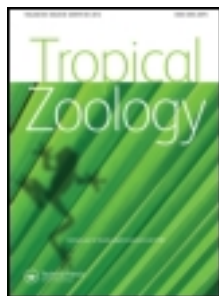


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## Social biology of *Parischnogaster striatula* (Hymenoptera: Stenogastrinae)

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The social biology of the wasp *Parischnogaster striatula* has been studied in Peninsular Malaysia. This species shows the main characteristics of hover wasps (Vespidae, Stenogastrinae) which set them aside from the other social Vespidae. These include the use of an abdominal secretion in brood rearing, the three-phase egg deposition and the presence of only four larval instars. Colonies are invariably small sized and on average 1.92 females on the nests present developed ovaries. Colony life revolves around a dominant female, which has developed ovaries and spends most of her time on the nest. Since visual cues were recently found in two different species of *Liostenogaster* (Stenogastrinae), we also looked for a possible badge of status in these wasps but with negative results.

**Keywords:** Stenogastrinae; tropical hover wasps; nesting biology; sociality; social insects; visual communication; status badge

### Introduction

The Stenogastrinae represent a sub-family of social wasps belonging to the family Vespidae (Carpenter 1982; Hines et al. 2007; Turillazzi 2012). Stenogastrinae are endemic to the Indo-Malaysian area and comprise 57 described species belonging to seven genera with *Eustenogaster* van der Vecht, 1969, *Liostenogaster* van der Vecht, 1969 and *Parischnogaster* Schultess, 1914 showing the highest number of species described so far. The peculiar features of the Stenogastrinae (reviewed by Turillazzi 1989, 1991, 1996, 2012; Hunt 2007) are the absence of a nest pedicel, the use of poor-quality nest material and the use of an abundant glandular secretion for the rearing of the larvae characterized by a very long developmental time. Due to the primitive social organization and the generalized small size of the colonies, exceeding a dozen of individuals only in few species (Baracchi et al. 2009), these organisms have been proposed as a key group for understanding the origin of social evolution in insects (West-Eberhard 1978; Turillazzi 1991). However, published information based on direct observation of living colonies is restricted to a small number of species: one species of the genus *Stenogaster* Guérin-Méneville, 1831 (*Stenogaster concinna* van der Vecht, 1975) and one of *Metischnogaster* van der Vecht, 1977 [*Metischnogaster drewseni* (de Saussure, 1857)], four species of the genus *Parischnogaster* [*Parischnogaster mellyi* (de Saussure, 1852), *Parischnogaster*

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*nigricans serrei* (Cameron, 1902), *Parischnogaster alternata* (Sakagami, 1969), *Parischnogaster gracilipes* (van der Vecht, 1977)], three species of *Liostenogaster* [*Liostenogaster flavolineata* (Cameron, 1902), *Liostenogaster vechti* Turillazzi, 1988, *Liostenogaster topographica* Turillazzi, 1999] and *Anischnogaster* van der Vecht, 1972 (*Anischnogaster laticeps* Smith, 1859; *Anischnogaster iridipennis* Smith, 1859), three of *Eustenogaster* [*Eustenogaster calyptodoma* (Sakagami and Yoshikawa, 1968), *Eustenogaster eximia* (Bingham, 1890), *Eustenogaster fraterna* (Bingham, 1897); see also Turillazzi 2012].

*Parischnogaster striatula* (du Buysson, 1905) is a common species belonging to the “*striatula* group” together with *P. alternata* characterized by a petiole of the gaster only about as long as wide, clypeus narrowly separated from eye and posterior ocelli separated at most one ocellus diameter. The very close relationship between *P. striatula* and *P. alternata* was recently confirmed by comparing the shape of their forewings (Baracchi et al. 2011). Colonies of this species are common in sheltered niches along forest roads or trails, or under the vaults of caves and human buildings, mainly small gazebos, where thread-like suspensions are present.

Given the great need for continued detailed studies on social biology in this group of wasps our purpose here was to extend the present scanty knowledge of this species. Some aspects of the biology of *P. striatula* have been recently studied, such as the ability of nest mates recognition on the basis of cuticular hydrocarbons (Zanetti et al. 2001), the strong relationship between fertility and cuticular profiles (Turillazzi et al. 2004) and the use of antimicrobial polar compounds of the venom as antiseptic tools on the cuticles (Baracchi et al. 2010; Baracchi, Mazza, et al. 2012).

The purpose of this work was to investigate the main social biology traits of this species, both at the colonial and individual levels. We thus focused on colony demography, reproductive skew and morphophysiological traits of colony members. As Stenogastrinae are heavily exposed to predation and they evolved specific defence adaptations, we investigated their defence strategies against two of the most common sympatric threats. Finally, as two species of *Liostenogaster* have been recently highlighted to use visual communication (Baracchi, Petrocelli, et al. 2012), we also looked for the possible existence of visual cues in *P. striatula*.

## Materials and methods

### *Colony collection and colony composition*

Eighty-four colonies of *P. striatula* were collected at dusk together with all the resident wasps (a total of 167 females and 80 males) and the immature brood from two different sites in the Pahang State of Peninsular Malaysia: Jeriau Waterfall, Bukit Fraser (BF; 3°43'N, 101°42'E, 1000 m above sea level; 49 colonies, 112 females, 52 males) and Genting Tea Estate (GTE; 3°21'N, 101°47'E, 610 m above sea level; 35 colonies, 55 females, 28 males). After collection, the number of males, females, cells and immature brood (eggs, larvae and pupae) was registered for each nest. All the immature brood from both sites and the adult wasps of BF were preserved in alcohol while the adults of GTE were released.

### *Morpho-physiological data of females*

The maximum head width of the larvae ( $N = 263$ ) was measured and the distribution of the head width was calculated. We dissected the ovaries of all females ( $N = 112$ ) under a

binocular microscope to determine their reproductive status. For each adult wasp, the ovarian status was quantified by using an ovarian index (OI) based on the measure of the length of the six largest oocytes present in the ovarioles. When possible, we also checked the spermatheca of each female for the presence of sperms ( $N = 70$ ). To categorize the facial patterns of females ( $N = 112$  for 49 colonies), we first took a picture of the “face” of each collected wasp with a Fuji-film digital camera (Figure 1) and then we measured the size of the black-pigmented area on the clypeus (facial marking size, FMS; see also Baracchi, Petrocelli, et al. 2012). The maximum width of the head was used as a body size estimate (Eickwort 1969). All measurements were made on digital photographs by using the free software ImageJ (<http://www.rsweb.nih.gov/ij/>). We took into account only the area corresponding to the clypeus (grey plus white area in Figure 1), as the inner and outer eye stripes and the mandibles were invariably black in all the analysed specimens.

### ***Behavioural observations***

Twenty-five additional colonies were studied for 1 week in the field. After marking all adult females with enamel colours for individual recognition, we carried out both direct observations and video recordings on different colonies (BF,  $N = 6$ ). The main behaviours were described: egg deposition, pap collection, activity on the nests and males’ and females’ off-nest activity (foragers). The hierarchical status of each adult female was assessed according to the proportion of time spent on the nest and, when possible, by dissecting their ovaries. The frequency of submissive behaviour (upon encounters, a subordinate female will usually yield place to a dominant) and the direction of aggressive acts were also taken into account to rank the females. We scored all interactions involving intense antennations, mounting, biting and fighting as aggressive and subdivided them on the basis of whether they were directed towards a moving individual or towards a resting individual, as suggested by Nonacs et al. (2004). For a total of  $N = 19$  colonies from BF, we scored the position of every female and male on the nest (or the time they spent away

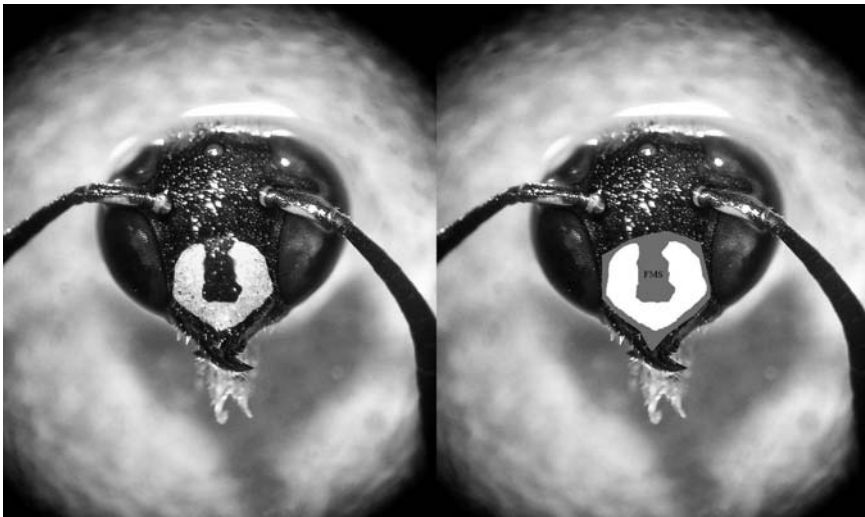


Figure 1. Head of females of *Parischnogaster striatula*. The pictures show the measurement of head width (left) and that of the black facial marking (FMS, right), which is represented by the grey area (FMS) on the clypeus.

from it) at 30-min intervals for 11 h, from 7.00 am to 6.00 pm. All the video recordings and observations took place exclusively on sunny days.

The main defensive strategies and reactions against common predators such as vespine wasps and ants were studied directly in the field (GTE site) on an additional 20 colonies. A dead hornet, tied to the tip of a 50-cm long thin stick, or a live ant, tied by a fishing line on the thorax, was presented to the nest residents of free-living colonies. According to the type of threat they represented (aerial vs. terrestrial), the two lures were, respectively, presented in front of the nest surface or directly put onto the nest surface. All the trials (20 for each lure, 40 in total) were conducted from 9.00 am to 6.00 pm in a single sunny day. Trials lasted 1 min and were carried out randomly alternated and 1 h apart. We counted the response showed by resident females (antennations, bites, abdomen bendings and stings, and dropping flights). Responses were also correlated with the content of the nest.

### *Statistical analyses*

The Pearson and Mann–Whitney tests were used to analyse the composition of the colonies (number of wasps per nest, number of females, males and broods).

Spearman and Wilcoxon tests were used for comparing different levels of reaction against the presented lures and for correlating aggressive reaction with the nest content. The Kolmogorov–Smirnov test was used to analyse the distribution of the larvae head width.

Finally, ANOVA design was used for comparing facial markings with physiological and morphological data. In the general linear model (GLM) analysis, head width and FMS were used as covariate variables, colony as a random factor and OI as dependent variable. These analyses were carried out using only individuals showing an OI > 0.30, which roughly corresponds to the division value of the two curves of the OI distribution (see also “Result” section). This choice was necessary to exclude those individuals who are still too young for having developed ovaries and to have reproduced from the analysis (Baracchi, Petrocelli, et al. 2012). All analyses were carried out using the statistical program SPSS 13.0 (SPSS Inc., Chicago, IL, USA) for Windows.

## **Results**

### *Characteristics of nesting sites*

Contrary to the close-related species *P. alternata*, *P. striatula* builds her nests in open places, attaching them to thread-like suspensions such as rootlets, thin stems and artificial wires, usually in positions sheltered from the rain. Sometimes it is possible to find several nests in close proximity but, as a rule, they do not form aggregations of the type of those formed by colonies of *P. alternata*.

### *Nest architecture*

The architecture of the nest of a species of the *P. striatula* complex was among the first to be accurately described of all stenogastrine wasps together with those belonging to *Eustenogaster luzonensis* (Rohwer, 1919), *Liostenogaster varipicta* (Rohwer, 1919) and to the species later described as *Parischnogaster timida* (du Buysson, 1910). Williams (1919) presented a detailed drawing of the nest of *Parischnogaster depressigaster* (Rohwer, 1919) from the Philippines; the nest consisted of cells spirally arranged around a central tube and it is quite similar to that of *P. striatula* (Figure 2). The nests of this species can be



Figure 2. Nest architecture of *Parischnogaster striatula*.

variously garnished with external decorations acquiring a “rough” aspect, otherwise the external surface can be smooth. Nest material is mainly organic (plant material), and inorganic components (mud) (calculated with the method of incineration) can vary between 17% and 24% on average (Bongiovanni 1998).

### ***Colony composition and morpho-physiological data***

#### *Sex ratio*

GTE colonies had on average  $1.57 \pm 1.19$  females and  $0.81 \pm 1.02$  males per colony (females range 1–5, males range 0–3, 35 colonies), whereas BF colonies had on average  $2.28 \pm 1.44$  females and  $1.07 \pm 1.05$  males (female range 1–8, male range 0–4, 49 colonies; Figure 3). The female/male ratio averages  $1.45 \pm 1.10$  at GTE and  $1.79 \pm 1.38$  at BF (see also Table 1); both are lower with respect to the ratio of 2.4 recorded in *L. flavolineata* (Samuel 1987) but higher than 1.27 recorded in *P. nigricans serrei* (Turillazzi 1985). GTE and BF colonies differed in the number of females per nest (Mann–Whitney *U*-test,  $U = 570$ ,  $P = 0.006$ ,  $N = 55, 112$ ), but not for number of males (Mann–Whitney *U*-test,  $U = 732$ ,  $P = 0.221$ ,  $N = 55, 112$ ). On average, we found  $3.34 \pm 1.90$  wasps per colony in BF and  $2.34 \pm 1.67$  wasps per colony in GTE (Table 1). The difference was statistically significant (Mann–Whitney *U*-test,  $U = 609$ ,  $P = 0.022$ ,  $N = 55, 112$ ). In the overall population, the number of males in each colony is not related to the number of females (Pearson correlation test,  $R = 0.165$ ,  $P = 0.133$ ,  $N = 84$ ).

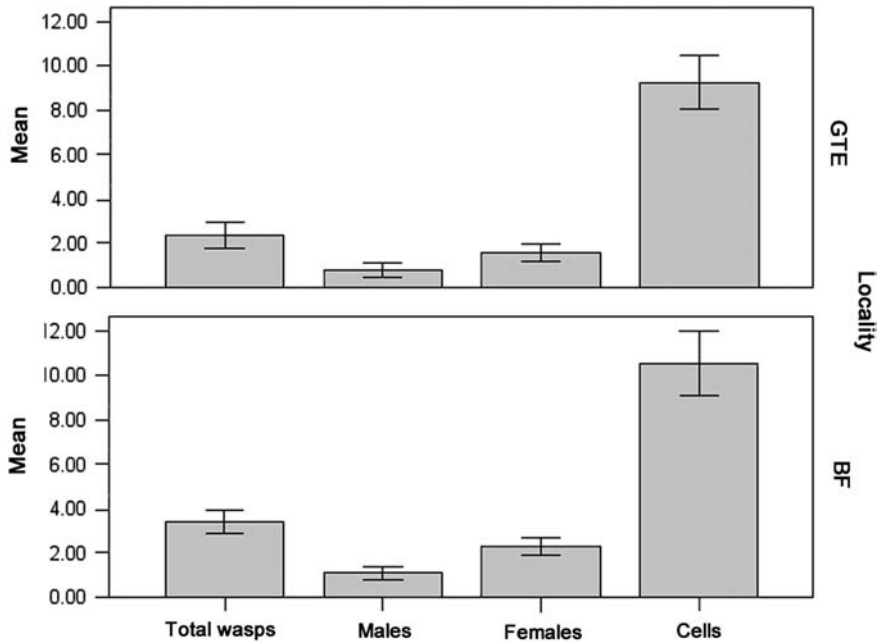


Figure 3. Comparison between the number of cells and the number of males and females of nests collected at GTE ( $N = 35$ ) and BF ( $N = 49$ ) sites.

*Nest and immature brood (number of cells, number of brood, developmental stages)*

The number of cells, eggs, larvae, pre-pupae and pupae found in 35 and 49 nests collected from GTE and BF, respectively, are given in Table 1, and they are not statistically different between the two sites (Figure 4). In the overall population, the total number of wasps in a colony is correlated with the number of cells in the nest (Pearson correlation test,  $R = 0.452$ ,  $P < 0.001$ ,  $N = 84$ ).

Larvae and pupae are correlated with the number of males and females on the nest (Pearson correlation test, female larvae:  $R = 0.281$ ,  $P = 0.010$ ,  $N = 84$ ; female pupae:

Table 1. Population differences between GTE and BF colonies.

	GTE (mean $\pm$ SD)	BF (mean $\pm$ SD)
Females per nest*	1.57 $\pm$ 1.19	2.28 $\pm$ 1.44
Males per nest	0.81 $\pm$ 1.02	1.07 $\pm$ 1.05
Sex ratio (nest)	1.45 $\pm$ 1.10	1.79 $\pm$ 1.38
Fertilized females per nest	Not recorded	1.93 $\pm$ 10.8
Non-fertilized females per nest	Not recorded	0.68 $\pm$ 0.46
Number of Cells	9.25 $\pm$ 3.64	10.5 $\pm$ 4.97
Empty cells	2.28 $\pm$ 2.50	3.38 $\pm$ 3.13
Eggs	2.00 $\pm$ 1.32	2.63 $\pm$ 1.88
Small larvae	1.42 $\pm$ 1.17	1.26 $\pm$ 1.22
Medium larvae	0.91 $\pm$ 0.98	0.85 $\pm$ 1.55
Large larvae	0.80 $\pm$ 0.96	0.85 $\pm$ 1.09
Pre-pupae	0.51 $\pm$ 0.91	0.63 $\pm$ 0.97
Pupae	1.22 $\pm$ 1.45	0.89 $\pm$ 1.43

\*Significant difference between the colonies of the two sites (Mann–Whitney  $U$ -test).

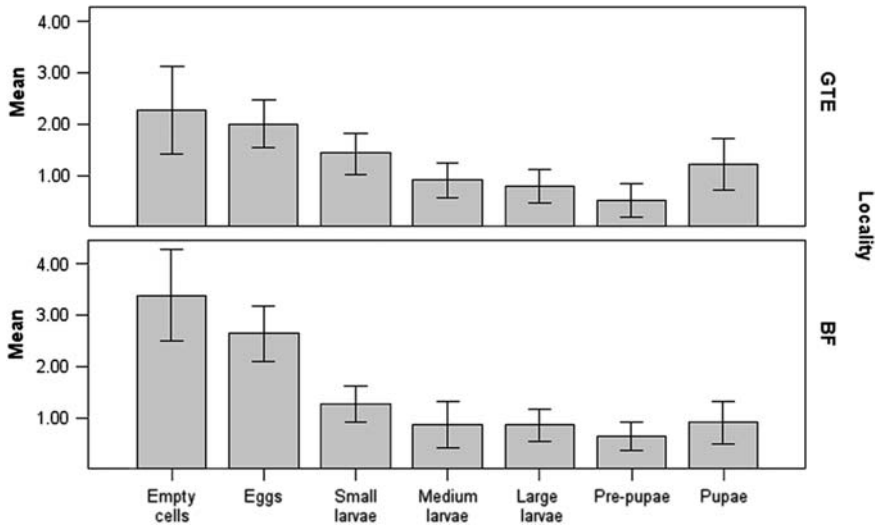


Figure 4. Number of cells and contents of nests collected at GTE ( $N = 35$ ) and BF ( $N = 49$ ).

$R = 0.316$ ,  $P = 0.003$ ,  $N = 84$ ; male larvae:  $R = 0.286$ ,  $P = 0.008$ ,  $N = 84$ ; male pupae:  $R = 0.246$ ,  $P = 0.024$ ,  $N = 84$ ) while the number of eggs are only correlated with the number of females (Pearson's correlation test, females:  $R = 0.309$ ,  $P < 0.004$ ,  $N = 84$ ; males:  $R = 0.010$ ,  $P = 0.928$ ,  $N = 84$ ).

The distribution of the maximum head width of 262 larvae extracted from various nests collected at BF and GTE and preserved in alcohol is given in Figure 5. The distribution shows four main clusters (not statistically different from normal distributions, Kolmogorov–Smirnov test,  $Z = 0.74$ ,  $P = 0.6$ ;  $Z = 1.17$ ,  $P = 0.11$ ;  $Z = 0.93$ ,  $P = 0.32$ ;  $Z = 0.63$ ,  $P = 0.78$ ) with averages around  $0.42 \pm 0.053$  mm ( $N = 54$ ),  $0.67 \pm 0.038$  mm ( $N = 37$ ),  $0.94 \pm 0.054$  mm ( $N = 40$ ) and  $1.41 \pm 0.76$  mm ( $N = 132$ ).

#### Ovarian development

The 79.7% of females was fertilized and the average number of wasps with developed ovaries (with OI higher than 0.030) per nest was [1.92 (77.14%), see Table 1].

The distribution of the overall female OIs is clearly bimodal with a cut around 0.30 (Figure 6). The mean ovarian development in unfertilized females ( $N = 14$ ) was around  $0.428 \pm 0.28$  SD, (arbitrary units) whereas in the fertilized females ( $N = 56$ ) it was  $0.57 \pm 0.29$  SD (arbitrary units). Only 6 of 14 non-fertilized females and 9 of 56 fertilized females had undeveloped ovaries (Fisher's exact test, two tailed,  $P = 0.0687$ ). However, unfertilized wasps had smaller ovary index with respect to fertilized wasps (Mann–Whitney  $U$ -test,  $Z = 5.7$ ,  $P = 0.001$ ).

#### Visual communication

In the population of females examined for visual communication collected in BF ( $N = 112$  for a total of 49 colonies), the head width and the FMS were normally distributed (Kolmogorov–Smirnov test:  $Z = 0.886$ ,  $N = 112$ ,  $P = 0.37$ ;  $Z = 0.539$ ,  $N = 112$ ,  $P = 0.90$ , respectively, Figure 7) and correlated with each other (Pearson correlation:  $r = 0.344$ ,  $P < 0.001$ ).

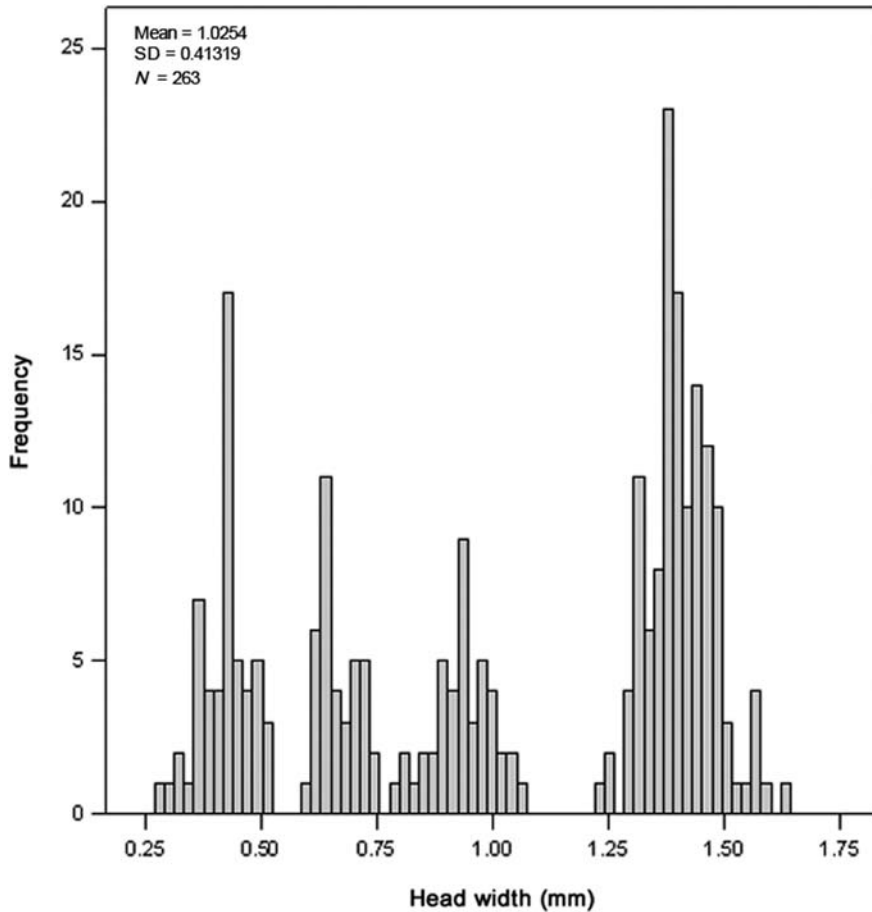


Figure 5. Distribution of the maximum head width for 263 *P. striatula* larvae collected from various nests.

When we examined the individuals with OI higher than 0.30 ( $N = 93$ ; see Figure 6), the GLM found no significant relationship of the ovarian development with FMS ( $F_{1,43} = 1.10$ ,  $N = 93$ ,  $P = 0.30$ ) or with head width ( $F_{1,43} = 0.287$ ,  $N = 93$ ,  $P = 0.59$ ; Figure 8). The colony membership, entered as a random factor into the model, had no significant relevance ( $F = 1.05$ ,  $N = 93$ ,  $P = 0.45$ ).

### ***Behavioural observations***

#### *Egg deposition and pap collection*

*Parischnogaster striatula*, like all the other Stenogastrinae, secretes a gelatinous substance (mainly produced by Dufour's gland; Turillazzi 1989) from the tip of its abdomen, which it uses to rear its brood and aid egg deposition. The sequence of egg deposition in *P. striatula*, based on direct observations of the female wasps on the nest, is very similar to that described for other species (Turillazzi 1985, 2012). After an initial inspection of the cell, the wasps collect a small quantity of abdominal substance in its mouth parts. Then, the wasp bends her abdomen, with the sting extruded, towards its mouth and collects the egg as it emerges allowing concave surface to adhere to the patch of abdominal substance

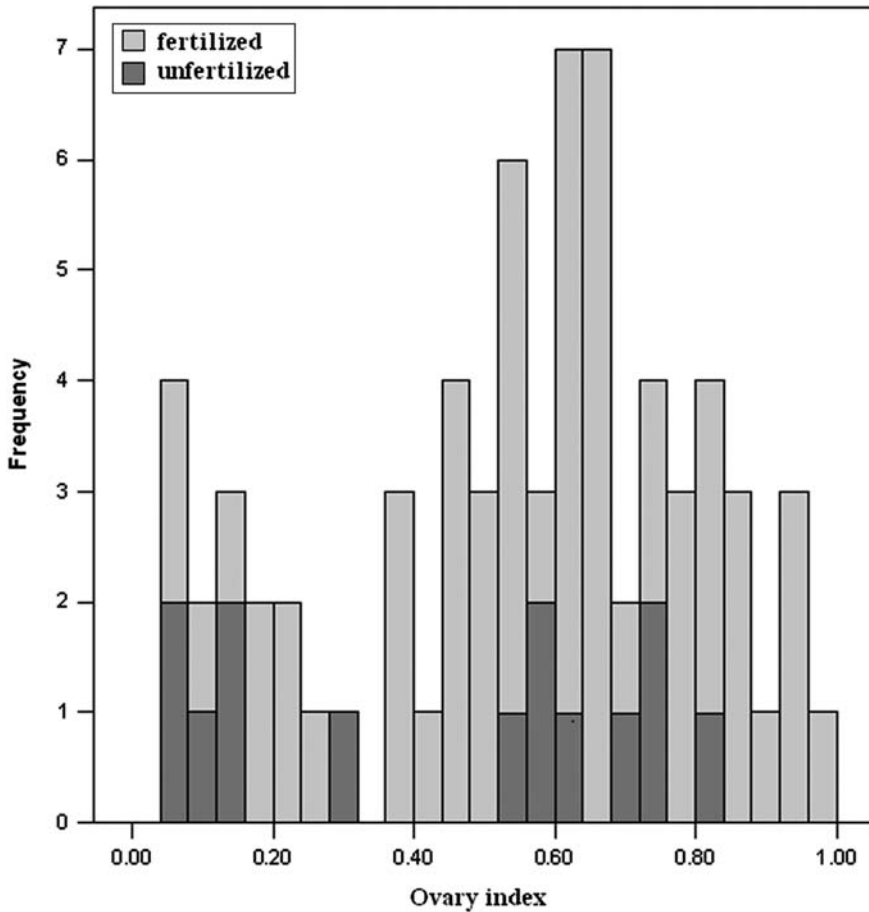


Figure 6. Distribution of ovarian index (reported in arbitrary units) in *Parischnogaster striatula* fertilized ( $N = 56$ ) and non-fertilized females ( $N = 14$ ).

it brought in its mouth parts. When the egg is expelled, the wasp does not touch it with its legs, but it places it directly onto the secreted bolus held in the mouth. The egg is then placed in the cell and stuck to the bottom by means of the secretion present on its convex surface. The first patch of abdominal substance is left on the concave surface of the egg. After placing the egg, the wasp bends the abdomen again and collects a larger patch of abdominal substance that is added to the previous patch on the egg. This last passage is often done by many wasps on the nests when they simply add pap to larvae. In all the directly observed egg depositions ( $N = 3$ ), the laying female was the dominant one (i.e. the wasp with the highest ovary index within the colony and the female that spent the majority of time on the nest, especially in the upper region of the nest). Other females beyond the dominant one add pap into the cells. In one nest, we observed a  $\beta$ -female collecting four times consecutively the pap in  $< 0.5$  h (10.00–10.30 am).

#### *Nest/off-nest activity of males and females*

Direct observation on 19 foundations located in BF from 7.00 am to 6.00 pm on two different days gave the daily off-nest activity of colony members (both males and females).

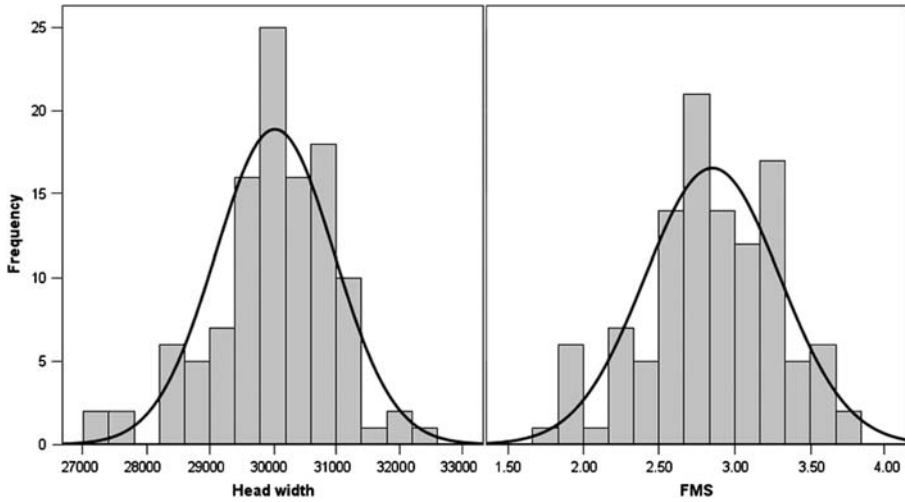


Figure 7. Distributions of head width (reported in arbitrary units) (on the left) and FMS (reported in area units, right) of 112 *Parischnogaster striatula* females.

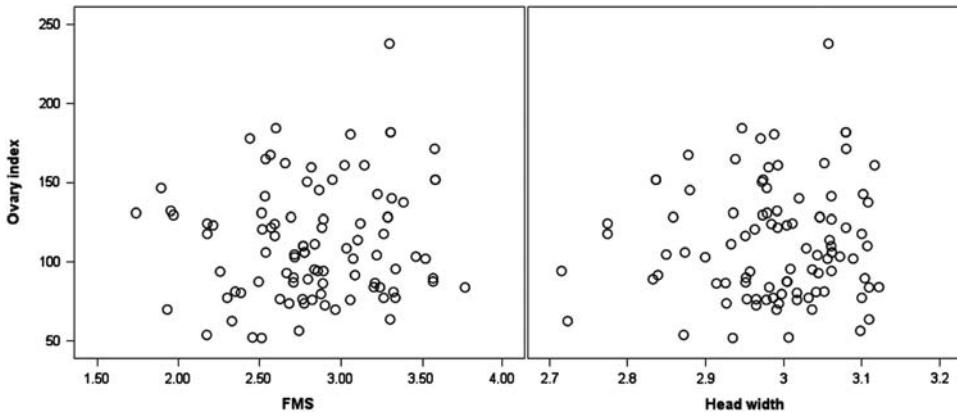


Figure 8. Correlations between ovarian development (OI) and FMS (reported in area units) in 93 *Parischnogaster striatula* females (left). Correlations between ovarian development (OI) and head width in 93 *P. striatula* females (right).

There are two distinct periods of major activity, one during the morning and one in the afternoon (Figure 9). The two peaks during the late morning and late afternoon are similar to those reported for other hover wasps (Turillazzi 1988; Baracchi et al. 2009).

Males ( $N = 18$ , belonging to 19 colonies – BF) rest most of the time near the entrance of the last cells (the lower part of the nest) except when soliciting food from returning foragers. On the contrary, females ( $N = 42$ , belonging to 19 colonies – BF) usually rest in the middle region of the nest and dominant ones rest in the upper part of the nest. The dominant female is the individual that almost always starts antennations and aggression with other returning females. Nest construction is mainly carried out by non-dominant females. It is commonly observed that wasp foragers with mud on the mouth parts start the construction of the nest soon after they return from outside. Social interactions were more frequent during the middle of the day, between the two off-nest

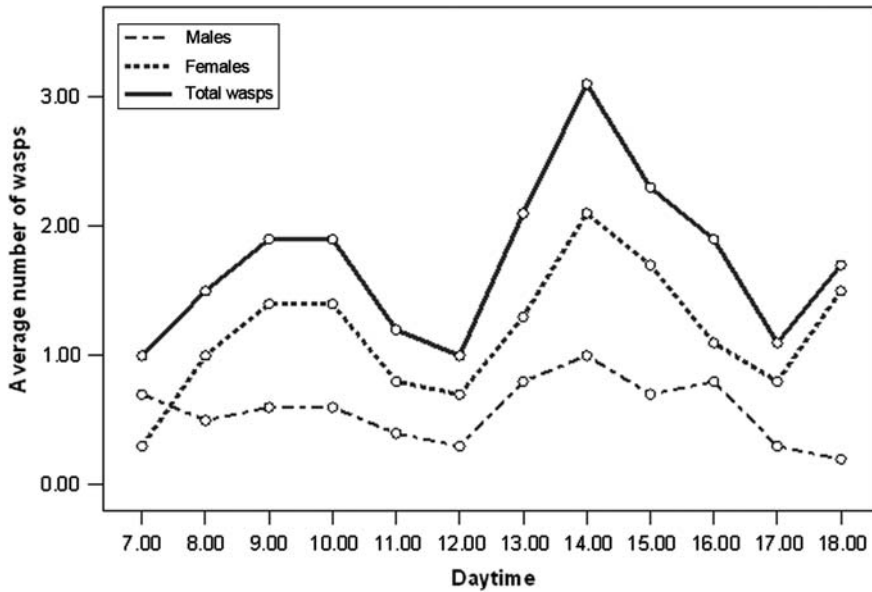


Figure 9. Daily off-nest activity of *Parischnogaster striatula* males and females reconstructed on the basis of direct observation on 19 colonies located in BF.

activity peaks, when many females return to the nest. Head-to-head contests, in which individuals push each other with their heads aided by their forelegs, occur both between females and between males and females. These sometimes develop into falling fights, in which grappling contenders fall from the nest and then immediately fly back. Females on the nest are usually very attentive to arriving wasps (foragers with food), which they seldom threaten with the abdomen bent to the side and more often with antennations and bites.

#### *Defence reaction against hornets and ants*

On average, the ants received significantly more bites and stings than hornets (Wilcoxon test: bites:  $Z = -3.72$ ,  $P < 0.001$ ,  $N = 20$ ; stings:  $Z = 2.87$ ,  $P = 0.001$ ,  $N = 20$ ). By contrast, the hornet lure triggered more abdomen bendings than ant (Wilcoxon test:  $Z = 3.92$ ,  $P < 0.001$ ,  $N = 20$ ; Figure 10). When presented with the hornet lure, nest was more often deserted by wasps (Wilcoxon test:  $Z = 2.32$ ,  $P = 0.016$ ,  $N = 20$ ) than when presented with the ant lure. No relation was found between the cumulative aggressive reactions towards both ants and hornet lures and the brood content of the nest (Spearman's correlation test, pupae:  $\rho = 0.25$ ,  $P = 0.075$ ,  $N = 20$ ; big larvae:  $\rho = 0.23$ ,  $P = 0.087$ ,  $N = 20$ ; medium larvae:  $\rho = 0.15$ ,  $P = 0.27$ ,  $N = 20$ ; small larvae:  $\rho = 0.02$ ,  $P = 0.88$ ,  $N = 20$ ).

## Discussion

*Parischnogaster striatula* presents the main behavioural and social features of the so far studied species of hover wasps: (1) small colony size, (2) use of abdominal substance in brood rearing and (3) presence of more than one potential reproductive female in the colony. Social life in the colonies revolves round one dominant individual, which has

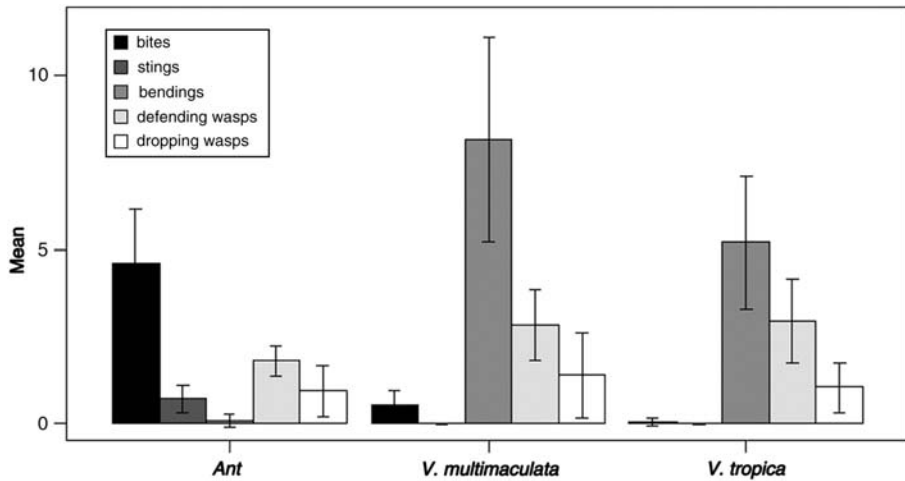


Figure 10. Defence reactions (bites, stings, abdomen bendings and dropping flights) of 20 colonies of *Parischnogaster striatula* elicited by presented hornet (*Vespa multimaculata* Perkins, 1910 and *Vespa tropica* Linnaeus, 1758) lures and ants.

well-developed ovaries and spends most of her time on the nest. Other individuals are away for most of the time and act as foragers; these females, however, do not necessarily have poorly developed ovaries and for the most part are fertilized. In *P. striatula*, 79.7% of females are inseminated; this last percentage is quite near to that found in *Parischnogaster jacobsoni* (du Buysson, 1913) (70%) but very different to those found in *P. alternata* (51%) and in *P. nigricans serrei* (37%). Most of the fertilized females have large ovaries, but ovarian development in some of them (8 out of 14) is comparable to that of non-fertilized females. No correlation was found between ovarian development index and facial marking, suggesting that a visual status badge does not exist in this species, which is different from *L. vechtii* where a status badge was recently described (Baracchi, Petrocelli, et al. 2012). Further experiments will be, however, necessary to exclude the possibility of the existence of visual cues used for nestmate or individual recognition among colony members as found in *L. flavolineata* (Baracchi, Petrocelli, et al. 2012). The presence of various females with developed ovaries in several colonies examined is probably a widespread feature in the Stenogastrinae and, in particular, this seems the rule in the genus *Parischnogaster*. However, in *P. alternata* and in *P. mellyi*, colonial reproductive skew is almost always complete as only one female reproduces at a time (Fanelli et al. 2005; Bolton et al. 2006). Owing to the limited size of the colonies of *P. striatula* and despite the high percentage of females with developed ovaries, we could expect that also in this species only one female per colony is actually laying eggs (Fanelli et al. 2005; Bolton et al. 2006). The fact that the reproductive skew may be high is also supported by the low number of eggs and by the presence of at least an equal number of empty cells found in the analysed colonies, as it occurs in the other mentioned species. All these findings suggest that in the hover wasps, helping behaviour is probably required for a successful brood breeding and females may choose to remain as helpers on the natal nest for at least the first part of their life. As it happens in other stenogastrine species, egg-laying females add their Dufour's gland secretion to the just laid eggs (Hansell et al. 1982; Hansell 1984), but then other females can add their own secretion to the eggs and small larvae. In agreement with previous results in *P. mellyi* (Turillazzi et al. 2008), the

findings of this research suggest that also in colonies of *P. striatula* individuals are probably not able to discriminate between eggs covered by the pap produced by their nestmates or by alien females leaving open the problem of the actual cues used for the formation of the template necessary for nestmate recognition.

The difference in the number of females per nest between colonies from the BF and GTE is interesting as it could be related to differences in climate in the two localities (1000 and 610 m a.s.l., respectively). A similar situation was already found in *L. vechti* where colonies studied at both GTE and BF sites (Turillazzi 1988) differed for the number of potential egg layer females per colony, suggesting that the ecology could influence the helping behaviour. In particular, despite the number of females correlates with the content of the nest (i.e. brood), the number of females is the only aspect in which colonies of *P. striatula* from GTE and BF sites differed. This finding could suggest that BF is not an optimal site, and a helping behaviour among females is required to obtain the same result in terms of brood productivity. Indeed the number of empty cells is quite lower (even if not significantly) with respect to that found in GTE colonies. A possible explanation could also be to the fact that an extraordinary intense predation pressure by hornets was observed at BF (pers. observation).

Hornets and ants are the principal predators of hover wasps. The main defensive strategy used by *P. striatula*, like all other stenogastrinae wasps, is represented by the architecture of their nests. Nests are always small in size and highly mimetic. In some species nests are defended by a sticky ant-guard substance, but this is not the case for *P. striatula*. Active defence includes “dropping flights” from nests and attacks against approaching conspecifics and fierce aggression against ants and conspecifics found on nest.

The results of our experiments showed that females on the nest change the defence strategy depending from the predator. In particular, ants are rejected and attacked with bites, while hornets are mainly faced with the bending posture of the abdomen, although the dropping flight seems often the best strategy. Accordingly, during our studies in BF site, we observed several direct attacks of hornets that usually resulted in the destruction of the nests and the predation of a great part of the brood, without causing the death of any adult wasp.

Finally, data on the larval stages for *P. striatula* confirm previous findings in other genera of Stenogastrinae including *L. flavolineata* (Samuel, 1987), that is there are only four larval instars. Although Kojima (1990) hypothesizes that the Stenogastrinae have five larval instars (with the first instar inside the chorion, being the head width of the first instar larva smaller than the egg diameter) and insists on more accurate measurements, no evidence for a further instar has been found in this study.

Our study elucidated many fundamental features of the social life of *P. striatula* adding knowledge on these elusive and poorly studied wasps.

Future long-term observations should address other key aspects of the social biology of this species, such as the type of nest foundation, the presence of alternative reproductive strategies and the presence of a permanently sterile female caste. Studies on population dynamics focusing on colonies and on the relatedness between different individuals should furnish important information on the behavioural evolution of this species and could clarify the evolution of sociality in the hover wasps.

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