Can cuticular lipids provide sufficient information for within-colony nepotism in wasps?

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Inclusive fitness theory predicts that members of non-clonal societies will gain by directing altruistic acts towards their closest relatives. Multiple mating by queens and multiple queens create distinct full-sister groups in many hymenopteran societies within which nepotism might occur. However, the weight of empirical data suggests that nepotism within full-sister groups is absent. It has been suggested that a lack of reliable recognition markers is responsible. In this paper, we investigated whether epicuticular lipids could provide reliable cues for intracolony kin recognition in two species of social wasps, the paper wasp Polistes dominulus and the hornet Vespa crabro. Epicuticular lipids have previously been shown to be central to kin recognition at the nest level, making them excellent candidates for within-nest discrimination. We genotyped individuals using DNA microsatellites and analysed surface chemistry by gas chromatography-mass spectrometry (GC-MS). We find that in both species epicuticular lipids typically could provide enough information to distinguish related nest-mates from unrelated nest-mates, a difference which occurs in colonies with multiple queens. However, in V. crabro, where colonies may be composed by different lineages, information for discrimination between full sisters and half-sisters is weaker and prone to errors. Our data suggest that epicuticular lipids at best provide reliable information for intracolony nepotism in wasps.

Keywords: intracolony recognition; nepotism; cuticular lipids; social wasps; hornets; Vespa; Polistes

1. INTRODUCTION

Whether social animals are able to directly identify their closest relatives is a central question in the study of social evolution (Sherman et al. 1997). In social insects, the ability to discriminate between nest-mates and alien conspecifics is widespread and ensures colonies maintain themselves as coherent units. Furthermore, workers in some species have been shown to alter sex allocation (Sundstrom et al. 1996) and worker male production (Foster & Ratnieks 2000) in response to colony kin structure, suggesting that workers can estimate overall worker-worker relatedness. Evidence that workers directly discriminate between colony members of different relatedness, however, has been much more problematic.

Many social insect colonies are composed of multiple matrilines (progeny from different mothers and, typically, fathers) and/or patrilines (progeny from different fathers). Inclusive fitness theory (Hamilton 1964), therefore, predicts that nepotism within full-sister groups would be beneficial if this does not incur excessive costs. Evidence for the existence of intracolony nepotism has been reported in some specific contexts in the honeybee Apis mellifera (reviewed by Breed et al. 1994) and the ant Camponotus floridanus (Carlin et al. 1987). However, in their 1994 review Breed and coworkers have highlighted a wealth of negative evidence for nepotism in the honeybee and indeed argue that some positive results could be owing to experimental bias (Breed et al. 1994). A similar case has been made for the Camponotus data (Carlin et al. 1993).

Furthermore, research on several other species has provided no evidence for intracolony nepotism (Queller et al. 1990; Balas & Adams 1996; Bernasconi & Keller 1996; Strassmann et al. 1997; DeHeer & Ross 1997; Solis et al. 1998).

Two main arguments have been proposed for the absence of intracolony nepotism in the social insects (reviewed by Keller 1997). The first is that the benefit derived from nepotistic behaviour towards close relatives is outweighed by the costs incurred by less-related nest-mates (Ratnieks & Reeve 1992). The second suggests that nepotistic behaviours are disfavoured owing to costs associated with kinship assessment errors (Reeve 1989; Sherman et al. 1997). Accurate kin discrimination requires both reliable genetically based cues and a self-matching mechanism, where the self provides a template for comparison (Sherman 1991). The aim of this study was to...
In this study, we investigated the link between genetic relatedness within colonies and cuticular lipid composition in the hornet Vespa crabro and the paper wasp Polistes dominulus, two species with colony kin structures differing from each other. In the honeybee, the entire colony kin recognition has not been reported for these two species. Vespa crabro colonies typically consist of one to three patrilines and a single matriline, although occasionally two matrilines occur, owing to nest take-overs by usurping queens (Foster et al. 2000). Conversely P. dominulus queens are always singly mated (Queller et al. 2000), but colonies are often founded by several queens, which may often be unrelated (Queller et al. 2000).

Genetic relatedness and chemical analysis, therefore, allowed us to analyze differences in the cuticular lipids within colonies of the following genetic structures:

- (i) single patriline (V. crabro and Polistes);
- (ii) multiple patriline (V. crabro); and
- (iii) and multiple-matrilines colonies (V. crabro and Polistes).

2. MATERIAL AND METHODS

Polistes dominulus colonies were collected in July 1996 in a 2 ha area of countryside ca. 100 km southeast of Florence, when worker number in the colonies varies from 1 to 40 (Lohse 1999). Cervo, personal communication. Vespa crabro colonies were collected in the New Forest, Hampshire, UK in summer 1998, and contained at least 50 workers. In both cases the entire colony extractions of nest-mates were accepted whereas lures applied to contain at least 50 workers. In both cases the entire colony extractions of nest-mates were accepted whereas lures applied to contain at least 50 workers. In both cases the entire colony extractions of nest-mates were accepted whereas lures applied to contain at least 50 workers. In both cases the entire colony extractions of nest-mates were accepted whereas lures applied to contain at least 50 workers. In both cases the entire colony extractions of nest-mates were accepted whereas lures applied to contain at least 50 workers. 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(a) GC-MS analysis

For V. crabro, the concentration of 44 compounds was considered in the multivariate statistic analysis (table 1) and only 24 compounds were considered.

We used three methods to analyse the data: chemical distances, principal component analysis and discriminant analysis. The chemical distance data allow us to look for a general correlation between relatedness and chemical similarity, and examine whether chemical distance changes between groups of related and unrelated individuals. Principal component analysis (PCA) whirls the chemical data down to a few important variables allowing us to test if differences existed for those variables between nest-mate lines. Finally, discriminant analysis adds to PCA and asks whether it is possible to assign an individual to its genetic group based upon its chemical profile.

(c) Chemical distances

Squared Euclidean distances of the chemical concentrations (percentage data of 44 compounds for the Polistes and 20 compounds for V. crabro) were calculated for all possible pairs of individuals in both samples. This measure of chemical distance is the sum of the squared differences between the concentrations of each compound in the two species. Statistical analyses were performed with SPSS v. 7.5.

The relationship between chemical distance and relatedness was tested in two ways. First, a Spearman correlation was calculated between chemical distance and relatedness, estimated by the program RELATEDNESS v. 4.1.2 (Goodnight & Queller 1999).

Second, the chemical distance for non-nest-mate pairs and for nest-mate pairs with different degrees of relatedness (unrelated nest-mate, half-sisters, full-sisters) were compared using the Kruskal-Wallis test, considering the significance level based on an estimate of the exact distribution based on 10 000 sampling from the dataset (Monte Carlo Estimate, SPSS v. 7.5). Differences between each pair of groups were tested using the Mann-Whitney U-test, and the exact probability was compared with n = 1 - (1 - α)^K, being the number of comparisons (five for V. crabro and three for Polistes) and α being 0.05, 0.01 or 0.001 (Dunn-Sidak correction for multiple comparisons, Sokal & Rohlf 1995).

(d) Multivariate statistics

PCA was performed on both the V. crabro and Polistes datasets to reduce the number of variables and to obtain orthogonal components (by the Anderson Rubin method in SPSS). Variables showing low communalities (Norusis 1992) were removed from the PCA. The Kaiser-Meyer-Olkin (KMO) value was considered to evaluate the correlation matrix adequacy (values between 0.6 and 0.8 are considered indexes of acceptable communalities; Focardi 1993). For each mixed-line colony, the values of the variables excluded from the PCA and the scores obtained from each principal component (PC1, PC2, PC3), were compared using the Mann-Whitney U-test between the individuals of the two lines (and the exact probability was considered). Stepwise discriminant analysis was performed for each colony formed by two lines. The analysis was performed on the scores derived from the PCA for the Polistes colonies and for one of the two matriline V. crabro colonies, which had a low number of variables (table 2). For the other V. crabro colonies, a stepwise discriminant analysis was performed on the original data. Canonical correlations, Wilks’ λ significance and the percentage of correct assignments were considered to evaluate the validity of the discriminant function. A problem with discriminant analysis is that with small datasets, it may be able to find differences.

(b) Data processing and statistics

For V. crabro, 20 compounds producing peaks whose area was reliably integrated were considered in the data analysis. For Polistes, the concentration of 44 compounds was considered in the calculation of chemical distances (see § 2B.1.1). However, many of these compounds were only present in traces in some of the specimens and absent from most. As for these compounds, the distribution of the concentration values was markedly not normal; these compounds were excluded from the multivariate statistic analysis (table 1) and only 24 compounds were considered.

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between any groups that it is given. To test whether this was the case for our data, we randomly assigned individuals in a colony to one of two arbitrary groups of the same size as the real groups and tested to see if they could be separated through a discriminant analysis performed with the same procedure as on the real data. This was repeated for 50–100 permutations for the data of each colony. We then calculated the percentage of permutations for which the stepwise discriminant analysis gave a better discrimination than that on the real data. If the percentage is high then it casts doubt on the role of relatedness in obtaining the separation.

3. RESULTS

The compounds identified in the epicuticular lipids of V. crabro and *P. dominulus* are given in table 1. Compounds do not substantially differ from those already reported by previous authors (Butts et al. 1991, 1995; Ruther et al. 1998 for *V. crabro*; Bonavita-Cougourdan et al. 1991; Dani et al. 1996 for *Polistes*). However, in *V. crabro* we found some alcohols not reported in other studies, and hydrocarbons up to 32 carbon atoms, which is heavier than the 3-methyl heptacosane and s-nonacosane previously reported (Butts et al. 1995 and Ruther et al. 1998, respectively).

(a) Chemical distances

A significant decrease of the chemical distance when relatedness increased among nest-mates was found in *P. dominulus* (n = 307, r = 0.0091, p < 0.0001), but not in *V. crabro* (n = 975, r = 0.0322, n.s.).

The median of the chemical distances (figure 10) decreased from non-nest-mates through the unrelated nest-mates to full sisters for *P. dominulus* (Kruskal–Wallis, H = 150.07, d.f. = 2, p < 0.0001). Significant differences in chemical distance were found between non-nest-mate pairs and different matrilines pairs, and between the latter and full-sister pairs. For *V. crabro* chemical distance also differed among groups (Kruskal–Wallis, H = 92.88, d.f. = 3, p < 0.0001; figure 10). The main cause of this was the difference between unrelated and related individuals, with non-nest-mates and unrelated nest-mates having close median values, higher than those found for half-sisters and full sisters. An unexpected result was that full sisters were significantly more chemically different than half-sisters (p = 0.05, considering the Dunn–Šidak correction). However, this effect was slight with only a marginal difference between the medians of the two classes. Overall the chemical distance data suggest that significant chemical differences occur among matrilines but not among pariline.

(b) Principal component analysis and discriminant analysis

In *V. crabro*, PCA was performed on all the original variables except for tetracosane, 3-methyl heptacosane and 3-methyl pentacosane, which had the lowest communalities. The KMO test gave a value of 0.68. The analysis produced four PCs whose eigenvalues were higher than 1, which altogether accounted for 85.50% of the original variance. Significant differences were found between the two lines of the two-matriline (2M) colonies for the scores derived from some PCs and for some variables excluded from the PCA (colony 10: n = 6, n1 = 3; Mann–Whitney U-test = 1,000, p exact = 0.048 for PC4 scores; Mann–Whitney U-test = 0.000, p exact = 0.024 for 3-methyl heptacosane; colony 11: n1 = 12, n2 = 8; Mann–Whitney U-test = 22.000, p exact = 0.047 for PC1 scores; Mann–Whitney U-test = 12.000, p exact = 0.004 for PC4 scores; Mann–Whitney U-test = 17.000, p exact = 0.016 for 3-methyl pentacosane). No differences were found between the lines of the 2P colonies, again suggesting that chemical differences were limited to between-matriline comparisons.

The stepwise discriminant analysis on the 2M nest named 10, performed on PCs only, gave a percentage of correct classification equal to 77.8 (canonical correlation = 0.678, Wilks λ = 0.541, χ² = 3.996, d.f. = 1, p = 0.046). For the other *V. crabro* colonies, a discriminant analysis (on the original data) was calculated for the 2M colony 11 and for the two 2P colonies named 20 and 26, but not for the 2P colony 14 (table 2). The percentage of correct classification was 95% for colony 11 (canonical correlation = 0.820, Wilks λ = 0.327, χ² = 18.980, d.f. = 2, p = 0.000), 85% for colony 20 (canonical correlation = 0.675, Wilks λ = 0.544, χ² = 10.336, d.f. = 2, p = 0.006) and 70% for colony 26 (canonical correlation = 0.445, Wilks λ = 0.802, χ² = 3.869, d.f. = 1, p = 0.049).

Table 2a summaries for each colony the comparison between the results of the discriminant analysis performed on the real data and on the permuted data. For the 2M colony 10, a better discriminant analysis than on the real data was obtained for 14.28% of the permutations. A better discrimination was obtained than on the real data for only one of the 2M colonies 11. For the colonies 2P, 20 and 26, a better discriminant analysis was found respectively for 9 and 38% of the permutations. Therefore for only one of the 2M colonies (colony 11) we found a low percentage (1.9) of situations where discriminant analysis worked better than on the original data.

In *P. dominulus*, the dimethyl heptacosanes, 3-methyl heptacosane, 3-methyl nonacosane and the dimethyl triacontanes were excluded from the PCA. The PCA produced six components whose eigenvalues were higher than 1, which altogether accounted for 86.05% of the original variance. The KMO test gave a value of 0.624. For each colony, except one (14–31), significant differences were found between the two lines for the scores derived from some PCs and for the values of some of the variables excluded from the PCA (colony 32–1: n1 = 6, n2 = 4; Mann–Whitney U-test = 0.000, p exact = 0.010 for PC4 scores; colony 28–8: n1 = 7, n2 = 4; Mann–Whitney U-test = 0.000, p exact = 0.006 for PC2 scores; Mann–Whitney U-test = 0.000, p exact = 0.006 for PC3 scores; Mann–Whitney U-test = 2.000, p exact = 0.021 for PC5 scores; colony 14–22: n1 = 7, n2 = 4; Mann–Whitney U-test = 3.000, p exact = 0.042 for PC5 scores; Mann–Whitney U-test = 3.000, p exact = 0.042 for PC5 scores, Mann–Whitney U-test = 2.000, p exact = 0.042 for 3-methyl heptacosane; colony 14–31: n1 = 5, n2 = 3; Mann–Whitney U-test = 0.000, p exact = 0.036 for dimethyl heptacosane).

A discriminant function was obtained for each colony, except one (14–31), on the six PCs, obtaining a percentage of correct classification ranging from 90.9 to 100 (colony...
32-1, canonical correlation = 0.94, Wilks $\lambda = 0.119$, $\chi^2 = 14.86$, d.f. = 2, $p = 0.001$; colony 29-8, canonical correlation = 0.99, Wilks $\lambda = 0.10$, $\chi^2 = 18.35$, d.f. = 2, $p = 0.001$; colony 34-1, canonical correlation = 0.97, Wilks $\lambda = 0.05$, $\chi^2 = 13.49$, d.f. = 3, $p = 0.004$; colony 14-22, canonical correlation = 0.83, Wilks $\lambda = 0.31$, $\chi^2 = 9.36$ d.f. = 2, $p = 0.009$. Table 28 reports for each colony the results of the discriminant analysis performed on the real data and on the permuted data. For one colony no better results could be obtained on the permuted data; for the other three colonies the percentages were 7.54, 5.94 and 2.

4. DISCUSSION

In both species, related nest-mates are markedly more similar than unrelated nest-mates (figure 1). Thus, variation in cuticular hydrocarbons provides information that could be used for discrimination between unrelated and related nest-mates. However, our principal question concerns the possibilities for discrimination among differently related nest-mates. The general absence of within-colony nepotism could be caused by three factors: workers may not benefit from discrimination; genetic cues may be absent or unreliable; or workers may lack a self-referent

\[ \text{cuticular lipids and intracolony recognition in social wasps} \]

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Mechanism. Our study shows that there is genetic information available in hydrocarbon profiles. However, we also find that there may be limits to this information.

One hypothesis for why within-nest discrimination may be less effective than between-nest discrimination is blending of recognition cues. We find mixed results on this point. In *P. dominulus*, sharing the same nest seems to render cuticular lipid composition more similar, as demonstrated by a smaller difference in the chemical distance between nest-mates belonging to different matrilines than between non-nest-mates (figure 1a). The difference observed between the two species, could possibly be due to a different extent of allogrooming or trophallactic interactions, favouring epicuticle lipid homogenization between colony members. It is difficult to evaluate if the different methods used for the lipid extraction, superficial sampling from the cuticle in *V. crabro* and extraction of the abdomen in solvent in *P. dominulus*, may have affected the results. Although the Dufour’s gland and the ovaries (where hydrocarbons of the same kind as those found on the cuticle are present, see §6b) had been removed before *P. dominulus* abdomens were extracted in solvent, contamination with the internal hydrocarbons may have occurred, and this may have affected the results. However, preliminary results suggest that once the Dufour’s gland and the ovaries are removed, abdomen tissues release only a low amount of hydrocarbons when extracted.

The chemical distance results obtained for *P. dominulus* show a strong relationship between cuticular lipid composition and level of relatedness, as demonstrated by both the correlation between chemical distance and relatedness and by the differences in the chemical distance between nest-mates belonging to different matrilines and full sisters. The pattern for *V. crabro* is more complex. Both types of sisters are more similar than are unrelated nest-mates, as expected from closer relatedness. However, full sisters were found to be more dissimilar than half-sisters. This result is difficult to explain, and it contrasts with honey-

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**Table 2. Main results from the stepwise discriminant analysis.**

(Main results from the stepwise discriminant analysis between (a) the two lines of each *Vespa crabro* colony and per cent of better results (see §6b) obtained on the permuted data and (b) the two lines of each *Polistes* colony and per cent of better results (see §6b); 2M, two-matriline colony; 2P, two-patriline colony.)

<table>
<thead>
<tr>
<th>(a) <em>V. crabro</em> colonies</th>
<th>colony 10 (2M)</th>
<th>colony 11 (2M)</th>
<th>colony 14 (2P)</th>
<th>colony 20 (2P)</th>
<th>colony 26 (2P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentage of correct classifications and ( p ) associated with Wilks’ ( Λ ) in the DFA on the real data</td>
<td>77.8%, 0.046</td>
<td>95%, 0.0001</td>
<td>failed</td>
<td>85%, 0.006</td>
<td>70%, 0.049</td>
</tr>
<tr>
<td>number of possible permutations for which DFA produced a better separation than that on the real data (%)</td>
<td>14.28</td>
<td>1.90</td>
<td>9.00</td>
<td>38.00</td>
<td></td>
</tr>
<tr>
<td>(b) <em>Polistes</em> colonies</td>
<td>colony 32-1 (2M)</td>
<td>colony 29-8 (2M)</td>
<td>colony 34-1 (2M)</td>
<td>colony 14-22 (2M)</td>
<td>colony 14-31 (2M)</td>
</tr>
<tr>
<td>percentage of correct classifications and ( p ) associated with Wilks’ ( Λ ) from the DFA on the real data</td>
<td>100%, 0.001</td>
<td>100%, 0.0001</td>
<td>100%, 0.004</td>
<td>90.9%, 0.009</td>
<td>failed</td>
</tr>
<tr>
<td>number of possible permutations for which DFA produced a better separation than that on the real data (%)</td>
<td>2.00</td>
<td>0.00</td>
<td>7.54</td>
<td>5.94</td>
<td></td>
</tr>
<tr>
<td>number of permutations executed</td>
<td>209</td>
<td>329</td>
<td>55</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>number of permutations executed</td>
<td>49</td>
<td>48</td>
<td>53</td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>
Cuticular lipids and intracolony recognition in social wasps

F. R. Dani and others

Figure 1. Chemical distances (see §2b(i)) between pairs of (a) *Vespa crabro* workers divided into: non-nest-mates, unrelated nest-mates (workers belonging to different matrilines) and full sisters. Horizontal lines connect groups for which the exact probability *p* for the Mann–Whitney *U*-test was lower than probability applied with the Dunn–Sidak correction (see § 2b(i)). *p* < 0.05, **p** < 0.001.

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bees, where Page et al. (1991), using a method similar to ours, found that half-sisters were significantly more dissimilar for their epicuticular composition than full sisters.

The discriminant analyses (table 2) show more directly whether these kinds of differences could be used to effectively distinguish matrilines or patrilines within colonies.Arnold et al. (2000) showed that honeybee patrilines could often be distinguished in a discriminant analysis. Our results were variable from colony to colony, and generally went in the same directions as those obtained analysing the differences in the concentration of single compounds and the PC scores. In most 2M colonies, related workers could be grouped correctly based upon their chemical profiles, and this separation was most likely owing to relatedness because few random groups gave better results. For *V. crabro*, the best separation was found for one of the 2M colonies, but a mediocre discrimination was obtained for the other 2M colony. In the *P. dominulus* colonies, high separation was obtained for four out of the five 2M colonies. Alternatively, separation was obtained in two out of the three 2P *V. crabro* colonies, but it was far from being complete. This suggests that epicuticular lipid composition could be a reliable indicator of lines in most multiple matriline colonies, while only moderate information is available for discrimination between patrilines.

The weak discrimination between patrilines in *V. crabro* suggests therefore that there are limits to the level of information provided by surface chemistry and this is consistent with chemical distance being very similar among full and half-sisters. There are several possible explanations for the relatively weak patriline discrimination. It could result from their common environment blending any genetic differences, although, as noted in § 2b(i), the similar average chemical distance between non-nest-mates and unrelated nest-mates suggests that in *V. crabro* a common environment has little effect on epicuticular lipid composition. An alternative hypothesis is that weaker discrimination of patrilines than matrilines may derive from the smaller relatedness differences involved. Relatedness within patrilines is 0.75 and 0.25 between them. Relatedness within matrilines is also 0.75, but relatedness between unrelated matrilines is 0.

A final reason is that the genetic chemical differences between matrilines may be enhanced by environmental effects. If matrilines are produced at different times (as happens when colony usurpation occurs), other factors like age and larval diet could also contribute to the divergence of cuticle lipid composition. Recent research on *Polistes* supports the notion that cuticular lipid composition varies with age (Panek et al. 2001) and an effect of diet has been demonstrated in an ant species (Lian & Silberman 2000).
These results show that cuticular lipids could sometimes provide enough information to be reliable kin cues in colonies formed with unrelated lines, but are likely to lead to significant errors in discrimination among patrilines. The absence of within-patroline nepotism may, therefore, reflect the cost of using unreliable cues. Even if we assume that workers are capable of the sophisticated comparisons made by discriminant analysis, our data suggest that they would make regular mistakes in identifying their own patrilines using cuticular lipids. This conclusion is conservative because the discriminant analysis finds the best separation available using the data for each colony and workers may be unable to do it. Of course, workers may be able to use cues other than those we have considered. However, cuticular lipid composition has been shown to be a reliable indicator of nest origin, caste and reproductive status within castes (Lorenzi et al. 1996; Singer et al. 1998; Breed et al. 1999; Liebich et al. 2000; Sledge 2003), so it is significant that it appears to be a limited indicator of within-colony relatedness in wasps. Confirmation of this conclusion must come from additional studies examining a wide range of species and potential chemical cues.

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