

Maschwitz, 1985), *M. capitatus* (Grasso et al., 1998), *M. minor* and *M. wasmanni* (Grasso et al., 1999), the Dufour gland secretion of *M. barbarus* acts both as a recruitment pheromone which induces numerous nest exits and as an orientation substance which elicits a marked trail-following response over long distances. The interspecific variability in the ethological role of the Dufour gland observed within the Pheidolini tribe could be related to the foraging ecology of ant species. The Dufour gland produces persistent orientation cues and/or recruitment pheromones only in harvester ants as *Messor* spp. which are obligatory granivorous and which forage on persistent trunk-trails. By contrast, the Dufour gland secretion does not show trail-related activity in the phylogenetically close *Aphaenogaster* and *Pheidole* species, which are omnivorous or occasional seed-eating ants (Hölldobler and Wilson, 1990). It should also be noticed that the only *Messor* species, *M. pergandei*, in which the Dufour gland is devoid of any trail-following activity, is characterised by rotating and labile trails, which regularly change direction (Rissing and Wheeler, 1976). The anatomical location of trail pheromone within the Dufour gland seems thus to be coupled with the need for ant species to produce relatively persistent chemical cues and to forage on long-lasting trails exploiting stable food resources as seed patches.

Concerning agonistic behaviour, harvester ant species differ considerably in their defensive gear. *Pogonomyrmex* ants possess a highly effective sting and a proteinaceous burning venom (Blum, 1974) while *Messor* workers have only a vestigial sting. We demonstrate that, in *M. barbarus*, the secretion of the poison gland elicits aggressiveness among nestmates. A possible role of poison gland in alarm and defence has also been suggested for five other *Messor* species though not based on quantitative data (Hahn and Maschwitz, 1985; Coll et al., 1987; Grasso et al., 1998, 1999). Besides its role in alarm and defence, the poison gland secretion may be a gustatory repellent when contacted orally or ingested by predators. Indeed, alkaloids such as anabasine or anabaseine are the main volatile components present in the poison gland of three *Messor* species [*M. ebeninus* (Coll et al., 1987), *M. bouvieri* (Jackson et al., 1991) and *M. capensis* (Brand and Mpuru, 1993)] as well as in the taxonomically related *Aphaenogaster* species (Wheeler et al., 1981; Attygalle et al., 1998). These defensive compounds could make up for the lack of effective sting in members of the Pheidolini tribe. However, this is not a general rule since, contrary to the species above mentioned, *M. barbarus* workers do not manufacture anabasine or anabaseine in their poison glands (Leclercq et al., submitted). The absence of alkaloids in the poison gland of *M. barbarus* workers could be somewhat compensated by their high degree of worker polymorphism [body size range: 3.4–12.1 mm (Chrétien, 1996)] compared to alkaloids-producing ants which are often monomorphic (*Aphaenogaster* spp.) or less polymorphic species [e.g. *M. bouvieri:* 4–8.5 mm (Cerda et al., 1998)]. The existence of large-sized workers might be advantageous in aggressive encounters, though no data are available on whether body size of *M. barbarus* workers influence their fighting performances.

Where polymorphism is associated with a caste-based polyethism, a chemical and/or morphological specialisation of exocrine glands can be found between castes. This has been demonstrated in strictly dimorphic ants like *Pheidole* (Law et al., 1965; Hölldobler and Möglich, 1980; Detrain and Pasteels, 1987; Detrain et al., 1987) and *Camponotus* species (Ali et al., 1988) or in highly polymorphic ants with multiphasic allometry like leaf-cutting ants (Moser and Blum, 1963; Wilson, 1980). Information is however lacking for ant species characterised by a more primitive "polymorphism" sensu Wilson (1953). In this respect, *M. barbarus* provides a good example since it is characterised by a monophasic allometry while showing a wide range of worker body sizes (3.4-12.1 mm) larger than in other species such as *M. bouvieri* or *M. sanctus* [4–8.5 mm (Bernard, 1968; Cerda et al., 1998)]. Regarding the glandular source of the trail pheromone, Dufour glands of *M. barbarus* workers increase monophasically with worker body size though at a slightly lower rate. The intensity of the trail-following response is however not related to the volume of the Dufour gland. Indeed, the highest response elicited by glands from media workers reflects the task specialisation of this size class in most of foraging activities and trail recruitment (unpublished observations on *M. barbarus*). Concerning defensive behaviour, *M. barbarus* poison gland also grows monophasically with worker body size. The higher aggressive responses to poison glands from medium and larger-sized workers can be explained by the increased glandular volume and thus the larger amount of available defensive compounds. As for *M. pergandei* in which fighting tasks were not related to worker body size (Rissing, 1987), our results on *M. barbarus* do not evidence any morphological or chemical specialisation of exocrine glands of larger workers to nest defence. Further investigations are however needed on how each worker size classes participate in *M. barbarus* defence.

Acknowledgments

We warmly thank J.M. Pasteels and J.C. de Biseau for their critical reading of the manuscript. We thank C. Collingwood for the identification of *M. barbarus* specimens. This study was supported by the Belgian Fund for Joint Basic Research (grants n° 2451393 and 2451001). The Van Buuren Foundation financially supported A. Heredia. C. Detrain is research associate from the Belgian National Fund for Scientific Research.

References

Ali, M.F., J.P.J. Billen, B.D. Jackson and E.D. Morgan, 1988. Secretion of the Dufour gland of two African desert ants, *Camponotus aegyptiacus* and *Cataglyphis savignyi*. *Biochem. Syst. Ecol. 16*: 647–654.

Ali, M.F., J.P.J. Billen, B.D. Jackson and E.D. Morgan, 1989. The Dufour gland contents of three species of Euro-African *Messor* ants and a comparison with those of North-American *Pogonomyrmex*. *Biochem. Sys. Ecol. 17*: 469–477.

Attygalle, A.B., F. Kern, Q. Huang and J. Meinwald, 1998. Trail pheromone of the myrmicine ant *Aphaenogaster rudis* (Hymenoptera: Formicidae). *Naturwissenschaften 85*: 38–41.

Bernard, F., 1968. *Les fourmis (Hymenoptera: Formicidae) d'Europe occidentale et septentrionale*. Masson et Cie, Paris. 411 pp.

Blum, M.S., 1974. Myrmicine trail pheromones: specificity, source and significance. *J. N.Y. Entomol. Soc. 82*: 141–147.

Brand, J.M. and S.P. Mpuru, 1993. Dufour gland and poison gland chemistry of the myrmicine ant, *Messor capensis* (Mayr). *J. Chem. Ecol. 19*: 1315–1321.

Cerda, X., J. Retana and S. Cros, 1998. Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Funct. Ecol. 12*: 45–55.

Cerdan, P., L. Borel, J. Palluel and G. Delye, 1986. Les fourmis moissonneuses et la végétation de la Crau (Bouches-du-Rhone). *Ecol. Medit. 12*: 15–23.

Chrétien, L., 1996. Organisation spatiale du matériel provenant de l'excavation du nid chez *Messor barbarus* et des cadavres d'ouvrières chez *Lasius niger* (Hymenoptera: Formicidae). Ph.D. diss., Université Libre de Bruxelles, Bruxelles, 119 pp.

Coll, M., A. Hefetz and H.A. Lloyd, 1987. Adnexal glands chemistry of *Messor ebeninus* Forel (Formicidae: Myrmicinae). *Z. Naturforsch. 42c*: 1027–1029.

Davidson, D.W., 1977. Species diversity and community organization in desert seed-eating ants. *Ecology 58*: 711–724.

Davidson, D.W., 1978. Size variability in the worker caste of a social insect (*Veromessor pergandei* Mayr) as a function of the competitive environment. *Am. Nat. 112*: 523–532.

Detrain, C. and J.M. Pasteels, 1987. Morphological and biochemical differences in the abdominal glands of *Pheidole pallidula* (Myrmicinae). In*: Chemistry and Biology of Social Insects* (J. Eder and H. Rembold, Eds.), Peperny, München. pp. 447–448.

Detrain, C. and J.M. Pasteels, 2000. Seed preferences of the harvester ant *Messor barbarus* in a Mediterranean mosaic Grassland (Hymenoptera: Formicidae). *Sociobiology 35*: 35–48.

Detrain, C., J.M. Pasteels, J.C. Braekman and D. Daloze, 1987. (Z, E)-α-farnesene, main constituent of the hypertrophied Dufour gland of the major workers of *Pheidole pallidula* (Formicidae). *Experientia 43*: 345–346.

Detrain, C., O. Tasse, M. Versaen and J.M. Pasteels, 2000. A field assessment of optimal foraging in ants: trail patterns and seed retrieval by the European harvester ant *Messor barbarus*. *Insectes soc. 47*: 56–62.

Grasso, D.A., A. Mori and F. Le Moli, 1998. Chemical communication during foraging in the harvesting ant *Messor capitatus* (Hymenoptera, Formicidae). *Insectes soc. 45*: 85–96.

Grasso, D.A., A. Mori and F. Le Moli, 1999. Recruitment and trail communication in two species of *Messor* ants (Hymenoptera, Formicidae). *Ital. J. Zool. 66*: 373–378.

Hahn, M. and U. Maschwitz, 1985. Foraging strategies and recruitment behaviour in the European harvester ant *Messor rufitarsis* (F.). *Oecologia 68*: 45–51.

Hölldobler, B. and M. Möglich, 1980. The foraging system of *Pheidole militicida* (Hymenoptera: Formicidae). *Insectes soc. 27*: 237–264.

Hölldobler, B., R.C. Stanton and H. Markl, 1978. Recruitment and food-retrieving behavior in *Novomessor* (Formicidae, Hymenoptera) I. Chemical signals. *Behav. Ecol. Sociobiol. 4*: 163–181.

Hölldobler, B. and E.O. Wilson, 1970. Recruitment trails in the harvester ant *Pogonomyrmex badius*. *Psyche 77*: 385–399.

Hölldobler, B. and E.O. Wilson, 1990. *The Ants*. The Belknap Press of Harvard University Press, Cambridge, Mass., 732 pp.

Jackson, B.D., P.J. Wright and E.D. Morgan, 1991. Chemistry and trail following of a harvester ant. In: *Insect Chemical Ecology* (I. Hrdy, Ed.), Academia Prague and SPB Acad. Publ., The Hague. pp. 109–112.

Law, J.H., E.O. Wilson and J.A. Mc Closkey, 1965. Biochemical polymorphism in ants. *Science 149*: 544–546.

López, J.R. and J.F. Haeger, 1999. Sequential co-operative load transport in the seed-harvesting ant *Messor barbarus*. *Insectes soc. 46*: 1–7.

Moser, J.C. and M.S. Blum, 1963. Trail marking substance of the Texas leaf-cutting ant: source and potency. *Science 140*: 1228.

Rissing, S.W., 1987. Annual cycles in worker size of the seed-harvester ant *Veromessor pergandei* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol. 20*: 117–124.

Rissing, S.W. and G.B. Pollock, 1984. Worker size variability and foraging efficiency in *Veromessor pergandei* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol. 15*: 121–126.

Rissing, S.W. and J. Wheeler, 1976. Foraging responses of *Veromessor pergandei* to changes in seed production. *Pan-Pacific Entomol. 52*: 63–72.

Traniello, J.F.A. and S.N. Beshers, 1991. Polymorphism and size-pairing in the harvester ant *Pogonomyrmex badius*: a test of the ecological release hypothesis. *Insectes soc. 38*: 121–127.

Wheeler, J.W., O. Olubajo, C.B. Storm and R.M. Duffield, 1981. Anabaseine: venom alkaloid of *Aphaenogaster* ants. *Science 211*: 1051–1052.

Wilson, E.O., 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*) I. The overall pattern in *A. sexdens*. *Behav. Ecol. Sociobiol. 7*: 143–156.

Wilson, E.O., 1953. The origin and evolution of polymorphisms in ants. *Q. Rev. Biol. 28*: 136–156.