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Are you my mother? Kin recognition in the ant *Formica fusca*

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Abstract

In social insects, workers trade personal reproduction for indirect fitness returns from helping their mother rear collateral kin. Colony membership is generally used as a proxy for kin discrimination, but the question remains whether recognition allows workers to discriminate between kin and nonkin regardless of colony affiliation. We investigated whether workers of the ant *Formica fusca* can identify their mother when fostered with their mother, their sisters, a hetero-colonial queen or hetero-colonial workers. We found that workers always displayed less aggression towards both their mother and their foster queen, as compared to an unfamiliar hetero-colonial queen. In support of this finding, workers maintain their colony hydrocarbon profile regardless of foster regime, yet show modifications when exposed to different environments. This indicates that recognition entails environmental and genetic components, which allow both discrimination of kin in the absence of prior contact and learning of recognition cues based on group membership.

Introduction

In group-living animals, reliable parent–offspring recognition systems are expected to evolve (e.g. Beecher, 1991), as this decreases the risk that a parent will direct care to unrelated young and prevents the offspring from receiving aggressive behaviour from unrelated adults. Precise mother-offspring recognition, based on different communication channels, is well documented in many animals, from reptiles (e.g. lizards, Main & Bull, 1996) to birds (e.g. swallows, Leonard *et al.*, 1997; penguins, Searby *et al.*, 2004) and mammals (e.g. humans, Porter, 1991; sheep, Searby & Jouventin, 2003). Although mutual recognition is beneficial to both parties, parent–offspring conflict theory (Trivers, 1974) predicts that the young should be under stronger selection to develop recognition abilities towards their parents as failing to do so would increase offspring mortality. Indeed, pups of northern fur seals have been shown to put more effort in the reunion process with their mothers than vice versa (Insley, 2001).

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In social insects, workers trade personal reproduction for indirect fitness returns from helping their mother to rear collateral kin (Hamilton, 1964a,b; Trivers & Hare, 1976). Colony membership is generally used as a proxy for discriminating between kin and nonkin. Failure to discriminate colony members (i.e. their mother queen and/or her offspring) from unrelated hetero-colonial individuals (i.e. individuals from a different colony), and consequently exclude intruders, will significantly reduce the inclusive fitness returns from helping. In the extreme case, social parasites are able to enter the colony exploiting the host worker force to rear their own young (Lenoir *et al.*, 2001). The process of recognition is thought to involve matching a specific set of detected cues (the label) against a neural representation of these cues (the template), both of which may comprise environmentally and genetically determined components (e.g. Hepper, 1991; Lenoir *et al.*, 1999). Recognition in social insects is mainly mediated by chemical compounds, mostly cuticular hydrocarbons (Lahav *et al.*, 1999; Wagner *et al.*, 2000; Guerrieri *et al.*, 2009). Lenoir *et al.* (1999) suggested that template acquisition and label development are facilitated by a lack of hydrocarbons on newly emerged individuals (callows). This is followed by a period of chemical integration, when callows learn the

colony odour and attain their own hydrocarbon profile through synthesis, grooming and the exchange of liquid food.

Between-colony recognition has been well established by several studies (reviewed in Lenoir *et al.*, 1999; van Zweden & d'Ettorre, in press). The question remains, though, whether genetically determined cues, effectively generating individual signatures, alone are sufficient for recognition, e.g. whether worker ants can recognize relatives despite being raised by unrelated individuals. Such recognition would require a strong enough genetic component and would represent true kin recognition (*sensu* Grafen, 1990). However, theory predicts that selection tends to erode cue diversity in genetic recognition systems, as this allows within-colony nepotistic behaviour and, hence, social disruption (Keller, 1997; Boomsma *et al.*, 2003; Dani *et al.*, 2004). This may explain why within-colony kin recognition has been notoriously difficult to demonstrate in social Hymenoptera (De Heer & Ross, 1997; Strassmann *et al.*, 2000; Goodisman *et al.*, 2007; but see Hannonen & Sundström, 2003). Nonetheless, extrinsic selection pressures may maintain cue diversity, thereby catering for the maintenance of a genetic recognition system (Rousset & Roze, 2007). Social parasitism, wherein queens enter heterospecific host colonies and exploit these, may provide one such extrinsic selection force by selecting for enhanced recognition abilities in host species, and consequently the maintenance of high diversity in recognition cues.

The ant *Formica fusca* has previously been used to study recognition systems, both with respect to colony closure and brood recognition (Wallis, 1962; Helanterä & Sundström, 2005). The species is also subject to extensive temporary social parasitism by ants of the *Formica rufa* s.str. group. Parasitized colonies die within a year (Collingwood, 1979; Czechowski *et al.*, 2002), and this is likely to select for enhanced recognition abilities. Hannonen & Sundström (2003) found evidence for workers favouring closely related brood over less related brood in multi-queen colonies. Workers of this species also discriminate against eggs laid by hetero-colonial queens (Helanterä & Sundström, 2007), yet previous experience with hetero-colonial eggs can enhance their acceptance (Helanterä *et al.*, 2007). This suggests that workers of *F. fusca* have very precise recognition abilities, but to date no studies have experimentally tested whether recognition involves a component of kinship independent of familiarity.

In this study, we use a cross-fostering design to test whether workers can recognize their mother in the absence of contact during adult life. We raised workers with their own mother or sisters, or with a hetero-colonial foster queen or workers. We then staged aggression bio-assays to assess the behaviour of these workers towards their mother, the foster queen and an unfamiliar hetero-colonial queen. We also analysed the cuticular chemical profile of the workers raised in the different regimes. Altogether, this allowed us to examine the degree to which recognition depends on an individual's genetic background and social environment.

Materials and methods

Study species

Formica fusca is a boreal ant with colonies of 500–2000 workers which nest in decaying tree stumps or under rocks in semi-open habitat (Savolainen & Vepsäläinen, 1988). Approximately 26% of the queens are doubly mated and 60% of the colonies are weakly polygynous, with a median queen number of three (Hannonen *et al.*, 2004). Mean queen turnover in polygynous colonies has been estimated at 0.35 per year, resulting in an average worker–worker relatedness of 0.27 in polygynous colonies (compared with 0.63 in monogynous colonies in the same population, Bargum *et al.*, 2007).

Experimental setup

Fifty-nine colonies of *Formica fusca* were collected on the Hanko peninsula (SW Finland) before the onset of egg-laying in early spring (April 2007). Single-queen laboratory nests were established from each field colony in plastic trays lined with peat, some of the original nest material, *Sphagnum* moss to maintain humidity and a 15×15 cm ceramic tile to serve as a nest site. Colonies were fed a standard Bhatkar-Whitcomb diet (Bhatkar & Whitcomb, 1970) daily and moistened from a spray can as needed. The queens were allowed to lay eggs and the workers to raise the brood until pupation (cocoons). To ensure that the newly emerged workers were naïve with respect to colony odour we removed the cocoons from their maternal nest before eclosion (when they had begun to darken) and introduced 50 cocoons per colony to the five treatments described below. In total, 17 colonies produced enough brood to be used as focal colonies (see below), the rest provided foster queens and workers, or did not produce enough brood to be used.

All experimental nests were established in $12 \times 12 \times 12$ cm plastic boxes, the sides of which were coated with Fluon® (DeMonchy, Rotterdam, The Netherlands). The boxes contained a plaster base with a depression to serve as a nest site covered by a piece of translucent red plastic. The ants were fed daily on a standard Bhatkar-Whitcomb diet and water was provided *ad libitum*. After the fostering period the new workers were entered into the aggression assay described below. No workers were used in more than one treatment and replicate. The fostering queen and workers (treatments 2 and 4) were always from the same colony and fostering colonies were not reused across replicates. It is not known whether the queens were singly or multiply mated.

Naïve treatment: reared without adult individuals. The cocoons of 17 colonies were opened manually and the

newly emerged workers placed in groups of on average nine individuals (range 6–11) in nest boxes, without contact with adult workers or queens. To minimize the probability of cue acquisition from their newly emerged sisters and provide a naïve recognition template, these workers were entered into the aggression assay 0–3 days after emergence and immediately frozen for hydrocarbon extraction.

Treatments 1 and 2: reared with a queen. Cocoons were allowed to emerge with a queen. A few cocoons were opened manually; the young workers then opened the remainder. On day 4 after the 50 cocoons had been introduced, all unemerged cocoons were removed to standardize the age of the new workers. The newly emerged workers were then left to be fostered with their respective queen for nine additional days. On average 24 young workers (range 12–48) were fostered in each treatment. In treatment 1 (own queen), cocoons from 14 colonies were fostered with their own mother; in treatment 2 (foster queen), cocoons from 12 colonies were fostered with a hetero-colonial queen.

Treatments 3 and 4: reared with workers. Cocoons were allowed to emerge with adult workers. The adult workers in these treatments were marked with a paint marker for identification. On day 4 after the 50 cocoons had been introduced, all remaining cocoons were removed to standardize the age of the focal workers. The newly emerged workers were then left to be fostered by the adult workers for nine additional days. On average 25 young workers (range 15–43) were fostered with an average of 40 adults (range 30–50). In treatment 3 (Own workers), cocoons from nine colonies were fostered with adults from their own colony. In treatment 4 (Foster workers), cocoons from eight colonies were fostered with adults from an alien colony.

Aggression assays

Workers from each treatment were assayed for their response to three different queens: their mother (henceforth, 'own'), the foster queen (henceforth, 'foster') and an entirely unfamiliar hetero-colonial queen (henceforth, 'alien'). The assays were conducted on neutral ground in a circular plastic container with a plaster base 7.0 cm in diameter. The focal queen was placed in the container and allowed to settle for approximately 1 min. Workers were introduced individually and observed until they had completed an interaction with the queen, at which point they were removed; the behaviour of the queen during the assay was not recorded. The next worker was introduced after a pause of approximately 10 s (max. 15 s). After each test sequence (workers from the same colony and treatment) the queen was removed and the arena was left empty for 1 min before a different queen was introduced; the next test sequence was then started. The order was randomized with respect to queen type (mother, foster or alien). On average 7 ± 2.5 (range 2–11) workers were tested per colony and treatment. The observer was blind to the identity of the focal queen.

An interaction was considered complete once the test worker disengaged from the queen or when she began biting the queen. Interactions were recorded according to the following scale of increasing aggression: (1) antennal contact; (2) sustained antennation; (3) avoidance or antennation followed by sudden flight; (4) opening of mandibles; (5) jerking; and (6) flexing of gaster or biting. For each worker, the most aggressive action (i.e. highest score) observed was recorded for the entire interaction (e.g. opening of mandibles followed by biting would be recorded as '6'). Only the actions of the worker were recorded; the queen was passive during the interaction, as queens of *Formica fusca* generally avoid aggressive interactions with workers (S. El-Shack, personal observation). Following the aggression assay, workers were individually placed in clean glass vials (Supelco, Z291706, Supelco Inc., Bellefonte, PA, USA) and frozen for chemical analysis.

Chemical analysis

We used workers from five of the original colonies to analyse the cuticular hydrocarbon patterns across all five treatments. We first analysed five adult workers per colony to establish the degree of among-colony variation in hydrocarbon profiles. Then, to study the effect of treatments, we analysed 2–5 new workers per treatment and colony. Cuticular hydrocarbons were extracted by immersing workers individually in 200-μL high-performance liquid chromatography-grade pentane (Sigma-Aldrich, Brøndby, Denmark) for 10 min. The solvent was then allowed to evaporate in a laminar flow cupboard. The extract was re-dissolved in 50 μL of pentane, of which 2 μL was injected in an Agilent 6890N gas-chromatograph (Agilent Technologies, Waldbronn, Germany), equipped with a HP-5MS capillary column (30 m $\times$ 250 μm $\times$ 0.25 μm), a *split-splitless* injector and a Flame Ionisation Detector. The carrier gas was helium at 1 mL min^{-1}. After an initial hold of 1 min at 70 °C, the temperature rose to 210 °C at a rate of 30 °C min^{-1}, then to 280 °C at 2 °C min^{-1} and then again at 30 °C min^{-1} to 320 °C, with a final hold of 2.5 min. The areas of forty-one compounds (or mixtures of compounds, because of co-elution) found on the cuticles of workers were integrated for further analysis (Fig. S1a). The compounds corresponding to each peak had previously been determined by gas-chromatography mass-spectrometry with a 5975 Agilent Technologies Mass Spectrometer, using 70eV electron impact ionization (Table S1).

Statistics

The behavioural responses of naïve workers were only tested against their own mother and an alien queen. Therefore, we used a paired *t*-test to test whether they were more aggressive towards the alien queen, than their

mother. Given the *a priori* H_0 hypothesis of no difference, and H_1 hypothesis that aggression is higher towards the alien queen we used a one-sided test. In the remaining cases, we used repeated measures ANOVA to allow for nonindependence between treatments, as fragments of the same colony were used in the different treatments. We ran separate analyses for each fostering regime (naïve, own queen, foster queen, own workers and foster workers). Separate analyses were unavoidable as one or two aggression assays were missing for a considerable fraction of the colonies, so that very few complete sets with all treatments and all different assays were available. In case where workers had been fostered with their own mother or nestmate workers both the 'foster' and the 'alien' queen were unfamiliar and alien. The two categories were none the less entered separately, as we could not rule out the possibility that recognition cues may have been transferred from the workers to the foster queen.

The repeated measures ANOVA was carried out on the average aggression scores per colony, the residuals of which, obtained with a General ANOVA with treatment and assay as main factors, were normally distributed (Wilk-Shapiro's $W = 0.98$, $n = 63$, $P = 0.28$). Each treatment was tested for differences in behaviour against the three queen types ('own', 'foster' and 'alien'), with queen type as the within-subjects factor, colony of origin as the subject factor, and the aggression score averaged for each colony as the dependent variable. We also tested each set of assays separately for differences between the fostering regimes, with fostering regime (mother/nest mate vs. alien foster queen/workers) as the within-subjects factor, colony of origin as the subject factor and the aggression score averaged for each colony as the dependent variable. *Post hoc* pairwise comparisons were performed using Tukey's method complemented with general contrasts to examine differences between single means against all other means. All calculations were performed using Statistix® 9.0 (Analytical Software, Tallahassee, FL, USA).

The chromatographic peak areas of hydrocarbons were log-normalized using the formula $z_j = \ln[x_j/g(x)]$, in which z_j is the normalized peak area of the jth hydrocarbon, x_j is the absolute peak area of the jth hydrocarbon, and $g(x)$ is the geometric mean of all peak areas (Aitchison, 1986). They were then further analysed using principal component analysis (PCA) and MANOVA in STATISTICA 7.1 (StatSoft Inc., Tulsa, OK, USA). Euclidean distances are calculated as the square root of the sum of squared differences between two samples.

Results

In general, workers fostered in the different treatments showed less aggression towards their mother than towards other queens, and their cuticular hydrocarbon profile, although affected by the fostering environment, retained some measure of colony integrity. In the Naïve treatment, workers were significantly more aggressive towards an alien queen than towards their mother (average scores: 2.51 ± 0.59 and 2.19 ± 0.46 respectively, mean ± SD; paired t-test, one-sided: $t = 1.89$, d.f. = 16, $P = 0.039$).

Comparisons within treatments

Workers reared with their mother (treatment 1) were significantly more aggressive towards unfamiliar queens (i.e. queens from the 'alien queen' and the 'foster queen' assays) than towards their mother (Fig. 1a; repeated measures ANOVA: $F_{2,25} = 3.97$, $P = 0.03$, general contrasts: own vs. alien and foster queen combined, Scheffe's $F_{25} = 3.8$, $P = 0.036$; to avoid pseudo-replication, we used the average aggression score per colony, rather than the scores of the individual ants). Aggression towards the foster queen for the paired fragment of each colony was intermediate between that towards the mother and the unfamiliar alien queen, and not significantly different from either, whereas aggression towards the unfamiliar alien queen was significantly higher than towards the mother queen (Tukey's *post hoc* test: $Q_{crit} = 3.52$, SE for comparison 0.16–0.17; critical value for comparison 0.40–0.42, and mean values 2.31, 2.25, and 1.87 for 'alien', 'foster' and 'own' queen respectively).

Workers reared with a foster queen (treatment 2) were significantly more aggressive towards the unfamiliar alien queen than towards either their own mother or their foster queen (Fig. 1b; repeated measures ANOVA: $F_{2,19} = 13.7$, $P = 0.0002$, general contrasts, unfamiliar alien queen vs. foster queen and own mother: Scheffe's $F = 13.22$, $P = 0.0003$). However, there was no significant difference in aggression towards the mother vs. the foster queen (Tukey's *post hoc* test: $Q_{crit} = 3.59$, SE for comparison 0.27–0.29; critical value for comparison 0.69–0.74, and mean values 3.14, 2.04, and 1.81 for 'alien', 'foster' and 'own' queen respectively).

Workers reared with workers from their own colony (treatment 3) were significantly less aggressive towards their mother than towards either the alien or the foster queen (Fig. 1c; repeated measures ANOVA: $F_{2,14} = 4.70$, $P = 0.03$, general contrasts ('own' vs. 'alien' and 'foster' queen combined: Scheffe's $F = 4.67$, $P = 0.03$; to avoid pseudo-replication, we used the average aggression score per colony, rather than the scores of the individual ants). Aggression towards both the alien and the foster queens was significantly higher than that towards the mother (Tukey's *post hoc* test: $Q_{crit} = 3.70$, SE for comparison 0.25–0.28; critical value for comparison 0.66–0.73, and mean values 2.59, 2.54, and 1.81 for 'alien', 'foster' and 'own' queen respectively).

When reared with foster workers (treatment 4), aggression towards the mother queen was on average lower than towards either alien or foster queens, but the level of aggression did not differ significantly among treatments (Fig. 1d; repeated measures ANOVA,

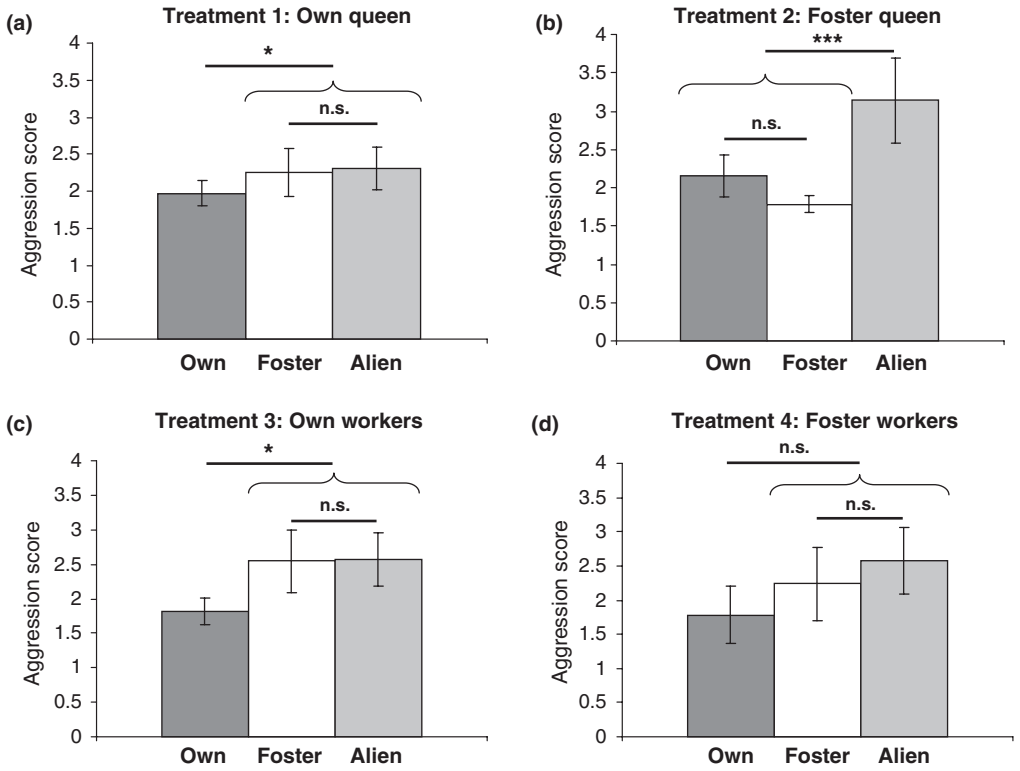

Fig. 1 Average aggression level towards queens, displayed by workers reared with (a) their own queen, (b) a foster queen, (c) workers of their own colony and (d) foster workers. Aggression level towards three types of queens are shown: their mother (Own), the foster queen (Foster) and an unfamiliar alien queen (Alien). In the case of treatment 1 and 3, the Foster came from the foster queen treatment of their sisters (treatment 2). Repeated measures ANOVA was carried out on the average aggression scores per colony. Mean and 95% confidence intervals are reported for the full set of replicates within each treatment.

$F_{2,12} = 1.11$, $P = 0.36$; mean values 2.57, 2.24, and 1.94 for 'alien', 'foster' and 'own' queen respectively).

Comparison between treatments

Workers reared with their mother (treatment 1) were significantly less aggressive towards alien queens than those reared with a foster queen (treatment 2; repeated measures ANOVA: $F_{1,11} = 7.94$, $P = 0.02$), but were more aggressive against the foster queen, compared with those that had been reared with it (repeated measures ANOVA: $F_{1,12} = 7.49$, $P = 0.02$). No significant differences in aggression towards the mother queen were found between workers from the two rearing regimes (repeated measures ANOVA: $F_{1,13} = 0.69$, $P = 0.43$). No significant differences were found between the corresponding treatments in the worker-fostering regimes (repeated measures ANOVA: $F_{1,6–8} = 0.00–1.73$, $P = 0.94–0.23$).

Cuticular hydrocarbons

The cuticular profile of *F. fusca* comprises over 40 hydrocarbons, including both linear and methyl-branched alkanes (Fig. S1a and Table S1). As expected, workers from field colonies showed colony-specific hydrocarbon patterns (Fig. S1b). However, the cross-fostered workers also expressed their colony-specific odours despite being fostered with hetero-colonial workers or queens. PCA on all five treatments (seven PC with an eigenvalue > 1) explained 84.36% of the total variation, and showed a significant effect of 'colony' (two-way MANOVA, Wilks' $\lambda = 0.0002$, $F_{28,289.87} = 96.90$, $P < 0.0001$). Nonetheless, regardless of colony origin, the chemical profile of workers was also influenced by the foster environment, as shown by a significant effect of 'treatment' (Wilks' $\lambda = 0.0032$, $F_{28,289.87} = 40.54$, $P < 0.0001$; Fig. S2 and Table S2), although this effect was mostly because of the Naïve treatment. The interaction between 'colony' and 'treatment' was also significant (Wilks' $\lambda = 0.0003$, $F_{112,526.88} = 11.33$, $P < 0.0001$), which shows that the hydrocarbon profiles of workers responded differently under different treatments depending on their colony of origin. This can be explained by the fact that each pair of colonies was unique and thus that the adult workers or queen in the foster treatments influenced the hydrocarbon profiles of the callow workers in a different way.

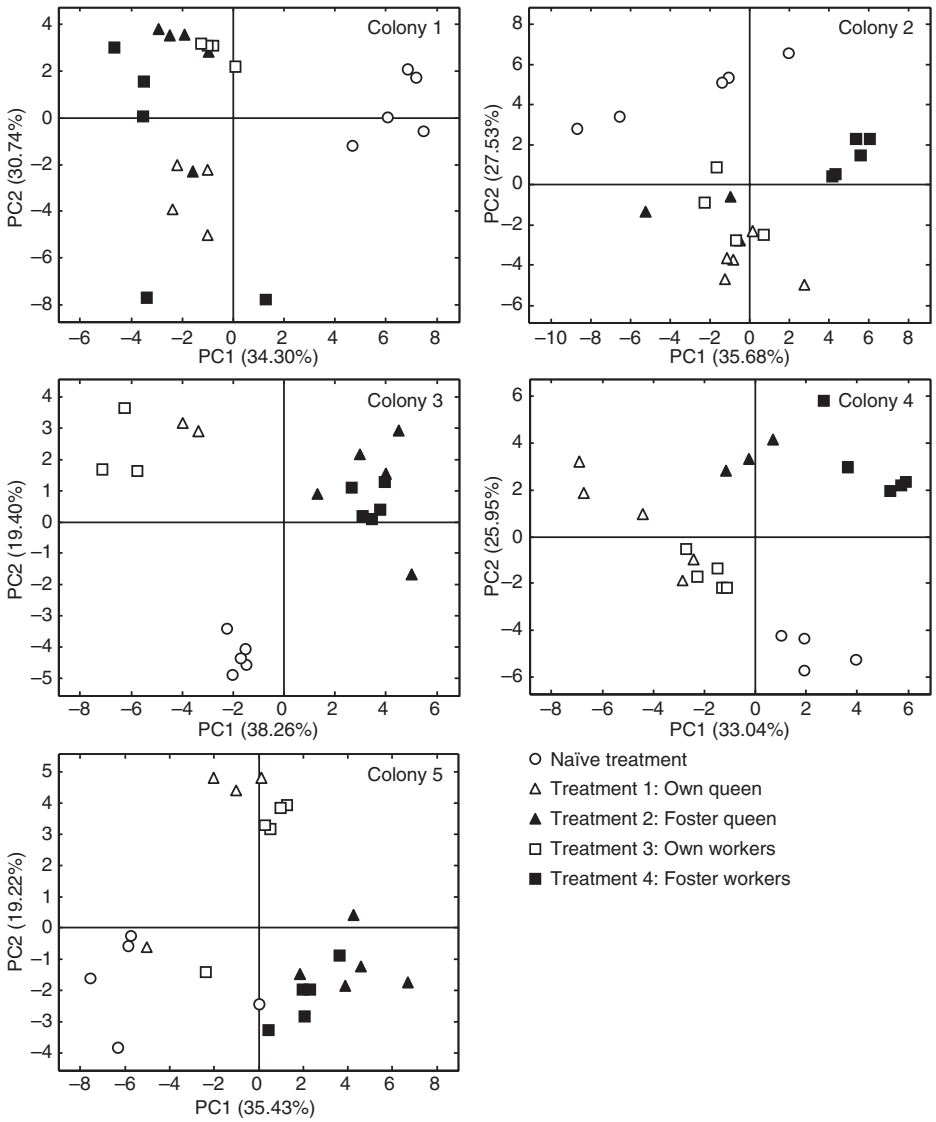

Fig. 2 Principal component analysis per colony, based on the cuticular hydrocarbon profiles of young *Formica fusca* workers, showing the effect of treatments (naïve treatment and treatments 1–4) among the five colonies.

When colonies were analysed separately (Fig. 2), the largest difference was between the Naïve treatment and all other treatments. The next largest variation was between Own and Foster treatments. In a formal analysis based on Euclidean distances between the respective group centroids, the pairwise distances between Naïve and Own was not significantly different from that between Naïve and Foster treatments (Fig. 3a; one-way ANOVA, $F_{3,16} = 1.16$, $P = 0.355$). This result, however, may be confounded by compounds that are not used in recognition. When only the peaks that have been suggested to be involved in nestmate recognition, i.e. methyl-branched alkanes (e.g. Dani *et al.*, 2001 showed that methyl-branched alkanes are more important than linear alkanes, Martin *et al.*, 2008 suggested x,y-di-MeC25s as important for *F. fusca*) were selected, the distance between naïve workers and workers in own treatments was indeed significantly shorter than that between naïve workers and workers in the foster treatments (Fig. 3b; one-way ANOVA, $F_{3,16} = 4.07$, $P = 0.025$; planned contrasts, Own vs. Foster treatments, $F_{1,16} = 9.69$, $P = 0.007$). Hence, naïve workers synthesized these latter compounds in a ratio that is similar to that of adult workers of their own colony, whereas this did not hold for the hydrocarbon profile as a whole.

Discussion

Our behavioural results show that workers of *F. fusca* are able to recognize their own mother despite being raised

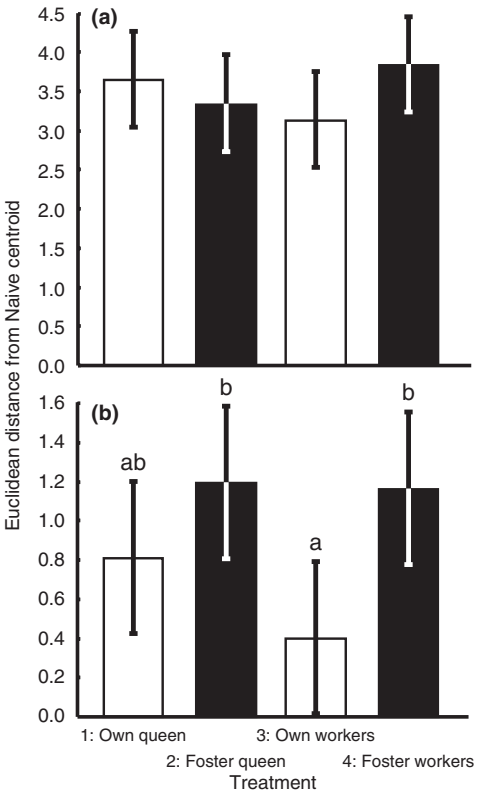

Fig. 3 Average chemical distance from naïve workers depending on treatment, based on (a) all hydrocarbons and (b) x,y-dimethyl C_{25} hydrocarbons. Chemical distances are calculated as the Euclidean distance between the centroid of workers in the naïve treatment and that of workers in the respective treatment (treatments 1–4). Whiskers depict 95% confidence limits; significant differences at $P < 0.05$ are depicted by different lower case letters.

with a foster queen. Indeed, regardless of foster environment, workers always treated their mother with less aggression than an unfamiliar and unrelated queen, and never treated their mother with more aggression than a foster queen. Furthermore, naïve workers, void of any contact with adult queens or workers, were more aggressive towards hetero-colonial queens than towards their mother. On the other hand, the reduced aggression of workers towards their foster queen compared with an unfamiliar alien queen suggests that familiarity can be as important as kinship. In agreement with the behavioural results, chemical analyses showed that workers retain some colony integrity with respect to their cuticular hydrocarbon profile regardless of whether they were fostered with their own mother, a hetero-colonial foster queen, or workers. When examined by colony, however, individuals also segregated according to treatment in all cases, which shows that workers both express (synthesize) their own hydrocarbon profile and acquire cuticular chemicals from their nestmates.

The observed recognition patterns are consistent with several modes of development of recognition, including a genetically determined recognition system, maternal effects, pre-imaginal learning and/or learning during adulthood. Of these, only genetic or maternal effects would generate a recognition system robust enough to allow true kin recognition *sensu* Grafen (1990), e.g. within-colony discrimination among matrilines in multi-queen colonies. Learned recognition cues, either at the pre-imaginal stage (Isingrini *et al.*, 1985), or during early adulthood (Errard, 1986) would generate a recognition system contingent on the identity of the fostering individuals. The cues thus learned could subsequently be used as a reference (phenotype matching) for discriminating against hetero-colonial intruders.

The naïve workers expressed a distinct hydrocarbon profile, but given that they were in contact with their full sisters during 3 days after eclosion, these sisters may have served as a source for recognition cues for their mothers. Thus, the naïve workers may have used their own (self-referent) or their sisters' hydrocarbon profiles for comparison when assessing kinship. However, the workers raised with a foster queen or foster workers were exposed to recognition cues from unrelated individuals, yet they were no more aggressive towards their mother than the foster queen. Conversely, they were significantly more aggressive towards unfamiliar unrelated queens. This, in conjunction with the retention of colony integrity in their cuticular chemistry, strongly suggests the presence of genetically or maternally determined recognition cues, in addition to learned ones. At present we cannot, however, rule out the possibility that cue acquisition occurred during the larval stage.

Naïve workers synthesized colony-specific hydrocarbon profiles but they were quite distinct from all other treatments and clustered equally far from treatments involving their nestmates vs. non-nestmates (Fig. 2). This indicates the presence of qualitative differences in the chemical profile, which may reflect changes during maturation. Naïve workers were tested after only 3 days in isolation, compared with 9–12 days for workers in the other treatments. The lack of a colony-specific pattern in the cuticular profiles, when based on all detectable cuticular hydrocarbons (Fig. 3a), could be as a result of the fact that some compounds are not used in nestmate recognition. When only those hydrocarbons suggested to act as nestmate recognition cues in this species were considered (Martin *et al.*, 2008), the chemical profile of naïve workers was significantly closer to that of workers raised with sisters than to that of workers in either of the foster treatments (Fig. 3b). For workers fostered with their mother, the trend was similar but not significant, perhaps indicating that the acquisition of colony odour labels is weaker when interacting with a single individual (the queen). This could also explain why workers in the

behavioural assays were significantly less aggressive towards their mother than towards an unfamiliar alien queen in all treatments, except when reared with foster workers. In the latter case, the pattern was identical, but differences in aggression were not significant owing to greater variation in aggression scores and lower power of the test. This suggests that cues acquired from workers were present but involved more noise, which may be as a result of a greater diversity of cues presented by workers than by a single queen, especially if the foster workers stem from several matrilines, as may be the case here.

In conclusion, our results show that recognition can be contingent on both genetically determined cues and prior exposure to prospective group members. The recognition cues of *F. fusca* are flexible, given that their hydrocarbon profile was modified by interaction with both workers and queens. This flexibility in recognition cues may have added complexity to recognition cues, rendering discrimination more difficult, as indicated by the relatively small differences in aggression between treatments. The integration of cues from multiple sources allows the recognition system in young workers to be modulated according to contextual information, perhaps including factors not examined in this study, such as fertility signals of queens (Hannonen & Sundström, 2002; Hannonen *et al.*, 2002). The exact interaction between familiarity and genetic recognition will have to be studied in a setting with multiple queens at the same time.

Workers recognize and treat foster queens as group members likely because of the learning of odour cues during early adulthood. Learned recognition cues have been shown to underpin the ability of offspring to recognize their parents, for example in fallow deer (Torriani *et al.*, 2006) and long-tailed tits (Sharp *et al.*, 2005), as well as recognition of siblings in lizards (O'Connor & Shine, 2006). More importantly, our study is among the first to demonstrate recognition of kin in the absence of learning opportunities during adulthood, as worker ants recognized their mother even without prior exposure during adult life. However, pre-imaginal learning (Isingrini *et al.*, 1985) cannot be excluded, although this is still a controversial phenomenon (Fénéron & Jaisson, 1995). To date only one recent cross-fostering study has demonstrated the presence of learning and genetic cues in parallel in sibling recognition in Belding's ground squirrel (Mateo, 2009).

If recognition in *Formica fusca* indeed includes a genetic component to recognition cues, the question arises how enough diversity in recognition cues is maintained. Precise kin recognition is thought to be associated with high variability in cues and should lead to social disruption via nepotism (Keller, 1997), and selection at the colony level tends to erode genetic variation for recognition cues (Ratnieks, 1991; Boomsma *et al.*, 2003; Rousset & Roze, 2007). However, Rousset & Roze (2007) also showed that under some conditions, if there is a strong enough advantage for rare variants of genetically determined odour cues relative to the fitness costs of nepotism, genetic recognition can prevail. Indeed, the diversity of chemical compounds is exceptionally high in *F. fusca* (S. Martin & H. Helanterä, personal communication), perhaps indicating independent fitness benefits of high genetic odour cue diversity. Whether the recognition capacity of *F. fusca* workers, is precise enough to allow discrimination among queens within the same colony remains, however, to be tested.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 The cuticular hydrocarbon profile of *Formica fusca* and natural variation among colonies.

Figure S2 The interaction between colony of origin and treatment in their effect on cuticular hydrocarbon profiles of *Formica fusca*.
Table S1 Identity of cuticular hydrocarbons found on the cuticles of workers of *Formica fusca*.
Table S2 The effect of treatments on young worker hydrocarbon profiles.

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