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Do Cuticular Hydrocarbons Provide Sufficient Information for Optimal Sex Allocation in the Ant *Formica exsecta*?

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Abstract Split sex ratio theory predicts that when kin structure varies among colonies of social insects, in order to maximize the inclusive fitness, colonies with relatively high sister-sister relatedness should specialize in producing reproductive females, whereas in those with relatively low sister-sister relatedness workers should bias their sex ratio towards males. However, in order to achieve this, workers need to be able to reliably assess the type of colony in which they live. The information on colony kin structure may be encoded in cuticular hydrocarbons (CHCs), assuming that genetic variability translates accurately into chemical variability. However, in genetically heterogeneous colonies, too accurate information may encourage the pursuit of individual interests through nepotistic behavior and reduce colony efficiency or cause social disruption. In this study, we estimated how well variability of CHC recognition cues reflects colony kin structure in the ant *Formica exsecta*. Our results show that CHC variability does not covary with kin structure or the overall genetic diversity of the colony, and that patrilines and matrilines can have distinct CHC profiles in some but not all colonies. However, within-colony relatedness remains the key determinant of colony sex ratios. Based on our results, CHC variability cannot serve as accurate information on within-colony relatedness, kin structure, or full-sib affiliation, nor do workers seem to use colony CHC variability as a proxy for sex-ratio adjustment. The use of this type of information thus could lead workers to make mistakes, and it remains unclear how colonies of *Formica exsecta* adjust offspring sex ratio to their optimal value.

Key Words Insects · Social evolution · Nepotism · Kin selection · Levels of selection · Genetic variance · Alkanes · Alkenes

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Introduction

Reproductive division of labor is the key contributor to the ecological success of social insects (Wilson, 1971). However, despite an impressive degree of cooperation, social insect colonies are rife with potential conflict, which arises because colony members, not being clonal, each have an "incentive" to pursue their individual fitness interests (reviewed in Ratnieks et al., 2006). The worker-queen conflict over sex ratio is contingent on the fact that the haplodiploid system of sex determination of Hymenoptera

creates asymmetric genealogies, such that full sisters are more closely related to each other, than they are to their brothers (Trivers and Hare, 1976). This relatedness asymmetry, however, decreases when the queen has mated more than once (polyandry) or if colonies contain several related queens (polygyny) (Boomsma, 1993). Consequently, when kin structure varies among colonies in the same population, theory predicts that workers can enhance their inclusive fitness by specializing on the sex to which they are most related relative to the population average, i.e., females in monogynous/monandrous colonies (with high relatedness asymmetry), and males in polyandrous or polygynous colonies (with low relatedness asymmetry) (split sex ratio theory; Boomsma and Grafen, 1990, 1991). Indeed, empirical studies, mainly of ant species, have found sex allocation patterns consistent with worker control in response to variation in relatedness asymmetry (Chan and Bourke, 1994; Sundström, 1994; Sundström et al., 1996), although other studies have found sex allocation patterns inconsistent with worker control in response to variation in relatedness asymmetry (Brown and Keller, 2000; Liautard et al., 2003; Bonckaert et al., 2011).

Precise adjustment of sex ratios by colony workers in response to colony kin structure requires a mechanism by which they can assess the genetic diversity within colonies, and manipulate brood composition accordingly, e.g., by removing male larvae (Chapuisat et al., 1997). A parsimonious hypothesis is that recognition cues are at least partly genetically determined, so that within-colony recognition cue variability to a certain extent reflects genetic diversity and, thus, relatedness asymmetry. Ants and other social insects use cuticular hydrocarbons (CHCs) for discriminating colony members from strangers to maintain colony integrity. Typically, each colony is characterized by specific proportions of a set of CHCs (reviewed in van Zweden and d'Ettorre, 2010), the exact relative compositions of which can have strong genetic underpinnings (e.g., Stuart, 1988; van Zweden et al., 2009, 2010). On the other hand, if the individuals' blends of CHCs accurately reflect their genetic lineage within a colony, this could allow workers in colonies that contain different patrilines or matrilines to favor close kin to the detriment of more distant kin, and so promote their own evolutionary interests. Such nepotistic discrimination is expected to carry costs with reduced colony efficiency and social disruption in its wake (Keller, 1997), and may lead to selection against highly diverse recognition cues (Ratnieks et al., 2007). Therefore, the level of information encoded in social insect recognition cues is likely to be the result of these two opposing evolutionary forces, being good enough to allow efficient discrimination against non-nestmates but noisy enough to prevent precise discrimination within colonies (van Zweden et al., 2010). Evidence to date indicates that this is indeed the case in the ant *Formica truncorum* (Boomsma et al., 2003) and the wasp *Vespa crabro* (Dani et al., 2004), where CHC variation explained by patriline affiliation is variable among colonies, although a recent study on *Acromyrmex octospinosus* shows higher kin specificity (Nehring et al., 2010).

Not all components of the CHC profile are of equal importance in inter- and intra-colony recognition. Methyl branched alkanes and alkenes have been especially implicated in nestmate recognition, whereas the role of linear alkanes in nestmate recognition is less clear (Dani et al., 2001, 2005; Akino et al., 2004; Greene and Gordon, 2007; Martin et al., 2008; Guerrieri et al., 2009). Conversely, the relative abundance of linear alkanes appears, for example, to be involved in within-colony recognition of (worker) castes in harvester ants (Wagner et al., 2001; Greene and Gordon, 2003; Martin and Drijfhout, 2009). Therefore, we hypothesize that CHCs associated with nestmate recognition may be uniform within colonies, whereas those involved in the assessment of colony kin structure may be more variable (c.f., van Zweden et al., 2010).

In the ant *Formica exsecta*, both the number of queens that head the colony (monogyny vs. polygyny) and the number of males that mate with a queen (monandry vs. polyandry) vary. A previous study showed sex ratio specialization consistent with the predictions from split sex ratio theory (Sundström et al., 1996). This implies that workers can assess colony kin structure also when the relatedness asymmetry varies solely due to queen mating frequency. The CHC profile of *F. exsecta* typically consists of five to six linear alkanes (*n*-C_{21}: *n*-C_{31}) and five to six alkenes ((*Z*)-9-$C_{21:1}$: (*Z*)-9-$C_{31:1}$), of which the latter have been implicated as nestmate recognition cues (Martin et al., 2008). In this study, we compared the CHC variability among workers in monogynous/monandrous, monogynous/polyandrous, and polygynous colonies of *F. exsecta*, to assess whether workers have enough information to act in their best inclusive fitness interests. We predicted that CHC variability would be greater in polyandrous and polygynous than in monogynous/monandrous colonies, thus reflecting lower relatedness asymmetry. The second prediction was that greater CHC variability corresponds to colonies with male-biased sex ratios. We then combined the information on CHC and genetic diversity with sex ratio data for the same colonies to evaluate the degree to which sex allocation corresponds to colony relatedness on the one hand and CHC variability on the other.

Methods and Materials

Study Organisms and General Procedures Workers of F. exsecta were collected from 9 colonies in June 2007 and from 6 colonies in June 2009 (Table 1), on islands off the

Tvärminne Zoological Station, southwestern Finland. The population comprises largely monogynous colonies headed by queens mated with 1 to 3 males, with the exception of a few polygynous colonies (Haag-Liautard et al., 2009). This is the same population where Sundström et al. (1996) found that colonies headed by a singly mated queen specialize in female brood, and those headed by a multiply mated queen specialize in male production. Altogether we chose 5 colonies with a single queen that had mated once (monogynous-monandrous, MG/MA), 5 colonies where the queen had mated multiply (monogynous-polyandrous, MG/PA) and 5 colonies with multiple queens (polygynous, PG) (Table 1). From each colony, 18–24 workers were collected and killed by freezing at −20°C for later genetic and chemical analysis.

Sex ratios of the study colonies were assessed by sampling 50–60 sexual pupae from each colony, in June-July 2007 and 2009 after all the larvae had pupated and before any sexuals had emerged. The sex of the pupae was determined based on morphology, and the sex ratio was calculated as the proportion of queens of all sexual brood (*c.f.*, Vitikainen et al., 2011).

Genetic Analysis To confirm the kin structure of the sampled colonies and to determine patriline or matriline affiliation, the 18–24 workers of each colony were genotyped at ten loci: Fe11, Fe13, Fe17, Fe37, Fe38, Fe42, Fe49 (Gyllenstrand et al., 2002), Fl21 (Chapuisat, 1996), P22 (Trontti et al., 2003), and Fy3 (Hasegawa and Imai, 2004). Further details of PCR conditions are equal to Haag-Liautard et al. (2009). The PCR-products were separated by using automated capillary sequencer (MegaBACE 1000) and sized against ET400-R standard (GE Healthcare). The genotypes were scored with the program Fragment Profiler v1.2 (GE Healthcare), and allele calling was confirmed manually.

We used maximum likelihood methods to confirm the genetic composition of the colonies, and to assign workers into groups of full- and half-sisters, as implemented in the program COLONY 1.3. (Wang, 2004). Average within-colony relatedness was calculated as the pedigree relatedness, based on the equations in Bourke and Franks (1995), assuming r=0.75 for MG/MA colonies, and using the observed paternity shares in MG/PA colonies. For the PG colonies, we inferred the number of matrilines from the genotype data assuming the queens were unrelated (r=0). The true value may be higher, so we ran each test also with higher values without finding any differences in the outcome. Pairwise relatedness estimates between individuals and colonies were calculated based on individual genotypes at each of the ten loci using Relatedness 5.0.8 (Goodnight and Queller, 1999), and colony inbreeding (HL) following the procedure by Aparicio et al. (2006). We used the background population genetic data from 102 colonies (c.f. Haag-Liautard et al., 2009) to enhance the accuracy of allele frequency and relatedness estimates.

Table 1 Basic data for the sampled colonies

Colony	Year	*N*	Sex ratio	Inbreeding coefficient	Gene diversity	Allelic richness	Nr. of matri/ patrilines	Pedigree relatedness	All compounds	(Z)-9-Alkenes	*n*-Alkanes
MG/MA 1	2007	20	0.85	0.29	0.513	2.275	1 m/1p	0.750	1.664	1.021	1.525
MG/MA 2	2007	18	0.17	0.04	0.548	2.279	1 m/1p	0.750	1.676	0.838	1.469
MG/MA 3	2007	19	1.00	0.04	0.513	2.214	1 m/1p	0.750	1.860	0.843	1.214
MG/MA 4	2009	24	0.96	0.26	0.479	2.130	1 m/1p	0.750	1.871	0.423	1.154
MG/MA 5	2009	23	0.00	0.00	0.585	2.462	1 m/1p	0.750	1.365	0.569	1.017
MG/PA 1	2007	20	0.13	0.26	0.516	2.194	1 m/2p	0.503	2.817	1.337	1.562
MG/PA 2	2007	19	0.55	0.39	0.408	1.983	1 m/2p	0.540	1.946	1.191	1.259
MG/PA 3	2007	19	1.00	0.16	0.647	2.805	1 m/2p	0.590	2.701	1.308	1.970
MG/PA 4	2009	24	0.00	0.12	0.577	2.488	1 m/2p	0.674	1.499	0.534	1.074
MG/PA 5	2009	23	0.00	0.11	0.583	2.415	1 m/2p	0.516	1.531	0.277	1.262
PG 1	2007	18	0.15	0.25	0.718	3.373	8 m	0.094	1.940	0.759	1.063
PG 2	2007	19	0.00	0.28	0.622	2.930	7 m	0.107	1.914	0.856	1.368
PG 3	2007	20	0.00	0.28	0.714	3.305	9 m	0.083	2.774	0.848	1.890
PG 4	2009	23	0.29	0.33	0.446	2.180	4 m	0.083	1.475	0.579	1.343
PG 5	2009	23	0.04	0.40	0.604	2.933	7 m	0.048	1.321	0.683	1.129

Colony name also reflects colony type (*MG/MA* monogynous-monandrous, *MG/PA* monogynous-polyandrous, *PG* polygynous). *N* equals the number of individuals screened per colony. Sex ratio is expressed as the number of females as a proportion of the total number of sexual offspring. Pedigree relatedness is calculated based on the assumption that queen relatedness is zero. All compounds, (Z)-9-Alkenes, and *n*-Alkanes refer to cuticular hydrocarbon (CHC) variability measures based on the respective sets of compounds.

Colony-specific gene diversity and allelic richness were calculated with colony defined as population using Fstat 2.9.3 (Goudet, 2001). As the two measures correlated strongly (r=0.94, df=15, P<0.001), we used the factor scores from the first axis in a PCA encompassing the two measures. Comparisons between the different categories of colonies were done with a one-way ANOVA.

Chemical Analysis Cuticular lipids were obtained by individually immersing the 18–24 freshly killed workers (before genotyping them) in 200 µl HPLC-grade pentane for 10 min, with gentle vortexing for 15 sec at the start and end of the 10 min. The pentane was left to evaporate at room temperature under a laminar air-flow hood. Extracts were resuspended in 50 µl pentane, 2 µl of which were injected into an Agilent Technologies 6890 N gas chromatograph (GC), connected to an Agilent Technologies 5975 mass selective detector (MS), using 70 eV electron impact ionization. The GC was equipped with an HP-5MS capillary column (30 m×0.25 mm ID, 0.25 µm film thickness) and a split-splitless injector. The carrier gas was helium at 1 ml/min. After an initial hold of 1 min at 70°C, the temperature rose to 180°C at a rate of 30°C/min, and then to 320°C at 4°C/min with a final hold of 5 min.

The areas of five (*Z*)-9-alkenes and five linear alkanes found on the cuticle of all workers (c.f. Martin et al., 2008) were integrated for further analysis with Agilent ChemStation v. D.02.00.237. Peak areas were normalized for each individual by using a Z-transformation (Aitchison, 1986).

Statistics We estimated within-colony CHC variability both for single compounds and for sets of multiple compounds [all compounds (10 variables), (*Z*)-*9*-alkenes only (5 variables), and linear alkanes only (5 variables)]. For single compounds, we calculated the within-colony standard deviation from the Z-transformed values. For sets of multiple compounds, we first scaled the Z-transformed variables to zero mean and unit variance, and then calculated the absolute Euclidean distance to the mean of the colony. CHC variability then was expressed per colony as the mean distance of its individuals.

To assess which factors may influence within-colony CHC variability, we used ANCOVAs with within-colony CHC variability as the dependent variable, and colony kin structure (MG/MA, MG/PA, PG) or within-colony relatedness, year of sampling (2007 or 2009), within-colony genetic diversity (as captured in the factor scores for gene diversity and allelic richness combined), and colony inbreeding (HL) as the predictor variables. We included HL in the analysis, given that two earlier studies have indicated a significant effect of inbreeding on sex ratios (Haag-Liautard et al., 2009; Vitikainen et al., 2011). To test the effects of within-colony CHC variability, relatedness, genetic diversity, and inbreeding on colony sex ratio we used a multiple regression with stepwise backward elimination. These analyses were done in Statistix 9 (Analytical Software, USA).

To test for an association between genetic and CHC similarity of colonies and individuals within colonies, we created distance matrices, one with the pairwise genetic relatedness based on the ten genotyped loci, and one with the pairwise Euclidean CHC distance. Pairwise Euclidean CHC distance was calculated for sets comprising either all compounds (10 variables), (*Z*)-*9*-alkenes only (5 variables), or linear alkanes only (5 variables). The correlation between the genetic and CHC matrices then was tested using Mantel tests in the program Genodive (v. 2.0b20) (Meiermans, 2010). Correlations between relatedness and CHC distances were performed both between individuals within colonies (i.e., for each colony separately) and between colonies.

Finally, to estimate variation among hydrocarbon profiles explained by patriline or matriline affiliation, we performed a PCA using the MGPA and PG colonies, based on the three sets of multiple compounds [all compounds, linear alkanes only, and (*Z*)-*9*-alkenes only]. This was followed by a MANOVA in the program R (v. 2.12.0) with those PCs explaining >95% of the variation as dependent variables, and colony and patri- or matriline nested within colony as the explanatory variables. We repeated this analysis for each colony separately, except that colony was not used as an explanatory variable in the MANOVA. We corrected the above analyses for multiple testing by using the false discovery rate as appropriate (Benjamini and Hochberg, 1995).

Results

All putative MG/MA colonies were confirmed to be composed of full sisters only. All five MG/PA colonies had two patrilines, and the samples from PG colonies contained between 4 and 9 matrilines (Table 1). The diversity of (*Z*)-*9*-alkenes was in both sampling years significantly lower than that of *n*-alkanes, and the diversity of both was lower in 2009 than in 2007 (full factorial ANOVA, compound type: $F_{1,26}$=46.3, P<0.001, year: $F_{1,26}$= 23.5, P<0.001, compound type x year: $F_{1,26}$=1.08, P=0.31). We found no significant differences in CHC variability between PG, MG/PA, and MG/MA colonies, neither when all compounds were considered together, nor when the subsets of (*Z*)-*9*-alkenes or *n*-alkanes were considered separately [Fig. 1; one-way ANOVA with colony type as explanatory variable: (*Z*)-9-alkenes: $F_{2,12}$=0.57, P=0.58,

n-alkanes: $F_{2,12}$=0.31, P=0.74, all compounds: $F_{2,12}$=0.84, P=0.46]. Similarly, in the more comprehensive model, neither within-colony relatedness, genetic diversity (factor scores for the combined effects of allelic diversity and gene diversity), nor colony inbreeding were significantly associated with CHC variability in any of the three multi-compound sets [all compounds, (*Z*)-*9*-alkenes, *n*-alkanes; Table 2], or any of the individual compounds (Appendix 1). The outcome was qualitatively the same when colony type (PG, MG/PA, and MG/MA) was used instead of within-colony relatedness (Appendix 1). Indeed, within-colony CHC variability was not significantly correlated to any of the other measures, except for sampling year (Table 2). However, genetic diversity did not significantly differ among the three colony types either (PG, MG/PA, MG/MA) ($F_{2,12}$=3.25, P=0.07), suggesting that the increased number of genetic lineages in MG/PA and PG colonies did not necessarily increase genetic diversity.

We found a portion of CHC variation was explained by patri- and matrilines when all compounds or (*Z*)-*9*-alkenes only were considered (all compounds: Wilks' λ=0.333, approx. $F_{148,632.1}$=1.36, P<0.01; (*Z*)-*9*-alkenes: Wilks' λ=0.420, approx. $F_{111,477.1}$=1.44, P<0.01; *n*-alkanes: Wilks' λ=0.521, approx. $F_{111,477.1}$=1.05, P=0.367), although these differences were only pronounced in some of the colonies (Table 3). Matrilines differed significantly in their CHCs in one of the five PG colonies, regardless whether all compounds or only (*Z*)-*9*-alkenes were used. Similarly, in one of the five MG/PA colonies patrilines differed significantly with respect to the (*Z*)-*9*-alkenes (all corrected for false discovery rate; Table 3). We also found a negative correlation between pairs of colonies for pairwise relatedness and (*Z*)-*9*-alkene distance after correction for false discovery rate (r=−0.342, P=0.001), but not for *n*-alkanes or when all compounds were considered (r=−0.021, P=0.401, and r=−0.213, P=0.016, respectively) (Fig. 2). This shows that the relative abundance of (*Z*)-*9*-alkenes has a strong genetic component with respect to between-colony variation. However, within colonies, we found no significant associations between genetic and CHC distances for any of the considered compound sets (Appendix 2).

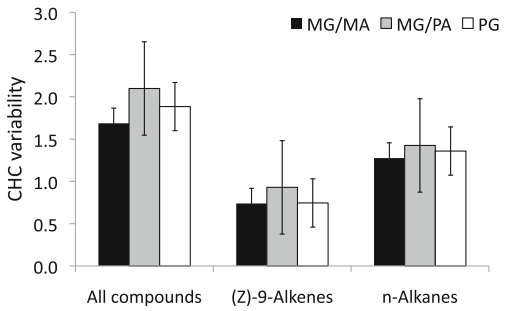

Fig. 1 CHC variability, colony type and CHC group (mean ±95%CI; *MG/MA* monogynous-monandrous, *MG/PA* monogynous-polyandrous, *PG* polygynous)

Table 2 Ancova results for effects on CHC variability

Effect	All compounds		(*Z*)-*9*-Alkenes		*n*-Alkanes	
	F	*P*	*F*	*P*	*F*	*P*
Year	5.63	0.04	20.09	0.001	3.71	0.08
Relatedness	0.12	0.73	0.33	0.58	0.01	0.92
Genetic diversity	0.39	0.55	0.12	0.73	0.07	0.80
Inbreeding	0.25	0.63	1.64	0.23	0.06	0.81

Within-colony relatedness was calculated assuming zero relatedness among queens, and genetic diversity as the combined effects of allelic richness and gene diversity captured in their factor scores. *df*1=1, *df*2=10 in all cases.

The sex ratios of all PG colonies were highly male biased, as expected based on their low relatedness (Fig. 3). The monogynous colonies were more variable. Two of the five MG/PA colonies produced a female biased sex ratio, and two of the five MG/MA colonies produced mainly males (Fig. 3). When the effects of colony-specific CHC variability on sex ratio were considered in conjunction with within-colony relatedness and colony inbreeding (HL), the only significant determinant of colony sex ratios was within-colony relatedness (full model including relatedness and HL as explanatory factors: R^2=0.40, $F_{2,12}$=4.04, P=0.045; HL: $F_{2,12}$=3.13, P=0.102; relatedness $F_{2,12}$=8.07, P=0.015; all other variables, year, all compounds, (*Z*)-*9*-alkenes, *n*-alkanes: $F_{2,12}$<0.35, P>0.50).

Discussion

In this study, we showed that in *Formica exsecta* the within-colony CHC variability is not contingent on colony kin structure, yet genetically related colonies have more similar chemistry, showing a genetic component to the cuticular chemistry without a direct link with the genetic composition of the colony. Nonetheless, genetic lineages were chemically distinct in some but not all colonies. This suggests that chemical cues by which workers can assess colony kin structure are occasionally available, but that these cues generally are unreliable, so that workers would make too many mistakes when using them as a proxy for the sex ratio to be produced by the colony.

Polygynous and monogynous/polyandrous colonies showed no higher amounts of CHC variability than monogynous/monandrous ones. This is in agreement with the result obtained by S.J. Martin et al. (unpublished data) for the same species, but stands in stark contrast with the general expectation of greater CHC variability in genetically more heterogeneous, i.e., polygynous and/or mono-

Table 3 Discriminant analyses between genetic lineages within MGPA and PG colonies

Colony	All compounds (3 PCs)			*(Z)-9*-Alkenes (2 PCs)			*n*-Alkanes (2 PCs)		
	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*
All colonies	0.333	$F_{148,632}=1.36$	0.007	0.420	$F_{111,477}=1.44$	0.005	0.521	$F_{111,477}=1.05$	0.367
MG/PA 1	0.795	$F_{3,16}=1.38$	0.285	0.891	$F_{2,17}=1.05$	0.373	0.858	$F_{2,17}=1.41$	0.272
MG/PA 2	0.502	$F_{3,14}=4.63$	0.018	0.810	$F_{2,15}=1.76$	0.206	0.577	$F_{2,15}=5.49$	0.016
MG/PA 3	0.730	$F_{3,15}=1.85$	0.182	0.711	$F_{2,16}=3.25$	0.065	0.851	$F_{2,16}=1.40$	0.276
MG/PA 4	0.732	$F_{3,20}=2.44$	0.094	0.539	$F_{2,21}=8.99$	0.002	0.829	$F_{2,21}=2.16$	0.140
MG/PA 5	0.718	$F_{3,19}=2.49$	0.092	0.821	$F_{2,20}=2.18$	0.140	0.902	$F_{2,20}=1.09$	0.355
PG 1	0.098	$F_{20,20.7}=1.23$	0.323	0.482	$F_{14,16}=0.50$	0.899	0.221	$F_{14,16}=1.29$	0.310
PG 2	0.290	$F_{18,28.8}=0.88$	0.606	0.255	$F_{12,22}=1.80$	0.113	0.325	$F_{12,22}=1.38$	0.246
PG 3	0.220	$F_{24,26.7}=0.76$	0.746	0.413	$F_{16,20}=0.70$	0.767	0.379	$F_{16,20}=0.78$	0.690
PG 4	0.088	$F_{9,41.5}=7.92$	<0.001	0.023	$F_{6,36}=33.52$	<0.001	0.468	$F_{6,36}=2.77$	0.026
PG 5	0.323	$F_{18,40.1}=1.09$	0.394	0.351	$F_{12,30}=1.72$	0.111	0.436	$F_{12,30}=1.29$	0.277

A PCA was performed on the Z-transformed CHC data of either all colonies together or single colonies; PCs explaining >95% of the variation were then included as the dependent variable in a MANOVA with genetic lineage (nested within colony, when all colonies were considered together) as the explanatory variable.

gynous/polyandrous societies, which has formed the foundation for assuming lower discrimination abilities in polygynous societies (e.g., Fletcher and Michener, 1987; Starks et al., 1998). However, we also found that the within-colony genetic diversity was not higher in the polygynous and the monogynous/polyandrous colonies than in the monogynous/monandrous ones. Indeed, although within-colony relatedness varied 15-fold, the corresponding ranges for both gene diversity and allelic richness were only 1.7 fold in both cases (Table 1). Thus, the genetic basis for a greater diversity is not present, which may explain the lack of correlation between colony kin structure and CHC variability.

The (*Z*)-*9*-alkenes overall were less variable within colonies than *n*-alkanes. This supports the existing evidence that mainly alkenes mediate nestmate recognition in *F. exsecta*, whereas alkanes are less informative in this respect (Martin et al., 2008). Moreover, the between-colony CHC distance for (*Z*)-*9*-alkenes, but not the *n*-alkanes, decreased significantly with increasing between-colony pairwise relatedness. This, in conjunction with the result that within-colony CHC variability showed no clear association with genetic diversity, suggests a strong genetic foundation of the (*Z*)-*9*-alkene profile that is to a large extent blurred within colonies by odor cue transfer between nestmates (c.f. van Zweden et al., 2010). It also suggests unequal mixing

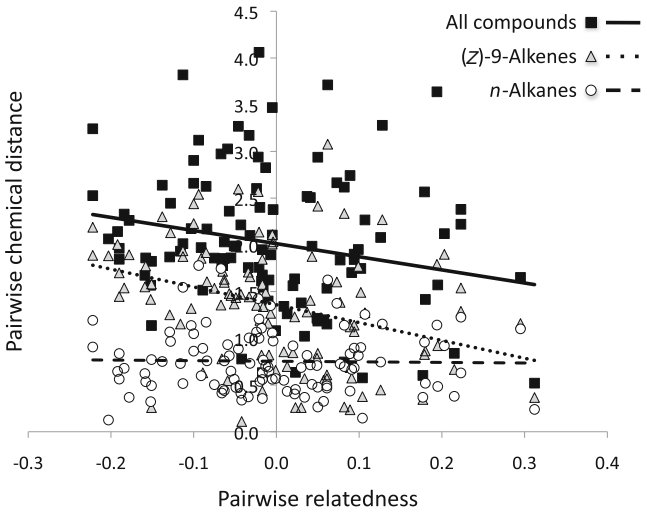

Fig. 2 Correlations between colony pairwise relatedness and colony CHC distances. Association is significant, after correction for false discovery rate, for *(Z)*-9-alkenes (Mantel's test; *(Z)*-9-alkenes, $r=-0.342$, $P<0.001$), but not when *n*-alkanes or all compounds were considered (*n*-alkanes, $r=-0.021$, $P=0.401$; all compounds, $r=-0.213$, $P=0.016$)

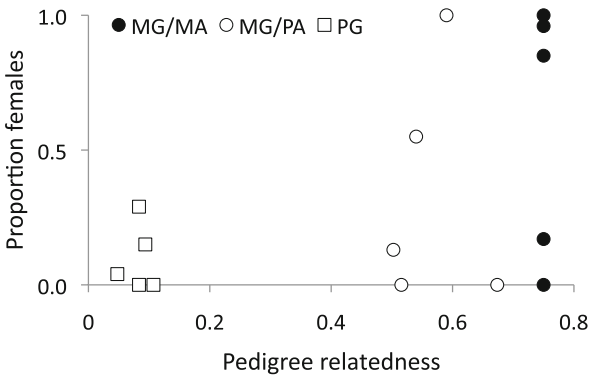

Fig. 3 Sex ratios (numerical proportion of females in the brood) as a function of within-colony relatedness, grouped according to colony type (*MG/MA* monogynous-monandrous, *MG/PA* monogynous-polyandrous, *PG* polygynous)

of CHCs, which could be achieved by accumulating alkenes to a greater extent than alkanes in the postpharyngeal gland (PPG; Soroker et al., 1994). The PPG does not synthesize hydrocarbons, but acts as a reservoir for these compounds to be homogenized and exchanged via grooming and trophallaxis (Soroker et al., 1994). Thus, inequalities in diversity could arise if alkenes are released more readily, or workers metabolize alkanes more rapidly, with alkenes becoming better mixed than alkanes among colony members. Altogether, these results suggest a pattern similar to that observed in *F. rufibarbis*, where cues used in nestmate recognition are genetically determined but preferentially mixed among nestmates (van Zweden et al., 2010).

Although the within-colony CHC variability showed no correlation with colony kin structure, relatedness, or any of the measures of genetic diversity, we found significant CHC divergence between genetic lineages within colonies. This was, however, only the case in two of the ten colonies that comprised more than one lineage. Earlier analyses in the wasps *Polistes dominulus*, *Vespa crabro* (Dani et al., 2004), and *Dolichovespula saxonica* (Bonckaert et al., 2011), and the ant *Acromyrmex octospinosus* (Nehring et al., 2010), found more consistent CHC differences between matrilines and patrilines. However, the CHC profile of *F. exsecta* exhibits far fewer hydrocarbons with fewer structural groups than these species (*P. dominulus*: 44 compounds; *V. crabro*: 25 compounds; *D. saxonica*: 56 compounds; *A. octospinosus*: 38 compounds), and so offers less scope for both divergence and statistical discrimination between genetic lineages. However, in *F. truncorum*, Boomsma et al. (2003) nonetheless were able to discriminate a greater fraction of patrilines although the analysis entailed only nine compounds.

At least two mutually non-exclusive explanations for the relative lack of CHC discrimination among genetic lineages are possible. First, active cue scrambling may render the colony odor more uniform or blur the recognition code. This may be an evolved response that either mitigates the opportunities for workers to selfishly favor close kin and disfavor more distant kin with social disruption in its wake, or increases discrimination between members of different colonies (Keller, 1997; van Zweden et al., 2010). Theory predicts depauperate CHC profiles and cue mixing when potential conflicts are rife in the colony (e.g., Sundström and Boomsma, 2001). This is indeed the case in *F. exsecta*, but many more compounds have been found in *F. truncorum* (Akino, 2006), which goes against this interpretation. Second, given that inbreeding is rife in the population of *F. exsecta* studied here (Sundström et al., 2003; Haag-Liautard et al., 2009; Vitikainen et al., 2011), genetic variation for cue diversity may have been purged. We found no significant effects of inbreeding on CHC variability, but the measure we used as a covariate in the analyses does not reflect the absolute level of inbreeding, only the relative level. Thus, a significant effect of inbreeding may not be detectable.

Within-colony CHC variability had no significant predictive value on sex ratio, but colonies with higher relatedness produced more females, when also accounting for colony inbreeding. This suggests that workers do not use the CHC variability of adult individuals as a proxy for sex ratio decisions, in contrast to the results obtained by Boomsma et al. (2003) for *F. truncorum*. It begs the question by what mechanism this is achieved. All polygynous colonies produced a male-biased brood, but against expectations based on the kin value of brood, one polyandrous colony produced only female brood and one monandrous colony only male brood. In the case of polyandrous colonies, errors in the assessment of colony kin structure may occur if the two fathers are closely related, and so mediate similar CHC profiles to their offspring. However, the polyandrous colony that produced an all-female brood had the lowest relatedness between fathers, and not an exceptionally unequal contribution by the two fathers (Table 1). It remains to be shown what information the workers use to assess colony kin structure, and why such apparent errors occur. A possibility is that CHC information on eggs or larvae more accurately reflects the genetic make-up of the colony, owing to no or less transfer of cues between genetic lineages, and that nurse workers bias the sex ratio accordingly. Another possibility is that nurse workers can assess the number of queens directly or by colony egg production, and use this as a proxy to adjust sex ratio in polygynous colonies. This is consistent with split-sex ratio theory (Boomsma, 1993), because polygynous colonies will tend to have higher egg-production and a lower than population-level-average relatedness asymmetry, and also with the idea that specialization in the cheaper sex (males weigh less in *F. exsecta*; Vitikainen et al., 2011) requires greater initial egg-production for equal total biomass production. On the other hand, this does not explain the tendency of monogynous/polyandrous colonies to specialize in male production. Environmental factors also are likely to influence the optimal sex ratio for a colony, and hence may explain the discrepancies between the observed pattern and the outcome expected based on purely inclusive fitness arguments (Nielsen et al., 1999; Liang and Silverman, 2000).

In summary, we found no support for the suggested association between colony kin structure and variability of recognition cues (c.f. Fletcher and Michener, 1987), but this lack of relationship may be explained by the uniform levels of genetic variation we found among the different colony types. The breeding patterns and the genetic structure of the population, which indicate non-trivial levels of inbreeding (Sundström et al., 2003; Haag-Liautard et al., 2009; Vitikainen et al., 2011), may have contributed to the

depauperate chemical profile through genetic purging, a question which will have to be addressed in future studies. We also found that the within-colony CHC variability does not allow accurate assessment of colony kin structure, and that workers do not seem to use any single compound or set of compound as a proxy for sex ratio adjustments. Rather, genetically informative cues appear to be mixed among workers to create a colony odor that is used in between-colony discrimination. The cuticular hydrocarbon variability of *Formica exsecta* may thus be subject to balancing selection on the accuracy of genetic variability, allowing colony members to serve their collective but not selfish fitness interests, possibly blended with loss of genetic diversity owing to inbreeding in this population.

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