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# On status badges and quality signals in the paper wasp *Polistes dominulus*: body size, facial colour patterns and hierarchical rank

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To establish a dominance order, social animals often rely on indicators of fighting to avoid costly aggressive encounters. In some species, individuals use colour patterns to signal their social status. Recent studies claimed that facial markings in the eusocial paper wasp *Polistes dominulus* are status badges that allow co-foundresses to form a linear hierarchy based on individual quality. Here, we evaluated facial patterns in natural populations of *P. dominulus*, in its native range, to observe whether the marks reflect overall wasp quality in different contexts. We used the same measures of clypeus patterns used by earlier studies, but did not find that they functioned as status badges. Our analyses showed no evidence that visual markers are related to: (i) size, (ii) probability of surviving winter, (iii) social rank in spring associations, or (iv) health status (assessed by the presence of strepsipteran endoparasites). Size, however, is important. Larger wasps are more likely to survive the winter and to acquire the dominant position in spring associations. Larvae infected with endoparasites become smaller adult wasps. These findings suggest that body size is a reliable quality indicator on which wasps build their social networks, and that clypeus patterning is not involved.

**Keywords:** dominance hierarchy; *Polistes* wasps; status badges; body size; winter survival; Strepsiptera

## 1. INTRODUCTION

Dominance hierarchies are widespread in group-living animals (see Theraulaz 2001). Stable rank order reduces the costs of group living by substituting inter-individual overt conflicts with a network of non-aggressive dominance–submission relationships among group members (Chase 1974). However, in the initial phase of hierarchy establishment, potentially costly fights may arise among group contenders. Usually, individuals with better fighting abilities have higher probabilities of reaching the dominant position and several characters directly linked to strength (including body size, weapons, etc.) are often evaluated by opponents before contests.

According to the 'status-signalling hypothesis' (Rohwer 1975), contestants may be advantaged if they can rely on additional signals of fighting ability to predict the outcome of the agonistic contests and thereby avoid costly interactions. Conspicuous colour asymmetry between contestants has been proposed as a 'badge of status' evolved to communicate differences in competitive ability. Several studies have demonstrated a positive correlation between colour patches, size and dominance status in many vertebrates, especially in birds and lizards but also in mammals (for reviews, see Senar 1999; Whiting *et al.* 2003; Setchell & Wickings 2005), as well as in some invertebrates (e.g. butterflies, Shreeve 1987; wasps, Tibbetts & Dale 2004). In some avian species, individuals displaying the largest melanin-based badges are behaviourally dominant to those having smaller colour patches (see Senar 1999). However, other studies on birds failed to find a clear correlation between colour patch and status (see Whitfield 1986; Belthoff *et al.* 1994; McGraw & Hill 2000). A further controversial topic is the honesty of status badges. When the production of these badges is too expensive for low-quality individuals, they should be considered to be honest signals (Zahavi 1975; Grafen 1990). In some cases, such badges are cheap to produce and are then vulnerable to use by dishonest individuals that lie about their status (Rohwer 1975; Maynard Smith & Harper 1988). However, their maintenance in the social network could be too costly for low-quality individuals ('social control hypothesis'; Rohwer 1977; Rohwer & Rohwer 1978). Recent studies (for a review, see Jawor & Breitwisch 2003) suggest that rank markings must be costly to their bearer, and only high-quality individuals can support the social cost of aggressive interactions directed towards high-ranking individuals.

Social wasps of the genus *Polistes* are the first invertebrate taxon in which a linear dominance hierarchy was reported (Pardi 1942). Future queens of various species of *Polistes* living in temperate areas (the so-called 'gynes' or future foundresses) hibernate in aggregations to increase winter survival. Winter represents a bottleneck selecting the individuals that will reproduce in the following year, as about half of future queens die during hibernation (see Dapporto & Palagi (2006) and references therein). In early spring, surviving wasps exit from diapause and start a new colony either alone or associated

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with other females. Foundresses, before nest foundation, engage in violent fights to establish hierarchical relationships; with time, the aggression diminishes and is replaced with low-cost ritualized postures between dominant and subordinate individuals (Pardi 1942). There is some evidence that the order of arrival at the nest site is also important in establishing dominance; the first to arrive is dominant (Field *et al.* 1998; Seppä *et al.* 2002). Once an individual's rank in the queue is established, it may be maintained for a prolonged period without additional aggression. Moreover, according to recent findings (Dapporto *et al.* 2006), dominance interactions even occur in *Polistes dominulus* in autumn pre-hibernating clusters of wasps, where they are predictive of spring rank though not having an immediate link with direct reproduction.

Pardi (1942), describing this spring social organization in associative foundations of *P. dominulus* (formerly *Polistes gallicus*), observed that the dominance order mirrors a reproductive skew as well as a division of labour among co-foundresses: the dominant female (α female) assumes the queen position, becoming the principal colony egg-layer, while the other foundresses (subordinates) perform different tasks linked with colonial development (nest building, foraging behaviour, brood care and colony defence) and progressively relinquish opportunities of direct reproduction. As all overwintered females are mated and are potentially egg-layers, the competition for the high-rank position is expected to be strong (Hart & Monnin 2005). Moreover, in *P. dominulus*, it seems crucial to become the dominant individual in a group of foundresses, because relatedness among co-foundresses can sometimes be low and reproductive skew is high (the dominant foundress lays more than 90% of the eggs; Queller *et al.* 2000). As a consequence, when co-foundresses are unrelated, subordinates obtain neither direct nor indirect fitness.

Much work has been done on the determinants of dominance in colonies of *Polistes* wasps to understand which factors are important for nest initiation and for acquiring a dominant rather than a subordinate position in a group. Body size (see Reeve 1991) as well as neuroendocrine activity and oogenesis (see Röseler 1991) play a role in establishing the outcome of dominance order in founding associations. Dominant individuals are usually larger, have better developed ovaries and have more active *corpora allata* (endocrine glands that produce gonadotropic juvenile hormone) than their subordinates. The larger size clearly facilitates fighting ability, allowing individuals to reach the dominant position among co-foundresses. The chemical signature of cuticular hydrocarbons is the principal cue used in nestmate recognition in *Polistes* wasps (Gamboa 2004) and may function as a dominance signal among co-foundresses of *P. dominulus* (Dapporto *et al.* 2007). However, chemical differences between dominant and subordinate individuals are lacking at the beginning of an association (Sledge *et al.* 2001). The appearance of distinctive chemical patterns among co-foundresses occurs only after the hierarchy has been established, suggesting that it is the result and not the cause of dominance hierarchy position. Moreover, direct experimental evidence is still lacking on whether or not cuticular odour plays a role in the maintenance of dominance order.

In line with status-signalling and social control hypotheses (*sensu* Rohwer), a visual facial pattern has recently been proposed as a rank indicator in *P. dominulus* (Tibbetts & Dale 2004). In fact, foundresses of North American populations show a wide range of variation in the black spots on their yellow clypeus in terms of spot number, proportion of black area and irregularity in spot shape (reported as 'the brokenness index'; see Tibbetts & Dale 2004). Tibbetts & Dale (2004) explored the signal value of clypeus patterns measuring the variation in number, size and shape of the black spots on the clypeus in relation to rank in the group and to overall size. According to these authors, the facial pattern of the foundresses strongly predicts both overall body size and social dominance. First, larger females have a higher proportion of black on their clypeus as well as more broken up black spots than smaller ones. Second, in short-term contests between pre-nesting unfamiliar gynes matched for body size, the winners had significantly more black and more broken spots than losers. Third, Tibbetts & Dale (2004) manipulated the status signals of wasps by masking their presumed badges of status with black and yellow paint on their clypeus, to furnish an experimental support for the social control hypothesis. As they predicted, wasps painted to look more dominant received more aggression, but only after hierarchy establishment was complete, not before; furthermore, the same pattern of increased aggression was directed towards wasps marked to look *less* dominant, against all predictions. Honest signalling requires that subordinates with false dominant signals be punished, not females with false subordinate signals (see also Strassmann 2004).

More recently, Tibbetts (2006), using scores to categorize both wasp size and 'brokenness' of black clypeus spots, reported a difference in the distribution of facial patterns in *P. dominulus* between workers and gynes emerging at mid-summer. The facial pattern of wasps seems to be related to either their emergence time or body size, and gynes show more broken clypeus patterns than do workers. Moreover, Tibbetts & Curtis (2007) showed that *P. dominulus* females that were food supplemented during the larval stage had more disrupted black spots than unsupplemented females. However, in contrast to a previous study (Tibbetts & Dale 2004), they found a negative correlation between body size and clypeus spot brokenness. They interpreted these results to mean that nutritional factors could influence clypeus black spot development in *P. dominulus* wasps, so that this trait—rather than body size—is a costly and reliable quality indicator.

In the present work, we repeated the study carried out by Tibbetts and co-workers to determine whether their results could be replicated using a more long-term measure of dominance in the native range of *P. dominulus*. We measured clypeus marks on the faces of *P. dominulus* foundresses collected in the field. If facial patterns reflect overall wasp quality in a natural context, the following predictions should be true. First, if facial markers are reliable indicators of caste (Tibbetts 2006) and nutritional intake at the larval stage (Tibbetts & Curtis 2007), we expect that wasp winter survival—and its physical/behavioural predictors—should be related to the clypeus pattern. Second, if the clypeus pattern does function as a pivotal badge of status, then we predict a conspicuous asymmetry in the number, size and shape of black spots among spring co-foundresses in relation to high/low social dominance on the nests, in agreement with the pre-nesting dyadic contests in small arenas carried out by Tibbetts & Dale (2004). Third, we predict that facial patterns of healthy overwintered females differ from those of 'stylopized' ones (i.e. parasitized by *Xenos vesparum*, Strepsiptera, Stylopidae). This endoparasite infects wasp larvae and grows at the host's expense until the wasp reaches adulthood (Giusti *et al.* 2007). Parasitized wasps are castrated, have no active role in colony life and desert their nest early to form aberrant summer extra-nidal aggregations, where the wasps are inactive (Beani 2006).

We also checked for the predictive importance of body size as a more reliable quality indicator than facial pattern. As for facial marks, we predict that body size is correlated with winter survival, rank in spring associations and health status.

## 2. MATERIAL AND METHODS

### (a) *Study on overwintering clusters*

In September, we collected four clusters of *P. dominulus* females (i.e. groups of females preparing to overwinter) from Reggello (Florence, Italy; C1, $n=40$; C2, $n=48$; C3, $n=51$; C4, $n=43$). The wasps were individually marked, then caged in four containers (50×50×50 cm) with water and sugar ad libitum and maintained under natural light and temperature conditions. We recorded from 10.00 to 16.00 hours between 5 September and 1 November (85 hours in all) the frequency of six behavioural acts using the 'all occurrences sampling method' (Altmann 1974): dominance performed and received; attacks performed and received; and trophallaxis transferred and received. Moreover, we recorded individuals foraging on the sugar by the 'scan sampling method' (Altmann 1974) at 5 min intervals. We calculated the hourly frequencies of each behaviour, and we added a variable to quantify the total food intake calculated for each individual as (as trophallaxis was measured by all occurrences and sugar foraging by scan),

$$\text{food intake} = \text{sugar foraging} + \text{trophallaxis obtained} - \text{trophallaxis given}. \tag{2.1}$$

Frequencies were normalized. The four cages were then transferred to an unheated room with natural outdoor temperature and photoperiod. At the beginning of March, i.e. the end of hibernation, we recorded which individuals survived the winter and we evaluated body size and clypeus patterns on saved individuals (see below).

Wasp aggregations are characterized by the presence of some individuals that perform most of the foraging activity and do not survive until spring (Dapporto *et al.* 2005). These individuals should probably be classified as 'late workers' (Hunt & Amdam 2005). In order to determine which females of overwintering clusters should have been considered late workers and not future foundresses, we used a *K*-means test on the hourly frequencies of all the behaviours imposing the formation of two clusters. *K*-means divided the wasps of each aggregation into two clusters based on trophallaxis given and sugar foraging. This allowed us to separate future foundresses from late workers. By cluster membership, we identified 28 late workers (seven in C1 and C2, six in C3 and eight in C4; *K*-means, trophallaxis given and sugar foraging $p<0.001$ for each cluster) and they were removed from the following analyses. We removed them because we are interested in the differential dominance and winter survival of future foundresses, not late workers. Thereafter, we performed a multiple regression using a dominance index as the dependent variable. Dominance index ($D_\mathrm{i}$) was calculated for each wasp as

$$D_\mathrm{i} = \frac{D_\mathrm{p} - D_\mathrm{r}}{D_\mathrm{p} + D_\mathrm{r}}, \tag{2.2}$$

where $D_\mathrm{p}$ is the dominance performed and $D_\mathrm{r}$ is the dominance received. Head width, the number of black spots, the percentage of black area on the clypeus and the brokenness index were used as independent predictors. Finally, we performed LogRA to discover the influences of the 10 covariates on the binary-dependent variable, wasp survival. The covariates were entered into the model after selection by the forward stepwise method. We used SPSS v. 13.0 for all statistical analyses.

### (b) *Study on foundresses of established spring colonies*

In April and May, we collected 74 nests of *P. dominulus* (26 solitary and 48 associative foundations, 14 with more than two foundresses) from the field. Colonies were collected from five different sites: four located in Central Italy (Tuscany) and one in northeast Italy (Veneto). All the colonies were in the late pre-worker stage, i.e. when dominance hierarchies were well established. Colonies were collected early in the morning to assure the capture of all the foundresses. Solitary foundresses were frozen at −20°C upon arrival at the laboratory. Multiple colonies were transferred to the laboratory and housed in glass boxes (15×15×15 cm). Each box was provided with food (sugar and fly maggots), water and paper material for nest building. Before housing the wasps in the boxes, we individually marked the foundresses of each association with coloured marks on the thorax. After some days of acclimatization to the laboratory conditions, behavioural observation sessions were performed independently by more than one observer to establish the dominance hierarchy among co-foundresses of each colony. Hierarchical ranks in each colony were checked for stability in two subsequent observations, spaced by a few days, and in the warmest hours of the day to assure a high activity level. The following behavioural patterns were considered to be indicators of rank: dominance–submission posture; aggressive behaviour; presence–absence on the nest; foraging behaviour; egg-laying; and nest-building activity (Pardi 1942). Once the linear dominance hierarchy was established for every colony, all the individuals were killed at −20°C for body size and clypeus measurements (see below).

### (c) *Study on healthy and stylopized wasps*

Between February and March, we collected mixed hibernating clusters of healthy and parasitized wasps from two areas in Tuscany (Italy) characterized by high parasitism levels: Impruneta (Florence) and S. Gimignanello (Siena). Wasps were caged in laboratory conditions until April, when the first aggressive interactions occurred among unparasitized wasps. All individuals were then killed at −20°C. We evaluated body size and clypeus patterns (see below) of 38 female wasps that were infected by one *X. vesparum* female (with the exception of two infected by two *Xenos* females) and 74 healthy (i.e. not stylopized) female wasps as controls.

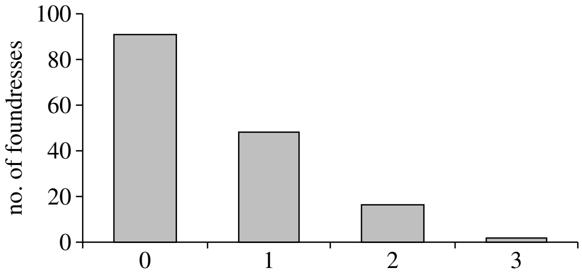

Figure 1. Number of *P. dominulus* spring foundresses with 0, 1, 2 and 3 black spots on the clypeus ($n=157$).

### (d) *Morphological measurement of wasps*

The head of each dead female wasp in the three studies was separated from the body and a picture was taken of its front view. Each picture was imported into Adobe Photoshop. Body size estimates were obtained by measuring the maximum width of the head (Eickwort 1969). To compare co-foundresses differing in social rank, as well as healthy/stylopized wasps, we counted the number of black spots and measured the percentage of black pigmented area on the clypeus using the software ImageJ. Finally, for overwintering gynes and for each alpha and beta female of the 48 associative foundations, we calculated the 'badge brokenness index' as defined by Tibbetts & Dale (2004): 'the standard deviation of amount black pigment deposited along the horizontal gradient located between two peaks'. We calculated the brokenness index using the software (named 'black spot') we developed for this purpose.

To compare clypeus pattern characteristics (number of spots, percentage of black spots and brokenness index) between different groups (dominants versus subordinates and healthy versus parasitized wasps), we used non-parametric tests for both independent and paired data (Mann–Whitney $U$-test and Wilcoxon test). For body size data (head width), we used Student's $t$-test for both independent and paired samples after testing for normality and equality of variance. LogRA was performed to search for influences of clypeus pattern characteristics and body size on the rank of each wasp (binary dependent). The covariates were entered into the model after selection by the forward stepwise method. Finally, we tested for correlations between clypeus pattern characteristics and head width using the Pearson correlation test. All analyses were performed using the statistical program SPSS v. 13.0.

## 3. RESULTS

### (a) *Study on overwintering clusters*

Multiple regression analysis showed that the autumnal dominance index ($D_i$), which measures the relative number of dominance actions performed and received, is not related to any predictors (head width, $\beta=0.061$, $p=0.448$; percentage of black area, $\beta=-0.059$, $p=0.527$; spot number, $\beta=-0.067$, $p=0.651$; brokenness, $\beta=-0.061$, $p=0.681$). Wasp survival differed among the four aggregations: C1=60.6; C2=29.3; C3=82.2; and C4=64.4% ($G$-test, $G=28.24$, $p<0.001$). The overall survival percentage was 59.8%, in line with previous studies (see Dapporto & Palagi (2006) for references). Finally, logistic regression analysis showed that the only variables correlated with winter survival were head width ($B=4.21$, Wald=13.59, $p<0.001$) and, to a lesser extent, trophallaxis given ($B=-0.91$, Wald=5.74, $p=0.017$). However, LogRA assigned only 68.52% of the cases to their correct group. Particularly, the analysis correctly attributed to their own group 81.25% of surviving wasps, but 50% of the wasps that LogRA predicted to die actually survived winter.

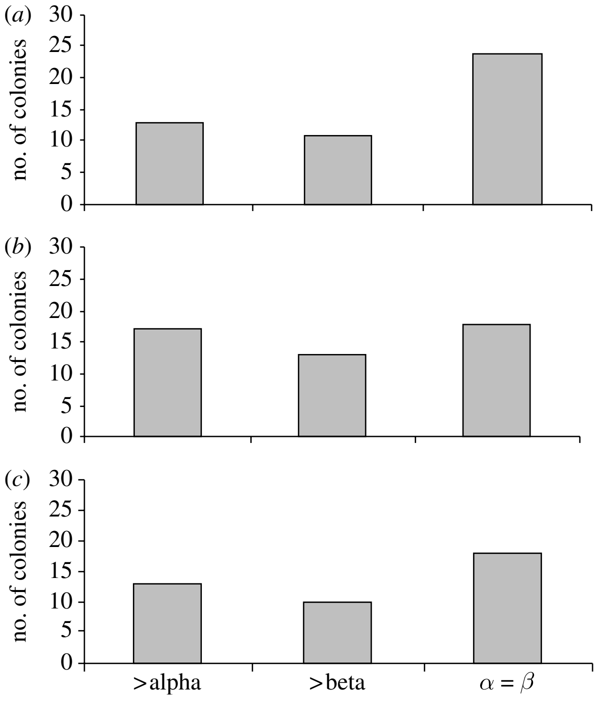

Figure 2. Number of associative foundations ($n=48$) where the dominant female had a higher, a lower or the same ($a$) number of spots, ($b$) brokenness index and ($c$) percentage of black on the clypeus, than or as her own subordinate female.

### (b) *Study on foundresses in spring colonies*

Overall, the average head width of alpha females ($3.42\pm0.11$ mm, $n=48$) is larger than that of subordinate females ($3.31\pm0.11$ mm, $n=69$; $t$-test, $t=5.01$, $p<0.001$). Moreover, the head width of each alpha was larger than that of her own beta female (paired $t$-test, $t=5.61$, $p<0.001$, $3.43\pm0.12$ and $3.33\pm0.12$ mm, respectively). In 35 out of the 48 colonies, the dominant female was larger than the beta female. In 11 out of the 48 cases, the alpha and beta females were of the same size, and the beta female was larger than the alpha female on only 2 out of the 48 nests ($\chi^2$-test, with Yates' correction $\chi^2=34.05$, $p<0.001$).

The clypeus pattern in *P. dominulus* foundresses is variable, as previously reported by Tibbetts & Dale (2004) for a North American population of this species. However, in our populations, approximately 60% of 157 foundresses (131 from foundress groups and 26 from single foundress nests) have a completely yellow clypeus (figure 1). The average number of spots on the clypeus did not significantly differ between alpha ($0.56\pm0.71$, $n=48$) and subordinate females ($0.55\pm0.79$, $n=69$; $U$-test, $U=1599$, $p<0.72$). Moreover, the mean number of spots of each alpha was not higher than that of the beta female on the same nest (Wilcoxon test, $Z=-0.241$, $p=0.81$). In 24 out of the 48 colonies, the dominant female had the same number of spots as her own beta female; in the remaining 13 and 11 cases, alpha foundresses had a higher and a lower number of spots than their own beta ones, respectively ($\chi^2=25.17$, n.s.). These results support a random distribution of black spots among the foundresses of different ranks (figure 2*a*).

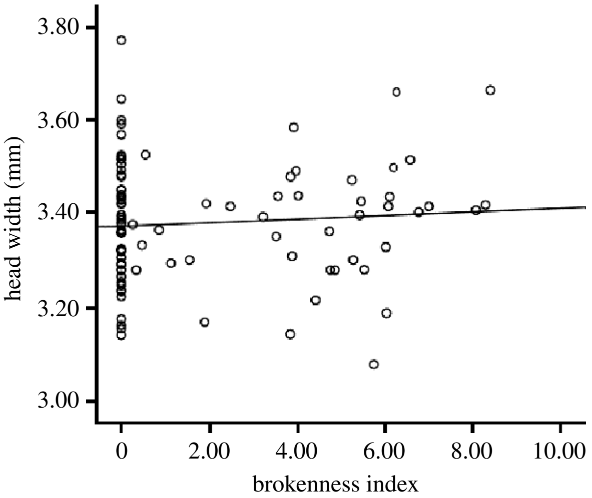

Figure 3. Relationship between head width (mm) and brokenness index ($n=157$, $r^2=0.006$, $F=0.563$, $p=0.45$).

Overall, the badge brokenness index was not different comparing all the alpha females (2.1 ± 2.7, $n=48$) versus all the beta females (1.6 ± 2.4, $n=48$; $U=1078$, $p<0.55$), even with a test where alpha and beta were paired by nest ($Z=-1.04$, $p=0.29$, $n=48$). In 18 out of the 48 colonies, the dominant female had the same brokenness index as her own beta female (both had a solid yellow clypeus), while in 17 and 13 colonies, respectively, the alpha had a higher and a lower brokenness index than her own beta female ($\chi^2=0.547$, n.s.; figure 2*b*).

The black pigmented area was not different between alpha and subordinate females: this is true comparing either all alpha females (1.9 ± 4.7, $n=42$) versus all subordinate females (2.9 ± 6.5, $n=43$; $U=899$, $p=0.97$), or alpha and beta females from the same nest ($Z=-0.24$, $p=0.81$, $n=41$). In 18 out of the 41 colonies, the dominant female had the same proportion of black clypeus as her own beta female (both had a solid yellow clypeus), while in 13 and 10 associative foundations, respectively, the alpha had a higher and a lower proportion of black than her own beta female ($\chi^2=1.81$, n.s.; figure 2*c*). Moreover, logistic regression analysis showed that the only predictor of rank was head width ($B=-6.707$, Wald$=9.028$, $p<0.001$).

According to Tibbetts & Dale (2004), the brokenness index is a reliable general predictor of dominance in *P. dominulus* and was positively correlated with body size in their sample. Our analysis of 131 associated and 26 solitary foundresses of the same species (figure 3) showed that this index was not correlated with body size ($n=157$, $r^2=0.006$, $F=0.563$, $p=0.45$). Moreover, we found no correlation between spot number and body size ($n=157$, $r^2=0.001$, $F=0.01$, $p=0.92$).

### (c) *Study on healthy and stylopized wasps*

Figure 4 shows the distribution of both healthy and stylopized overwintering females with different numbers of spots on their clypeus. The average number of black spots was not different between 38 stylopized and 74 healthy wasps ($U=1261$, $p=0.3$). Moreover, healthy wasps did not have a higher proportion of black area on the clypeus than stylopized wasps ($U=1130$, $p=0.6$). Even though healthy wasps had no more pigmented area on their clypeus than stylopized wasps, they were larger overall than stylopized wasps ($t=-3.12$, d.f.$=100$, $p<0.005$, 3.36 ± 0.16 and 3.26 ± 0.1 mm, respectively). No correlation was found between body size and proportion of black on the clypeus of overwintering wasps ($n=101$, $r^2=0.019$, $F=1.922$, $p=0.169$).

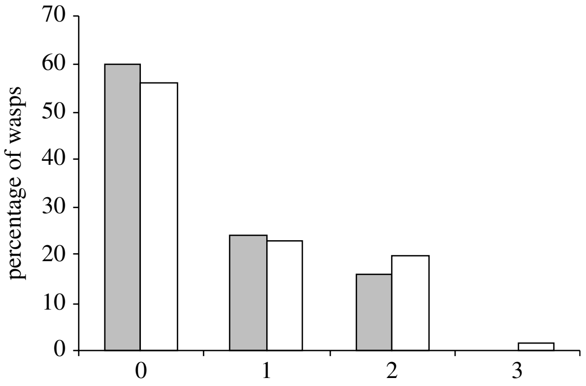

Figure 4. Percentage of stylopized ($n=39$, grey bars) and healthy ($n=74$, white bars) wasps with 0, 1, 2 and 3 black spots on the clypeus.

## 4. DISCUSSION

### (a) *Clypeus patterns as a signal of rank: unlikely*

We did not find support for the hypothesis that the clypeus markers proposed by Tibbetts & Dale (2004) and Tibbetts & Curtis (2007) work as status badges or quality signals in foundresses of *P. dominulus* in its native area. Our analyses showed no evidence that visual markers on the clypeus of *P. dominulus* foundresses are related to: (i) size, (ii) probability of surviving the winter, (iii) social rank in spring associations collected in the field, after a dominance hierarchy had been established, and (iv) health status assessed by the presence of strepsipteran endoparasites. Conversely, all these characteristics are largely predicted by body size. Indeed, larger wasps have a higher probability of surviving the winter and of acquiring the dominant position in a colony in the subsequent spring. Moreover, larvae suffering from the endoparasite presence emerged as smaller adult wasps.

Food intake in the adult stage and facial patterns do not predict future foundress survival during winter. In fact, our results suggest that autumnal feeding (both by foraging and trophallaxis) does not increase the probability of surviving the winter, but perhaps meets immediate needs. Conversely, *P. dominulus* survival was positively related to body size (large wasps have a higher probability of surviving) and negatively with trophallaxis given to others. However, 50% of the wasps predicted to die by logistic regression analysis actually overwintered, thus demonstrating that a large number of future foundresses of small size giving food to cluster mates, not recognized as late workers (helpers), survived and were present in spring colonies probably with a very low hierarchical rank.

The large number of cases incorrectly assigned by logistic regression analysis also suggests that other variables probably play a role in wasp survival. One of these is the quantity and the quality of fat bodies the foundresses have at their emergence as adults, which are determined during the larval stage by feeding (Hunt & Amdam 2005). This parameter was not measured in this study because measuring it requires that we kill the wasps before their winter fate could be recorded, something that was incompatible with the other goals of the study.

However, the lack of a correlation between winter survival and facial pattern, as well as the lack of a difference in visual markers between individuals in good condition and those carrying parasites, was unexpected. Indeed, Tibbetts & Curtis (2007) stated that *P. dominulus* females reared with supplemental food during the larval stage have more disrupted facial patterns, suggesting that this parameter may convey information about quality linked to early nutrition. In our study, both winter-survived and non-stylopized wasps (from areas with high parasitism levels) can be considered to be high-quality individuals. Clearly, most of their good condition was acquired during the larval stage, when the host defence mechanisms play a role against parasites (Manfredini *et al.* 2007). Recent data demonstrate that food depletion by *Xenos* parasites stops when the parasite protrudes from the host abdomen, i.e. a few days after the wasp becomes an adult (Giusti *et al.* 2007). As a whole, in hibernating wasps, clypeus patterns do not seem to represent any kind of quality signal.

In line with these data, at the beginning of the annual colonial cycle, when foundresses interact frequently to assess their position in the linear hierarchy, we found that none of the visual indicators of status and quality previously proposed by Tibbetts & Dale (2004) were significantly correlated with social dominance order for wasps in their native range. In our sample of well-established spring associations, dominant individuals have neither a higher number of black spots on the clypeus, nor a higher proportion of black, nor a higher 'brokeness index' when compared with their subordinates, suggesting that these facial visual markers are not indicators of rank.

There are two main differences between our study and those of Tibbetts and collaborators. First, in our samples, approximately 60% of foundresses had a completely yellow clypeus, whereas only 20% of American *P. dominulus* foundresses had a completely yellow clypeus (Tibbetts & Dale 2004). In our test sites, clypeus marks are not variable enough to make them very good candidates for dominance decisions. In half of our 48 natural co-foundresses pairs, both individuals had the same number of spots and, in 18 pairs, the clypeus was entirely yellow. The recent invasion of the European *P. dominulus* into North America (Cervo *et al.* 2000) could be the reason for this difference in clypeus marks (bottleneck effect). However, the relatively high genetic variability in these introduced populations suggests multiple independent introductions of *P. dominulus* into North America (Liebert *et al.* 2006). Moreover, as we have studied *P. dominulus* foundresses in their natal range, what we found there is the most likely to be biologically real; it is very unlikely that they would have evolved such sophisticated new abilities in the short time since the 1970s when they came to America (Cervo *et al.* 2000).

Second, the experimental design of two-player contests (Tibbetts & Dale 2004) seems too brief to establish stable dominance interactions. At the beginning of interactions for dominance order establishment, the wasps composition in the associations is very unstable and could change rapidly (Strassmann *et al.* 2004). Our dominant and subordinate individuals, collected in the field and when their rank positions were well established, were not bearers of high and low status badges, respectively, on their clypeus. Moreover, the trials carried out for 2 hours by Tibbetts & Dale (2004) furnished no clear-cut results: in dominance trials between foundresses paired for similar size, the dominant individuals have, on average, more spots and higher brokenness index but a lower percentage of black on their clypeus than the subordinate ones (Tibbetts & Dale 2004). These dominance trials were performed during pre-nesting period, when it has been suggested that physiological traits could be more important than body size (Röseler *et al.* 1984). Even if Tibbetts & Dale's wasp pairs were controlled for body size, other uncontrolled physiological factors might have influenced the outcome of the contests.

Moreover, black spots on the clypeus are permanent patches of colour, while the status of a female cannot be so; whenever a queen dies, she is replaced by a co-foundress or by the oldest worker (Pardi 1946) that, according to Tibbetts (2006), should bear a low status badge.

Interesting future work would include examining recognition and colour marks, and repeating this study on the same population that Tibbetts and colleagues used.

### (b) *The role of body size and other hypotheses*

Body size is a morphologically significant though not absolute predictor of rank in *Polistes*, and probably confers competitive ability (Reeve 1991). Our study confirmed this.

Through direct conflicts, an individual could estimate its own competitive ability from previous wins and losses and 'build' an expectation of the outcome of future competitions, regardless of the opponent (Barnard & Burk 1979). Even if a mechanism based on 'loser and winner effects' could be sufficient to explain rank-order formation in *Polistes* wasps (Markiewicz & O'Donnell 2001), the evolution of signals for status recognition could be advantageous for both the opponents. However, we did not find any correlations between body size of foundresses and facial pattern. Moreover, the correlation between visual markers and head width found by Tibbetts & Dale (2004) is very weak (accounting for a small percentage of the variance), as was noted by Strassmann (2004).

Although intraspecific colour variability is very common in many animal species, it does not always have a communicative function. Face colour polymorphism in *Polistes* wasps was documented by various authors in the past (see Enteman 1904; Guiglia 1972) for different species, but it was never found to have a functional explanation. However, colour patterns might serve as individual identity markers rather than status badges (Whitfield 1987) with hierarchy then maintained through individual recognition. In small colonies, co-foundresses might be able to recognize each other based on facial markings and memorize the fighting ability of each foundress. Although only few cases of rank-order maintenance based on individual recognition are reported in the arthropod literature (Hazlett 1969; Caldwell 1979; Karavanich & Atema 1998; Gherardi & Atema 2005), this could be a way that *Polistes* co-foundresses assess hierarchical relationships. As visual recognition has been reported for *Polistes fuscatus* (Tibbetts 2002), co-foundresses could use visual patterns for individual recognition rather than for rank recognition. However, individual recognition based on visual cues is likely to work only in the very beginning phase of the colony cycle when few individuals are in the colony; it is highly unlikely that many wasps in a mature colony would be able to or benefit from individual recognition based on visual cues. Definitive studies are necessary to demonstrate that *Polistes* wasps are capable of individual recognition, i.e. the capacity of discrimination of multiple individuals according to their unique features (Thom & Hurst 2004).

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## REFERENCES

Altmann, J. 1974 Observational. Study of behavior: sampling methods. *Behaviour* **49**, 227–267.

Barnard, C. J. & Burk, T. 1979 Dominance hierarchies and the evolution of 'individual recognition'. *J. Theor. Biol.* **81**, 65–73. (doi:10.1016/0022-5193(79)90081-X)

Beani, L. 2006 Crazy wasps: when a parasite manipulates *Polistes* phenotype. *Ann. Zool. Fenn.* **43**, 564–574.

Belthoff, J. R., Dufty, A. M. & Gauthraux, S. A. 1994 Plumage variation, plasma steroids and social dominance in male house finches. *Condor* **96**, 614–625. (doi:10.2307/1369464)

Caldwell, R. L. 1979 Cavity occupation and defensive behaviour in the stomatopod *Gonodactylus festae*; evidence for chemically mediated individual recognition. *Anim. Behav.* **27**, 294–301. (doi:10.1016/0003-3472(79)90139-8)

Cervo, R., Zacchi, F. & Turillazzi, S. 2000 *Polistes dominulus* (Hymenoptera: Vespidae) invading north America; some hypotheses for its rapid spread. *Insectes Soc.* **47**, 155–157. (doi:10.1007/PL00001694)

Chase, I. D. 1974 Models of hierarchy formation in animal societies. *Behav. Sci.* **19**, 374–382. (doi:10.1002/bs.3830190604)

Dapporto, L. & Palagi, E. 2006 Wasps in the shadow: looking at the pre-hibernating clusters of *Polistes dominulus*. *Ann. Zool. Fenn.* **43**, 583–594.

Dapporto, L., Palagi, E. & Turillazzi, S. 2005 Socially outside the nest: helpers in pre-hibernating clusters of *Polistes dominulus*. *Ann. Zool. Fenn.* **42**, 135–139.

Dapporto, L., Palagi, E., Cini, A. & Turillazzi, S. 2006 Prehibernating aggregations of *Polistes dominulus*: an occasion to study early dominance assessment in social insects. *Naturwissenschaften* **93**, 321–324. (doi:10.1007/s00114-006-0104-3)

Dapporto, L., Dani, F. R. & Turillazzi, S. 2007 Social dominance molds cuticular and egg chemical blends in a paper wasp. *Curr. Biol.* **17**, r502–r503. (doi:10.1016/j.cub.2007.05.002)

Eickwort, K. 1969 Separation of the caste of *Polistes exclamans* and notes on its biology (Hymenoptera: Vespidae). *Insectes Soc.* **16**, 67–72. (doi:10.1007/BF02224464)

Enteman, W. M. 1904 *Coloration in Polistes*. Washington, DC: Carnegie Institution of Washington publication 19. 1–88.

Field, J., Solís, C. R., Queller, D. C. & Strassmann, J. E. 1998 Social and genetic structure of paper wasp cofoundress associations. Tests of reproductive skew models. *Am. Nat.* **151**, 545–562. (doi:10.1086/286140)

Gamboa, G. J. 2004 Kin recognition in eusocial wasps. *Ann. Zool. Fenn.* **41**, 789–808.

Gherardi, F. & Atema, J. 2005 Memory of social partners in hermit crab dominance. *Ethology* **111**, 271–285. (doi:10.1111/j.1439-0310.2004.01060.x)

Giusti, F., Dallai, L., Beani, L., Manfredini, F. & Dallai, R. 2007 The midgut ultrastructure of the endoparasite *Xenos vesparum* (Rossi) (Insecta, Strepsiptera) during post-embryonic development and stable carbon isotopic analyses of the nutrient uptake. *Arthropod Struct. Dev.* **36**, 183–197. (doi:10.1016/j.asd.2007.01.001)

Grafen, A. 1990 Biological signals as handicaps. *J. Theor. Biol.* **144**, 517–546.

Guiglia, D. 1972 *Les guepes sociales (Hymenoptera: Vespidae) d'Europe occidentale et Settentrionale. Faune de l'Europe et du bassin Mediterranen. 6*. Paris, France: Masson et cie.

Hart, A. G. & Monnin, T. 2005 Conflict of the timing of breeder replacement in vertebrate and invertebrate societies. *Insectes Soc.* **53**, 375–389. (doi:10.1007/s00040-005-0895-4)

Hazlett, B. A. 1969 'Individual recognition' and agonistic behaviour in *Pagurus bernhardus*. *Nature* **222**, 268–269. (doi:10.1038/222268a0)

Hunt, J. H. & Amdam, G. V. 2005 Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* **308**, 264–267. (doi:10.1126/science.1109724)

Jawor, J. M. & Breitwisch, R. 2003 Melanin ornaments, honesty, and sexual selection. *Auk* **120**, 249–265. (doi:10.1642/0004-8038(2003)120[0249:MOHASS]2.0.CO;2)

Karavanich, C. & Atema, J. 1998 Individual recognition and memory in lobster dominance. *Anim. Behav.* **56**, 1553–1560. (doi:10.1006/anbe.1998.0914)

Liebert, A. E., Gamboa, G. J., Stamp, N. E., Curtis, T. R., Monnet, K. M., Turillazzi, S. & Starks, P. T. 2006 Genetics, behavior and ecology of a paper wasp invasion: *Polistes dominulus* in north America. *Ann. Zool. Fenn.* **43**, 595–624.

Manfredini, F., Giusti, F., Beani, L. & Dallai, R. 2007 Developmental strategy of the endoparasite *Xenos vesparum* (Strepsiptera, Insecta): host invasion and elusion of its defense reactions. *J. Morphol.* **268**, 588–601. (doi:10.1002/jmor.10540)

Markiewicz, D. A. & O'Donnell, S. 2001 Social dominance, task performance and nutrition: implications for reproduction in eusocial wasps. *J. Comp. Physiol. A* **187**, 327–333. (doi:10.1007/s003590100204)

Maynard Smith, J. & Harper, D. 1988 *Animal signals*. Oxford, UK: Oxford University Press.

McGraw, K. J. & Hill, G. E. 2000 Carotenoid-based ornamentation and status signalling in the house finch. *Behav. Ecol.* **11**, 520–527. (doi:10.1093/beheco/11.5.520)

Pardi, L. 1942 Ricerche sui Polistini V. La poliginia iniziale di *Polistes gallicus* (L.). *Boll. Ist. Entomol. Univ. Bologna* **14**, 1–106.

Pardi, L. 1946 Ricerche sui Polistini. VI. La "dominazione" e il ciclo ovarico annuale in *Polistes gallicus* (L.). *Boll. Ist. Entomol. Univ. Bologna* **15**, 28–84.

Queller, D. C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M. T., Santorelli, L. A. & Strassmann, J. E. 2000 Unrelated helpers in a social insect. *Nature* **405**, 784–787. (doi:10.1038/35015552)

Reeve, H. K. 1991 *Polistes*. In *The social biology of wasps* (eds K. G. Ross & R. W. Matthews), pp. 99–148. Ithaca, NY: Cornell University Press.

Rohwer, S. 1975 The social significance of avian winter plumage variability. *Evolution* **29**, 593–610. (doi:10.2307/2407071)

Rohwer, S. 1977 Status signalling in harris' sparrows. *Behaviour* **61**, 107–129.

Rohwer, S. & Rohwer, F. C. 1978 Status signalling in harris sparrows: experimental deceptions achieved. *Anim. Behav.* **26**, 1012–1022. (doi:10.1016/0003-3472(78)90090-8)

Röseler, P. F. 1991 Reproductive competition during colony establishment. In *The social biology of wasps* (eds K. G. Ross & R. W. Matthews), pp. 309–335. Ithaca, NY: Cornell University Press.

Röseler, P. F., Röseler, I., Strambi, A. & Augier, R. 1984 Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp *Polistes gallicus*. *Behav. Ecol. Sociobiol.* **15**, 133–142. (doi:10.1007/BF00299381)

Senar, J. C. 1999 Plumage colouration as a signal of social status. *Proc. Int. Ornithol. Congr.* **22**, 1669–1686.

Seppä, P., Queller, D. C. & Strassmann, J. E. 2002 Reproduction in foundress associations of the social wasp, *Polistes carolina*: conventions, competition, and skew. *Behav. Ecol.* **13**, 531–542. (doi:10.1093/beheco/13.4.531)

Setchell, J. M. & Wickings, J. 2005 Dominance, status signals and coloration in male mandrills (*Mandrillus sphinx*). *Ethology* **111**, 25–30. (doi:10.1111/j.1439-0310.2004.01054.x)

Shreeve, T. G. 1987 The mate location behaviour of the male speckled wood butterfly, *Parage aegeria*, and the effect of phenotypic differences in hind wing spotting. *Anim. Behav.* **35**, 682–690. (doi:10.1016/S0003-3472(87)80104-5)

Sledge, M. F., Boscaro, F. & Turillazzi, S. 2001 Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. *Behav. Ecol. Sociobiol.* **18**, 165–174.

Strassmann, J. E. 2004 Rank crime and punishment. *Nature* **432**, 160–161. (doi:10.1038/432160b)

Strassmann, J. E., Queller, D. C., Emerson, J. J., Stagi, M., Cervo, R. & Turillazzi, S. 2004 Comparing the costs and benefits of grouping with non-relatives in the social amoeba *Dictyostelium discoideum* (Amoebazoa) and the social wasp *Polistes dominulus* (Hymenoptera Vespidae). *Redia* **LXXXVII**, 145–148.

Theraulaz, S. 2001 Dominance hierarchies in paper wasps. In *Self-organization in biological systems* (eds S. Camazine, J.-L. Deneubourg, N. R. Franks, J. Sneyd, G. Theraulaz & E. Bonabeau), pp. 443–481. Princeton, NJ: Princeton Unversity Press.

Thom, M. D. & Hurst, J. L. 2004 Individual recognition by scent. *Ann. Zool. Fenn.* **41**, 765–787.

Tibbetts, E. A. 2002 Visual signals of individual identity in the paper wasp *Polistes fuscatus*. *Proc. R. Soc. B* **269**, 1423–1428. (doi:10.1098/rspb.2002.2031)

Tibbetts, E. A. 2006 Badge-of-status in worker and gyne *Polistes dominulus* wasps. *Ann. Zool. Fenn.* **43**, 575–582.

Tibbetts, E. A. & Curtis, T. 2007 Rearing conditions influence quality signals but not individual identity signals in *Polistes* wasps. *Behav. Ecol.* **18**, 602–607. (doi:10.1093/beheco/arm013)

Tibbetts, E. A. & Dale, J. 2004 A socially enforced signal of quality in paper wasp. *Nature* **432**, 218–222. (doi:10.1038/nature02949)

Whitfield, D. P. 1986 Plumage variability and territoriality in breeding *Turnstones arenaria* interpres: status signalling or individual recognition. *Anim. Behav.* **34**, 1471–1482. (doi:10.1016/S0003-3472(86)80218-4)

Whitfield, D. P. 1987 Plumage variability, status signalling, and individual recognition in avian flocks. *Trends Ecol. Evol.* **2**, 13–18. (doi:10.1016/0169-5347(87)90194-7)

Whiting, M. J., Nagy, K. & Bateman, P. W. 2003 Evolution and maintenance of social status-signaling badges: experimental manipulations in lizards. In *Lizard social behavior* (eds S. F. Fox, J. K. McCoy & T. A. Baird), pp. 47–82. Baltimore, MD: John Hopkins University Press.

Zahavi, A. 1975 Mate selection—a selection for a handicap. *J. Theor. Biol.* **53**, 205–213. (doi:10.1016/0022-5193(75)90111-3)