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Cuticular Chemistry of Males and Females in the Ant *Formica fusca*

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Abstract Communication between organisms involves visual, auditory, and olfactory pathways. In solitary insects, chemical recognition cues are influenced mainly by selection regimes related to species recognition and sexual selection. In social insects, chemical recognition cues have an additional role in mediating recognition of society members and, thereby, allowing kin selection to operate. Here, we examined whether cuticular hydrocarbon profiles are sex-specific and whether males and young queens of the ant *Formica fusca* have colony-specific profiles. We also investigated whether there is a relationship between genetic relatedness and chemical diversity within colonies. We demonstrated that female and male sexuals do not have unique sex-specific compounds, but that there are quantitative chemical differences between the sexes. Out of the 51 cuticular hydrocarbon compounds identified, 10 showed a significant quantitative difference between males and females. We also showed that both males and females have a significant colony-specific component in their profiles. Finally, we found a negative correlation between within-colony relatedness and within-colony chemical diversity of branched, but not linear compounds. This suggests that colonies with multiple matri- or patrilines also have a significantly greater chemical diversity.

Keywords Social · Mating · Cuticular hydrocarbons · Ant · Relatedness · Chemical diversity · Hymenoptera · Formicinae

Introduction

Communication occurs at multiple levels from cells to societies, and is found in all domains of life. Communication at the cellular level involves chemical signalling pathways, whereas in whole organisms visual, auditory, and olfactory pathways dominate (Tomecek, 2009). In insects, olfactory communication can be mediated by a wide range of volatile compounds, such as esters or terpenes, or non-volatile compounds, such as cuticular hydrocarbons (Blomquist and Bagnères, 2010). Although a principal role of cuticular hydrocarbons is to prevent desiccation and protect against environmental pressures, they also function extensively in species recognition, division of labor in social insects, predator avoidance, parasitism and as fertility and dominance signals (Howard and Blomquist, 2005; Blomquist and Bagnères, 2010). Moreover, they also are involved in recognition of sexual partners (Ferveur and Cobb, 2010) and nest mates (van Zweden and d'Ettorre, 2010).

In many insect species, cuticular hydrocarbons are sexually dimorphic with some compounds present in only one sex, or quantitative differences in compounds shared between the sexes (Blomquist and Bagnères, 2010 and references therein; Layton et al., 1994; Yasui et al., 2008; Thomas and Simmons, 2008 and references therein). Unique sex-specific compounds often are involved in mate recognition and sexual selection, at least in non-social insects (Thomas and Simmons, 2008; Ferveur and Cobb,

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2010). However, in social insects, this explanation may be less likely as pre-copulatory sexual selection is thought to be comparatively limited (Boomsma et al., 2005). To date, eight published studies have analyzed sex differences in cuticular hydrocarbon profiles, and among these, seven have found quantitative, but not qualitative differences in profiles between males and young queens (*Ectatomma vizottoi*, Antonialli et al., 2007; *Camponotus japonicus* Hojo et al., 2009; *Chalepoxenus muellerianus* Beibl et al., 2007; *Melipona bicolor*, Abdalla et al., 2003; *Diacamma ceylonense*, Cuvillier-Hot et al., 2001; *Cardiocondyla obscurior*, Cremer et al., 2002), and males and resident queens (*Polistes metricus*, Layton et al., 1994). In *Formica truncorum*, a few compounds were present in young queens, but absent in males (Johnson and Sundström, 2012).

Cuticular hydrocarbons are perhaps best known for their role in nest mate recognition in social insects (van Zweden and d'Ettorre, 2010). Most studies to date have focused on workers, for which acceptance of relatives and rejection of aliens is the main objective of recognition. However males and young queens also are subject to other selective forces. For example, sex-specific compounds, which facilitate mate choice, species-specific compounds, which guarantee correct species-recognition, and intra- or intersexual selection may oppose selection for colony-specific odor profiles. As a result, one might expect sexuals to show a less distinct colony-specific chemical profile than workers.

Precise between-colony recognition requires that the diversity of recognition cues within colonies should be lower than that between colonies. However, given that the chemical component in recognition cues often appears to have a genetic component (van Zweden and d'Ettorre, 2010), cue diversity may co-vary with kin structure. Thus, assuming that low relatedness is associated with high genetic diversity (Giraud et al., 2001; Helanterä et al., 2011, but see Trontti et al., 2007; van Zweden et al., 2011), low-relatedness societies may have a greater diversity of recognition cues (Hölldobler and Wilson, 1977; van Zweden and d'Ettorre, 2010). To date, six studies have demonstrated colony-specific profiles in resident queens (*Formica fusca*, Hannonen et al., 2002; *Polistes metricus*, Layton et al., 1994; *Vespa crabro*, Butts et al., 1995), or young sexuals (*F. truncorum*, Johnson and Sundström, 2012; *P. metricus*, Layton et al., 1994; *Leptothorax gredleri*, Oppelt et al., 2008). However, the link between cuticular chemistry and colony kin structure has to date only been explored in workers of *F. fusca* (Helanterä et al., 2011).

In our study species, *F. fusca*, queen number varies from one to over 20 (Hannonen et al., 2004). Single- and multi-queen colonies occupy the same habitat, and are not genetically differentiated from each other (Hannonen et al., 2004; Bargum et al., 2007). This allows us to analyze the link between colony kin structure and chemical diversity in the absence of confounding pressures on the recognition systems of colonies. Several earlier studies have analyzed the cuticular chemistry of workers of *F. fusca* (Martin et al., 2008, 2011; Helanterä et al., 2011).

Here, we examined whether cuticular hydrocarbon profiles are sex-specific, and quantified the extent to which males and young queens of *F. fusca* have colony-specific profiles. We then linked this information to the kin structure of the colony to investigate whether there is a relationship between genetic relatedness and chemical diversity within colonies. Our results provide new insight to the degree to which males, young queens, and workers differ in their cuticular chemistry, and on the impact of colony kin structure on chemical diversity.

Methods and Materials

Study Colonies To obtain young queens and males for chemical analysis we collected a total of 12 colonies of *F. fusca* in late April 2009 in the vicinity of Tvärminne zoological station in southern Finland. Seven of these colonies contained one or more mature queens and 5 contained none. From this material we established 7 laboratory nests with 1 mature queen and 5 orphaned nests in plastic trays (30×30×40 cm), the walls of which were coated with Fluon™ to prevent ants from escaping. The bottom of the trays was lined with peat, and a ceramic tile was added for shelter. The colonies were kept at 25–27 °C, fed with Bhatkar–Whitcomb diet (Bhatkar and Whitcomb, 1970), and moistened daily. The queen-right laboratory nests were used to produce young queens and the orphaned ones to produce males. In addition we collected sexual pupae and ca 100 workers from one field colony in July in the same area and established it as described above; this colony produced only males.

Once a queen-right nest contained over 100 eggs, it was divided into a queen-right and an orphaned fragment, each with ca 200 workers, and maintained in the conditions described above. We then transferred all eggs laid by the mature queen (min 100 eggs) to the corresponding orphaned fragments, and discarded the queen-right fragments. Under these conditions the workers raise the diploid brood into new queens rather than new workers. Given that the egg sex ratio in queen right colonies is highly female-biased, most eggs would develop into new queens (Helanterä and Sundström, 2005). Colonies that did not have any queens upon collection were used to produce males; workers of *F. fusca* readily lay eggs when orphaned and these develop into fully functional males (Helanterä and Sundström, 2005).

After emergence, young queens and males were allowed to mature in their natal fragments for ca 2 wk. Once they became positively phototactic and were found on the top of

the nest, indicating readiness for mating flights, we collected 5–7 young queens and 6–8 males from each laboratory nest (N=7 for queens, and N=6 for males) for chemical analysis and genotyping.

Chemical and Genetic Analyses We first extracted the surface chemicals from all males and young queens in 150 μl HPLC-grade pentane (Sigma-Aldrich, Brøndby, Denmark) in a 2 ml glass vial. Ants were immersed in pentane for 10 min, the extract was transferred to a 200 μl glass insert (Supelco) and evaporated. The extract was rediluted in 60 μl pentane, 2 μl of which were injected into an Agilent Technologies 6890 N gas-chromatograph (GC) (capillary column: Agilent HP-5MS, 30 m×25 μm×0.25 μm; split-splitless injector; carrying helium gas at 1 ml/min) using an Agilent 7683B auto-sampler. The temperature program began at 70 °C, for 1 min, then rose to 210 °C at 30 °C/min^{-1} to 280 °C at 2 °C/min^{-1}, and finally to 320 °C at 30 °C/min (hold of 2.5 min) (El-Showk et al., 2010). The order in which samples were processed was randomized. The compound(s) comprising each peak were identified by eye, by inspection of the diagnostic ions present in mass spectra produced by an Agilent 5975 mass selective detector (70 eV) coupled with GC. This was done for every peak in each sample. We selected a set of peaks for inclusion in the study by choosing the maximal number of chemicals that we could reliably separate (some chemicals have similar retention times, and thus co-elute). These peaks were integrated using MSD Chemstation software (Agilent).

We genotyped all sexuals used in the chemical analyses (77 individuals in total) at 8 highly polymorphic DNA-microsatellite loci (FL12, FL20: Chapuisat, 1996; FE13, FE17, FE19, FE21, FE51: Gyllenstrand et al., 2002; FY7: Hasegawa and Imai, 2004). DNA-extraction, PCR amplification, and fragment analysis followed protocols described in Chernenko et al. (2011).

Statistical Procedures Prior to factor analysis, the peak areas were transformed by $ln(P_i/g(P))$ (Aitchison, 1986), where P_i is the area of the focal peak and $g(P)$ is the geometric mean of all peak areas for that individual. We then used factor analysis to reduce the number of variables, and to identify peaks associated with the factors carrying the most comprehensive explanatory power (Eigenvalues >0.70).

We tested whether young queens and males differ in their the overall profiles or some specific compounds, first using discriminant analysis on the factor scores for the first ten factors with sex as a predictor, followed by t-tests on sex differences for all ten factors. In addition we tested for sex differences for all compounds (relative peak areas) using t-tests. In these analyses, we adjusted the significance levels for multiple testing with Bonferroni corrections. We then tested for colony specificity in the chemical profile jointly for males and young queens again with a discriminant analysis and classification matrix on the factor scores of the first ten factors. This outcome was verified with a multivariate one-way ANOVA on colony and the first four factors, followed by Tukey's *post-hoc* tests for each pair of colonies and each factor (Supplementary Table S1). We also analyzed branched and linear compounds separately using the same procedure.

To test for genetic effects on profile diversity we estimated the within-colony relatedness based on the Queller and Goodnight (1989) algorithm as implemented in the software Coancestry (Wang, 2011). We estimated the average chemical distance between individuals within each colony based on Mahalanobis distances (Mahalanobis, 1936), and then tested for an association between within-colony genetic relatedness as a proxy for colony kin structure and genetic diversity, and chemical diversity with standard correlation analysis (Pearson). All statistical analyses were conducted in Statistica 10 (Statsoft).

Results

In total, 51 hydrocarbon compounds or co-eluting compound combinations were found in the profiles (Table 1, Fig. 1). We found no unique compounds for either males or young queens. The factor scores from the first 10 factors explained 86 % of the data variation. Young queens and males were correctly classified according to sex based on the first ten factors (Table 1). The first factor correctly classified 78 % of the young queens, but only 39 % of the males. When the first four factors were considered, 100 % of the young queens and 89 % of the males were classified correctly with respect to sex, and when the first six factors were included, all individuals were classified correctly. However, not all factors contributed to the correct classification, as significant effects were found only for factors 1, 2, 4, and 6 (t-test, factor 1: T_{75}=4.25, P<0.001; factor 2: T_{75}=−2.31, P=0.02; factor 3: T_{75}=1.56, P=0.12; factor 4: T_{75}=8.19, P<0.001; factor 5: T_{75}=0.06, P=0.63; factor 6: T_{75}=2.05, P=0.04). After a Bonferroni correction, only the effects of factors 1 and 4 were significant.

Significant differences between males and young queens in relative peak areas were found for 10 different compounds or their co-eluted components (Table 1, Figs. 1 and 2). Eight of these compounds (peaks 1, 24, 30, 33, 39, 47, 50, and 51) were highly correlated (factor loading >0.50) with either factor 1 or factor 4. All but one (C_{23}) of the ten compounds were branched alkanes, with one or more methyl groups. A further seven compounds had P-values of less than 0.05 but greater than the Bonferroni-corrected P-value threshold of P=0.005. Six of these were branched alkanes, and one a linear alkane (C_{29}, Table 1). When branched and

Table 1 Comparison of relative peak areas of cuticular hydrocarbon compounds (or combinations of co-eluting compounds) in males and females

Compound	Males Mean (SD)	Females Mean (SD)	T_{75}-value	P
1) C23	0.47 (0.5)	1.36 (0.72)	−6.34	**<0.001**
2) 11- & 9-MeC23	0.73 (0.38)	0.59 (0.47)	1.45	0.15
3) 7-MeC23	−0.87 (0.49)	−0.68 (0.89)	−1.14	0.26
4) 5-MeC23	−1.55 (0.63)	−1.69 (0.5)	1.02	0.31
5) 9,13-diMeC23	−2.49 (0.73)	−3 (1.27)	2.19	0.03
6) 3-MeC23	0.84 (0.32)	0.42 (0.83)	2.97	0.004
7) 7,15-diMeC23	−2.36 (1.64)	−2.17 (1.65)	−0.52	0.60
8) 5,9 & 5,13-diMeC23	−1.13 (0.7)	−1.55 (0.73)	2.60	0.01
9) C24	−0.01 (0.66)	−0.2 (0.6)	1.30	0.20
10) 3,11-, 3,9- & 3,7-diMeC23	0.8 (0.57)	0.46 (0.97)	1.88	0.06
11) 12-, 10- & 8-MeC24	1.07 (0.35)	0.64 (0.38)	5.22	**<0.001**
12) 6-MeC24	−1.39 (0.33)	−1.55 (0.74)	1.26	0.21
13) 5-MeC24	−2.77 (0.58)	−3.03 (0.74)	1.76	0.08
14) 4-MeC24	−0.69 (0.42)	−0.99 (0.35)	3.33	0.001
15) 10,14- and 8,12-diMeC24	−0.47 (0.63)	−0.64 (0.69)	1.11	0.27
16) 6,10-diMeC24 and 3-MeC24	−0.9 (0.47)	−0.98 (0.6)	0.66	0.51
17) 5,11-diMeC24	−1.77 (0.5)	−1.64 (0.43)	−1.17	0.25
18) 4,12-, 4,10- & 4,18-diMeC24	0.08 (0.48)	0.27 (0.56)	−1.54	0.13
19) C25	2.24 (0.8)	2.18 (0.57)	0.37	0.71
20) 2,12-, 2,10-, 2,8-diMeC24	−1.24 (0.53)	−1.24 (0.57)	−0.03	0.97
21) 13-, 11- & 9-MeC25	3.2 (0.25)	3.05 (0.21)	2.96	0.004
22) 7-MeC25	0.25 (0.47)	0.59 (0.86)	−2.22	0.03
23) 5-MeC25	1.1 (0.36)	0.93 (0.7)	1.37	0.18
24) 11,15- and 9,13-diMeC25	1.37 (0.69)	0.3 (0.86)	6.03	**<0.001**
25) 3-MeC25	2.12 (0.25)	1.47 (1.33)	3.05	0.003
26) 7,15-diMeC25	−1.38 (1.06)	−0.26 (1.92)	−3.22	0.002
27) 5,17-, 5,15- and 5,13-diMeC25	1.99 (0.75)	1.85 (1.32)	0.57	0.57
28) C26	0.06 (0.87)	−0.24 (0.51)	1.81	0.07
29) 3,13-, 3,11- and 3,9-diMeC25	2.22 (0.6)	1.76 (0.64)	3.26	0.002
30) 13-, 12- and 8-MeC26	1.63 (0.31)	1.43 (0.14)	3.54	**<0.001**
31) 6-MeC26	−0.72 (0.36)	−0.59 (0.73)	−0.96	0.34
32) 4-MeC26	−1.66 (0.84)	−1.25 (0.38)	−2.69	0.01
33) 10,14- and 8,12-diMeC26	0.62 (0.57)	−0.09 (0.61)	5.26	**<0.001**
34) 6,10-diMeC26 and 3-MeC26	−0.01 (0.49)	0.21 (0.95)	−1.27	0.21
35) 5,13-diMeC26	−1 (0.57)	−1.13 (0.71)	0.90	0.37
36) 4,12 and 4,10-diMeC26	−0.42 (0.71)	−0.48 (0.68)	0.37	0.71
37) C27	1.05 (0.92)	1.32 (0.76)	−1.39	0.17
38) 13-, 11- and 9-MeC27	2.06 (0.32)	2.03 (0.29)	0.47	0.64
39) 7-MeC27	−0.23 (0.53)	0.72 (0.51)	−8.00	**<0.001**
40) 5-MeC27	−0.13 (0.6)	−0.32 (0.53)	1.46	0.15
41) 11,15- and 9,15-diMeC27	0.94 (0.65)	0.2 (1.2)	3.44	**<0.001**
42) 7,11-diMeC27	0.57 (0.97)	0.59 (1.12)	−0.12	0.90
43) 3-MeC27	−0.12 (0.91)	−0.75 (1.53)	2.25	0.03
44) 5,15-, 5,13- and 5,11-diMeC27	0.75 (0.71)	0.81 (0.77)	−0.33	0.74
45) C28	−1.14 (0.82)	−1.15 (0.62)	0.11	0.92
46) 3,15-, 3,13-, 3,11- and 3,9-diMeC27	0.22 (1.13)	0.26 (0.67)	−0.19	0.85
47) 13-, 12-, 10- and 8-MeC28	−0.09 (0.32)	0.84 (0.5)	−9.81	**<0.001**
48) 8,12-diMeC28	−0.18 (0.74)	−0.85 (1.44)	2.64	0.01
49) C29	−0.41 (1.01)	0.25 (0.75)	−3.24	0.002
50) 15-, 13-, 11-, 9-MeC29	−0.14 (0.52)	1.69 (0.5)	−15.77	**<0.001**
51) 7-MeC29	−1.15 (0.47)	0.25 (0.54)	−12.23	**<0.001**

P-values in boldface are significant after a Bonferroni correction

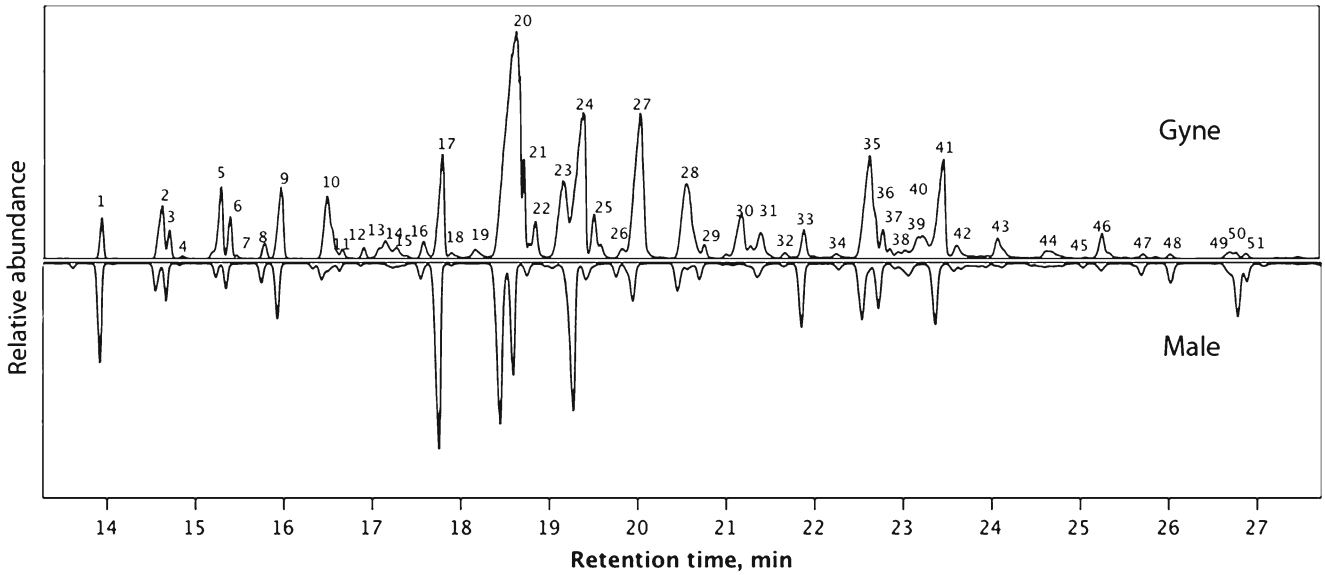

Fig. 1 Cuticular hydrocarbon profile of *Formica fusca* male and female sexuals (peak numbers as referred in Table 1)

unbranched alkanes were analyzed separately, 97.5 % of the young females and 88.9 % of the males were correctly assigned to sex based on the three first factors for branched alkanes. Only factors 1 and 3 contributed significantly to the correct classification after a Bonferroni correction (factor 1: T_{75}=3.79, P=<0.001; factor 2: T_{75}=−2.37, P=0.02; factor 3: T_{75}=8.75, P<0.001). For the linear alkanes, the first factor explained 77.9 % of the variance and correctly assigned 85.4 % of the young females and 27.8 % of the males. All compounds were strongly correlated negatively with this factor (r>−0.70), but this factor did not contribute significantly to the correct classification according to gender (T_{75}=0.83, P=0.41).

The cuticular hydrocarbon profiles had a significant colony-specific component, both for males and young queens, as shown by the discriminant analysis on the first ten factors (Table 2). The classification matrices showed that the combined information from the first four factors correctly assigned all but three males (out of 36), and one young queen (out of 41) to their colony of origin. Overall 95 % of the individuals were correctly assigned to colony (Fig. 3). All four factors also contributed to the separation between colonies (ANOVA, factor 1: $F_{12,\ 64}$=53.45, P<0.001; factor 2: $F_{12,\ 64}$=11.67, P<0.001; factor 3: $F_{12,\ 64}$=52.96, P<0.001; factor 4: $F_{12,\ 64}$=15.59, P<0.001). Nonetheless, the *post-hoc* tests indicated that some of the colonies were not significantly distinct in their profiles (Supplementary Table S1). For the young queens, we found two groups in which three and two colonies, respectively, were indistinct, and for males, we found one group in which two colonies were indistinct. In two groups, each with two colonies, young queens and males were indistinct. When branched and unbranched alkanes were analyzed separately, 96 % of the individuals were correctly assigned to their corresponding colonies based on the three first factors for branched alkanes. All three factors contributed to the correct colony classification (ANOVA, $F_{12,64}$=168.53, P<0.001; factor 2: $F_{12,64}$=44.50, P<0.001; factor 3: $F_{12,64}$=54.70, P<0.001; 56 out of 78 *post-hoc* comparisons were significant). The field-collected colony did not stand out from the laboratory-reared ones. For the linear alkanes, the first factor captured 78 % of the total variance (all peaks were strongly negatively correlated with this factor, r>−0.70), but correctly assigned only 21 % of all individuals to their corresponding colonies. Nevertheless, the first factor significantly contributed to the separation of the colonies (ANOVA, factor 1: $F_{12,\ 64}$=3.65, P, 0.001), although only 8 out of 78 *post-hoc* comparisons indicated significantly distinct colony profiles. Six of these comparisons involved the field-collected colony, but also with this colony excluded the first factor significantly separated the colonies (ANOVA, Factor 1: $F_{11,\ 59}$=2.11, P=0.033), yet only one out 66 *post-hoc* comparisons were significant.

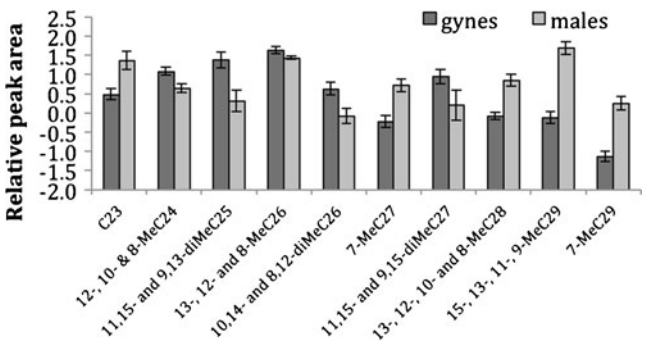

Fig. 2 Peaks that significantly differentiated between males and young queens. (Mean ± 95 % CI)

Table 2 Discriminant analysis on factor scores for colony and sex separately

Colony			*P*	Sex		*P*
Factor	Wilks'	$F_{12, 54}$		Wilks'	$F_{1, 65}$	
1	0	389.38	<0.001	0.32	99.82	<0.001
2	0	109.21	<0.001	0.19	34.00	<0.001
3	0	96.00	<0.001	0.16	16.19	<0.001
4	0	74.92	<0.001	0.60	242.51	<0.001
5	0	29.03	<0.001	0.13	1.55	0.22
6	0	30.47	<0.001	0.18	27.26	<0.001
7	0	59.95	<0.001	0.13	1.81	0.18
8	0	14.99	<0.001	0.17	23.69	<0.001
9	0	18.71	<0.001	0.13	0.07	0.79
10	0	8.97	<0.001	0.13	0.52	0.47

Finally, we found that colonies with a relatively low within-colony relatedness also showed significantly greater chemical diversity. The higher the within-colony relatedness, the shorter the average within-colony chemical distances were (r=−0.59, P=0.03; Fig. 4). Removing the ten sex-biased compounds (Fig. 2) from the analysis did not change the results (r=−0.58, P=0.04). Furthermore, when only branched compounds were considered, the pattern remained (r=−0.57, P=0.04), but not when only linear compounds were considered (r=0.02, P=0.94).

Discussion

In this study, we showed that both males and young queens of the ant *Formica fusca* have distinct colony signatures encoded in their cuticular chemistry. Furthermore, the degree to which cuticular chemistry varied within colonies was associated with colony kin structure such that low-relatedness colonies showed greater chemical diversity. We also found significant differences in cuticular hydrocarbon profiles between males and young queens. However, in agreement with most earlier studies on social insects (Layton et al., 1994; Cuvillier-Hot et al., 2001; Cremer et al., 2002; Abdalla et al., 2003; Antonialli et al., 2007; Beibl et al., 2007; Oppelt et al., 2008; Hojo et al., 2009), no unique sex-specific compounds were found. Chemical differences were quantitative, and significantly discernible in only 10 of the 51 compounds detected. In contrast, unique sex-specific compounds have been reported in solitary insects such as flies (*Drosophila sp*., Ferveur and Cobb, 2010), crickets (*Teleogryllus oceanicus*, Thomas and Simmons, 2008), and long-horn beetles (*Xylotrechus colonus*, Ginzel et al., 2003; *Anoplophora melasiaca*, Akino et al., 2001).

Some sex-specific compounds may have remained undetected in our study. For example, running analyses at higher temperatures could have revealed heavier compounds (Martin and Drijfhout, 2009). However, only approximately 10 % of the cuticular hydrocarbons have chain lengths above C_{29} in *F. fusca* (S.J. Martin, personal communication), which is considerably fewer than in other ant species (Martin and Drijfhout, 2009). Further differences between males and females may have been present among the compounds that co-eluted, especially among the branched compounds with two methyl groups. Indeed, earlier studies of *F. fusca* identified nine different C25-dimethylalkanes (Helanterä et al., 2011; Martin et al., 2008, 2011). We did not separate all nine of these compounds because some of them co-eluted, preventing reliable quantification of each separate compound. Even with this lower resolution, we found significant differences among both colonies and sexes. Separating these compounds could, therefore, only strengthen our conclusions that hydrocarbons are colony and sex-specific in *F. fusca* sexuals. The chemical profile may also be affected by diet and environment (Gibbs, 1998; Liang and Silverman, 2000; Buczkowski et al., 2005). However, given that all laboratory colonies were kept in largely identical conditions and fed the same food, major environmental divergences between the sexes are unlikely (Sorvari et al., 2008).

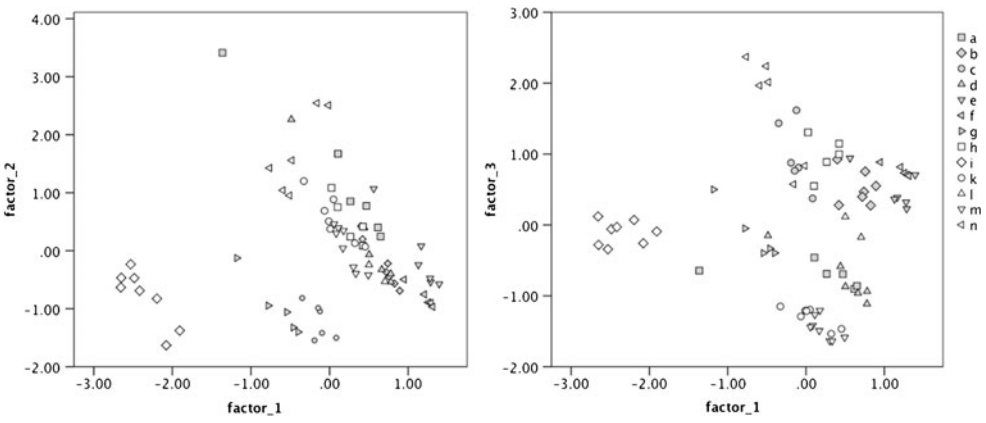

Fig. 3 Principal component analysis based on the cuticular hydrocarbon profiles of all colonies. Closed symbols indicate females, open symbols males. "N" is the field colony

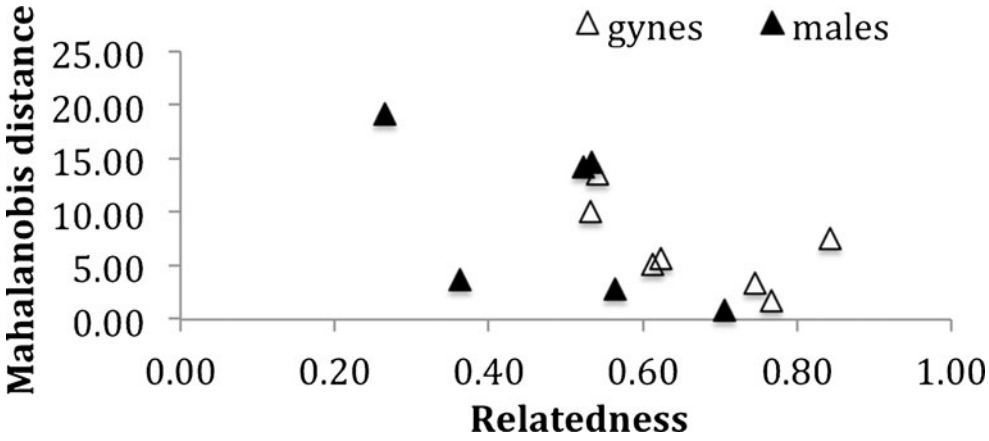

Fig. 4 Within-colony chemical diversity as a function of within-colony genetic relatedness

The disparity in sex-specific cues in social vs. solitary insects may be due to the relative importance that sexual selection presumably plays in many solitary vs. social insects. Indeed, sexual selection mediated by distinct compounds plays a crucial role in many solitary insects (Blomquist and Bagnères, 2010)), whereas in social insects, pre-copulatory sexual selection is considered to be limited (Boomsma et al., 2005). Conversely, if a distinct chemical colony identity is beneficial also for sexuals due to e.g., inbreeding avoidance, and if signals for mate choice, species recognition, and sexual selection interfere with these cues, less distinct sex-specific cues based on quantitative differences may emerge. In principle, selection should favor the profile that allows both species-, and sex-discrimination, as well as precise nest mate recognition (Martin et al., 2009). Our results suggest that this is indeed the case, as we found both sex-specific differences and colony-specific profiles for both males and females. Nevertheless, some of the colonies were not significantly distinct in their profiles, and in one pair of colonies young queens and males were indistinct. This may in part be due to the fact that all laboratory colonies were maintained under similar conditions, which may have homogenized chemical profiles through effects of diet and similar nest material on the odor profiles (Lenoir et al., 2009; van Zweden et al., 2009; Bos et al., 2011).

In this study, we analyzed only sexuals, and found more compounds in the chemical profile compared to an earlier study on *F. fusca* workers (Martin et al., 2008). For example, Martin et al. (2008) found fewer heavier compounds (>C27) in workers. This could reflect either a genuine absence of these chemicals in workers, or a failure to detect them because they compose a small proportion of the total chemical profile, and workers are smaller than sexuals. In support of the former interpretation, long-chain hydrocarbons denote higher fertility in *Harpegnathos saltator* (Liebig et al., 2000), and mean chain length increases during reproductive development in queen *L. niger* (Holman et al., 2010); this suggests that we might expect queens to have longer cuticular hydrocarbons than workers. Altogether, our results suggest that selection has not reduced the complexity of the chemical profiles of young queens or males relative to workers.

We found that colonies in which relatedness was low showed significantly greater chemical diversity. An earlier study on *F. fusca* workers found no association between kin structure and the diversity of odor cues (Helanterä et al., 2011). Similarly, studies on workers of other *Formica* species also failed to find such a correlation (Martin et al., 2009; van Zweden et al., 2010, 2011), with one exception (Boomsma et al., 2003). The discrepancy between the present study and these others implies that the chemical signatures of worker and reproductive castes may depend to a different degree on the genetic composition of individuals. In ants, chemical recognition cues are mixed throughout the colony by means of trophallaxis, and exchange of glandular products (Soroker et al., 1995). However, if workers engage in trophallaxis much more than sexuals, female and male sexuals may receive updated colony odors at a lower rate than workers. This may help preserve the chemical integrity of the sexes, as well as preserve chemical diversity among genetically dissimilar nest mate sexuals. Future studies designed to assess the chemical profile, not only of sexuals but also of workers from the same colonies, should add more information to this topic. Nevertheless, the removal of sex-specific compounds from the analysis did not alter the association between within-colony chemical- and genetic diversity.

The diversity of cuticular hydrocarbons makes them one of the major agents involved in chemical communication (Blomquist and Bagnères, 2010). Nevertheless, branched and unbranched compounds seem to be fundamentally different with respect to nest mate recognition. Here we show that linear alkanes do not provide enough information for either correct colony or sex classification, in contrast to branched compounds. Earlier studies on honeybees (*Apis mellifera)*, paper wasps (*Polistes dominulus)*, and several ant species (including *F. fusca*; Martin et al., 2008, 2011; Helanterä et al., 2011) also suggest that branched compounds seem to be more important in nest mate recognition,

and indicate a lack of information in linear compounds (Dani et al., 2001; Akino et al., 2004; Châline et al., 2005; Dani et al., 2005; Martin et al., 2008, but see Greene and Gordon, 2007). For example, both *Camponotus herculeanus* ants and *Apis mellifera* honeybees are unable to discriminate or learn linear compounds (Châline et al., 2005; Guerrieri et al., 2009), but can learn branched compounds. In contrast, *Camponotus aethiops* workers discriminate between different hydrocarbons efficiently regardless of their structure (Bos et al., 2012). This suggests that the type of cuticular hydrocarbons, e.g., branched vs. unbranched, differ in the information they convey, so that the compound types relevant for recognition varies among species, with some more relevant for protection against environmental factors, e.g., desiccation (d'Ettorre and Moore, 2008).

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