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Polistes dominulus (Hymenoptera, Vespidae) Larvae Show Different Cuticular Patterns According to their Sex: Workers Seem Not Use This Chemical Information

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Abstract

During reproductive phase, larvae of male and female are intermingled in nest of social wasps. Workers care for and feed larvae that gives them an opportunity to bias investment with respect to sex, or even to kill some larvae, if they can distinguish between immature males and females. Cuticular hydrocarbon (CHC) mixtures are the most studied cues for species, nestmate, and caste recognition in social Hymenoptera. In this study, we investigate the paper wasp *Polistes dominulus* to see if male and female larvae show different patterns of CHCs and if workers are able to discriminate between male and female larvae on this basis. We performed gas chromatography-mass spectrometry analysis on cuticular extracts of larvae, and then we genotyped them to assign sex. We found sex-based variation in CHC-profiles sufficient for discrimination. However, our behavioral assays do not support the view that adults discriminate between male and female larvae within nests.

Key words: cuticular hydrocarbons, haplodiploid system, larvae, *Polistes dominulus*, sex ratio, wasps

Introduction

Hamilton's rule predicts that an individual will help a relative when the individual can pass on more shared genes that are identical by descent in this way (Hamilton 1964). This kind of cooperation is called kin selection and has been much studied in the social Hymenoptera (ants, wasps, and bees). In the social Hymenoptera, a colony is composed of at least one mated queen and her offspring, many of whom are unmated females (workers) that stay in the colony and help care for their mother's progeny. Hymenoptera have a haplodiploid reproductive system: females are diploid and develop from fertilized eggs, whereas males are haploid and develop from unfertilized ones. The haplodiploid reproductive system causes asymmetries in relatedness among colony members and queen–worker conflict over resource allocation in reproductive brood of the 2 sexes (Trivers and Hare 1976; Ratnieks and Reeve 1992; Crozier and Pamilo 1996; Arévalo et al. 1998). The queen could control the primary sex ratio by laying an equal number of male and female reproductive-destined eggs. The workers, however, could control the secondary sex ratio, the proportion of reproductive male and female at later developmental stages (larvae or pupae), by differentially caring and feeding the brood of the 2 sexes or by selective elimination of one of the 2 sexes.

The ability of workers to bias colony sex ratio toward the sex that is more related to them is dependent on their ability to distinguish between male and female brood. This ability has been reported for some ants (Aron et al. 1995; Passera and Aron 1996; Sundström et al. 1996) and not others (Nonacs and Carlin 1990). Queens could prevent workers from culling male larvae by hiding male sex identity during the early stages of development (''sexual deception hypothesis,'' Nonacs 1992). Moreover, males themselves should be the principal actors of this deception by mimicking the odor of their sisters to avoid being suppressed or underfed by workers, as hypothesized by Keller and Nonacs (1993).

In temperate species of social wasps of the genus *Polistes*, discrimination between male and female larvae has never been investigated. Nevertheless, this is one of the most studied genera on social recognition generally (Gamboa 2004). Adults of different colonies and reproductive stages are

distinguished by chemical cues present on their cuticle (reviewed in Howard and Blomquist 2005; Dani 2006). Most studies are on adult females because they are the most active in colony life. Very few studies have investigated adult male discrimination, probably because adult males are not helpful colony members, instead usually leaving to mate elsewhere. As a consequence, we do not even have clear evidence that *Polistes* females can recognize even their adult brothers (Ryan and Gamboa 1986). Because the secondary sex ratio is likely to be determined at an early developmental stage and workers are the principal dispensers of brood care, the ability to recognize the sex of brood could allow workers to eliminate male larvae. Although this kind of discrimination is an implicit assumption that underlies all models and theoretical suppositions about the potential conflict between queen and workers (Hamilton 1964; Trivers and Hare 1976), experimental studies have never definitively shown that workers are able to distinguish male and female larvae (Nonacs and Carlin 1990; Jemielity and Keller 2003).

Larvae of *Polistes dominulus* present a distinctive cuticular chemical pattern, which is characteristic of their colony, and adults are able to perceive this cue (Cotoneschi, Dani, et al. 2007). In the present study, we investigated whether there is a chemical difference between male and female larval cuticular hydrocarbons (CHCs). Moreover, we tested if wasps behave differently toward larvae of the 2 sexes by performing observations on natural nests and experiments where larval chemical odors only were presented to adults.

Materials and methods

Chemical analyses, collection of the specimens

Verona population

In June 2002, we collected 3 colonies of *P. dominulus* in Northeast Italy (near Verona). They were transported to the laboratory, transferred into glass cages (15 × 15 × 15 cm), and provided with food (fly maggots and sugar) and water ad libitum and with blotting paper as nest-building material, until they were used for chemical analyses. At the end of July, when male larvae start to appear (Pardi 1942), we collected CHCs from both small (2–3° instars) and large larvae (4–5° instars). We did not collect first instar larvae because they were too small for study. We collected a total of 26 larvae from the 3 nests: 8, 14, and 4, respectively. Each larva was extracted from the nest using soft tweezers and immediately frozen. CHCs were then extracted by immersing each single larva in a vial containing 100 μL of heptane, in the case of large larvae, or in 50 μL in the case of small ones. Each vial was then kept for 2 min in an ultrasonic bath, and larvae were removed from the vials soon after.

Florence population

In July 2004, we collected 7 colonies of *P. dominulus* near Florence (Italy) and maintained them in the laboratory as described above. At the beginning of August, we collected CHCs from a total of 53 large larvae, 9, 13, 4, and 3 from 4 nests and 8 from each of the other 3 nests. We put each larva in 100 μL of pentane for 30 s. We used a shorter time than previous CHC extractions as some preliminary analyses showed that this is a sufficient extraction time (data not presented).

Chemical analyses, methods

The extract of each single larva was dried under a nitrogen stream and resuspended in 50 μL of heptane.

Analyses of the cuticular compounds were performed using a Hewlett Packard 5890A gas chromatograph (GC) coupled to an HP 5971 mass-selective detector (using a 70-eV electron impact ionization). A fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coated with 5% diphenyl–95% dimethyl polysiloxane (Rtx-5MS, Restek, Bellefonte, PA) was installed in the GC. The injector port and transfer line were set at 280 °C, and the carrier gas was helium (at 12 PSI [83 kPa]). The temperature program was as follows: from 70 °C to 150 °C at a rate of 30 °C/min, 150 °C for 5 min, and from this temperature to 320 °C at 5 °C/min, this last temperature was held for 13 min. Identification of the cuticular compounds was performed on the basis of their mass spectra (Nelson 1978; Bonavita-Cougourdan et al. 1991) and of their retention time (Carlson et al. 1998).

All statistical analyses were performed using SPSS 11.5. For each chromatogram, the area of each single peak was transformed as the percentage of the total area of all the peaks present. Before conducting statistical analyses, we reduced the data set by eliminating hydrocarbons representing less than 1% in more than 80% of the specimens. In order to verify if larvae belonging to the 2 sexes presented different CHC profiles, we performed a stepwise discriminant analysis (DA). The significance of Wilks's λ and the percentage of correct assignments were used for evaluating the validity of the discriminant functions. When the sample size was lower that 30 (Verona population), we evaluate the results by randomly assigning the specimens to arbitrary groups having the same size as the actual sex groups and tested whether these artificial groups could be separated by a DA performed with the same procedure as used on the original data (Dani et al. 2003).

Genetical analysis

After CHC extraction, each larva (from 2002 and 2004) was preserved individually in 96% ethanol in a small vial and each larva was given a number. In 2002, in order to assess the sex of the 26 larvae, we genotyped them using 6 microsatellite loci previously identified for *P. dominulus* (Henshaw 2000): Pdom7, Pdom117, Pdom122, Pdom140, Pdom2, and Pdom20. In 2004, the 53 larvae were genotyped using 4 microsatellite loci previously identified for *P. dominulus* (Henshaw 2000): Pdom7, Pdom117, Pdom122, and

Pdom140. We used the same procedure and data analysis reported in Cotoneschi, Scognamiglio, et al. (2007).

Behavioral experiments

Odor assays with a binary choice

In July 2004 and 2005, 25 and 15 *P. dominulus* colonies, respectively, were collected near Florence, Italy. These were transported to the laboratory and caged as described above. At the beginning of August, we collected epicuticular hydrocarbons by extracting larvae from their nest using soft tweezers and putting them on clean aluminum foil. Then, excluding the head to avoid collecting drops of saliva, we rubbed the entire body for 2 min with a glass capillary with a rounded point. This method allowed us to collect CHCs sample from larval cuticle (Cotoneschi, Dani, et al. 2007). Two capillaries, inserted on a forked rod, were presented simultaneously, for 3 min, to the wasps of a colony. During the presentation, we recorded the number of aggressive behaviors (bites) and the time, in seconds, that wasps spent in exploratory behaviors (antennal contacts), toward the 2 capillaries. We tested: 1) odors of nestmate female and male larvae (n = 15), 2) odors of nestmate and alien female larvae (n = 10), 3) odors of nestmate and alien male larvae (n = 18), and 4) odors of alien female and male larvae (n = 24). All the experiments were conducted blind and recorded with a video camera for the duration of the experiment. Data obtained were then analyzed with the nonparametric Wilcoxon matched-pairs signed-rank test, and we applied the exact tests as suggested by Mundry and Fischer (1998). We excluded from the analysis the colonies where the wasps did not explore both the presented capillaries because in these cases the wasps could not perform an odor comparison. After odor collection, larval sex was determined by typing the immature gonads (Cotoneschi, Scognamiglio, et al. 2007) and not by using genetical method as Cotoneschi, Scognamiglio, et al. (2007) have recently showed that the first method is a reliable one to sex assignment.

Visits to female and male larvae in natural nests

In late July 2005, when we were quite sure that both female and male larvae were present in the nests, we recorded the activity in 2 large colonies. Nest 1 was composed of 260 cells, 25 adults, a total of 92 larvae (68 small and 24 large ones), and 16 eggs. Nest 2 was composed of 200 cells, 20 adults, a total of 58 larvae (27 small and 31 large ones), and 37 eggs. One frame per minute for 4 h was recorded in order to investigate if adult wasps showed differential treatment toward male and female larvae. After the recording, we drew a map of the nests indicating the position of the larvae. Then, all the large larvae were dissected to determine their sex (Cotoneschi, Scognamiglio, et al. 2007). The videotapes were watched blind with respect to the sex of the larvae, and all the inspections that wasps performed toward the large larvae were noted. The data were analyzed both separately by colony and together. In the latter case, we normalized the number of inspections by dividing by the number of wasps active in that colony (N = 8 in nest 1 and N = 12 in nest 2). Data were compared using the nonparametric Mann–Whitney signed-rank test.

Results

Genetical analyses

In the Verona population, we assigned 13 larvae (8 large and 5 small) to the male sex and 13 (6 large and 7 small) to the female sex. In Florence population, we assigned 39 larvae to the male sex and only 14 to the female sex.

Chemical analyses

In the larval epicuticular mixture, we found n-alkanes, methyl-alkanes, and n-alkenes just as were reported by Cotoneschi, Dani, et al. (2007). However, we found no qualitative and only relative quantitative differences in CHC profiles between male and female larvae. Because the larvae were collected from 2 different localities and analyzed at 2 different times, we considered them separately in the statistical analyses, to avoid potential biases due to slight differences in instrumental settings and to the different colony origins (Dapporto et al. 2004). Tables 1 and 2 report the list of CHCs found in male and female larvae for both localities.

In colonies where we found a similar number of male and female larvae (the 3 from Verona and the 1 from Florence; in the other collected nests, we founded larvae belonging only to one of the 2 sexes or a not comparable number of male and female larvae), we applied the function generated by DA to the profile of male and female larvae to determine to which group (male or female) they were more similar to. This comparison was performed within colony both because Cotoneschi, Dani, et al. (2007) have showed a clear chemical difference among larvae belonging to different colonies and because workers have to discriminate between female and male larvae present in their own nest.

Verona population

For Verona population, stepwise DA performed on the total larva sample (both small and large ones) separated male (N = 13) and female (N = 13) larvae on the basis of 4 cuticular compounds: C_{25}, 5-meC_{27}, 9-meC_{27}, and 13-,15-meC_{31} with a 96.2% correct classification (Figure 1; Wilks's $\lambda = 0.429$, $P = 0.001$, explaining 100% variance). For these colonies, the results of DA performed on the real data were better than those obtained on all the 50 permutations, performed randomly.

Figure 2a,b,c shows discriminant scores obtained by DA between male and female larvae and applied to 3 different colonies separately. Also in this case, most of the larvae were

Table 1 List of chemical compounds found on the cuticle of male and female larvae of *Polistes dominulus* collected from nests of Verona population

Chemical compounds	Male larvae Mean ± SD (%)	Female larvae Mean ± SD (%)
C_{23}	0.34 ± 0.39	0.31 ± 0.42
C_{25}	2.44 ± 1.99	4.29 ± 1.71
9-meC_{25}	0.25 ± 0.43	0.35 ± 0.56
C_{26}	0.21 ± 0.36	0.17 ± 0.34
C_{27}	36.4 ± 7.00	41.31 ± 11.25
13-meC_{27}	4.72 ± 2.95	4.42 ± 1.89
9-meC_{27}	2.86 ± 1.78	3.66 ± 2.15
5-meC_{27}	0.35 ± 0.47	0.14 ± 0.27
9-,13-dimeC_{27}	5.39 ± 5.03	7.22 ± 3.43
3-meC_{27}	6.01 ± 2.17	6.82 ± 2.93
C_{28}	1.44 ± 1.44	0.73 ± 1.13
C_{29}	14.90 ± 6.47	13.08 ± 8.45
13-,15-meC_{29}	11.57 ± 4.55	12.09 ± 6.43
7-meC_{29}	1.50 ± 1.17	1.07 ± 1.84
C_{31}	1.92 ± 2.61	1.38 ± 2.52
13-,15-meC_{31}	3.70 ± 3.25	0.93 ± 1.80
7-meC_{31}	0.53 ± 0.66	0.18 ± 0.35
7,y-meC_{31}	5.45 ± 8.38	1.83 ± 4.87

Mean percentage (±SD) of CHCs of male (N = 13) and female (N = 13) larvae are given.

correctly assigned according to their sex (only one female larva was incorrectly assigned).

Florence population

In the Florence population, we excluded from the analyses 2 colonies where we did not find any female larvae. In the colonies analyzed, the DA separated male (N = 39) and female (N = 14) larvae on the basis of the following compounds: 13-,14-meC_{28}, 11,y-dimeC_{29}, and 3-meC_{27}. We obtained 87.9% correct classification (Figure 1; Wilks's $\lambda = 0.390$, $P < 0.000$, explaining 100% variance).

The plot of the discriminant scores, performed on the only colony where larvae of the 2 different sex were in comparable number (female N = 7 and male N = 6), shows a 100% correct classification (Figure 2d).

Behavioral experiments

Odor assays with a binary choice

i) In the 15 colonies where wasps interacted with the glass capillaries rubbed on nestmate larvae (Table 3), no statistical differences were found in the interactions with capillaries covered with male and female larval odors, neither in the time spent in antennal contacts (Wilcoxon paired test, $Z = -0.483$, not significant [NS]) nor in the number of aggressive acts (Wilcoxon paired test, $Z = -1.133$, NS).

ii) In the 10 colonies where wasps interacted with the glass capillaries rubbed on female larvae (Table 3), wasps performed more antennal contacts toward the capillary covered with alien odor than toward those covered

Table 2 List of chemical compounds found on the cuticle of male and female larvae of *Polistes dominulus* collected from nests of Florence population

Chemical compounds	Male larvae Mean ± SD (%)	Female larvae Mean ± SD (%)
C_{23}	1.12 ± 0.88	1.01 ± 0.66
C_{25}	3.98 ± 1.34	3.83 ± 0.44
C_{26}	1.85 ± 0.84	2.00 ± 1.23
C_{27}	31.62 ± 5.34	26.60 ± 2.71
11-,13-meC_{27}	6.06 ± 1.76	6.55 ± 1.45
7-meC_{27}	0.49 ± 0.29	0.51 ± 0.17
5-meC_{27}	0.58 ± 0.31	0.67 ± 0.16
9-,13-dimeC_{27}	7.08 ± 2.55	8.51 ± 2.82
3-meC_{27}	6.10 ± 1.64	5.67 ± 1.23
C_{28}	3.23 ± 1.49	3.69 ± 2.13
13-,14-meC_{28}	0.46 ± 0.39	0.54 ± 0.23
$C_{29:1a}$	0.51 ± 0.39	1.50 ± 0.44
$C_{29:1b}$	0.73 ± 0.45	0.82 ± 0.30
C_{29}	11.37 ± 2.82	9.30 ± 2.05
11-,13-meC_{29}	7.18 ± 5.30	7.33 ± 3.71
7-meC_{29}	1.40 ± 0.92	1.64 ± 0.56
5-meC_{29}	0.75 ± 0.40	0.67 ± 0.34
11,y-dimeC_{29}	0.52 ± 0.72	0.80 ± 0.77
3-meC_{29}	1.30 ± 0.63	1.61 ± 0.49
C_{30}	2.33 ± 2.19	2.50 ± 2.24
$C_{31:1}$	0.66 ± 0.38	1.64 ± 0.39
C_{31}	1.97 ± 1.35	2.73 ± 1.67
13,15-meC_{31}	2.96 ± 2.36	2.70 ± 2.26
7-meC_{31}	0.75 ± 0.52	0.68 ± 0.48
7,y-dimeC_{31}	0.47 ± 0.40	0.67 ± 0.32
C_{32}	0.77 ± 0.86	1.25 ± 1.10
C_{33}	0.54 ± 0.63	0.83 ± 0.93

Mean percentage (±SD) of CHCs of male (N = 39) and female (N = 14) larvae are given.

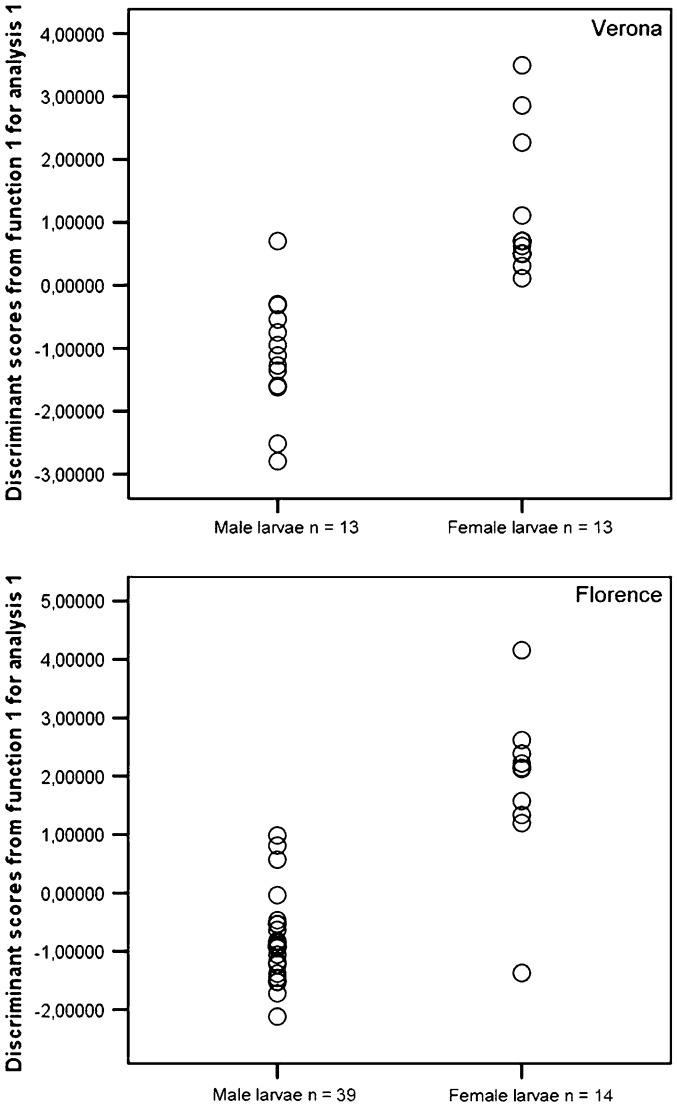

Figure 1 Discriminant scores produced by DA of male and female larval odors belonging to the Florence (larvae collected from 7 colonies) and Verona populations (larvae collected from 3 colonies). In Verona population, we obtained 96.2% correct classification. In Florence population, we obtained 87.9% correct classification.

with nestmate odor (Wilcoxon paired test, $Z = -1.994$, $P = 0.049$). However, no difference was found in the number of bites (Wilcoxon paired test, $Z = -1.383$, NS).

iii) In the 18 colonies where wasps interacted with the glass capillaries rubbed on male larvae (Table 3), wasps did not show differences in the antennal contacts between alien and nestmate larval odors (Wilcoxon paired test, $Z = -0.480$, NS). However, capillaries covered with the odor of alien larvae received more aggressive acts than those covered with nestmate larval odor (Wilcoxon paired test, $Z = -3.195$, $P = 0.001$).

iv) In the 21 colonies (we eliminated from the analysis 3 colonies because they did not react toward both the 2 presented capillaries) where wasps interacted with the glass capillaries rubbed on alien male and female larvae (Table 3), we did not find any difference in either antennal contacts (Wilcoxon paired test, $Z = -0.174$, NS) or number of bites (Wilcoxon paired test, $Z = -0.841$, NS).

Visits to female and male larvae in natural nests

By dissecting the large larvae, we found a total of 40 males (16 on nest 1 and 24 on nest 2) and 14 females (8 on nest 1 and 6 on nest 2). From the analysis of the frames recorded, wasps performed the same number of visits to the larvae of the 2 sexes. This was evident both when colonies were considered together (40 males and 14 females; mean ± standard deviation [SD] of inspections per larva/number of active wasps for each nest, males = 0.622 ± 0.338, females = 0.631 ± 0.643; Mann–Whitney test, $U = 219.500$; $Z = -1.198$, NS) and separately (colony 1: mean ± SD of inspections per male larva = 4.81 ± 3.04, female larva = 5.50 ± 6.28; Mann–Whitney test, $U = 56.00$, $Z = -0.494$, NS; colony 2: males = 7.73 ± 3.79, females = 6.67 ± 5.35; Mann–Whitney test, $U = 51.50$, $Z = -1.068$, NS).

Discussion

In *P. dominulus* wasps, male and female larvae have the same CHCs, but they differ in relative quantities. This difference, confirmed by DA performed on the 2 populations, suggested that a sex profile exists at the level of species. Differences in the relative amount of chemical compounds found in larvae belonging to different populations parallel those found in adult females of the same species by Dapporto et al. (2004). These chemical differences between localities could be due both to genetic and environmental components, as it is known that chemical cuticular composition can be influenced by both of them (Gamboa, Reeve, Ferguson, and Wacker 1986, Gamboa, Reeve, and Pfenning 1986). Moreover, our analyses indicate that male and female larvae belonging to the same colony can be discriminated on the base of their CHC profiles. These chemical results suggest that CHCs that cover the larval body could provide reliable cues within colonies to allow workers to discriminate between female and male larvae. Of course, we cannot exclude the possibility that larvae have additional cues other than those we investigated.

Although larval CHC profiles differ between males and females, all bioassays indicate that such differences are not used by the adult wasps for favoring one of the 2 sexes. In fact, in the binary choice odor assays with capillaries rubbed on male and female larvae belonging to their colony, adults performed both exploratory and aggressive behaviors at the same level toward the 2 odors. By contrast, in the binary choice odor assays with alien and nestmate larvae, adults recognize and differentially treat alien odors, confirming results previously reported by Cotoneschi, Dani, et al.

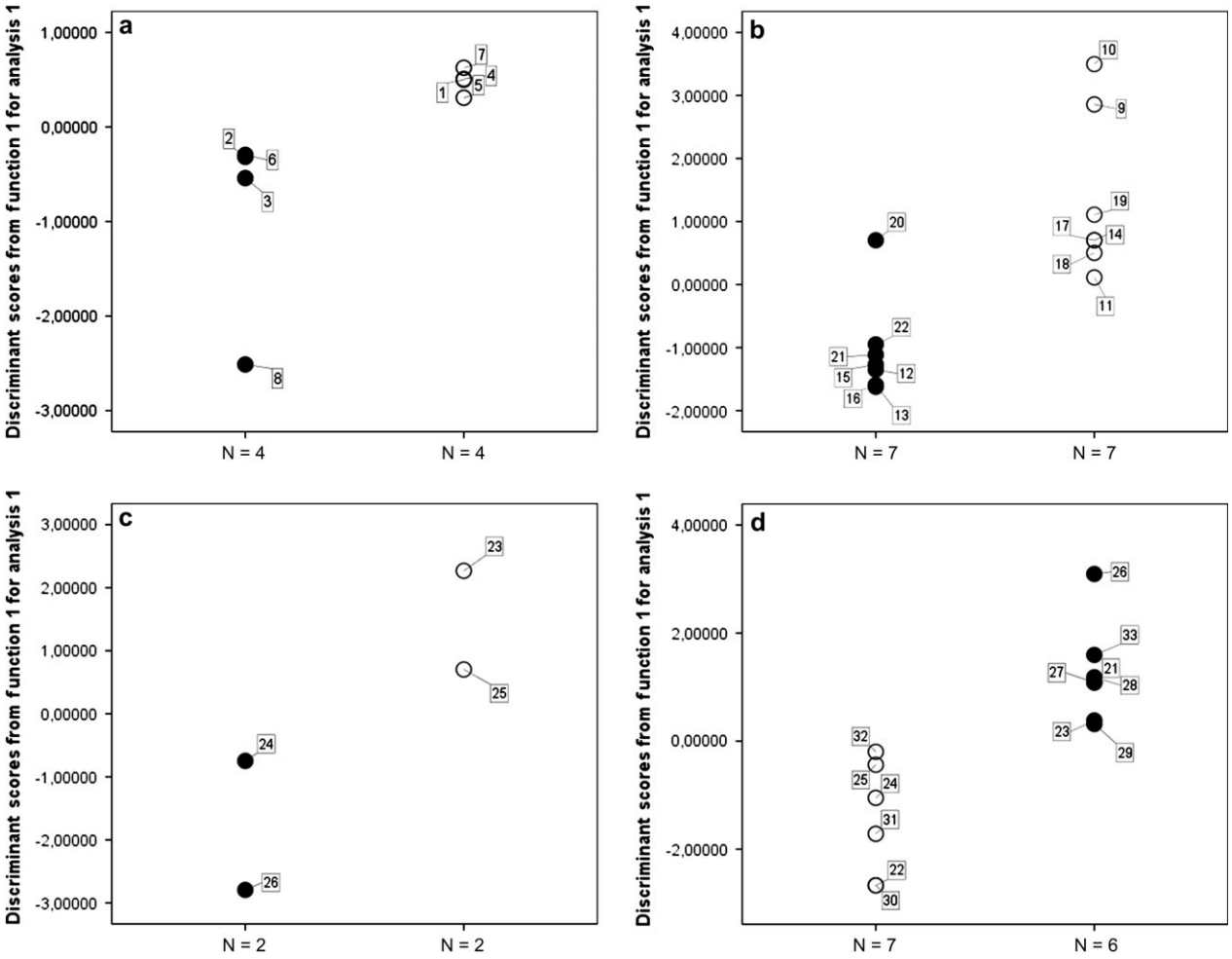

Figure 2 Discriminant score obtained by DA between male (empty circles) and female larvae (solid circles) per colony (**a**, **b**, **c**: Verona colonies; **d**: Florence colony; the sample sizes are given on abscissa axis. Labels are reported to show every sample). We performed the intracolony comparison only for those colonies that had a comparable number of male and female larvae.

(2007). In fact, when male odor was presented, capillaries applied with alien odors received more aggressive interactions than those covered with male nestmate odors. In the female assays, wasps spent significantly more time inspecting the alien odors than the nestmate ones, and no difference was found with regard to aggressive interactions, though this test lacked power because of the few colonies used.

These results show that workers are better at distinguishing nestmate and alien larvae than they are at distinguishing male and female larvae in their own nest. So, in our binary choice tests, *P. dominulus* females differentiate between nests by origin but they do not differentiate between sexes based on chemical cues. Nevertheless, the lack of discrimination in our behavioral assays does not demonstrate failure to recognize. Adult females could recognize larvae belonging to different sex and perform a different resource allocation toward one of the 2 sexes, as hypothesized in ant species (Deslippe and Savolainen 1995). However, our observations on the natural nests did not show differences in the number of visits to male and female larvae, although we cannot exclude differences in the amount or quality of food supplied.

It could be that discrimination happens much earlier in larval development, not at the late instars we studied behaviorally. We used late instars where we could use larval morphological characteristics to determine their sex. It could be that at this late stage, the best strategy for workers is to complete rearing the larvae to adulthood, even if they are males. Selective elimination of male larvae could be advantageous only at an early stage of their development; at a late stage, this selection could be disadvantageous and costly as a consequence of resources already invested on them (Reuter et al. 2004). In ant *Camponotus floridanus* (Nonacs and Carlin 1990), workers seem not able to sex brood until the pupal stage, when males have received the major part of the total energy to be invested in them. Unfortunately, these authors did not investigate on the nature of the cues, neither in larval nor in pupal stages, and hypothesized that the lack of discrimination in this species could be linked with the

Table 3. Results of the binary odor choice behavioral experiment

		Time spent in antennation (s ± SD)	Wilcoxon paired test, *P* exact	Number of bites ± SD	Wilcoxon paired test, *P* exact
Nestmate female and male larvae (15 colonies)	Nestmate female	28.07 ± 14.33	$Z = -0.483$, NS	1.40 ± 1.68	$Z = -1.133$, NS
	Nestmate male	29.53 ± 15.31		1.93 ± 2.15	
Nestmate and alien female larvae (10 colonies)	Nestmate female	11.80 ± 3.82	$Z = -1.994$, $P = 0.049$	2.50 ± 2.07	$Z = -1.383$, NS
	Alien female	17.40 ± 7.15		4.00 ± 3.09	
Alien and nestmate male larvae (18 colonies)	Nestmate male	11.61 ± 6.82	$Z = -0.480$, NS	0.89 ± 1.84	$Z = -3.195$, $P = 0.001$
	Alien male	12.11 ± 7.54		4.00 ± 6.10	
Alien female and male larvae (21 colonies)	Alien female	16.00 ± 10.82	$Z = -0.174$, NS	2.29 ± 2.00	$Z = -0.841$, NS
	Alien male	15.76 ± 10.66		2.90 ± 2.51	

In rows: the 4 different combinations of larval cuticular odors presented to adults of *Polistes dominulus* colonies. In columns: means (±SD) of time spent by wasps antennating the capillaries, the number of bites (±SD) that wasps performed toward capillaries and results of statistical analysis.

sexual deception hypothesis (Nonacs 1992; Keller and Nonacs 1993). In *Apis mellifera*, workers are able to discriminate and selectively eliminated male larvae at an early stage of development using chemical cues (Sasaki et al. 2004). Conversely, our data demonstrated the existence of a possible chemical cue presented by the last larval stages that could allow adults to discriminate between male and female larvae in *P. dominulus*, which could be presented at earlier stages too. Thus, workers might eliminate males at an early stage when it might be advantageous. We could not exclude that other chemical recognition cues could be involved. However, larvae of the 2 sexes appear to be morphologically undistinguishable (Cotoneschi, Scognamiglio, et al. 2007).

In conclusion, our study shows evidence of possible cues allowing discrimination between male and female CHC larvae in *P. dominulus* but at the same time does not support the hypothesis that wasps discriminate between older larvae of the 2 sexes. Work on younger larvae might reach a different conclusion.

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