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Blending in with the crowd: social parasites integrate into their host colonies using a flexible chemical signature

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Social parasites are able to exploit their host's communication code and achieve social integration. For colony foundation, a newly mated slave-making ant queen must usurp a host colony. The parasite's brood is cared for by the hosts and newly eclosed slave-making workers integrate to form a mixed ant colony. To elucidate the social integration strategy of the slave-making workers, *Polyergus rufescens*, behavioural and chemical analyses were carried out. Cocoons of *P. rufescens* were introduced into subcolonies of four potential host species: *Formica* subgenus *Serviformica* (*Formica cunicularia* and *F. rufibarbis,* usual host species; *F. gagates*, rare host; *F. selysi*, non-natural host). Slave-making broods were cared for and newly emerged workers showed several social interactions with adult *Formica*. We recorded the occurrence of abdominal trophallaxis, in which *P. rufescens*, the parasite, was the donor. Social integration of *P. rufescens* workers into host colonies appears to rely on the ability of the parasite to modify its cuticular hydrocarbon profile to match that of the rearing species. To study the specific *P. rufescens* chemical profile, newly emerged callows were reared in isolation from the mother colony (without any contact with adult ants). The isolated *P. rufescens* workers exhibited a chemical profile closely matching that of the primary host species, indicating the occurrence of local host adaptation in the slave-maker population. However, the high flexibility in the ontogeny of the parasite's chemical signature could allow for host switching.

Keywords: social parasitism; chemical integration; mimicry; host specificity; *Polyergus rufescens*

1. INTRODUCTION

Parasites are believed to be one of the strongest forces driving evolution (Schmid-Hempel 1998) and social parasitism—the coexistence in the same nest of two species of social insects, one of which is parasitically dependent on the other—represents a unique model system for studying a number of fundamental problems in evolutionary biology (D'Ettorre & Heinze 2001). Obligate social parasites exploit entire societies from the first step of their life cycle. They are not able to found a colony independently, but must enter a colony of a host species. Hence, effective mechanisms to overcome the ability of hosts to discriminate nestmates should have been selected for. A diversity of social integration strategies have been described, which vary with the parasite life history. In particular, newly mated females of social parasitic inquilines gain acceptance from entire host colonies, coexisting with the resident queen(s) and producing only sexual offspring (Buschinger & Klump 1988). By contrast, young queens of temporary parasitic ants (which need the host only for colony foundation) and almost all slave-making ants eliminate and replace the host queen(s) with a colony foundation by usurpation (Buschinger 1986; Hölldobler & Wilson 1990). In the case of an aggressive usurpation, almost all of the adult workers are driven out of the host nest and only the host brood is kept. Once the host workers eclose, they imprint on the parasite's odour and

become its worker force (Stuart 1988; Jaisson 1991). Other usurping slave-making queens succeed in being adopted by the adult host workers present in the nest. This is the case with *Polyergus* newly mated queens (Topoff *et al.* 1988; Mori *et al.* 1995).

Overcoming the nestmate discrimination code, thus reducing the consequent aggression by the host, is a key element for entering a host colony. By exploiting the mechanisms underlying nestmate recognition, social parasites can socially integrate. Recognition cues are predominantly chemicals and members of the same nest share a common 'colony odour' (the gestalt odour (Crozier & Dix 1979)). Cuticular hydrocarbons are thought to play a crucial role in recognition (reviewed by Singer 1998; Vander Meer & Morel 1998; Lenoir *et al.* 1999). There is evidence for chemical mimicry as a social integration strategy in several social parasites (reviewed by Lenoir *et al.* 2001*a*). Several studies of the slave-making ants *Polyergus* have attempted to elucidate the strategy of social integration into the host colony. The Japanese species *P. samurai* shows the same cuticular hydrocarbon pattern as the host present in the colony (*Formica japonica* or *F. hayashi*) (Yamaoka 1990). Chemical similarity was found in established colonies of *P. breviceps* and the *Formica* hosts (Howard & Akre 1995). According to Habersetzer & Bonavita-Cougourdan (1993) and Bonavita-Cougourdan *et al.* (1996, 1997), the cuticular hydrocarbon patterns of *P. rufescens* in mature mixed colonies seem not to correspond entirely to that of its *Formica* host, but the parasite modifies the relative amounts of hydrocarbons to match the host's profile.

Behavioural and chemical studies have clarified the

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mechanisms underlying a successful takeover of the host nest by the slave-making queen. After mating (e.g. Mori *et al.* 1994*a*), the *Polyergus* queen finds and penetrates a *Formica* nest and eliminates the host queen in order to obtain the care of the host mature workers by forming permanent social bonds (Emery 1911; Forel 1920; Zaayer 1967; Topoff *et al.* 1988; Mori *et al.* 1995). *Polyergus rufescens* newly mated queens break their host's nestmate recognition code and succeed in being adopted by the host workers. It has been shown that *P. rufescens* queens rely on a double chemical strategy: the secretion of a repellent to avoid aggressive interactions with the host workers (D'Ettorre *et al.* (2000) and references therein for an additional interpretation) and chemical neutrality ('chemical insignificance') to elude the host recognition system and easily acquire the host queen pattern, probably by chemical camouflage (D'Ettorre & Errard 1998; Lenoir *et al.* 2001*a*). Similar results, supporting chemical mimicry, were recently found in the American species *P. breviceps* (Johnson *et al.* 2001). The newly mated *P. breviceps* queen has only few peaks on the cuticle (*n*-alkanes) before usurpation. Profiles of *P. breviceps* queens that had attacked a *Formica* queen were virtually identical to the queen profile of the species killed.

Once the parasitic queen is accepted and cared for, she starts to lay eggs and her brood must be reared by the slaves. Several studies have investigated the mechanisms underlying the acceptance of *Polyergus* cocoons by the hosts (Zimmerli & Mori 1993; Mori *et al.* 1996; Johnson 2000), but newly emerged workers have received little attention. How could slave-making callow workers socially integrate to form a mixed colony? We investigated the behavioural and chemical strategies for social integration employed by callow workers of *P. rufescens* by inducing adoption of cocoons in four different *Formica* (*Serviformica*) species: *F. cunicularia* and *F. rufibarbis* (usual hosts), *F. gagates* (rare host) and *F. selysi* (not reported as a host, but living in biotopes where *P. rufescens* is absent). We also compared the chemical profile of *P. rufescens* workers reared by these different *Formica* species with that of *P. rufescens* workers soon after emergence from their cocoon and artificially reared without any contact with adult workers.

2. METHODS

(a) *Animals and housing*

Polyergus rufescens broods, workers and host workers were collected in the summer of 1999 from natural mixed colonies (*P. rufescens*/*F. cunicularia*) situated in an open field (Tours, France). Workers of *F. cunicularia*, *F. rufibarbis* and *F. gagates* were collected in the spring and summer of 1999 from free-living colonies coming from the same environment. Workers of *F. selysi* stemmed from free-living colonies located in the French Alps (Morillon, altitude 800 m).

Ants were housed in the laboratory in artificial nests. Subcolonies were removed from the mother colonies and placed in plaster nests (18 cm × 12 cm × 5 cm) with two chambers, regularly moistened and kept in the dark. This nest was connected to a circular Fluon (Whitford GmbH)-lined plastic box (diameter 10 cm) representing the foraging arena, and kept under the natural photoperiod cycle. Colonies were maintained under con-

trolled conditions (25 °C, 45% humidity, natural photoperiod) and fed with honey and meal worms three times a week.

(b) *Behavioural experiments*
(i) *Callow workers*

In the laboratory, *P. rufescens* callow workers were removed from the mother colony within 1 h following their emergence from the cocoon. Some ($n = 12$) were immediately killed by freezing for chemical analysis. The remaining callows ($n = 20$) were separated from the mother colony and kept together in glass tubes (18 cm × 1.5 cm) fitted at one end with a water container. In this way, we avoided the effect of individual social isolation on hydrocarbon dynamics (Boulay *et al.* 2000; Lenoir *et al.* 2001*b*). Many callows died, as slave-makers need the care of host workers, but eight of them survived until the age of five days, when they were killed by freezing for chemical analysis (hereafter named 'isolated *P. rufescens*').

(ii) *Induced adoption*

Subcolonies of each *Formica* species and of the natural mixed nest ($n = 15$, three replicates for each species), consisting of 50 workers each, were housed as described above. After three days of acclimatization, cocoons ($n = 10$ for each subcolony) of *P. rufescens* from the original mother colony were introduced in each *Formica* subcolony and in the natural mixed nest fragments (control) (see table 1). The rate of acceptance of the cocoons was recorded. As soon as the first *P. rufescens* workers started to emerge in the rearing *Formica* colonies, the behaviour of the ants was videotaped (two sessions of 30 min day^{-1}) until the slave-making workers reached the age of five days, when they were killed by freezing for chemical analyses. Five *Formica* workers from the rearing subcolony were also killed by freezing for chemical analyses.

Behavioural analysis quantified the frequency and the duration of peaceful social interactions (antennal contact, allogrooming and trophallaxis) and of possible aggressive interactions (threat, biting, biting with gaster-flexing) between the *P. rufescens* callows and the rearing *Formica* workers. Data were analysed using Pearson's chi-square and Kruskal–Wallis analysis of variance (using S<small>TATISTICA</small> v. 5.1 for Windows, Statsoft, Inc., Tulsa, OK, USA).

(c) *Chemical analyses*

The cuticular compounds of whole ants killed by freezing were extracted by immersing each in 500 µl of pentane for 10 min. An internal standard (n-C$_{15}$) was added to each extract. Following drying under nitrogen, extracts were redissolved in 40 µl of pentane and 1 µl of this solution was analysed on a Varian 3300 gas chromatograph, with a flame ionization detector, equipped with a capillary column (Supelco, 30 m × 0.25 mm). The injector was the 'on-column' type, the carrying gas helium at a pressure of 4 bars and the temperature was programmed to rise from 80 to 160 °C at 10 °C min^{-1} and from 160 to 280 °C at 3 °C min^{-1}. The chromatograph was coupled to a Delsi Enica 10 computer to record data.

To determine or verify the identity of cuticular components, extracts were analysed by combined gas chromatography/mass spectrometry (GC–MS). Analyses were carried out on a benchtop Perkin-Elmer Turbomass system with a split-splitless injector and a fused-silica capillary column (25 m × 0.32 mm) with a 0.5 µm polydimethylsiloxane coating. The carrier gas was helium (99.99%) and the column temperature programme was 5 °C min^{-1} from 70 to 250 °C. The injection port temperature

Table 1. Response of the different *Formica* subcolonies to *Polyergus rufescens* cocoons and callow workers.

rearing subcolony	number of nests	total number of cocoons introduced	acceptance of cocoons (%)	survival of callow workers (%)
Formica cunicularia	3	30	40	83
Formica rufibarbis	3	30	40	100
Formica selysi	3	30	47	79
Formica gagates	3	30	20	67
natural nest (*Formica cunicularia/Polyergus rufescens*)	3	30	90	100

was 200 °C. Total ion chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV. The transfer line and the source temperature were maintained at 150 °C. Compound identifications were based on retention times and comparison with published data (Bagnères *et al.* 1991; Hefetz *et al.* 1992; Doolittle *et al.* 1995; Bonavita-Cougourdan *et al.* 1996) or with spectra of available authentic compounds.

The hydrocarbon profiles of *P. rufescens* callows separated from adult workers and those reared in different *Formica* subcolonies, as well as the hydrocarbon profiles of the *Formica* rearing workers, were compared. For each cuticular profile, the relative value of each identified peak with respect to the total was calculated and expressed as a percentage. We used a multivariate statistical analysis (using STATISTICA v. 5.1 for Windows) to estimate the similarity (or the divergence) of the chemical profiles of the different subcolonies. This statistical analysis was performed on 43 peaks and 52 workers who were extracted from the subcolonies: *P. rufescens* ($n = 6$) reared by *F. selysi* and *F. selysi* ($n = 4$); *P. rufescens* ($n = 8$) reared by *F. cunicularia* and *F. cunicularia* ($n = 7$); *P. rufescens* ($n = 5$) reared by *F. rufibarbis* and *F. rufibarbis* ($n = 5$); *P. rufescens* ($n = 5$) reared by *F. gagates* and *F. gagates* ($n = 7$); *P. rufescens* ($n = 5$) reared in isolation from adult workers. Peaks were first analysed with principal component analysis (PCA) to reduce the number of variables subsequently used in a discriminant analysis (DA). The standardized discriminant function coefficient and the factor loading (more than 0.7) were used to assess the importance of individual compounds. DA was performed to determine whether predefined groups could be discriminated on the basis of their chemical profiles and to assess the degree of similarity among groups. The correct classification of individuals to the respective groups was verified and groups were also compared by calculating the squared Mahalanobis distances between the group centroids (the larger the differences, the further the respective groups are apart from each other).

3. RESULTS

(a) *Behaviour*

Overall, the slave-making brood was accepted by all of the *Formica* species. *Polyergus rufescens* cocoons and callows were cared for in each *Formica* subcolony (table 1). The acceptance rate of *P. rufescens* cocoons in the rearing *Formica* subcolonies was significantly lower compared with the acceptance in the natural mixed colony ($\chi^2 = 10.51$, $p = 0.033$, d.f. = 4), but there was no significant difference in the acceptance rate among the different rearing *Formica* species ($\chi^2 = 2.58$, $p = 0.46$, d.f. = 3). Aggressive interactions were never observed. Some *P. rufescens* callows were found dead in rearing subcolonies

of *F. cunicularia*, *F. selysi* and *F. gagates* (table 1), but the survival rate of *P. rufescens* callows after adoption in the various *Formica* subcolonies was not statistically different ($\chi^2 = 0.58$, $p = 0.96$, d.f. = 4).

Soon after eclosion, *P. rufescens* callows interacted actively with *Formica* adult workers. We observed several instances of antennal contact, allogrooming and oral trophallaxis (during which *Formica* workers were the donors). Even if the amount of time spent by *P. rufescens* callows in each type of social interaction seems to vary with the rearing species (table 2), there is no significant difference in the durations of social interactions of callows with adult workers of the different *Formica* species ($H = 4.96$, $p = 0.29$ for antennal contact; $H = 2.13$, $p = 0.71$ for trophallaxis; $H = 5.36$, $p = 0.25$, for allogrooming; Kruskal–Wallis analysis of variance, d.f. = 4). For the first time in a Formicinae species, to our knowledge, we observed three instances of abdominal trophallaxis (passage of fluid from the abdominal tip of one individual to the mouthparts of another) in which the *P. rufescens* callows were the donors. The abdominal secretion of slave-making workers appeared to be very attractive to *Formica* workers. They quickly picked up the droplet from the *P. rufescens* abdomen and shared it.

(b) *Chemical analyses*

Polyergus rufescens callow workers lack a veritable chemical cuticular signature at emergence. They possess only traces of few compounds (individual total amount (mean ± s.d.): 10.03 ± 1.08 ng, ranging from 0.8 to 2.7 ng per compound). Typically, these are linear alkanes (n-C$_{23}$, n-C$_{25}$, n-C$_{27}$), alkenes (C$_{23:1}$, C$_{25:1}$, C$_{27:1}$) and methyl branched alkanes (11- +13-meC$_{25}$, 3-meC$_{25}$, 11- +13- +15-meC$_{27}$, 7-meC$_{27}$). The presence of very low amounts of hydrocarbons on the cuticle is also typical of *Formica* freshly emerged workers (Lenoir *et al.* 2001*a*). Preliminary analyses (results not shown) demonstrated that five-day-old *F. cunicularia* callows reared in isolation develop a cuticular profile qualitatively matching that of the mother colony.

Isolated *P. rufescens* workers, separated from the mother colony soon after their emergence from the cocoon (without any contact with adult workers), exhibit a more complex chemical cuticular signature at the age of five days, which is characterized by 31 products. The total amount of hydrocarbons detected per individual was 780 ± 94 ng. When adopted by *Formica* workers, five-day-old *P. rufescens* workers exhibited a composite chemical profile. The *P. rufescens* workers reared by rearing *Formica* subcolonies came from the same mother colony, so that

Table 2. Amount of time spent by *Polyergus rufescens* callows in each type of social interaction in the different rearing subcolonies, as a proportion of the total interaction time.

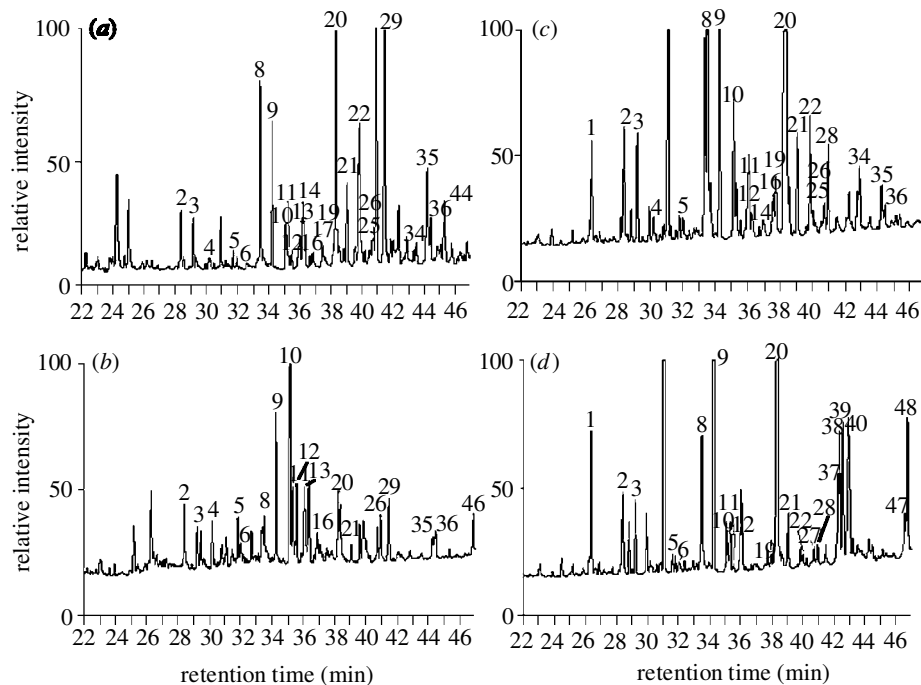| rearing colony	antennal contact (%)	trophallaxis (%)	allogrooming (%)	aggression
Formica cunicularia	16.8	26.0	57.2	0
Formica rufibarbis	56.7	12.3	31.1	0
Formica gagates	10.2	7.8	81.9	0
Formica selysi	37.6	24.2	38.2	0
natural nest	65.6	14.4	20.00	0

Figure 1. Cuticular hydrocarbon profiles of *Polyergus rufescens* reared by (*a*) *Formica cunicularia*, (*b*) *F. gagates*, (*c*) *F. rufibarbis* and (*d*) *F. selysi*. ((1) $C_{22:1}$; (2) $C_{23:1}$; (3) n-C_{23}; (4) 11- +9-meC_{23}; (5) n-C_{24}; (6) 10- +9-meC_{24}; (7) $C_{25:1}$; (8) $C_{25:1}$; (9) n-C_{25}; (10) 13- +11-meC_{25}; (11) 7-meC_{25}; (12) 5-meC_{25}; (13) 3-meC_{25}; (14) $C_{26:1}$; (16) n-C_{26}; (17) 10-meC_{26}; (18) x,y-dimeC_{26}; (19) $C_{27:1}$; (20) $C_{27:1}$; (21) n-C_{27}; (22) 11- +13-meC_{27}; (25) 7-meC_{27}; (26) 3-meC_{27}; (27) 5,21-dimeC_{27}; (28) $C_{28:1}$; (29) 10-meC_{28}; (30) 8,12-dimeC_{28}; (31) 9,21-dime$C_{29:3}$; (32) 9,21-dime$C_{29:3}$; (33) 9-me$C_{29:1}$; (34) n-C_{29}; (35) 15- +13- +11-meC_{29}; (36) 7-meC_{29}; (37) 9,19-dime$C_{31:1}$; (38) 9,21-dime$C_{31:3}$; (39) 9,23-dime$C_{31:3}$; (40) 9-me$C_{31:1}$; (41) 5-meC_{29}; (42) 11,15-dimeC_{29}; (43) 3-meC_{29}; (44) 5,x-dimeC_{29}; (45) n-C_{30}; (46) 15- +13- +11-meC_{31}; (47) 11,x-dimeC_{31}; and (48) 5,x-dimeC_{31}. x, y, unknown position of the methyl group.)

all the young slave-making workers were sisters, but they developed a different cuticular signature when reared by different *Formica* species (figure 1*a–d*). In the presence of *F. cunicularia*, *P. rufescens* workers had a total amount of 1430 ± 98 ng hydrocarbons. *P. rufescens* callows reared by *F. rufibarbis* had a total of 1054 ± 105 ng, whereas those reared by *F. gagates* had 1366 ± 260 ng of hydrocarbons on the cuticle. When *F. selysi* was the rearing species, the profile of *P. rufescens* had 1131 ± 449 ng of hydrocarbons. It appears that *P. rufescens* workers reared by *Formica* species possess a greater amount of hydrocarbons than when reared in isolation ($H = 12.19$, $p = 0.016$; Kruskal–Wallis analysis of variance, d.f. = 4).

To compare the chemical profiles, a PCA was performed on 43 compounds. This produced eight principal components with eigenvalues of more than 1, explaining 90.7% of the total variance. The DA performed on 18 variables (factor-loading more than 0.7) significantly dif-

ferentiated the chemical profiles of *P. rufescens* reared in different social environments (Wilks' $\lambda = 0.000\,01$, $F_{72,29} = 78.73$, $p < 0.000\,01$). All *P. rufescens* young workers were 100% correctly assigned to their group (four groups reared by different *Formica* species and one group of callows isolated from adult workers). On the basis of their proportions of cuticular compounds, *P. rufescens* workers reared by *F. selysi* were clearly separated from all of the other groups by function 1, which accounted for the 95.7% of the total variance. All of the other groups were discriminated by the function 2 (3.6% of the original variance). The group of *P. rufescens* that was isolated was closer to the group of *P. rufescens* reared by *F. cunicularia* than to any other group, showing the smallest Mahalanobis *F*-ratio for distances ($F = 10.9$, $p = 0.0017$).

Because the profile of *P. rufescens* appeared to change depending on the rearing species, we performed an additional analysis to determine whether their chemical
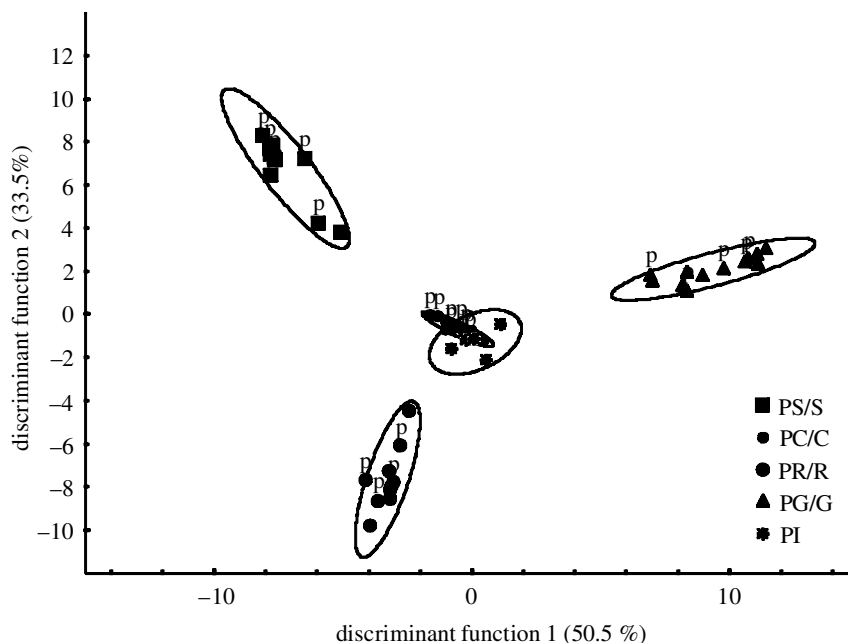
Figure 2. DA of *Polyergus rufescens* in different rearing conditions and their rearing *Formica* workers, on the basis of proportions of cuticular compounds. In parenthesis the percentage of variance is explained by each of the two main functions. (PS/S, *Polyergus rufescens* reared by *Formica selysi* and *F. selysi*; PC/C, *P. rufescens* reared by *F. cunicularia* and *F. cunicularia*; PR/R, *P. rufescens* reared by *F. rufibarbis* and *F. rufibarbis*; PG/G, *P. rufescens* reared by *F. gagates* and *F. gagates*; and PI, *P. rufescens* reared in isolation from adult workers.) p indicates *P. rufescens* in the different rearing groups.

profile matches those of the rearing workers. A PCA performed on 43 variables produced nine principal components with eigenvalues of more than 1, explaining the 86.5 % of the total variance. The DA was performed by assigning *P. rufescens* workers and their respective rearing *Formica* species to the same group and *P. rufescens* isolated to a separate group (15 variables with factor-loading more than 0.7), Wilks' $\lambda = 0.000\,061$, $F_{60,131} = 24.04$, $p < 0.0001$). The analysis confirmed that these five groups were 100% correctly classified, functions 1 and 2 explaining together 84% of the variance (figure 2). This demonstrates that the cuticular profiles of the slave-making workers were quantitatively similar to those of their rearing species and divergent from the others. Interestingly, the *P. rufescens* workers separated from the mother colony at emergence and reared without any contact with adult workers, exhibited a cuticular profile similar to that of *F. cunicularia* workers (the host species present in the natural nest). The squared Mahalanobis distances between the group centroids revealed that the profile of *P. rufescens* reared without any host species does not significantly differ from that of *P. rufescens* reared by *F. cunicularia* ($F = 1.32$, $p = 0.2$). It is of critical importance to note that the parasite develops the chemical signature of the primary host species in the absence of social interactions. All of the other distances between groups were statistically significant and it is worth pointing out that the groups of *P. rufecens* with *F. cunicularia* and *F. rufibarbis*, the usual host species, are closer to each other in comparison with the groups of *F. gagates* and *F. selysi*, non-usual hosts (figure 3).

4. DISCUSSION

Social parasites have evolved strategies to overcome the nestmate recognition code of their hosts and to allow them
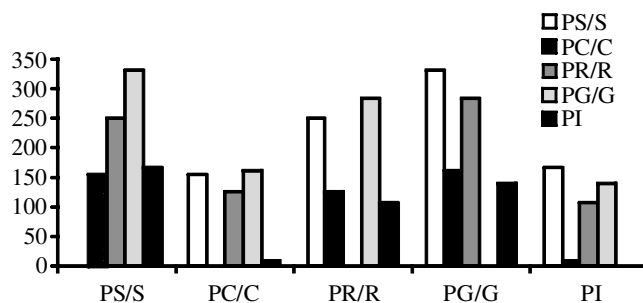

Figure 3. Squared Mahalanobis distances between the group centroids (referring to DA of figure 2; legend as in figure 2).

to penetrate a host colony and to achieve social integration. Several studies have shown that social parasites rely on chemical mimicry to gain acceptance into the host colony (reviewed by Lenoir *et al.* 2001*a*). Our results provide what we believe to be new evidence for the ontogeny of the chemical profile and the mechanisms underlying social integration in parasitic ants. It appears that the slave-making ant *P. rufescens* has not only evolved to match most closely the chemical profile of its most important host ant species but it has also evolved the ability to modify its profile should it penetrate any other host species. In previous studies, *P. rufescens* has been reported to adjust its chemical signature to match its host's, even if the cuticular hydrocarbon profile of parasite and host was found not to match closely (Bonavita-Cougourdan *et al.* 1996). However, these studies were performed only on adult ant workers from established colonies, and experimental host species exchanges were done under unusual laboratory conditions (Bonavita-Cougourdan *et al.* 1997). We can show that young slave-making *P. rufescens* workers lack a chemical signature at emergence. This is not surprising, as chemical insignificance appears to be a general

feature of callow workers, lacking hydrocarbons both on the cuticle and the post pharyngeal gland (*see* Lenoir *et al.* 1999). When reared in isolation from adult host workers, young *P. rufescens* workers exhibit a complex chemical profile at the age of five days. This indicates that the parasite does not necessarily need to interact with adult workers to perform its own hydrocarbon synthesis. However, when reared by workers of different *Formica* species, *P. rufescens* callows engage in several social interactions (antennal contact, allogrooming, trophallaxis). Slave-makers appear to be behaviourally specialized in gaining acceptance and we observed several instances of abdominal trophallaxis in which the slave-making callow was the donor. This behaviour has never, to our knowledge, been described previously for *Polyergus* workers, nor any other Formicinae ant. Among slave-makers, abdominal trophallaxis was observed in *Protomognathus americanus* (Stuart 1981), but its role is also not clear. In *P. rufescens*, the secretion appeared to be very attractive to the host workers, but unfortunately the origin and the composition of this secretion is unknown and further studies are needed to clarify the role of this behaviour in the integration process. Social interactions are likely to play a key part in the formation of the colony odour (Lenoir *et al.* 1999) and this could be the mechanism allowing the *P. rufescens* callows to change their chemical signature to that of the rearing host species. Indeed, allogrooming occurs more frequently in experimentally mixed groups compared with natural nests.

Our results show clearly that *P. rufescens* young workers adopt a different cuticular signature, both qualitatively and quantitatively, when reared by different *Formica* species. This is reinforced by the fact that the cocoons introduced into the different rearing *Formica* subcolonies came from the same mother colony, so that all of the young *P. rufescens* workers were sisters. They appear to be effective at chemically 'blending in with the crowd' and it seems that their cuticular profile can be altered to match that of a species rarely encountered as a host (*F. gagates*) and even to one that is never enslaved under natural conditions (*F. selysi*). Chemical congruency with the host seems to be a common strategy of social parasites: for example, in the xenobiotic ant *Formicoxenus provancheri* (Lenoir *et al.* 1997), in the inquiline ant *Doronomyrmex kutteri* (Franks *et al.* 1990) and also in slave-makers *Harpagoxenus sublaevis* (Kaib *et al.* 1993) and *Rossomyrmex minuchae* (C. Zamora-Muñoz, F. Ruano, C. Errard, A. Lenoir, A. Hefetz & A. Tinaut, unpublished data). The ability to match the host species' profile is not exclusive of parasitic ants but has also been demonstrated in parasitic *Polistes* wasps (Bagnères *et al.* 1996; Turillazzi *et al.* 2000; Sledge *et al.* 2001). Which could be the mechanisms by which the parasite achieves a successful integration into the life of a host colony? Chemical similarity between the parasite and the host queen could be achieved either by camouflage, mimicry or a combination of both (see Dettner & Liepert 1994). Camouflage implies the acquisition of chemicals through social interactions, both passively (through physical contacts with ants and nest material) and actively (by allogrooming, trophallaxis). Mimicry means a *de novo* biosynthesis of host-specific chemicals (*sensu* Howard *et al.* 1990). The mechanism underlying chemical integration of *P. rufescens* workers is

most probably camouflage, a fine tuning soon after emergence to match their host's profile, even if this could also be combined with the synthesis of appropriate compounds. The chemical signature of *P. rufescens* reared in isolation from adult workers is very close to that of their natural host *F. cunicularia*. The possibility of a contamination by *F. cunicularia* hosts in the natal colony during *P. rufescens* emergence is weak; indeed, *P. rufescens* workers analysed soon after emergence from their cocoon revealed only traces of cuticular hydrocarbons. It was also shown that the quantity of topically applied hydrocarbons decreases dramatically within a few hours (Meskali *et al.* 1995). Therefore, the isolated five-day-old *P. rufescens* workers exhibited their own specific hydrocarbon pattern. The similarity between the parasite and its primary host indicates the occurrence of an adaptation process in the parasite population, which has been selected to mimic the recognition cues of *F. cunicularia*. Studies based on the selective acceptance of parasitic brood by different potential host species and aggression tests provided some evidence for host specificity in a population of *P. rufescens* (Mori *et al.* 1994*b*, 1996). This process appears to be crucial in colony foundation: the newly mated *Polyergus* queen is more successful in usurping a host colony of the same species present in the natal nest (Mori *et al.* 1995). This host fidelity can be reinforced by early learning, the parasite imprinting on the host species of its natal nest (Goodloe & Sanwald 1985; Schumann & Buschinger 1994). The present study strongly indicates that host preference in *P. rufescens* is genetically mediated, as the parasite produces the hydrocarbons characteristic of its usual host species. This can lead to the formation of host-specific races or 'gentes' in the parasitic species, host choice being influenced by the interaction of innate preferences and early experience (Schumann & Buschinger 1994, 1995). This phenomenon is comparable with the variations in adaptations of cuckoos (*Cuculus canorus*) to a particular passerine host species. (Marchetti *et al.* 1998; Gibbs *et al.* 2000).

Chemical flexibility and the ability of the parasite to integrate into host species' colonies other than that of the rearing species could facilitate the change to a different host species. As for other slave-makers, differences in host use within and between parasite populations might reflect different host densities (Heinze *et al.* 1992). If the main host species becomes rare, the parasite might switch over to other potential host species (c.f. one observation of host switching reported for *P. rufescens* by A. Forel is cited in Hölldobler & Wilson (1990), p. 456). The other reported common host species of *P. rufescens* is *F. rufibarbis*, and, interestingly, the chemical profiles of *F. cunicularia* and *F. rufibarbis* are more similar compared with the profiles of *F. gagates* and *F. selysi*. Moreover, *F. cunicularia* and *F. rufibarbis* are phylogenetically close relatives (R. Savolainen, personal communication). The question arises about the suitability of *F. gagates*, which can be also found in habitats where *P. rufescens* is present, but is not reported as a common host species. In one study of a *P. rufescens* population (usual host *F. cunicularia*), the species *F. cunicularia* and *F. rufibarbis* co-occurred with *F. gagates*, but the latter was not abundant (Le Moli *et al.* 1994). Ecological factors (c.f. Seifert 1996), together with a dissimilarity in the chemical profile, the higher level of

aggression and a possible lower capability for rearing the slave-making brood (low rate of cocoon acceptance), could account for the low suitability of *F. gagates* as a host for *P. rufescens*. The high rate of cocoon acceptance in the allopatric *F. selysi* (non-host) might be due to a lower non-nestmate rejection threshold because of the absence of the parasite (Davies *et al.* 1996; Brooke *et al.* 1998).

Slave-makers can have a severe impact on host populations and act as a strong selective force for their hosts (D'Ettorre & Heinze 2001). Counter-measures are therefore expected to be selected, as recently shown by Foitzik *et al.* (2001) studying three populations of the pair *Protomognathus americanus*/*Leptothorax longispinosus*. As a consequence of higher parasite pressure, the host population becomes more effective in defending against raiders. In a coevolutionary arms race (Dawkins & Krebs 1979), host species are expected to develop a more effective recognition system. The present study does not provide evidence for this phenomenon. Nestmate recognition is at the core of insect societies and a more strict recognition system might prevent host colonies from social parasitism, but workers might also be more disposed to misidentify their own nestmates. Acceptance of social parasites could be a result of the adaptive cost–benefit balance of establishing defences: acceptance errors should persist if the cost of correct discrimination exceeds the benefit (Zahavi 1977; Lotem 1993; Takasu 1998). There is evidence for non-nestmate acceptance thresholds (c.f. Reeve 1989) being flexible: honeybee guards' acceptance of other bees into the hive decreases when the frequency of non-nestmate contact and the cost of accepting non-nestmates, due to food-robbing, is high (Downs & Ratnieks 2000). Similarly, for avian brood parasites, host defences might reflect adaptive phenotypic flexibility rather than evolutionary change: egg rejection rate appears to be related to the probability of parasitism (Davies *et al.* 1996; Brooke *et al.* 1998). If a social parasite is sufficiently rare, hosts are not expected to shift their acceptance threshold and the parasite can integrate into their colonies. Further studies are needed to investigate the relative importance of genetic change and conditional responses in host defences induced by social parasites.

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