

## Hydrocarbon rank signatures correlate with differential oophagy and dominance behaviour in *Polistes dominulus* foundresses

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### SUMMARY

**Social life offers animals increased fitness opportunities. However, the advantages are not evenly distributed and some individuals benefit more than others. The ultimate advantage of reaching the highest rank in a dominance hierarchy is the achievement of reproduction monopoly. In social insects, dominant individuals and queens keep their reproductive control through differential oophagy of unwanted eggs (egg policing). Egg recognition is the main proximate mechanism for maintaining reproductive dominance. In the social wasp *Polistes dominulus*, subordinate queens often lay eggs in the presence of the dominant individual. Combining gas chromatography–mass spectrometry (GC-MS) analysis and laboratory bioassays, we found that chemical differences between eggs of subordinate and dominant foundresses can explain the differential success in oophagy enjoyed by dominant individuals. We propose that dominance behaviour is an investigative behaviour as well as a ritualized agonistic behaviour. In fact, the frequency of dominance acts increases with the chemical similarity of the surfaces of dominant- and subordinate-laid eggs. Therefore, dominant individuals probably perform dominance behaviour to test the cuticular signatures of subordinates and so better assess the chemical profiles of subordinate eggs. Finally, we provide evidence that in particular social contexts, subordinate *Polistes* foundresses can develop ovaries as large as those of dominant individuals but nevertheless lay very few eggs. The subordinates probably lay a limited number of eggs to avoid unnecessary energy loss, as a result of efficient queen policing, but will start laying eggs as soon as the queen fails.**

Key words: oophagy, egg policing, dominance behaviour, egg recognition, *Polistes dominulus*.

### INTRODUCTION

In social insect colonies, reproductive success is skewed in favour of one or a few dominant individuals. Helpers (subordinate foundresses and workers) usually obtain minor benefits from (i) the chance to lay eggs, (ii) indirect fitness and/or (iii) nest inheritance. Hamilton's theory furnished an elegant model to explain the benefit of the helpers' efforts (Hamilton, 1964). However, indirect fitness often appears too low to compensate for helper costs (Ratnieks and Wenseleers, 2007). Accordingly, helpers will lay eggs in an attempt to obtain direct reproduction but queens and/or workers will eat helper-laid eggs (queen and worker policing) (Ratnieks, 1988). In this scenario, egg recognition is a major proximate mechanism in social regulation. Diversification of the chemical cues in eggs should be a fundamental feature that allows the queen to attain her reproductive optimum (Beekman and Ratnieks, 2003). Chemical cues of cuticle and eggs are known to differ between dominants/queens and subordinates/workers in many social insects (Endler et al., 2004; Monnin, 2006).

*Polistes* wasps are one of the most studied organisms in terms of the evolution of social behaviour (Starks and Turillazzi, 2006). *Polistes dominulus* presents associative colony foundation with several totipotent and often unrelated individuals (Queller et al., 2000), and linear hierarchies are established through dominance behaviours (Pardi, 1946). As in other social insects, dominance takes the form of lengthy inspections involving antennation, biting and mouthing by the dominant female on the subordinates, but never the reverse (Pardi, 1946). To recognize subordinate eggs, the alpha female may compare the odour of the eggs found in the nest directly

with her own cuticular odour (Jackson, 2007); however, it can also be supposed that the continuous antennal inspections performed during dominance interactions may help dominants to verify the degree of chemical diversification between their own cuticular signatures and those of their subordinates. By comparing this chemical information with that of the egg surface, dominants can improve their ability to distinguish between their own eggs and those of their subordinates, leading to more efficient differential oophagy. The subordinates will attempt to lay their own eggs, and consequently several mechanisms that enforce their altruistic efforts, be they physical or egg policing, are well known in this species (Pardi, 1946; Gervet, 1964; Röseler, 1991; Queller et al., 2000; Liebig et al., 2005; Smith et al., 2009). Differential oophagy of dominant foundress and worker eggs has been reported in *P. dominulus* (Gervet, 1964; Liebig et al., 2005). The cuticle and egg surface of the same individual exhibit similar hydrocarbon profiles (Dapporto et al., 2007), and both the cuticle (Sledge et al., 2001) and eggs (Dapporto et al., 2007) of dominant and subordinate foundresses show a distinctive chemical blend. This chemical cue has been proposed as a 'fertility signal' indicating the high reproductive capability of the dominant female and the opportunity for helpers to gain indirect fitness (Keller and Nonacs, 1993; Monnin, 2006). Moreover, this cue could be a marker for egg recognition (Dapporto et al., 2007). Several studies have analysed the occurrence, emergence and dynamics of the queen signal in *P. dominulus* (Sledge et al., 2001; Dapporto et al., 2005; Dani, 2006). However, no evidence has so far been found that this cue is involved in social organization. If egg surface hydrocarbons are egg markers, then we should expect a correlation between the number

of subordinate eggs eaten by the dominant foundresses and the level of differences in egg signatures. Indeed, in *P. dominulus*, despite the distinctive signatures on the cuticle and eggs, dominant females do not necessarily perform total differential oophagy and in some cases even avoid eating subordinate eggs (Gervet, 1964). Moreover, in this species the queen signal is linked to social status and is relatively independent of fertility (Dapporto et al., 2007). This implies that subordinate wasps may become fertile in the presence of the dominant individual without changing their signature and lay eggs which lack the dominant signal. Therefore, in *P. dominulus* colonies with dominant and subordinate co-foundresses, the eggs may well exhibit different chemical profiles. The same scenario, however, does not occur in other species of social insects where hydrocarbon signatures are strictly linked to fertility and the helpers never become fertile in the presence of queens (Monnin, 2006; Smith et al., 2008; Smith et al., 2009).

If, in paper wasps, dominant individuals use chemical signatures and dominance behaviour to recognize and destroy subordinate eggs, we can expect that: (i) differential oophagy should strictly depend on chemical diversification between dominant- and subordinate-laid eggs rather than on other physical and physiological traits (e.g. size, ovarian development and corpora allata) of the egg layers and (ii) dominant individuals may perform accurate inspection of a subordinate (part of their dominance behaviour) to check the subordinate's cuticular signature (thus predicting the chemical profile of their eggs). In this respect, this behaviour should occur more frequently in alpha and beta pairs sharing similar egg and cuticular profiles, since it would take longer to accurately assess the differences in their chemical signatures. An increase in dominance acts may also enhance the physiological loops determining submissive behaviours (Markiewicz and O'Donnell, 2001) and increase divergence in chemical signatures.

#### MATERIALS AND METHODS

Sixteen pre-emergence associations of three foundresses of *P. dominulus* (Christ) were collected near Florence at the end of April 2007. In the field the beta females of multiple (more than two foundresses) associations often show ovaries only slightly less developed than those of the alpha females (Turillazzi et al., 1982). The foundresses were marked and the colonies were reared in glass cages in the laboratory. Each colony was observed for 3 h before and after the experiments to record the number of dominance acts and aggressive behaviour frequency per hour and to establish dominance hierarchies in each colony. During dominance interactions the dominant wasp climbs onto the subordinate, antennates her and often demands trophallaxis by mouth-to-mouth contact. Attacks include lunging, biting, aggressive mountings, pursuits, stinging and falling fights. The brood was removed from 50% of the cells every 2 days to induce ovary development in the subordinates (Liebig et al., 2005). Bioassays began 15 days after brood removal, when subordinates started to lay eggs (Dapporto et al., 2007), and continued for 3 days. On day 1, all the eggs and subordinate wasps were removed from each comb, to obtain future eggs that had definitely been laid by the dominant female. The following day (day 2) we noted the cell and the position inside the cell (12 possible laying positions in each cell considering edges and walls) of each new egg laid by the alpha foundresses. The dominant females were subsequently removed and the beta subordinates returned to their nests to obtain eggs laid by beta subordinates. We collected at least one dominant-laid egg per nest for chemical analysis. The following morning (day 3), new eggs found in different cells and/or eggs deposited in the same cells previously used by alpha females but in different positions

(replacements) were taken as subordinate-laid eggs. At least one subordinate-laid egg was collected per colony for chemical analysis. The subordinates were again removed and the alpha foundresses were allowed to return to their nests to observe next morning which eggs had been eaten and/or replaced. On the fourth day, we noted the number of alpha- and beta-laid eggs eaten or replaced by alpha foundresses. It was impossible to misidentify the eggs employed for chemical analysis, because all the eggs we collected could be ascribed to specific individuals (those laid in previously empty cells and/or laid in previously occupied cells but in different positions). Conversely, there is a slight risk of misidentification of eggs used to assess alpha oophagy, because if on day 4 a wasp had replaced an egg by laying a new one in exactly the same position, the egg would not be ascribed as a replacement. However, there is only one possibility of misidentification out of 12 different positions and it would be evenly distributed over the colonies, and therefore would not influence comparative analyses.

Differential oophagy (*sensu* Gervet, 1964) of dominant females was determined as:

$$DO = \frac{\beta_{e,e} - \alpha_{e,e}}{\beta_{e,1} - \alpha_{e,1}}, \quad (1)$$

where  $\beta_{e,e}$  and  $\alpha_{e,e}$  are the number of subordinate- and dominant-laid eggs eaten by dominant females and  $\beta_{e,1}$  and  $\alpha_{e,1}$  are the number of subordinate- and dominant-laid eggs present in the comb at the beginning of day 3.

At the end of the experiment, the wasps were frozen and their head width measured under a stereomicroscope. The average length of the six largest eggs in the ovaries was used as an index of ovarian development. The corpora allata were extracted from the heads and their approximate volume determined by measuring their relative area in a 0.1 mm deep blood corpuscle counting chamber (Turillazzi et al., 1982).

We calculated the relative difference of the three traits (corpora allata size, ovarian development and head width) between dominant and subordinate individuals in each colony using the following formula:

$$RM = \frac{M_{\alpha} - M_{\beta}}{M_{\alpha} + M_{\beta}}, \quad (2)$$

where RM is the relative measure for one of the three traits, and  $M_{\alpha}$  and  $M_{\beta}$  are the measurements of the same trait for dominant and subordinate foundresses, respectively.

Each egg was extracted in 20  $\mu$ l heptane; 2  $\mu$ l of the solution was injected into a Hewlett Packard (Palo Alto, CA, USA) 5890A gas chromatograph coupled with an HP 5971A mass selective detector. A column coated with 5% diphenyl-95% dimethyl polysiloxane (ZB-5, 30 m  $\times$  0.25 mm  $\times$  0.1  $\mu$ m; Zebtron, Phenomenex, Torrance, CA, USA) was used. The injector port and transfer line were set at 300°C and helium (12 p.s.i., 82.7 kPa) was used as the carrier gas. The temperature protocol was: 70–150°C at a rate of 12°C min<sup>-1</sup> (held for 2 min), and 150–320°C at 8°C min<sup>-1</sup> (held for 5.08 min). Analyses were performed in splitless mode. The cuticular compounds were identified on the basis of their mass spectra produced by electron impact ionization (70 eV), then the areas of the chromatogram peaks were transformed into percentages.

Mean chemical distances for each pair of dominant- and subordinate-laid eggs were calculated as:

$$MCD = \frac{\sum_{i=1}^n |p_{i\alpha} - p_{i\beta}|}{n}, \quad (3)$$

where  $p_{i\alpha}$  and  $p_{i\beta}$  are the percentage values of the peak  $i$  of alpha and beta foundress eggs and  $n$  is the total number of peaks.

An initial exploration of the relationships among physical, physiological and chemical characteristics was carried out by principal components analysis (PCA). Compared with successive general linearized models (GLZ) analyses, PCA does not aim to search for predictors explaining variance of any *a priori* determined dependent variables, but highlights correlation patterns between all the variables involved. Our PCA included all the variables measured in each pair of dominant/subordinate foundresses (differential oophagy, dominance and aggression frequencies, mean chemical distances of eggs, relative differences in corpora allata, head and ovary size). We considered PCs with eigenvalues of more than 1 and variables with factor loadings higher than 0.5. Subsequently, we performed three stepwise GLZ to determine which factors were specifically related to (i) differential oophagy, (ii) dominance frequency and (iii) aggressive behaviours. The same predictors (relative differences in the number of eggs laid and corpora allata, head and ovary size) were used in GLZ. Differences in ovarian development, corpora allata size and number of eggs laid between alpha and beta foundresses were assessed by the Wilcoxon non-parametric paired test.

## RESULTS

Differential oophagy distribution for the 16 colonies proved to be bimodal. In five colonies, the dominant females ate all the subordinate-laid eggs and none of their own (DO=1); in five colonies the dominant females did not eat any subordinate-laid eggs (DO=0) or ate more of their own eggs than subordinate ones (DO<0) (Fig. 1). In two other colonies the alpha ate one of her own eggs. In seven colonies, the alpha females ate less than half of the beta-laid eggs.

In total, we found 49 hydrocarbons on the egg surfaces (see Dapporto et al., 2007). PCA extracted seven PCs. PC1, PC2 and PC3 had eigenvalues of more than 1 (PC1=2.52, PC2=1.31, PC3=1.25) while the other PCs had eigenvalues of less than 1. PC1, explaining 35.99% of variance, was positively represented by dominance and differences in ovarian development, and negatively represented by mean chemical distance and oophagy efficacy; PC2,

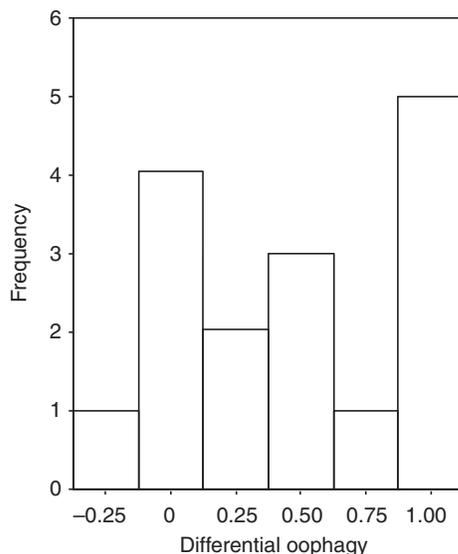


Fig. 1. Frequency distribution of differential oophagy in the 16 experimental colonies.

Table 1. Factor loadings of the three principal components (PCs) with eigenvalues higher than 1

	PC1	PC2	PC3
Dom.	<b>0.757</b>	-0.087	-0.076
DO	<b>-0.845</b>	-0.121	0.430
Att.	0.306	-0.362	<b>0.650</b>
MCD	<b>-0.833</b>	0.209	0.307
HW	0.358	<b>0.607</b>	<b>0.510</b>
CA	0.248	<b>0.799</b>	0.119
OvD	<b>0.505</b>	-0.323	0.337

Factors with loadings >0.5 are in bold.

Dom., dominance frequency; MCD, mean chemical distance; DO, differential oophagy; HW, differences in head width; Att., attacks; CA, differences in corpora allata; OvD, differences in ovarian development.

explaining 18.75% of variance, was positively represented by differences in head width and corpora allata size; PC3, explaining 17.90% of variance, was positively represented by differences in head width and number of attacks (Table 1, Fig. 2).

GLZ revealed that differential oophagy was positively related only to the chemical distance of laid eggs (Fig. 3, Table 2). Moreover, GLZ showed that dominance behaviour frequency was negatively correlated with the chemical distance of the laid eggs (Fig. 4, Table 2). Finally, aggression frequency was correlated with differences in corpora allata size and head width (Table 2).

Dominant foundresses had larger corpora allata and laid a higher number of eggs than subordinates ( $Z=-2.379$ ,  $P=0.017$  and  $Z=-2.222$ ,  $P=0.026$ , respectively), while there was no significant difference in their ovarian development ( $Z=-1.396$ ,  $P=0.163$ ).

## DISCUSSION

Our results confirm that dominant females do not always destroy subordinate eggs to the same extent. Indeed, in about half the colonies, the dominant female ate less than half of the subordinate-

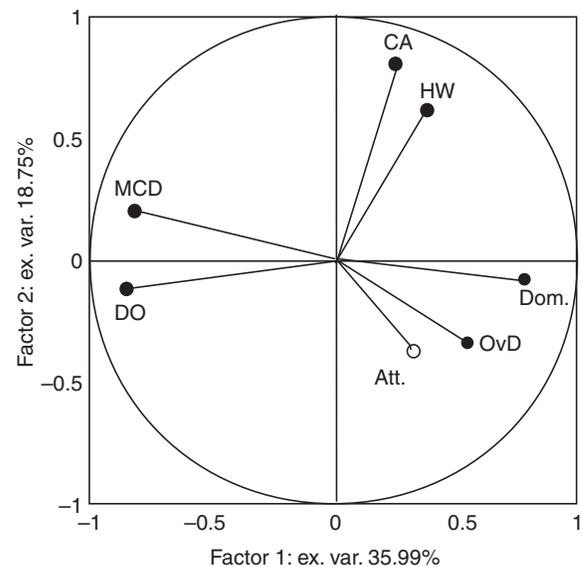


Fig. 2. Representation of factor loadings for the first two principal components (PC1 and PC2). Dom., dominance frequency; MCD, mean chemical distance; DO, differential oophagy; HW, differences in head width; Att., attacks; CA, differences in corpora allata; OvD, differences in ovarian development.

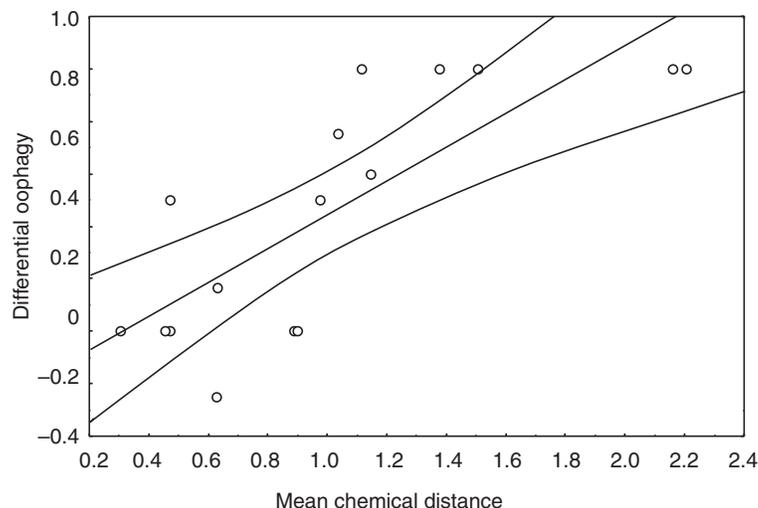


Fig. 3. Correlation between differential oophagy and mean chemical distance. Lines indicate regression fit and 95% confidence interval.

laid eggs. Differential oophagy was highly related to differences in the chemical signatures between dominant- and subordinate-laid eggs, indicating the importance of these cues as egg markers. Chemical distances between eggs also predicted the dominance frequency of the alpha female towards her subordinate partner, suggesting that this behaviour is involved in social recognition. Finally, after brood removal, *P. dominulus* subordinates developed ovaries as large as those of dominant individuals, but maintained smaller corpora allata and laid fewer eggs than dominant females.

In most social insects, fertility has been shown to determine hydrocarbon profiles (Monnin, 2006). Conversely, in *Polistes* wasps, cuticular hydrocarbon signatures depend on social status rather than fertility (Dapporto et al., 2005; Dapporto et al., 2007). At the beginning of association, foundresses engage in continuous interactions to establish hierarchical ranks (Pardi, 1946). In this early phase physiological differences among co-foundresses are small (Turillazzi et al., 1982; Markiewicz and O'Donnell, 2001; Sledge et al., 2004). In particular, slight differences both in the size of the corpora allata, secreting juvenile hormone (Turillazzi et al., 1982; Sledge et al., 2004), and in cuticular signatures exist (Sledge et al., 2001; Sledge et al., 2004). Intriguingly, at the beginning of associations, the dominant foundresses seem to be less able to perform egg recognition and often eat their own eggs (Gervet, 1964). In this phase cuticular signatures seem to be correlated only with fertility (Sledge et al., 2004). After about 1 month of hierarchical interactions, ranks among foundresses are finally established and clear differences in corpora allata size (Turillazzi et al., 1982; Sledge et al., 2004) and in chemical signature (Sledge et al., 2001) between dominant and subordinate foundresses emerge. Under our experimental procedure betas increased their

fertility and the number of eggs they laid was not significantly different from the number laid by pre-treatment alphas (Dapporto et al., 2007). However, they did not change their chemical profiles to match the former alpha signatures as would be expected if fertility was the main factor determining hydrocarbon composition (Dapporto et al., 2007).

In several insects juvenile hormone is responsible for the hydrocarbon composition of both cuticle and eggs (Cuvillier-Hot et al., 2004; Monnin, 2006) suggesting that in *P. dominulus*, after a dominance hierarchy is established, cuticular signatures are honest signals of corpora allata size (i.e. dominance) and to a smaller extent of ovary size (i.e. fertility). The present study confirms this pattern because the difference in corpora allata size [not evaluated in our previous study (Dapporto et al., 2007)] is maintained under our experimental procedure whilst the difference in egg hydrocarbon signature appeared inversely related to differences in ovarian development (Table 1 and Fig. 2). This feature may allow a certain degree of egg recognition when subordinates become fully fertile and start laying their own eggs. However, as predicted by Ratnieks and Wenseleer (Ratnieks and Wenseleer, 2007), subordinates probably develop full-sized ovaries in order to be ready for queen replacement and to try laying some eggs in the empty cells. Nevertheless, the subordinate may lay a limited number of eggs, probably because high egg laying activity could be a pointless cost thanks to the efficiency of queen policing. Actually, we did not find any significant difference in ovarian development between alphas and betas, but we did find a difference in the number of eggs laid, in cuticle and egg chemical compounds (see also Dapporto et al., 2007) and in corpora allata size. Although chemical diversity is always present in foundress associations, it can vary greatly in

Table 2. GLZ testing for the influence of mean chemical distance and differences in head width, corpora allata and ovarian development on differential oophagy, dominance and aggressive behaviour by alpha females

Predictor	Differential oophagy			Dominance behaviour			Aggression		
	Wald	s.e.	P	Wald	s.e.	P	Wald	s.e.	P
HW	0.263	3.870	0.607	0.402	0.456	0.526	<b>5.738</b>	<b>1.302</b>	<b>0.017</b>
CA	0.581	0.955	0.446	0.140	0.715	0.709	<b>4.194</b>	<b>9.237</b>	<b>0.041</b>
OvD	0.017	2.275	0.897	0.042	0.940	0.837	3.772	4.755	0.053
MCD	<b>10.598</b>	<b>29.541</b>	<b>0.001</b>	<b>6.322</b>	<b>40.097</b>	<b>0.012</b>	0.614	110.69	0.433

Significant effects are in bold. N=16.

MCD, mean chemical distance; HW, differences in head width; CA, differences in corpora allata; OvD, differences in ovarian development.

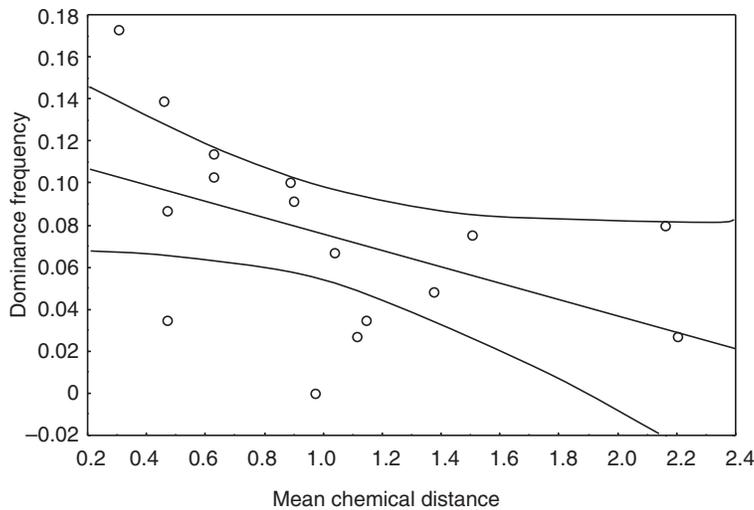


Fig. 4. Correlation between dominance frequency and mean chemical distance. Lines indicate regression fit and 95% confidence interval.

degree. Our data show that the extent to which these rank signatures diverge can explain the differential oophagy in alphas and allows them to recognize subordinate eggs and so maintain their reproductive dominance. Relatedness (not measured in the present study) may be a factor determining hydrocarbon differences (Dani et al., 2004); however, we previously (Dapporto et al., 2004) assessed the influence of social dominance and locality of origin (thus implicitly relatedness) on hydrocarbons, and showed that the latter is ineffective compared with the former in colonies collected in a range of 15 km.

Behavioural dominance also seems to be related to chemical recognition. The increased dominance behaviour between chemically similar co-foundresses suggests a different mechanism from the well known phenotype matching used in nestmate recognition, in which large chemical differences induce increased agonistic interactions (Gamboia, 2004). Jackson (Jackson, 2007) hypothesized that *P. dominulus* dominant females may become accustomed to their own odour, thus enabling them to detect different eggs laid by subordinates. Since eggs and cuticle have the same chemical signature (Dapporto et al., 2007) and some patterns of dominance behaviour appear to be in-depth inspection, we suggest that one function could be to improve the possibility of the alpha female predicting the signature of subordinate-laid eggs. When chemical distances are small, alpha females may need to inspect their partners more frequently and thoroughly in order to recognize the subordinate-laid eggs. At the beginning of the colony cycle, when there are no chemical differences between co-foundresses (Sledge et al., 2001), the alpha females perform more dominance interactions (Pardi, 1946) and often eat their own eggs (Gervet, 1964). The negative correlation between dominance behaviour and chemical distances may be explained by another, not necessarily alternative, hypothesis. When a dominant foundress perceives only a slight chemical difference in her beta, she may increase dominance frequency to further subjugate the subordinate. This would enhance the physiological loops linking dominance status with chemical signatures, thus increasing chemical diversification and the consequent possibility of recognizing subordinate-laid eggs. However, in this case, aggressive interactions should also be correlated with chemical differences, but our results did not show this. Moreover, our data do not support the idea that dominance behaviour increases as a punishment for rising fertility in the subordinates, as suggested for other social species (Monnin et al., 2002; Monnin, 2006; Smith et al., 2009). Indeed, we did not find

any evidence of a negative correlation between the ratio of ovarian development in dominant and subordinate females and dominance frequency. Paradoxically, the opposite seemed to be true (Fig. 2, Table 1). Future specific bioassays modifying the cuticular profile of beta females by chemical supplementation are needed to validate these hypotheses.

Finally, as demonstrated in other social insects, the 'queen signature' in *P. dominulus* is the fundamental cue allowing physical and egg policing (Monnin et al., 2002; D'Ettoire et al., 2004; Endler et al., 2004; Endler et al., 2007; Smith et al., 2008; Smith et al., 2009). In another species in which the 'queen signal' functions as the egg discrimination cue, the helper hydrocarbon signatures do not change with increased fertility [*Camponotus floridanus* (Endler et al., 2004; Endler et al., 2007)]. From this perspective, a dominant signature could be a more reliable egg discrimination cue for dominant individuals and workers than a signal directly linked to ovarian activity. Before worker emergence, a rank signal could favour reproductive dominance in dominant individuals over subordinates. After worker emergence, this type of signal would also facilitate the collective control of worker policing (Ratnieks, 1988; Queller et al., 1997; Ratnieks and Wenseleer, 2007). Indeed, in Hymenopteran societies, workers are predicted to prefer rearing the queen brood rather than that of subordinates and sister workers, and thus police for unwanted eggs (Ratnieks, 1988). This is also predicted and demonstrated in single mated *Polistes* wasps in the impossibility of recognizing male from female eggs [e.g. *P. dominulus* (Liebig et al., 2005)]. Thus, a dominance signal, allowing reliable attribution of eggs to the single highly related dominant individual, could enable workers to obtain higher indirect fitness benefits.

The evidence of hydrocarbon signatures as an egg discrimination cue and the apparent lack of correlation between surface hydrocarbons and ovarian activity do not imply that queen fertility is of little use as information for helpers. Dominant females and workers certainly seem to benefit from hydrocarbon information linked to dominance instead of fertility, in order to unequivocally recognize helper-laid eggs. However, subordinates and workers can accurately assess the fertility of the dominant females by ascertaining the presence of dominant eggs in the comb. Indeed, when the dominant eggs are removed (Liebig et al., 2005; Dapporto et al., 2007) or the dominant female is unable to lay her own eggs (Röseler, 1991), ovary size starts to increase in both subordinates and workers, and they start challenging for direct reproduction.

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