DOTTORATO DI RICERCA IN ETOLOGIA, ECOLOGIA ANIMALE E ANTROPOLOGIA

(XXVI CICLO)

The recognition system in social wasps: morphological traits, chemical cues and visual signals

Tesi di

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(2013)

CONTENTS

1. Summary

The ability to recognize and classify conspecifics is associated with the evolution of social behaviour. Individuals belonging to a given group usually cope with collective and expensive investment in resources located in the colony and all possible exploitations of such resources by unrelated individuals must be avoided. Accordingly, almost all social groups are capable of complex forms of recognition and social insects are no exception in this regard. Recognition can be carried out by different sensory channels, in insects mainly through olfaction and vision.

In this thesis I explored the recognition system of different social wasps belonging to Polistinae (*Polistes* genus) and Stenogastrinae subfamilies, focusing on different aspects: 1) morphological and behavioural adaptations related to an efficient olfactory recognition, 2) visual recognition abilities in different taxa and cohorts of individuals, 3) integration between olfactory and visual cues in the recognition process.

In the first section I studied the functional variability of an abdominal gland (Van der Vecht's organ) used for marking the nest by dominant individuals of *Polistes* wasps. I found that the studied European species show invariably a different developmental trajectory in the size and the functionality of this gland in queens and workers. Queens, which monopolize reproduction within colonies, are equipped with a more efficient organ with respect to workers. Van der Vecht's organ is also involved in the camouflage strategies of *Polistes* social parasites which have to enter and exploit the colonies of their con-generic hosts in order to reproduce. The parasites *Polistes sulcifer* and *Polistes semenowi* - in need of a quick camouflage to be accepted as dominants by the host's workers next to emerge at the time of usurpations - show an enlarged abdominal gland. By contrast the social parasite *Polistes atrimandibularis*, specialized on a mountain host with a delayed workers' emergence, showed a slow chemical integration and a regressed gland. In the genus *Polistes* selective pressures toward an efficient chemical rank recognition affects the functionality of the morphological traits associated with olfactory recognition causing an incipient divergence between castes and parasites and their hosts as well.

In the second section I studied visual cues and ther involvement in recognition process of two species of tropical hover wasps (Stenogastrinae) and the solitary founding European paper wasps *Polistes gallicus.*

I found that facial pattern represent a visual signal used by nestmate to recognise individuals as own group members in *Liostenogaster flavolineata*. In this species olfactory recognition, although existing, seems to be almost neglected at least in the nestmate recogniton context. In *Liostenogaster vechti* facial pattern is a reliable and honest signal of status and conveys information on agonistic ability. Indeed dominant individuals bear high ranked facial patterns, gain reproduction but deal with social costs. Visual cues seems to be

effective and reliable signals useful for a quick recognition in small groups characterized by a primitively eusocial behaviour such as Stenogastrinae wasps. I finally demonstrated that queens of *P. gallicus* are able to use facial pattern - which is linked with body size and possibly with competitive abilities - in order to modulate their level of aggression while facing potential usurper conspecifics. Strikingly, workers from the same species ignore facial patterns both in the context of rank inheritance within orphaned nest and rival assessment out of the colony. Despite that, queens with high quality facial patterns also produce workers with high quality facial pattern. Therefore, visual communication maintains its reliability in some *Polistes* species, especially during the early foundation phase and the ability to use such cues seems to be caste biased.

2. Introduction

The recognition system in social wasps

Social wasps are capable of multiple forms of recognition which are essential for living in a group. Nestmate recognition (i.e. the ability of a given individual to discriminate between own group members and outsiders) is widespread in social Hymenoptera (Gamboa et al, 1986a). Indeed, the ability to recognise nestmates is a key feature of social insects and it aims at avoiding intrusions by other conspecific individuals which could exploit, parasitize or prey colony resources. Some social wasps are also capable of more complex kinds of class-level recognition: status recognition is used to assess hierarchical rank, quality, fertility or agonistic ability of conspecific females (Downing and Jeanne, 1985; Sledge et al, 2001; Tibbets and Dale 2004; Tibbets and Sheehan 2011). while a recent study showed that some species could be also capable of kin recognition (Dani et al, 2004). Recognition systems in social wasps are mainly based on two different sensory channels, the olfactory and the visual one.

Chemical recognition in paper wasps

Recognition among social wasps mainly bases on chemical stimuli: in particular epicuticular hydrocarbons, which form a film on the outermost layer of the cuticle, are useful to reduce the loss of water and responsible for recognition in almost all social insects (Holldobler and Michener, 1980; Espelie et al, 1990; Ruther et al, 2002; van Zweden and d'Ettorre, 2010). Individuals belonging to a particular colony shared a similar odour that differed from those of alien individuals.

In paper wasps the chemical signature of a given colony is detectable on the nest surface and is responsible for nestmate recognition (Dani et al, 2001). Actually, the dominant female smears large amounts of its own cuticular chemical signature on the nest surface using specialised abdominal exocrine glands (Dani et al, 1992, 2003, Singer and Espelie, 1992). Newly emerged individuals form their template on the specific odour of their own colony in the first phase of their life, becoming later able to recognise individuals as familiar or unfamiliar (Gamboa et al, 1986b).

Chemical nestmate recognition is also found in primitively eusocial wasps belonging to the Stenogastrinae subfamily (Cervo et al, 1996; Zanetti et al, 2001; Cervo et al, 2002). In some of these species chemical cues also act as fertility signals and are associated with hierarchical rank (Turillazzi et al, 2004).

Epicuticular hydrocarbons are also used by paper wasps as pheromones, operating as fertility signals and being involved in rank recognition. For example, *Polistes dominula* co-foundresses show qualitative and quantitative differences in their chemical signature which is associated with hierarchical rank (Sledge et al. 2001; Dapporto et al. 2004). Furthermore the presence of a reproductively dominant female with its own chemical signature smeared on the cuticle and on the nest surface, has a major role in the ovary suppression of subordinate females (Dapporto et al, 2005; Dapporto et al, 2007a,b).

It is thought that social parasitism is the strongest selective pressure towards the selection of recognition abilities in social insects. Social parasites are species that have to exploit to various extents the colonies of their hosts in order to reproduce. Social parasitism is common in ants, wasps and bees and represent a peculiar case of brood parasitism in which parasites not only exploit the brood care behaviour, but also many aspects of the social system of another social insect (Brandt and Foitzik, 2004; Scharf et al, 2011). Parasites actually break up the chemical recognition system of their host to enter the colony and often show physiological, morphological and behavioural adaptations useful for an efficient sensory deception of the host. In this regard, social parasites of *Polistes* paper wasps have been extensively studied (Cervo, 2006).

Therefore, social wasps and social insects in general mainly communicate by means of olfaction which is a very effective and reliable way of communication, especially when the colony size increases a lot.

Visual recognition in paper wasps

In the last decade a huge amount of literature showed that *Polistes* paper wasps are also able to use visual communication. Tibbets (2002) proved that females *Polistes fuscatus* are able to visually recognise their nestmates as individuals using individual identity signals, such as facial and abdominal markings. This is the unique case of visual individual recognition among insects. Despite that, at least two species of *Polistes* (*P. dominula* and *Polistes exclamans*) show a visual badge of status based on the melanin facial markings which correlate with fighting and agonistic abilities (Tibbets and Dale 2004; Tibbets and Sheehan 2011). This is a conventional quality signal used for the assessment of agonistic ability during dyadic encounters among females or during the establishment of hierarchies within associative foundations. Such signals are thought to be honest since they are maintained via social costs: individuals displaying high quality have to withstand increasing aggressions by opponents due to their own signal (Rohwer 1975, Maynard-Smith and Harper 2003). As a consequence, low quality individuals, are unable to cope with an increased rate of aggression and fail in cheating on their actual status. Further evidences showed that badge development in *P. dominula* is essentially resource-dependent (Tibbets and Curtis, 2007). Individuals that received a surplus of food during larval stages showed high quality facial pattern (Tibbets and Curtis, 2007). By contrast, workers of the same species with low quality facial patterns probably received less food during larval stages with respect to potential queens (Tibbets, 2006).

Since conflicting results emerged, especially among different *P. dominula* populations, conventional signals of agonistic ability in paper wasps are still a highly debated issue. Indeed, no correlation was found between facial pattern and social rank in associative foundations of both Italian and Spanish populations of *P. dominula* (Cervo et al. 2008; Zanette and Field, 2009, Green et al, 2013). Some recent works suggested that these controversial results might be due to different climatic conditions experienced by different *P. dominula* populations. Increased expression of melanin on the cuticle is actually linked with a more efficient thermoregulation within populations experiencing colder climates. As a result *P. dominula* populations living in warm climates (i.e. Italy and Spain) do not show enough variability to favour the evolution of a status badge, which is more likely to evolve in populations inhabiting colder climates (Tibbets et al. 2011; Green et al. 2012).

An intrasexual conventional signal has been also found in an hover wasp (Stenogastrinae): in males of *Parischnogaster mellyi,* the abdomen stripes display performed during patrolling behaviour is a conventional signal of agonistic ability and it is also associated with mating success (Beani and Turillazzi, 1999).

Visual communication in social wasps is a recent line of research and further studies are required to clarify some neglected aspects, such as the influence of visual cues on linear hierarchies and on social organization in natural condition, their development mechanisms and their adaptive value.

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3. Aims and organization of the thesis

This PhD thesis concerns communication and recognition abilities among different groups of social wasps focusing on olfactory and visual cues. The primary aim of my PhD was to deepen the knowledge about some complex recognition abilities shown by social wasps focusing on different models and social contexts. I studied recognition in wasps at two different levels of investigation. In the first part of my thesis I studied morphological adaptations related to the efficient olfactory recognition in *Polistes* paper wasps. In the second part I studied different kinds of visual recognition abilities in both Stenogastrinae and Polistinae (i.e. *Polistes* genus) wasps using a behavioural approach. One more aim of the second section has been to understand the relationship between olfactory and visual recognition at different degree of sociality.

In the first section of my thesis (chapters IV, V and VI) I measured the wide size variability (i.e. the differences in functionality) of an exocrine organ involved in secreting hydrocarbons within European *Polistes* species. This organ is located in the abdomen of Polistinae and Vespinae female wasps and is called Van der Vecht's organ. Such a morphological trait is strictly linked with olfactory recognition in *Polistes* paper wasps as it is used by dominant individuals and social parasites to actively mark the comb and to be recognized as reproductively dominant. In chapters IV and V, I compared the relative size and the shape of the Van der Vecht's organ between queens and workers in five European *Polistes* species, with the aim of investigating differences in the functionality of this trait between reproductive and non (i.e. queens and workers), due to their different roles within social groups. Such a divergence would also point out an incipient caste bias within *Polistes* genus which is traditionally thought to show very plastic castes.

In chapter VI, I compared the relative size of the Van der Vecht's organ between *Polistes* social parasites and their respective host species to investigate possible host-parasites coevolutionary differences in the functionality of this organ and to clarify the relationship between behavioural and morphological adaptation observed in several parasites species.

The second section of my thesis (chapters VII, VIII, IX and X) concerns some case studies of visual recognition in three species belonging to Polistinae and Stenogastrinae subfamilies.

In chapter VII I studied the variable facial pattern of the solitary founding European *Polistes gallicus* to investigate whether this is a conventional signal associated with agonistic ability, useful for rival assessment among queens attempting nest usurpations. In chapter VIII, I also studied facial pattern variability in workers of the same species to investigate whether this could be also associated with competitive abilities in this caste (i.e. in the context of rank inheritance within orphaned nest or rival assessment out of the colony). Since conventional signals in paper wasps are thought to be environment-dependent while genetic influences are excluded, I also check for any correspondence, both in wild and laboratory reared colonies, between the quality signals of queens and that of their worker daughters. Visual recognition abilities among *Polistes* workers are traditionally neglected as researchers usually focus on queens: however, focusing on workers gives the opportunity to deep the knowledge of status signalling in advanced stages of the colony cycle and to investigate open questions about badge development.

In chapter IX and X, I finally studied visual communication in two species of tropical hover wasps belonging to Stenogastrinae subfamily, *Liostenogaster flavolineata* and *Liostenogaster vechti*: these amazing South-East Asian wasps are characterized by small colonies (counting no more than a dozen of inhabitants), often built in huge aggregates of hundreds of nests, in which females are organized in a linear hierarchy of dominance..

Both these two species show high variable facial patterns: in chapter IX I investigated possible roles of face recognition in determining social organization in *L. vechti* and *L. flavolineata*. We first check for correspondence between facial pattern and social rank in wild colonies, then, based on the results obtained, we tested whether (1) *L. vechti* females use their facial markings as quality signals to assess the value of conspecifics, and (2) *L. flavolineata* females are able to use facial markings to recognize visually nestmates.

In a second instance I further investigate the nestmate recognition process in *L. flavolineata.* In chapter X I study how chemical and visual senses are combined during the perceptual processing and to which extent wasps prioritize one channel over the other to discriminate conspecifics. Recognition ability is one of the most intriguing feature of social insects and a full understanding of both the behavioural dynamics and the perceptive mechanisms underlying discrimination process is a crucial goal for sociobiology. Furthermore using different models and a comparative approach also allows to delineate the extent to which recognition abilities are affected by the degree of sociality, by the different eco-ethological context and by the life history of insect colonies .

4. Incipient morphological castes in *Polistes gallicus* (Vespidae, Hymenoptera)

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Zoomorphology, **130** (2011), 197-201.

Abstract

The occurrence of a pre-imaginal caste determination represents a sort of "point of no return" to eusociality. In some social insect taxa, including *Polistes* species, the occurrence of distinct queen and worker castes is still debated. Before this report, no clear morphological divergences between reproductive and non-reproductive individuals were known. Here we show that several pre-imaginal morphological differences of the Van der Vecht organ occur between foundresses (queens) and workers. Geometric morphometrics revealed that queens are characterized by shape deformations of this organ, which is responsible for a typical allometric growth of the secretory area. This organ is predicted to be larger in foundresses compared to workers because its secretion is involved in defense against ants, in nestmate recognition and in preventing workers from challenging for direct reproduction. The results presented here indicate the existence of an incipient morphological caste determination *Polistes gallicus* and suggest that this species may have passed the "point of no return" for eusociality.

Keywords: *Polistes gallicus*, Pre-imaginal caste determination, Van der Vecth organ, Geometric morphometrics, Allometry

Introduction

The occurence of morphological castes of high phenotypic diversity among individuals with low genotypic differences is one of the most spectacular characteristics of social insects (Darwin 1859; Wilson 1971). Yet, the development of individuals before the adult stage (pre-imaginal) into different castes is not universally recognized as a prerequisite for identifying eusociality. One widely accepted definition indientifies as eusocial insects those species with two or more overlapping generations, cooperative care of the colony's young and the reproductive division of labour (Wilson 1971). According to this definition insect taxa characterized by individuals relinquishing direct reproduction for a part or over their complete lifespan should be considered as eusocial even in the absence of morphological castes. These species would include some Ponerinae (Formicidae), Stenogastrinae (Vespidae) and Halictidae, which are primitively eusocial for some authors (e.g. Batra 1966).

Other authors suggest that the clear occurrence of castes, together with the division of labour, should be considered as a third condition (see for example Wilson and Hölldobler 2005; Boomsma 2007).

In some taxa the occurrence of clear pre-imaginal caste determination is still debated. *Polistes* species are a typical example and show intermediate characteristics (Dapporto et al. 2005; Starks and Turillazzi 2006; Hunt et al. 2010). At temperate latitudes, future foundresses emerge and mate in late summer. Then after overwintering at the adult stage they found new colonies in spring (Reeve 1991). Foundresses rear their first cohort of offspring, which are usually called "workers", despite the fact that there is no apparent morphological diversification other than smaller size (Dani 1994). In the following months, the size of emerging females gradually increases until they are recognized as future foundresses (reproductive individuals) (Reeve 1991). In most species, there is not a clear size cut off between foundresses and workers which are also characterized by a behavioural plasticity, which allows them, in some cases, to mate and to found new colonies (Strassmann 1981; Reeve et al. 1998). In the absence of a clear morphological diversification many authors consider the capability to overwinter as the best discriminatory characteristic (e.g. Strassmann 1985; Solís and Strassmann 1990; Hunt and Amdam 2005). Overwintering and successive oogenesis clearly require a set of physiological characteristics and it is well known that foundresses possess well developed (multistratified), fat bodies, which are usually reduced in workers (e.g. Eickwort 1969; Toth et al. 2009). Moreover, Hunt et al. (2003, 2007, 2010) showed that by comparison individuals emerging in summer are characterized by differential gene expression, prolonged larval development and the presence of hexamerinae proteins.

Recently, another trait linked to caste diversification may have been found in the chemical secretions of *Polistes gallicus* (Dapporto et al. 2007). Females of Vespinae and Polistinae species possess particular glands associated with the so called Van der Vecht organ (VdVo), which is visible as a hairy, slightly sclerotized cuticular area at the anterior edge of the last gastral sternite (Fig. 1). The ducts of numerous tegumental glands are clustered in two lateral masses and open on the external cuticle of this area (Turillazzi 1979). VdVo was shown to be involved in the production and application of ant-repellent secretions on the nest pedicel of various species (London and Jeanne 2000; Dani et al. 2003). Moreover, in many social insects, blends of cuticular hydrocarbons function as recognition cues of reproductive individuals (Liebig 2010). In *Polistes gallicus*, a clear difference between secretion of VdVo between foundresses and workers exists, but orphaned workers maintain their original hydrocarbon signature (Dapporto et al. 2007). This finding suggested that, in *Polistes gallicus*, the chemical signature was dependent on early caste determination. These authors also showed that marking of the comb by the queen can deter workers from direct reproduction.

In this sense, it could be possible that the VdVo also shows morphological diversification between foundresses and workers and emerged in response to a particular need for queens to perform an eYcient chemical defense and a strong marking activity (Dapporto et al. 2007).

In this research, we applied both surface measurements of VdVo and geometric morphometrics to search for dimensional and shape variations, which could identify two different ontogenetic trajectories between foundresses and workers. In particular, we aimed to verify if (i) the size of the VdVo increases with wasp size and if this change differs among castes and (ii) if queens and workers show VdVo with different allometric trajectories in the shape of the VdVo.

Materials and methods

During April 2008, 21 pre-emergence colonies of *Polistes gallicus* (Linnaeus, 1761) (Vespidae, Hymenoptera) were collected in an area of about 200 km^2 around Florence. Solitary individuals collected in early spring can be identified as foundresses since workers do not survive winter and emerge in late spring. Foundresses were marked with paint at their colonies and caged in the laboratory waiting for the mergence of workers. The colonies were supplied with fly maggots, water and paper until the end of their cycle in August. The First workers are usually much smaller than foundresses but differences tend to be reduced later in the season (Dani 1994). We thus discriminated late workers from future foundresses by the absence or the presence of well developed and multistratiWed, fat bodies (e.g. Strassmann 1985; Reeve 1991; Toth et al. 2009; Hunt et al. 2010). At the end of the sampling we were able to categorize 130 individuals (51 workers and 79 foundresses). The wasps were then killed by freezing at 18°C, then the last sternite was dissected in all specimens and mounted on water between microscope slides and cover slips. The shape of the last sternite in *Polistes gallicus* is almost flat, and the distortion caused by this procedure can be considered as negligible. The sternites were photographed by coupling a bionocular microscope with a digital

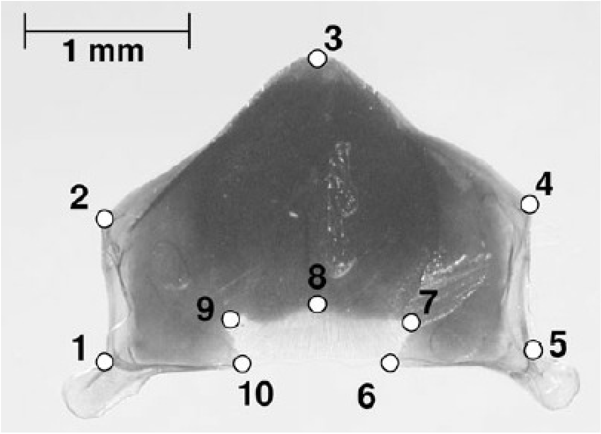

Fig. 1 The last sternite of *Polistes gallicus* and landmarks (*white circles*) considered in geometrical morphometric analyses. The Van der Vecht secretory area is enclosed by landmarks 6–10

camera. The area of the VdVo, that of the entire seventh abdominal sternite and the head, taken in frontal view, were measured with imageJ software (available at http://rsbweb.nih.gov/ij/). We log transformed the area of the VdVo and that of the sternite. Then we tested for normality of data and the homogeneity of variances as requested by successive parametric analyses. We tested if the area of the sternite could be considered as a measure of the overall size of the individuals by searching for correlation between sternite and head areas, which is considered to be one of the best measures of size in *Polistes* species (Eickwort 1969).

The area of VdVo was entered as the dependent variable in an ANCOVA using caste membership as a factor and sternite area as a covariate. The interaction between caste and sternite area was tested to verify if there is a difference in slope of the relationship between the area of the sternite and the area of the VdVo among foundresses and workers. In case of a non-significant result, the area of the sternite was entered as a controlled covariate and caste membership as a factorial predictor to test for differences in the intercepts between the two castes. We used STATISTICA to run the ANCOVA.

The geometric morphometric landmark-based approach to the description of size and shape variation was applied by using the TPS (thin-plate spline) series of softwares to recognize differences in shape (Rohlf 2009, 2010 available at http://life.bio.sunysb.edu/morph/). We recognized as landmarks ten points that can be precisely identified (type II and type III landmarks, Bookstein 1997) (Fig. 1). Points 1–5 refer to the sternite while points 6–10 refer to the VdVo. Digitalizing of landmarks on photographs was carried out using TPSDIG 2.16. Shape variables were extracted from the raw landmark coordinates using TPSREGR 1.37. Landmark configuration for each specimen were scaled to a unit centroid size and optimally aligned using a General Procrustes Analysis (GPA). The hypothesis of differential allometry between castes was tested by multivariate regression of shape variables and size (centroid size) using Wilks' lambda as a test statistic. Differences in allometries were evaluated by comparing the slopes and intercepts of multivariate regressions using partial warp scores as variables in the MANCOVA methods provided by TPSREGR. The thin plate spline was used to visualize overall shape changes according to size and caste membership.

Results

After logarithmic transformation variables did not show significant deviations from the normal distribution ($\chi2 = 3.245$, $P = 0.197$, $\chi2 = 4.480$, $P = 0.214$, $\chi2 = 5.632$, $P = 0.060$ for sternite, VdVo and head size, respectively). Variances among foundresses and workers were homogeneous (Levene stat. = 0.391, $P = 0.533$; Levene stat. = 0.759, $P = 0.385$, Levene stat. = 3.732, $P = 0.056$ for sternite, VdVo and head sizes, respectively). Sternite area was highly correlated with head area (Pearson $R = 0.960$, $P < 0.001$ for queens, Pearson R

= 0.951, P < 0.001 for workers) and thus could be used as an overall measure of wasp size. Foundresses showed a larger sternite than workers (df = 128, t = 4.337, P < 0.001). ANCOVA revealed that the relationship between sternite and VdVo size did not show any difference in slope between the two castes (sum of squares = 0.001, F = 0.584, P = 0.446). Nevertheless, a second ANCOVA, performed after verifying the non significant effect of the size £ caste interaction, revealed that the secretory area of the VdVo size is correlated with both sternite area (sum of squares = 1.690, F = 1,012.117, P < 0.001) and caste membership (sum of squares = 0.079, F = 47.250, P < 0.001) (Fig. 2).

Multivariate regression of shape on size and caste membership results indicated that queens and workers differed in allometric slopes (Wilks' Lambda = 0.793, P = 0.041). Patterns of variation linked to caste and allometric shape are shown in Fig. 3. Workers and smaller individuals show a similar reduction in the VdVo area (contraction of area comprised by landmarks 6–10) compared to the sternite area (expansion of the area comprised among landmarks 1–5) (Fig. 3). Permutation test (2000 replicates) for the regression model supports the significance of the results (permutation test, 0.1% of Lambda values less than the observed). This means that shape differences between morphs reflect more than just allometric projections of size differences (Adams and Funk 1997).

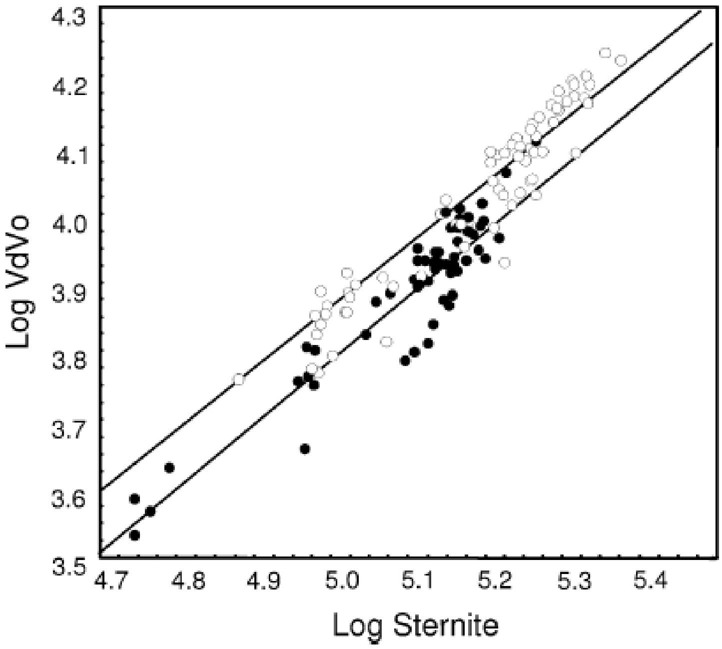

Fig. 2 The relationship between Van der Vecht organ (VdVo) and sternite areas. Foundresses, *white circles*; workers, *black circles*. The linear regressions are indicated for the two groups separately

Discussion

The results presented here indicate the existence of an incipient morphological caste determination in *Polistes gallicus*. We confirmed the tendency in this species for foundresses to be larger than workers (Dani 1994). A similar size change of the VdVo surface with respect to the sternite area would lead foundresses to have a larger secretory area than workers. Nonetheless, the existence of an effect for caste in determining the area of the VdVo emerged from the different intercepts in the sternite-VdVo relationship. Differences between foundresses and workers in the shape of VdVo were also revealed by geometric morphometrics. Larger individuals tend to show shape changes which increased the area of the VdVo compared to the sternite. One hypothesis is that this variation could be accounted for simply by the larger size of foundresses with respect to workers. However, the allometric relationship also showed different slopes revealing that workers and foundresses have different developmental trajectories, with foundresses having larger secretory area with respect to sternite area.

All together, our results suggested that overall size differences among individuals produced an immediate and strong effect on the emergence of a diversification between the two castes and it also represent the main determinant for the size of the VdVo. However, the advantage of a large secretory area for foundressess has probably favoured the evolution of different developmental trajectories between foundresses and workers (different intercept in the VdVo-sternite relationship and different slopes in the multivariate regression of shape on size) conferring a further increase of VdVo secretory area.

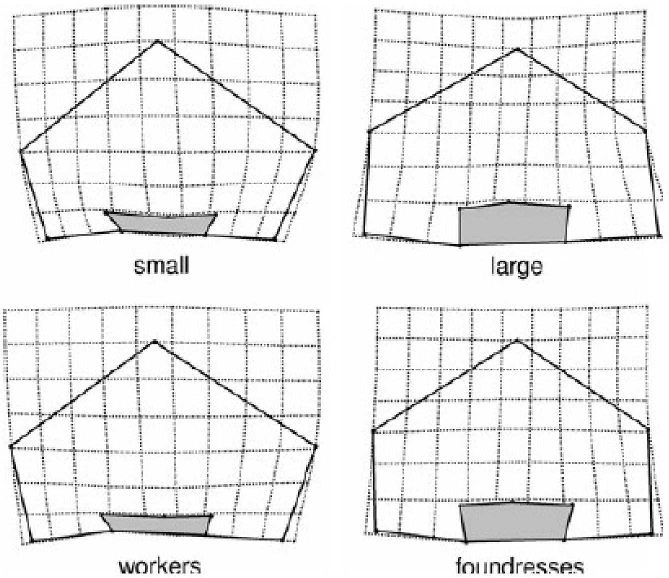

Fig. 3 *Right* deformation grids for shapes predicted by the multivariate regression of the VdVo shape onto size for the smallest and largest specimens and onto caste between foundresses and workers

During the colony cycle, the first function of the VdVo secretions are to protect the comb nest from ant attacks (London and Jeanne 2000). A high efficiency of such secretions is particularly important for foundresses that are the sole defenders of the colonies in spring. VdVo sections are smeared on the comb (stroking behaviour) by a behavior that involves rubbing the abdomen on the nest paper (Dani et al. 1992; Van Hooser et al. 2002). After workers emerge stroking is performed at the highest frequency by the queen. It was shown that individuals react more aggressively toward secretions belonging to alien conspecifics compared to nestmates (Dapporto et al. 2007). This fact provides evidence that VdVo secretions contribute to the reference colony odour, learned by workers, when they emerge, as a template for nestmate recognition (Cervo and Turillazzi 1989; Gamboa 2004). Finally, foundresses and workers have different VdVo secretion compositions (Dapporto et al. 2007). These differences suggest that a third function of secretions is as a primer pheromone (queen signal). Actually, in orphaned colonies, workers start to lay their own eggs in a few days. However, if the queen is removed from the colony, but allowed to stay alone on the nest for 2 h a day, egg deposition by workers is delayed due to the queen's stroking activity on the nest (Dapporto et al. 2007).

In similarity to the morphological differences we report here, it has been found that chemical profiles of VdVo secretions in orphaned *P. gallicus* workers do not match those of the former foundresses, but maintain a worker specificity even after the development of ovaries and the beginning of the egg laying activity (Dapporto et al. 2007). It is interesting to note that a similar phenomenon does not occur in the congeneric and sympatric *P. dominulus* (Christ, 1791) where workers' hydrocarbon blends depend on a combination of factors tied to social status (Dapporto et al. 2005). It is also noteworthy that *P. gallicus*, contrary to many *Polistes* species, is characterized by colonies invariably founded by a single foundress. *Polistes* foundresses are usually inseminated by a single male (Strassmann et al. 1989).

The monogyny of foundation in *P. gallicus* results in a high relatedness of workers with the other colony members (foundress, future foundresses and males); the highest among 13 congeneric species (Strassmann et al. 1989). It can be hypothesized that the high intra-colonial relatedness could have allowed *P. gallicus* to pass the "window to eusociality" (Boomsma 2007), an evolutionary transition which is predicted to occur when a monogamous and monogynic species has a Hamiltonian benefit-cost ratio equal to 1. This window to eusociality usually represents a "point of no return" showing that the role of relatedness in shaping social organization largely prevails over other factors (Wilson and Hölldobler 2005). According to most authors, the best marker indicating that a species passed the "point of no return" is the occurrence of a morphologically distinct worker caste (Wilson and Hölldobler 2005; Boomsma 2007). We demonstrated here that *Polistes gallicus* shows incipient characteristics of advanced eusocial species in an important characteristic involved in nest defence and chemical communication. Intriguingly, differences in social organization among various *Polistes* species (as we suggested for *Polistes dominulus*) could be responsible for diverse outcomes in similar species. Future comparative research might reveal if this genus actually lies astride the "point of no return" for eusociality.

Acknowledgments

Research performed with funds from theUniversity of Firenze (60% Dept of Evolutionary Biology and Centro di Servizi per la Spettrometria di Massa (CISM) of the University of Firenze). We thank Thomas Bartolomaeus, Andrea Cardini and two anonymous referees for improving an early version of the manuscript and Prof. Roscoe Stanyon of the University of Firenze for the revision of the English text.

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5. The morphology of Van der Vecth's organ as a tool to measure caste dimorphism in *Polistes* paper wasps: a comparative approach

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Journal of Zoological Systematics and Evolutionary Research, **51** (2013), 274-278

Abstract

Given the centrality of chemical communication in social insects, there are many selective pressures acting on morpho-functional traits that mediate chemical pheromones. On the last gastral sternite of *Polistes* females, there is an important exocrine surface secreting chemical pheromone, named Van der Vecht's organ. It is involved in chemical defence of the nest, in rank and nestmate recognition, preventing workers from direct reproduction. Allometric differential growth of phenotypic traits between castes of social insects is generally considered as an indication of incipient physical castes. European *Polistes* present different nesting strategies and reproductive choices. Here, we carry out a comparison of Van der Vecht's organ size between castes of four European *Polistes* to provide a general measure of dimorphism. We show that Van der Vecht's organ of *Polistes dominula* and *Polistes nimphus* foundresses shows an allometric development being enlarged with respect to workers. Otherwise, no allometries have been highlighted in the other two studied species (i.e. *Polistes associus* and *Polistes biglumis*). Therefore, our data show that neither rigid monogyny nor specific nesting habits foster the evolution of true morphological castes in primitively eusocial taxa. Thus, at least two other species of Ezuropean *Polistes* show real evidence of incipient morphological castes.

Key words: Social wasps – geometric morphometrics – morphological castes

Introduction

The evolution of castes and their polymorphism is one of the most intriguing aspects of social biology of insects. A caste is a subgroup of colony members, genetically but not physiologically for anatomically homogeneous, that specializes on particular tasks for prolonged periods of time (Wilson 1979). In primitively eusocial taxa, such as some Ponerinae ants and Stenogastrinae wasps, morphological castes lack and reproductive and helping individuals only show divergent behavioural patterns (Jeanne 1995). Within the genus *Polistes*, both the presence of preimaginal differentiation and morphological castes is still debated (reviewed in O'Donnel 1998). Recent researches showed that differential genes expression prolonged larval development and the presence of storage hexamerinae proteins, clearly typify foundresses from workers (Hunt et al. 2003, 2007, 2010; Toth et al. 2009).

At temperate latitude, *Polistes* paper wasps present a common nesting strategy. Future foundresses and males emerge in late summer and mate. During spring, after winter diapause, one or more foundresses found a new colony and raise their first generation of workers (Reeve 1991). European *Polistes* present different nesting strategies and reproductive choices. Poligyny is common in *Polistes dominula* (Pardi 1948), *Polistes nimphus* (Cervo and Turillazzi 1985) and *Polistes associus* (Petrocelli I and Turillazzi S, pers. obs.) but has never been observed in *Polistes biglumis* (Lorenzi and Turillazzi 1986). The last one is a strictly mountain species nesting over 1000–1200 metres on rocky outcrops along pastures (Lorenzi and Turillazzi 1986). *Polistes associus* (Petrocelli I and Turillazzi S, pers. obs.) and *P. nimphus* (Cervo and Turillazzi 1985) frequently nest on shrubs and saplings, but both are also found on buildings.

Epicuticular hydrocarbons (CHCs) are status-dependent in *Polistes* wasps, being moulded by social dominance; accordingly, the presence of an egg-layer dominant female with its characteristic chemical signature is clearly involved in the ovary inhibition of subordinates (Dapporto et al. 2007a,b). Actually, the dominant female smears large amounts of its own cuticular chemical signature on the nest surface using Van der Vecht's organ (VdVo) during abdominal stroking behaviour (Dani et al. 1992, 2003). Van der Vecht organ is a hairy, hyaline cuticular area on the anterior edge of the last gastral sternite of Polistinae and Vespinae female wasps which is in connection with several tegumental glands (Post and Jeanne 1980). Van der Vecht organ size seems to be in relation with an increased production or with a more efficient smearing of secretion on the nest surface (see also Post and Jeanne 1980). Van der Vecht organ secretion is a complex blend of hydrocarbons which is applied on the nest pedicel during an abdominal rubbing for its ant-repellent effect (London and Jeanne 2000). However, the dominant female applies it on the nest surface to induce dominance recognition by subordinates and inhibit their ovary development (Dani et al. 1992, 2003; Dapporto et al. 2007a,b).

A recent work showed that in the solitary nest founding *Polistes gallicus*, a clear chemical difference in the secretion and a dimensional divergence of the VdVo between foundresses and workers exist (Dapporto et al. 2007b, 2011). Foundresses of *P. gallicus* show an ipermetric allometry of VdVo (Stern and Emlen 1999; Shingleton et al. 2007) (i.e. an increased functionality) with respect to workers as a response to the need of an efficient chemical defence of the nest and dominance recognition by workers; as a consequence, incipient morphological castes have been proposed for this species (Dapporto et al. 2011). Furthermore, a high variability of VdVo size is widespread in *Polistes*, and it has been showed in *Polistes* social parasites as a response to the adaptation on their congeneric host species (Petrocelli and Turillazzi 2013). Actually in social insects, the presence of static allometries between castes (i.e. an intraspecific scaling relationship between individuals), particularly in the growth of some abdominal anatomical structures, has been pointed out as a good indicator of morphological castes (O'Donnel 1998; Stern and Emlen 1999; Shingleton et al. 2007).

Here, we carry out a comparison of VdVo size among foundresses and workers of four European *Polistes* using the same approach used for *P. gallicus* in Dapporto et al. 2011; to investigate the degree of caste dimorphism among the genus. Whether specific characteristics, shared by a group of species, precede the evolution of physical castes, we predict to find a similar pattern in species more closely related with *P. gallicus* (i.e. *P. biglumis* and *P. associus*; Carpenter 1997) and a different one in species more distant (i.e. *P. dominula* and *P. nimphus*). Our study could thus provide a general measure of dimorphism between castes of such a key genus and could point out the ecological and social constraints driving this phenomenon.

Material and method

Four species of Polistes from central Italy (Tuscany) were analysed: *P. dominula* Christ, 1791, *P. nimphus* Christ, 1791, *P. associus* Kohl, 1898 and *P. biglumis* Linnaeus, 1758. Specimens were collected in the field during Spring and Summer 2011 and 2012, at the beginning and at the end of the colony cycle, to have overwintered foundresses, workers from different generations and future foundresses. In temperate *Polistes*, the first workers are very small in size and quite distinguishable from foundresses on the colonies, while size differences are less pronounced later in the season (West Eberhard 1969 for *Polistes fuscatus*; Haggard and Gamboa 1980 for *Polistes metricus*; Miyano 1983; for *Polistes chinensis antennalis*; Dani 1994 for *P. gallicus*). The presence/absence of multi stratified fat bodies was used to discriminate late workers from future foundresses (West Eberhard 1969; Strassmann 1985; Reeve 1991; Toth et al. 2009; Hunt et al. 2010). Thirty foundresses and

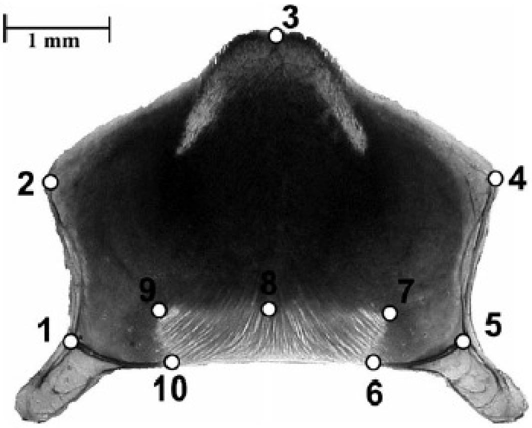

Fig. 1. The last gastral sternite of a *Polistes* paper wasp with landmarks (*white circles*), used in the geometric morphometrics analysis. The VdVo is enclosed by landmarks 6–10

workers were collected for *P. dominula*, *P. nimphus* and *P. associus*; unfortunately, only 20 foundresses and 11 workers of *P. biglumis* were available for measurements. All specimens were killed by freezing at -18°C, and the last abdominal sternite was dissected. The dissected parts were mounted on a microscope slide and photographed with a digital Fujifilm camera mounted on a binocular microscope. We measured the area in pixels of the entire sternite and that of the VdVo using IMAGE-J software (available at http://rsbweb.nih.gov/ij/); then, we log-transformed, and we performed parametric statistical tests on the measurements after testing them for normality and homogeneity of variances. In a subset of the sample, we search for correlation between sternite area and head width–which is thought to be the best measure of size in *Polistes* (Eickwort 1969) – to test whether sternite area could be actually considered a reliable indicator of the overall size of the individuals in the studied species (as done in Dapporto et al. 2011 for *P. gallicus*).

We compared VdVo size between foundresses and workers using two consecutive GLM tests where VdVo size was the dependent variable, caste membership was the factor, and body size (sternite area) was the covariate variable. The first GLM was to look for possible factor covariate interactions, where no interactions emerged we made a second ANCOVA GLM for testing differences in intercepts between the samples.

Shape variability of sternite and VdVo between foundresses and workers was also described using the TPS (thin-plate spline) (Department of Ecology and Evolution, State University of New York, Stony Brooks, NY, USA) series of software (Rohlf 2009, 2010; available at http://life. bio.sunysb.edu/morph/), a geometric morphometrics landmark-based tool for studying two-dimensional variability of shape. We chose as landmarks ten points that are specifically identifiable (type II and type III landmarks; Bookstein 1997). Points 1–5 refer to the sternite, points 6–10 refer to the VdVo (Fig. 1). Landmarks were digitalized on photographs using TPSDIG 2.16 (Department of Ecology and Evolution, State University of New York, Stony Brooks, NY, USA). Landmark configurations for each specimen were scaled and optimally aligned using a General Procrustes Analysis (GPA); shape variables were computed on landmark coordinates using TPSREGR 1.37 (Department of Ecology and Evolution, State University of New York, Stony Brooks, NY, USA). A multivariate regression (MANCOVA) of shape and size variables (partial warps scores and centroid size), provided by TPSREGR, was carried out to investigate possible differential allometry (both in slope and in intercept) between the two castes using Wilks' λ as a test statistic. Thin-plate-spline deformation grids were used to visualize changes in shape due to body size and caste membership. All tests and graphs were performed using SPSS 13.0 for Windows (Apache Software Foundation, Wilmington, DE, USA).

Results

In all the species, sternite area was highly correlated with head width (Spearman's Rho = 0.822, $p < 0.0001$, n = 39 for *P. dominula*; Spearman's Rho = 0.931, $p < 0.0001$, n = 30 for *P. nimphus*; Spearman's Rho = 0.913, $p < 0.0001$, n = 57 for *P. associus*; Spearman's Rho = 0.624, $p < 0.0001$, n = 28 for *P. biglumis*) and thus could be used as a reliable indicator of wasps overall size.

Results of the comparison tests between foundresses and workers are listed in Table 1. Larger individuals has larger VdVo in all species we have measured; thus, body size (sternite area) positively correlates with VdVo size. Caste membership affects VdVo size in *P. dominula* and *P. nimphus*, with foundresses having an enlarged VdVo with respect to workers. Furthermore, in *P. dominula* and *P. nimphus* emerge a significant interaction between factor (caste membership) and body size (sternite area; Fig. 2). On the contrary, VdVo size do not differ between the two castes in *P. associus* (Fig. 2) and *P. biglumis*.

Multivariate regression of shape on body size and caste membership indicates that queens and workers of *P. dominula* actually differ in allometric intercept (Wilks' $\lambda = 0.506$, $p = 0.011$). Smaller individuals and workers show the same allometric contraction of VdVo (landmarks 6–10), as opposed to the enlargement of VdVo showed by larger individuals and foundresses (Fig. 3). Landmarks 1–6, which enclose VdVo's area, explain more than 90% of the last gastral sternite shape variance. *P. associus* (Wilks' $\lambda = 0.792$, $p = 0.847$), *P. biglumis* (Wilks' $\lambda = 0.559$, $p = 0.838$) and *P. nimphus* (Wilks' $\lambda = 0.725$, $p = 0.528$) do not show any significant differential allometry related to the VdVo among foundresses and workers.

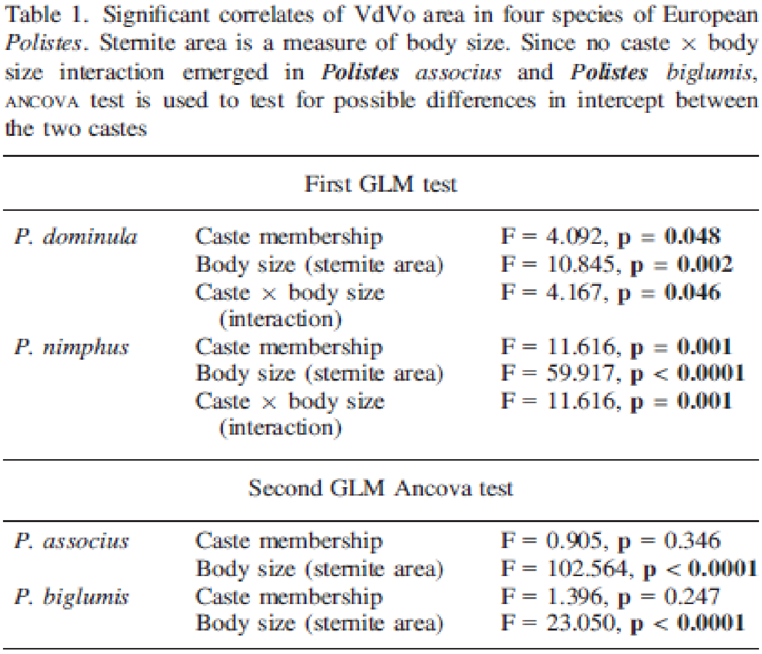

Table 1. Significant correlates of VdVo area in four species of European *Polistes*. Sternite area is a measure of body size. Since no caste × body size interaction emerged in ***Polistes associus*** and ***Polistes biglumis***, ANCOVA test is used to test for possible differences in intercept between the two castes

First GLM test		
P. dominula	Caste membership	F = 4.092, **p = 0.048**
	Body size (sternite area)	F = 10.845, **p = 0.002**
	Caste × body size (interaction)	F = 4.167, **p = 0.046**
P. nimphus	Caste membership	F = 11.616, **p = 0.001**
	Body size (sternite area)	F = 59.917, **p < 0.0001**
	Caste × body size (interaction)	F = 11.616, **p = 0.001**
Second GLM Ancova test		
P. associus	Caste membership	F = 0.905, p = 0.346
	Body size (sternite area)	F = 102.564, **p < 0.0001**
P. biglumis	Caste membership	F = 1.396, p = 0.247
	Body size (sternite area)	F = 23.050, **p < 0.0001**

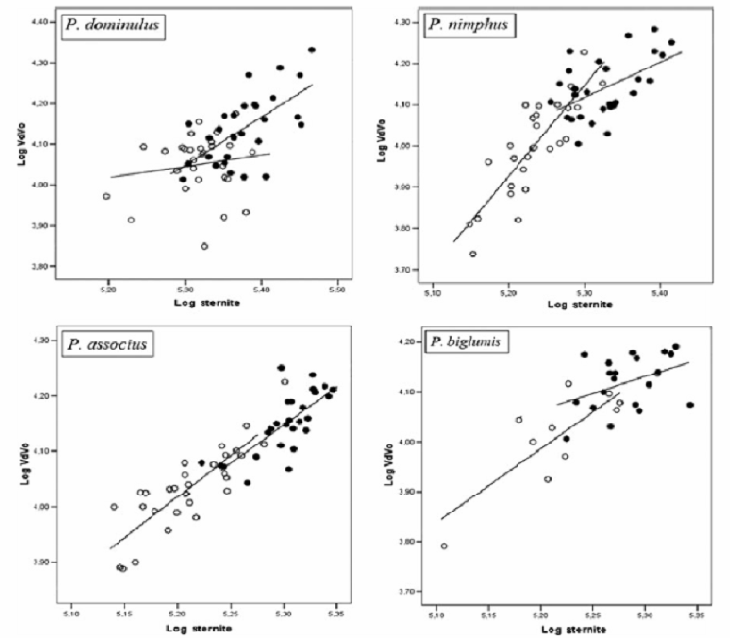

Fig. 2 The relationship between VdVo area and sternite area in foundresses and workers of four European Polistes species. Foundresses, black circles; workers, white circles. The linear regression is indicated for foundresses and workers separately

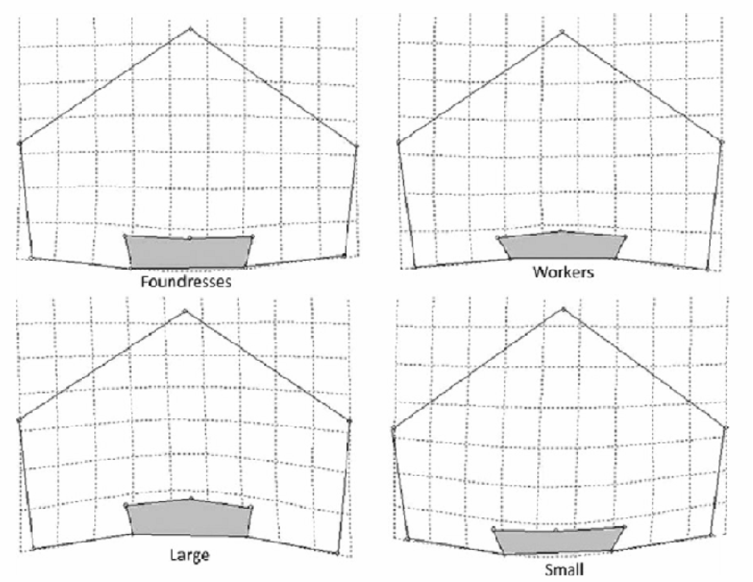

Fig. 3 Results for the multiple regression of partial warps (sternite shape) on caste membership (foundresses versus workers) and body size (large versus small) in *Polistes dominula*

Discussion

Tendency of foundresses to be larger in body size than workers was confirmed for all analysed species and actually produces a strong isometric effect on VdVo size. The sternal gland secretion, frequently spread on the comb by the dominant female, functions as a queen or a dominance pheromone, being able to inhibit subordinates' ovary development (Dani et al. 1992, 2003; Dapporto et al. 2007b). So, there is a clear advantage of foundresses in having a large secretory area that probably has leaded towards different developmental trajectories between the two castes in some *Polistes* species. Moreover, an enlarged VdVo in foundresses is an effective weapon for the defence by ants in the pre-emergence phase, when queens are the sole defenders of the colony, mostly in monogynic nests (London and Jeanne 2000; Dapporto et al. 2011).

Geometric morphometrics analysis reveals that VdVo is insomuch enlarged in *P. dominula* foundresses as to cause a deformation of the entire last gastral sternite with respect to workers. On the other hand, in foundresses and workers of *P. nimphus*, different developmental trajectories highlighted in the dimensional measurements of the VdVo, were not confirmed by geometric morphometrics analysis, suggesting a functional but not a morphological diversification between the two castes. In *P. associus* and *P. biglumis*, no static allometry (Stern and Emlen 1999) but probably only differences in mean body size between the two castes make foundresses to have an isometrically larger VdVo than workers; therefore, no diversification arose in these species.

There is an overlapping area between foundresses and workers of *P. dominula* and *P. nimphus* (Fig. 2) which means that the two castes are only incipiently but not fully separated unlike some swarming Polistinae wasps Turillazzi et al. 1994). Hence, foundresses and workers represent two gradually diverging extreme in a distribution of forms still in continuity. However, the difference in slope between VdVo-sternite relationship of foundresses and workers in these two species shows that an allometric differential growth affect VdVo development in the two castes.

Non-isometric growth (growth leading to difference in body shape), which are especially pronounced in the structures of the gaster among social wasps (Jeanne and Fagen 1974; Turillazzi et al. 1994), is usually recognized as the clearest evidence of incipient morphological castes. Only behavioural, physiological and metabolic differences have been reported so far between foundresses and workers of temperate *Polistes*, mainly as a result of the queens adaptation to the winter diapause (West Eberhard 1969; Strassmann 1985; Toth et al. 2009; Hunt et al. 2010). Our work on VdVo reveals that some other selective pressures, linked with communication and nest defence, are responsible for an incipient physical divergence between the two castes in *Polistes.*

Unexpectedly, our data show that species belonging to the same clade of *P. gallicus* (i.e. *P. biglumis* and *P. associus*; Carpenter 1997), thus presumably closer with it, do not share the same incipient allometric divergence between castes. Otherwise, species that are phylogenetically more distant from *P. gallicus* (i.e. *P. dominula* and marginally *P. nimphus*; Carpenter 1997) show an incipient divergence in the size and shape of VdVo between the two castes. Apparently in *Polistes*, reproductive choices and ecological features do not influence the degree of caste dimorphism of each species. Thus, there are no specific features increasing the likelihood to evolve physical castes in a given clade (i.e. *P. gallicus*, *P. associus* and *P. biglumis*) compared with another one. Moreover, because this group of species are substantially sympatric, we can assume the same ant predatory rate, so different rate of divergence in VdVo size between castes cannot be attributed to different predatory pressure on different species.

The onset of some static allometries (Stern and Emlen 1999) clearly shows that, in some *Polistes* species, different developmental trajectories are present between the two castes. Such a comparison among four species shows that strong evolutionary pressures act towards a general adaptive enlargement and a consequent hypertrophy of VdVo in foundresses with respect to workers. For most of the analysed species, the mean difference in body size between the two castes is sufficient to ensure to the foundresses an efficient spreading of CHC's on the nest surface. Otherwise, in *P. dominula*, this trend is extreme and two different allometric trajectories emerge in the two castes.

The evolution of allometric castes in social species has been interpreted as a 'point of no return' to the solitary life which is more likely to occur in monogamous and monogynic species showing a high intra-colony relatedness (Wilson and Holldobler 2005). However, our data show that rigid monogyny is not an obligate prerequisite for the evolution of incipient morphological castes among social insects which can occur also in poligynic species (i.e. *P. dominula*).

Therefore, also *P. dominula*, as has been previously established for *P. gallicus* (Dapporto et al. 2011), shows incipient characteristics of advanced eusociality, with a clear static allometry (Stern and Emlen 1999; Shingleton et al. 2007) between the two castes.

By the evolutionary point of view, both *P. dominula* and marginally *P. nimphus* seem to be clearly addressed to an increase in morphological gap between foundresses and workers as a response to the strong selective pressures acting on foundresses VdVo. The genus *Polistes* is therefore on the threshold of the evolution of true morphological castes, and this makes it a really excellent model for understanding how and when castes begin to diverge. The present study focuses on one character only and, although it has a considerable adaptive significance, further efforts to look for and describe other features showing dimorphism between castes may provide a more complete vision on the whole *Polistes* genus.

As a rule, differential allometric growth of morpho-functional traits involved in communication, coordination and reduction in fights between nestmates may be the primary drivers towards the onset of incipient physical castes in social insects at each level. In this regard, further wide morphological studies on key groups of social insects, focusing on the function of certain structure, will clarify the role of social constraints in passing the window of eusociality (Wilson and Holldobler 2005).

Acknowledgements

We thank Dr. David Baracchi (Università di Firenze) for patiently improving an early version of the manuscript. We thank especially Dr. Leonardo Dapporto, Dr. Alessandro Cini and Dr. Rita Cervo for several valuable suggestions and critical reviews of the manuscript. We also thank two anonymous referees for the helpful suggestions. This research has been funded by the Università degli Studi di Firenze.

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6. Comparative morphology of Van der Vecht's organ in *Polistes* social parasites: host ecology and adaptation of the parasite

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Biological Journal of the Linnean Society, **109** (2013), 313-319

Abstract

Camouflage strategies are common in insect social parasites. Being accepted into an alien colony as a dominant nestmate favours behavioural and morphological adaptations to mimic a specific odour. In *Polistes* social parasites, abdominal tegumental glands are involved in this camouflage strategy. These glands secreting cuticular hydrocarbons are connected with a modified cuticular area of the last gastral sternite of female wasps, named Van der Vecht's organ, whose secretion is involved in rank and dominance recognition. The size of this exocrine area has been demonstrated to be under selective pressure in *Polistes*, as a response to an efficient dominance recognition. Because chemical and behavioural integration differs between parasitic species, we carried out a comparison of Van der Vecht's organ size between the three *Polistes* social parasites and their respective hosts. The parasites *Polistes sulcifer* and *Polistes semenowi*, capable of a rapid chemical mimicry and specialized to exploit a lowland host, also show an enlarged Van der Vecht's organ. Conversely, the parasite *Polistes atrimandibularis*, specialized on a mountain species and showing a slow chemical integration, has a smaller organ. The time available for the parasite to tune up its chemical mimicry, before the emergence of workers to be accepted as a dominant nestmate, appears to be the most important selective pressure acting on the size of this abdominal organ.

Additional Keywords: allometry, chemical mimicry, host–parasite interactions, social wasps.

Introduction

Much biodiversity is a result of host–parasite antagonistic interactions, which result in a continuous coevolutionary output among species (Thompson, 1994). Brood parasites, such as cuckoo birds, utilize the brood care behaviour of other species to raise their offspring and are a well studied category of parasites (Rothstein & Robinson, 1998; Kilner & Langmore, 2011). Social parasitism is common in ants, wasps and bees and is a peculiar case of brood parasitism in which parasites not only exploit the brood care behaviour, but also many aspects of the social system of another social insect (Brandt & Foitzik, 2004; Scharf et al., 2011).

Only three species of obligate social parasites are known in *Polistes* paper wasps: *Polistes sulcifer*, *Polistes semenowi*, and *Polistes atrimandibularis*. These parasites have lost the capability to build a nest and to produce worker offspring and depend on congeneric colonies for brood care. Even if very different behavioural patterns are shown by *Polistes* social parasites during the usurpation of the host nests (Fanelli, 2001; Cervo, 2006), chemical mimicry is always crucial for exploiting the host. All *Polistes* social parasites have to perform an integration strategy by mimicking the host cuticular chemical profile to be accepted as dominant colony members (Bagnéres et al., 1996; Turillazzi et al., 2000; Lorenzi et al., 2004). *Polistes sulcifer* and *P. semenowi* are specialized parasites of *Polistes dominula*, whereas *P. atrimandibularis* is mainly specialized on *Polistes* specific circumstances, it has enlarged its original niche being able to exploit almost all sympatric *Polistes* species (Fanelli et al. 2001; Cervo, 2006).

A key role in the process of chemical deceiving of the hosts during the first phase of the usurpation is played by the abdominal glands associated with the Van der Vecht's organ (VdVo) (Turillazzi et al., 2000; Lorenzi, 2006). The VdVo is a hairy, hyaline cuticular area on the anterior edge of the last gastral sternite of Polistinae and Vespinae female wasps (Post & Jeanne, 1980) and its size is related to an increased production and more efficient spreading of its glandular secretion (Post & Jeanne, 1980). The structure is used to apply a complex mixture of hydrocarbons (Dani et al., 2003) both on the pedicel of the nest as ant-repellent (Turillazzi & Ugolini, 1978; London & Jeanne, 2000) and on the entire nest surface for inducing dominance recognition by workers and inhibiting the ovary development of the subordinates (Dani, Cervo & Turillazzi, 1992; Dapporto, Dani & Turillazzi, 2007a, Dapporto et al., 2007b). Recently, Dapporto et al. (2011) showed that foundresses of the solitary nest founding *Polistes gallicus* have an extremely enlarged VdVo compared to workers; this condition is adaptive for queens to enable the efficient spread of their own cuticular hydrocarbons on the nest surface to control workers.

An intense abdomen stroking on the comb during usurpation is reported for *P. sulcifer* and *P. semenowi* females (Turillazzi et al., 2000; Cervo, 2006). By means of stroking behaviour, the parasite acquires the smell (cuticular hydrocarbons) of the host and, at the same time, applies its own odour to the nest; this behaviour must be therefore crucial to attain chemical mimicry (Turillazzi et al., 2000; Lorenzi, 2006). By contrast, *P. atrimandibularis* does not perform abdominal stroking when usurping *P. biglumis* colonies (Lorenzi, 2006); however, some evidence shows that, when this parasite usurps colonies of lowland host species, it sometimes performs abdominal stroking on the back of the nest (Fanelli, 2001).

A recent study (Dapporto et al. 2011) showed that foundresses of the solitary nest founding *P. gallicus* have an extremely enlarged VdVo compared to workers, showing a static allometry (i.e. a size scaling relationship between individuals of the same species) (Stern & Emlen, 1999); because this abdominal organ is apparently under strong selective pressure in *Polistes*, we carried out a comparison of the relative size of VdVo in *Polistes* social parasites and in their host species aiming to understand whether the divergent behavioural patterns observed in the three parasites account for divergent morphological adaptations (i.e. evolutionary interspecific allometries) (Stern & Emlen, 1999; Shingleton et al., 2007). We predicted that two parasites performing intense stroking behaviour (i.e. *P. sulcifer* and *P. semenowi*) would show an adaptive enlargement of VdVo with respect to their host species. By contrast, we predicted that there would be no divergence between the VdVo size of the parasite *P. atrimandibularis*, which rarely shows abdominal stroking, and most of its hosts.

Matherial and mehtods

All specimens were collected in the field during 2011 and 2012 in various localities of Central Italy (Tuscany, Marche). Specimens of the three *Polistes* social parasites were collected in the Spring and Autumn in various high mountain locations (central Apennines) where these species usually mate and overwinter (Cervo, 2006). We collected 17 females of *P. atrimandibularis*, 26 of *P. sulcifer*, and 21 of *P. semenowi*. All foundresses of each host species (workers were excluded from the analysis) were collected during Spring on several newly-founded nests: *P. biglumis* (*N* = 20) in various sites of Central Apennines (above 1000 m a.s.l.), *P. gallicus* (*N* = 7), *Polistes associus* (*N* = 28), *Polistes dominula* (*N* = 29), and *Polistes nimphus* (*N* = 28) in various lowland localities of Tuscany. All specimens were killed by freezing at -18 °C and their last abdominal sternites were dissected. These were then mounted on a microscope slide and photographed with a digital Fujifilm camera under a binocular microscope (Fig. 1). All specimens were measured at x25 magnification. We measured

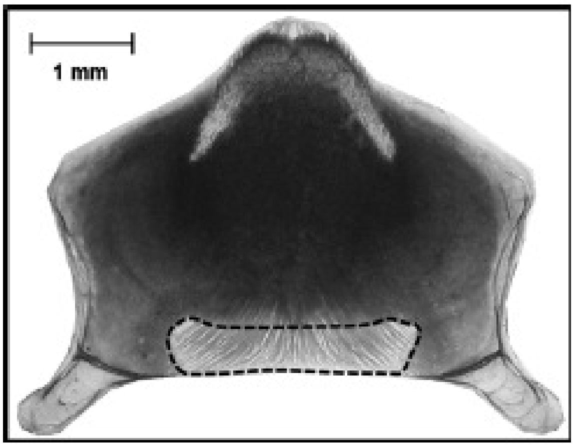


Figure 1. The last gastral sternite of a *Polistes* pape wasp; the Van der Vecht's organ (VdVo) is the hyaline anc hairy area highlighted by the dashed line on the lowe edge of the sternite.

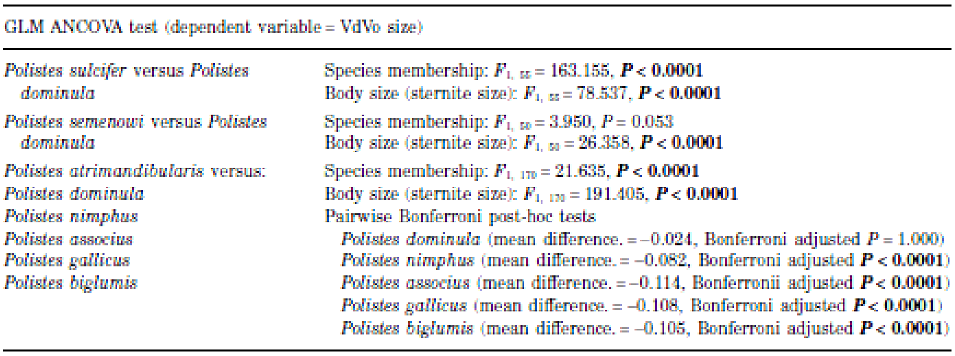
(in pixels) the area of the entire sternite and that of the VdVo using the IMAGEJ software (http://rsbweb. nih.gov/ij/) and then we log-transformed the measures and used the sternite area as a predictor of the overall size of the individuals (Dapporto et al., 2011). We performed parametric statistical tests on the measurements after testing them for normality and homogeneity of variances.

We entered the VdVo area as a dependent variable of a general linear model (GLM) for each parasite– host pair, using species membership as a factor and sternite area as a covariate, to test for possible factor x covariate interactions. Because no significant interaction emerged, we performed another analysis of covariance (ANCOVA) GLM analysis (dependent: VdVo area; covariate: sternite area; factorial predictor: species membership) to test for differences in VdVo size intercepts between each parasite and its respective host species. We also compared VdVo size between the three parasites using another ANCOVA GLM test (dependent: VdVo area; covariate: sternite area; factorial predictor: species membership) to obtain further evidence of different evolutionary trends between them. To compare *P. atrimandibularis* and its potential hosts, we performed pairwise comparisons (Bonferroni) based on estimated marginal means provided by ANCOVA GLM. All statistical analysis was performed using SPSS, version 13.0 (SPSSInc.).

Results

We compared VdVo size between parasites and their host species using two consecutive GLM tests where VdVo size was the dependent variable, species membership was the factor, and body size was the covariate variable. The first GLM excluded possible factor x covariate interactions and the second ANCOVA GLM tested differences in intercepts between the samples. An identical approach was adopted for comparing VdVo size among the three species of parasites.

Table 1. Result of the generalized linear model (GLM) analysis of covariance (ANCOVA) comparing the Van der Vecht's organ (VdVo) area of the three obligate *Polistes* social parasites and their respective host species



GLM ANCOVA test (dependent variable = VdVo size)	
Polistes sulcifer versus *Polistes dominula*	Species membership: $F_{1,\,55} = 163.155$, $\mathbf{P < 0.0001}$ Body size (sternite size): $F_{1,\,55} = 78.537$, $\mathbf{P < 0.0001}$
Polistes semenowi versus *Polistes dominula*	Species membership: $F_{1,\,50} = 3.950$, $P = 0.053$ Body size (sternite size): $F_{1,\,50} = 26.358$, $\mathbf{P < 0.0001}$
Polistes atrimandibularis versus:	Species membership: $F_{1,\,170} = 21.635$, $\mathbf{P < 0.0001}$
Polistes dominula	Body size (sternite size): $F_{1,\,170} = 191.405$, $\mathbf{P < 0.0001}$
Polistes nimphus	Pairwise Bonferroni post-hoc tests
Polistes associus	*Polistes dominula* (mean difference. = –0.024, Bonferroni adjusted $P = 1.000$)
Polistes gallicus	*Polistes nimphus* (mean difference. = –0.082, Bonferroni adjusted $\mathbf{P < 0.0001}$)
Polistes biglumis	*Polistes associus* (mean difference. = –0.114, Bonferronii adjusted $\mathbf{P < 0.0001}$)
	Polistes gallicus (mean difference. = –0.108, Bonferroni adjusted $\mathbf{P < 0.0001}$)
	Polistes biglumis (mean difference. = –0.105, Bonferroni adjusted $\mathbf{P < 0.0001}$)

Sternite area is a measurement of body size. Significant results ($P < 0.05$) are shown in bold.

The first GLM test showed no factor x covariate (species membership x body size) interactions in VdVo–sternite relationships of parasites and foundresses of their host species: *P. sulcifer* versus *P. dominula* (F1,55 = 0.0001, P = 1.000); *P. semenowi* versus *P. dominula* (F1,50 = 0.019, P = 0.890); *P. atrimandibularis* versus *P. dominula*, *P. nimphus*, *P.gallicus*, *P. associus*, and *P. biglumis* (F5,170 = 0.657, P = 0.657). The results of the

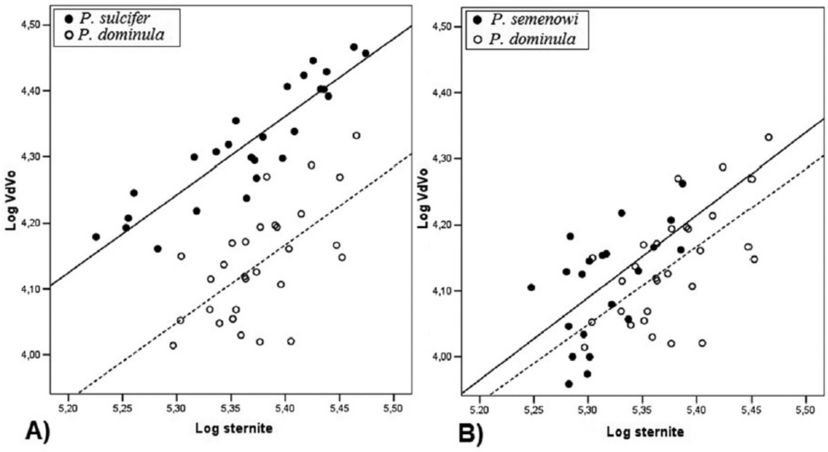


Figure 2. The relationship between the Van der Vecht's organ (VdVo) area and sternite area in two obligate social parasites, *Polistes sulcifer* (A) and *Polistes semenowi* (B), with respect to their usual host species *Polistes dominula*. The linear regressions are shown separately for each species.

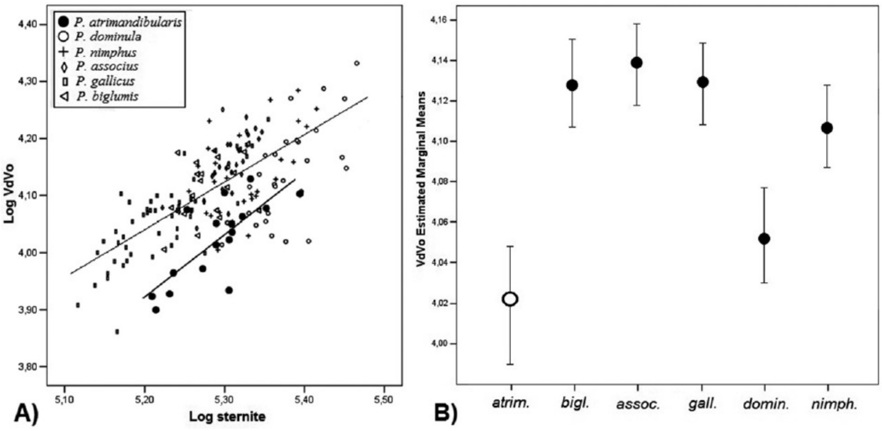


Figure 3. A: the relationship between the Van der Vecht's organ (VdVo) area and sternite area in *Polistes atrimandibularis* with respect to its possible host species (*Polistes dominula*, *Polistes nimphus*, *Polistes associus*, *Polistes gallicus*, and *Polistes biglumis*). The lower linear regression represents the parasite, the upper one represents the group of host species. B: the profile linear plot shows estimated marginal means of the VdVo area (the mean VdVo area corrected for the mean sternite area) for *P. atrimandibularis* and for all its possible host species. Error bars represent lower and upper bound of the 95% confidence interval.

subsequent ANCOVA GLM tests are shown in Table 1. VdVo size is significantly influenced by body size (sternite area) in all social parasites and hosts. Both *P. sulcifer* (Fig. 2A) and *P. semenowi* (Fig. 2B) females show an enlarged VdVo with respect to females of *P. dominula* (their usual host species); this enlargement is much more evident in *P. sulcifer* females than in *P. semenowi* females. By contrast, *P. atrimandibularis* females show a significant decrease of VdVo size with respect both to the foundresses of its original host *P. biglumis* and to the three lowland species that can be exploited by this parasite (Fig. 3).

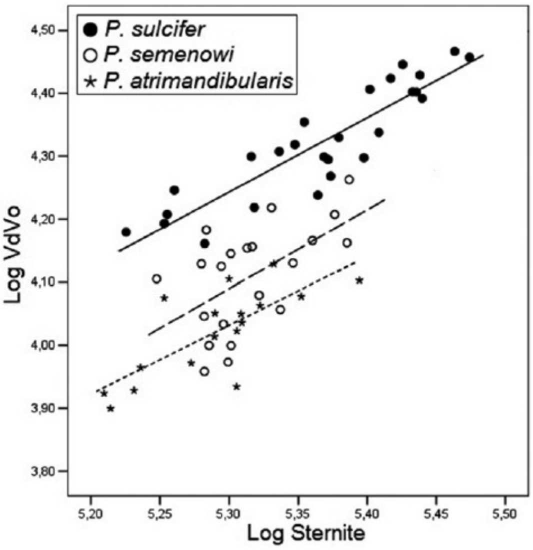


Figure 4. The relationship between the Van der Vecht's organ (VdVo) area and sternite area among *Polistes* obligate social parasites (*Polistes sulcifer*, *Polistes semenowi*, and *Polistes atrimandibularis*); linear regressions are shown separately for each parasite.

No factor x covariate interaction emerges when comparing each other the three parasites (species membership x sternite area interaction: *F*2,63 = 0.077, *P* = 0.926); however, *P. sulcifer* and *P. semenowi* show an enlarged VdVo with respect to *P. atrimandibularis*, and *P. semenowi* is intermediate between the other two parasites (*F*2,63 = 68.241, *P* < 0.0001). Consistently, for the three species, larger individuals have a larger VdVo (*F*2,63 = 91.461, *P* < 0.0001) (Fig. 4).

Discussion

The secretion of VdVo has a dual function in *Polistes*: (1) defence against predation by ants when it is applied on the nest pedicel Dani et al., 1992; London & Jeanne, 2000) and (2) dominance recognition when it is applied on the nest surface (Dani et al., 2003; Dapporto et al., 2007b). These two selective pressures are responsible for the variability of VdVo size observed in *Polistes* (Dapporto et al., 2011). Abdominal rubbing behaviour on the pedicel has never been reported in *Polistes* social parasites; therefore, there is no evidence of direct defence from ant predation by these parasites (Cervo, 2006). Accordingly, the variability of VdVo size that arose in these species must be attributed only to the selective pressures deriving from its involvement in chemical deception of the host.

Polistes social parasites show an enlarged VdVo in the species *P. sulcifer* and *P. semenowi* that are specialized on a single lowland host, and significantly reduced in the species *P. atrimandibularis* that was originally adapted to exploit the strictly mountain host *P. biglumis* but is capable of exploiting multiple secondary species. However, the enlargement trend of VdVo is much more pronounced in *P. sulcifer* than in *P. semenowi*.

Once a *Polistes* social parasite has conquered the host colony, it should be accepted by its hosts on mimicking their chemical cuticular profile (Lorenzi, 2006), although mechanisms for such camouflage differ among species (Lorenzi et al., 2004; Cervo, 2006; Lorenzi, 2006). Intense abdominal stroking on the nest is reported for *P. sulcifer* and *P. semenowi* usurper females, together with a quick change in their chemical profiles that become more similar to that of the replaced dominant female Turillazzi et al. 1990; Zacchi et al. 1996; Turillazzi et al., 2000; Sledge et al. 2001). For *P. sulcifer* and *P. semenowi*, chemical mimicry comprises a way of being accepted as a colony member and being recognized as a dominant individual by workers, which will be tricked to rear their brood; by contrast, the host queen is subdued by force and generally driven off the nest as it is not useful for the parasite (Cervo, 2006; Lorenzi, 2006). Furthermore, quick mimicry is crucial for these two parasites because they invade host nest just before workers emergence (Cervo, 2006); thus, a very functional exocrine structure (i.e. VdVo) helps parasites to produce and spread own hydrocarbons on the nest paper to be accepted as the dominant colony member by workers (Turillazzi et al., 2000; Lorenzi et al., 2004). These needs resulted in an evolutionary trend towards phylogenetic allometry (Stern & Emlen, 1999; Shingleton et al., 2007) of VdVo in *P. sulcifer* and *P. semenowi* with respect to their host species. Some possible explanations for the slight differences emerged in *P. semenowi* are discussed below.

On the other hand, females of *P. atrimandibularis* show a clear reduction of VdVo size compared to both its original host *P. biglumis* and to its secondary host species. Females of *P. atrimandibularis* rarely perform abdominal stroking (Cervo et al. 1990) and their chemical mimicry is quite slow (Lorenzi et al., 2004; Lorenzi, 2006). Unlike the other two *Polistes* parasite species, *P. atrimandibularis* has a long time available for camouflaging because it invades *P. biglumis* colonies 1 month before the emergence of workers (Lorenzi, 2006). Furthermore, preventing the flight of the *P. biglumis* host foundress is very important for this parasite because it needs cooperation to rear host workers that usually are still immature at the time of usurpation. Unlike the other parasites, it is advantageous for *P. atrimandibularis* to adopt a very cautious and sneaky strategy of usurpation because it needs to be accepted as a dominant colony member not only by host workers but also, at once and succesfully, by the host queen.

Some recent evidence shows that, when invading the lowland host *P. gallicus*, the chemical mimicry of this parasite can be faster and its stroking behaviour is more frequent than when it exploits its usual host *P. biglumis*, probably as a response to the short time available before the emergence of workers (Fanelli, 2001; Fanelli et al., 2001). Moreover, the host foundress is not retained by the parasite when usurping *P. gallicus* nests and it is often fought off because the workers will emerge and start work some time later (Fanelli, 2001; Fanelli et al., 2001).

Morphofunctional traits related to a quick chemical deception of the host (i. e. VdVo) are thus regressed in *P. atrimandibularis*. This regression of VdVo is also consistent with the concealing identity strategy via a blank state adopted by the parasite during usurpation, as made possible by a limited amount of cuticular hydrocarbons (Lorenzi & Bagnéres, 2002). An enlarged VdVo might be counter-adaptive for such a sly parasitic strategy. Our comparative findings show that the parasite probably had a long process of coevolution with its original mountain host, and its ability to exploit other lowland hosts should be considered as a secondary and contingent strategy (Fanelli et al., 2001). Even if *P. atrimandibularis* does not form genetically distinct host races (Fanelli et al., 2005), we might expect an enlargement trend of VdVo size in populations of this parasite usually exploiting lowland species as an adaptation to the need for rapid chemical mimicry and efficient dominance recognition.

Comparison among the three parasites, regardless of their hosts species, shows that different evolutionary trends affect the size of VdVo in these species. *Polistes sulcifer* females show an evolutionary trend towards an extreme enlargement of VdVo, whereas *P. atrimandibularis* shows a contrary trend, having a VdVo that is regressed with respect to the other two parasites. *Polistes semenowi* presents some intermediate characteristics between the other two parasites, possibly as a result of a less species-specific host specialization. For example, it is able to exploit *P. nimphus* colonies besides those of *P. dominula* (Demolin & Martin, 1980) and it can cohabitate with the host queen for some time (Zacchi et al., 1996). These findings, together with the results reported in the present study, suggest that specialization on the same host tends to drive the two parasites toward morphological and behavioural convergent evolution.

Achieving chemical mimicry and dominance odour approximately at the time when workers emerge from colonies is crucial for all *Polistes* social parasites (Lorenzi, 2006), and this must be the main selective pressure acting on the size of VdVo in all of these species. In *Polistes*, the timing of emergence of the first workers is strongly affected by the timing of nest foundation; the higher the altitude of the nesting site, the more the foundation and emergence of the first workers is delayed. This could explain the convergent enlargement of VdVo in species that are specialized on a lowland host and the opposite trend in species that usually exploit a high mountain species.

Polistes social parasites represent a monophyletic group: *P. semenowi* and *P. sulcifer* are sister species, whereas *P. atrimandibularis* is less close to the other two parasites (Carpenter, 1997). Even though *Polistes* social parasites are a highly related group of species, we found an evolutionary allometry in VdVo size that represents a distinctive feature among them. Our findings suggest that the phenotype of obligate parasites is primarily shaped by adaptation on a specific host and by host–parasite coevolutionary interactions (Brooks, 1979) rather than by phylogenetic constraints alone. Therefore, variability of morphofunctional traits in parasites appears to be highly predictable when considering the biology and behaviour of the respective host species.

Acknowledgements

We thank Dr David Baracchi for patiently improving an early version of the manuscript. We especially thank Dr Leonardo Dapporto, Dr Alessandro Cini, Dr Rita Cervo, and three anonymous referees for several valuable suggestions and critical reviews of the manuscript. This research has been founded by the Università degli Studi di Firenze.

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7. Visual assessment of fighting ability among queens in a solitary founding European paper wasp

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Manuscript submitted for publication

Abstract

Conventional signals are maintained via social costs and are commonly used in animal kingdom to assess conspecifics' fighting ability during the disputes over resources. In the last decade many experimental studies showed that visual conventional signals are also common in social wasps, being good rank predictors in some social contexts tied with the definition of hierarchical ranks. Here we show, using both behavioural and morphological approaches, that visual conventional signals are also used by queens of the solitary founding European *Polistes gallicus* to assess conspecifics' fighting ability during intraspecific parasitism. Usurpation of a conspecific nest (i.e. intraspecific parasitism) is a very common strategy among *P. gallicus* queens, as nests usually suffer high predation from birds, especially before the workers' emergence. Queens having a large spot on their clypeus are larger in body size and show a higher winter survival rate. Besides, potential usurpers with large black spot on the clypeus are more promptly and aggressively rejected by resident queens than usurpers with a little black spot or a totally yellow clypeus. Such findings show that queens are able to assess potential usurpers' fighting ability and modulate their response basing on this visual cue: this actually allows them to save costly energies focusing their effort on rejecting more dangerous usurpers. Our study reveals that visual recognition abilities seem to be widespread among social wasps regardless of their social biology, especially among taxa which are facing similar selective pressures within a competition context.

Keywords: *Polistes gallicus*, status badge, intraspecific parasitism, conventional signals.

Introduction

In the animal kingdom resources can be limited in time and space and competition over them is often costly. A good breeding or feeding territory or a sexual partner are the most common subjects of fierce disputes between conspecifics. Actually these contentions come at a price for the contenders in terms of spent energy or potential injuries. Thus, some animals can reduce the costs of exhausting disputes over resources by using honest signals, useful to assess rivals during competitions (Maynard-Smith and Harper 2003). There are many signals having high production costs, i.e. carotenoid-based plumages of some passerines birds or powerful roars of some large cervids (Hill 1991; McComb 1991, Briefer et al. 2010).The quality of the cue bearer and the honesty of the signal are thus guaranteed by the considerable energy investment linked to the production and maintenance of these signals.

Other kind of signals, which are called conventional signals or badge of status, do not show production costs (Guilford and Dawkins, 1995) but are often associated with fighting ability and social status. For example, the melanin-based achromatic plumage of a passerine bird (Mennill et al. 2003), the ultraviolet emissions of male European lizards (Bajer et al. 2011) and the ornamentations of some females spiny lizards (Weiss et al. 2009). Visual conventional signals have also been described in some social wasps belonging to the Polistinae and Stenogastrinae subfamilies. In males *Parischnogaster mellyi* the abdomen stripes display, performed during patrolling behaviour, is a conventional signal of agonistic ability (Beani and Turillazzi 1999). In the American invasive population of *Polistes dominula* and in *Polistes exclamans*, females facial patterns are visual quality signals used for rival assessment (Tibbets and Dale 2004; Tibbets and Sheehan 2011). Furthermore in females *Liostenogaster vechti*, facial markings are a status badge, both linked with hierarchical rank within colonies and used for rival assessment (Baracchi et al. 2012). Accuracy of conventional signals is maintained via social costs: individuals showing a high status badge must withstand increased aggression rates by other testing individuals. As a consequence, low quality individuals, which are unable to withstand an increased rate of aggressions, cannot cheat on their actual status (Rohwer 1975, Maynard-Smith and Harper 2003).

Since conflicting results emerged, especially among different *P. dominula* populations, conventional signals of agonistic ability in paper wasps is still a highly debated issue. In fact no correlation was found between facial pattern and social rank in associative foundations of both Italian and Spanish populations of *P. dominula* (Cervo et al. 2008; Zanette and Field, 2009). Some recent works suggest that these controversial results might be due to different climatic conditions experienced by different *P. dominula* populations. Increased expression of melanin on the cuticle is actually linked with a more efficient thermoregulation within populations experiencing colder climates. Thus, *P. dominula* populations living in warm climates (i.e. Italy and Spain) do not show enough variability to favour the evolution of a status badge, which is more likely to evolve in populations inhabiting colder climates (Tibbets et al. 2011; Green et al. 2012).

Polistes gallicus, a congeneric sympatric species of *P. dominula*, is a common circum-Mediterranean species showing colonies always started by a single queen and usually founded in open locations (Cervo and Turillazzi 1989). A high rate of intraspecific usurpations (theft of one nest by a foreign queen) has been ascertained in *P. gallicus* as a response to the high rate of nest predation (up to 90% of colonies fail), especially before the emergence of the first workers (Dani and Cervo 1992). It is actually a profitable strategy for a foundress, which has had its nest destroyed, to usurp an already started nest, instead of building a new one (Dani and Cervo, 1992). *P. gallicus*, shows a great variability in the black markings on the clypeus (Fig. 1): this marking always appears as an undivided round spot but it shows a great size variability among individuals. Moreover, some individuals show an entirely yellow clypeus.

Here we explore whether size variability in the clypeus markings has a biological relevance in this strictly monogynic *Polistes* species, focusing on intraspecific parasitism as a suitable context to test our hypothesis. A previous work on interspecific usurpation of a *Polistes* obligate social parasites has actually shown that facial pattern may be relevant during usurpation attempts (Ortolani et al. 2010). Also intraspecific usurpation context of *P. dominula* seems to be influenced by facial pattern of the two challengers (Tibbets and Shorter 2009). We tested, by some behavioural essays, whether clypeus markings represent a conventional signal useful for rival assessment during intraspecific usurpation attempts, then we also inquired whether this visual cue is actually related to quality features or to fighting ability of its bearer.

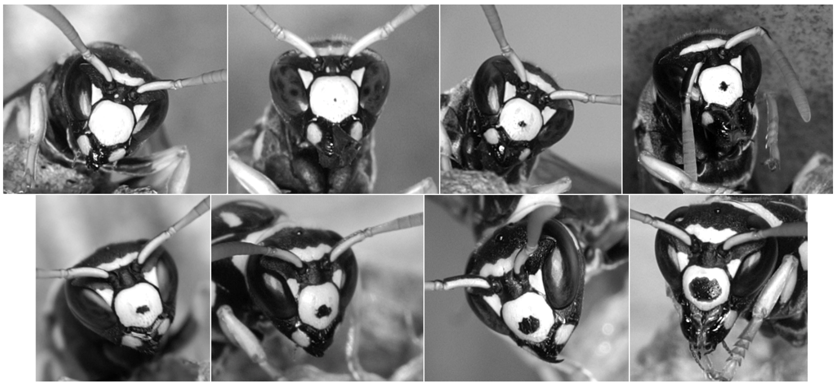

Figure 1. Some portraits of *P. gallicus* queens showing the natural size variability of the black spot on clypeus.

Materials and Method

Sample collection, morphological measurement and facial pattern categorization

Two groups of *P. gallicus* queens, from two different locations near Florence (Tuscany), were collected at different times during 2012 and 2013. We collected the first group of foundresses (N = 58) in April, as soon as they started to build the very first cells of their nest. The second group was only composed of future foundresses (N = 58 from 10 colonies), i.e. reproductive females that usually mate at the end of the Summer and are able to overwinter. This group of foundresses were collected in August, when reproductive individuals (potential foundresses and males) usually emerge on the colonies. Future foundresses were distinguishable from late workers as they show more developed and multistratified fat bodies in their abdomens (Strassmann 1985; Reeve 1991; Toth et al. 2009; Hunt et al.2010; Dapporto et al. 2011).

The face of each collected wasp was photographed with a digital camera and an optical microscope using a standardized procedure. The head width and the size of the black spot on clypeus (BSS henceforth) were measured on the pictures using the software Image-J (available on line at http://rsbweb.nih.gov/ij/). Head width is a good indicator of body size in *Polistes* (Eickworth 1969) and it was measured at the level of the antennal insertion for all the samples. Since BSS was not normally distributed in the sample, we used a generalized linear model (GLZ) for comparing facial markings with head width in the two groups of queens. In the GLZ analysis BSS was the dependent variable, group membership (i.e. queens vs future queens) was the factor and head width was the covariate. All analyses were performed using the Statistical program SPSS® 13.0 for Windows®.

Behavioural assays

To test whether foundresses of *P. gallicus* actually use the black spot on their clypeus as a visual cue to assess conspecifics, we designed a specific behavioural experiment to simulate, in a natural contest, an intraspecific usurpation attempt. Forty-eight nests founded in a semirural lowland area, near Florence Airport (Peretola), were chosen for this experiment. The experiment was done during the pre-emergence phase as we wanted to test foundresses in the period of the highest rate of usurpation (Dani and Cervo 1992).

Ten foundresses were collected several kilometres away from the focal wasps nesting, to ensure that they had never met before, and were used as lures. Before trials, the wasps were killed by freezing and rinsed in 500 µl of pentane for 24 hours in order to eliminate possible chemical cues. The lures were paired for comparable head width (Tibbetts and Dale 2004; Baracchi et al. 2012) but one of them had an entirely yellow clypeus while the other one had an highly pigmented clypeus (mean BSS of the pigmented lures: ~ 9105,9 arbitrary units; SD = 733,5). During the experiments we presented the two lures for 30 s alternately and approximately half an hour apart to the focal wasps on their nests (N = 48) with a procedure similar to that followed by Baracchi and coworkers (2012). To control for order effects, half of the colonies was first presented with the pigmented clypeus lure, while the other half were first presented with the yellow clypeus lure. The number of aggressive interactions of the focal queens with the lures was counted.

The lures, attached to a thin iron wire, were slowly approached to the focal foundresses on their nests, simulating an usurpation attempt. Lures were held for 30 seconds, at the minimum distance from the nest so that the wasps could interact with it. We considered the number of bites directed towards the lures and the latency of attack (time in seconds elapsed until the first bite) to evaluate the elicited aggressiveness. All the trials were videotaped and the videos were analysed with a blind procedure. We used a Wilcoxon signed rank test to analyse these data, so that each colony was compared for its own reaction when facing the two opposite lures.

For a sub-sample of twenty-four focal queens we collected additional data to control for possible other factors influencing resident responses. To test this hypothesis, we performed a GLZ using the cumulative responses of the wasps to the lures as a dependent variable, individual lure identity and ranked BSS of the focal wasps as factors.

We performed a further behavioural experiment to test whether the size or the shape variability of the black spot on the clypeus alone are sufficient to trigger different reactions in the resident wasps. We presented four natural-size micropictures of a female face with four artificially increased levels of BSS (see Baracchi et al. 2012) to 42 resident foundresses on their nest in the wild during the pre-emergence phase (Fig 7a). Micropictures were obtained using a software (Photoshop CS©, version 8.0.1.) by increasing BSS of the same female wasp picture according to a geometric series (BSS range 0 - 10000 arbitrary units).

We also presented, to 30 additional natural colonies, two different micropictures of a female face having the same BSS but different shape complexity of the clypeal spot (a circle and a six pointed star of the same area) to ascertain if different shapes elicited different reactions (Fig 7b).

Micropictures were presented on the top of a thin iron wire in a random order for 20 s and approximately half an hour apart to the focal wasps. All the trials were videotaped and videos were analyzed with a blind procedure. With the specific aim of evaluating just the response of the wasps due to the visual signal we considered the aggressive reactions (number of bites) during the very first phase of each trial (10 seconds) and the latency of response (time in second elapsed until the first bite). Data of increasing BSS experiment were analyzed using a repeated measures Anova design, while the data of different shape experiment were analyzed using a Wilcoxon signed rank test. All analyses were performed using the Statistical program SPSS® 13.0 for Windows®.

Results

Body size and visual markings measurements

The GLZ results showed a highly significant relationship between BSS and head width (i.e. body size) in the entire sample (Chi Square = 55.492, N = 116, P < 0.0001) (Fig. 2). Furthermore, BSS was significantly different in the two groups of females with Spring queens having a higher BSS and being generally larger in size than future Autumn queens (Chi Square = 7.257, N = 116, P = 0.007) (Fig. 2, Fig. 3). This result means that larger queens also show larger black spots on their clypeus, moreover, queens that have successfully overwintered and have founded a nest show, on average, larger spots and body size than queens collected before the Winter.

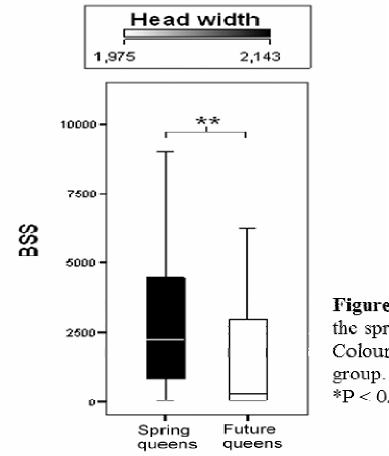

Figure 2. Distribution of BSS values (black spot size) in the spring queens and in the future queens of *P. gallicus*. Colour of boxes represent the mean head width for each group. Box plots show medians, 25th and 75th percentiles. *P < 0.006; **P < 0.0001.

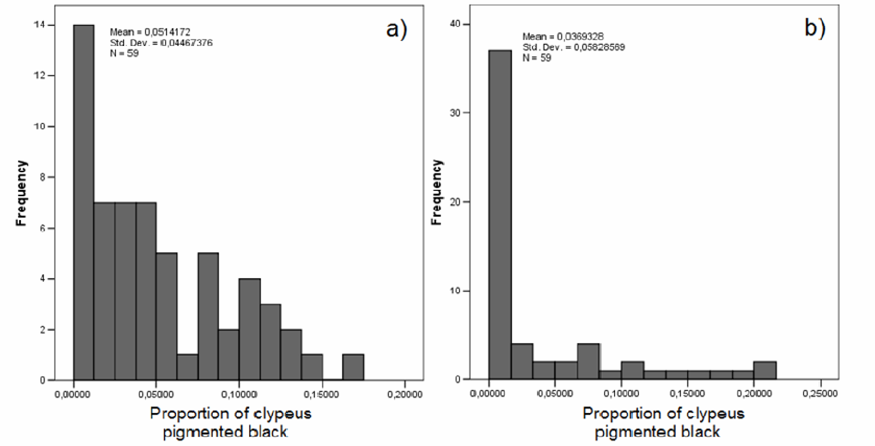

Figure 3. Frequency distribution of different facial patterns in Spring foundresses (a) and Autumn potential queens (b) of *P. gallicus*. The facial pattern of queens is expressed as the proportion of clypeus pigmented black.

Behavioural assays

Lures presentation experiment: Lures with a large black spot on the clypeus received significantly more bites than lures with a totally yellow clypeus (N = 48; Wilcoxon test; bites: Z = - 3.144, P = 0.002) (Fig. 4a) but the latency of attack does not differ between the two lures (N = 48; Wilcoxon test; bites: Z = - 0.950, P = 0.342) (Fig. 4b). The GLZ shows that focal wasps' responses to the lures are not influenced by their own ranked BSS (Chi Square = 2.031, N = 24, P = 0.362) or by the individual lure identity (High BSS lure: Chi Square = 3.030, N = 24, P = 0.220; yellow clypeus lure: Chi Square = 2.509, N = 24, P = 0.474;).

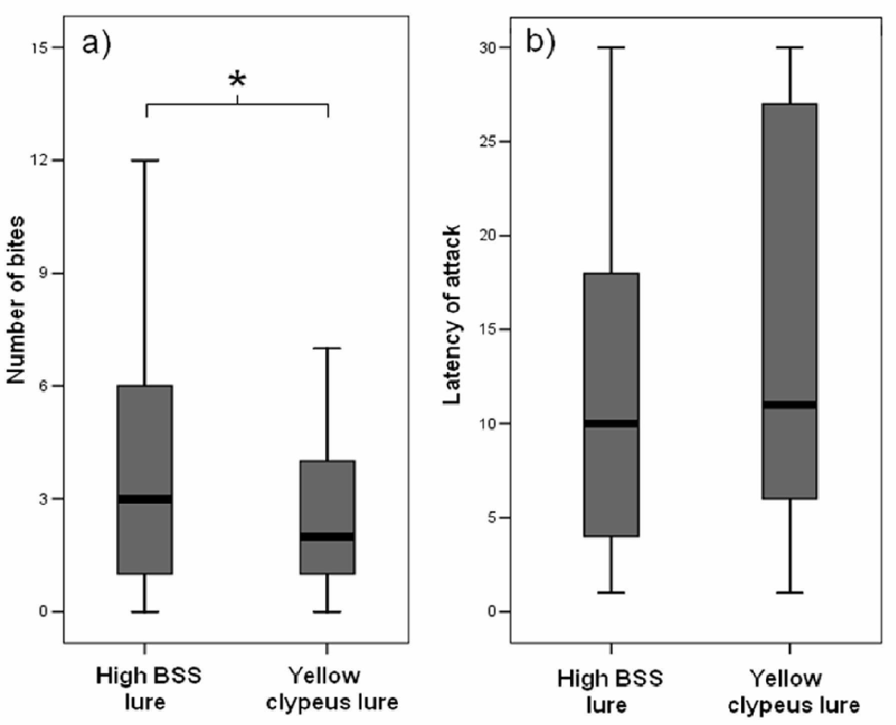

Figure 4. Number of bites (aggressive reactions) (a) and latency of attack (b) of the resident wasps towards the lures with high BSS values (black spot size) compared to the one with a totally yellow clypeus. Box plots show medians, 25th and 75th percentiles. *P < 0.006; **P < 0.0001.

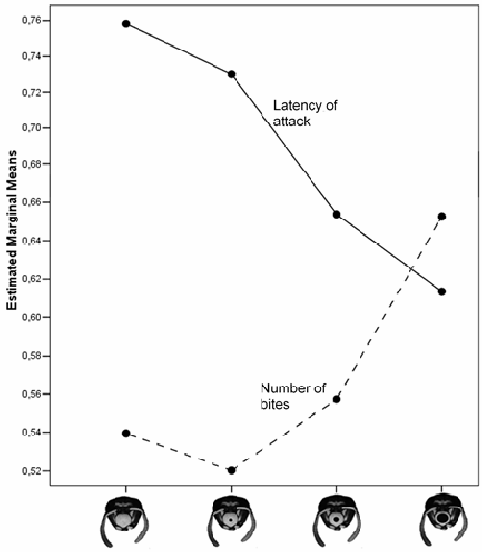

Figure 5. Number of bites and latency of attack shown by the resident wasps when presented with four micropictures of female faces of *P. gallicus* with increasing value of BSS.

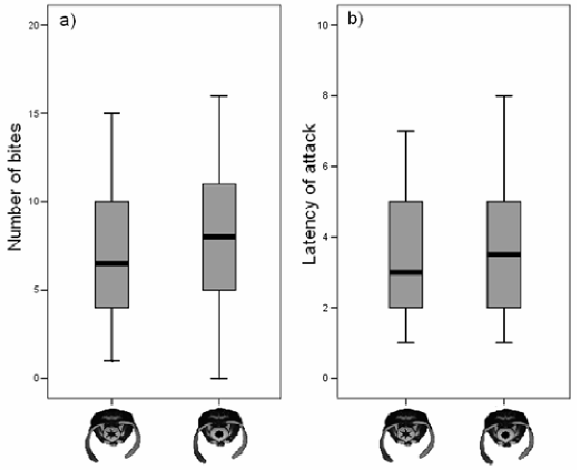

Figure 6. Number of bites (a) and latency of attack (b) shown by the resident wasps when presented with two micropictures of female faces of *P. gallicus* with the same value of BSS but having different shapes. Box plots show medians, 25th and 75th percentiles. *P < 0.006; **P < 0.0001.

Micropictures presentation experiments: When micropictures of female wasp faces were presented to the foundresses on their nest, the number of bites increased significantly with the BSS of the presented micropicture (GLM for repeated measures, N = 42: F3 = 2.694, P = 0.049), while the latency of response decreased (GLM for repeated measures, N = 42: F3 = 2.968, P = 0.035) (Fig. 5). On the contrary, when presenting micropictures of female wasp faces having the same BSS but different shape of the spot we did not find any difference both in the number of bites (N = 30; Wilcoxon test; bites: Z = - 0.816, P = 0.414) (Fig. 6a) and in the latency of attack (N = 30; Wilcoxon test; latency of attack: Z = - 0.371, P = 0.711) (Fig. 6b).

Discussions

When a cue is linked with some competitive ability, such as body size or fighting ability it is said to be a badge of status. The ability of an animal to gain, maintain or defend a particular resource ("resource holding potential" sensu Parker 1974) is often, directly or indirectly, linked with body size and what is known as fighting ability (Rohwer 1982). Also among invertebrates, social hierarchies are regulated by different cues and top ranked individuals are often easily recognizable by distinctive cues (Gherardi et al. 2012). In the temperate paper wasp *Polistes dominula*, body size is the main predictor of rank in the associative spring foundations, although in the north American population the facial pattern is a visual badge of status and a rank predictor (Turillazzi and Pardi 1977; Tibbets and Dale 2004; Cervo et al. 2008; Zanette and Field 2009).

Our findings show that queens of the solitary nesting paper wasp *Polistes gallicus* show a pronounced variability in the size of a black spot on the clypeus. We also found that this variability is strictly linked both with body size and with the probability of successfully overwintering. Indeed, Spring foundresses collected on their recent foundations show larger black spot on their clypeus and relatively higher body size than future foundresses collected before hibernation. Therefore queens which have a large black spot are favoured in overwintering, perhaps because they also have more abundant lipid stores, and these individuals are possibly favoured also in maintaining and protecting their brood as they are larger in body size. Variability in clypeal pigmentation pattern is a candidate to be a honest quality signal for *P. gallicus* queens which is presumably related to fighting ability and resource holding potential of its bearer, as it has been reported for other social Vespid wasps (Tibbets and Dale 2004; Tibbets and Sheehan 2011; Baracchi et al. 2012).

Since in *P. gallicus* there is no pleometrosis and colonies are single founded, there are no linear hierarchies between cofoundresses (Cervo and Turillazzi 1989). Despite this, there is a certain time when queens frequently fight each other and compete for resources. During pre-emergence phase most of the nest (up to 90 %) are preyed upon by birds and several queens lose their brood and nest: in this stage, competition over nests that survived predation is so ruthless and usurpation rate can reach 30% (Dani and Cervo 1992). Furthermore, surviving nests are often built in a semi-protected or mimetic place so they assume a higher value and competition for them is relevant (S. Turillazzi, personal observation). We experimentally proved that potential usurpers are differently faced by resident queens basing on the size of their black spot on the clypeus. Usurpers with a large black spot were more aggressively rejected by resident queens than those with a totally yellow ones. Furthermore, a set of printed faces of potential usurpers with an increasing size of the black spot, elicited increasing aggressiveness and more prompt response in the resident queens. While, difference in the shape and in the jaggedness of the black spot on the clypeus (for a given size of the spot) did not elicit different reactions. Therefore, the visual cue remains meaningful for the wasps also when other possible information about the opponent has been eliminated or standardized (i.e. additional body regions with variable pigmentation, limbs position, general body size) (see Sheehan and Tibbets 2011). As evidence, as far as the latency of attack of the resident wasps, we did not find any difference between the two lures but we did find differences only when presenting printed micropictures with the isolated visual cue.

Resident wasps are more aggressive and reactive towards alien queens with a larger black spot as they actually represent a greater threat and have many chance to usurp a nest. Therefore resident wasps are able to assess potential usurpers' fighting ability and modulate their response basing on this visual cue, this actually allows them to save costly energies. They can easily and immediately recognize high quality individuals and concentrate their

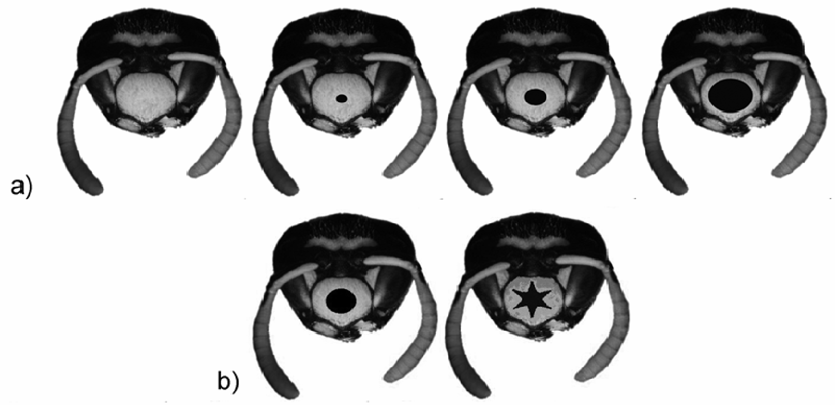

Figure 7. Micropictures of female faces of *P. gallicus* with size (a) and shape (b) of the black spot on the clypeus artificially altered using a computer software. These were used in the behavioural experiments.

aggressive efforts towards them, avoiding long and exhausting aggressions toward low quality individuals which have little chance to usurp their nest (see Baracchi et al. 2012). The density of foundation is usually very high for this species (Dani e Cervo 1992), so it is also possible that queens after losing their nests, make several usurpation attempts towards surviving nests to determine which one are less risky to usurp. Indeed, when many competing individuals have frequent dyadic encounters and/or have quick and inexpensive shift systems (i.e. flight), the evolution of class-level recognition is highly probable, as a response to the need of promptly assess an opponent's fighting ability (Rohwer 1982; Shreeve 1987; Zulandt Schneider et al. 1999).

It may appear counterintuitive that high ranked potential usurper showing a larger black spot on the clypeus are strongly rejected despite the high risk associated for resident queens. However, during a dyadic encounter, the owner of a given resource is the one who has more to lose and is highly motivated to respond according to the perceived threat, because of its considerable investment upon the resource (Rowher 1982). Furthermore, conventional signals such as "badge of status" are maintained via social costs (Rowher 1975; Maynard-Smith and Harper 2003), thus any *P. gallicus* usurper queen which cheats on its actual status could not suffer the proportional reaction of the resident owner. We did not find any differential rate of aggressiveness among resident queens during behavioural experiments. Foundresses showing a larger spot on the clypeus do not behave more aggressively toward intruders than foundresses with a yellow clypeus but differences were only due to the presented stimuli. Accordingly, high fighting ability and resource holding potential of top-ranked queens do not imply an higher level of aggressiveness.

We could conclude that having a large black spot on the clypeus gives some competitive abilities to *P. gallicus* queens which are larger in body size, more often survive to the winter diapause and are a great threat for their conspecifics in a biologically relevant context (i.e. intraspecific parasitism). Further experimental studies might clarify the main features influencing such a quality signal in *P. gallicus* and its adaptive value, for example whether high quality queens actually show a higher fitness (see Green et al. 2013). Until now, visual badge of status were only described in poligynic species of paper wasps (Tibbets and Dale 2004; Tibbets and Sheehan 2011), while we report the first case of class level recognition based on visual cue in a solitary nesting *Polistes* species. Therefore, such recognition abilities seems to be widespread among Vespidae, regardless of their nesting habits and social biology.

Acknowledgements

We thank Dr David Baracchi for many fruitful discussions about these research. We also thank all members of the sociobiology research team at the Biology Department - University of Florence. This research has been founded by the Università degli Studi di Firenze.

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8. Visual cues, age and body size: dominance hierarchies and rank inheritance among workers of a solitary founding paper wasp

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Manuscript in preparation

Abstract

Visual status signalling is widespread in social wasps but most of the studies focused on its role in mutual assessment among queens. However, in species showing distinct castes, few studies have investigated the importance of visual status signalling in regulating dominance hierarchies, rank inheritance and social conflict among workers. Here we show that workers of a solitary founding European paper wasp, whose queens use facial markings for visual assessment of alien usurper females, also have variable facial markings associated with body size. Workers signal a lower level of quality with respect of queens, despite that, queens signalling a higher level of quality actually produce workers signalling higher quality, suggesting a broader involvement of genetic factors in the badge development. In spite of the high agonistic abilities associated with such a visual signal, top rank inheritance among orphan workers is clearly ruled by a gerontocracy. Moreover workers, unlike queens, seem to be unable to assess fighting ability of a conspecific alien female by using visual cues in a dyadic encounter on a food source. Our data deepen the previous knowledge about visual recognition in social wasps showing that communication systems may vary significantly with respect to different phases of the colony cycle, different cohorts of individuals and different natural context.

Introduction

Status signalling is widespread in the animal kingdom aiming to ritualize aggressions and avoid expensive direct fights among contenders. Dominant individuals often show some distinctive characteristics, i.e. status signals, for an easy rank recognition. Some of these cues (e.g. carotenoid-based plumage of passerine birds, roaring behaviour of some cervids) have high production and maintenance costs, thus only high quality individuals can bear these charges (Hill, 1991; Briefer et al., 2010). On the contrary, conventional signals, i.e. badge of status, are also associated with rank and fighting ability but their accuracy is maintained via social costs (Guilford and Dawkins, 1995). Some examples of status badge in vertebrates are the melanin-based plumage of chickadees (Mennil et al., 2003) and the ultraviolet emissions of male European lizards (Bajer et al., 2011). High ranked individuals, i.e. the cue bearers, suffer high social costs and must withstand increased aggression rates by other testing individuals. Thus, low quality individuals, which are unable to withstand such a rate of aggression, cannot cheat on their actual status (Rohwer 1975, Maynard-Smith and Harper 2003).

Recognition systems based on visual conventional signals are also widespread among invertebrates, especially among social wasps. In the American population of *Polistes dominula* and in *Polistes exclamans*, females facial patterns are visual quality signals used for rival assessment (Tibbetts & Dale, 2004; Tibbetts and Sheehan, 2011). In the tropical social hover wasp *Liostenogaster vechti* high ranked females show more pigmented face with respect to subordinates females (Baracchi et al., 2012). Also in the solitary founding *Polistes gallicus* high quality queens show a larger black spot on the clypeus, have a larger body size and are more aggressively and promptly rejected by resident females during nest usurpation attempts (Petrocelli et al., submitted). Recently some controversial results emerged about visual conventional signalling in *P. dominula*, which has not been confirmed for the European population (Cervo et al. 2008; Zanette and Field, 2009) and do not show any adaptive value in the wild (Green et al. 2013).

Linear hierarchies and queues for dominance are a common feature of group living in animals (Wilson, 1975). Queues for dominance in social wasps are usually age-based or based on body size (Strassmann, 1983; Nonacs and Reeve, 1995; Bridge and Field, 2007; Cervo et al, 2008). Conventional signals are expected to correlate with body size and fighting ability but experimental evidence of their influence on dominance hierarchies among social wasps are conflicting (Zanette and Field, 2009; Baracchi et al, 2012 but see also Cervo et al, 2008). Therefore, the role of status signalling in determining social queues is a still open issue as many researches only focused on the relationship between status badge and fighting ability within a dyadic encounter context.

Most of research about conventional signals in social wasps concern queens, especially in their nest-founding associations, while only few studies focused on status signalling among workers. According to Tibbetts 2006, facial pattern of *P. dominula* workers signal a low level of quality with respect to queens. This finding shows that badge development is influenced mainly by environmental factors. However, nothing is known about the role of status badge in dominance hierarchies among workers, moreover it is a still open issue whether it is an heritable trait or if it is influenced only by the environment and available resources.

Since queens of the solitary founding European paper wasp *P. gallicus* use facial markings for a visual assessment of alien usurper females (Petrocelli et al. submitted), here we explore if workers also show variable facial markings comparable with those of the queens. We also inquire whether these markings i) are correlated with some competitive ability among workers (for instance body size), ii) have a relevance in rank inheritance within orphaned colonies and/or iii) are used for the assessment of alien females' fighting ability during dyadic encounters outside a colonial context. Furthermore, we investigated whether queens signalling a high level of quality actually produce workers showing high quality facial markings: in recent researches about quality signalling among paper wasps this still represents an underinvenstigated issue.

Matherials and method

Morphological analysis

A total of 263 wasps were collected from 20 different colonies of *P. gallicus*. Wasps were collected in a semirural area near Florence (Italy) at two different times during summer 2012: eleven nests in mid June and nine in mid July, in order to have workers from different generations. Colonies were always collected early in the morning in order to gather all the workers and the queen. All the wasps were killed by freezing and were dissected in order to establish their caste. Mid June workers were easily distinguished by the queen as this has a larger body size, shows very fried wing tips and has fully developed ovaries. Mid July workers were sometimes as large as the queen, but they usually have no sperms in the spermatheca and show no fried wing tips (Dani, 1994, Reeve 1991). Three mid July colonies were orphan and had not a queen. In five mid July colonies we also find some future queens, i.e. reproductive individuals with very abundant and multistratified fat bodies in the abdomen (Strassmann 1985; Reeve 1991; Toth et al, 2009; Hunt et al, 2010; Dapporto et al, 2011; Petrocelli et al, 2013). A total of 187 workers were analyzed since sure or putative future queens (59) have been excluded from the analysis. The head of each wasp was photographed in frontal view using a standardized procedure in order to measure the head width and the size of the black spot on the clypeus (BSS henceforth).

The queen of each nest and nineteen additional Spring queens (collected in April 2013 in the same area at the beginning of the breeding season) were dissected and photographed to measure head width and BSS (for a total of 36 queens). Head width were used as a measure of body size (Eickworth, 1969).

We made a comparison between BSS of queens and workers: both within each nest - to investigate possible correlations between worker's BSS and that of their putative mother - and in the whole sample to investigate possible differences between castes. Since BSS was not normally distributed in the sample we performed a generalized linear model (GLZ) using BSS as dependent variable, body size and respective mother queen’s BSS as covariates. To test for difference in the relative size of the black spot among castes we used a Mann-Whitney U test.

Orphaned colonies experiment

Twenty-one nests of *P. gallicus* were collected with the respective queen in may 2013, in a semirural area near Florence during the pre-emergence phase. In order to obtain a successful laboratory rearing and avoid queen abandonment, we only collected nests having at least four pupae and some big larvae. Queens were caged in laboratory with their own nest and supplied with sugar, proteins (maggot fly) and paper ad libitum. Every morning for a period of three weeks each newly emerged worker was marked with enamel paints, using a unique code of colours. After this period each nest was orphaned by removing the queens, regardless of the number of workers on the colony and their age. We waited another week for giving the workers enough time to develop ovaries and then all the wasps were killed by freezing. Workers emerged during this week were excluded from the analysis. All the workers were dissected in order to establish the ovary development using an ovary index (i.e. the average length of the six most developed eggs in the ovary) (Röseler et al, 1984), then the heads were photographed in frontal view using a standardized procedure to measure head width and BSS. Head width was used as measure of body size (Eickworth, 1969). Also the queens were dissected and measured. Since ovary index was not normally distributed in the sample, we used a generalized linear model (GLZ) to investigate whether body size, BSS (as covariates) and age (as factor) are good predictors of the ovary index (dependent variable) among orphan workers.

Dead guard experiment

In order to establish whether BSS has a biological meaning during a dyadic encounter we observed the wasps behaviour in relation to a source of food guarded by a dead conspecific. This experiment was performed in a round arena (20 cm in diameter). Two same sized pieces of sugar were placed in the arena. Two dead wasps of approximately the same size were placed as guards above each piece of sugar, one of them had a high BSS value, the other one a totally yellow clypeus.

The sugar cubes were placed at the same distance from an opening through which experimental wasps were introduced. Necessarily, focal wasps had to face the dead guards before approaching and feeding on the sugar cubes (see Tibbets et al., 2008). Focal wasps were kept in the dark until the beginning of the trial and had 20 min to make a choice, we considered that a wasp had made his choice when it started feeding on a specific piece of food.

One-hundred workers of *P. gallicus* (up to four for each colony) were collected from 30 colonies in June 2012 in a semi-rural area near Florence to be used as focal wasps. To avoid collecting freshly emerged individuals only workers that actively defended the nest were collected. The wasps were kept overnight in small groups in a dark room and were only supplied with water. The day after they were all tested.

Several pairs of guards were collected in a hilly area near Florence, far enough from the area of the focal wasps to ensure that they had never met before. Guards were all workers belonging to several different colonies. Guards were all killed by freezing and then were rinsed in pentane (300 microliters) overnight to remove any possible chemical cue. The pairs of guards were used randomly during the experiments.

The first choice of focal workers, regardless of subsequent moves, were analyzed using a Chi-square test.

Results

Morphological analysis

Head width is strongly associated with BSS in *P. gallicus* workers (GLZ: N = 187, Wald Chi-square = 26.461, $p < 0.0001$). Larger workers have larger black spots on their clypeus (Fig. 1a). This confirms that, what have been recently found among the queens of the same species (Petrocelli et al., in press), also applies to workers.

On average, workers show a smaller black mark on the clypeus than

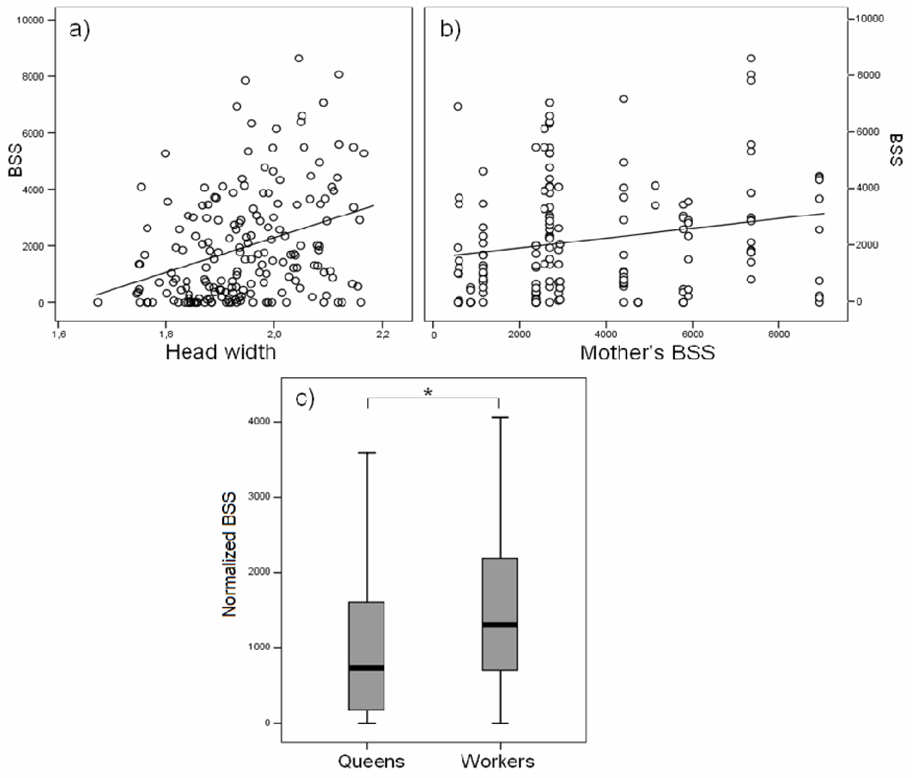

Figure 1. BSS values of *Polistes gallicus* workers from wild colonies are plotted with respect of head width (a) and of respective queen mother's BSS (b). Box plot (c) shows the difference in normalized BSS values between workers and spring queens of *P. gallicus* (Box plot show medians, 25th and 75th percentiles. $^{*}P < 0.006$; $^{**}P < 0.0001$).

queens (Mann Whitney test: N = 223; U = 2322,5; Z = -2,9; p < 0.003) (Fig. 1c). Furthermore, queens showing higher values of BSS produce worker offspring with high value of BSS (GLZ: N = 170, Wald Chi-square = 9.803, p = 0.002) (Fig. 1b).

Orphaned colonies experiment

The mean number of workers per colony at the end of the laboratory rearing period was 5.14.

A week after the removal of the queen, on 12 out of 21 colonies the oldest worker showed the highest ovary index, while on 7 out of 21 the second worker in order of birth showed the highest ovary index. In the two remaining colonies the third worker in order of birth showed the highest ovary index (Fig 2).

The GLZ test confirmed that age was strongly associated with ovary development among orphan workers (GLZ: N = 107, Wald Chi-square = 19.92, p = 0.003), while no link was found both with BSS (GLZ: N = 107, Wald Chi-square = 2.94, p = 0.086) and head width (GLZ: N = 107, Wald Chi-square = 2.98, p = 0.084). The analysis of this further sample confirmed that BSS is strongly associated with the body size of workers (GLZ: N = 107, Wald Chi-square = 1397,77, p < 0.0001) and that queens showing higher values of BSS

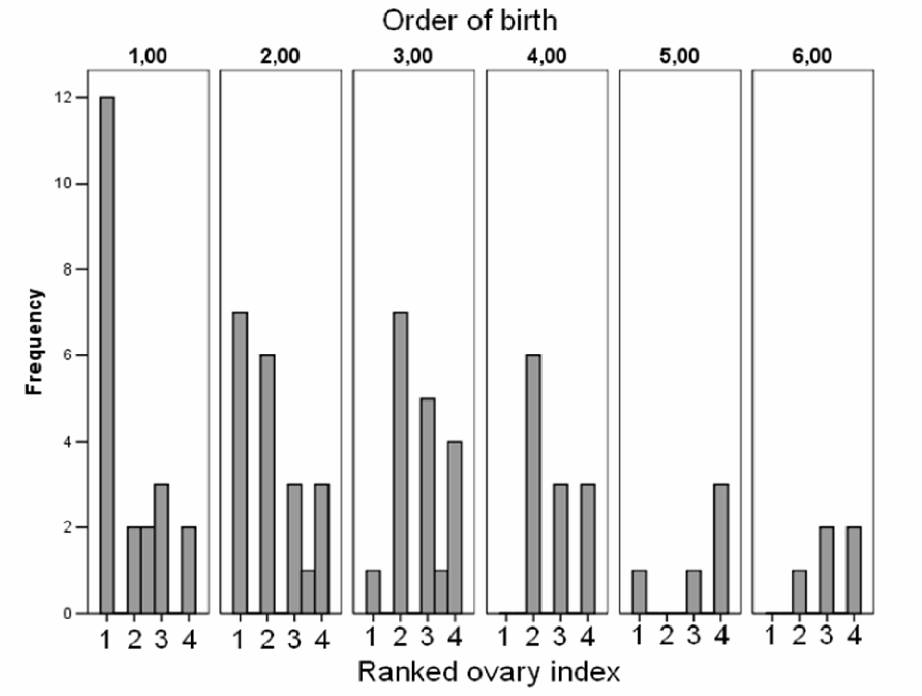

Figure 2. The histogram shows the experimentally orphaned workers ranked for ovarian index (only the first four ranks are indicated) and for order of birth in each of the colony examined (N = 21).

produce putative worker offspring with high value of BSS (GLZ: N = 107, Wald Chi-square = 25.21, p < 0.0001) (Fig 3).

Figure 3. BSS values of experimentally orphaned workers are plotted with BSS values of their respective queen mothers.

Dead guard experiment

Focal workers did not prefer feeding on a specific sugar cube, regardless of the guard's BSS (N = 100, Chi square = 0.160, p = 0.689). Fifty-two workers fed at the piece of sugar headed by the high BSS guard, while 46 fed at the one headed by a totally yellow clypeus guard (Fig. 4). This means that BSS is totally irrelevant for workers to make a choice on a specific food resource outside the colony context.

Discussion

The size of the black round spot on the clypeus is strongly associated with body size and is a conventional signal (i.e. badge of status) used by the queens of the solitary founding *P. gallicus* for assessing fighting ability within an intraspecific parasitism context (Petrocelli et al., submitted). Our data proved that also workers show a high inter-individual variability in the size of the black clypeal markings. This variability is highly associated with body size, with

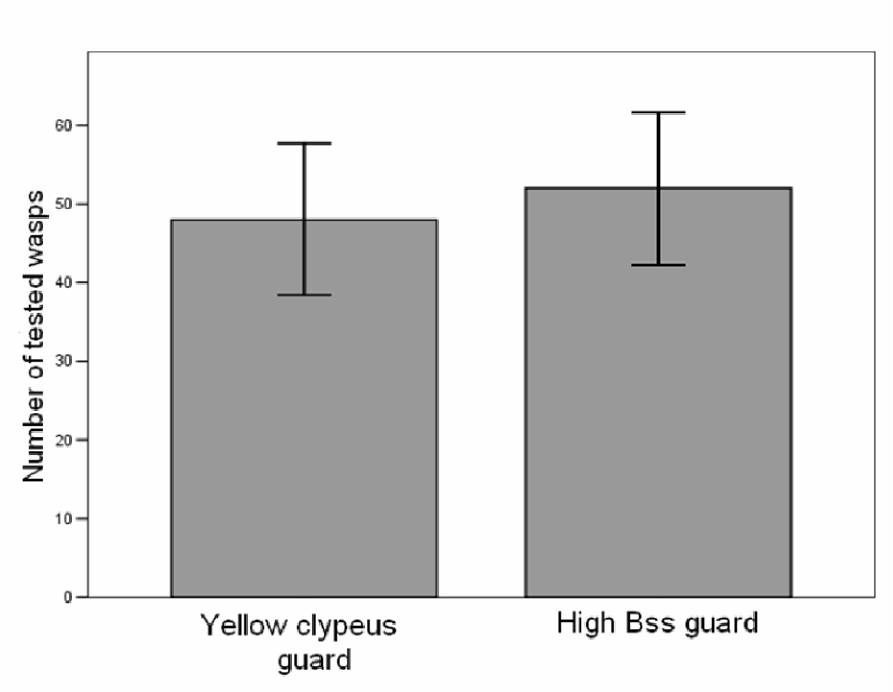

Figure 4. Choices made by 100 tested workers when introduced in an experimental arena with two source of food defended by two dead guard having the same body size but different clypeal pattern.

larger individuals having larger black spots and smaller ones with a small spot or a totally yellow clypeus. *P. gallicus* workers tend to increase in body size during the breeding season and indeed first generations of workers, which receive less food, are usually very smaller than the workers emerged later in the season, which are cared for by many more females (Dani, 1994). Thus, it logically follows that, on average, later emerging workers show larger black clypeal markings than first workers. This findings confirm that badge development in *Polistes* species is highly influenced by environmental factors (i.e. the available food at the larval stage), as reported by Tibbets and Curtis (2007).

We also found differences in the size of clypeal markings of queens and workers: queens showed larger black spots than workers, displaying a higher quality. Tibbetts (2006) proved that also workers of the American population of *P. dominula* actually display a lower level of quality with respect of queens. According to Dapporto et al, (2011) and Petrocelli and Turillazzi (2013), morphological differences between workers and queens are actually deeper in *P. gallicus* than in other congeneric species and a pre-imaginal divergence of castes has been hypothesized. The difference in the displayed quality among castes actually confirms the presence of a distinct bias between workers and queens.

However, our data show something new about badge development in paper wasps as we found a strong association between the badge of a queen and that of its putative daughters. Thus, queens displaying a high quality also have daughters displaying a high quality and vice versa. This data are fairly reliable as they regard two different samples of colonies: one set of free living colonies, collected at the mid and at the end of the breeding season, and a set of laboratory reared colonies. No genetic analysis were done to assess maternity of each queen and some nests may have suffered intraspecific usurpation: this possibility, however, is restricted to the pre-emergence phase colonies when usurpation rate is very high while in the post-emergence phase is zero (Dani e Cervo, 1992). We actually found the same result in the colonies of the post-emergence phase as in the pre-emergence ones, so we are reasonably sure that this data has an objective relevance. Moreover, all the colonies were collected at a low usurpation rate site, where colonies are frequently built in sheltered places and thus suffering little predation (Dani e Cervo, 1992). Even so, according to our data, we cannot precisely weigh how genetic or environmental factors actually influence the badge development in *P. gallicus*. Indeed, it may be possible that queens displaying high quality are also more adept at finding food, feeding more frequently their larvae and producing high-quality daughters. More likely, badge development in *Polistes* wasps is the result of complex interactions between genetic and environmental factors.

When present, conventional signals might be tied with linear dominance hierarchies and individuals displaying a high quality might play a reproductively dominant role within colonies (see Zanette and Field 2009; Baracchi et al. 2012). Instead, our data show that queues for dominance among *P. gallicus* orphan workers are based neither on visual conventional signals nor on body size but only on age, as it has been shown for other social wasps (Strassmann, 1983, Bridge and Field, 2007). Older workers, which are smaller due to the linear increase in workers' body size, usually become dominant after queen removal. The age is the primary social convention within *P. gallicus* orphaned colonies and this excludes larger putative high quality workers from the top ranks. Furthermore, unlike queens, workers seem to be unable to use conventional signals to assess fighting ability of a conspecific female out of the colony context, when the resource is, for instance, a source of food. Thus, the size of clypeal markings is not used by workers of *P. gallicus* as a badge of status, even if this signal is highly associated with body size and perhaps with fighting ability. Given the presence of a pre-imaginal bias between castes of *P. gallicus* (Dapporto et al 2011), it is possible that workers have a lower visual perceptual ability with respect of queens so they cannot see differences in the size of clypeal markings of their conspecifics. After all, *Polistes* species that are able to use visual signals seem to show some neural specialization with respect of other species (Gronenberg et al, 2007). However, workers could be able to perceive difference in clypeal markings but not to correctly use this cue or to perceive its connection with some individual qualities (i.e. fighting ability).

Actually, when colonies become large, more effective and inexpensive cues (i.e. chemical or vibrational signals) become crucial in the recognition process (Wilson, 1965), while other signals (for instance visual cues) could be set aside because of their ineffectiveness, this simple paradigm could explain the lack of use of visual signals among workers of *P. gallicus*.

Our data substantially deepen the previous knowledge about visual recognition in social wasps showing that communication systems may vary significantly with respect to different phases of the colony cycle, different cohorts of individuals and different natural contexts.

Acknowledgements

We thank Dr David Baracchi and Prof Laura Beani for many fruitful discussions about these research. We also thank all members of the sociobiology research team at the Biology Department - University of Florence. This research has been funded by the Università degli Studi di Firenze.

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9. Facial markings in the hover wasps: quality signals and familiar recognition cues in two species of Stenogastrinae

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Animal Behaviour, **85** (2012), 203-212

Abstract

Both recognition and conventional signals are widespread in the animal kingdom. Chemical communication plays a major role in invertebrates, and especially in social insects. In the last decade, observational and experimental evidence has shown the existence of visual quality signals and individual recognition cues in *Polistes* paper wasps, meaning that visual communication might also be common in insect societies. Here we show that two species of facultatively eusocial hover wasps (Vespidae, Stenogastrinae) use the visual channel for social communication. By combining morphoanatomical measurements and behavioural assays, we found that the size of the dark facial markings was related to reproductive status and dominance in colonies of *Liostenogaster vechti*, thus representing a badge of status. By contrast, no correlation between facial coloration and reproductive status was found in *Liostenogaster flavolineata*, which instead used facial markings as familiar visual recognition cues. Our results reveal that visual communication in social insects might be more widespread than previously thought and has evolved independently in distinct wasp taxa facing similar selection pressures.

Keywords: animal communication, badge of status, conventional signal, dominance hierarchy, hover wasp, *Liostenogaster flavolineata*, *Liostenogaster vechti*, quality signal, recognition system, social behaviour

Introduction

Members of animal societies need to cooperate and coordinate group behaviours. Efficient communication systems as well as recognition abilities are required for almost all social behaviours. Nestmate recognition, that is, the ability of an individual to recognize its colony mates, is critical to prevent outsiders from exploiting colony resources (Hölldobler & Wilson 1990). Discriminating between different colony mates allows individuals to adjust their behaviours, which is fundamental for assessing and maintaining dominance hierarchies and regulating parenteoffspring interactions. Most social species can discriminate between conspecifics for several characteristics, which include reproductive status, hierarchical rank, familiarity, group membership, kinship and individual identity (Thom & Hurst 2004).

In class-level recognition, conspecifics are assigned to the appropriate class by using a relatively simple distinction between limited alternatives, such as ‘male’ versus ‘female’, ‘familiar’ versus ‘unfamiliar’, ‘group member’ versus ‘nongroup member’. Signals that are relatively uniform for individuals within a class, but relatively distinct from the overall population, are usually involved in these processes (Tibbetts & Dale 2007). For individual recognition the evaluator learns the individually distinctive characteristics of the cue-bearer, which are predicted to be (1) variable enough to allow accurate discrimination between individuals and (2) not dependent on health (Dale et al. 2001). In contrast to identity signals, quality signals require high, differential costs to maintain an honest association with true quality (Zahavi 1975; Getty 2006). Unlike most sexual signals, which have production-related costs, several fighting ability signals have only social costs associated with their maintenance (Rohwer 1975; Maynard Smith & Harper 1988; Senar 1999, 2006; Gil & Gahr 2002; Whiting et al. 2003; Tibbetts & Dale 2004). These ‘area’ signals are often termed ‘conventional’ or ‘badges of status’ (Guilford & Dawkins 1995). Recent studies suggest that rank markings must be costly to their bearers because only high-quality individuals can support the social costs of increased aggression from other individuals (reviewed in Jawor & Breitwisch 2003).

Both recognition and conventional signals are common in the animal kingdom and they occur in many different modalities, including olfactory, visual and acoustic (reviewed in Dale et al. 2001). Several studies have shown that visual signals evolved as badges of status or recognition cues in many vertebrates (reviewed in Senar 1999; Whiting et al. 2003; Setchell & Wickings 2005). Furthermore, even if invertebrates, and in particular social insects, primarily use chemical cues for communication (reviewed in Vander Meer et al. 1998; Howard & Blomquist 2005) both observational and experimental evidence has shown that they are also able to use the visual channel (Shreeve 1987; Karavanich & Atema 1998; Beani & Turillazzi 1999; Tibbetts 2002; Tibbetts & Dale 2004). The paper wasps *Polistes fuscatus* and *Polistes dominulus* have variable cuticular markings that are used for social communication. Despite their similarities, the signals of these two species apparently convey completely different information. Experiments in which facial and abdominal markings of *P. fuscatus* females were manipulated suggest that workers and gynes use these features to recognize individual nestmates (Tibbetts 2002). In contrast, according to Tibbetts & Dale (2004) the complexity (brokenness) of facial markings of *P. dominulus* females in a North American population conveys information on their agonistic qualities. Tibbetts & Dale (2004) suggested that the cost of this badge is not due to pigment production, since it represents only 1% of the total body pigment, but to the social costs that it entails. In contrast, this badge of status is absent in the nesting foundresses of an Italian population (Cervo et al. 2008). Furthermore, although Zanette & Field (2009) did report a correlation between facial markings and dominance in a Spanish population, this was no longer significant when other factors (e.g. intragroup relatedness) were also considered. In this case the hypothesis is that badges of status are one of various factors that can influence dominance hierarchies and reproductive skew (Zanette & Field 2009). Owing to the differences between these populations, many controversies in the literature revolve around the role of visual signals in wasps (see Strassmann 2004; Cervo et al.2008). Information on visual communication is also available for other genera not closely related to *Polistes* wasps. For example, a status signal was described and studied by Beani & Turillazzi (1999) in males of *Parischnogaster mellyi* (Vespidae, Stenogastrinae), which use it during flying duels for winning a perch in aerial leks. Beani & Turillazzi (1999) found that males were increasingly challenged by contenders when given an extra visual signal represented by an additional white stripe painted on the back of their gasters.

The Stenogastrinae, or hover wasps, represent a taxon of 58 described species in seven genera inhabiting the forests of South-East Asia (Turillazzi 1991; Carpenter & Kojima 1996). They have small colonies (maximum 10 females) in which recognition, up to the individual level, might be useful for social organization. In the genera *Liostenogaster*, *Eustenogaster* and *Parischnogaster* various species present highly variable facial markings, potentially allowing the use of visual cues. Both *Liostenogaster vechti* and *Liostenogaster flavolineata* (two of the most common species) have a peculiar nesting biology with many colonies forming large aggregations, sometimes with hundreds of nests built within 5 cm of one other. As a consequence, colonies in clusters experience continuous landing attempts by neighbouring, alien individuals, which are sometimes received peacefully (Samuel 1987; Turillazzi et al. 1997; Coster-Longman 1998). Zanetti et al. (2001) observed how the hesitant approaching flights of landing individuals can elicit an alarm reaction from the residents of colonies of *Parischnogaster* striatula, just before recognition based on chemical cues could trigger aggressive reactions. These reports suggest a possible involvement of facial markings in the recognition process in both *L. vechti* and *L. flavolineata*. The aim of our study was to determine whether visual communication could be important in the social organization of *L. flavolineata* and *L. vechti*. We first measured the head width (a reliable indicator of body size) and size of facial markings of female wasps collected in the field, checking for a relation of these features with ovarian development and social rank. Based on the results obtained in the first part of the study we tested whether (1) *L. vechti* females use their facial markings as quality signals to assess the value of conspecifics, and (2) *L. flavolineata* females are able to use facial markings of nestmates as visual recognition cues.

Methods

Studied Species

Liostenogaster vechti forms colonies with up to seven females on a bracket-like or ringed nest (Turillazzi 1990). Colonies of this species are usually found in large clusters (up to more than 600 nests) on thewalls of buildings, caves or overhanging rocks. For this species, Cervo et al. (1996) reported a nestmate recognition ability based on chemical cues, and Turillazzi (1990) found that, only one or two potentially egg-laying females per nest are usually present.

Liostenogaster flavolineata is one of the best-known species in the group. It builds mud comb nests with up to more than 100 cells, sometimes in huge aggregates (Hansell 1982; Samuel 1987). Similarly to *L. vechti*, chemical nestmate recognition in this species has been reported and studied by Cervo et al. (1996, 2002). Social groups are relatively small (up to 10 females) and newly emerged females may disperse or become helpers on their natal nests. Bridge (2005) and Bridge & Field (2007) reported the existence of a queue for dominance based on gerontocracy (dominance order is age-based: in a nest a subordinate inherits dominance only when all her older relatives have disappeared). Field & Foster (1999) and Sumner et al. (2002) found on average only one egg-laying female per nest and only 8% of nondominant females with developed ovaries.

Sample Collection

Twenty-five colonies of *L. vechti* with a total of 76 females (mean: 3.0 females per colony, range 2-5) and 30 colonies of *L. flavolineata* with a total of 97 females (mean: 3.2 females per colony, range 2-5) were collected from two different sites: Bukit Fraser (1000 m above sea level) and Genting Tea Estate (610 m above sea level) in the Pahang State of Peninsular Malaysia. We killed all the wasps at -20°C before measuring head width, ovarian development and facial markings (see below).

Morphological Measurements and Facial Pattern Categorization

We took a picture of the ‘face’ of each collected wasp with a Fujifilm digital camera (Fig. 1). A body size estimate was then obtained by measuring the maximum width of the head (Eickwort 1969). We dissected the ovaries of all females under a binocular microscope to determine their reproductive status. For each individual, the ovarian status was quantified by using an ovarian index (OI) based on the development of the ovaries. This was performed by taking a picture using a Fujifilm camera and by measuring the length of the six largest oocytes present in the ovarioles. Since usually only the dominant female lays eggs, she possesses the most developed ovaries in her colony. The OI is thus a meaningful rank estimator. We also checked the spermatheca of each female for the presence of sperm. To categorize the facial patterns of females in both species we measured the size of the brown pigmented area on the faces (facial marking size, FMS; see also Zanette & Field 2009). All measurements were made on digital photographs by using the free software ImageJ (http://rsbweb.nih.gov/ij/). We took into account only the area corresponding to the clypeus plus eyebrows (dorsal to the antennal sockets region) since it includes most of the variability in the wasps' facial markings. Inner and outer eye stripes, antennae and mandibles were ignored as they were yellow in all the specimens of both species (see Appendix Fig. A1). To avoid the bias from individuals with a high FMS but still too young to develop ovaries and reproduce, further statistical analyses were performed only for individuals showing an OI higher than 100 (*L. vechti*: N = 38; *L. flavolineata*: N = 58), which roughly corresponds, in both species, to the division value of the two curves of the OI distribution (see also Appendix Fig. A2a,b). ANOVA designs were 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. All analyses were performed using the statistical program SPSS 13.0 (SPSS Inc., Chicago, IL, U.S.A.) for Windows.

Behavioural Assays

On the basis of the absence of correlation between facial pattern and morphoanatomical measurements, we designed a specific behavioural experiment for each species. We tested whether *L. vechti* wasps use facial markings as signals to assess the quality of conspecifics and whether *L. flavolineata* wasps are able to use them as visual cues (i.e. to discriminate familiar from unfamiliar individuals). The experiments were carried out in the field on two nest clusters of *L. flavolineata* and on two clusters of L. *vechti* located at Genting Tea Estate and Bukit Fraser, always on sunny days between 10:00 and 18:00 hours.

L. vechti

We prepared several pairs of unfamiliar lures (consisting of female wasps taken from different clusters). Before trials, thewasps were killed by freezing and then rinsed in 500 μl of pentane for 24 h to eliminate possible chemical cues. The lures were paired for comparable head width (Tibbetts 2002) but selected for different size of the brown facial marking (mean FMS of the paired lures: ca. 1900 and ca. 2500 area units, respectively). During the experiments we presented the two lures (for 1 min) alternately and approximately 1 h apart to the nest residents of free-living colonies (N = 60) with a procedure similar to that followed by Cervo et al. (2002). We counted the interactions of the residents with the lures. To control for order effects, half of the colonies were first presented with the lures with a large brown area while the other half were first presented with the lures with a small brown area. To verify that the brown facial marking of *L. vechti* is an actual visual stimulus for the wasps, we performed a test in which we presented a natural-size micropicture of a female face with five artificially increased levels of FMS edited with a picture manager software (range 1800-2600, in steps of about 160) to the residents of 30 field colonies (see Appendix Fig. A3). A brown tag and a yellow tag of the same dimension as the wasp face were also shown to each colony to ascertain whether the face models triggered a larger or smaller reaction than a nonspecific stimulus. To control for order effects, the seven stimuli were presented in a random sequence.

L. flavolineata

Before the trials, two nondominant females (foraging females according to Samuel 1987) were collected from each experimental colony (N = 40) that had at least three wasps; they were killed by freezing andthenrinsedin 500 µl of pentane for 24 h to eliminate any possible chemical cues. We then applied an experimental protocol in which the facialmarkings of focalwaspswere altered with paint, and control treatments in which focal wasps were painted without altering their markings (Tibbetts 2002). In the experimental treatments, the facial markings were changed either by adding brown paint (on average 1.6 ± 0.5% of the brown area) to the yellow surface contiguous to the brown area (N = 20) and by adding yellow paint (on average 1.6 ± 0.5% of the brown area) to the brown surface contiguous to the yellow area (N = 20). For the control, a similarsized brown area and a similar-sized yellow area of the wasps' face were painted with brown (N = 20) and yellow paint (N = 20), respectively. This design allowed us to test whether experimental responses were caused by alteration of the facial marking, and not by the covering of specific facial areas with paint (Tibbetts 2002). The two lureswere presented alternately and approximately 1 h apart to the resident females. To control for order effects, half of the experimental treatments were performed before the controls.

During the experiments on both species we held the lure for 1 min at about 1-2 cm away from the nest, starting when at least one of the resident females showed signs of reaction. To avoid excessive disturbance, wasps of experimental colonies were not marked. The number of responses by all colony members was normalized for the number of females on the nest. For both species the reactions of resident females considered were antennation (nonaggressive reaction), bending of the abdomen (a behaviour related to alarm/defence of the nest) and bites towards the lure (aggressive reaction). Ambiguous behaviours were excluded from analyses to minimize subjectivity. All the experiments were videotaped and the videos were later analysed with a blind procedure. All the data were analysed with the Wilcoxon signed ranks test: so that each colony was compared with its own reaction when facing the two different lures. For data evaluation in the 'photograph' complementary test conducted on *L. vechti*, we used a repeated measures ANOVA design (GLM for repeated measures), with the size of the brown facial markings as within-subject variables. As the data were not normally distributed and homogeneity of variances and sphericity could not be assumed in several cases, we performed corrections according to Huynh-Feldt epsilon. All analyses were performed using the statistical program SPSS 13.0 for Windows.

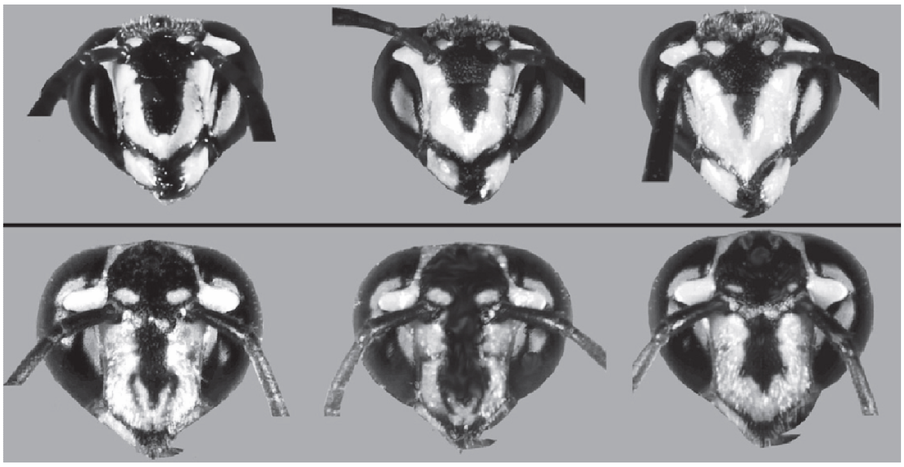

Figure 1. (a) Heads of females of *Liostenogaster vechti* with large, medium and small facial markings. (b) *L. flavolineata* females' portraits representing some examples of different facial patterns present in the species.

Results

Head Width, Ovarian Development and Visual Markings

L. vechti

Head width and FMS were normally distributed in the population (Kolmogorov-Smirnov test: Z = 0.43, N = 76, P = 0.98; Z = 0.54, N = 76, P = 0.93, respectively) and correlated with each other (Pearson correlation: r_{75} = 0.26, P = 0.023). By contrast, the distribution of OI in the overall population was evidently bimodal with a clear cut around value 100 (area units; see Appendix Fig. A2a); all the unfertilized females (N = 24) and 25% of fertilized females (13 of 51) had an OI below this level.

When we examined individuals with an OI higher than 100, the GLM analysis found a significant relationship of ovarian development with the FMS (F1,12 = 7.538, N = 38, P = 0.002) and weakly also with head width (F1,12 = 4.095, N = 38, P = 0.044; Fig. 2a). The colony membership, entered as a random factor into the model, had no significant relevance (F23,12 = 1.137, N = 38, P = 0.422). These results indicate that in theoverall population aswell aswithinanycolony theindividuals with the highest FMS, regardless of their body size (head width), were often the reproductively dominant ones. These results are also visualized in Fig. 3a which ranks the relative position of females for ovarian development and FMS values in each colony.

L. flavolineata

The distribution of OI in the overall population of females examined (N = 97) is given in Appendix Fig. A2b. The distribution is not so different from that of *L. vechti* with a cut around value 100; almost all the unfertilized females (34 of 39) and some of the fertilized females (three of 56) had an OI below this level (the spermathecae of two females were not found). Head width and FMS were normally distributed in the population (Kolmogorov-Smirnov test: Z = 0.57, N = 97, P = 0.89; Z = 0.66, N = 97, P = 0.76, respectively) and not correlated with each other (Pearson correlation: r_{96} = 0.151, P = 0.127). When we examined the individuals with OI higher than 100, the GLM found no significant relationship of the ovarian development with FMS (F1,27 = 1.562, N = 58, P = 0.222) or with head width (F1,27 = 1.895, N = 58, P = 0.180; Fig. 2b). The colony membership, entered as a random factor into the model, had no significant relevance (F28,27 = 0.537, N = 58, P = 0.946; see also Fig. 3b, which ranks the relative position of females for ovarian development and FMS values in each colony). To be sure to capture the variation in the facial markings adequately, additional parameters apart from the FMS area were measured. However, no significant correlation was found between these parameters and the ovarian development (see Appendix Fig. A4 legend). These results indicate that the reproductive status of a wasp is independent from its FMS value.

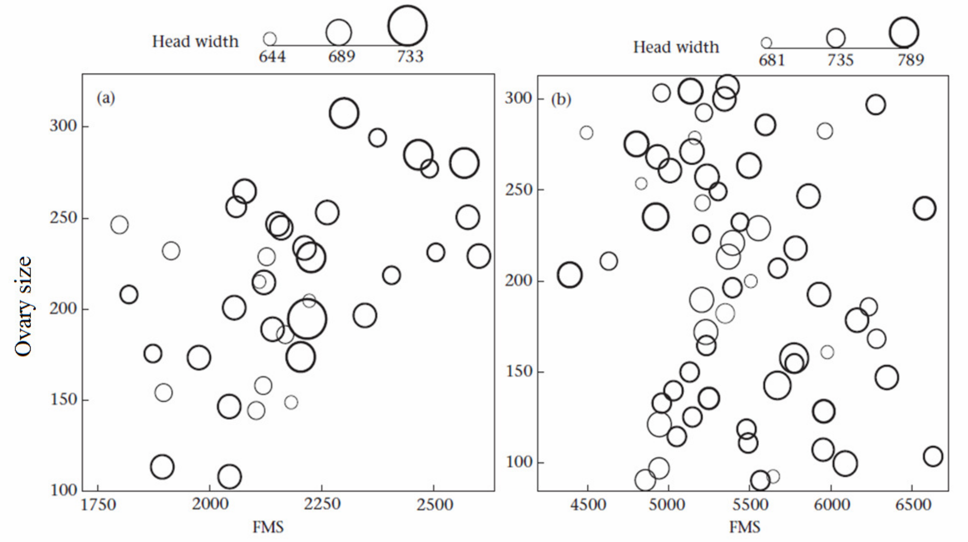

Figure 2. Correlations between ovary development (ovarian index, OI) and facial marking size (FMS, reported in area units) for different head widths (black dot size) in (a) *L. vechti* and (b) *L. flavolineata* females.

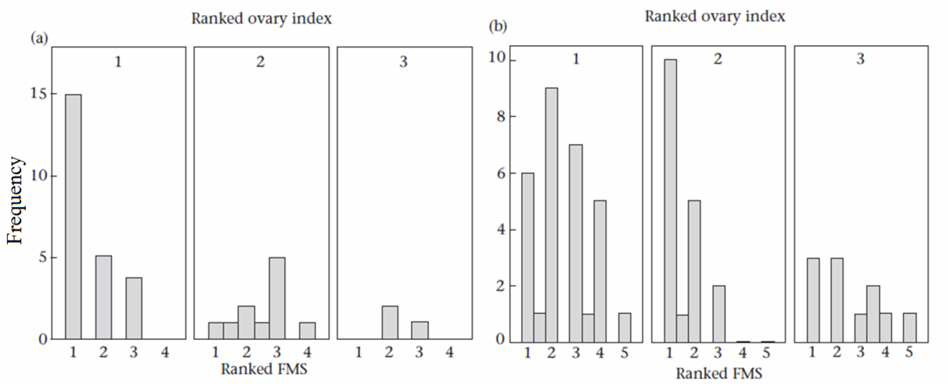

Figure 3. (a) Females of *L. vechti* (N = 38) ranked for ovarian index and FMS (i.e. the FMS decreases from 1 to 4) in each of the colonies examined (N = 25). (b) Females of *L. flavolineata* (N = 58) ranked for ovarian index and FMS in each of the colonies examined (N = 30).

Behavioural Assays

L. vechti

On average, the lures with a larger FMS received significantly more inspections and aggression than the lures with a smaller FMS (N = 60; Wilcoxon test: antennations: Z=-3.13, P < 0.001; bites: Z=-3.93, P < 0.0001). Similarly, larger FMS lures triggered the bending of the abdomen much more than smaller FMS lures (Wilcoxon test: Z=-4.65, N = 60, P < 0.0001; Fig. 4).

When the micropictures of the wasps' faces were presented to the colonies, we observed that the larger the FMS value was, thehigher the elicited explorative and aggressive responses were (GLM for repeated measures corrected according to Huyn-Feldt's epsilon, N = 30; antennations: F4 = 4.06, P = 0.04; abdomen bends: F3.9 = 8.96, P = 0.001; bites: F2.9 = 6.11, P = 0.001; Fig. 5). Contrarily, the reactions elicited by the brown and yellow tags were lower even than those elicited by the photograph of the face with the smaller FMS value (N = 30; Wilcoxon test: yellow tag: Z = 3.38, P < 0.001; brown tag: Z = 3.73, P < 0.001). These results indicate that the levels of aggression of the residents of the tested colonies increased with the proportion of brown pigment on the face of the experimentally presented wasp. This also suggests that awasp with a browner face should represent a greater threat for the dominance order of the colony than a wasp with a yellower face.

L. flavolineata

Neither browner nor yellower experimental lures elicited significantly more antennations and bites than the controls (N = 20; Wilcoxon test: browner lures: antennations: Z=-0.08, P = 0.94; bites: Z=-0.846, P = 0.46; yellower lures: antennations: Z=-1.77, P = 0.079; bites: Z=-1.34, P = 0.17; Fig. 6).

However, the number of abdomen bends triggered by the experimental lures was significantly higher than that triggered by the controls both when brown (Wilcoxon test: Z=-2.65, N = 20, $P < 0.006$) and when yellow (Wilcoxon test: Z=-3.24, N = 20, $P < 0.0001$). These results indicate that the residents of the tested colonies were alarmed by a wasp that was unfamiliar owing to altered (increased and diminished) facial markings.

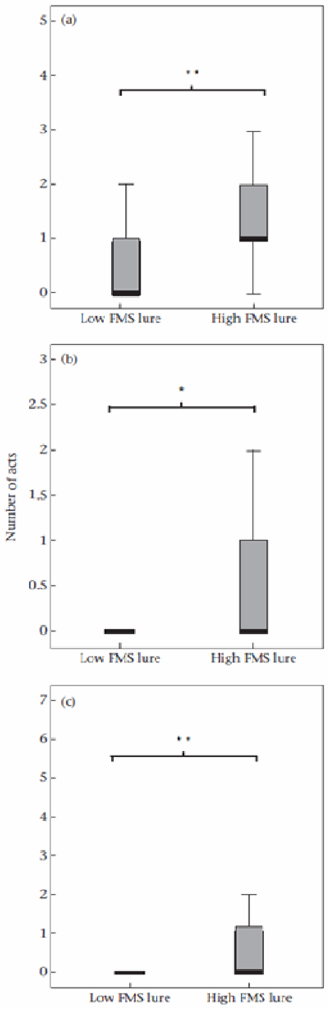

Figure 4. Comparison between the number of (a) abdominal bends, (b) antennations and (c) bites directed by L. vechti resident females against low FMS and high FMS lures. Box plots show medians, 25th and 75th percentiles. *P < 0.001; **P < 0.0001.

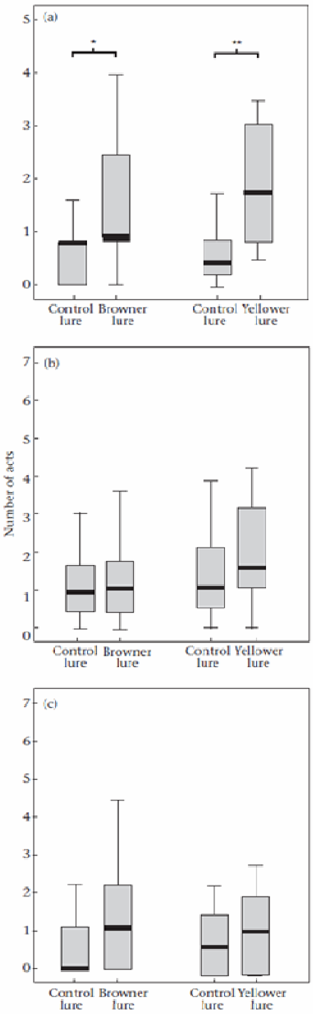

Figure 6. Comparison between the number of (a) abdominal bends, (b) antennations and (c) bites directed by L. flavolineata resident females towards experimental and control lures. Box plots show medians, 25th and 75th percentiles. *P < 0.006; **P < 0.0001.

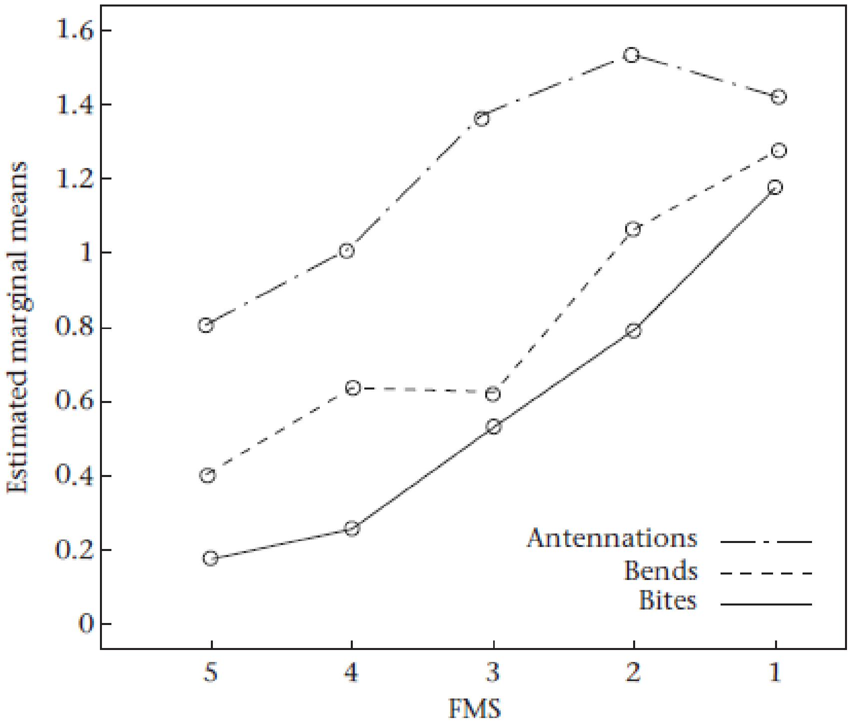

Figure 5. Explorative and aggressive responses elicited when the micropictures of the female faces with five increased levels of FMS were presented to 30 *L. vechti* colonies.

Discussion

In *P. dominulus*, a temperate wasp species, body size is a significant, although not absolute, predictor of rank in associative foundations, probably because it confers somecompetitive ability (Turillazzi & Pardi 1977; Reeve 1991; Cervo et al. 2008; Zanette & Field 2009). In hover wasps, mainly because of the relatively aseasonal tropical environment and complete absence of caste dimorphism, the range of body size of individuals is less pronounced than in temperate *Polistes* wasps. As a consequence, the fighting ability of females may be less influenced by body size. This difference can account for our results: aweak influence of body size (head width) in the dominance hierarchy of *L. vechti* and no influence in that of *L. flavolineata*. Our behavioural experiments showed that the variable brown facial pattern functions as a visual signal in these two Stenogastrinae species. Dissections andmeasurements provided evidence that these visual signals can convey different information in the two species.

In *L. vechti* the size of the brown facial markings was correlated with head width and was a more important predictor of ovarian development than body size. Furthermore, it was a decisive element in rank determination and was used as a status badge by wasp females. In a colony, individuals had larger facial markings the bigger their ovaries. These findings suggest that individuals choosing different strategies (e.g. to stay in their native nest or migrate) may lead to a redistribution of dominants and subordinates among nests of their own or neighbouring clusters. In fact, in this species individuals have a strong tendency to shift between different nests (Coster-Longman 1998). Several females leave their nests to join other colonies or become floaters as happens in *L. flavolineata* (Samuel 1987; Field et al. 1998). Cervo et al. (1996, 2002) demonstrated nestmate recognition ability in *L. vechti* as well as in *L. flavolineata*; this ability allows residents to discriminate conspecifics from alien colonies. However, Cervo et al. (1996, 2002) also noted a high percentage of 'erroneous' acceptances of alien females in both *L. vechti* (24%) and *L. flavolineata* (30%), and hypothesized that the discrimination ability in clustering species might be lower than in other hover wasps (e.g. *Parischnogaster jacobsoni*, Cervo et al. 2002). However, if not all alien females represent a real threat for a colony because not all of them will try to reproduce, their acceptance could be not an 'error' but a choice based on visual cues. This could account for the significant number of alien females that are peacefully received on the nests by resident females (Samuel 1987; Turillazzi et al. 1997; Coster-Longman 1998). In our study, aggression was observed towards high-ranking and not towards lower-ranking experimentally presented individuals. This behaviour allows resident females to reduce the costs of continuously rejecting alien nonfertile individuals approaching or landing on their nest, at the same time maintaining an efficient control system. Floaters and alien females, on the other hand, often approach alien nests with uncertain and explorative flight (Turillazzi 1990) and status signalling by facial marking may help these wasps to present themselves to alien colonies, testing the immediate response of residents and exploring the possibility of joining or not in relation to the level of aggression received.

Independent nesting is very unsuccessful in these wasps (in *L. flavolineata*, for example, more than 50% of the single female foundations fail: Samuel 1987; Field et al. 1998). Thus, a female is better off if it has help in brood rearing. A female with large facial markings (high status badge) may incur two sources of social costs: it is challenged when it tries to join an alien colony and, if it founds a nest alone, it may be less likely to be joined by other potential helper individuals.

The fact that wasps with higher status badges were subjected to more aggression by alien females could appear counterintuitive. Signals of agonistic ability are expected to reduce the cost of aggressive conflicts by allowing opponents to avoid costly fights that have predictable outcomes (Senar 2006). However, attacked individuals are never predicted to give up immediately when competing over something as valuable as their nest. Furthermore, our result makes more sense if we consider that signals of fighting ability will remain evolutionarily stable and exempt from cheating only if receivers of signals aggressively 'test' the sender's true behavioural status (Rohwer 1977). Receivers may test signal accuracy mainly during landings of potentially threatening alien wasps (i.e. an egg-layer female), but accept the signal without continuous aggressiveness in a linear hierarchy inside the colony (andthis also represents abenefit thatbalances the high social costs, in aggression, suffered at the beginning by the bearer of a higher status badge). Maynard Smith & Harper (1988) suggested a similar scenario in a nonsocial contest and Tibbetts et al. (2010) demonstrated that in *P. dominulus* wasps the receiver's responses to signals of agonistic ability are context dependent.

A different situation occurs in *L. flavolineata* where the visual signal could convey completely different information. No relationships were found between the size of facial markings and body size (head width) or ovarian development, either in the overall sample or within single colonies. Consequently, we exclude the existence of visual cues as quality signals in this species. The lack of status badges in *L. flavolineata* is consistent with the existence of dominance hierarchies based on gerontocracy in which dominance order is agebased (Bridge 2005; Bridge & Field 2007). Even if all helpers have the potential to become egg-layers (Field & Foster 1999), only the highest quality individuals, and thus the most likely to survive long enoughto inherit, are effectively selectedto bedominants by queuing (Field et al. 1998). Still, our behavioural experiment indicates that resident wasps respond with an alarm/defence posture (bending) towards nestmates whose facial markings have been experimentally altered, suggesting that *L. flavolineata* wasps use the visual channel for communication and discriminate between familiar and unfamiliar individuals. Visual cues in this species could represent an additional useful recognition tool because nests often occur in very dense clusters. Beyond the use of visual signals for recognition of familiar individuals, one potential benefit of visual signals in *L. flavolineata* might be the reduction of aggression among nestmates. If facial markings can be learned as an individually distinctive characteristic, they may permit an accurate discrimination of individuals on the nest, allowing newly emerged wasps to recognize each nestmate as an individual and learn and memorize its position in the dominance queue (Bridge & Field 2007). This could help a subordinate individual to assess the length of the queue and to decide whether to leave or to remain on the natal nest. Unfortunately, as often occurs, it is very difficult to distinguish between true individual recognition (where the receiver learns the signaller's individually distinctive characteristics and associates them with the signaller itself) and class-level recognition (where the learned signaller's distinctive characteristics are associated with the inferred classspecific information about the signaller; Sherman et al. 1997; Tibbetts & Dale 2007). In both cases, wasps learn the individually distinctive facial pattern of the signaller. Our behavioural experiments did not allow us to discriminate between these two kinds of recognition and further research is needed to clarify this aspect of the hover wasps' biology. In conclusion, our results showthe relevance of visual communication in both these species of hoverwasps. The size of the dark facial markings of female *L. vechti* is related to reproductive dominance and represents a badge of status. On the other hand, *L. flavolineata* use facial markings as visual recognition cues. Our results on two different species of stenogastrine wasps suggest that communication systems based on visual recognition cues might be a primitive feature of the genus. Moreover, our study reveals that visual communication might be widespread among theVespidae and other social insects, and that similar selection pressures might have produced similar communication systems in two distinct wasp subfamilies, the Polistinae and Stenogastrinae.

Acknowledgments

We thank Dr Leonardo Dapporto, Dr Rita Cervo (Università di Firenze, Italy) and Professor Jeremy Field (University of Sussex, U.K.) for critically reading and improving the manuscript and the anonymous referees for their helpful comments.We also thank Professor Rosly Bin Hashim (University of Malaya, Malaysia), Mr Henry Barlow and Mr Simon Hok for their support in Malaysia. We thank also Professor Roscoe Stanyon for revising the English text. This research was funded by the Università degli Studi di Firenze.

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Appendix

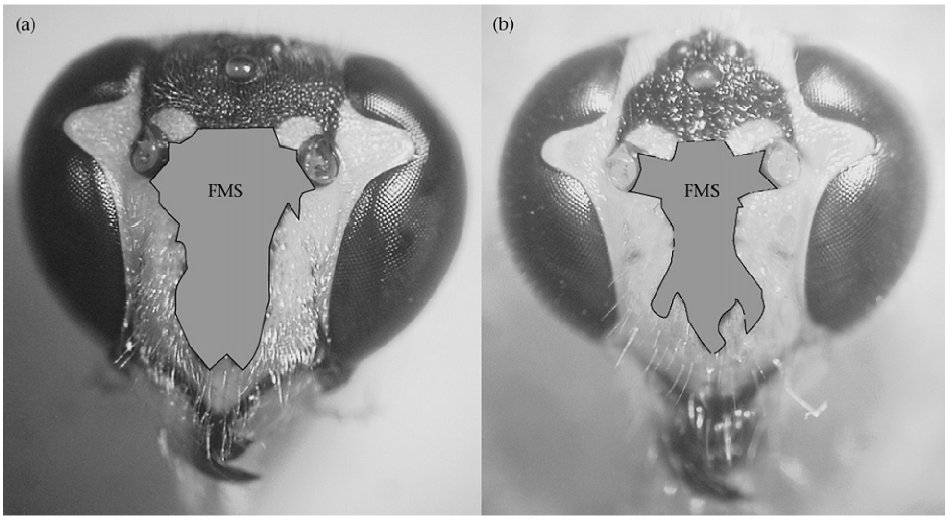

Figure A1. The brown facial marking (FMS) is represented by the grey area (FMS) reported in the picture of the face of both (a) *L. vechti* FMS: mean ± SD = 2168 ± 199, range 1750-2600 area units) and (b) *L. flavolineata* (FMS: mean ± SD = 53 683 ± 4951 area units, range 4000-66 500) females.

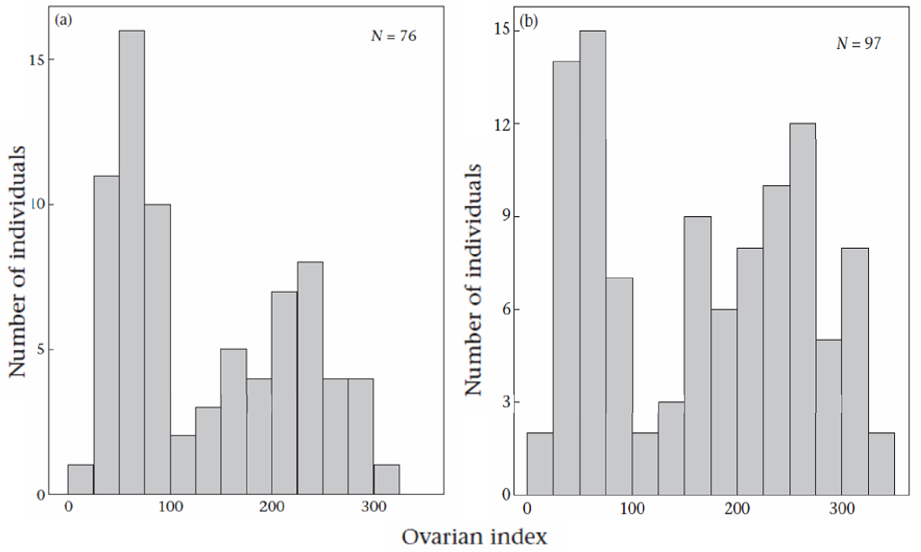

Figure A2. Distributions of ovarian index in the overall sampled population of (a) L. vechti females and (b) L. flavolineata females.

Figure A3. Micropictures of faces of female *L. vechti* experimentally altered in the brown facial markings with computer software and then reduced to natural size. The brown area was organized in five increased levels of FMS (range 1800-2600 area units, by steps of ca. 160 area units). All the pictures were presented in a random order to the residents of 30 field colonies.

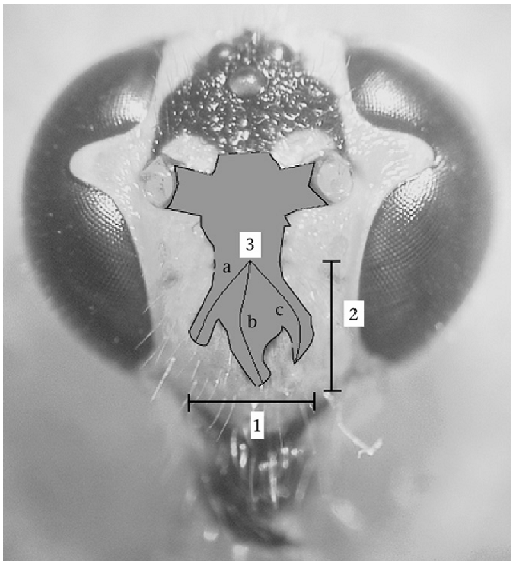

Figure A4. Additional parameters of visual marking measured in *L. flavolineata*: 1: maximum width of the median pronged shape; 2: maximum length of the facial marking; 3: median and SD of the length of the three pronged shapes of the facial marking (a, b, c)). No significant correlations were found between the ovarian development and the maximum length of the median pronged shape (Spearman correlation: $rS=0.003$, $N = 58$, $P = 0.77$), the maximum width of the facial marking (Spearman correlation: $rS = 0.009$, $N = 58$, $P = 0.94$) or the mean and SD of the length of the three pronged shapes of the facial marking (a, b, c; Spearman correlation: $rS = 0.034$, $N = 58$, $P = 0.78$; $rS=0.205$, $N = 58$, $P = 0.101$, respectively). Even transforming all these variable values within each nest into ranks, we found that highly ranked individuals for ovaries were not those showing highest ranked values for the maximum length of the median pronged shape (Spearman correlation: $rS = 0.041$, $N = 58$, $P = 0.76$), the maximum width of the facial marking (Spearman correlation: $rS = 0.141$, $N = 58$, $P = 0.26$) or the mean and SD of the length of the three pronged shapes of the facial marking (a, b, c; Spearman correlation: $rS = 0.126$, $N = 58$, $P = 0.32$; $rS=0.175$, $N = 58$, $P = 0.16$, respectively).

10. Swift recognition in a social wasp: the face matters, the rest is ignored

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Manuscript in preparation

Abstract

Social insects have evolved highly developed recognition systems enabling them to accept nestmates but reject alien conspecifics. Chemical communication plays a crucial role in this ability. Recently it was discovered that visual quality signals and individual recognition play crucial play crucial roles in some species of social wasps. In the primitively eusocial wasp *Liostenogaster flavolineata* (Stenogastrinae), individuals differ in their cuticular hydrocarbons profiles according to their colony membership and each female also possesses a unique face. Since both chemical and visual channels mediate the recognition abilities in these wasps, this species represents a unique model to understand how these senses are integrated during the perceptual processing and the extent to which wasps prioritize one channel over the other to discriminate aliens and nestmates. Here we reveal that although *L. flavolineata* females are able to discriminate between alien and nestmate females using only visual or chemical cues, the chemical profiles become redundant when the visual and chemical stimuli are presented together. Our findings indicate that resident wasps regulate the level of aggression on the basis of the opponent's faces regardless of their cuticular hydrocarbons. This is the first report that shows visual channels prevail over odour pathways in a nestmate recognition context suggesting that the use of visual communication could reduce the importance of olfactory communication.

Key words: visual cues, chemical cues, multimodal sensory cues, nestmate recognition, cognitive abilities, visual cognition, Stenogastrinae.

Introduction

Cooperation and information flow among colony members are the main drivers of social insects' ecological success (Wilson 1985, Maynard Smith & Szathmary 1995). Efficient communication systems and recognition abilities are required for almost all social behaviours. In particular, the ability of an individual to recognise its colony mates (i.e. nestmate recognition) is crucial to prevent outsiders from exploiting colony resources (Hölldobler & Wilson, 1990). Indeed social insects have evolved highly developed recognition systems enabling them to behave altruistically towards nestmates and to reject alien conspecifics (Lenoir et al. 2001).

Chemical communication plays a major role in social insects' recognition systems (Wyatt, 2003). Cuticular hydrocarbons (hereafter CHCs) together with pheromones are assumed to regulate almost all social interactions, implying the chemical sense as the predominant channel of communication in insect societies (Lenoir et al 2001; Howard & Blomquist, 2005). In the last two decades, however, visual communication abilities have been discovered in two subfamilies of social wasps (Tibbetts, 2002; Tibbetts & Dale 2004; Baracchi et al. 2012; Chittka & Dyer 2012; Avarguès-Weber 2012). A pioneering experiment demonstrated that males of the stenogastrine wasp *Parischnogaster mellyi* use a visual status badge during flying duels for winning a perch in aerial leks (Beani & Turillazzi 1999). It was since found that visual communication plays a key role in the social interactions between colony members and indeed, a facial badge of status has been shown to convey information on the agonistic qualities in foundresses of the North American population of *Polistes dominula* and to regulate dominance hierarchies in the stenogastrine wasp *Liostenogaster vechti* (Tibbetts & Dale 2004; Baracchi et al. 2012; but see also Cervo et al. 2008; Zanette & Field 2009). *P. fuscatus* wasps are more aggressive to individuals with unfamiliar appearances landing on the nest (Tibbetts 2002) and they are able to remember the individual identity of partners after one week of interactions with several other wasps (Sheehan & Tibbetts 2008). Remarkably the visual communication seems to be developed to the extent that specialized face learning is associated with individual recognition ability in paper wasps (Sheehan & Tibbetts 2011).

Despite those extraordinary cognitive abilities, no brain specialisation such as an increase in the size of the primary visual centres was found in species that posses the faculty to recognize one another individually (Gronenberg et al. 2009). On the other side however, wasps showing face recognition abilities possess concomitant smaller antennal lobes suggesting that the use of visual communication could reduce the importance of olfactory communication (Gronenberg et al. 2009). Even if brain size may have only a tiny relationship with behavioural repertoire and cognitive capacity (Chittka & Niven 2009) high communication abilities might be cognitively demanding and required concomitant bigger brains. As a consequence, small brain invertebrates may should have to balance between different abilities, especially avoiding redundancy in the involvement of different senses to get identical information. So far, many studies on chemical and visual communication in social wasps have been done, but no information exists about the relationship between them. The study of multimodal sensory cues deserves more attention.

In the primitively eusocial wasp *Liostenogaster flavolineata*, individuals differ in the CHCs profiles according to their colony membership and each female has also a unique facial marking (Cervo et al. 2002; Baracchi et al. 2012) (fig.1). It has been shown by experimental manipulation that in these wasps both chemical and visual channels mediate nestmate recognition ability, and opponent wasps presenting unfamiliar odours or faces are rejected aggressively from the nests by evaluators (Cervo et al. 1996; Cervo et al. 2002; Baracchi et al. 2012). *L. flavolineata* represents a unique opportunity to understand how these senses are combined during the perceptual processing and to which extent wasps prioritize one channel over the other to discriminate aliens and nestmates. In the present work we answered this question by quantifying the relative importance of visual and chemical channels in recognising opponents' colony membership when visual and chemical cues are presented alone or combined.

Materials and methods

Study organism

Liostenogaster flavolineata is one of the best-known species in the Stenogastrinae group. It builds mud comb nests with up to more than 100 cells, sometimes in huge aggregations of about 10-150 nests in close proximity (Samuel 1987). Both chemical nestmate recognition and visual recognition cues have been reported and studied by Cervo et al. (1996; 2002) and Baracchi et al. (2012) respectively. Social groups are relatively small (up to 10 females) and newly emerged females may disperse or become helpers on their natal nests. Bridge (2005) and Bridge and Field (2007) reported the existence of a queue for dominance based on gerontocracy (dominance order is age-based: in a nest a subordinate inherits dominance only when all her older relatives have disappeared). Field and Foster (1999) found on average only one egg-laying female per nest and only 8% of non-dominant females with developed ovaries.

Sample collection & lures preparation

The experiments were carried out in the field on one nest cluster of *L. flavolineata* located at Bukit Fraser (1000 m a.s.l.; 03° 42.774 N - 101° 46.319 E) in the Pahang State of Peninsular Malaysia, always on sunny days between 10 am. and 5 pm. Overall, 50 colonies of *L. flavolineata* with a total of 233 females (mean: 4.6±1.6 females per colony, range: 2-8) were studied. The day before the trials, a non-dominant female (foraging female according to (Samuel 1987)) was collected from each experimental colony (first experiment n = 20; second experiment n = 30) that had at least three wasps. The same day an equivalent number of alien females (n = 50) were also collected from a different cluster located at 20 km apart as the crow flies but in the other side of the mountain (3° 38' 0" N - 101° 42' 0" E) to ensure individuals had not previously interacted with tested colonies. Each wasp was killed by freezing and then rinsed in 1 ml of pentane for 24 hours in order to eliminate all cuticular lipids (epicuticular hydrocarbons, CHCs). Pentane extracts were then dried at room temperature (roughly 26 ^{o}C), re-suspended in 200 µl of pentane and split in two aliquots of 100 µl each. Aliquots were then placed, by means of a micropipette, on pre-washed wasps (head and thorax) or square pieces of clean filter paper (5mm side length) depending on the experiment (see below). The aliquots were applied on lures 15 min before presentations in order to ensure the evaporation of the solvent.

In order to test whether a visual stimulus alone and a chemical stimulus alone was able to allow the resident wasps to discriminate between alien and nestmate females we presented, in a first assay, four stimuli to 30 colonies: a nestmate visual stimulus (nestmate wasp deprived of its CHCs), alien visual stimulus (alien wasp deprived of its CHCs), a nestmate chemical stimulus (filter paper drenched with a full aliquote of a nestmate CHCs extract) and an alien chemical stimulus (filter paper drenched with a full aliquote of an alien CHCs extract). A preliminary assay had shown that resident wasps do not react to a piece of filter paper applied with the solvent only (Friedman test, $\chi 2 = 20.77$, d.f. = 2, $p = 0.0001$, n = 30 colonies; post hoc test (Wilcoxon Monte Carlo Exact test), solvent-alien odour: n = 30, $Z = -3.79$, $p = 0.0001$; solvent-nestmate odour: n = 30, $Z = -2.39$, $p = 0.017$).

To quantify the relative importance of the two communication channels presented alone in the nestmate recognition process, we calculate two indexes as: 1) chemical index: the n° of bites elicited by an alien CHCs minus those elicited by a nestmate CHCs; 2) visual index: the n° of bites elicited by an alien face minus those elicited by a nestmate face. This procedure was preferred since we could not directly compare the bites elicited by a "face" stimulus and a "piece of paper" stimulus regardless the conveyed information (i.e. alien and nestmate).

To understand whether one stimulus prevails over the other in the nestmate recognition context we conducted a second assay in which we presented four stimulus combinations to 20 colonies: a full nestmate stimulus (nestmate washed wasp re-coated with an half of own CHCs extract), a full alien stimulus (alien washed wasp re-coated with an half of own CHCs extract) and two crossed lures (nestmate washed wasp reapplied with a half of an alien CHCs extract and an alien washed wasp reapplied with a half of alien CHCs extract). Preliminary GC-MS analyses have shown that this protocol does not alter the chemical profile or the quantity of cuticular lipids present on the head of female wasps (Cini et al. 2008).

Behavioural assays

In both assays, the stimuli were presented to the resident females at approximately one hour intervals. To control for order effects, the stimuli were presented in a random sequence. During the experiments we held the lure about 1 cm away from the nest for 30 seconds. In order to avoid excessive disturbance, wasps of experimental colonies were not marked. The number of responses presented by all colony members was normalized for the number of females on the nest as done in previous experiments (Baracchi et al. 2012). The aggressive reaction of resident females considered were the bites towards the stimulus. Ambiguous behaviours were excluded from analyses in order to minimise subjectivity. All the experiments were videotaped and the videos were later analysed with a blind procedure in which the watcher did not know the nature of the stimulus presented (alien vs nestmate). When the data were not normally distributed and homogeneity of variances could not be assumed, they were analysed with a non-parametric test (i.e. Friedman and Wilcoxon signed rank test, so that, for each colony, we compared the reactions towards the different presented stimuli. All analyses were performed using the statistical program SPSS® 13.0 for Windows®.

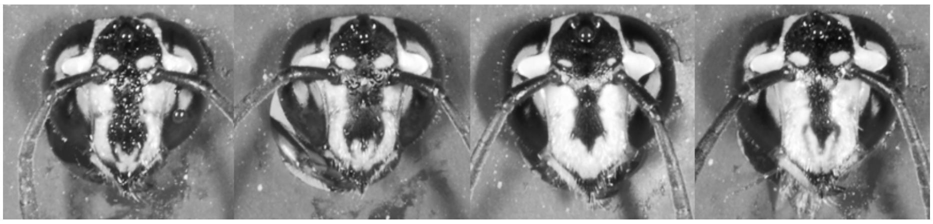

Figure 1. *L. flavolineata* females' portraits representing some examples of different facial patterns present in the species.

Results

When resident females of *L. flavolineata* (n = 30 colonies; Exp 1) were presented with CHCs extracts or faces belonging to nestmates and alien females, the alien cues invariably sparked significantly more aggressiveness (Friedman test, $\chi 2 = 31.83$, d.f. = 3, p = 0.0001, n = 30 colonies; post hoc test (Wilcoxon Monte Carlo Exact test), visual stimuli: n = 30, $Z = -2.21$, p = 0.021; chemical stimuli: n = 30, $Z = -2.31$, p = 0.018; figure 2). These results indicate that the residents of the tested colonies were alarmed by a wasp which was unfamiliar due to its face and its chemical odour. Quantifying the relative importance of the role of the visual and chemical channel, we found that the information about "alien status" is better conveyed by the visual cue than by the chemical one (paired t-test: t = 2.37, df = 29, n = 30, p = 0.025; figure 3). Indeed on average, a visual cue triggered an aggressive reaction about four times more than the chemical cues (mean aggressive reaction (mean ± s.d.), visual index: 2.32 ± 3.69, chemical index: 0.60 ± 1.37, n = 30; figure 3).

When resident females (n = 20 colonies, Exp 2) were allowed to use visual *and* chemical stimuli to evaluate the presented lures, chemical cues were no longer considered by the wasps to discriminate between alien and nestmate females (one way Anova, F = 7.18, df = 3, n = 20, p = 0.0001; figure 4). Indeed the difference existing in the aggression level directed towards a full alien and a full nestmate lure (i.e. washed wasp reapplied with its own CHCs extract) was equal to that existing between the "crossed" lures (i.e. washed nestmate reapplied with alien CHCs extract and vice versa), (Tukey HSD post hoc test, n = 20, full-full, mean diff. = 3.03, p = 0.02; cross-cross, mean diff = -3.50, p = 0.003; figure 4). More importantly, the aggression level directed towards a full alien female was very similar to that directed towards a "crossed" lure displaying an alien face but with a nestmate odour (Tukey HSD post hoc test, n = 20, p = 0.98; figure 4). Similarly, the aggression level directed towards a full nestmate female was very similar to that directed towards a "crossed" lure displaying a nestmate face but with an alien odour (Tukey HSD post hoc test, n = 20, p = 0.99; figure 4). These findings indicate that resident wasps regulate the level of aggression on the basis of the opponent's faces regardless of their CHCs.

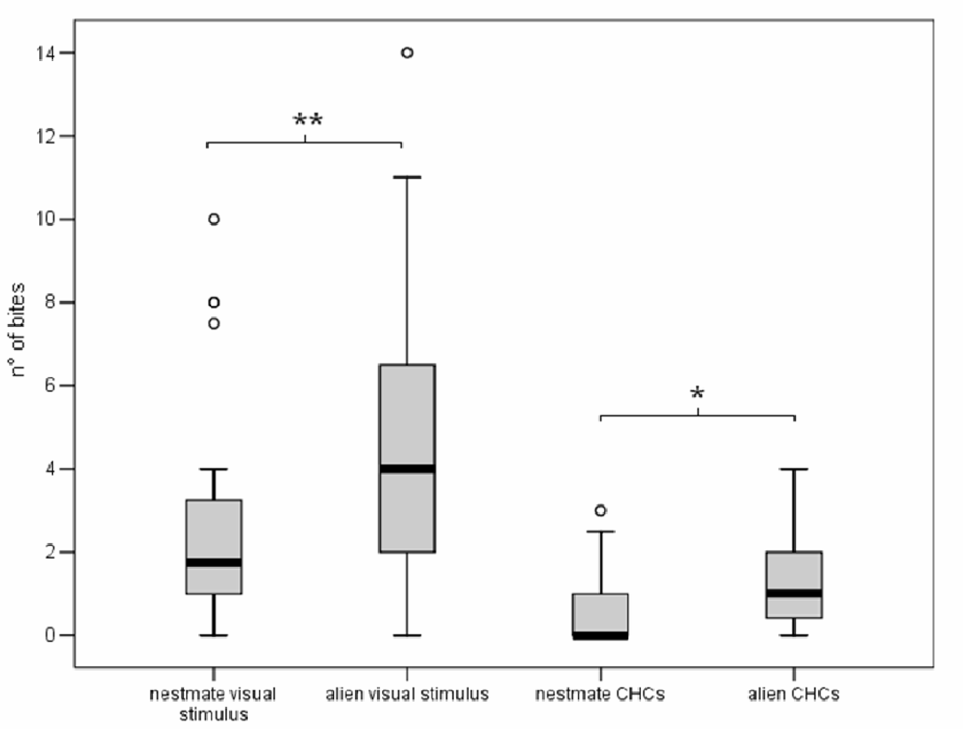

Figure 2. Comparison between the number of bites directed by *L. flavolineata* resident females towards a nestmate visual stimulus (nestmate wasp deprived of its CHCs), alien visual stimulus (alien wasp deprived of its CHCs), a nestmate chemical stimulus (nestmate CHCs extract applied on a square piece of filter paper) and an alien chemical stimulus (alien CHCs extract applied on a square piece of filter paper). Box plots show medians, 25th and 75th percentiles. (* = $P < 0.021$; ** = $P < 0.018$).

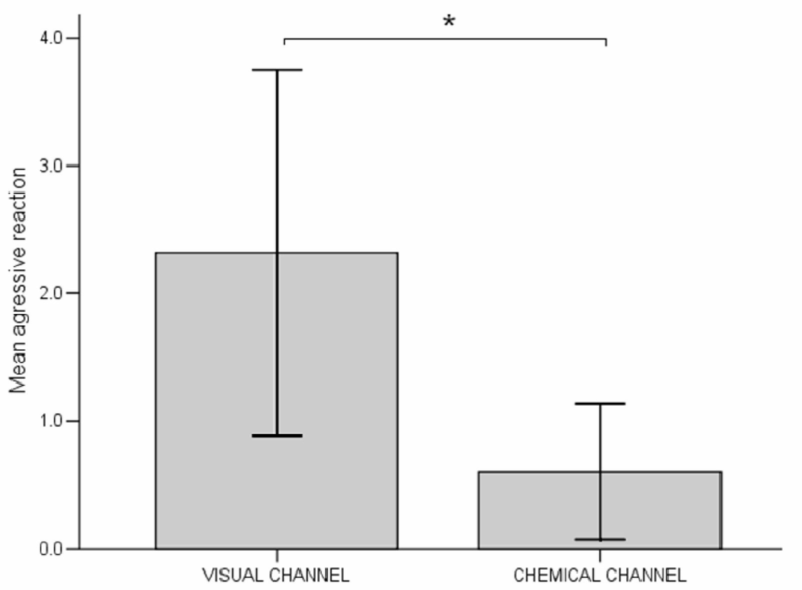

Figure 3. Comparison between the average number of bites showed by *L. flavolineata* resident females reacting towards and alien stimulus convey by the visual and the chemical channel. The average number of bites for each channel are obtained by subtracting the number of bites elicited by a nestmate stimulus from those elicited by an alien stimulus. (* = $P = 0.025$).

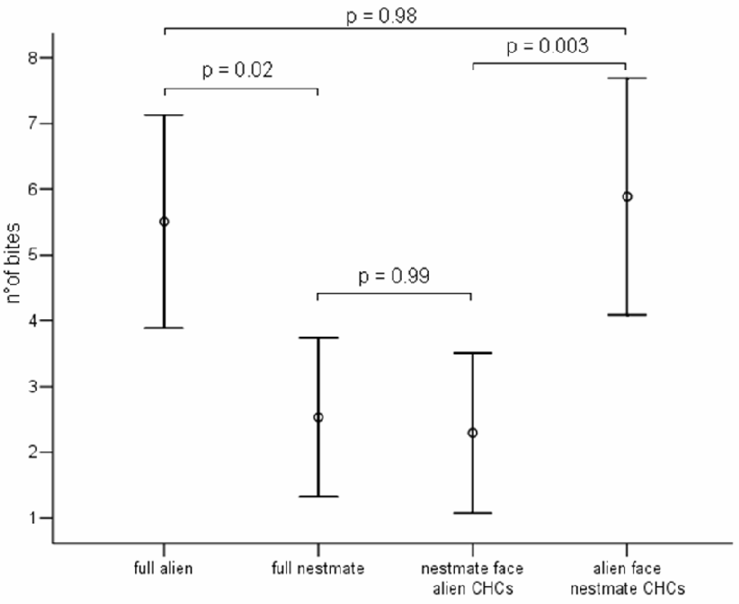

Figure 4. Comparison among number of bites showed by *L. flavolineata* resident females (N = 20 colonies) reacting towards a full alien and a full nestmate lure (alien and nestmate washed wasp reapplied with own CHCs extract respectively), and two crossed lures (a nestmate washed wasp reapplied with alien CHCs extract and an alien washed wasp reapplied with alien CHCs extract).

Discussion

Here we experimentally demonstrated that when resident females of *L. flavolineata* were presented with odours or faces belonging to nestmates and alien females, the alien cues always spark significantly more aggressiveness. Accordingly to previous findings (Cervo et al. 1996; Cervo et al. 2002; Baracchi et al. 2012) these results clearly indicate that *L. flavolineata* wasps are able to assault and reject alien conspecifics appearing unfamiliar due to their facial markings or their chemical odour. In a previous paper, Cervo et al. (2002) found that alien wasps deprived of their CHCs and added with those of nestmates were treated less aggressively than those with their own CHCs, concluding that CHCs were used to recognise alien females. However, more recent analyses (Baracchi et al. 2012) based on higher resolution observations (i.e. considering normalized data for number of wasps on the nest and considering the level of aggression of each single colony instead of just the frequency of colonies accepting the presented lures) demonstrated that it is not the case. Cervo et al. (2002) presented lures for longer than 30 seconds starting from the first wasp reaction, probably giving the resident wasps more opportunity to integrate chemical information during the encounters. Focusing on the very first 30 seconds of response we showed that when resident females are allowed to use visual and chemical stimuli together, the latter were not considered by the wasps to evaluate the lures. Indeed the aggression level directed towards an alien lure was very similar to that directed towards a “crossed” lure displaying an alien face and a nestmate odour. Similarly, a nestmate lure and a “crossed” lure displaying a nestmate face and an alien odour received the same peaceful treatment. The only explanation for our results are that resident wasps regulate the level of aggression on the basis of the opponent’s faces regardless of their CHCs, at least when the encounter is relatively brief. The absence of an additive effect on the aggressiveness induced by mismatch visual and chemical stimuli suggests that the nestmate recognition cue actually is not an instance of a multimodal sensory cue. Even if a visual cue seems to prevail in *L. flavolineata*, a deficit in chemical perception cannot explain the results because resident wasps were still perfectly able to detect and discriminate between self and non-self CHCs profiles when visual information was not available. Instead, a selective attention process might explain the complete predominance of sight over smell when both cues were available. It seems likely that during the recognition process attention of receivers is selectively focused on the faces of opponent females while odours are ignored. It is known that bees cannot process all the available visual information at once, unless after extensive training (Zhang and Srinivasan, 1994), but perform a serial search to extract the relevant information (Spaethie et al. 2006). Even if we cannot exclude the possibility that wasps are able to use parallel search in differents sensory modalities, our results might reflect a similar attention mechanism in *L. flavolineata*. Resident wasps therefore would choose the most clearly displayed cue avoiding division of attention between two forms of input and responding better and faster to the opponent. Despite no studies exist on the cognitive abilities in wasps dealing contemporaneously with more than one cue, it is known that other Hymenoperan species (i.e. pollinators) can at least in principle juggle multiple tasks, albeit cope efficiently with only one task at a time (Wang et al. 2013; Chittka & Thomson 1997).

The fact that aggressive reaction towards an alien female occurs in the very first instants, indicates that also the recognition process must occur very quickly. The readiness of the recognition process is quite evident as resident wasps often react to approaching unfamiliar wasps assuming an alert posture before their landing (Turillazzi 1990). *L. flavolineata* present a peculiar nesting biology with many colonies forming large aggregations, sometimes with hundreds of nests built within five centimetres of one other. Individuals have a strong tendency to shift between different nests (Coster-Longman 1998) and several females leave their nests to join other colonies or become floaters (Samuel 1987). As a consequence, colonies in clusters experience continuous and exhausting landing attempts by alien and neighbouring individuals, raising the costs of nest defence. In fact, even if it is not completely clear what is the goal of those landing females they are rejected in more than the 70% of the cases costing time and efforts and diverting attention from other possible dangers (Samuel 1987; Coster-Longman 1998). In this ecological context visual communication could have been favoured by natural selection, more than chemical one, as a mechanism able to reduce the time and the cost of direct fights between opponents. Indeed, while CHCs require a physical contact between senders and receivers to allow information flow, the ability to visually recognize approaching females as aliens allows residents to assume an alarm posture and to deter conspecific without entering in contact with them. Furthermore, alien females often approach nests with uncertain and explorative flights (Turillazzi 1990), and hover just in front of the colonies, which could facilitate face recognition even more.

This is the first report that the visual channel prevails on chemical one in the nestmate recognition context, proving that sight is not secondary to smell in the regulation of this key process of social life in the species tested. *L. flavolineata* societies are very small groups that never exceed a dozen of females, and this condition probably boosts the benefits of a recognition system based on visual communication. The primitively eusocial hover wasps are at the boundary of the sociality, making them a unique model to understand how different sensory channels evolved into complex communication systems in the transition towards more complex societies. It is known that more eusocial wasps belonging to Polistinae subfamily possess even more complex visual and chemical cognitive abilities and Stenogastrinae wasps could thus reveal how chemical and visual communication and their relationship have been shaped by social requirements.

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11. Discussions and conclusions

My thesis focused on different aspects of class-level recognition in social wasps: 1) morphological and behavioural adaptations related to efficient olfactory recognition, 2) visual recognition abilities in different taxonomic groups as well as in different cohorts of individuals, 3) integration between olfactory and visual cues in the recognition process.

First I study the functional variability of the abdominal structure (Van der Vecht's organ) used to mark the nest by dominant individuals of *Polistes* paper wasps and I found that this structure is enlarged in queens with respect to that of the workers. This finding seem to be the rule for all the seven studied species representing a general trend of the genus. Furthermore, some European species clearly show a different developmental trajectory in the size and the functionality of this structure in queens and workers. Queens, which usually have a reproductively dominant role within *Polistes* colonies, are actually equipped with a more efficient secretory organ with respect to that of workers. Furthermore, this secretory organ is enlarged in two *Polistes* social parasites that specialized on a lowland host which use to perform intense marking behaviour soon after nest usurpation to be rapidly accepted by workers of the host species next to emerge. By contrast, in a different social parasite species the Van der Vecht's organ shows a regression in size and in the frequency of use during usurpation. The difference lies in the fact that the latter parasite has more time available to be accepted as it is specialized on a mountain host characterized by delayed workers' emergence. Selective pressures toward an efficient chemical class-level recognition in the genus *Polistes* affects the functionality of the morphological traits associated with olfactory recognition causing an incipient divergence between castes and parasites and their hosts as well.

I then focused on visual cues and their importance on class-level recognition process in two species of hover wasps (Stenogastrinae) and in the solitary founding European paper wasp *P. gallicus.* I found that visual cues convey two different information in the hover wasps *L. flavolineata* and *L. vechti*: in the former facial pattern represents a visual signal, more reliable than olfactory cues which is used to recognise individuals approaching the nest as familiar or unfamiliar. In the latter facial pattern is a reliable signal of status and conveys information on agonistic ability, since dominant individuals bear high ranked faces. Working on *P. gallicus* I also found that queens are able to use faces appearence , while facing potential conspecific usurpers, to modulate their level of aggression. Given the relationship between facial pattern and body size, also face probably convey information on wasps' fighting ability. Workers from the same species were also investigated during my studies and my results demonstated as queens with high quality facial patterns also produce workers with high quality facial pattern. Strikingly *P. gallicus* workers do not use faces appearence to assess conspecifics value in an out-nest context and hierarchical ranks in queenless colonies can be only predicted by workers age, regardless face and body size.

The recognition process is one of the most intriguing topics in social species and a wide comparative approach represent a powerful way to reveal different evolutionary trends in social wasps. To conclude my PhD thesis provided new and unifying results that shed light on proximate and ultimate mechanisms of social wasp communication system.. Visual communication is common in two different groups of social wasps and it is an effective recognition system in small colonies (i.e. some Stenogastrinae) or in the early foundation-phase of the more social *Polistes* wasps (i.e. pre-emergence phase of *P. gallicus*). Visual cues dramatically lose their effectiveness when individuals receiving the signal become numerous.By contrast olfactory cues become more effective as the group size increases, being transmitted at a distance and indirectly. Indeed facial pattern is not a predictor of rank among workers of *P. gallicus* but it is still a quality signals used by queens for rival assessment. It seems that social wasps start to use olfactory communication in place of visual one as the degree of sociality and the number of per-colony individuals grow up. *Polistes* wasps, which are halfway between the highly eusocial Vespinae and the facultatively eusocial Stenogastrinae, are able to use complex chemical informations showing several morphological adaptations to an efficient olfactory recognition (i.e. caste biased development of the Van der Vecht's organ). Despite that some species still use visual cues to recognise conspecifics. My findings also suggest that highly complex social groups are mainly ruled by olfactory communication while less complex social groups can also be successfully ruled by other cues (i.e. visual cues). Much evidence show that when sociality crosses a given threshold, some cohorts of individuals start to show physiological and anatomical specializations to better cope with different requirements associated with their own social role. My thesis point out that morphological traits and olfactory communication are first to be shaped by natural selection when such a threshold is exceeded, proving that, when the complexity of social groups increases an hyper-efficient recognition system is strongly required.