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 creates 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. We find that in both species epicuticular lipids typically could provide enough information to distinguish related nest-mates from unrelated nest-mates, a difference that occurs in colonies with multiple queens. However, in *V. crabro*, where colonies may be composed by different patrilineages, 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 nepotistic discrimination in multiple-queen colonies composed of unrelated lines.

**Keywords:** intracolony recognition; nepotism; cuticular lipids; social wasps; *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 that colonies maintain themselves as coherent units. Furthermore, workers in some species have been shown to alter sex allocation (Sundström 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 matrilineages (progeny from different mothers and, typically, fathers) and/or patrilineages (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* (Carlín et al. 1987). However, in their 1994 review Breed and coworkers have highlighted a plethora of negative evidence for nepotism in the honeybee and indeed argue that some positive results could be due to experimental bias (Breed et al. 1994). A similar case has been made for the *Camponotus* data (Carlín 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 examine whether reliable genetically based cues exist in...
Insect societies. Reliable genetic cues may be confounded by mixing of cues among colony members either accidentally or deliberately (Carlin et al. 1993; Keller 1997). Deliberate scrambling of genetic cues will be favoured by colony members that stand to lose from nepotism, such as members of minor patrilines or matrilines, and the queen (Keller 1997). Moreover, homogenization of recognition cues between colony members may be necessary for efficient nest-mate recognition.

In social insects, epicuticular lipids are considered to be central cues in nest-mate recognition, with a wealth of evidence showing that epicuticular lipids are more similar among nest-mates than non-nest-mates (reviewed in Lorrenzi et al. 1996; Singer et al. 1998; Breed 1998). However, less is known about the structure of the epicuticle lipid signature used in recognition. This has been studied in honeybees (Breed 1998) and in Polistes (Dani et al. 2001), but the results obtained may not apply to other social insects. Which classes of lipids act as recognition pheromones is controversial. Some experimental work found that hydrocarbons are the only class of lipids acting as recognition cues (in the ant Cataglyphis niger: Lahav et al. 1999), while other studies have found that the oxygenated constituents are more relevant (in the honeybee: Breed 1998; Fröhlich et al. 2001).

In studies on epicuticular lipids, multivariate statistics are most commonly used to demonstrate differences among groups of individuals. Whether differences in lipid profiles discerned by these methods can also be detected by the insects can be determined only through acceptance experiments. Studies based on bioassays showed that manipulation of cuticular lipid composition significantly affects nest-mate acceptance (Breed 1998; Dani et al. 2001; Ruther et al. 2002). Lures applied with cuticle extracts of nest-mates were accepted whereas lures applied with extracts of non-nest-mates were rejected (Dani et al. 1996; Lorenzi et al. 1997). Moreover, the capacity to discriminate between complex mixtures of cuticular lipids was demonstrated for honeybees by using a conditioning assay that takes advantage of the proboscis extension reflex (Gettz et al. 1988; Fröhlich et al. 2001).

Cuticular lipids also differ at the intracolony level among castes and physiological states, suggesting that they may play a role in caste recognition or as a signal of reproductive status (Lorenzi et al. 1996; Peeters et al. 1999; Liebig et al. 2000; Sledge et al. 2001). Epicuticular lipids, therefore, seem to be a likely candidate for intracolony kin recognition.

Differences in the lipid composition have been shown between two worker patrilines in honeybee colonies headed by artificially doubly mated queens (Page et al. 1991). Workers in a honeybee colony with a single queen mated 16 times could be correctly assigned to their patriline on the basis of their cuticular lipid composition when the workers were isolated and when they were allowed to remain in their colony (Arnold et al. 1996). Honeybee queens normally mate 7–20 times (Estoup et al. 1994) so a 16-patriline colony is not unusual. In a following paper, Arnold et al. (2000) reanalysed the same data with mixed results showing that within-hive homogenization of cuticular hydrocarbons occurs among some patrilines but not in others.

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 and from honeybees. Evidence of intracolony 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 analyse 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) multiple matriline (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 (R. 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 colonies were immediately killed by freezing after collection and kept at −20 °C until genetic and chemical analysis. We did not isolate individuals to eliminate any effects of sharing hydrocarbons because we were interested in what cues are present in natural colonies. Age of the individuals was unknown.

Worker thoraces were used for the genetic analysis, and worker abdomens for chemical analyses. For both species, up to 10 workers, randomly chosen, from each colony were analysed. For V. crabro, up to 10 additional workers were analysed in colonies with more than one matriline or patriline, except for one mixed-matrilinie colony. Six DNA microsatellite loci (Paco3434AAT, Pan80AAT, Pbe102TAG, Pbe128TAG, Pbe269AAG, Pbe430AAG; see Queller et al. (2000)) were analysed for P. dominulus and four (Foster et al. 1999) for V. crabro. Workers cohabiting the same colony were identified as full sisters when:

(i) all shared one allele at each locus (from the haploid father);
(ii) when all collectively had no more than two other alleles at each locus (from the mother, who could be a heterozygote); and
(iii) when all the likelihood ratios between the members of the group, estimated through the program KINSHIP v. 1.3 (Queller & Goodnight 1989), were higher than the ratio needed to reject the null hypothesis.

Using these criteria, five mixed-matrilinie colonies and one one-matrilinie (1M) colony were identified in P. dominulus. In V. crabro we chose two two-matrilinie (2M) colonies, three two-patriline (2P) colonies and four one-patriline (1P) colonies.

(a) Gas chromatography–mass spectrometry analysis

For V. crabro, cuticular lipids were analysed for a total of 129 workers. Lipid sampling was performed with the protocol of
Turlilazzi et al. (1998), i.e. by gently rubbing the hornet wasp abdomen for 1 min using a cotton wool bud and then extracting the cotton wool in 1 ml of pentane for 15 min in an ultrasonic bath. This method did not give a satisfactory result for _P. dominulus_, since the signal obtained by gas chromatography–mass spectrometry was too weak. Instead, the 86 _Polistes_ workers were analysed by directly extracting the abdomen in 1 ml of pentane for 10 min in the ultrasonic bath. Before cuticle extract preparation, the ovaries and the Dufour’s gland were removed by carefully pulling out the sting. In this species, the Dufour’s gland is known to produce the same hydrocarbons as those found on the cuticle (Dani et al. 1996). We also removed the ovaries because in other insects they have been reported as one of the body organs where a large proportion of internal hydrocarbons of the same kind as those found on the cuticle are accumulated (Schal et al. 1998).

For both species, pentane extracts were evaporated under a gentle nitrogen stream, and then resuspended in 100 µl of heptane. Chemical analysis was performed on a HP5890 II series gas chromatograph coupled to a HP 5971A quadrupole mass spectrometer (using 70 eV electron impact ionization), controlled by an HP G1034C chemstation. The gas chromatograph was installed with a Restek Rtx-5MS column (30 m × 0.25 mm, 95% polydimethylsiloxane and 5% phenyl, phase thickness 0.5 µm) with helium as a carrier gas. The injector port was set at 280 °C. Resuspended extracts (2 µl) were injected in splitless mode. The oven temperature was raised from 70 °C to 150 °C at a rate of 30 °C min⁻¹, and after 5 min at 150 °C, it was raised to the final temperature of 320 °C at 5 °C min⁻¹. In _V. crabro_, the alkene double bond position was determined through chemical ionization on an ion-trap mass spectrometer using acetoniitrile as reagent gas (Moneti et al. 1997). Determination of the branching position of the methyl alkanes was based on the fragmentation pattern reported in literature (Nelson 1978) and on the comparison of their retention times with those of linear alkanes (Carlson et al. 1998). Citronellyl citronellate and citronellyl geranate, found in the _V. crabro_ samples, have already been found for the tergal glands of this species by Wheeler et al. (1982). The mass spectra in our samples matched those reported by these authors. The spectra of hexadecan-1-ol and octadecan-1-ol found in the hornet samples matched with those reported in the electronic libraries and their identification was confirmed through injection of standards. In _V. crabro_, two compounds eluting late in the chromatogram were identified as octacosan-1-ol and triacontan-1-ol on the basis of

(i) their mass spectra corresponding to a primary alcohol and showing an ion corresponding to M-(392 for octacosan-1-ol and 420 for triacontan-1-ol); and
(ii) of their retention times close to that of the linear alkane bearing three more carbon atoms.

(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(i)). However, many of these compounds were only present in traces in some of the specimens and absent from most. As the distribution of the concentration values for these compounds was markedly abnormal, these compounds were excluded from the multivariate statistic analyses (table 1) and only 24 compounds were considered.

We used three methods to analyse the data: chemical distances, principal component analysis (PCA) 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. PCA whittles 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 (DA) 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 _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 concentration of each compound in the two specimens. 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-mates, 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 α = 1 – (1 – α)²K, 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–Sidák 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 indices of acceptable matrices; Focardi 1993). For each mixed-line colony, the values of the variables excluded from the PCA and the scores obtained from each principal component (PC) 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 small number of specimens (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 between any groups that it is given. To test whether this was the case for our dataset, 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) and Ruther et al. (1998) for *V. crabro*; Bonavita-Cougourdan et al. (1991) and Dani et al. (1996) for *Polistes*). However, in *V. crabro* we found some alcohols not reported in other studies, and hydrocarbons containing up to 32 carbon atoms, which is heavier than the 3-methyl heptacosane and n-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* = −0.329, *p* = 0.001), but not in *V. crabro* (*n* = 975, *r* = 0.032, n.s.).

The median of the chemical distances (figure 1b) 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* = 0.000). Significant differences in chemical distance were found between non-nest-mate pairs and different matriline 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 1a). 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–Šidák 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 patrilines.

**b) Principal component analysis and discriminant analysis**

In *V. crabro*, PCA was performed on all the original variables except for tetracosene, 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 two-matriline (2M) colonies for the scores derived from some PCs and for some variables excluded from the PCA (colony 10: *n* = 6, *n* = 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: *n* = 12, *n* = 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 PC scores only, gave a percentage of correct classifications 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 2a). 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 summarizes 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 permutations on the 2M colony 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 permutations 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 tritricontanes 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: *n* = 6, *n* = 4; Mann–Whitney *U*-test = 0.000, *p* exact = 0.010 for PC4 scores; colony 29–8: *n* = 7, *n* = 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: *n* = 7, *n* = 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 = 0.024 for 3-methyl heptacosane; colony 14–31: *n* = 5, *n* = 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 PC scores, obtaining a percentage of correct classification ranging from 90.9% to 100% (colony 32–1, canonical correlation = 0.94, Wilks’ *λ* = 0.119, *χ²* = 14.88, d.f. = 2, *p* = 0.001; colony
Table 1. Constituents of *Vespa crabro* and *Polistes dominulus* cuticular lipids.

(For *V. crabro*, only the compounds in italics were considered in the computation of chemical distances—see § 2b—and in multivariate statistics. For *P. dominulus*, all the compounds were considered in the computation of chemical distances, while only those in bold were considered in multivariate statistics.)

<table>
<thead>
<tr>
<th><strong>V. crabro</strong></th>
<th><strong>P. dominulus</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>hexadecan-1-ol</td>
<td>n-pentacosane</td>
</tr>
<tr>
<td>citronellol</td>
<td></td>
</tr>
<tr>
<td>x-octadec-1-ol</td>
<td></td>
</tr>
<tr>
<td>x-octadecen-1-yl A</td>
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</tr>
<tr>
<td>octadec-1-ol</td>
<td></td>
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<td>citronellyl geranate</td>
<td>n-hexacosane</td>
</tr>
<tr>
<td>9-tricosene</td>
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<tr>
<td>n-tricosane</td>
<td>2-methyl hexacosane</td>
</tr>
<tr>
<td>3-methyl tricosane</td>
<td>n-heptacosane</td>
</tr>
<tr>
<td>9-tetraosane</td>
<td></td>
</tr>
<tr>
<td>n-tetraosane</td>
<td>3-methyl heptacosane</td>
</tr>
<tr>
<td>9-pentacosane</td>
<td>9,17; 9,13; 9,15- and 11,17-dimethyl heptacosane</td>
</tr>
<tr>
<td>n-pentacosane</td>
<td></td>
</tr>
<tr>
<td>3-methyl pentacosane</td>
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</tr>
<tr>
<td>x-hexacosene</td>
<td>n-octacosane</td>
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<td>10;14;12;13;14-methyl octacosane</td>
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<tr>
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<td>n-heptacosane</td>
<td>x,y-dimethyl octacosane</td>
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<tr>
<td>3-methyl heptacosane</td>
<td>x-nonacosane</td>
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<td>n-nonacosane</td>
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<td>n-nonacosane</td>
<td>7-methyl nonacosane</td>
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<td>n-dotriacontane</td>
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</tr>
<tr>
<td>triacontan-1-ol</td>
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29-8, canonical correlation = 0.95, Wilks’ $\lambda = 0.10$, $\chi^2 = 18.35$, d.f. = 2, $p = 0.000$; 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 2b 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 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
Table 2. Main results from the stepwise discriminant analysis. (Main results from the stepwise discriminant analysis (DA) between (a) the two lines of each *Vespa crabro* colony and percentage of better results (see § 2d) obtained on the permuted data and (b) the two lines of each *Polistes* colony and percentage of better results on the permuted data. 2M, two-matriiline colony; 2P, two-patrilines colony.)

(a) *V. crabro* colonies

<table>
<thead>
<tr>
<th>Colony</th>
<th>PC scores only</th>
<th>Percentage of correct classifications and $p$</th>
<th>Wilks’ $\lambda$ in the DA on the real data permutations for which DA produced a better separation than that on the real data (%)</th>
<th>Number of possible permutations executed</th>
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<tbody>
<tr>
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<td>77.8, 0.046</td>
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<td>original variables</td>
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<tr>
<td>26 (2P)</td>
<td>original variables</td>
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(b) *Polistes* colonies

<table>
<thead>
<tr>
<th>Colony</th>
<th>PC scores only</th>
<th>Percentage of correct classifications and $p$</th>
<th>Wilks’ $\lambda$ in the DA on the real data permutations for which DA produced a better separation than that on the real data (%)</th>
<th>Number of possible permutations executed</th>
</tr>
</thead>
<tbody>
<tr>
<td>32-1 (2M)</td>
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<td>100, 0.001</td>
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<td>209</td>
</tr>
<tr>
<td>29-8 (2M)</td>
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<td>100, 0.0001</td>
<td>0.00</td>
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<tr>
<td>34-1 (2M)</td>
<td>PC scores only</td>
<td>100, 0.004</td>
<td>7.54</td>
<td>48</td>
</tr>
<tr>
<td>14-22 (2M)</td>
<td>PC scores only</td>
<td>90.9, 0.009</td>
<td>5.94</td>
<td>53</td>
</tr>
<tr>
<td>14-31 (2M)</td>
<td>PC scores only</td>
<td>failed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

render cuticular lipid composition more similar, as demonstrated by a lower chemical distance between nest-mates belonging to different matrilines than between non-nest-mates (figure 1b). On the contrary, sharing the same nest does not seem to have an effect on cuticular lipid composition in *V. crabro*, where the average distance between unrelated nest-mates is similar to that 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 are removed, abdomen tissues release only a small 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 honeybees, 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 probably due 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 blanding any genetic differences, although, as noted before, 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 & Silverman 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-patriline 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 1998; Peeters *et al.* 1999; Liebig *et al.* 2000; Sledge 2001), so it is significant that it appears to be a limited indicator of within-colony relatedness in social wasps. Confirmation of this conclusion must come from additional studies examining a wide range of species and potential chemical cues.

Funding for this work was obtained through the research network ‘Social evolution’ of the Universities of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) Programme (ERBFMRXCT960072); through the research network ‘Insects’ of the Universities of Copenhagen, Firenze, Keele, Lausanne, Oulu, Regensburg, Sheffield and the ETH Zürich, financed by the European Commission via the Research Training Network established under the Improving Human Potential Programme (HPRN-CT-2000-00052); through the Italian MIUR (Cofin 2001 ‘Comunicazione Animale: studio integrato sulla comunicazione chimica negli Insetti Sociali’) and the University of Florence, and through US NSF IBN-9975351 to D.C.Q. and J.E.S.

**Figure 1.** Chemical distances (see § 2c) between pairs of (a) *Vespa crabro* workers divided into non-nest-mates, unrelated nest-mates (workers belonging to different matrilines), half-sisters and full sisters, and (b) *Polistes dominulus* 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–Šidák correction (see § 2c). *** *p* < 0.05; ** *p* < 0.001.
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F. R. Dani and others


As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.