Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer*

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Colonies of the polistine wasp *Polistes dominulus* are parasitized by the permanent worker-less social parasite *Polistes sulcifer*. After usurpation of the host colony, parasite females are characterized by a change in the relative proportions of their cuticular hydrocarbons to match those of the host species. In this paper we present evidence from field data and laboratory experiments that *P. sulcifer* females adopt a colony-specific host odour that facilitates their acceptance by host females of the usurped colony. Presentation experiments demonstrate that parasite females are recognized as foreign individuals by workers of other parasitized nests. We show that the modification of parasite cuticular compounds is sufficient for this recognition. This provides evidence that, after invasion, *P. sulcifer* queens do not require appeasement or propaganda substances for their acceptance by host colonies. Furthermore, multivariate discriminant analysis of the cuticular hydrocarbon proportions of the parasites after usurpation assigns the parasites together with *P. dominulus* females of their own host colony. To the authors' knowledge, this is the first confirmation that social parasites adopt colony-specific host odours.

Keywords: social parasitism; *Polistes* wasps; cuticular hydrocarbons; Hymenoptera

1. INTRODUCTION

Social parasitism is generally considered as a principal example of evolutionary arms races (Davies *et al.* 1989; Soler & Soler 2000). Usurpation of host nests by parasites often requires complex adaptations, which in turn elicit the evolution of defensive adaptations in the hosts in order to counter this exploitation. This has been well studied in the cuckoo where females from host-specific races (or *gentes*) lay eggs that mimic the distinctive egg patterning of the host (de L. Brooke & Davies 1988; Cherry & Bennett 2001). In response, hosts have evolved greater discrimination abilities in order to allow them to reject eggs of foreign origin (Davies and de L. Brooke 1989; Lotem *et al.* 1992). Colonies of social Hymenoptera (ants, bees and wasps) have also provided excellent examples of the evolution of a parasitic mode of life. Many types of parasitism exist in the social insects, ranging from simple kleptobiosis (stealing of food) and temporary parasitism (both intra- and interspecific) through to species that have lost the ability to establish nests on their own and are completely dependent on their hosts for the raising of their offspring. This latter group includes the well-known slave makers (species that raid host nests for worker brood) and the inquilines (species that produce sexual offspring only and no worker caste) (Wilson 1971; Hölldobler & Wilson 1990; D'Ettorre & Heinze 2001). Despite the seemingly numerous advantages of a parasitic mode of life, permanent inquilinism is rare in the social Hymenoptera and only *ca.* 100 species are known to date (R. M. Lowe and M. F. Sledge, unpublished data).

The life cycle of the parasite commences with newly mated queens searching for and usurping host nests. In

doing so, the parasites must overcome the host defence system, and a variety of usurpation strategies are known to exist. Parasites, for example, may possess several morphological adaptations, such as a thickened cuticle and strengthened mandibles, which allow them to enter nests successfully (Reed & Akre 1982; Ondricek-Fallscheer 1992; Cervo 1994). In some species, parasites aggressively resist the attacks of host workers and search for the resident queen, who they then proceed to kill (Hölldobler & Wilson 1990; Cervo & Dani 1996; Lenoir *et al.* 2001). In the slave-making ants *Polyergus breviceps* and *Polyergus rufescens*, parasite queens quickly locate and kill the host queen by biting her (Topoff & Zimmerli 1993; Mori *et al.* 1995). Similarly, in two social wasp parasites of the genus *Polistes*, *Polistes sulcifer* and *Polistes semenowi* (which are parasites of *Polistes dominulus*), the female parasites aggressively overcome the host nest defences. In the case of *P. sulcifer* the dominant host foundress is killed or driven off, while *P. semenowi* usually tolerates the presence of the host foundress (Turillazzi *et al.* 1990; Zacchi *et al.* 1996). There are several species in which aggression has not been observed and the parasites enter nests in an apparently peaceful manner. The parasitic sweat bee *Paralictus asteris* enters nests of its host *Lasioglossum imitatum* without any obvious signs of aggression (Wcislo 1997). Similarly, *Polistes atrimandibularis* adopts a submissive posture on entering a host nest and tolerates attacks from the host foundress (Cervo *et al.* 1990). Parasite queens may also produce pheromones that aid in usurpation by repelling or appeasing host workers, as has been found in many ant species (see Hölldobler & Wilson 1990; Lenoir *et al.* 2001 for reviews). For example, the contents of Dufour's gland in *P. breviceps* and *P. rufescens* have been found to reduce aggression in host workers (Topoff *et al.* 1988; Mori *et al.* 2000; D'Ettorre *et al.* 2001). Compounds released by the

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same gland are also important in nest usurpation in *Harpagoxenus sublaevis* and *Leptothorax kutteri* (Allies *et al.* 1986; Ollett *et al.* 1987).

The principal host defence in social insects is an intricate recognition system based on cuticular odours (Howard 1993). This system allows for the rejection of non-nest-mates from a colony and, consequently, prevents usurpation by unrelated individuals. Social parasites have evolved mechanisms that exploit this phenomenon and facilitate their acceptance in a colony. This is hypothesized to occur by the parasite adopting a cuticular signature after usurpation to match that of the host, thus ensuring their acceptance within the colony (Lenoir *et al.* 2001). Little is known of the mechanisms involved in these changes, but parasitic species are either able to synthesize host hydrocarbons (which is termed mimicry and is, for example, hypothesized to occur in *Polistes atrimandibularis*) (Bagnères *et al.* 1996) or acquire them from host individuals or the host nest itself (which is termed camouflage, e.g. in *Leptothorax kutteri*) (Franks *et al.* 1990). We showed in a previous paper that, after aggressively eliminating the dominant host foundress, the cuticular signature of the parasite *P. sulcifer* changes to match that of the host within 3 days after usurpation (Turillazzi *et al.* 2000). This presumably allows for parasite acceptance, although this has not been experimentally demonstrated. Furthermore, it is unknown whether the parasite adopts the signature of the specific colony it has usurped. Invading *P. sulcifer* females also perform a rapid stroking of the abdomen over the host nest surface after usurpation (Turillazzi *et al.* 1990). This behaviour probably accounts for the presence of the parasite species-specific hydrocarbon 9,15-dimethyl nonacosane (a carbon backbone of 29 carbon atoms with two methyl branches in positions 9 and 15) on the material of usurped nests (Turillazzi *et al.* 2000). The function of this is unknown, although it may modify the host recognition system, as the material of the nest is essential for nest-mate recognition in *Polistes* wasps (reviewed by Singer *et al.* 1998). In this paper we describe the results of experiments aimed at determining the role of cuticular compounds in the integration of *P. sulcifer* parasites in host colonies and to what extent cuticular signatures of parasites match that of the host colony.

2. METHODS

(a) *Study species and general methods*

Polistes sulcifer is an obligate worker-less social parasite of the social wasp *P. dominulus* that is found distributed through areas around the Caspian Basin. At the end of May, mated females emerge from hibernation and search for host nests (Cervo & Turillazzi 1990). Their emergence from hibernation is delayed with respect to nest founding by their hosts (end of April). This lag ensures that the host nests contain sufficient numbers of pupae, thereby eventually providing an adequate worker force for raising the parasite's offspring (Cervo & Dani 1996). We performed experiments over a 5 year period on parasitized and non-parasitized nests of *P. dominulus* collected during the last weeks of May and first weeks of June. In addition, we collected *P. sulcifer* females in flight during the month of May and these parasites were thus considered not to have previously usurped any host colony. We collected two sets of data (behavioural and

chemical) that were aimed at determining the extent to which parasites are chemically integrated in host colonies. The behavioural experiments consisted of presentations of live parasite females to foreign parasitized colonies, as well as the presentation of dead host females that had solvent extracts of parasite cuticular compounds applied to them. Compositional data on the cuticular and nest lipids acquired through gas chromatography–mass spectrometry (GC-MS) were analysed by multivariate statistical techniques in order to determine whether parasite queens could be assigned to the host colony that they had usurped.

(b) *Behavioural experiments*

We collected 18 parasitized nests of *P. dominulus* (10 from northeastern Italy during the first week of June 1998 and eight from western central Italy during the last week of May 2000) (mean $\pm$ s.e. number of host individuals = 5.9 ± 0.68 and range = 2–11). These were transported to the laboratory in glass bottles and housed in glass cages (15 cm $\times$ 15 cm $\times$ 15 cm) and provided with supplies of food and water. Nests of similar size (number of cells) and host female number were organized into pairs. Parasites from each of the colonies were removed and placed in a refrigerator at 4 °C in order to slow their movement. Each parasite was then presented to each of the nests of a pair. Individuals were grasped by the legs with forceps and slowly moved through a hole in the side of the glass cage and held at 1 cm from the nest for 1 min. The responses of host individuals were observed and the totals of the two responses (biting and stinging) that were considered to indicate an unambiguous aggressive reaction were counted. Controls were performed in a similar manner using a single host individual from each of the colonies. All experiments were performed blind and video recorded in order to ensure standardized interpretations of the wasps' responses.

Subsequent to these experiments, the role of epicuticular compounds in parasite acceptance by hosts was investigated in 10 of the original 18 nests. All parasites from the nests were sacrificed and frozen at -20 °C. The abdomen of each parasite was removed (in order to avoid contamination from exocrine glands) and the rest of the corpse placed in 2 ml of hexane for 24 h in order to remove cuticular compounds. We performed the same procedure for a host worker from each colony for controls. In addition, two host foundresses from each of the 10 nests were sacrificed and rinsed in hexane for 24 h without prior removal of the abdomen in order to remove all cuticular hydrocarbons. These foundresses were then used as lures for the experiment. In order to ensure that all cuticular compounds were removed, we performed preliminary presentations of lures to foreign nests and assessed the responses of host females. In no case were lures reacted to aggressively and the cuticular compounds were thus considered as being removed. One millilitre of cuticular extracts was applied to lures with a Pasteur pipette and then left for 3 min in order to ensure evaporation of the hexane before presentation to the nests. As for the previous experiments, we paired nests of similar size and presented the lures with cuticular extracts of the parasite from the original host nest and the foreign one. Lures were presented in a similar way, held at 1 cm from the nest for 1 min. Control experiments were performed using cuticular extracts from *P. dominulus* host individuals applied to the lures and presented as for the experiments described above. All experiments were blind.

We performed an additional presentation experiment (on six of the nests not used in the experiment described above) that

involved only epicuticular compounds and not the entire body of individuals. This was possible by using the method established by Turillazzi *et al.* (1998) in which cuticular substances are removed by rubbing small pieces of cotton wool on the thorax of the wasp. Each piece of cotton wool containing parasite ($n=6$) cuticular compounds was then presented in the same way as described for previous presentations.

(c) Chemical analyses

We collected seven parasitized (mean $\pm$ s.e. number of host individuals = 2.57 ± 0.37 and range = 1–4) and six non-parasitized nests (mean $\pm$ s.e. number of host individuals = 2.17 ± 0.31 and range = 1–3) of *P. dominulus* from Lessinia in Northern Italy during the last weeks of May and June 1995. The nests were placed in glass bottles and maintained alive until transported to the laboratory where they were immediately stored at -20°C . Cuticular compounds were extracted from females by washing individuals in 1 ml of pentane. We removed the abdomens of the females before this procedure in order to ensure that there was no contamination from exocrine glands (e.g. Dufour's gland) within the abdomen. Individual females were rinsed in the pentane for 2 min, after which their bodies were removed and the remaining pentane taken to dryness under a nitrogen stream. We then added 30 μl of heptane, of which 2 μl was injected into a gas chromatograph–mass spectrometer.

A further five parasites in flight and five non-parasitized nests of *P. dominulus* were collected in May 1997 from the same locality. After transportation to the laboratory, we extracted cuticular hydrocarbons from live females using non-destructive contact solid-phase microextraction (SPME) (Monnin *et al.* 1998). This method consists of rubbing the SPME fibre on the thorax of individual females in order to extract cuticular substances. The fibre is then injected into a gas chromatograph–mass spectrometer. We sampled the cuticular profiles of parasite females and hosts before usurpation in the laboratory and again at set time-intervals (from 3 to 40 days) after each parasite was allowed to usurp a host colony. Sampling was performed with a 7 μm polydimethylsiloxane fibre (Supelco Inc., Bellefonte, PA, USA).

Identification of the cuticular compounds was performed on the basis of their mass spectra, which were produced by electron ionization mass spectrometry using a Hewlett Packard (Palo Alto, CA, USA) 5890A gas chromatograph coupled to an HP 5971A mass selective detector (using 70 eV electron impact ionization). A fused silica capillary column (30 m $\times$ 0.25 mm $\times$ 0.5 μm) coated with 5% diphenyl–95% dimethyl polysiloxane (Rtx-5MS, Restek, Bellefonte, PA, USA) was used. The injector port and transfer line were set at 280°C and the carrier gas was helium (at 12 lbf in $^{-2}$ (83 kPa)). The temperature protocol for the solvent injections was as follows: 70 – 150°C at a rate of $30^{\circ}\text{C min}^{-1}$ (held for 5 min) and 150 – 320°C at $5^{\circ}\text{C min}^{-1}$ (held for 13 min). The temperature protocol for the SPME injections was 150 – 200°C at a rate of $5^{\circ}\text{C min}^{-1}$, 200 – 260°C at $2^{\circ}\text{C min}^{-1}$ and 260 – 310°C at $10^{\circ}\text{C min}^{-1}$. The injections were performed in splitless mode.

(d) Statistical analyses

All statistical analyses were performed using SPSS versions 7.5 and 9.05 for Windows. The areas of 41 peaks (field data) and 47 peaks (laboratory data), representing one or more cuticular compounds, were subjected to principal components analysis (PCA) and stepwise discriminant analysis (DA) for analysis of the hydrocarbon profiles. PCA was used for reducing the

number of variables subsequently used in the DA. Exclusion of the variables was performed based on the communality value of each variable. Those with communalities of less than 0.8 were excluded (and included as independents in the DA) (Norusis 1992). The Kaiser–Meyer–Olkin (KMO) test was used for evaluating the sampling adequacy (values between 0.6 and 0.8 are considered indexes of good sampling). DA was performed on the scores obtained from the PCA and the excluded variables in order to determine whether the predefined groups could be discriminated on the basis of the cuticular lipid compositions of the individuals constituting the groups. The significance of Wilks' λ and the percentage of correct assignments were used for evaluating the validity of the discriminant function. Each peak area was transformed before the multivariate tests (in order to avoid the complications present in analysing compositional data) according to the formula (Aitchison 1986)

$$\tilde{z} = \ln[A_p/g(A_p)], \quad (2.1)$$

where A_p is the area of the peak, $g(A_p)$ is the geometric mean of all peaks in the females to be considered in the multivariate analysis and $\tilde{z}$ is the transformed peak area.

We estimated the variation for each individual in the laboratory colonies over the experimental period by calculating the sum of the squared differences of the percentage values for each compound (which is termed the 'chemical distance') (Turillazzi *et al.* 2000) between its cuticular hydrocarbon composition before and 3 days after usurpation.

3. RESULTS

(a) Behavioural experiments

Polistes sulcifer parasites from usurped nests were recognized as foreign individuals by the host females of other parasitized nests of *P. dominulus* (figure 1). The host wasps were significantly more aggressive towards parasite females from foreign nests than 'nest-mate' parasites, biting and stinging them in all cases (Wilcoxon signed ranks test, $\tilde{z} = -3.729$, $p = 0.001$ and $n = 18$). Similar results were obtained for the presentation of non-nest-mate and nest-mate host individuals (Wilcoxon signed ranks test, $\tilde{z} = -3.558$, $p = 0.001$ and $n = 17$). Epicuticular compounds were found to be important in the recognition and acceptance of parasites. When cuticular extracts from 'foreign' parasites were applied to them, *P. dominulus* lures evoked significantly more aggressive responses than lures containing extracts from resident parasites (Wilcoxon signed ranks test, $\tilde{z} = -2.807$, $p = 0.005$ and $n = 10$) (figure 2). Indeed, lures that had cuticular extracts from resident parasites applied to them were accepted by host workers and aggressive responses were rare or absent. Controls (the presentation of lures that had non-nest-mate and nest-mate host extracts applied to them) evoked similar responses in host colonies (Wilcoxon signed ranks test, $\tilde{z} = -2.675$, $p = 0.007$ and $n = 10$) (figure 2). Confirmation of the importance of cuticular compounds in parasite acceptance was attained by the presentation of pieces of cotton wool containing parasite cuticular hydrocarbons. Host colonies responded more aggressively to cotton wool containing foreign parasite extracts than their own parasite extracts (Wilcoxon signed ranks test, $\tilde{z} = -2.214$, $p = 0.027$ and $n = 6$).

Figure 1. Aggressive responses of workers in colonies of *P. dominulus* parasitized by the social parasite *P. sulcifer* when presented with the parasite from their own colony and a foreign parasite as well as foreign and nest-mate workers. Asterisks indicate differences at $p < 0.01$.

Figure 2. Aggressive responses of workers in parasitized *P. dominulus* colonies when presented with lures (*P. dominulus* foundresses) applied with cuticular extracts (HC) from 'nest-mate' and foreign *P. sulcifer* parasites and nest-mate worker and foreign workers. Asterisks indicate differences at $p < 0.05$.

(b) Integration in host nests: chemical analyses

PCA was performed separately on the data obtained in the field (by solvent extraction) and in the laboratory (on live individuals by SPME). PCA was performed on all except three variables for the field colonies (which had communalities of less than 0.8). The KMO test gave a value of 0.609. The analysis produced eight principal components with eigenvalues higher than unity, accounting for 90.9% of the original variance. PCA was performed on all except 12 variables for the laboratory analyses (which had communalities of less than 0.8). The KMO test produced a value of 0.868. The analysis produced seven principal components with eigenvalues higher than unity, accounting for 92.2% of the original variance. A DA was performed on the scores obtained from the principal components and on the excluded

Figure 3. Canonical discriminant functions. DA of (a) non-parasitized and (b) parasitized field-collected colonies of *P. dominulus* on the basis of the proportions of the epicuticular compounds that were identified using GC-MS. *Polistes sulcifer* parasites (indicated by arrows in (b)) were all correctly assigned to the colony that they had usurped. The percentages of the variance explained by each of the two main functions are given in parentheses.

variables for both experiment types. Hydrocarbon extracts of the nest itself were excluded from the analyses in order to avoid complications arising from differences in the sample matrices (nest material and wasp bodies).

Stepwise DA of females collected in the field confirmed the behavioural evidence of parasite integration into host nests (figure 3). Females in non-parasitized *P. dominulus* colonies were all correctly classified (figure 3a) (function 1, canonical correlation = 1.000, Wilks' $\lambda = 0.001$, $\chi^2 = 100.123$ and $p = 0.001$, function 2, canonical correlation = 0.996, Wilks' $\lambda = 0.001$, $\chi^2 = 54.235$ and $p = 0.001$ and function 3, canonical correlation = 0.985, Wilks' $\lambda = 0.007$, $\chi^2 = 27.026$ and $p = 0.029$). All *P. sulcifer* females in parasitized nests were correctly assigned to their colony on the basis of their proportions of cuticular compounds, with three (12%) host females being incorrectly classified (function 1, canonical correlation = 0.922, Wilks' $\lambda = 0.061$, $\chi^2 = 54.655$ and $p = 0.001$ and function 2, canonical correlation = 0.771, Wilks' $\lambda = 0.405$, $\chi^2 = 17.622$ and $p = 0.003$) (figure 3b). This demonstrates that the

Figure 4. Canonical discriminant functions. DA of *P. sulcifer* parasites and host females before and 14 days after usurpation of the colony on the basis of the proportions of the epicuticular compounds that were identified using GC-MS. Post-uruspation parasites (indicated by circles filled with their respective colony symbol and Pxpost) were assigned using the discriminant function produced from the DA of host and parasite individuals pre-uruspation (see the text for details). All individuals were correctly assigned to their respective groups both pre- and post-uruspation. The percentages of the variance explained by each of the two main functions are given in parentheses.

cuticular profiles of the parasites were quantitatively similar to the colony that they had usurped. Further confirmation was obtained from five parasites in the laboratory, with *P. sulcifer* females pre-uruspation (one group) and each of the five host colonies (five groups) all being correctly assigned prior to usurpation (figure 4) (function 1, canonical correlation = 0.999, Wilks' λ = 0.001, χ^2 = 143.365 and p = 0.001, function 2, canonical correlation = 0.996, Wilks' λ = 0.001, χ^2 = 90.071 and p = 0.001 and function 3, canonical correlation = 0.987, Wilks' λ = 0.002, χ^2 = 50.448 and p = 0.001). We performed a DA on parasite females at set intervals after takeover of the colony using the discriminant function from this analysis (pre-uruspation). All but one of the parasites (from colony 1) were correctly assigned to their colony 3 days after usurpation. However, the misclassified individual was correctly assigned 14 days post-uruspation, as were the remaining parasites and hosts (100% correct classification) (figure 4). This situation was maintained at both 25 and 40 days following usurpation.

The matching of host and parasite cuticular odours after usurpation was due to changes in the parasite females. The chemical distances between the hydrocarbon profiles of each individual (host and parasite) in the laboratory colonies before usurpation and 3 days afterwards were significantly higher for the parasites than for the hosts (Mann-Whitney U -test, U = 5.00 and p = 0.19). One of the most apparent changes in the parasites was the appearance of compounds 3 days after usurpation that were present in the host colony, but absent in the parasites before usurpation (table 1). In no case did we

Table 1. Qualitative changes in the cuticular profiles of *P. sulcifer* parasites after usurpation of host colonies.

(The compounds listed were absent from the parasites before usurpation, but were found in all or some *P. dominulus* colonies (right-hand column). Each parasite presented those compounds specific to the host colony 3 days after usurpation. x -meC_{29:1} and x -meC_{31:1} were identified on the basis of a mass fragmentation spectrum only.)

compound	colony code
n -C _{27:1}	all
x -meC _{29:1}	1
9,17-dimeC ₂₉	1 and 4
7-meC ₃₀	2, 3 and 5
n -C ₃₁	all
x -meC _{31:1}	1
11,15-dimeC ₃₁ /11,19-dimeC ₃₁	all
13,17-dimeC ₃₃	all
7, γ -dimeC ₃₃	2–5
5, γ -dimeC ₃₃	2–5

observe a compound present in the host (and absent in pre-uruspation parasites) that was not displayed by the parasite after usurpation. In addition, we observed considerable variation in the quantities of several compounds for each parasite that were present in both the *P. dominulus* colonies and pre-uruspation parasites. The following were those that were more consistent between the five parasites: 9-, 7-meC₂₇, 13,17-dimeC₂₉ and 14-, 13-, 12-, 11-meC₃₂ increased after usurpation, while 9,15-dimethylC₂₉ and 9,15-dimethylC₃₁ decreased. We consistently found 9,15-dimethylC₂₉ on host nest material after usurpation in parasitized colonies, but it was only present in trace amounts on a few isolated host females from field-collected colonies.

4. DISCUSSION

Our results demonstrate that *P. sulcifer* females modify their cuticular hydrocarbon profiles in order to match those of the usurped host colony and that this is sufficient for their recognition and acceptance as nest-mates by host individuals. To the authors' knowledge, this is the first compelling evidence that parasites not only adopt host species-specific odours, but also a colony-specific signature. Both our statistical analyses and behavioural results show that integration within colonies is exact and that parasite females are recognized as foreigners by foreign host colonies, but not by their own colonies. Our results also show that epicuticular compounds are responsible for this. Application of foreign parasite cuticular extracts to *P. dominulus* females resulted in aggressive responses from host workers of their own usurped colonies. We further suggest that these compounds are non-volatile hydrocarbons. The presentation of cotton wool containing only parasite cuticular hydrocarbons (which was confirmed by GC-MS analysis of solvent extractions of the cotton wool pieces) evoked aggressive reactions in the host workers. This is supported by the fact that direct behavioural evidence for hydrocarbons as recognition discriminators

now exists in ants (Lahav *et al.* 1999). However, the use of appeasement or propaganda pheromones cannot be ruled out during the actual act of usurpation. This occurs in many ant species, with pheromones released from exocrine glands being used for reducing worker aggression during the initial phases of usurpation, while the cuticle is modified subsequently (Lenoir *et al.* 2001).

It is not yet known whether true mimicry (the biosynthesis of host compounds) or camouflage (the active or passive acquisition of compounds from the host) occurs in *P. sulcifer*. Notwithstanding this, our results suggest that camouflage is the most likely mechanism. The ability of *P. sulcifer* to match the host colony signatures in such an exact quantitative manner as well as the exhibition of characteristic compounds present in the various host colonies makes it likely that the colony signature is acquired from the host. It is known that, in several ant and termite species, hydrocarbons are readily transferred among individuals (even of differing species) during acts such as grooming or trophallaxis. Both species in mixed colonies of the ants *Formica selysi* and *Manica rubida* produce species-specific hydrocarbons, but entire mixed colonies present a mixture of the two species, the formation of which is the result of transfer between individuals (Bagnères *et al.* 1991; Vienne *et al.* 1995). Social parasites may exploit the same mechanism in order to adopt the host colony odour actively. This has been proposed to occur during killing of the host queen, as in the slave-making ants of the genus *Polyergus* (Topoff & Zimmerli 1993; Le Moli *et al.* 1994; Lenoir *et al.* 2001), extensive grooming of and/or by parasites (Franks *et al.* 1990; Kaib *et al.* 1993; Dettner & Liepert 1994; Lenoir *et al.* 1997) or trophallaxis (Franks *et al.* 1990). This is also conceivable in *P. sulcifer*, as there is considerable grooming and licking of host foundresses and workers by the parasites, as well as exchange of regurgitated liquid droplets (true trophallaxis is absent in *Polistes* wasps) during dominance interactions (Turillazzi *et al.* 1990). Host odour could also be acquired in social wasp parasites from the nest material itself. The surface of the nest is covered by hydrocarbons that are important for nest-mate recognition (Singer & Espelie 1992, 1996; reviewed by Singer *et al.* 1998). This would account for the vigorous stroking of the abdomen over the nest by invading social parasites (Turillazzi *et al.* 1990; Turillazzi 1992), but also by intra-specific usurping foundresses (Cervo & Dani 1996). Therefore, host hydrocarbons are probably transferred to the parasite during this behaviour in *P. sulcifer*. At the same time, it is a mechanism by which the addition to the nest of the parasite-specific 9,15-dimethylC₂₉ occurs, which may allow for the modification of the host recognition system (Turillazzi *et al.* 2000). We also showed (Turillazzi *et al.* 2000) that low quantities of host-specific compounds are observable on the cuticle as soon as 90 min after usurpation, but that the host cuticular signature is only fully developed 3 days after usurpation. From our current analyses, it appears that the full colony-specific odour is also attained at this time (all but one parasite were correctly classified in their colonies after 3 days). The transfer of hydrocarbons within such a short period of time seems plausible. Heterospecific hydrocarbons in mixed colonies of the termites *Reticulitermes santonensis* and *Reticulitermes lucifugus grassei* were acquired by each species

as early as 2 h after being placed together (Vauchot *et al.* 1996), while Meskali *et al.* (1995) have shown that topical applications of some hydrocarbons to the cuticle in the ant *Camponotus vagus* can result in quick transfer to nest-mates (between 30 min and 7 days after application).

True cuticular hydrocarbon mimicry has been conclusively demonstrated (by the use of radioactive markers) in several species of termitophiles (Howard *et al.* 1980, 1982) and a myrmecophilous fly (Howard *et al.* 1990). The synthesis of host-specific compounds has also been suggested to occur in the slave-making ant *P. rufescens* (Bonavita-Cougourdan *et al.* 1996, 1997) and *P. atrimandibularis* (Bagnères *et al.* 1996). This would presumably involve stimulation and/or inhibition of the genetic control of selected enzymes in hydrocarbon synthesis pathways (Adams *et al.* 1995; Wicker & Jallon 1995; Bagnères *et al.* 1996). Queens in both *P. sulcifer* and *P. atrimandibularis* are at least able to halt synthetic pathways. The proportion of 9,15-dimethylC₂₉ in *P. sulcifer* drops drastically after usurpation (Turillazzi *et al.* 2000; this study), while in *P. atrimandibularis* a whole suite of unsaturated hydrocarbons disappears following takeover of a colony. However, these reappear in the queen later in the colony cycle (Bagnères *et al.* 1996). As we have mentioned above though, it appears more likely, in *P. sulcifer* at least, that adsorption of host hydrocarbons occurs after usurpation and cessation of this synthetic pathway.

Dufour's gland in *P. dominulus* contains the same hydrocarbons (and in the same proportions) as the cuticle and has been shown to be involved in nest-mate recognition (Dani *et al.* 1996a,b). It is not known whether hydrocarbons are synthesized by Dufour's gland in wasps, but it is known that the hydrocarbon constituent of Dufour's gland in the honeybee is produced elsewhere in the body and is sequestered by the gland (Katzav-Gozansky *et al.* 1997, 2000). From analyses of Dufour's gland in *P. sulcifer* queens before and after takeover of the colony, we have evidence that the glandular contents change in the same manner as the cuticle, but that the modification is delayed with respect to the cuticle (M. F. Sledge, F. R. Dani, R. Cervo and S. Turillazzi, unpublished data). This further suggests that hydrocarbons are acquired and then stored in Dufour's gland rather than synthesized.

Although social parasites and their hosts are hypothesized to be engaged in a coevolutionary arms race, little or no information exists on host defences or whether encounters with parasites produce subsequent behavioural changes in hosts, as is evident in the cuckoo (Davies *et al.* 1989; Lotem *et al.* 1992; Soler & Soler 2000). Host defences could include better recognition of non-nest-mates, discrimination of parasite brood and resistance to appeasement pheromones. Indeed, it has recently been demonstrated that defence mechanisms against social parasites can evolve (Foitzik *et al.* 2001). Our results show that parasites are able to match their host colony's odour precisely and, with an aggressive behavioural strategy, this may explain the seeming inability of *P. dominulus* colonies to prevent usurpation or recognize parasite queens after usurpation. Future work in both the field and laboratory addressing the coevolutionary relationships between *P. sulcifer* and its host will clarify whether strategies that reduce the probability of parasitism exist in *P. dominulus*.

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