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

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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 European *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 or 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'Donnell 1998). Recent researches showed that differential genes expression prolonged larval development and the presence of storage hexamerin 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. Polygyny 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 isometric 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'Donnell 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.

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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 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 \times 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

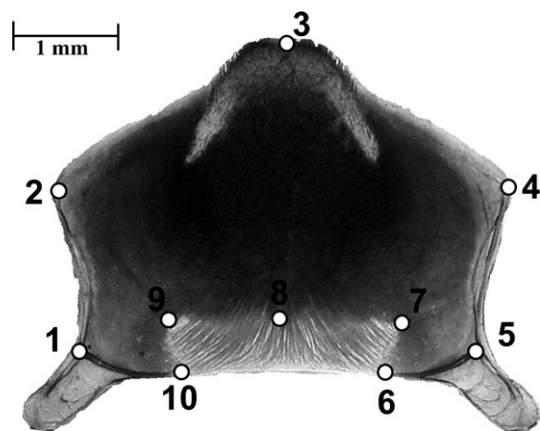


Fig. 1. The last gastral sternite of a *Polistes* paper wasp with landmarks (white circles), used in the geometric morphometrics analysis. The VdVo is enclosed by landmarks 6–10

(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. *Polistes 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.

Discussion

Tendency of foundresses to be larger in body size than workers was confirmed for all analysed species and actually produces a

Table 1. Significant correlates of VdVo area in four species of European *Polistes*. Sternite area is a measure of body size. Since no caste \times 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		
<i>P. dominula</i>	Caste membership	F = 4.092, p = 0.048
	Body size (sternite area)	F = 10.845, p = 0.002
	Caste \times body size (interaction)	F = 4.167, p = 0.046
<i>P. nimphus</i>	Caste membership	F = 11.616, p = 0.001
	Body size (sternite area)	F = 59.917, p < 0.0001
	Caste \times body size (interaction)	F = 11.616, p = 0.001
Second GLM Ancova test		
<i>P. associus</i>	Caste membership	F = 0.905, p = 0.346
	Body size (sternite area)	F = 102.564, p < 0.0001
<i>P. biglumis</i>	Caste membership	F = 1.396, p = 0.247
	Body size (sternite area)	F = 23.050, p < 0.0001

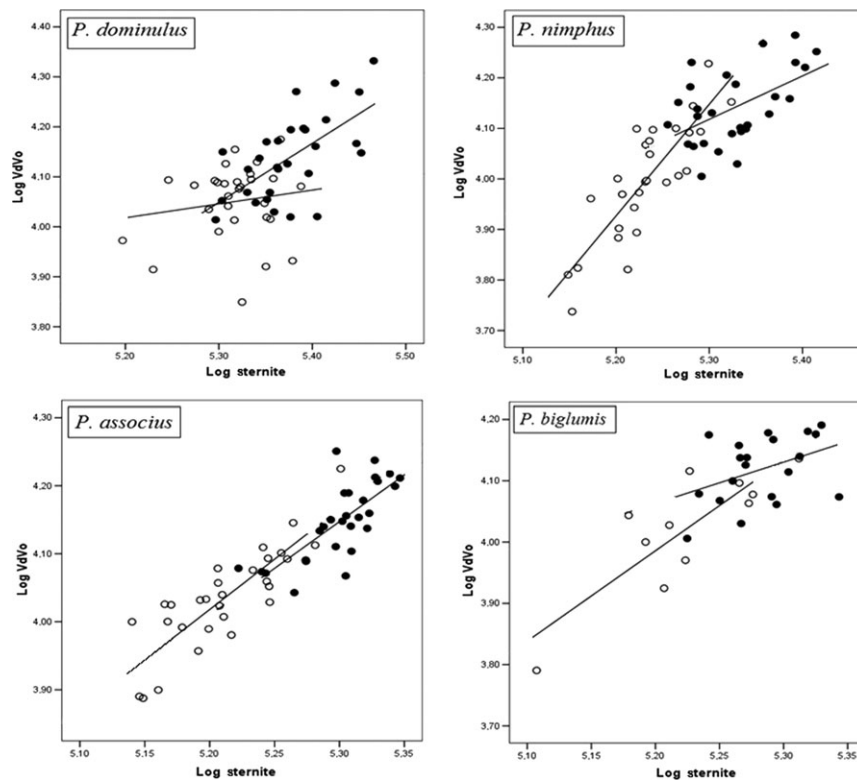


Fig. 2. The relationship between VdVo area and sternite area in foundresses and workers of four European *Polistes* species. Foundresses, black circles; workers, white circles. The linear regression is indicated for foundresses and workers separately

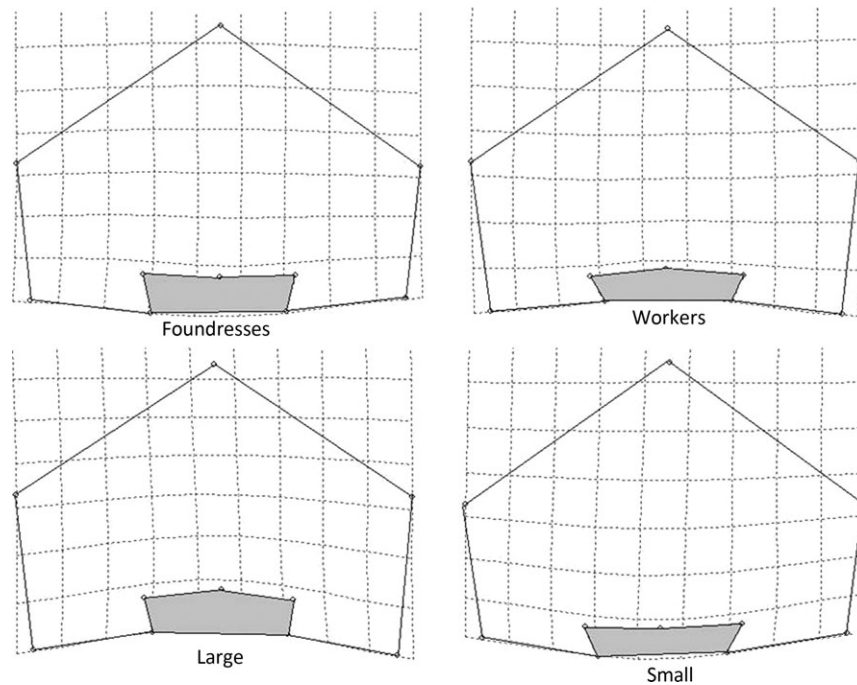


Fig. 3. Results for the multiple regression of partial warps (sternite shape) on caste membership (foundresses versus workers) and body size (large versus small) in *Polistes dominula*

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 led

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 inso-much 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 Hölldobler 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 polygynic 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 Hölldobler 2005).

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