

Chemical mimicry in an incipient leaf-cutting ant social parasite

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Abstract Some social parasites of insect societies are known to use brute force when usurping a host colony, but most use more subtle forms of chemical cheating either by expressing as few recognition cues as possible to avoid being recognized or by producing similar recognition cues to the host to achieve positive discrimination. The former “chemical insignificance” strategy represents a more general adaptive syndrome than the latter “chemical mimicry” strategy and is expected to be characteristic of early evolutionary stages of social parasitism. We tested this hypothesis by experimentally analyzing the efficiency by which *Acromyrmex echinator* leaf-cutting ants recognize intruding workers of the incipient social parasite *Acromyrmex insinuator*. The results were consistent with the parasite being “chemically insignificant” and not with the “chemical mimicry” hypothesis. Gas chromatography–mass spectrometry analysis of cuticular hydrocarbon profiles showed that social parasite workers produce significantly fewer hydrocarbons overall and that their typical profiles have very low amounts of hydrocarbons in the “normal” C29–C35 range but large quantities of unusually heavy C43–C45 hydrocarbons. This suggests that the C29–C35

hydrocarbons are instrumental in normal host nestmate recognition and that the C43–C45 compounds, all of which are dienes and thus more fluid than the corresponding saturated compounds, may reinforce “chemical insignificance” by blurring any remaining variation in recognition cues.

Keywords *Acromyrmex echinator* ·
Acromyrmex insinuator · Parasitism · Chemical mimicry ·
Dienes

Introduction

The colonial life of social Hymenoptera (ants, bees, and wasps) has allowed many evolutionary developments toward social parasitism, ranging from cleptobiosis and temporary parasitism to conditions in which the parasite species is completely integrated and dependant on a single host colony for survival and reproduction. Most of the ca. 100 known species of permanent social parasites (inquilines) are ants, where they express two distinct life histories: slave making and inquilinism (obligate permanent social parasitism) (Wilson 1971; Hölldobler and Wilson 2005; D’Ettorre and Heinze 2001). While temporary social parasites in ants are often as widely distributed as their hosts, the inquilines and slave makers are normally rare and locally distributed (Hölldobler and Wilson 2005).

Inquiline social parasites normally have a series of specific adaptations for exploiting the resources provided by a host colony (Hölldobler and Wilson 2005). These are normally derived from ancestral host traits (Emery 1909) because social parasites are sister clades (the “loose” version of Emery’s rule) or even sister species (the “strict” version of Emery’s rule). Emery’s rule has been hypothesized to be

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consistent with sympatric speciation (Elmes 1978; Buschinger 1986), but allopatric explanations for the evolution of social parasitism cannot be excluded (Wilson 1971; Lowe et al. 2002). A recently discovered example of the strict version of Emery's rule is the Panamanian leaf-cutting ant *Acromyrmex insinator*, which is an inquiline social parasite of its host and sister species *Acromyrmex echinator* (Schultz et al. 1998; Sumner et al. 2004a).

A. insinator is peculiar because its queens still produce a worker caste, unlike most inquiline parasites, a characteristic considered to represent a primitive parasitic condition (Buschinger 1986; Hölldobler and Wilson 2005; Bourke and Franks 1995) as expected for a host–parasite sister species pair that fits the strict version of Emery's rule (Sumner et al. 2004a). *A. insinator* queens are slightly smaller than *A. echinator* queens and differ somewhat in pigmentation and sculpture of the cuticle (Schultz et al. 1998), but are otherwise very similar, as expected for an incipient social parasite. However, in spite of its recent origin, the social parasite has almost completely reverted to single mating, whereas the host and all other *Acromyrmex* species studied so far have multiply mated queens (Sumner et al. 2004b). While the *A. echinator* host has both large and small worker castes, *A. insinator* produces mostly small workers and very rarely large workers (Schultz et al. 1998; Sumner et al. 2003a). Workers of host and parasite can only be distinguished by measuring the distance between the bulla of the metapleural gland and the propodeal spiracle (Sumner et al. 2003a). This single diagnostic character reflects that parasite workers have significantly smaller metapleural glands than host workers, which suggests that they are less competent in hygienic tasks (Sumner et al. 2003a). However, the presence of parasite workers is apparently essential for initiating inquiline reproduction (Sumner et al. 2003b). Once this happens, the host no longer reproduces, confirming that inquiline workers play a vital role in suppressing the host queen and promoting the rearing of parasite sexual brood (Sumner et al. 2003b).

Recognition between social insect colony members relies on the secretion and perception of specific blends of cuticular lipids, which are determined by genetic, physiological, and environmental factors (Howard and Blomquist 2005; Lorenzi et al. 1996; Lahav et al. 1999; Lenoir et al. 1999). This recognition system is almost ubiquitous in ants but can be selfishly exploited by social parasites. For example, parasites can either express host-specific cues (chemical mimicry) or refrain from expressing any identification cues (chemical insignificance) (Howard et al. 1990; Dettner and Liepert 1994; Lenoir et al. 1989, 2001). Some ant species have further evolved chemical “weaponry” to actively distract or confuse the hosts during brood raiding or nest usurpation (Regnier and Wilson 1971; Mori et al. 2000; D'Ettore et al. 2000).

Chemical insignificance might be acquired by retaining the almost nonexistent cuticular profiles of newly hatched individuals. This implies that newly hatched individuals can normally be introduced in and accepted by alien colonies (see Stuart 1992 for *Leptothorax* ants; Gamboa et al. 1986 for wasps; and Breed et al. 1988 for honeybees). Some social parasites shift from temporary chemical insignificance at usurpation to chemical mimicry after having become established in the host colony (see Lorenzi and Bagnères 2002 and Lorenzi et al. 2004 for *Polistes* wasps), which further supports the hypothesis that chemical insignificance tends to precede chemical mimicry, both during ontogeny and during evolutionary elaborations of social parasitism. To determine the extent to which *A. insinator* uses chemical mimicry, chemical insignificance, or a combination of both strategies to become integrated in a host colony, we analyzed the aggressive reactions of host (*A. echinator*) workers toward conspecific and parasite workers from other colonies. We use these data to evaluate the ability of the host to distinguish between parasite and nonparasite intruders. We also performed chemical [gas chromatography–mass spectrometry (GC–MS)] analyses of the cuticular hydrocarbon blends of host and parasite workers to identify the differences between the two species and between colonies of the same species.

Materials and methods

Collection and maintenance

Both parasitized and unparasitized colonies of *A. echinator* were collected in May 2003 in and around Gamboa, Panama, and set up in plastic boxes as laboratory colonies. They were kept for several weeks in Copenhagen to acclimatize and to redevelop a reasonably large fungus garden, and were subsequently transported to Florence, where they were kept in 30×30×15 cm boxes and provided with fresh leaves (mainly *Ligustrum vulgare*) and water. Colonies were kept at an almost constant temperature of 25°C and RH of 70% for the entire period of the study. Two of the parasitized colonies (colony Ae225 and colony Ae228) produced a large number of parasite workers that were used as intruders for the behavioral experiments, but the other three parasitized colonies (Ae190, Ae218, and Ae222) did not contain enough parasite queens to provide a sufficient number of parasite workers, consistent with the earlier findings by Sumner et al. 2003b). Four of the unparasitized colonies (Ae212, Ae215, Ae217, and Ae229) were used as host colonies to be confronted with experimentally introduced workers of both the social parasite and other host colonies. We also introduced social parasite workers into the other parasitized colony (Ae225 into

Ae228 and vice versa) so that the total number of colonies used for the experiments was 6. The remaining unparasitized colonies remained too small to be included in the experiments.

Behavioral experiments

Alien workers were presented to specific host colonies with a device that made it possible to have two intruders (one non-nestmate conspecific intruder and one non-nestmate parasite intruder) presented at the same time. These data are suitable for a Wilcoxon signed-rank test without having to consider the “size” of the colonies (i.e., the number of workers present in the nest). Two randomly selected experimental ants (both always from the same parasitized source colony) were placed on a plastic plate ($20 \times 40 \times 0.5$ mm) and fixed with a thin copper wire over the petiole (Fig. 1). This allowed the intruders to freely move their heads and appendages and to display their typical interaction behaviors when presented to the experimental laboratory colonies.

A total of 106 workers (56 from colony Ae225 and 50 from colony Ae228) were presented to six host colonies (four unparasitized: Ae212, Ae215, Ae217, and Ae229; two parasitized: Ae225 and Ae228) in 53 tests. Our worker–worker comparisons have the advantage that we could compare host and parasite individuals of the same size, status, and colony of origin. If we would have followed the natural situation, we would have had to compare newly mated intruding parasite queens (which are impossible to sample in quantities) with intruding host workers from other colonies, as host queens do not move between colonies. However, this would not have allowed us to control for colony of origin and to disentangle the possible effects of body sizes on one hand and of hydrocarbon profiles on the other hand. Another alternative would have been comparing virgin host queens and virgin parasite queens raised in the same colony, but such mixed broods almost never happen (Sumner et al. 2003b). Given that we know that cuticular hydrocarbon profiles of parasite queens and parasite minor workers are similar (see results), we thus believe to have pursued the best possible comparative experiment for testing whether the parasite relies on chemical insignificance or chemical mimicry.

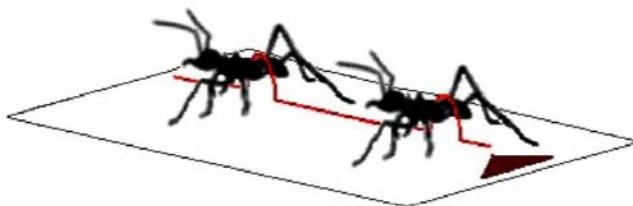


Fig. 1 The experimental device used for the behavioral tests. A single host and a parasite worker from the same source colony were introduced side-by-side in an unrelated receiving host colony

After carefully fixing the two ants on the plate, they were allowed to calm down for a few minutes before placing the entire device near the fungus garden of a receiving colony. The plate and all resident ant behaviors toward the two introduced ants were video-recorded for 5 min to ensure standardized interpretations. The videos were analyzed by the same person without prior knowledge of the identity of the experimental ants. Reactions of the resident colony workers toward the two intruders were quantified by scan sampling the video every 30 s to obtain records of the positions and behaviors of the resident workers that were in contact with the intruders. All scan samples were classified according to the following scale of increasing aggressiveness:

- *Antennation*: Supposedly neutral inspection with fast tapping of the body and head of the intruders by the antennae of resident workers.
- *Menace*: Resident worker raises her head and threatens with wide open mandibles.
- *Biting*: Including both single rapid “snaps” and “leg pulling”, i.e., persistent bites that are normally performed to drag an intruder out of the nest.
- *Attack*: The most aggressive interaction possible where workers tightly grasp each other while biting and trying to cut off legs, antennae, or even the abdomen or the head. This interaction often ended with the death of one or both of the ants involved.

In addition, we scored the latency time between introducing the experimental pair of ants and the first aggressive behavior (i.e., menace, biting, and attack) in every experiment.

Chemical analyses

Single specimens of both species (taken from three parasitized colonies that were not used in the behavioral essays: colonies Ae190, Ae218, and Ae222) were washed in pentane for 2 min after freezing. Solvent was evaporated under a flow of nitrogen, and extracts were redissolved in heptane containing a known quantity of 1-hexadecanol as internal standard. The respective pentane and heptane quantities used were 50 and 10 μ l for small workers, 75 and 15 μ l for medium workers, 200 and 40 μ l for large workers and queens.

The gas chromatograph used was a Varian Star 3400 Cx, with a Zebron ZB-5 capillary column (30 m \times 0.25 mm ID) coated with 5% diphenyl–95% dimethyl polysiloxane (0.10 μ m thickness). The injection port and transfer line were set at 300°C, and the carrier gas was hydrogen (at 15 psi). The initial oven temperature of 70°C increased to 150°C at a rate of 12°C min⁻¹. This temperature was kept for 2 min and then increased by 8°C min⁻¹ until the final temperature of 320°C, which was maintained for 15 min. A Varian

autosampler (8200 Cx) was used to inject 3 μ l of each sample in the GC.

For compound identification, we used concentrated extracts of 40 workers per species per colony in 1 ml of pentane. Analyses were performed on an HP5890 II series GC coupled to an HP5971A quadrupole mass spectrometer using 70 eV electron impact ionization. The registered spectra were compared with those reported in electronic libraries (Wiley 275 and Nist 2.0). The observed retention times registered for the natural compounds were compared with those obtained from a set of synthetic saturated and unsaturated aliphatic hydrocarbons (injected under the same experimental conditions) to confirm the identification of most compounds. Methyl alkanes were identified from their mass spectra and retention time according to Nelson (1993).

Statistical analyses

Wilcoxon signed-rank tests were used to analyze the results of the behavioral experiments. The differences between the total amounts of hydrocarbons in the sample subsets were analyzed with Mann–Whitney tests. For each specimen, the relative abundance of each compound was expressed as the ratio between the area given by each compound and the sum of the areas of the peaks present in the chromatogram. Values were normalized (dividing the area of each compound by the area of the internal standard in the same chromatogram) and then analyzed with a stepwise discriminant analysis. Canonical correlations, Wilks' lambda, and the percentage of correct assignments were used to evaluate the validity of the discriminant functions. All statistical analyses were performed with the program SPSS.

Results

All categories of resident ant behavior toward intruders except the most neutral antennation interaction were significantly

more frequent toward conspecific intruders than toward parasite intruders (antennation: $Z=-0.767$, $P=0.443$; threat: $Z=-4.768$, $P<0.001$; bites: $Z=-5.689$, $P<0.001$; complete attack: $Z=-5.393$, $P<0.001$; $N=106$) (Fig. 2). In addition, the latency time until the first behavioral reaction was significantly longer toward parasite (5.64 ± 0.97 s) than toward conspecific intruders (2.92 ± 0.77 s) ($Z=-6.191$, $P<0.001$; $N=106$).

In 27 of the experiments, the intruders were not removed from the colonies after the 5 min of video recording but were left in the nests for 1 h more to assess whether aggressiveness toward intruders was concentrated in the first minutes after introduction or continued so that it could actually cause serious damage to the intruders. As a result, 12 out of the 27 non-nestmate conspecific intruders were found dead, while just 1 of the 27 non-nestmate parasite intruders was killed by the resident ants (G test: $G=13.404$, $P<0.001$).

We identified 46 compounds with GC–MS analysis (23 linear alkanes, 15 alkenes and alkadienes, and 8 methylalkanes) (Table 1). The host had a rather "standard" cuticular profile with many peaks in the C_{29} – C_{33} range, but the major compounds in the parasite cuticular blend were mono- and diunsaturated alkenes in the C_{37} – C_{45} range (Fig. 3). The parasite also had a significantly lower total amount of cuticular hydrocarbons ($8.6\times 10^5\pm 8.0\times 10^4$ arbitrary units) than the host ($1.2\times 10^6\pm 1.6\times 10^5$ arbitrary units) (Mann–Whitney U test=647; $P=0.004$, $N=91$). When each caste was analyzed separately, only the minor workers were significantly different (Mann–Whitney U test=184.0; $P=0.038$; $N=49$; Fig. 4). Large workers, medium workers, and queens of *A. insinuator* were characterized by similar cuticular profiles as the minor parasite workers (i.e., an abundance of heavy C_{41} , C_{43} , and C_{45} alkenes and a distinct lack of C_{29} – C_{35} hydrocarbons; see Fig. 3a) and also showed lower overall quantitative amounts of hydrocarbons compared to the host although sample sizes were too small to obtain significant differences

Fig. 2 The mean number (\pm SE) of resident colony workers reacting to host intruders (white bars) and social parasites intruders (gray bars) in spot samples during 5 min of observation

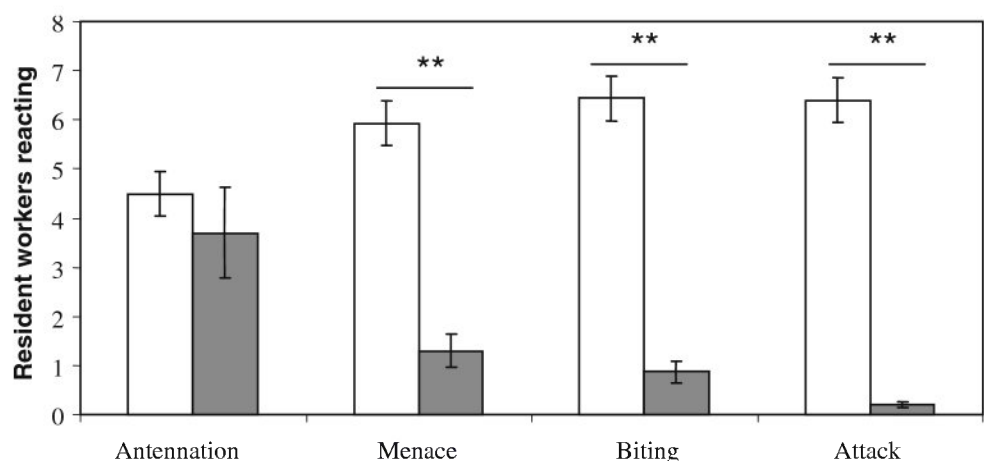


Table 1 Relative abundance±SD of the cuticular compounds identified through GC–MS

	Compound	<i>A. echinator</i>	<i>A. insinator</i>
1	<i>n</i> -C ₁₅	3.0±3.3	2.9±2.0
2	<i>n</i> -C ₁₆	2.6±2.4	3.1±1.8
3	<i>n</i> -C ₁₇	4.1±3.0	4.7±1.9
4	<i>n</i> -C ₁₈	1.9±2.3	2.3±1.4
5	<i>n</i> -C ₁₉	3.9±2.8	6.0±9.1
6	<i>n</i> -C ₂₀	1.5±1.3	1.8±0.9
7	<i>n</i> -C ₂₁	0.8±1.2	X
8	<i>n</i> -C ₂₂	0.6±0.8	1.2±1.1
9	<i>n</i> -C ₂₃	1.0±1.5	0.8±1.2
10	<i>n</i> -C ₂₄	3.5±2.5	3.6±1.5
11	<i>n</i> -C ₂₅	1.8±4.6	0.7±1.8
12	<i>n</i> -C ₂₆	3.4±2.6	3.4±2.0
13	<i>n</i> -C ₂₇	4.7±6.9	3.3±3.7
14	11-MeC ₂₇	2.8±2.7	3.2±2.3
15	<i>n</i> -C ₂₈	1.3±2.2	0.8±1.3
16	C _{29:1}	2.9±2.6	1.3±1.8
17	<i>n</i> -C ₂₉	3.9±4.8	1.6±3.1
18	13-MeC ₂₉	3.8±2.6	2.5±1.6
19	C _{30:1}	2.7±2.9	0.8±1.8
20	<i>n</i> -C ₃₀	2.8 ± 2.6	1.4±1.3
21	13-MeC ₃₀	2.2±2.2	1.1±1.0
22	C _{31:2}	0.5±0.8	0.6±0.9
23	C _{31:1}	X	X
24	<i>n</i> -C ₃₁	1.0±1.9	X
25	13-,15-MeC ₃₁	1.1±1.8	X
26	C _{33:2}	2.9±4.1	0.8±1.6
27	C _{33:1}	1.5±1.5	0.6±0.9
28	<i>n</i> -C ₃₃	1.6±1.5	0.9±1.1
29	13-,15-MeC ₃₃	1.6±1.5	1.5±1.1
30	C _{35:2}	1.6±1.9	X
31	<i>n</i> -C ₃₅	1.4±2.2	X
32	13-MeC ₃₅	4.8±4.2	1.9±1.4
33	13-,15-MeC ₃₅	7.3±7.0	8.2±5.0
34	C _{37:2a}	1.1±1.9	0.8±1.6
35	C _{37:2b}	0.5±1.3	X
36	<i>n</i> -C ₃₇	1.3±2.0	1.0±1.4
37	13-,15-MeC ₃₇	2.4±2.2	1.9±1.8
38	<i>n</i> -C ₃₉	2.2±2.3	1.7±1.7
39	C _{41:2}	1.6±2.1	3.8±2.9
40	C _{41:1}	2.3±2.1	4.7±2.4
41	<i>n</i> -C ₄₁	X	2.7±3.5
42	<i>n</i> -C ₄₂	2.8±2.6	3.0±1.7
43	C _{43:2a}	1.7±2.0	6.3±3.4
44	C _{43:2b}	1.5±1.7	5.2±2.3
45	C _{45:2a}	1.0±1.4	3.9±2.3
46	C _{45:2b}	0.7±1.2	2.4±1.3

Compounds absent in more than 90% of the samples of one of the two species or present in proportions <0.5% on average are omitted (marked with X).

for the separate castes (Fig. 4). However, a two-way ANOVA showed that there was both a significant effect of species ($F=4.339$; $df=1$; $df_{wg}=83$; $P=0.04$) and a significant effect of caste (rank numbered as minor workers=1, medium workers=2, large workers=3, queens=4;

$F=22.622$; $df=3$; $df_{wg}=83$; $P<0.001$) but no significant interaction term ($F=2.408$; $df=3$; $df_{wg}=83$; $P=0.073$). This shows that the overall amounts increase with body size at similar rates in both species and confirms that all female castes of the parasite have lower total amounts of cuticular hydrocarbons than the host (Fig. 4).

A species-level stepwise discriminant analysis selected seven variables (the relative abundances of C_{45:2a}, C_{45:2b}, *n*-C₁₉, *n*-C₂₅, 11-meC₂₇, *n*-C₂₇, *n*-C₂₈) as being sufficient to obtain a discriminant function that correctly separated 100% of the 30 host and 19 parasite minor workers (Wilks' lambda=0.091, $P<0.001$; $N=49$) (Fig. 5). A colony-level stepwise analysis selected seven other variables (13-,15-meC₃₅, *n*-C₃₇, *n*-C₂₉, *n*-C₃₉, *n*-C₂₆, *n*-C₂₃, *n*-C₂₁) to obtain 100% separation. When using only the first and second discriminant functions, 51 of the 54 minor and major workers (94.4%) from the three unparasitized colonies could be assigned to their respective colonies (Wilks' lambda=0.065 and 0.355 for the respective first and second functions, $P<0.001$ in both cases; $N=54$) (Fig. 6).

Discussion

We have shown that *A. echinator* host workers are significantly less aggressive toward intruding social parasite workers than the alien conspecific control workers (Fig. 2). At the same time, our comparison between cuticular hydrocarbons of hosts and parasites revealed that: (1) parasites generally have a lower amount of cuticular hydrocarbons (Fig. 4) and that (2) parasites have a significantly different spectrum of cuticular hydrocarbons (Figs. 3 and 5), lacking, in particular, many of the host's cuticular hydrocarbons in the C₂₉–C₃₅ range while expressing a number of unusual and very heavy unsaturated cuticular hydrocarbons in the C₄₃–C₄₅ range (Table 1, Fig. 3). In the sections below, we will discuss the implications of these findings for understanding how surface chemistry helps *A. insinator* inquilines to be effective social parasites.

The six *A. echinator* colonies used in our experiments were all quite aggressive to each other's workers so that introductions of alien conspecific workers usually ended with the death of the intruders. However, the same colonies seldom killed the parasite worker next to the alien host worker and often seemed to rather ignore this intruder after initial inspection. Given the similarity of cuticular profiles of parasite workers and queens, this result strongly suggests that chemical insignificance, rather than chemical mimicry, is the decisive factor in parasite workers not being recognized. While parasite queens have to gain access after their mating flight to a host colony different from the one in which they hatched (i.e., different also in cuticular

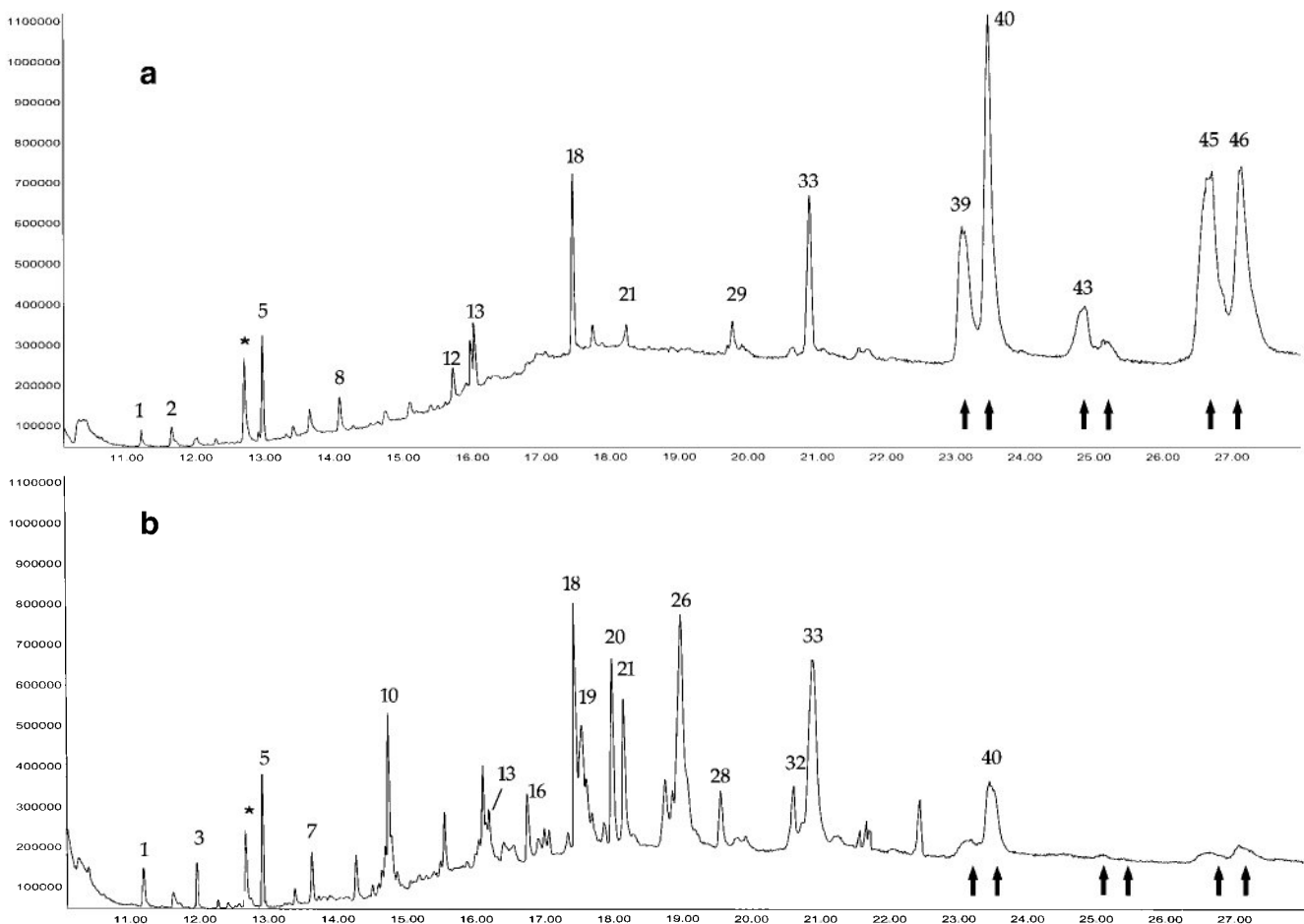


Fig. 3 Typical GC profiles of a social parasite worker (**a**) and a host worker (**b**) showing the greater abundance of heavy alkenes (C_{41} , C_{43} , and C_{45} ; marked with black arrows) and the overall lack of C_{29} – C_{35}

hydrocarbons in the social parasite. Peaks are numbered as in Table 1, while the asterisk marks the internal standard used for normalization of peaks' areas (see text and Table 1 for details)

hydrocarbon profile; Fig. 6), parasite workers are raised in their host colony and obtain the same food as the host workers. If cuticular hydrocarbon profiles had an exclusively environmental origin, parasite workers should thus have adopted the cuticular profiles of their host workers so that they should have been met with the same levels of aggression when introduced side-by-side into another host colony. Our results clearly show that this was not the case.

Our inference that the inquiline workers are chemically insignificant is reinforced by the GC and GC–MS analyses showing that the parasites both have a lower total amount of cuticular hydrocarbons (Fig. 4) and a remarkably different cuticular hydrocarbon profile (Table 1, Fig. 3). Low overall quantities of cuticular hydrocarbons have also been reported in other ant inquilines (Lenoir et al. 2001) and in social parasites of *Polistes* wasps (Lorenzi and Bagnères 2002). In many ways, the *A. insinuator* cuticular chemistry is reminiscent of a neotenic hydrocarbon profile, as newly hatched adults normally have a quite poor chemical profile, which increases in peak volume and peak diversity during the first days of maturation (see Lorenzi and Bagnères 2002 for *Polistes* wasps; Dahbi and Lenoir 1998 for ants).

The abundance of higher molecular weight alkenes in the social parasites is more difficult to explain. These compounds, which are more liquid than the corresponding linear alkanes (Gibbs and Pomonis 1995), may function as a kind of “sponge” to absorb traces of lighter hydrocarbons that might be used as recognition cues. If *n*-alkenes of this size are not involved in the recognition system, they might thus help to hide the information from the shorter hydrocarbons so that the parasite becomes even more chemically insignificant. Moreover, higher chain-length alkenes would offer better waterproofing to the parasites with fewer hydrocarbon molecules. Such compounds would then function best if they were alkenes and not alkanes, as alkanes with that chain length might not be liquid enough to spread easily over the cuticle when ants groom themselves or nestmates. It is interesting to note that the *Polistes* social parasite *Polistes atrimandibularis* also has a great quantity of alkenes when it usurps a host colony, but these are lost shortly afterward when the parasite queen adopts the host profile and thus starts to rely on chemical mimicry (Bagnères et al. 1996). Another possibility is that these heavy compounds are used as appeasing substances

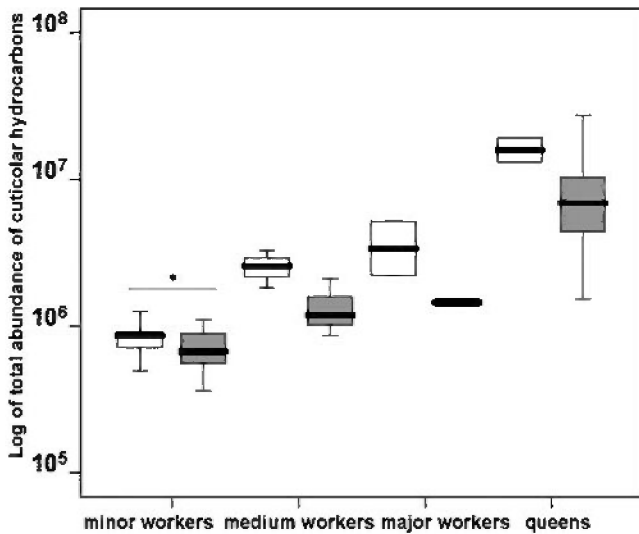


Fig. 4 The logarithm of the total cuticular hydrocarbon quantities in the different female castes of *A. echinator* (white bars) and *A. insinator* (gray bars). Boxes indicate median (black bars), lower and upper quartiles (boxes' bounds), and extremes (whiskers). Minor workers: $N=49$ (30 hosts, 19 parasites, Mann–Whitney U test=184.0; $P<0.05$); medium workers: $N=6$ (3 hosts, 3 parasites, Mann–Whitney U test=0.0; $P=0.121$); major workers: $N=4$ (2 hosts, 2 parasites, Mann–Whitney U test=1.0; $P=0.127$); queens: $N=12$ (2 hosts, 10 parasites, Mann–Whitney U test=7.0; $P=0.081$)

as has been shown in the slave making ant *Polyergus rufescens* (D’Ettorre et al. 2000). However, this explanation seems less likely as the appeasing pheromone of *Polyergus* workers (decyl butanoate) is secreted from the Dufour’s gland and is much more volatile than the heavy cuticular alkenes of *A. insinator*.

The most common strategy used by social parasites to become integrated in the host colony seems to be camouflage by chemical mimicry (Lenoir et al. 2001). The parasite can often acquire the host odor by mechanical contact with the host itself or with nest material. Sometimes, intense

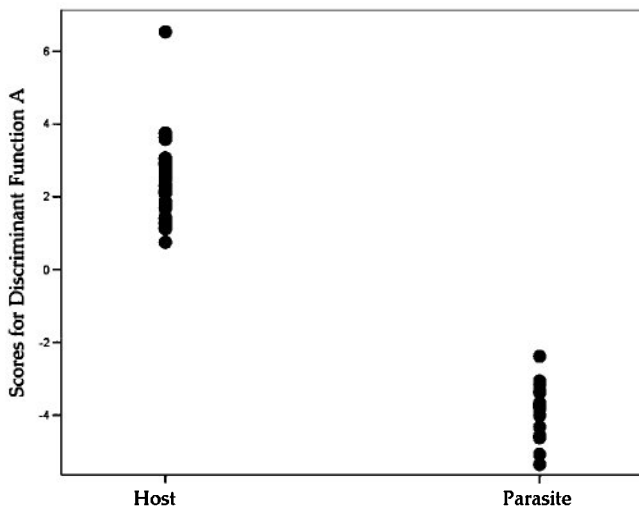


Fig. 5 Scores for the first discriminant function of cuticular hydrocarbon profiles for workers of the host and parasite

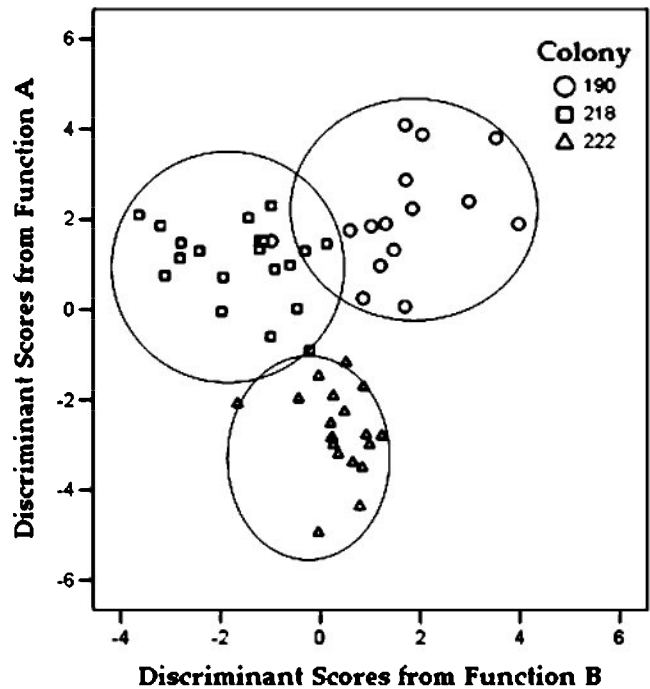


Fig. 6 Discriminant analysis showing that the three unparasitized host colonies (indicated by different symbols and ellipses) differed significantly in their cuticular hydrocarbon profiles (see text for details)

grooming is required for maintaining the new odor (see Lenoir et al. 1989 for *Formicoxenus provancheri*). In other cases, the parasite can affect the chemical pattern of the host with substances that are usually absent in nonparasitized colonies (e.g., the socially parasitic paper wasp *Polistes sulcifer* deposits 9,15-dimethyl C₂₉ on the nest of its host, *Polistes dominulus*; Turillazzi et al. 2000).

So far, only a single ant species (*Formicoxenus nitidulus*; Lenoir et al. 2001) has been shown not to express at least some form of chemical mimicry with its host, and it is doubtful whether *F. nitidulus* is a genuine social parasite as its workers are much smaller than the workers of their *Formica rufa* hosts. Also, the biomass of the entire *F. nitidulus* colonies is negligible compared to the mass of the host colony so that the damage that they impose must be very small. A chemically insignificant cuticular hydrocarbon profile therefore seems adaptive because it enables these tiny ants to merge with the background nest material in the *Formica* mound so that there is no need to evolve any form of active mimicry. This strategy might be specific for the entire genus *Formicoxenus*, as also *Formicoxenus diversipilosus* is ignored by its *Formica* host workers (Alpert and Akre 1973; Francoeur et al. 1985). If the workers of *A. insinator* would follow the same strategy, their cuticular hydrocarbon profiles should merely make sure that they merge with the fungus garden of the host colony. However, we must also keep in mind that almost all studies on social parasite mimicry have focused on usurping queens and that the scarcity of relevant compar-

ative data is also due to hardly any social parasite having maintained a worker caste (Wilson 1971) except when they are slave makers (D’Ettorre et al. 2002). The host–parasite system of *A. echinator* and *A. insinator* is thus providing very interesting opportunities to study inquiline social parasitism in its incipient evolutionary stage.

Recent studies have suggested that the fungal symbiont of *A. echinator* also has a hydrocarbon profile similar to that of the ants (Richard et al., unpublished), and that *Acromyrmex* workers instantaneously recognize nonresident fungal symbionts of neighboring colonies (Bot et al. 2001). Also, the fecal droplets that the ants use to fertilize newly inoculated parts of their fungus garden carry the manurial imprint of the resident fungus and are directly involved in detecting alien fragments of fungus that the ants may occasionally import into the nest (Poulsen and Boomsma 2005). Incidental observations during the present study seem to corroborate that the fungal symbiont and the fecal droplets may also play a role in the assessment and recognition of intruding workers. We observed 21 times (in a total of 106 experiments) that resident host workers released a fecal droplet over the body of the intruder, mainly during the complete attack phase. In four instances (in the same 106 experiments) we further observed that some host workers tore away a 2- to 5-mm fungus fragment from the garden, held it between their mandibles, and “rubbed” the intruder over the head and appendages during inspection or attack. None of these behaviors was frequent enough to allow statistical analysis so that their significance remains unclear. However, they deserve further study, as they might be of interest for the development of colony gestalt odor in leaf-cutting ants, because recent findings suggest that colony odor is partly due to the hydrocarbons present on the fungus garden (Richard et al., unpublished).

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