

## Determinants of immature brood and nest recognition in a stenogastrine wasp (Hymenoptera Vespidae)

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*Parischnogaster mellyi* is a common species of hover wasp which lives in the Oriental Region. In this research we wanted to achieve a deeper understanding of some aspects of its social biology and chemical ecology considering the composition of colonies, the reproductive potential of the female nest-mates, the chemical similarities between the adult cuticular hydrocarbons, the nest paper, the Dufour's gland secretion of the females and the pap placed on the eggs as a support for larval development. We were able to assess with nest exchange experiments that this species is capable of immature brood recognition. Neither the pap placed on eggs, nor the nest odour are used in this recognition process, despite the potential discrimination cues shown by chemical analyses.

KEY WORDS: recognition, hover wasps, *Parischnogaster*, Dufour's gland secretion, cuticular hydrocarbons.

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## INTRODUCTION

It is well known that nestmate recognition is a main characteristic of social animals which was favoured by natural selection as a device to limit the exploitation of colony resources and worker force by unrelated conspecific usurpers or social parasites (FLETCHER & MICHENER 1987). Epicuticular hydrocarbons have been demonstrated to be the cues upon which recognition is carried out in social insects (see LORENZI et al. 1996, SINGER et al. 1998). Also for social wasps there is much evidence that cuticular hydrocarbons (CHCs) are the main factors of nestmate recognition (GAMBOA 2004). The cuticular chemical mixture is more similar among individuals belonging to the same colony than among individuals belonging to other conspecific colonies. If this has been demonstrated for adult nestmates a small number of studies exist for the recognition of immature nestmates, including all stages of development from eggs to pupae. Concerning *Polistes* Latreille 1802 wasps, for example, recent studies demonstrated the recognition of eggs and larvae [PANEK & GAMBOA 2000 in *P. fuscatus* (Fabricius 1793); LORENZI & FILIPPONE 2000 in *P. biglumis* (Linnaeus 1758)] but we should note that basic ethological researches on these wasps had already indicated a “differential oophagy” (and thus an egg discrimination capacity) of dominant alpha females on eggs recently laid by subordinates and workers in colonies of various species [for example PARDI (1942) on *P. dominulus* (Christ 1791), WEST-EBERHARD (1969) on *P. fuscatus* and *P. canadensis* (Linnaeus 1758), etc.]. BROWN et al. (1991) demonstrated that larvae and adults of *Vespula germanica* (Fabricius 1793) have different chemical cuticular patterns and ongoing researches of COTONESCHI et al. (2007) showed similar differences in adults and larvae of *Polistes dominulus*. STRASSMANN et al. (2000), on the other hand, highlighted that in the colonies of *Polistes carolina* (Linnaeus 1767) adults cannot discriminate between larvae of different matriline.

Owing to the particular size and architecture of their nests the experimental technique of nest exchange was used by various researchers to demonstrate recognition of nest and immatures in many independent founding polistine wasps [(PARDI 1948, in *P. dominulus*; KLAHN & GAMBOA 1983, in *P. fuscatus*; CERVO & TURILLAZZI 1989, in *P. gallicus* (Linnaeus 1767); LORENZI & CERVO 1992, in *P. biglumis*; FRANCESCATO et al. 1994, in *Belonogaster juncea* (Fabricius 1781)]. In the Stenogastrinae, the most primitive of all social wasps (CARPENTER 1982), nest exchange was employed to demonstrate colony recognition in *Parischnogaster jacobsoni* (Du Buysson 1913) (CERVO et al. 1996). Recently ZANETTI et al. (2001) demonstrated that adult nestmate recognition in a species of hover wasps [*Parischnogaster striatula* (Du Buysson 1905)] is carried out on the basis of CHCs.

In all the species of hover wasps females add to the eggs a large amount of a whitish gelatinous substance — the “pap” — secreted by the Dufour’s gland, just after deposition and continue for some time to provide the developing larva with the same secretion mixed with nectar (HANSELL 1982, TURILLAZZI & PARDI 1982, TURILLAZZI 1985, KEEGANS et al. 1993). This substance serves as a protective substrate for the food collected by the adults for the larvae but it is not a larval food in itself and is mainly composed by

hydrocarbons (KEEGANS et al. 1993, SLEDGE et al. 2001) which, at least in some species, are similar to those found on the cuticle of the adults (SLEDGE et al. 2001, CERVO et al. 2002). To this we can add that BEANI et al. (2002) found a similar mixture also in the hindgut contents of males and females of *Parischnogaster mellyi* (De Saussure 1852) which seem to use it to mark mating hotspots in the jungle.

From the researches of Gamboa and co-workers (reviewed in GAMBOA 2004) it is well known that in a *Polistes* wasp colony the nestmate recognition template is acquired by each adult individual from the "odour" (hydrocarbons blend) of the nest paper (which is similar to that of the wasps living on it) as soon as it emerges from its pupal cell. We do not know if this is true also for other social wasps. Decisive experiments similar to those performed by the American researchers are made problematic in the Stenogastrinae by the limited population of the colonies and by the difficulty of rearing these wasps in captivity. CERVO et al (2002) performed a series of experiments which demonstrated a limited discriminatory capacity by females of *Liostenogaster flavolineata* (Cameron 1902) of adults treated with Dufour's gland secretion obtained from nestmates and non nestmates; the same was observed when these individuals were smeared with gelatinous secretion taken from larvae or eggs. They suggested that brood secretion placed on eggs and added by the various females of the colony could represent the source of the cues which newly emerged wasps learn to form their recognition template.

*Parischnogaster mellyi* is a common species of hover wasp. Notes on its biology can be found in HANSELL (1981, 1982, 1983), YAMANE et al. (1983), TURILLAZZI (1991). It is frequent in Peninsular Malaysia. It builds nests with chewed vegetable material implanting groups of cells on thread like, natural or artificial substrata. It can nest on thin roots hanging from earth trenches along forest trucks as well as in human buildings. Its colonies reach a maximum of 35 cells and 5 females per colony (TURILLAZZI 1996). Resuming our knowledge about chemical recognition in this species there is first of all evidence of the presence of a chemical nestmate recognition ability based on the mixture of HCs (probably branched alkanes particularly numerous with respect to other species) of the epicuticle (DESTRI et al. 2002). The same HCs can be found also in the hindgut contents of female and male adults, in their Dufour's gland and in the secretion placed on the immature brood (SLEDGE et al. 2000, BEANI et al. 2002). We still do not know if a recognition of immature brood exists, if the same HCs can be found also on the nest material and if it correlates with colony membership. Also we do not know from where the colony members get the cues for developing the internal template for nestmate recognition. The main goal of the present research was to ascertain, by means of behavioural observations and experiments and with the help of GC-MS techniques, if immature brood recognition exists. In this case, we wanted to assess if the secretion placed on immature brood could serve as a cue for the acquisition of the template used in nestmate recognition or in the recognition of the maternity of freshly laid eggs.

## MATERIALS AND METHODS

### *Collection of colonies and samples for chemical analyses*

We collected five colonies with more than two females near the Ulu Gombak Field Study Center (UGFSC) of the University of Malaya, 25 km NE of Kuala Lumpur, Selangor State, Peninsular Malaysia. The colonies were collected at night and females present on the nests were measured for their head width and dissected in order to measure their ovarian development (a cubic Ovarian Index, O.I., was calculated averaging the length cubed of the four largest eggs in the ovary) and to remove their Dufour's glands. The glands were immediately placed into 2 cm glass capillaries (2 mm in section) then sealed with flame at the two extremities [according to the method developed by MORGAN (1990)]. In the same way the pap placed on different eggs was collected with a clean pin and placed in different glass capillaries. The wings of the females were cut away and preserved in aluminium foil. We did the same with small pieces of nest material.

### *Chemical analyses*

The capillaries with the Dufour's glands and with the egg pap were inserted in a glass vial (2 ml) and broken. Each vial was then immediately closed and heated in an aluminium heater block at 170 °C. The same treatment was performed with the wing and nest material samples. Headspace sampling was then performed using a solid phase microextraction (SPME) fibre covered with a 7 µm thick polydimethylsiloxane film (SUPELCO®). The fibre was inserted into the vial (kept at 170 °C) through the cap and then exposed in the headspace for 10 min as described by MONETI et al. (1997). For gas-chromatographic-mass spectrometric analyses the fibre was inserted in the injection port of a HP5890 II series gas-chromatograph coupled to a HP 5971A quadrupole mass spectrometer. A Restek Rtx 5-MS column (30 m × 0.25 mm, 95% polydimethylsiloxane and 5% phenyl, phase thickness 0.5 µm) was installed on the gas chromatograph. The temperature programme used was the following: the initial temperature of 150 °C was maintained for 2 min and then raised by 5 °C min<sup>-1</sup> until 200 °C, then by 2 °C min<sup>-1</sup> until 260 °C and, finally by 10 °C min<sup>-1</sup> until 310 °C. This temperature was maintained for 13 min.

### *Behavioural experiments*

*Study site.* Experiments and observations were conducted at the UGFSC. Observed colonies were situated under the lifted floor of the main building of the Center or hanging from the ceilings of old jungle barracks in disuse about 300 m apart.

*Nest substitution experiments.* Previous works analyzed the nest and brood discriminatory ability of social wasps with substitution experiments (in Polistinae wasps: KLAHN & GAMBOA 1983, CERVO & TURILLAZZI 1989, LORENZI & CERVO 1992, FRANCESCATO et al. 1994; in Stenogastrinae wasps: CERVO et al. 1996). In our work we performed a series of tests with an experimental protocol similar to the one used by CERVO et al. (1996). After forced adult removal we cut the thread substratum and exchanged 10 experimental nests (main building) with 10 other experimental nests placed at a distance of about 300 m (jungle barracks) fixing with mastic adhesive the part of the substratum carrying the nest to the stump of the substratum of the exchanged nest 1 hr after removal. The pairs of exchanged nests were similar in size and in adult and brood abundance. Ten more colonies were used as negative controls, with a similar treatment

(adult removal and nest detachment) but the same nest was replaced, after 1 hr, in its original position. We observed all the colonies for a few minutes to be sure that the wasps accepted the treatment.

For each colony we checked for adults, eggs, larvae and pupae content before the experiments and after 1, 24, 48 and 96 hr from the substitution.

*Pap substitution experiments.* We first chose 5 + 5 colonies located in the two sites 300 m apart (main building and jungle barracks). The nests were relatively large with at least 5 eggs each and larvae in different developmental stages. We marked in each nest (with a coloured dot on the respective cell) the position of 3 experimental eggs and 2 control eggs. We removed the pap with a glass capillary tube from each experimental egg, and placed it on a correspondent experimental egg of a nest from the other site. The pap from each control egg was removed and then placed back on the same egg.

We checked the presence of the treated eggs in the nests after 1, 24 and 48 hr from the beginning of the experiment.

*Alien nest odour application experiments.* The aim of this experiment was to ascertain how odours from different nests could affect the behaviour of resident wasps in a colony of *P. mellyi*. We prepared first an hexane extract of the material, reduced in small particles, of three conspecific nests collected in a locality some kilometres away. We then chose 20 experimental nests at the UGFSC main building. We placed two small squares of thin blotting paper (1 × 1 cm) along the thread-like substratum of each nest just in contact with the higher and lower extremities of the nest itself. The squares of blotting paper were put in position 1 day before the experiment in order to make the wasps accustom themselves with the new nest situation. The next day we applied a 10 µl drop of hexane extract on each of the two blotting paper squares just after having forced the wasps to abandon the nest. On their return we videotaped their reactions against the blotting paper for 1 min. Control observations with pure hexane drops were performed on each nest; control and experimental treatments were performed on each nest in a random order. The tapes were then watched by an observer, blind to the treatments, who recorded the behaviour of the resident females.

Changes in the number of immature brood from the beginning of the experiment were registered in each colony at 24 and 48 hr.

## RESULTS

### *Colony composition*

As seen in Table 1, which reports several characteristics of the female population of the five colonies used for chemical analyses, in all the colonies examined more than one female had eggs almost ready to be laid in her ovaries even if differences in the overall ovarian index were present. It was not possible to ascertain the status of the spermatheca for all the females, however; in two colonies (C and NN) at least two females had been fertilized. As we know that the dominance hierarchy in a colony is tied to the ovarian development of the colony members (TURILLAZZI & PARDI 1982, HANSELL 1983) we scored in hierarchical ranks the various individuals of each colony according to their respective O.I. (see below).

Table 1.

Size (head width), reproductive characteristics and estimated hierarchical rank of the females belonging to the five colonies used for chemical analyses. All the measure are given in arbitrary units (a.u.).

Nest code	Female	Head width (a.u.)	Cubic Ovarian Index (a.u.)	Rank (according to O.I.)	Max egg length (a.u.)
46	1	65	8288.50	1	30
46	2	62	5166.50	2	25
46	3	59.5	810.25	3	10
63	1	60.5	14735.00	2	30
63	2	61.5	15932.00	1	30
63	3	62	405.75	4	10
63	4	62.5	5001.75	3	23
70	1	62	17206.00	1	32
70	2	62	13202.50	3	33
70	3	62	14803.25	2	37
C	1	62	9938.75	1	32
C	2	60.5	64.00	3	4
C	3	63	9633.53	2	31
NN	1	61	230.2	4	9
NN	2	60	5653.00	3	25
NN	3	60.5	11176.00	1	30
NN	4	62	9479.75	2	31

### *Chemical analyses*

The compounds found on the wing cuticle (N = 16), Dufour's secretion (N = 15), egg pap (N = 18) and nest material (N = 5) of various females and colonies are reported in Table 2 together with the number of samples in which the same compounds were found for each component.

We identified a total of 31 peaks, a thirty second was detected but not identified. Eleven of the 31 peaks were linear alkanes, 15 branched alkanes, 3 alkenes, 1 amide and 1, probably, the alcohol of C18. For various branched alkenes it was not possible to establish the position of the methyl groups. As is clear from Table 2 not all the components present all the identified compounds and not all the analysed samples of a same component presented all the identified compounds. Compounds present in all the samples of all the components were only four n-alkanes with an odd number of C (nC25, nC27, nC29 and nC31).

The n-alkanes were in percentage terms the most represented group of compounds in all the components. In the Dufour's gland secretion, egg pap and nest material they represented more than 60% of all the compounds; in the cuticular mixture, however, they were similar in percentage to the branched alkanes. Alkenes represented very low percentage and were mainly present in the Dufour's gland secretion and in the egg pap (Fig. 1).

Table 2.

Compounds identified in the various components examined (wing cuticle, Dufour's gland secretion, egg pap and nest material). The numbers indicate the specimens in which the compound was actually found.

Peak no.	Compound	Wing cuticle (N=16)	Dufour's secretion (N=15)	Egg pap (N=18)	Nest material (N=5)
1	nC23:1 <sup>1</sup>	1	2	3	0
2	nC23:1 <sup>2</sup>	0	8	11	1
3	nC23	16	14	10	4
4	Amide	13	13	15	1
5	nC24	12	15	12	2
6	nC25:1	1	2	3	0
7	nC25	16	15	18	5
8	nC26	16	15	17	5
9	nC27	16	15	18	5
10	11 MeC27	10	6	3	2
11	x MeC27	4	4	4	0
12	nC28	16	14	18	4
13	nC29	16	15	18	5
14	11,13 MeC29	14	11	14	2
15	x MeC29	9	2	6	1
16	nC30	15	12	12	4
17	C18OH(?)	5	2	4	1
18	nC31	16	15	18	5
19	11,13,15 MeC31	16	14	14	3
20	11,15 DiMeC31	16	14	14	4
21	nC32	14	12	13	3
22	x MeC32	9	3	0	0
23	x,y DiMeC32	10	0	0	1
24	nC33	16	15	18	4
25	11,13,15 MeC33 <sup>1</sup>	16	14	12	4
26	11,15 DiMeC33	16	14	15	4
27	x,y DiMeC33	10	8	7	1
28	x,y DiMeC33 <sup>2?</sup>	8	8	12	1
29	x MeC35 <sup>1</sup>	4	1	3	1
30	x MeC35 <sup>2</sup>	11	9	2	1
31	x,y DiMeC35	13	10	2	2
32	unidentified	5	4	12	4

Concerning the MW of various compounds (linked to their respective retention times in the gas chromatographic column) we divided them into three fractions: a low MW fraction (up to and including nC26), a medium MW fraction (from nC27 to but not including nC31) and a high MW fraction (nC31

and upwards). Wing cuticle and nest material differentiate themselves from other components for they have a small proportion of low MW compounds. Nest material has the highest percentage of medium MW compounds while wing cuticle presents the highest percentage of high MW compounds (Fig. 2).

A Discriminant Analysis of the percentages of the chemical compounds including all the variables showed that the four components (wing cuticle, Dufour's gland, nest material and egg pap) are well discriminated (98.1% of the specimens correctly assigned to the predicted group) with the exception of wing cuticle and Dufour's gland secretion which show more chemical similarities (Fig. 3).

We observed that it is possible to discriminate individuals belonging to different colonies on the basis of their wing cuticle (Stepwise Discriminant Analysis, 100% of cases correctly assigned; Discriminant Function 1: Wilk's Lambda = 0.000, df = 36,  $P < 0.001$ , variance explained: 91.7%; Discriminant Function 2: Wilk's Lambda = 0.000, df = 24,  $P < 0.001$ , var. expl.: 6.9%) or Dufour's gland secretion compounds (Stepwise Discriminant Analysis, 86.7% of cases correctly assigned, Discriminant Function 1: Wilk's Lambda = 0.003, df = 16,  $P < 0.001$ , var. expl.: 73.2%; Discriminant Function 2: Wilk's Lambda = 0.076, df = 9,  $P < 0.005$ , var. expl.: 25.5%). It is also possible to discriminate eggs found in different nests on the basis of the chemical composition of the pap placed on them (Stepwise Discriminant Analysis, 94.4% of cases correctly assigned, Discriminant Function 1: Wilk's Lambda = 0.000, df = 20,  $P < 0.001$ , var. expl.: 84.4%; Discriminant Function 2: Wilk's Lambda = 0.007, df = 12,  $P < 0.001$ , var. expl.: 14.7%).

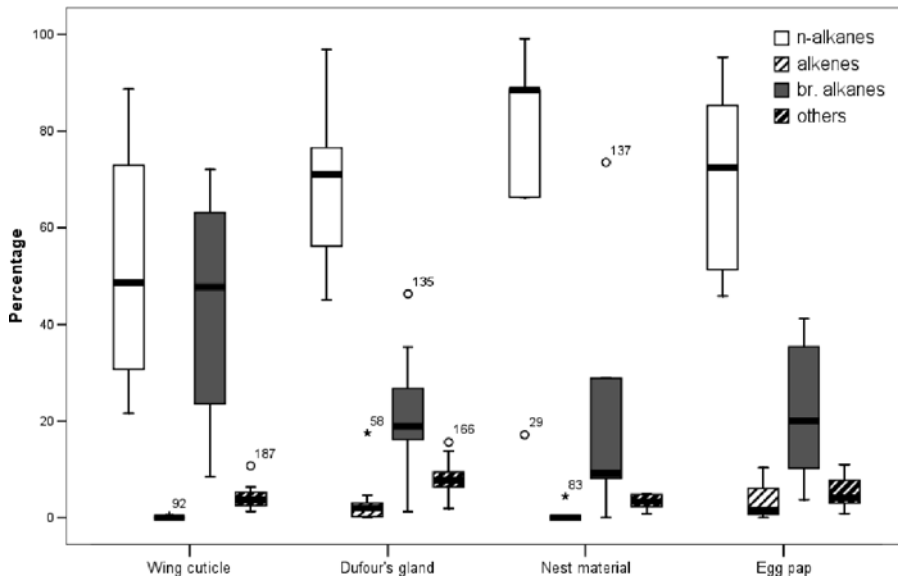


Fig. 1. — Percentages of CHCs of various groups present in the four colonial components examined.



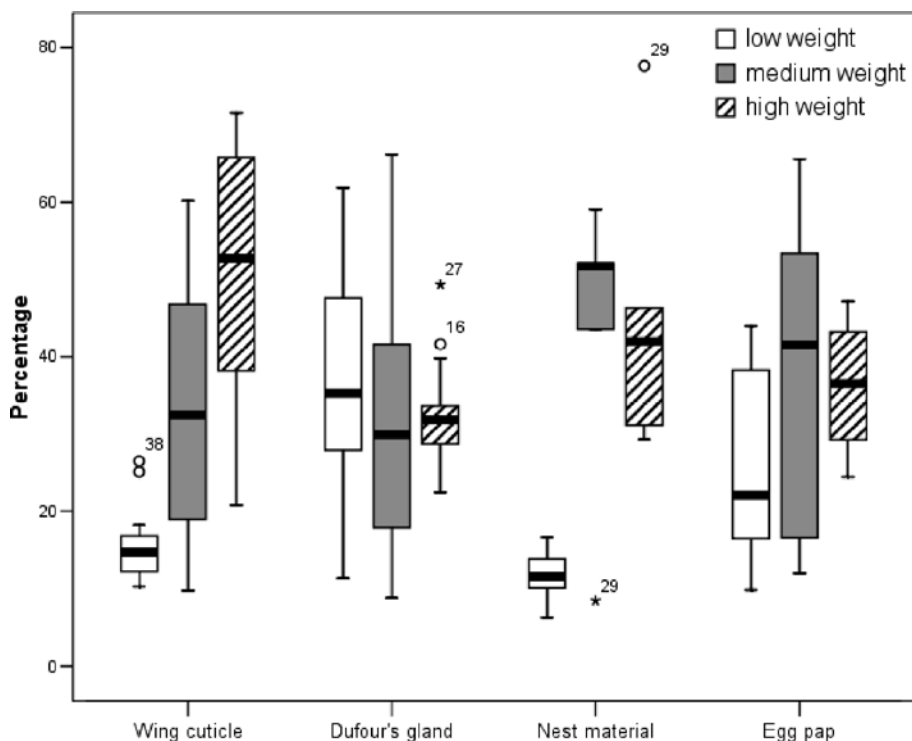


Fig. 2. — Percentages of the various MW fractions of compounds present in the various components.

The chemical composition of the Dufour's gland secretion of the various individuals varies with their ovarian development (as evaluated by their O.I.): in fact individuals with more developed ovaries present a higher percentage of branched alkanes and a lower percentage of n-alkanes with respect to individuals with less developed ovaries. Methyls of C31 (11,13,15 MeC31, and 11,15 DiMeC31) and some of C33 (11,13,15 MeC33 and 11,15 DiMeC33) increase significantly with ovarian development (11,13,15 MeC33 increases significantly also in the wing cuticle).

We also observed that in a discrimination analysis performed on Dufour's gland secretions of the various ranked individuals and on the pap placed on the eggs, without discriminating for colony membership, all the groups and females belonging to 1st, 2nd, 3rd and 4th ovarian rank are well discriminated. Moreover, the egg pap is more similar to the Dufour's gland secretion of low ranks (2nd and 3rd) individuals (that are the individuals with less developed ovaries) than to the Dufour's gland secretion of the first rank individuals (Fig. 4). As a confirmation of the previous finding we found also that in five out of five colonies the pap placed on the examined eggs had a shorter chemical distance with the Dufour's gland secretion of individuals with lower ovar-

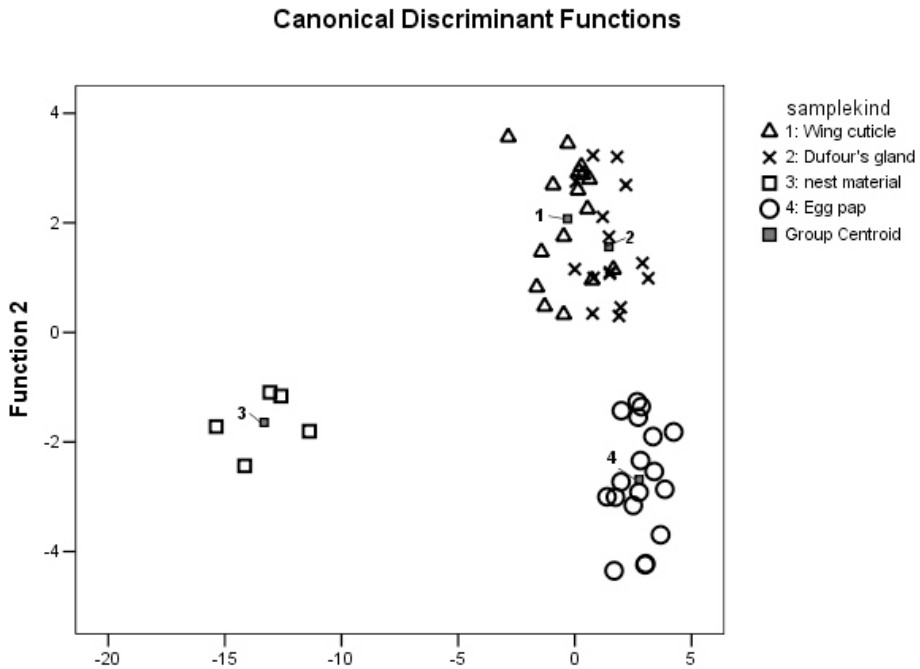


Fig. 3. — Graph of the two first discriminant functions obtained with a Discriminant Analysis performed with chemical compounds found on wing cuticle (1), Dufour's gland secretion (2), nest material (3) and egg pap (4).

ian development (2nd or 3rd rank) than from the Dufour's gland secretion of individuals with higher ovarian development.

Considering the colonies one by one we observe that in three out of 5 colonies (46, 70, NN) all the eggs examined had a pap with an extremely similar composition to the Dufour's gland secretion of a single individual (Fig. 5). In the other two colonies (63 and C) chemical proximity with pap deposited on the examined eggs was shared between two females. In all cases but one (colony 63) all the females had at least one mature egg (ready to be laid) in their ovaries even if they were not the female with more developed ovary in that colony.

#### *Behavioural experiments: Nest substitution experiments*

Table 3A reports the number of eggs and larvae that disappeared and the number of eggs laid after 1, 24, 48 and 96 hr from the first manipulation of nests (exchange of experimental nests and detachment and re-positioning of control nests). The average number of eggs and larvae that disappeared from the experimental nests was significantly higher than those that disappeared from the control nests after 24 and 48 hr from the first manipulation but the

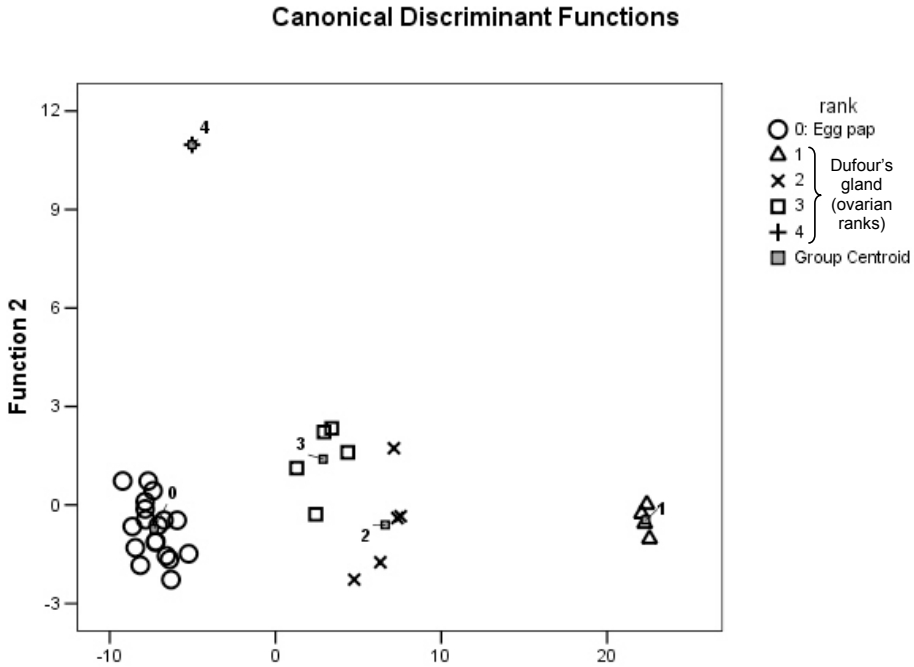


Fig. 4. — Graph of the two first Discriminant Functions obtained with a discriminant analysis of all the chemical compounds of egg pap of all the eggs examined and Dufour's gland secretion of the females belonging to various ovarian ranks.

differences are no more significant after 96 hr. The same is true if the values are normalized according to the different number of eggs present at the beginning of the experiment in each nest and according to the different average number of adults present on each nest (Table 3B).

#### *Behavioural experiments: Pap substitution experiments*

Table 4 reports the results of the experiment in which in 9 nests the pap present on some experimental eggs was substituted with pap taken from eggs of alien nests. Control eggs instead had their own pap removed and then repositioned. After 24 hr and 48 hr we noted no statistically significant difference in the disappearance of experimental and control eggs (Wilcoxon Signed Ranks test).

#### *Behavioural experiments: Alien nest odour application experiments*

The resident females reacted (by biting or repeatedly inspecting the square of blotting paper) similarly to hexane extracts of material taken from

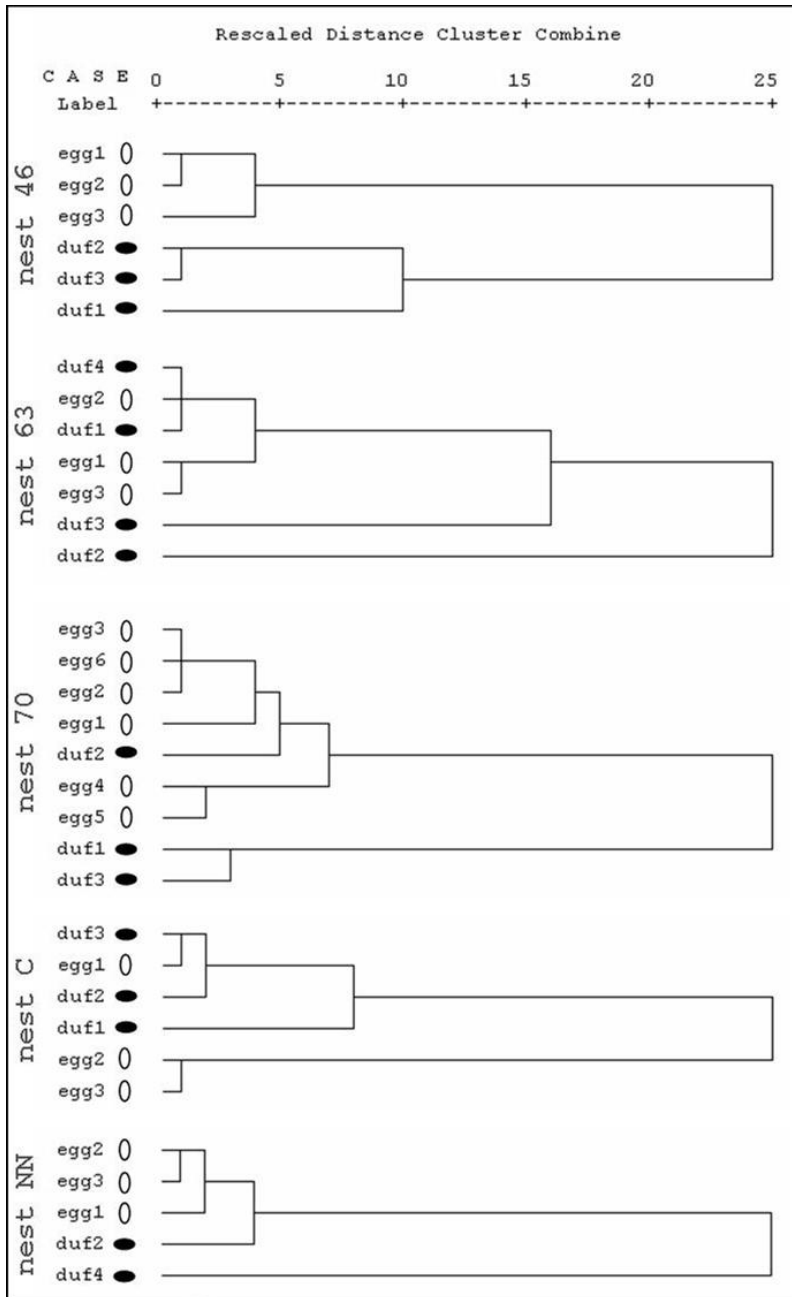


Fig. 5. — Dendrograms (generated by K mean cluster analysis) which indicate chemical similarities between pap deposited on the eggs (white dots, randomly numbered) and Dufour's gland secretion (black dots, numbered following the rank hierarchy) of the various females dissected in each of the five colonies examined.

Table 3A.

Number of eggs and larvae that disappeared and the number of eggs laid after 1, 24, 48 and 96 hr from the first manipulation of nests (exchange in experimental nests and detachment and re-positioning in control nests). (N = number of nests).

		1 hr	24 hr	48 hr	96 hr
Missing eggs	Exptal.	4 (N = 19)	31	48	56
	Control	0 (N = 10)	1	7	21
Missing larvae	Exptal.	3 (N = 18)	17	34	37
	Control	0 (N = 8)	0	0	5
Eggs laid	Exptal.	0 (N = 19)	5	14	27
	Control	0 (N = 10)	5	7	10

Table 3B.

Average number ( $\pm$  SD) of eggs and larvae that disappeared from experimental and control nests after 1, 24, 48 and 96 hr. from the treatment. Numbers are normalized for the number of eggs present at the beginning of the experiment and for the average number of adults on the nest recorded at the five checks.

		1 hr	24 hr	48 hr	96 hr
Missing eggs (mean)	Exptal.	$0.057 \pm 0.136$ (N = 19)	$0.334 \pm 0.317$	$0.535 \pm 0.491$	$0.654 \pm 0.569$
	Control	0 (N = 10)	$0.016 \pm 0.052$	$0.152 \pm 0.173$	$0.447 \pm 0.444$
Missing larvae	Exptal.	$0.098 \pm 0.258$ (N = 18)	$0.381 \pm 0.592$	$0.742 \pm 0.895$	$0.784 \pm 0.953$
	Control	0 (N = 8)	0	0	$0.239 \pm 0.529$
Eggs laid	Exptal.	0 (N = 19)	$0.349 \pm 0.913$	$0.732 \pm 1.002$	$1.21 \pm 1.258$
	Control	0 (N = 10)	$0.58 \pm 0.689$	$0.7 \pm 0.838$	$1.20 \pm 1.40$

Table 4.

Number of eggs (mean  $\pm$  SD) treated by substituting the pap with an egg pap coming from alien nests and their destiny in comparison with control eggs after 24 hr.

Exptal.	Control	Check 24 hr		Check 48 hr	
Eggs with alien pap	Eggs with their own pap removed and repositioned	Exptal. eggs missing	Control eggs missing	Exptal. eggs missing	Control eggs missing
$2.8 \pm 0.4$	$1.9 \pm 0.3$	$0.8 \pm 1.0$	$0.4 \pm 0.5$	$0.0 \pm 0.0$	$0.1 \pm 0.3$

alien nests or from their own nest and then dropped onto two square pieces of blotting paper placed at the top and at the base of their own nests. No statisti-

cal differences (Wilcoxon Signed Ranks test) were observed between experimental and control colonies in reaction intensity (bites and inspections: mean  $\pm$  SD  $0.4 \pm 0.5$  for experimental nests,  $0.3 \pm 0.5$  for control nests) and duration (in seconds: mean  $\pm$  SD  $52.1 \pm 14.6$  for experimental nests,  $29.0 \pm 40.7$  for control nests).

## DISCUSSION AND CONCLUSIONS

From a series of previous studies we know the basic social biology of *P. mellyi*. Colonies are small but usually their size is in line with that of other species. The architecture of the nests, quite mimetic, appears to be among the less elaborated of all the Stenogastrinae and it is based on an apparently disordered clustering of cells along a thread like substratum (HANSELL 1982). Various females in a colony can have developed ovaries (HANSELL 1983, YAMANE et al. 1983) but in most cases only one reproduces at a time (FANELLI et al. 2005). Egg laying females add their Dufour's gland secretion to the laid egg (HANSELL 1982) but then other females can add their own secretion to the eggs and small larvae (S. TURILLAZZI pers. obs.). Highly related and completely unrelated individuals can be members of the same colony (FANELLI et al. 2005). Thus, notwithstanding a documented nestmate recognition (DESTRI et al. 2002) based on cuticular hydrocarbons, the colonies of these hover wasps are open to intrusions by aliens.

Nest exchange experiments demonstrated that colonies of *P. mellyi* destroy immature brood present in an alien nest positioned in the place of their own: this selective destruction, which lasts only 2-3 days, can be seen as evidence of a discrimination between alien and nestmate immature brood. We may exclude that they are also due to the different shape and arrangement of the alien nest itself or to the different disposition, number and kind of the alien immature brood. The nests of this species are often exposed in their natural environment to relevant changes due to meteorological conditions and frequent manipulations by predators (such as *Vespa tropica* Linnaeus 1758, HANSELL 1982) or conspecifics or congenetics searching for construction materials. The same experiments demonstrated that alien nests are readily accepted as is shown by the similar number of new eggs laid in the experimental and control nests.

The experiments where we tried to alter the odour of the nest with an extract of the material from an alien nest did not give clear indications as the manipulation could have been too slight to trigger appreciable responses from the animals. However, judging from the results obtained in the nest exchange experiments it is also possible that nest odour (that is the HCs blend left on the nest) had only a slight influence on the discrimination of the immature brood. Actually old nests are continuously re-used by females coming from different sites and quite often females steal the material of old or active nests to re-cycle it for the construction of their own nest. Moreover no particular behaviour of the females which could be considered as clear evidence of the application procedure of secretions onto the nest material (such as the gaster stroking on

the nest of *Polistes* wasps; CERVO et al. 1990, DANI et al. 1992) has never been described in any hover wasps and was not noticed after nest exchange.

Analyses show a fundamental chemical homogeneity in each colony with regards to all considered sample groups: adult cuticular HCs, Dufour's gland secretion, the pap deposited on the eggs and the nest material. These four groups seem to be more similar if compared with the other samples from the same colony than if compared with samples from different colonies. Thus any colony component examined has potential chemical information to act as a cue for the recognition of the colony. Chemical similarity between CHCs and Dufour's gland secretion indicates a possible use of the later for spreading on the body, probably by cleaning movements (see collection of the ant guard secretion in *Parischnogaster nigricans serrei* (Du Buysson 1905) and *P. jacobsoni*, TURILLAZZI & PARDI 1981, TURILLAZZI 1985).

Focusing on the analyses performed on any single colony, we see that in 3 out of 5 cases the pap collected from the eggs was more similar to the Dufour's gland secretion of a single female, clearly the last one that had deposited the secretion. In the other two cases two females shared the attribution of the pap deposited on the eggs. We have no information on the actual pap deposition sequence but as we tried to sample the eggs with a very limited amount of pap there is a good probability that the eggs were laid by that particular individual. Actually all the females but one, found associated with the analysed eggs, were potential egg layers (judging from the largest egg found in their ovaries) even though they were not necessarily the ones with the more developed ovaries in that colony.

Actually individuals do not seem to discriminate between eggs covered by the pap produced by alien females or by their nestmates. This could be due to the fact that this secretion is supplied by different females in the colony in addition to the egg layer. However, the simplest explanation is that individuals recognize the eggs on the basis of the first secretion applied on it, produced by the egg layer female.

This research brings interesting information about the chemical communication of the common hover wasp species *P. mellyi*. However it does not completely answer the questions about the exact role of the Dufour's gland secretion and egg pap. In particular it demonstrates immature brood recognition ability in this species and brings evidence about the possible involvement of the colonial hydrocarbons (the main source of which seems to be the Dufour's gland secretion). At the same time the behavioural experiments were not sufficient to indicate the possible source of the recognition template. Further studies will be necessary to determine the characteristics of this phenomenon in hover wasps which, on the other hand, remain an important model for the evolution of chemical communication in social insects.

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