The Journal of Experimental Biology 211, 2442-2449
Published by The Company of Biologists 2008
doi:10.1242/jeb.017749

Caste differences in venom volatiles and their effect on alarm behaviour in the paper wasp *Polistes dominulus* (Christ)

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Accepted 12 May 2008

SUMMARY

Foundresses and workers of *Polistes* paper wasps show slight morphological and physiological differences. However, after the emergence of the workers, the castes can be readily discriminated by their behaviour: the dominant foundress is the principal egg-layer, whereas workers perform different tasks linked to colony development. Previous studies have demonstrated in this genus that defence of the colony by the workers is more effectively carried out by a collective response elicited by venom volatiles used as alarm pheromones. In the present study, gas chromatography–mass spectrometry analyses of the venom volatiles of foundresses and workers of *Polistes dominulus* (Christ) show predominantly quantitative differences. Spiroacetals, mainly (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, are significantly higher in the venom volatiles fraction of workers, whereas the amount of *N*-(3-methylbutyl)acetamide is almost double in foundresses. On the basis of the chemical results, behavioural assays were performed on fifteen field colonies to test the alarm response of the resident wasps to venom extracts from foundresses and workers. Our behavioural results suggest that worker venom has a stronger alarm effect on the colonies than that of the foundresses, which seems unable to elicit the complete alarm response ending with a final attack and sting. The venom volatiles of *P. dominulus* workers serve mainly to alarm the colony whilst those of foundresses may also be linked to additional functions related to conspecific interactions.

Key words: caste, paper wasp, *Polistes dominulus*, defence, alarm pheromone, venom volatile.

INTRODUCTION

In insect societies the reproductive division of labour plays an important role in the maintenance of the colony structure: workers cooperate in taking care of the colony while queens, usually morphologically distinguishable, maintain a reproductive role. In some social insects with large colonies, such as some species of bees, termites and ants, the worker caste is highly differentiated (polymorphisms) to perform specific tasks, such as colony defence, and there are elaborate communication systems to effectively coordinate the workers behaviour (Wilson, 1971).

Polistes paper wasps are a keystone genus for testing sociobiological theories, as there is little size difference between foundresses and workers [*P. fuscatus* (West-Eberhard, 1969); *P. dominulus* (Turillazzi, 1980); *P. metricus* (Haggard and Gamboa, 1980); *P. gallicus* (Dani, 1994)], and a reproductive division of labour is regulated by behavioural interactions among individuals that have (for most of their lives) an equivalent reproductive potential (Reeve, 1991). Indeed, an experimental study (Solis and Strassmann, 1990) on *P. exclamans*, demonstrated a caste plasticity in adult females that was dependent on brood availability in the nest.

There is some physiological evidence suggesting the existence of pre-imaginal mechanisms that divide foundresses and workers in the genus *Polistes* (see O'Donnell, 1998): females emerging in late summer (presumably future foundresses) possess a storage protein of the hexamerine family (Hunt et al., 2003) and also fat bodies (Pardi, 1939; Eickwort, 1969; Litte, 1977; Strassmann et al., 1984; Grechka, 1986). In addition, foundresses and workers of *P. dominulus* differ in their proportions of cuticular hydrocarbons (CHC) (Bonavita-Cougourdan et al., 1991; Sledge et al., 2001) and peptides (Dapporto et al., 2008). Dapporto and coworkers (Dapporto et al., 2005) were able to show that several workers in orphaned colonies of *Polistes dominulus* (Christ) developed ovaries and produced cuticular signatures characteristic of dominant foundresses, but the peptides and hexamerine proteins remained caste specific. However, even if there is still disagreement about the origin of the worker–foundress difference, what clearly discriminates the two castes is a strong behavioural separation in terms of the roles performed at the nest. Foundresses are responsible for egg production while workers perform nest-building, brood care, foraging activities and colony defence. Colony defence against predators is a risky duty, so foundresses will respond aggressively only at the beginning of the colony cycle, leaving this task to the workers after their emergence (Judd, 1998; Judd, 2000). Thus the need to communicate alarm within the colony should change depending on role and stage of the colony cycle. It seems likely that, at the beginning of the colony cycle with just one or a few foundresses on the nest, there is no need for chemical communication by means of alarm pheromones. Conversely, as the season progresses and the number of workers increases, they need to cooperate actively and rapidly by alerting their nestmates by means of chemical channels.

Many studies on vespine and polistine wasps have shown that volatile components of the venom function as alarm pheromones recruiting nestmates and eliciting attack towards the source of disturbance (Dani et al., 2000; Fortunato et al., 2004; Ishay et al., 1965; Jeanne, 1981; Jeanne, 1982; Kojima, 1994; Landolt and

Heath, 1987; Landolt et al., 1995; Maschwitz, 1964; Maschwitz, 1984; Maschwitz and Hanel, 1988; Moritz and Bürgin, 1987; Ono et al., 2003; Post et al., 1984; Saslavasky et al., 1973; Sledge et al., 1999; Veith et al., 1984). Our group (Bruschini et al., 2006a) has shown that, in *P. dominulus*, such venom volatiles are capable of eliciting an alarm response once the colony was stimulated both visually (moving targets) and chemically (worker venom extracts).

In the present study, in consideration of the different defensive role performed by foundresses on the colony, we analysed the composition of the venom volatiles of *P. dominulus* foundresses and workers, as well as the size of their venom reservoirs. In addition, we performed behavioural assays to investigate the effectiveness of foundress venom extracts as a stimulus for alarm within the colony.

MATERIALS AND METHODS

Studied species

P. dominulus (Christ) is the most common species of the genus *Polistes* in Mediterranean countries where it usually nests in lowlands or hills rarely reaching an altitude greater than 1000 m a.s.l. (Borsato, 1992). The species prefers to build unenveloped nests in artificial, sheltered structures (Pardi, 1980) with a mean nest size in mature colonies of about 200 cells. The colony cycle of *P. dominulus* begins in springtime (March–April) when one or more inseminated females emerge from the hibernacula and found a nest ('pre-emergence phase'). At the end of May, the 'worker phase' starts with the emergence of workers and that phase ends in late summer (July–August) with the emergence of reproductives (Pardi, 1996).

To unambiguously assign a caste to each wasp used, all the foundresses were collected (either on nests or in flight) before the emergence of workers, and the workers were collected at the very beginning of the worker phase on colonies where the foundresses had been previously marked.

Venom reservoir size

The sting apparatus (sting, venom glands and venom reservoir) from 31 foundresses and 35 workers collected in Trespiano (near Florence, Italy) was dissected under a microscope. Photographs of the sting apparatus of each female were taken using a digital camera (Olympus Camedia c-2500L, Japan) that was attached to the microscope in manual focus with 12× magnification. The area of the venom reservoir of each wasp was calculated in pixels using the program ImageJ 1.29x (Wayne Rasband, National Institute of Health, USA) and standardized by dividing the reservoir by the head area of the same wasp calculated in the same way. In the literature, head size is reported to provide a good estimate of total body size (Eickwort, 1969).

The morphological data obtained were tested for normality and equality of variance. Then a parametric Student's *t*-test (for independent samples) was used to investigate the differences in the venom reservoir dimension between workers and foundresses.

All the statistical analyses were performed using the program SPSS Smart viewer 13 (Field, 2005).

Venom volatiles analysis

A preliminary chemical analysis of the volatile components of the venom was conducted on 17 specimens, 10 workers (w.1) and 7 foundresses (f.1), collected from different nests in various Italian localities. We repeated the venom analysis on a sample of 10 workers (w.2) and 10 foundresses (f.2) that was homogeneous for locality of collection. The wasps were gathered from a restricted area of less than 1 km^2 (Trespiano) near Florence (Italy). The venom was collected directly from the sting with the aid of a glass capillary under a stereomicroscope. The venom from each wasp was stored in a 250 µl glass conical insert placed in a 2 ml glass vial, and kept at –20°C until the analysis. Each venom sample was subsequently analysed by gas chromatography–mass spectrometry (GC-MS) and its components were identified (see Bruschini et al., 2006b).

The peak areas of the venom gas-chromatogram of each wasp were transformed into percentages, which were analysed by stepwise discriminant analysis (DA). All components present in less than 75% of samples or in less than 75% of the individuals belonging to the same group were excluded from the analysis to reduce the number of variables for multivariate analysis. DA was used to determine whether the predefined groups of wasps (workers and foundresses) could be discriminated on the basis of their profiles, and which components were important for that discrimination. The significance of Wilks' lambda and the percentage of correct assignments were used to estimate the validity of the discriminant function. We also compared, with non-paramentric Mann–Whitney tests, the mean relative percentages of specific components in foundresses and workers. As shown in Table 1, for spiroacetals we summed components 4, 6, 7, 8, 10; for *N*-(3-methylbutyl)acetamide we used component 5; for acetates we summed components 3, 13, 29, 30, 34, 35, 36, 37, 38, 41, 42.

Behavioural experiments

Venom extract preparation

Fifty foundresses and fifty workers were killed by freezing immediately after collection and kept at –20°C until extraction. The pure venom from the sting was collected with the aid of a glass capillary under a stereomicroscope (around 0.4 µl per wasp). After collection of approximately 10 µl of pure venom, it was placed in separate vials, each containing 100 µl of dichloromethane (CH_2Cl_2). The venom extracts were stored in a freezer at –20°C until the experiments were performed. For each field experiment, 12 µl of the venom extract (corresponding to approximately three venom glands) were used.

Field bioassays

Experiments were conducted on 15 *P. dominulus* nests in the worker phase, that had been naturally founded inside open-ended plastic tubes (shelters) mounted to protect saplings in fields in different localities in the vicinity of Florence (seven nests in San Donato, four nests in Cascine del Riccio and four nests in Montevarchi; mean number of wasps on the nest ± s.d., 11.7±3.4, 14.2±6.4, 11.5±5.4, respectively). All the experiments were carried out in July 2006 during the hottest hours of the day, usually from 11:00 h. to 16:00 h, when the wasps were more active. To test if the venom of foundresses elicited alarm and consequently attack in a similar manner to the venom of workers (Bruschini et al., 2006a), a visual stimulus and a chemical one were presented simultaneously to each colony. A few days before the experiments we made two windows in the plastic shelters. A main window (6×8 cm) was cut in the side of the shelter facing the nest in order to present the visual stimulus in front of the comb. A second smaller window (0.5×1 cm), for the presentation of the chemical stimulus, was made in the side of the shelter, behind the nest close to the pedicel. The visual stimulus, oscillated rhythmically by a first experimenter for 1 min at a distance of 30 cm from the main window, consisted of a round target made of black porous neoprene attached to the top of a plastic rod (1.5 m). The target was always removed and changed before any new experiment. The wasps inside the shelter could see the black

Table 1. Mean percentages (± s.e.m.) of the 42 volatile components found in the venom of 20 *P. dominulus* workers (w.1+ w.2) and 17 *P. dominulus* foundresses (f.1+ f.2)

N	Compound	M_r	Worker (*N*=20)	Foundress (*N*=17)
1	Unidentified		0.27±0.15	0.00±0.00
2	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	150	0.77±0.26	0.65±0.23
3	**6-Methyl-5-hepten-2-yl acetate**	170	2.17±0.58	1.72±0.40
4	**(*E,E*)-2,8-Dimethyl-1,7-dioxaspiro(5.5)undecane**	184	6.49±1.60	1.55±0.48
5	***N*-(3-Methylbutyl)acetamide**	129	17.49±2.40	31.74±4.05
6	**2-Ethyl-7-methyl-1,6-dioxaspiro(4.5)decane**	184	1.30±0.24	0.53±0.31
7	2-Ethyl-7-methyl-1,6-dioxaspiro(4.5)decane	184	0.38±0.15	0.00±0.00
8	2-Methyl-7-ethyl-1,6-diaxospiro(4.5)decane	184	0.02±0.02	0.00±0.00
9	Unidentified		0.13±0.08	0.03±0.02
10	(*E,Z*)-2,8-Dimethyl-1,7-dioxaspiro(5.5)undecane	184	0.17±0.07	0.01±0.01
11	*N*-(3-Methylbutyl)propanamide	143	0.24±0.20	0.00±0.00
12	Unidentified		0.24±0.06	0.16±0.06
13	**2-Nonanyl acetate**	186	6.44±1.47	2.94±0.38
14	Spiroacetal		0.04±0.03	0.00±0.00
15	Undecen-2-ol	170	0.16±0.06	0.06±0.03
16	2-Undecanone	170	0.12±0.06	0.17±0.07
17	**2-Undecanol**	172	1.96±0.37	1.77±0.29
18	Unidentified		0.10±0.06	0.00±0.00
19	Unidentified		0.08±0.03	0.01±0.01
20	Unidentified		0.00±0.00	0.01±0.01
21	Unidentified		0.05±0.02	0.00±0.00
22	Unidentified		0.06±0.02	0.06±0.03
23	Unidentified		0.02±0.02	0.03±0.02
24	**Unidentified**		0.08±0.03	0.27±0.07
25	**Unidentified**		0.15±0.04	0.17±0.04
26	Unidentified		0.11±0.04	0.16±0.05
27	**Unidentified**		0.32±0.04	0.58±0.09
28	Unidentified		0.10±0.03	0.06±0.02
29	**2-Undecenyl acetate**	212	10.92±1.10	6.33±0.60
30	**2-Undecanyl acetate**	214	19.04±2.37	19.46±1.46
31	**6,10-Dimethyl-*(E)*-5,9-undecadien-2-one (geranyl acetone)**	194	3.25±0.47	3.54±0.72
32	Unidentified		0.08±0.04	0.05±0.02
33	Unidentified		0.12±0.05	0.06±0.02
34	**6,10-Dimethyl-(*Z*)-5,9-undecadien-2-yl acetate [(*Z*)-5-tangerinol]**	238	0.54±0.13	1.46±0.13
35	**6,10-Dimethyl-(*E*)-5,9-undecadien-2-yl acetate [(*E*)-5-tangerinol]**	238	12.79±1.14	21.69±3.01
36	**2-Tridecenyl acetate isomer A**	240	1.35±0.20	0.60±0.08
37	**2-Tridecenyl acetate isomer B**	240	0.85±0.10	0.46±0.05
38	**2-Tridecanyl acetate**	242	1.52±0.20	0.78±0.10
39	Unidentified		0.37±0.21	0.03±0.02
40	Unidentified		0.10±0.08	0.33±0.08
41	**2-Pentadecanyl acetate**	270	0.28±0.05	0.10±0.03
42	**3,7,11-Trimethyl-(*E*)6,(*E*)10-dodecatrien-2-yl acetate [(*E*,*E*)-farnesyl acetate]**	264	9.36±2.31	2.42±0.52

M_r, relative molecular masses of the compounds identified. Following established criteria (see Materials and methods), the 19 compounds in bold were included in the discriminant analysis performed on all workers (*N*=20) *versus* all foundresses (*N*=17).

target oscillating in front of the main window, but could not see the experimenters, who were hidden by the plastic shelter. The chemical stimulus was applied on a 1 cm^2 piece of filter paper held with long forceps by a second experimenter. The filter paper was introduced carefully, in order not to disturb the wasps, through the small window to a distance of less than 1 cm from the nest (see Bruschini et al., 2006a). Both windows were opened a few hours before performing the experiments in order to allow the colony to return to an undisturbed state. After the experiment, the main and the small windows were closed to avoid predation.

Each colony was tested with three different chemical stimuli: 12 µl of worker venom extracts, 12 µl of foundress venom extracts and 12 µl of the pure solvent (CH_2Cl_2) as a control. The interval between trials on the same colony was 2 h and the three chemical stimuli were presented to each colony in a random order to avoid any treatment position effect. The experiments were recorded using a video camera that was hidden from the wasps. The video recording was scored by one observer who was blind to the treatments employed. The number of wasps leaving the plastic shelter *via* the main window (leaving the nest; LN), the number of wasps landing on the target (LT) and the number of wasps bending the abdomen in the attempt to sting the target (BA) were recorded. The number of stings (S) was counted directly on the porous black target because drops of venom, released during the stinging, were visible. All the values were corrected by the number of wasps present on the nest at the beginning of each experiment.

The data for the four behaviours recorded (LN, LT, BA and S) under the three treatments (control, foundress venom extract and worker venom extract) were analysed with the Friedman non-parametric test for multiple comparisons of paired data. *Post-hoc* tests (Wilcoxon non-parametric tests using the Monte Carlo method) were used to assess if, and where, a significant difference existed between pairs of treatments with a P value of less than α/number of comparisons (0.05/3=0.0167) considered significant.

RESULTS

Venom reservoir size

The comparison of the mean (± s.d) venom reservoir area (in pixels) of foundresses (N=31, 8877.15±1463.82) and workers (N=35, 8594.98±1567.59) did not show any significant differences (t=0.753, P=0.454). However, the comparison of the venom reservoir areas of foundresses (0.026±0.004) and workers (0.040±0.007) normalised against the head area showed a significant difference, with the reservoir of the foundresses significantly smaller than that of the workers (t=–9.182, P<0.001).

Venom volatiles composition

Table 1 shows the 42 volatile components found in the venom of 20 workers (w.1+ w.2) and 17 foundresses (f.1+ f.2). Although several of the minor components are still to be identified, both quantitative and qualitative differences between the venom volatiles of the two castes can be seen. The spiroacetal, (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, is approximately four times more abundant in workers than in foundresses and there is twice as much *N*-(3-methylbutyl)acetamide in foundresses than in workers. In fact, as seen in Fig. 1, the venom volatiles fraction of workers (N=20) showed a higher proportion of spiroacetals (Mann–Whitney test, U=161.500, P=0.002) but a lower proportion of *N*-(3-methylbutyl)acetamide (Mann–Whitney test, U=79.000, P=0.005) than the venom volatiles fraction of foundresses (N=17). However, the relative proportion of acetates was not different in the venom volatile fraction of foundresses and workers (Mann–Whitney test, U=124.000, P=0.167; Fig. 1). These differences can be seen in the two typical chromatograms from single specimens of workers and foundresses shown in Fig. 2.

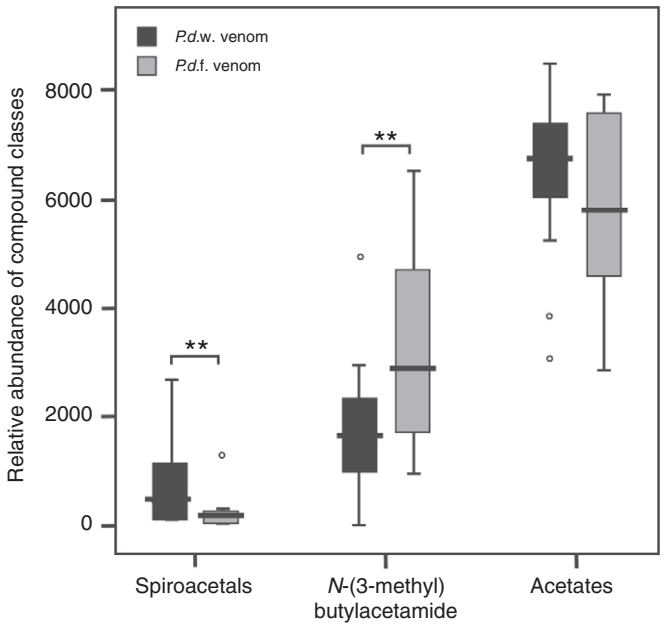

Fig. 1. Comparison of the mean relative percentages of specific groups of compounds [for spiroacetals we summed components 4, 6, 7, 8, 10; for *N*-(3-methylbutyl)acetamide, component 5; for acetates we summed components 3, 13, 29, 30, 34, 35, 36, 37, 38, 41, 42 as shown in Table 1] in the venom volatiles fraction of *P. dominulus* foundresses (N=17) and workers (N=20). **P≤0.005. Open circles indicate outliers. Box-plots show the 75th and 25th percentiles as the box, the median as the line in the box, and the extremes as the vertical lines.

DA performed on the first collection of w.1 (N=10) and w.2 (N=7) correctly assigned 100% of individuals to their original groups (Function 1, explained variance=100%; Wilks' lambda=0.112, χ^2=29.504, d.f.=3, P<0.001). Among the 24 components entered in the analysis (see Materials and methods for DA component selection), three were used to discriminate the two castes: 2-methyl-7-ethyl-1,6-diaxospiro(4.5)decane, unidentified component 33, and 2-tridecenyl acetate isomer A.

Even DA performed on the samples homogeneous for locality (10 w.2 and 10 f.2 from Trespiano), performed on 21 selected components, was able to discriminate fully all the individuals belonging to the same group (100% discrimination). (*E*)-4,8-dimethyl-1,3,7-nonatriene, *N*-(3-methylbutyl)acetamide, geranyl acetone, (*Z*)-5-tangerinol, 2-tridecanyl acetate were used in the analysis to generate Function 1 (explained variance=100%; Wilks' lambda=0.028, χ^2=55.371, d.f.=5, P<0.001).

Finally, DA performed on all the 20 workers (w.1+w.2) and 17 foundresses (f.1+f.2) using 19 out of 42 components, showed that they are, once again, fully distinguishable on the basis of their venom volatiles, correctly assigning 100% of individual to their original groups (Fig. 3). (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, unidentified 24 and 25, (*Z*)-5-tangerinol and (*E*,*E*)-farnesyl acetate were used in the DA to generate Function 1 (explained variance=100%; Wilks' lambda=0.206, χ^2=51.280, d.f.=5, P<0.001).

Field bioassays

The Friedman non-parametric test for multiple comparisons of paired data revealed a significant difference among the three treatments tested (foundresses venom extract, workers venom extract and control) in terms of all the four behaviours that were analysed (LN, χ^2=19.356, P<0.001; LT, χ^2=12.704, P=0.002; BA, χ^2=10.478, P=0.005; S, χ^2=15.209, P<0.001).

Post-hoc tests (with a P value less than α/number of comparisons 0.05/3=0.0167 considered as significant) indicate that the number of wasps leaving the nest after the presentation of both worker and foundress venom extracts is always higher than after the presentation of the solvent only (control) (Z=–3.408, P<0.001 and Z=–3.124, P=0.001 respectively), whilst there was no significant difference in the number of wasps leaving the nest when comparing worker with foundress venom extract (Z=–1.726, P=0.049; Fig. 4).

The number of wasps landing on the target and the number of wasps bending the abdomen in an attempt to sting were significantly higher after presentation of worker venom extract compared with the control (LT, Z=–2.542, P=0.005 and BA, Z=–2.353, P=0.009), whilst there was no difference in the two behaviours when comparing the two venom treatments (LT, Z=–1.335, P=0.095 and BA, Z=–1.363, P=0.098, respectively) or when comparing foundress venom extract with the control (LT, Z=–1.784, P=0.042 and BA, Z=–1.680, P=0.056; Fig. 4). Finally, the number of wasps stinging the target was significantly higher after presentation of worker venom extract than both foundress venom extract and the control (Z=–2622, P=0.003 and Z=–3062, P<0.001, respectively), but there was no difference in the number of wasps stinging the target when foundress venom extract was compared with the control (Z=–2.032, P=0.031; Fig. 4).

Moreover, failure to induce stinging was recorded for ten out of the 15 colonies tested when they were presented with foundress venom extract but for only three colonies when they were presented with worker venom extract (*G*-test with Williams correction, G=6.608, P<0.02).

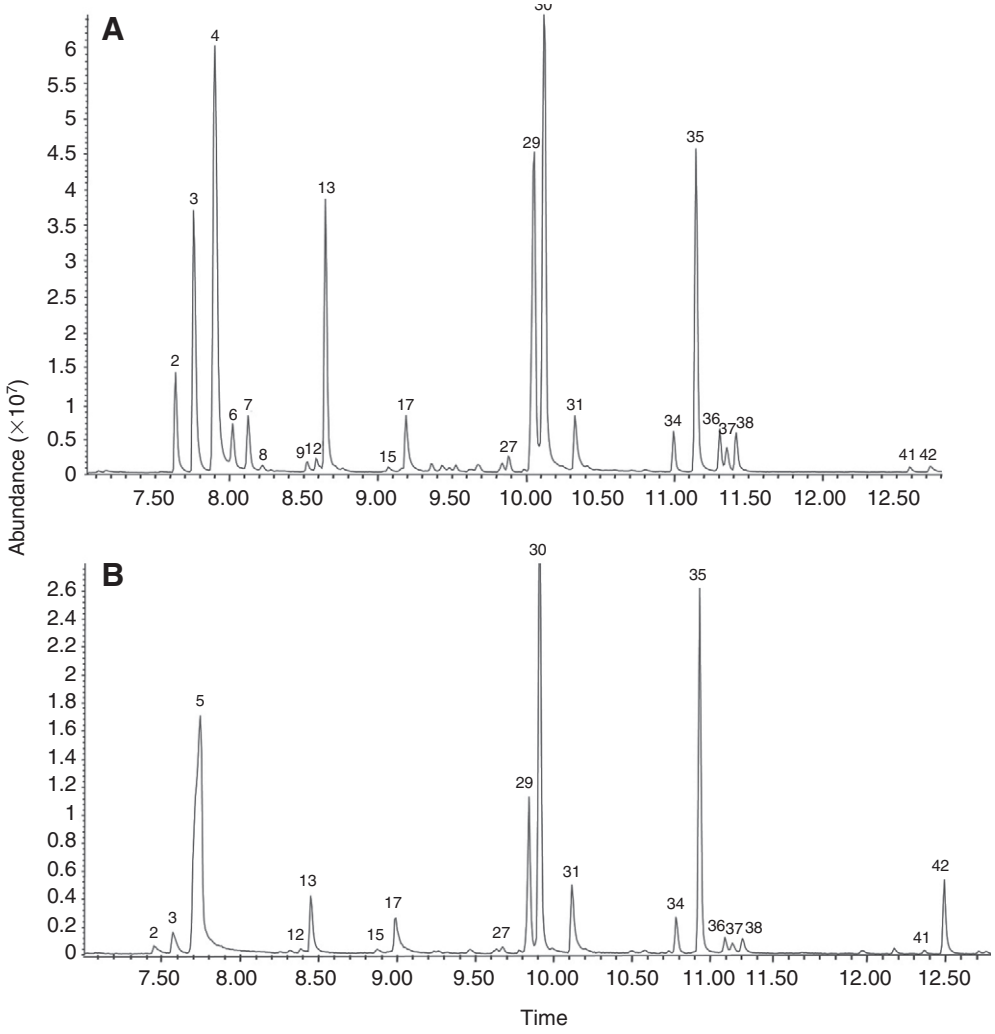

Fig. 2. Chromatograms of the venom volatiles from a single specimen of (A) a *P. dominulus* worker and (B) a *P. dominulus* foundress. The peak numbers indicate components as shown in Table 1.

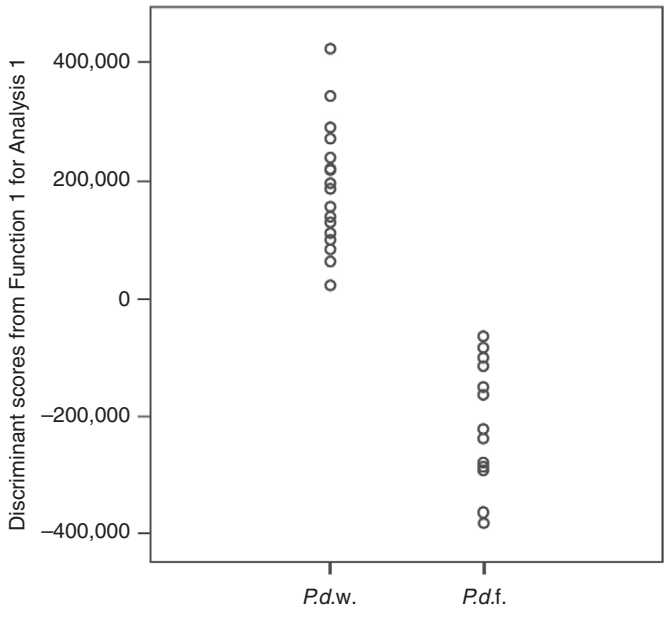

Fig. 3. Stepwise discriminant analysis (DA) separating 20 *P. dominulus* workers (w.1+w.2) and 17 *P. dominulus* foundresses (f.1+f.2) on the basis of the venom volatiles. DA correctly discriminated 100% of the females belonging to the two castes.

DISCUSSION

The morphometric analysis shows that *Polistes dominulus* workers possess a larger venom reservoir than foundresses in relation to the total body size. This result suggests that workers, notwithstanding their smaller body sizes, 'invest' more in the sting apparatus than do foundresses. However, our data do not allow us to determine if this differential investment takes place during development or after emergence. However, this size difference is in line with the different defensive roles that foundresses and workers play in the colony. The similarity of the non-standardized venom reservoir areas in foundresses and workers could be explained by the need, independent of caste and role, to actively defend themselves and the colony, through attacking and stinging any intruders when other warning behaviours have not been a sufficient deterrent. The proteic component (Pantera et al., 2003) is probably the major part of the reservoir content and it is important in both foundresses and workers.

The venom volatile fraction of *P. dominulus* workers is known to play a communicative role by inducing alarm behaviour in nestmates (Bruschini et al., 2006a). However, the chemical analysis of venom volatiles showed mainly quantitative differences between *P. dominulus* workers and foundresses, in line with the different roles played by the two castes in colony defence. DA showed that *P. dominulus* workers can be fully distinguished from *P. dominulus* foundresses on the basis of the profiles of their venom volatiles. Even foundresses and workers from different sets, i.e. in terms of

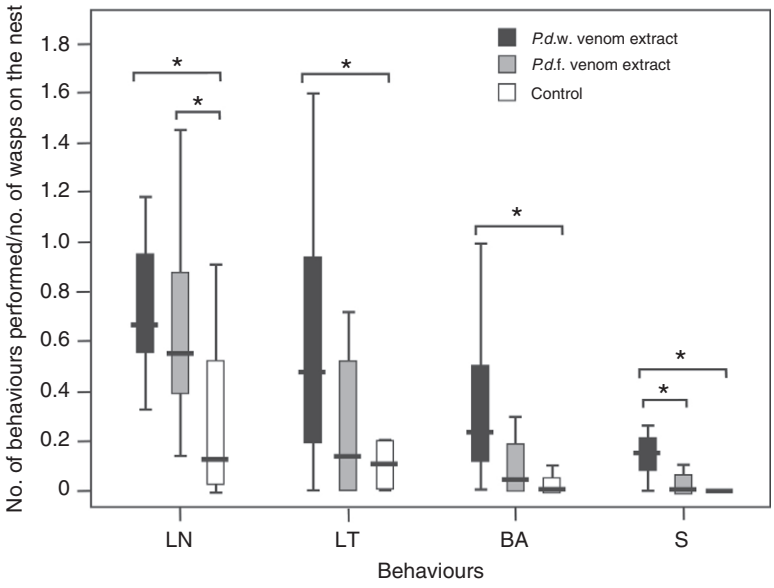

Fig. 4. The number of *P. dominulus* wasps from 15 nests that showed the four recorded behaviours (LN, leaving the nest; LT, landing on the target; BA, bending the abdomen in an attempt to sting the target; S, stings) after the simultaneous presentation of a visual and a chemical stimulus (black, worker venom extract; grey, foundress venom extract; white, CH_2Cl_2 as a control). The asterisk indicates significant differences within behaviours (with a P value less than α/number of comparisons 0.05/3=0.0167). Box-plots show the 75th and 25th percentiles as the box, the median as the line in the box and the extremes as the vertical lines.

the site of collection and period of analysis, were always clearly distinguishable. All the DA use different chemical components to discriminate between the two castes, indicating that probably it is not possible to identify specific compounds, or sets of components to separate workers and foundresses. In agreement with reports of the CHC mixture in different populations of *P. dominulus* (Dapporto et al., 2004), the relative proportion of the components of the venom volatiles fraction seems to differ in samples collected from different localities.

Despite this finding, some compounds seem to characterize the two castes. Spiroacetals were shown to be significantly higher in the venom volatile fraction of workers, whereas *N*-(3-methylbutyl)acetamide was significantly higher in the venom volatile fraction of foundresses. Spiroacetals and *N*-(3-methylbutyl)acetamide are widespread in glandular secretions of insects (see Francke and Kitching, 2001; Farine et al., 2002). In social wasps, spiroacetals have been reported in the venom of several species of Polistinae (Bruschini et al., 2006b; Bruschini et al., 2006c; Sledge et al., 1999; Dani et al., 2000) as well as in Vespinae (Weston et al., 1997) and in Stenogastrinae (Dani et al., 1998). Moreover, (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, the main spiroacetal found in *P. dominulus*, has been reported to elicit alarm behaviour in *Polybia occidentalis* (Dani et al., 2000). *N*-(3-methylbutyl)acetamide, has been found in the venom of Polistinae (Dani et al., 2000; Bruschini et al., 2006b; Bruschini et al., 2006c) and of Vespinae (Aldiss, 1983; Landolt et al., 1995). Although behavioural experiments have demonstrated its role as alarm substance in Vespinae (Aldiss, 1983; Landolt and Heath, 1987; Heath and Landolt, 1988) and in the cockroach *Therea petiveriana* (Farine et al., 2002), the same compound has also been reported in the male rectal gland secretion as a sexual attractant in fruit flies (Fletcher, 1969; Schultz and Bousch, 1971; Kobayashi et al., 1978; Bellas and Fletcher, 1979).

The results from the bioassays conducted on the *P. dominulus* colonies showed that there is an alarm response associated with the simultaneous presentation of a venom extract (chemical stimulus) and a moving target (visual stimulus), corroborating a previous study on the same species (Bruschini et al., 2006a). However, the strength and effectiveness of the response from colony members seem to be dependent on the chemical stimulus presented. The number of wasps exhibiting the four behaviours that we recorded was always higher after presentation of worker venom extracts compared with foundress venom, although this difference was significant only for the number of stings on the target. Furthermore, the four behaviours examined (leaving the nest, landing on the target, bending the abdomen in the attempt to sting and stinging the target) are hierarchically distributed in terms of an aggressive response to a danger. The exit of the wasps from the nest is a less specialized defensive behaviour, and it is also exhibited when the colony is slightly disturbed. Our results show that the venom extract presentation induces, on average, more than 70% of the colony population to leave the nest under both treatments (foundress and worker venom extract), compared with only about 30% of the population in control trials. Conversely, the other three behaviours (LT, BA, S) that are specifically linked to the implementation of an aggressive response to a danger, were differently performed depending on the experimental trial. The presentation of foundress venom extract did not lead to a significantly higher number of wasps landing on the target and bending the abdomen compared to the control. On the contrary, these two behaviours were performed by a higher number of wasps after the presentation of worker venom extract as compared with the control. Finally, our results suggest that worker venom is able to induce stinging of the target, as a significantly greater number of wasps performed the final stage of aggression only after the presentation of worker venom as compared with both foundress and control treatments.

The results of the behavioural assays seem to indicate that the presence of worker venom extract near the colony stimulates a higher number of workers to perform the entire alarm sequence ending in the attack and stinging of the target. The presence of foundress venom seemed to be less effective in this respect. In fact, the foundress venom extract induces the colony members to exit from the nest but it does not seem capable of eliciting all the sequence leading to the final aggressive act of stinging. Thus the venom of *P. dominulus* workers has a stronger alarm effect on the colonies than does the venom of *P. dominulus* foundresses.

Our results suggest that *P. dominulus* worker venom volatiles mainly have the function of alarming the colony (Bruschini et al., 2006c), whereas those of foundresses may have additional functions possibly linked to conspecific interactions. Post and Jeanne (Post

and Jeanne, 1983; Post and Jeanne, 1984) showed that in North American species, the venom of *P. fuscatus* and *P. exclamans* contains a sex pheromone. However, it is not clear which fraction of the venom is responsible of this sexual attraction. Our group has recently shown that the medium volatile fraction of the venom (peptides) is species specific (Turillazzi et al., 2007) and caste specific (Dapporto et al., 2008). Furthermore, these compounds can be perceived by the wasps (Turillazzi et al., 2006) indicating that peptides could be involved in communication. Future experiments on *Polistes dominulus* are needed to assess the role of the venom in sexual behaviour and subsequently to investigate which fraction of the venom functions as a sex pheromone.

LIST OF ABBREVIATIONS

BA	number of wasps bending the abdomen in the attempt to sting the target
CHC	cuticular hydrocarbons
DA	stepwise discriminant analysis
GC-MS	gas chromatography–mass spectrometry
LN	number of wasps leaving the nest
LT	number of wasps landing on the target
S	number of stings

We are grateful to the Istituto Sperimentale per la Zoologia Agraria (Cascine del Riccio, Firenze) for providing facilities and giving permission for us to perform some of the field experiments. We wish to thank Alessandro Michaelsson for assistance with the field experiments at Montevarchi. We also greatly thank the reviewers for their criticisms and Prof. G. Sanders for the English revision of the manuscript. This research was supported by the University of Florence.

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