

CHANGES IN THE CUTICULAR HYDROCARBON PROFILE
OF THE SLAVE-MAKER ANT QUEEN, *Polyergus breviceps*
EMERY, AFTER KILLING A *Formica* HOST QUEEN
(HYMENOPTERA: FORMICIDAE)

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Abstract—Queens of the slave-maker ant, *Polyergus breviceps*, take over nests of their *Formica* host species by fatally attacking the resident queen. As workers only begin grooming the *P. breviceps* queen once she has ceased her attack, we investigated whether a change in parasite queen chemistry may account for the change in worker behavior. Cuticular hydrocarbon profiles of newly mated *P. breviceps* queens and of queens of their two *Formica* host species were found to be species-specific. Profiles of newly mated *P. breviceps* queens that had attacked a *Formica* queen, however, were virtually identical to the queen profile of the species killed. Mass spectral analysis revealed that the hydrocarbons on the cuticles of newly mated *P. breviceps* changed from primarily normal alkanes to methyl and di-methyl branched alkanes after attacks. The results suggest that cuticular compounds from the host queen were transferred to the parasite queen during their aggressive interaction.

Key Words—Cuticular hydrocarbons, Formicidae, slave-maker, *Polyergus breviceps*, *Formica gnava*, *Formica occulta*, colony takeover, queen killing, chemical camouflage.

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INTRODUCTION

Species of the ant genus, *Polyergus*, are all obligate social parasites, unable to survive without the foraging, feeding, and brood care behaviors of their *Formica* hosts. *Polyergus* workers acquire host workers ("slaves") by robbing host species nests of their pupae during summer months. The *Formica* pupae mature in the slave-maker's nest and become full-functioning colony members. Queens of *Polyergus* also depend on the behaviors of their slaves, and new queens require a slave supply to establish their colonies. However, rather than raiding a host nest for brood, a new queen secures her initial slave supply by taking over a host species nest. Frequently, an alate *Polyergus* gyne will mate while running alongside nestmates that are advancing towards a *Formica* nest to raid. By the time the newly mated queen reaches the targeted *Formica* nest, the inhabitants have already abandoned it (temporarily) and the raiders have all but completed their pillaging and are making their way back to their natal nest. The newly mated *Polyergus* gyne enters the nest, as do the *Formica* soon thereafter, and locates and fatally attacks the resident queen (Wheeler, 1906; Topoff et al., 1988; Mori et al., 1995). In laboratory nests, *Formica* workers at first will attack the invading queen, but attacks diminish once the *Polyergus* queen has begun her attack on the *Formica* queen. *Formica* workers during this time seem to be either appeased (Topoff et al., 1988; Mori et al., 2000a,b) or repelled (D'Etorre et al., 2000) by a secretion from the Dufour's gland of the *Polyergus* queen. Immediately after the *Polyergus* queen ceases her attack on the host queen, approximately 25 min after attack onset, workers begin grooming the slave-maker queen. Results of laboratory experiments indicate that the aggressive interaction between the *Polyergus* and *Formica* queens is a key component in the ultimate acceptance of the *Polyergus* queen (Zaayer, 1967; Topoff et al., 1988, 1990; Topoff and Zimmerli, 1993; Mori et al., 1995). First, *Polyergus* queens are more successful in their attempts to take over a nest when a *Formica* queen is present (and the *Polyergus* queen kills her) than when the *Formica* nest is queenless. Worker attacks on an invading *Polyergus* queen in queenless colonies tend to be relentless and almost always result in her death (Zaayer, 1967; Topoff et al., 1988, 1990; Topoff and Zimmerli, 1993). Second, the associated change in worker behavior takes place immediately after attacks on the host queen cease, even if the *Formica* queen is still alive (although maimed, person. obs.). This is unlike the delayed and gradual decrease in worker aggression that is sometimes observed in other species when the resident queen is removed experimentally.

The prevailing hypothesis is that *Formica* workers adopt a *Polyergus* queen because she is "camouflaged" with the host queen cuticular chemicals, chemicals familiar to the workers (Topoff et al., 1988, 1990; Topoff and Zimmerli, 1993; Zimmerli and Topoff, 1994). The lipid layer of the insect cuticle is capable of absorbing other lipid soluble compounds (Soroker et al., 1994, 1995; Vienne et al., 1995), and it has been demonstrated that chemicals can be transferred among

individuals, even across higher level taxa, through social contact (see Vander Meer and Wojcik, 1982). Thus, while attacking a *Formica* queen, *Polyergus* queens may be absorbing chemicals involved in nestmate/queen recognition from the *Formica* queen. The recent results of Errard and D'Etorre (1998) support this contention; after a *Polyergus rufescens* Latreille queen kills a *Formica cunicularia* Latreille queen, her cuticular hydrocarbon profile resembles the hydrocarbon profiles of *F. cunicularia* queens.

In this study, we examined whether a newly mated *Polyergus breviceps* Emery queen also undergoes changes in hydrocarbon profile after killing a queen of her host species, and whether the resulting profile is then similar to the queen profile of the host species. By comparing profiles of *P. breviceps* queens that had killed either a *Formica gnava* Buckley or *Formica occulta* Francoeur host queen, we were further able to ascertain whether the changes in hydrocarbon profiles were specific to the species of *Formica* queen killed. Of the lipids found on the cuticle, a significant percentage tends to be hydrocarbons (Jackson and Blomquist, 1976). Numerous studies have demonstrated a correlation between hydrocarbon patterns and nestmate recognition (e.g., Bonavita-Cougourdan et al., 1987; Vander Meer and Morel, 1998). Recently, the importance of hydrocarbons in nestmate recognition has been confirmed for some ant species (e.g., Lahav et al., 1999), although other classes of compounds are undoubtedly also involved (Obin, 1986). The particular pattern of hydrocarbons, frequently characteristic of a species (nest [Nowbahari et al., 1990] or caste [Wagner et al., 1998]), is likely to reflect similarities or differences in other non-polar and polar cuticular lipids among species. Hydrocarbon profiles can, therefore, be used to investigate the transfer of cuticular lipids, which are likely to contain nestmate recognition cues. Certainly, the strong correlation between hydrocarbon profiles shared by other parasites and predator myrmecophiles and their hosts and adoption by the host colony is suggestive of this (e.g., Vander Meer and Wojcik, 1982; Franks et al., 1990; Vander Meer et al., 1989).

METHODS AND MATERIALS

Ant Collections and Housing. Ant colonies were collected during June and July of 1997 and 1998 from the Chiricahua Mountains of southeastern Arizona, U.S.A.. Fifteen queenright colonies of *F. gnava* were collected from the Arizona oak-alligator juniper woodlands of the Southwestern Research Station (SWRS) of the American Museum of Natural History (el. 1646 m). Thirteen queenright colonies of *F. occulta* were collected from an area just east of the Barfoot Peak trailhead (el. 2750 m) in Coronado National Forest populated with ponderosa pine. Colonies were brought into the laboratory at SWRS and kept in large Tupperware® boxes lined with Fluon® (Northern Products, Woonsocket, RI) to prevent escape.

Newly mated *P. breviceps* queens from three nests with *F. gnava* slaves ($N = 49$) and from two nests with *F. occulta* slaves ($N = 13$) were collected as they approached the *Formica* nest being raided by their non-reproductive nestmates and placed in individual 4 dram vials that contained a cotton ball moistened with water.

Solvent Extraction of Queens. Twelve established (with colony) *F. gnava* queens and ten established *F. occulta* queens were removed from their nests and placed in individual Tupperware boxes ($20.5 \times 45 \times 3.5$ cm) lined with a thin layer of soil. A single newly mated *P. breviceps* queen was introduced into the box and allowed to attack the *Formica* queen. Immediately after attacks ceased, *P. breviceps* and *Formica* queens were placed in individual 7 ml scintillation vials and a quantity of high purity hexane (GC² Grade [B & J, Muskegon, MI]) sufficient to cover the entire body (approximately 0.3 – 0.5 ml) was added to extract cuticular components (see Table 1). After 10 min, the solvent extract was transferred from the sample with a Pasteur pipette to a 2 ml scintillation vial and allowed to evaporate. In addition, cuticular chemicals from newly mated *P. breviceps* queens (that had not attacked a host queen) from nests containing *F. gnava* slaves and from nests containing *F. occulta* slaves, and from established *F. gnava* and *F. occulta* queens that had not been presented to *P. breviceps* queens for attack were extracted using the same protocol presented above (see Table 1). All specimens were preserved for voucher in 70% ethyl alcohol (maintained in personal collection—CAJ).

Chemical Analysis. The evaporated extracts were transported to the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida where they were reconstituted in 0.2 ml hexane, vortexed for 2–3 sec, and applied to a small silicic acid (70–230 mesh 60 Å, [Aldrich Chemical Co., Inc.]) Pasteur pipette

TABLE 1. SPECIES AND CONDITIONS OF QUEENS FROM WHICH CUTICULAR HYDROCARBONS WERE ANALYZED

Species	Condition	Host species in nest	Number of individuals
<i>Polyergus breviceps</i>	Newly mated	<i>F. gnava</i>	42
		<i>F. occulta</i>	5
	Killed <i>Formica</i> queen	<i>F. gnava</i>	6
		<i>F. occulta</i>	6
<i>Formica gnava</i>	Killed by <i>Polyergus</i>	—	12
	No interactions with <i>Polyergus</i>	—	3
<i>Formica occulta</i>	Killed by <i>Polyergus</i>	—	10
	No interactions with <i>Polyergus</i>	—	3

column. Hydrocarbons were isolated from other lipids by eluting the column with hexane. The eluent, containing purified hydrocarbons (ca. 6–7 ml, which from previous experience eluted all hydrocarbons), was concentrated to ca. 10 μ l under a stream of N₂. Samples were analyzed by gas chromatography (Varian 3700 [Varian Associates, Walnut Creek, CA] equipped with a split-splitless injector [Agilent Technologies, Palo Alto, CA], a capillary column [DB – 1, 30 m, 0.32 mm i.d., 0.25 μ m film thickness; J & W Scientific, Inc., Folsom, CA] and flame ionization detector). The injector and detector were set at 300°C; the oven temperature was programmed from 190°C to 290°C at 5°C/min, and then held at 290°C for 5 min. Hydrogen was used as the carrier gas and nitrogen was used as the makeup gas. Data were analyzed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer Corp., Norwalk, CT). Hydrocarbon standards (C24, C26, C28, C30, C32, #NP-MIX-H [Alltech Associates, Inc., Deerfield, IL]) were injected at regular intervals during sample analysis. The standards were used to calculate Kovat Indices (KI).

Mass Spectrometry. Representative samples of (a) newly mated *P. breviceps* queens from nests containing *F. gnava* slaves, (b) newly mated *P. breviceps* queens that had killed a *F. gnava* queen, (c) *F. gnava* queens, (d) newly mated *P. breviceps* queens from nests containing *F. occulta* slaves, (e) newly mated *P. breviceps* queens that had killed an *F. occulta* queen, and (f) *F. occulta* queens were analyzed by gas chromatography/mass spectroscopy. Electron impact-mass spectra (EI-MS) were obtained with an HP 5890 Series II GC (Agilent Technologies, Palo Alto, CA) connected to an HP 5988A MS instrument (Agilent Technologies, Palo Alto, CA) operated at 70 eV and tuned to accentuate the high mass fragments. The GC, equipped with an on-column injector and a SPB1 capillary column (0.25 μ m film; 30 m \times 0.32 mm i.d. [Supelco, Inc., Bellefonte, PA]), was held at 60°C for 2 min and then increased to 200°C at 30°C/min, and then to 280°C at 2°C/min. The MS was controlled and the data analyzed using Vector/Two software (ProLab Resources, Inc., Madison, WI).

Data Analysis. Data were analyzed as two separate groups on the basis of the host species present in the nests of newly mated *P. breviceps* queens. Group 1 compared: (a) newly mated *P. breviceps* queens from nests containing *F. gnava* slaves, (b) newly mated *P. breviceps* queens that had killed a *F. gnava* queen, (c) *F. gnava* queens, (d) and *F. occulta* queens. Group 2 compared: (a) newly mated *P. breviceps* queens from nests containing *F. occulta* slaves, (b) newly mated *P. breviceps* queens that had killed an *F. occulta* queen, (c) *F. occulta* queens, and (d) *F. gnava* queens. For multivariate analysis, it was necessary to preprocess the data. The relative proportions of cuticular hydrocarbons were computed by dividing the area given for each cuticular hydrocarbon by the total integrated peak area of the profile, and then autoscaling each peak to ensure that it had equal weight in the analysis. Principal component analysis (Jolliffe, 1986) was then conducted on 45 normalized variables from Group 1, or on 48 normalized variables

from Group 2. Invariant features were excluded from the principal component analysis, therefore some GC peaks were excluded from the analysis of data sets 1 and 2. In data set 1, one newly mated *P. breviceps* sample and one *F. occulta* sample were deleted because the generalized distance test (Schwager and Margolin, 1982) determined them to be outliers at the 0.01 probability level. In data set 2, one newly mated *P. breviceps* sample, one *F. occulta* sample, and one *F. gnava* sample were also determined to be outliers and were subsequently deleted from the analysis. The multivariate analyses described here were performed by using Pirouette (Infometrix, Woodinville, WA).

RESULTS

Five of the six significant hydrocarbon peaks isolated from the cuticle of newly mated *P. breviceps* queens from nests containing *F. gnava* slaves were determined to be normal alkanes from C25 to C29 (trace of n-C24). The sixth peak was identified as 2-methyl hexacosane. KI data indicate that other 2-methyl even carbon backboned homologues may be present, however, their concentration was inadequate for mass spectral verification. In contrast to the predominance of normal alkanes on the cuticle of newly mated *P. breviceps* queens, the hydrocarbon components of *F. gnava* queens were all methyl or di-methyl branched hydrocarbons (see Table 2). No normal alkanes were detected. Soon after attacking a *F. gnava* queen, the hydrocarbon profile of the newly mated *P. breviceps* queen changed dramatically to one that approximated the profile of a *F. gnava* queen (Figure 1). Mass spectral analysis indicates that the hydrocarbon peaks found on the cuticle of a *P. breviceps* queen that had attacked a *F. gnava* queen contained hydrocarbon components that were identical to those found on host *F. gnava* queens (Table 2, Figure 1).

A plot of the two largest principal components, which account for 58% of the total cumulative variance, for Group 1 (*P. breviceps* associated with *F. gnava*, see Figure 3a) shows that newly mated *P. breviceps* queens were clearly separated from *P. breviceps* queens that had attacked a *F. gnava* queen, *F. gnava* queens, and *F. occulta* queens. *Polyergus breviceps* queens that had attacked a *F. gnava* queen, however, clustered with *F. gnava* queens, indicating hydrocarbon profiles of newly mated *P. breviceps* queens changed after attacks. *Formica gnava* queens killed by *P. breviceps* had variable amounts of the saturated hydrocarbons associated specifically with *P. breviceps* newly mated queens, an indication that there was some reciprocal transfer of cuticular compounds during the interactions between parasite and host queens. *Formica occulta* queens were distinctly separated from the three groups defined above on the second principal component.

The chromatograms of newly mated *P. breviceps* queens reared in nests with *F. occulta* host workers are qualitatively similar to those of queens reared in nests

TABLE 2. COMPOUND STRUCTURES OF PEAKS IN HYDROCARBON PROFILES OF *P. breviceps* QUEENS FROM NESTS WITH *F. gnava* HOST WORKERS THAT EITHER WERE NEWLY MATED OR KILLED A *F. gnava* QUEEN, AND OF *F. gnava* QUEENS

Peak number	Carbon number	Structures	Kovat index	Queens		
				Newly mated <i>P. breviceps</i>	<i>P. breviceps</i> that killed <i>F. gnava</i>	<i>F. gnava</i>
1	25	11-;12-MeC ₂₄	2425		+ ^a	+
2	25	2-MeC ₂₄	2462		+	+
3	26	2,10-; 2,12-;2,14-DiMeC ₂₄	2492		+	+
4	25	n-C ₂₅ :0	2500	+		
5	26	9-;11-;13-MeC ₂₅	2528		+	+
6	27	9,13-;11,15-DiMeC ₂₅	2561		+	+
7	27	5,11-;5,13-;5,15-DiMeC ₂₅	2580		+	+
8	27	2,10-;2,12-;2,14-DiMeC ₂₅	2595		+	+
9	26	n-C ₂₆ :0	2600	+		
10	27	10-;11-;12-;13-MeC ₂₆	2625		+	+
11	27	2-MeC ₂₆	2669	+	+	+
12	28	2,12-;2,14-;2,16-DiMeC ₂₆	2697		+	+
13	27	n-C ₂₇ :0	2700	+		
14	28	9-;11-;13-MeC ₂₇	2734		+	+
15	28	n-C ₂₈ :0	2800	+		+
16	29	n-C ₂₉ :0	2900	+		

^aIndicates peak presence.

with *F. gnava* host workers, but the peaks differ in relative amounts (compare Figure 1a with Figure 2a). The cuticular hydrocarbons are composed primarily of normal hydrocarbons (C24 through C29). Minor components, 2-methyl branched hydrocarbons associated with even carbon backbones (C24, C26, C28), are also sometimes present in detectable amounts. The cuticular hydrocarbon profiles of *F. occulta* queens (Figure 2c) are more complex than those of *F. gnava* queens (Figure 1c). Like *F. gnava* queens, however, the cuticular hydrocarbons of *F. occulta* queens are composed exclusively of methyl and di-methyl branched compounds (Table 3). No normal alkanes were detected. Similar to *P. breviceps* queens that attacked a *F. gnava* queen, soon after attacking a *F. occulta* queen, the hydrocarbon profiles of the *P. breviceps* queens changed, mimicking the profile of *F. occulta* queens (Figure 2). Mass spectra of cuticular hydrocarbon peaks of the parasite *P. breviceps* queen after attacking a *F. occulta* queen were identical to

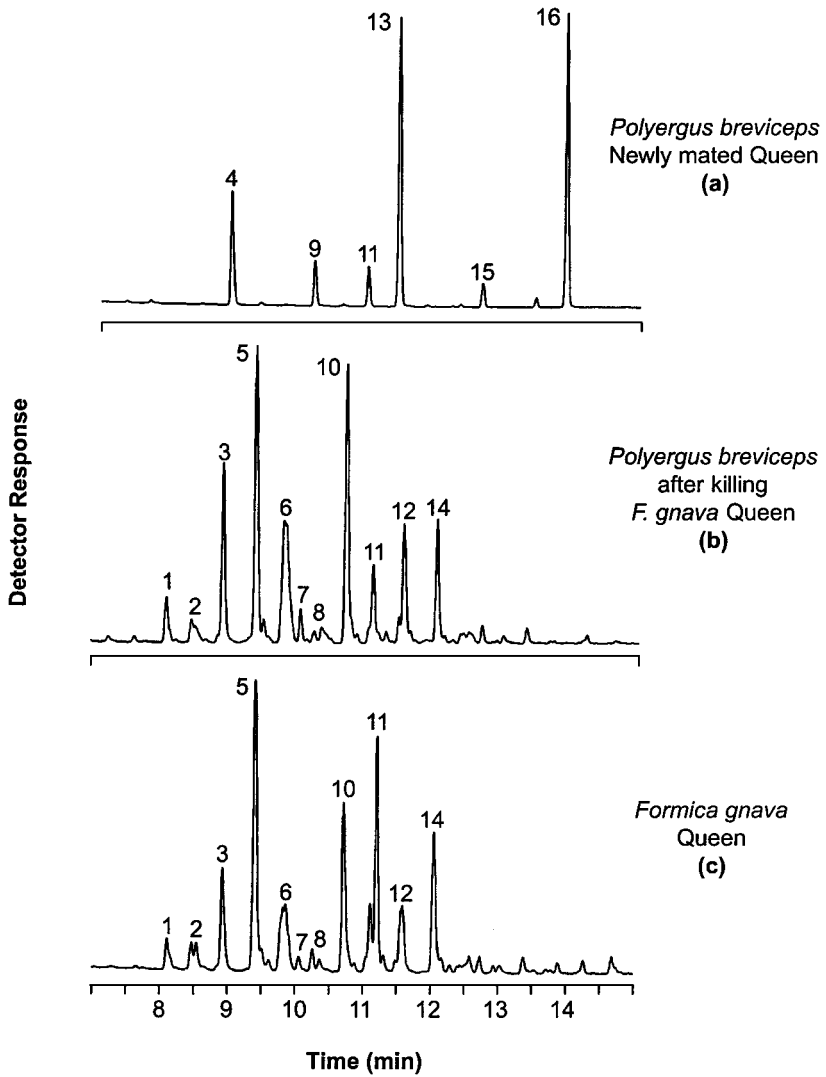


FIG. 1. Representative gas chromatographic profiles of cuticular hydrocarbons from *P. breviceps* and *F. gnava* queens. (a) Newly mated *P. breviceps* queen from nest containing *F. gnava* slaves. (b) *Polyergus breviceps* queen after attacking a *F. gnava* queen. Note the presence of chemical peaks that correspond to peaks in the profile of a *F. gnava* queen (c). Compare with Figure 2.

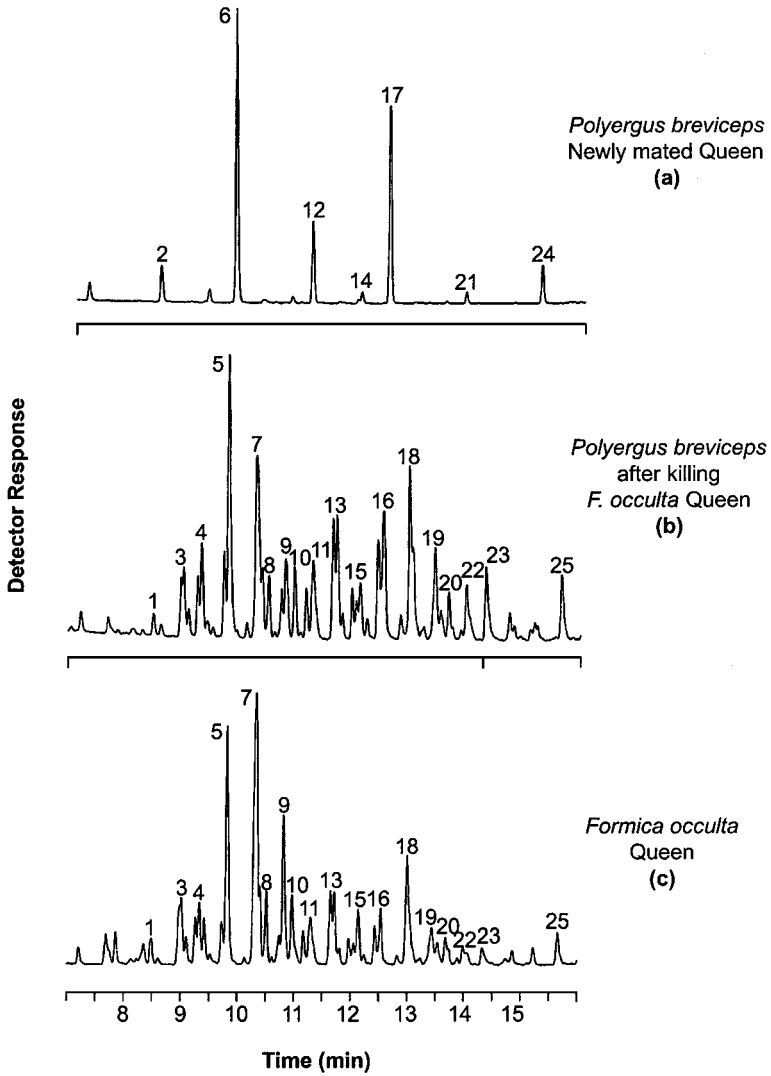


FIG. 2. Representative gas chromatographic profiles of cuticular hydrocarbons from *P. breviceps* and *F. occulta* queens. **(a)** Newly mated *P. breviceps* queen from nest containing *F. occulta* slaves. **(b)** *Polyergus breviceps* queen after attacking a *F. occulta* queen. Note the presence of chemical peaks that correspond to peaks in the profile of a *F. occulta* queen **(c)**. Compare with Figure 1.

TABLE 3. COMPOUND STRUCTURES OF PEAKS IN HYDROCARBON PROFILES OF *P. breviceps* QUEENS FROM NESTS WITH *F. occulta* HOST WORKERS THAT EITHER WERE NEWLY MATED OR KILLED A *F. occulta* QUEEN, AND OF *F. occulta* QUEENS

Peak number	Carbon number	Structures	Kovat index	Queens		
				Newly mated <i>P. breviceps</i>	<i>P. breviceps</i> that killed <i>F. occulta</i>	<i>F. occulta</i>
1	25	5,13-;5,15-; 5,17-DiMeC ₂₃ ; 2,6-DiMeC ₂₃	2380–2404		+ ^a	+
2	24	n-C ₂₄ :0	2400	+		
3	25	6-;8-;10-;12-MeC ₂₄	2425		+	+
4	25	4-;2-MeC ₂₄	2462		+	+
5	26	2,10-; 2,12-;2,14-; 2,16-DiMeC ₂₄	2492		+	+
6	25	n-C ₂₅ :0	2500	+		
7	26	9-;11-;13-MeC ₂₅	2528		+	+
8	26	7-MeC ₂₅	2545		+	+
9	27	7,11-;9,13-;11,15- DiMeC ₂₅	2561		+	+
10	27	5,11-;5,13-;5,15-;5, 17-;5,19-DiMeC ₂₅	2580			+
11	27	3,9-;3,11-;3,13- DiMeC ₂₅	2595–2604		+	+
12	26	n-C ₂₆ :0	2600	+		
13	27	6-;8-;10-; 12-;14-MeC ₂₆	2625–2631		+	+
14	27	2-MeC ₂₆ :0	2669	+	+	+
15	28	8,12-;10,14-DiMeC ₂₆	2657–2676		+	+
16	28	2,6-;2,8-;2,10-;2,12-; 2,14-DiMeC ₂₆	2690–2697		+	+
17	27	n-C ₂₇ :0	2700	+		
18	28	9-;11-;13-MeC ₂₇	2734		+	+
19	29	7,11-;9,13-;11,15- DiMeC ₂₇	2768–2776		+	+
20	29	5,12-;5,15-;5,17-; 5,19-DiMeC ₂₇	2785		+	+
21	28	n-C ₂₈ :0	2800	+		
22	29	3,11-;3,13-;3,15- DiMeC ₂₇	2802–2810		+	+
23	29	6-;10-;12-;14-MeC ₂₈	2825		+	+
24	29	n-C ₂₉ :0	2900	+		
25	30	11-;13-;15-MeC ₂₉	2930		+	+

^aIndicates peak presence.

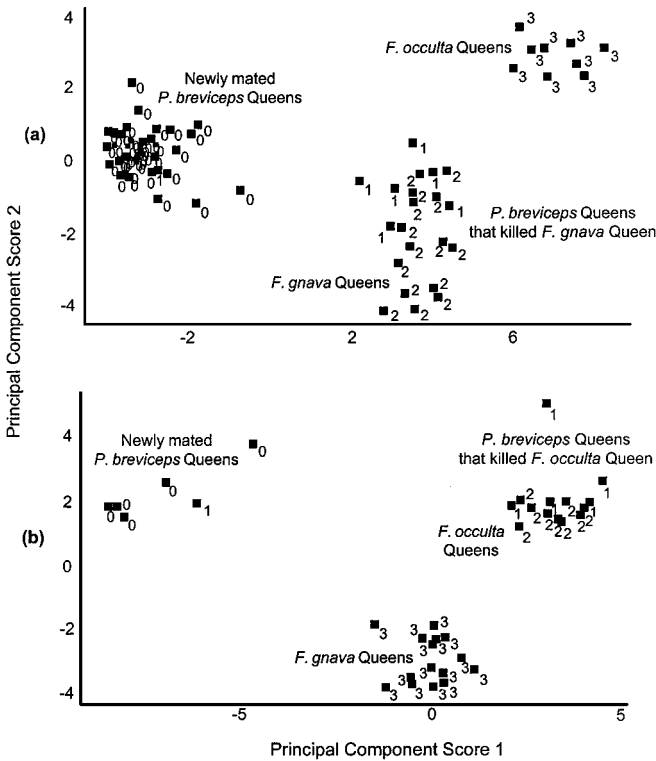


FIG. 3. Principal component maps of cuticular hydrocarbons from *P. breviceps*, *F. gnava*, and *F. occulta* queens. (a) 0 = newly mated *P. breviceps* queens from nests containing *F. gnava* slaves, 1 = killer newly mated *P. breviceps* queens from nests containing *F. gnava* slaves, 2 = *F. gnava* queens, 3 = *F. occulta* queens. (b) 0 = newly mated *P. breviceps* queens from nests containing *F. occulta* slaves, 1 = killer newly mated *P. breviceps* queens from nests containing *F. occulta* slaves, 2 = *F. occulta* queens, 3 = *F. gnava* queens.

those found on host *F. occulta* queens (Table 3, Figure 2). *Formica occulta* queens killed by *P. breviceps* queens seemed to acquire certain specific hydrocarbons associated with *P. breviceps* queens during the conflict, thus confirming again that there was some mutual transference of cuticular compounds.

A plot of the two largest principal components, which account for 61% of the total cumulative variance, for Group 2 (*P. breviceps* associated with *F. occulta*, see Figure 3b), shows that newly mated *P. breviceps* queens are well separated from newly mated *P. breviceps* queens that had just killed an *F. occulta* queen, *F. occulta* queens, and *F. gnava* queens. *Polyergus breviceps* queens that killed a *F. occulta* queen clustered among *F. occulta* queens. *Formica gnava* queens were distinctly separated from these groups.

DISCUSSION

Chemical Camouflage and Adoption of the Polyergus Queen. *Polyergus breviceps* queens, in taking over a *Formica* colony, must be accepted by adults that have not been exposed previously to her odor. It is clear from initial *Formica* worker aggression directed at *P. breviceps* queens invading a nest, that *P. breviceps* queens are not naturally candidates for adoption. The qualitative differences that we found in hydrocarbons between newly mated *P. breviceps* queens and host *Formica* queens was, therefore, none too surprising and indicate that *P. breviceps* and its host species have not co-evolved such that queen cuticular hydrocarbon profiles, and probably other relevant chemical profiles, are inherently similar. Instead, integration of parasite queen among host workers seems to require several diverse tactics, one of which is chemical camouflage. *Polyergus* queens first ward off attackers with secretions from the Dufour's gland while attacking the resident queen (Topoff et al., 1988; D'Ettoire et al., 2000; Mori et al., 2000 a,b). However, soon after the attack on the host queen ceases, host workers aggregate around the parasite queen and begin grooming her. Sampling of the cuticular hydrocarbons from newly mated queens and from attackers, just as attacks were completed, indicated that the newly mated queen profiles transformed dramatically, containing at first simple hydrocarbons and then harboring complex methyl and di-methyl branched hydrocarbons. These new profiles were virtually identical both in peak quality and relative proportion to the queen profiles of the species of queen attacked, a result similar to that discovered by Errard and D'Ettoire (1998) for *P. rufescens*. This, concomitant with the rapidity of the chemical change and modification in host worker behavior, suggests that the emergence of new chemical components (adoptive properties?) is not due to a biosynthetic change triggered by the aggressive interaction, at least initially, as has been proposed for other social parasites (Bagnères et al. 1996). Of course, only tracing the movement of labeled hydrocarbons could demonstrate chemicals were indeed transferred. It should be furthermore noted that, if this is a system of chemical transfer, it is not perfect. In two instances, profiles of *P. breviceps* queens that had killed a *Formica* queen (one *F. gnava* and one *F. occulta*) did not cluster with the respective *Formica* species, but clustered with newly mated *P. breviceps* (Figures 3a and b).

In the socially parasitic wasp, *Polistes atrimandibularis* Zimmermann, the profile change does undoubtedly seem to be the result of a change in metabolic pathway in addition to adsorption of host chemicals (Bagnères et al., 1996). Over a period of several months, unsaturated hydrocarbons of *P. atrimandibularis* queens that have invaded a nest of *Polistes biglumis bimaculatus* Geoffroy disappear from the parasite cuticle and are replaced with the saturated products of the host species. Generally, the invasion by *P. atrimandibularis* is a passive one and seems to be restricted to usurping incipient nests of its host. This is unlike the strategic invasion by queens of the socially parasitic wasp *Polistes sulcifer*

Zimmermann, which take over nests by expelling or killing the dominant queen of its host species, *Polistes dominulus* (Christ) (Turillazzi et al., 1990). Recently, it was demonstrated that *P. sulcifer* queens also change their chemical profile, resembling the host queen almost completely within 3 days (Turillazzi et al., 2000). The stroking and prolonged licking of host individuals suggest, furthermore, that *P. sulcifer* achieves host chemical similarity by obtaining chemicals directly from the host.

While the acquisition of new cuticular components by newly mated *P. breviceps* queens that have attacked a *Formica* queen is readily explainable, the apparent rapid loss of the n-alkanes in such a short time period is not. Most likely, the abundance of newly acquired material has overwhelmed the original components. Our data, however, were insufficient to determine if this indeed is the case. In the above mentioned species that undergo similar profile changes, pre-invasion or pre-attack profile components are also lost. Although the loss of particular components in these instances takes admittedly longer, one peak in the profile of *P. sulcifer* does show substantial decrease after only 90 min (Turillazzi et al., 2000).

Integration by Other Slave-Maker Species. Queens of other socially parasitic ant species also appear to infiltrate their host species colony by camouflaging themselves with host chemicals. For example, the slave-maker queen, *Leptothorax kutteri* Buschinger, aggressively grooms the host *Leptothorax acervorum* (Fabricius) queen and workers upon invading their nest, resulting in a chemical profile very similar to that of *L. acervorum*. Experimentally preventing a *L. kutteri* queen from grooming host individuals results in aggressive attacks, suggesting that a chemical transfer takes place and that this allows invading queens to become adopted by host workers (Franks et al., 1990). Some dependent colony founding queens, however, do not need to be adopted by adult host workers in order to establish a new colony. The facultative slave-maker, *Formica wheeleri* Creighton, usurps a *F. occulta* nest by invading a nest, inducing the adult inhabitants to flee, and appropriating the brood left behind (Topoff et al., 1990). The workers that emerge from the orphaned brood adopt the slave-maker queen and eventually rear her offspring. Presumably, these newly emerged workers, having been exposed to the *F. wheeleri* queen during early periods of development (Morel, 1983; Errard, 1984, Morel et al., 1988) or soon after eclosion (see Vander Meer and Morel, 1998), incorporate her odor into their nestmate recognition odor template and, thereby, accept her. A preliminary examination of cuticular hydrocarbon data revealed that the queen profiles of *F. wheeleri* and *F. occulta* were not similar, even after a new queen had been residing with *F. occulta* workers for one year (Johnson, 2000). These results promote the contention that the chemical change in *P. breviceps* is not fortuitous.

Newly Mated Queen Profiles. Acquisition of chemicals from a host species queen could facilitate integration of the *P. breviceps* queens among host workers, as well as allow the parasite queen to solicit the particular attention workers often display towards reproductive individuals. Nonetheless, the newly mated

P. breviceps profile should not be dismissed as inconsequential. The cuticular hydrocarbon profiles of *P. breviceps* queens here were clearly simple relative to the profiles of the host species queens. However, the parasite queen profiles were also qualitatively identical to the pupae hydrocarbon profiles of both *F. gnava* and *F. occulta* (Johnson, 2000; Johnson & Vander Meer unpublished data), but differed from pupae profiles of *P. breviceps*! The peaks in profiles of newly mated *P. breviceps* associated with *F. gnava* were, furthermore, of similar relative proportions. Others have noted previously that newly mated *Polyergus rufescens* queens (D'Ettorre et al., 2000) and callows belonging to other species (Lenoir et al., 1999) lack a chemical profile. This "chemical insignificance" is suggested to facilitate integration by minimizing aggression and allowing individuals to acquire the appropriate chemical suit (Lenoir et al., 2001). In the case of newly mated *P. breviceps* gynes, however, it seems that rather than being chemically insignificant, their pupa-like profile may be significant, having similar consequences as those gained by chemically insignificant profiles, such as diminishing aggressive reactions upon invading a nest and facilitating movement towards the host queen.

Visual inspection of hydrocarbon patterns of newly mated *P. breviceps* queens has revealed an additional interesting result. Hydrocarbon components of newly mated *P. breviceps* queens from nests with *F. gnava* slaves differed consistently in relative proportions from newly mated *P. breviceps* queens collected from nests with *F. occulta* slaves (compare Figure 1a with Figure 2a). Because *P. breviceps* are obligatorily dependent on their host species for survival, rearing *P. breviceps* without some chemical influence from their *Formica* host species seems unlikely. However, the clear differences in hydrocarbon profiles of *P. breviceps* female alates and their adult hosts are indicative of minimal chemical interaction. Thus, the consistent differences in cuticular hydrocarbon profiles of newly mated *P. breviceps* queens associated with the two host species could reflect (a) different environmental conditions in each host nest or (b) the emergence of two host races or species. It would be unduly worthwhile to clarify the relationship between these two populations of *P. breviceps*.

Previous Studies on Polyergus Worker Profiles. Not surprisingly, there are some similarities and differences in what we and Errard and D'Ettorre (1998) found with *Polyergus* queen profiles (and the respective changes) and what others have found examining worker profiles of *Polyergus* and other slave-makers and their hosts. The profile from workers of the Japanese slave-maker ant, *Polyergus samurai* Yano, was found to be qualitatively and quantitatively identical to the profile of their host species, *Formica japonica* Motschulsky (Yamaoko, 1990). Differences among colonies and sites for both parasite and host suggested that *P. samurai* synthesize very little of their own cuticular hydrocarbons, receiving the majority from the host species. Habersetzer and Bonavita-Cougourdan (1993) and Habersetzer (1993), on the other hand, found the European slave-maker, *Polyergus rufescens*, to maintain some species specificity in their cuticular

hydrocarbon profiles by retaining five major peaks not found in the profiles of their *Formica cunicularia* host. They also found that the *Formica* lost some of their colony characteristics, although this may be a confounding result of sampling workers from a mixed species nest. Bonavita-Cougourdan et al. (1996, 1997) also found *P. rufescens* to maintain some semblance of specificity by retaining compounds that were not on either of their two potential host species, *F. cunicularia* and *Formica rufibarbis* Fabricius. Their results, however, indicate a complex system of profile convergence that does not involve the transfer of chemicals across species. Instead, the proportions of cuticular hydrocarbons that are shared and normally synthesized by each species are adapted such that the other species profiles are rivaled. *Polyergus*, in the forefront, adjusted its profile the most, but the profile modification by both *Formica* species made them distinct from other monospecific, conspecific colonies.

One might expect similar results from another prominent slave-maker, *Harpagoxenus sublaevis* Nylander. However, *H. sublaevis*, which sometimes maintains two species of *Leptothorax* simultaneously, does not produce their own specific hydrocarbons (Kaib et al., 1993) but acquires constituents through allogrooming. Hence, they contribute nothing to the profiles of their host species. The two *Leptothorax* species, however, modify each other's signatures when both are in a single *H. sublaevis* nest (Kaib et al., 1993).

Differences in integration mechanisms of workers and slave-maker queens that usurp nests of worker adults are not surprising. Typically, both parasite and host workers in slave-maker nests are exposed to the nestmate recognition cues of the other species as immatures or callows, when much of the nestmate recognition template is being formed (see Jaisson 1975a,b; Le Moli and Passetti, 1977, 1978; Le Moli, 1980; Le Moli and Mori, 1982, 1987; Morel et al., 1988). They, therefore, need not rely on camouflage to be accepted by the other species. *Polyergus* queens as dependent colony founders, on the other hand, need an immediate chemical armor.

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