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Host queen killing by a slave-maker ant queen: when is a host queen worth attacking?

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A new colony of the slave-making ant *Polyergus breviceps* is initiated when a newly mated gyne invades a host nest and kills the resident queen. This process seems to result in chemical camouflage of the invading gyne and allows her to usurp the position of colony reproductive. Young, recently mated *Formica* gynes, however, are not attacked. To determine whether worker and/or immature presence is the basis for aggression, we placed eggs, larvae, pupae and workers from mature *F. gnava* queens with newly mated *F. gnava* queens and observed the responses of introduced *P. breviceps* queens. Because no newly mated gyne was attacked, we tested newly mated *F. gnava* queens (1) once they had produced eggs, (2) when the offspring reached the larval, pupal and callow stages of development, and (3) every 2 weeks until aggression ensued. Eventually all *F. gnava* queens were attacked but only 29 weeks after having mated. Thus, although offspring are the ultimate benefit from attacking an established *F. gnava* queen, *P. breviceps* queens detect mature queens using another time-dependent feature that is reliably indicative of reproductive status. The similarity of host queen hydrocarbon profiles, often correlated with reproductive status in other ant species, suggests that other compounds reflect queen fecundity and produce a kairomonal effect, or that another cue signals host queen and colony suitability. Our findings indicate *P. breviceps* gynes have evolved to respond aggressively to a host gyne cue that appears long after mating, preventing attacks on gynes without the workers necessary for colony founding.

Socially parasitic ant species rely on workers of other ant species to fulfil certain tasks that enable a colony to survive, such as foraging, feeding and brood care (Hölldobler & Wilson 1990). New gynes of socially parasitic ant species often lack the fat stores that most nonparasitic ants use to survive the isolation period before the first brood matures (Keller & Passera 1989) and are, therefore, typically incapable of independently founding new colonies. Occasionally, these queens initiate new colonies by departing from their natal nests with a supply of host workers (Marlin 1968, 1969, 1971; Buschinger et al. 1980; Kwait & Topoff 1983; Hölldobler & Wilson 1990). More commonly, they invade nests of other species and are accepted as members. Inquilines, which are socially parasitic species that tend to consist only of reproductives, are generally able to coexist with both the queen and workers of their host colony and frequently produce offspring that also remain, mate and reproduce within the host nest. Founding queens of many dulotic and temporary parasitic species, however, must dispose of the resident queen and sometimes the workers for interspecific bonds to form. Species that take over nests and appropriate only immature brood generally rid the colony of adult inhabitants indiscriminately (e.g. *Harpagoxenus sublaevis*: Buschinger 1974; *Formica wheeleri*: Topoff et al. 1990). In other species, the invader specifically targets the resident queen, either aggressively evicting her from the nest as does, for example, *Protomognathus americanus* (Wesson 1939) and *Harpagoxenus canadensis* (Stuart 1984), or killing her, as, for example, *Bothriomyrmex decapitans* (Santschi 1920) and *Lasius*

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reginae (Faber 1967). These tactics suggest a refined ability on the part of the parasite queen to distinguish between workers and queens, which is not surprising because queens tend to differ from workers both physically and chemically.

Queens of the slave-maker ant *Polyergus breviceps* Emery, however, distinguish between queens of their host species, *Formica gnava* Buckley, relative to their state of maturity. Whereas established (with nest) *F. gnava* queens are attacked almost immediately, *F. gnava* gynes that have yet to form colonies do not induce attacking behaviour (Zimmerli & Topoff 1994). *Polyergus* Latreille species are probably the most formidable of the dulotic taxa, regularly invading and stealing brood from nests of *Formica* Linné to replenish their slave supply. Colony take-overs by newly mated gynes are no less spectacular. Frequently, new gynes mate while running alongside advancing raiders and, apparently, use these raids to locate a *Formica* nest (Topoff & Greenberg 1988; Topoff 1990; Mori et al. 1995). By the time a newly mated *Polyergus* gyne arrives at the raided nest, the raiders have all but completed their pillaging and are returning home. The newly mated gyne then enters the *Formica* nest, and locates and attacks the resident queen. At first resident workers attack the invader, but this aggression diminishes as workers are either repelled (D'Ettorre et al. 2000) or appeased (Topoff et al. 1988; Mori et al. 2000a, b) by a Dufour's gland secretion. Almost immediately after her attack on the host queen ceases, workers begin grooming the slave-maker queen. Recent results suggest that host queen chemicals are transferred to the slave-maker queen quickly and relatively efficiently during attacks (Errard & D'Ettorre 1998; Johnson et al. 2001), and that this transfer may be responsible for the relatively permanent modification in host worker behaviour. Indeed, fatal attacks on *Formica* queens seem to contribute to the success rate of taking over a host nest. Workers are more likely to adopt a *Polyergus* queen if she has killed a queen conspecific to the workers than if she has attacked a queen of another *Formica* species or not attacked a *Formica* queen at all (Zaayer 1967; Topoff et al. 1988, 1990; Topoff & Zimmerli 1993). Denied the opportunity of killing a host queen, *Polyergus* queens tend to be killed by host workers (Topoff et al. 1988; D'Ettorre et al. 1997).

It is evident that established *F. gnava* queens differ from newly mated and alate gynes and that *Polyergus* queens have evolved the ability to detect and respond to these differences. What elicits the aggressive behaviour, however, is unknown and little has been done to investigate the problem. We investigated the nature of the stimulus that induces aggression in the slave-maker ant queen *P. breviceps*. The slave force is critical for the survival of founding *P. breviceps* queens and is something that only an established queen can supply. Hence, there are at least three potential stimuli for aggression: (1) workers, contact with which could reliably 'inform' the invading queen of the available slave supply; (2) immatures, contact with which could 'communicate' future slaves; (3) the queen herself. We first determined whether a newly mated *F. gnava* queen would be attacked by a *P. breviceps* queen when supplemented with foreign eggs, larvae, pupae and workers. Because no newly mated queens were attacked, we retested these queens once they had produced eggs, larvae, pupae and workers, and then every 2 weeks thereafter until aggression occurred, and examined the cuticular hydrocarbon (CHC) profiles of newly mated and established *F. gnava* queens.

Other parasites and parasitoids appear to use CHCs in locating their hosts, and CHCs trigger certain behavioural responses in the parasite that are maladaptive to the cue bearer (host), such as oviposition and feeding (Lewis et al. 1976; Conti et al. 1996; Meiners et al. 1997). Social insects tend to use chemicals as their primary form of communication and thereby maintain colony integrity (Hölldobler & Wilson 1990). The quality, quantity and relative proportions of cuticular hydrocarbons (CHCs) are often characteristic of a species or colony (Bonavita-Cougourdan et al. 1987; Vander Meer et al. 1989; Nowbahari et al. 1990; Dahbi et al. 1996). However, CHCs may also consistently differ within a colony between immatures and adults (Bonavita-Cougourdan et al. 1988, 1989, 1990), between immatures at different stages of development (Bagnères & Morgan 1991; Fénéron & Jaisson 1995; Johnson 2000) and between adults of different castes (Bonavita-Cougourdan et al. 1990) or task-related groups (Wagner et al. 1998). The great abundance of nonpolar compounds relative to other cuticular lipids (Jackson & Blomquist 1976) concomitant with within- and between-colony specificity could enable species and stage specialization by a social parasite.

METHODS

Ant Collections

During June and July 1998, 19 queenright colonies of *Formica gnava* were collected from the Arizona oak–alligator juniper woodland grounds of the Southwestern Research Station of the American Museum of Natural History in the Chiricahua Mountains of southeastern Arizona, U.S.A. (elevation 1646 m). All colonies were monogynous to ensure that the 'established' *F. gnava* queens used in this study were not virgin dealate gynes that are sometimes found in large polygynous colonies. Colonies were kept in large Fluon-coated Tupperware boxes (20.5 × 45 cm and 20 cm high) with original nest soil. We found newly mated *F. gnava* queens (N=32) by searching the ground during the beginning of July when the monsoon season began. Newly mated *P. breviceps* queens (N>90) from four colonies were collected into individual vials as they approached a nest of *F. gnava* being raided by the queens' nestmates during July and August.

Housing

Queens were kept in individual plastic vials with a small moist cotton ball until testing began. After testing, *F. gnava* queens were left in their testing boxes and *P. breviceps* queens were placed either back into their individual vials, if no aggression took place, or with the

Table 1. Percentage of trials in which *Polyergus breviceps* queens attacked established *Formica gnava* and newly mated *F. gnava* gynes, offspring number produced by *F. gnava* gynes at testing and number of days since mating

Formica gnava queen status	*Formica gnava* queen condition	Attacked (%)	Mean no. of offspring at testing	Days after mating
Established	Alone	100 (15) [10 naive:5 exp]*	Unknown	Unknown
Newly mated	Alone	0 (20) [15 naive:5 exp]	NA	1–3
	Foreign eggs, larvae, pupae, callows	0 (12) [10 naive:2 exp]	NA	1–3
	Own eggs (E) [queens retested]	0 (10) [5 naive:5 exp]	E=13	4–13
	Own eggs, larvae (L) [queens retested]	0 (10) [5 naive:5 exp]	E=5.46 L=8.19	14–30
	Own eggs, larvae, pupae (P) [queens retested]	0 (10) [all established; 8–1x/1–2x]†	E=8.43 L=2.17 P=8.61	31–50
	Own eggs, larvae, pupae, callows (C) [queens retested]	0 (10) [all established; 4–1x/3–2x]	E=6.95 L=5.23 P=3.86 C=8.23	51–65‡
	Own eggs, larvae, pupae, callows [queens retested]	100 (4) [all established; 4–1x]	E=4.00 L=2.00 P=5.30 C=19.0	204

NA: Not applicable. Numbers in parentheses=number of trials.
*Test history of *P. breviceps* gynes and their numbers: naive=first time tested; exp=tested earlier with no attacks; established=attacked a *F. gnava* queen and living with host workers.
†Numbers indicate number of times tested in this condition during this period. Different *P. breviceps* queens were used each time testing more than once was necessary.
‡From days 65 to 204, tested weekly for 2 months, then biweekly until aggression ensued.

workers of the *F. gnava* queen that had induced aggression and had been killed. Queens in individual vials were provided with a drop of 1:1 honey/water solution on the cotton ball. Otherwise, colonies received local insects when in Arizona or crickets (Fluker Farms) when in New York, along with the Bhatkar & Whitcomb (1970) diet, 1:1 honey/water solution and water when needed.

Behaviour Tests

We tested all *P. breviceps* queens with a newly mated *F. gnava* queen alone or with a full complement of foreign brood (five eggs, five larvae, five pupae, five callows; Table 1) within 24–72 h after mating. Only experienced *P. breviceps* queens, i.e. those that had been tested with and had killed an established *F. gnava* queen, were available for the remaining tests (Table 1) because only these queens, having been adopted by *F. gnava* workers, survived long enough for further testing. Queens without host workers died within 10–15 days after collection. However, because the newly mated *F. gnava* queens began producing eggs within 4 days, which matured into larvae within 14 days postmating, several of these trials were conducted with recently collected and newly mated *P. breviceps* queens as well as with experienced *P. breviceps* queens (Table 1). We counterbalanced the order in which we first tested *P. breviceps* queens with *F. gnava* queens. For the first trial, some were paired with an established *F. gnava* queen, and some were paired with a newly mated *F. gnava* queen that was alone or had foreign brood placed with her. For the second trial, some *P. breviceps* queens that had been tested already were tested with an *F. gnava* queen of a different state (established, lone or brood-supplemented newly mated; see Table 1).

Tests were conducted between 1500 and 1900 hours, during which raiding and colony take-over normally occur. After the first round of trials, in which no newly mated *F. gnava* queens were attacked, we conducted subsequent trials with the same gynes with the first emergence of individuals at every stage of development (Table 1). When newly mated queens still did not elicit aggression after workers had emerged, we conducted trials weekly for the first 2 months, then bimonthly thereafter, until newly mated *F. gnava* queens were attacked. We recorded the number of offspring produced by the newly mated *F. gnava* queens and the number of days from the date of collection (likely to be day of mating). Periodically, we tested *P. breviceps* queens with

an established *F. gnava* queen to verify that they would still attack an *F. gnava* queen long after having mated and usurped a small colony, which they did.

Thirty minutes before the first introduction of the *P. breviceps* queen, an *F. gnava* queen was placed in a Fluon-coated Tupperware box (20.5 × 35 cm and 9 cm high) lined with soil (thereafter the *F. gnava* queen remained in that box). When the condition included foreign brood, the brood was placed along with the *Formica* gyne in the test box. After testing we removed the foreign brood and the slave-maker gyne. The *F. gnava* gyne, however, was kept in her initial test box, where she eventually produced offspring, which were also left in the test box, and where she was subsequently tested with her offspring for the 'own brood' conditions. We observed queen interactions for 20 min unless an *F. gnava* queen was attacked; in these cases, the observation continued until the *P. breviceps* queen ceased her attack and began wiping her antennae. We had anticipated intermediate acts of aggression from the *P. breviceps* queens. However, because we observed no aggression from *P. breviceps* queens, we recorded attack or no attack for each 20-min test, as well as the latency to attack and attack duration for tests in which attacks occurred.

A chi-square test and, when appropriate, a Fisher's exact test were used to compare attack or no attacks. Mann–Whitney *U* tests were used to compare latencies to attack and total attack duration in minutes between established *F. gnava* queens and newly mated *F. gnava* queens when attacks occurred. Paired *t* tests were used to compare the total number of offspring, eggs, larvae, pupae, or workers produced by newly mated *F. gnava* queens 15 days before inducing aggression with the total number produced at the time aggression was observed. Statview was used to analyse all behavioural data (SAS Institute, Inc., Cary, North Carolina).

Solvent Extraction of Queens

We extracted cuticular components of newly mated and established *F. gnava* queens by placing queens into individual 7-ml scintillation vials and adding enough high-purity hexane (B & J, Muskegon, Michigan, U.S.A.; GC^2 Grade) to cover the entire body (ca. 0.3–0.5 ml). The hexane kills the ants as quickly as ethyl alcohol, a common insect-killing collecting agent. After 10 min, solvent extracts were transferred from the sample to a 2-ml scintillation vial and allowed to evaporate. *Formica gnava* queens attacked by *P. breviceps* queens were immersed immediately after the *P. breviceps* queen ceased her attack. All specimens were preserved for voucher in 70% ethyl alcohol (C.A.J., personal collection).

Chemical Analysis

The evaporated extracts were transported to the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, where they were reconstituted with 0.2-ml hexane, vortexed for 1–2 s, and applied to a small silicic acid (70–230 mesh, 60 Å, Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, U.S.A.) Pasteur Pipette column. Hydrocarbons were isolated from other lipids by eluting the column with hexane. The eluent, containing purified hydrocarbons (ca. 6–7 ml were collected, which from previous experience eluted all hydrocarbons), was evaporated to ca. 10 µl under a steady stream of nitrogen. Samples were analysed by gas chromatography (Varian 3700; Varian Associates, Walnut Creek, California, U.S.A.). The gas chromatograph was equipped with a split–splitless injector, a capillary column (DB–1, 30 m, 0.32 mm internal diameter, 0.25 µm film thickness; Agilent Technologies, J & W Scientific, Inc., Folsom, California, U.S.A.) and flame ionization detector. The injector and detector temperatures were set at 300°C; the oven was programmed from 190° 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 make-up gas. Hydrocarbon standards (C_{16}, C_{18}, C_{20}, C_{22}, no. NP-MIX-H; Alltech Associates, Inc., Deerfield, Illinois, U.S.A.) were injected at regular intervals during sample analysis and used to calculate Kovat Indices. Data were analysed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer Corp., Norwalk, Connecticut, U.S.A.).

Principal Components Analysis

The data were preprocessed for multivariate analysis. We computed the relative proportions of cuticular hydrocarbons by dividing the area given for each cuticular hydrocarbon by the total integrated peak area of the profile, then autoscaling each peak to ensure that it had equal weight in the analysis. Principal components analysis (Jolliffe 1986) was then conducted on 48 normalized variables. One alate *F. gnava* queen and one newly mated *F. gnava* queen were deleted from the data set because the generalized distance test determined them to be outliers ($P<0.01$). The multivariate analysis was performed with Pirouette (Infometrix, Woodinville, Washington, U.S.A.). Total hydrocarbon amounts were analysed with a Kruskal–Wallis test, because even after logarithmic transformation, variances were heteroscedastic (equality of variances *F* test, Statview, SAS Institute, Inc., Cary, North Carolina, U.S.A.).

RESULTS

Behaviour Tests

Newly mated *F. gnava* queens, either alone or with a full complement of foreign immature brood and a few workers, did not trigger aggression from *P. breviceps* queens. However, lone established *F. gnava* queens were attacked in 100% of the trials (chi-square test: $\chi^2_2=45.00$, $P<0.001$; Table 1). *Polyergus breviceps* queens placed with newly mated *F. gnava* gynes spent most of the 20-min trial running around the periphery of the test box or attempting to climb up the Fluon-coated sides. If the *P. breviceps* queen encountered the newly mated *F. gnava*

queen during her meandering, there was brief antennal contact (1–2 s) and an immediate retreat by the *P. breviceps* queen. If the *P. breviceps* queen encountered the *F. gnava* queen near her brood, the *F. gnava* queen sometimes struck at the *P. breviceps* queen with her mandibles. These strikes never resulted in attack by the *P. breviceps* queen, only in retreat. The results clearly indicate that neither immature nor adult *F. gnava* offspring alone triggered attacks by *P. breviceps* queens against established *F. gnava* queens. This conclusion is further supported by the fact that fatal attacks were directed against lone, established queens. In these trials, the established *F. gnava* queens did not strike at *P. breviceps* queens. They either ran away from an approaching *P. breviceps* queen or wiggled beneath the attacking *P. breviceps* queen, sometimes flexing the gaster.

For 190 days after mating, all newly mated *F. gnava* queens tested still failed to elicit aggression from *P. breviceps* queens, although they were producing offspring (Table 1). However, on the next test date, 204 days after mating, 100% of the newly mated *F. gnava* queens that were tested were attacked. Because *P. breviceps* are difficult to maintain in the laboratory without diapause, only four of the *P. breviceps* queens remained alive at this time. Therefore, we tested only four newly mated *F. gnava* queens. None the less, all of these newly mated *F. gnava* queens elicited aggression (Fisher's exact test: $P<0.001$).

In all four cases, attacks on *F. gnava* queens at 204 days postmating resembled attacks on established queens. Neither attack duration (established: $\bar{X} \pm \mathrm{SD} = 19.2 \pm 4.1$ min; newly mated: 24.85 ± 8.8 min; Mann–Whitney U test: $U=16$, $N_1=4$, $N_2=15$, NS) nor latency to attack (established: 0.51 ± 0.45 min; newly mated: 2.64 ± 2.7 min; Mann–Whitney U test: $U=18$, $N_1=4$, $N_2=15$, NS) differed significantly.

A statistical comparison of the number of eggs, larvae, pupae and workers produced by *F. gnava* queens on days 190 and 204 showed no significant differences, although workers had eclosed from existing pupae during this time (Table 2). Fewer larvae were present on day 204, presumably because they had metamorphosed into pupae.

Chemical Analysis

Cuticular hydrocarbon profiles of alate, newly mated and established *F. gnava* queens appear very similar (Fig. 1); mass spectral analysis revealed that the hydrocarbon profiles of the three queen types were qualitatively the same, consisting of methyl and dimethyl branched hydrocarbons (Table 3). None the less, the hydrocarbon profiles of alate *F. gnava* queens can be discriminated from newly mated and established *F. gnava* queens (which cluster together) in a plot of the first and second principal components of the data, which account for 57% of the total cumulative variance (Fig. 2).

The average total hydrocarbon amounts did not differ between the three groups (Kruskal–Wallis test: $H_{27}=2.45$, NS), although this result should be interpreted with discretion, because internal standards were not used during sample processing.

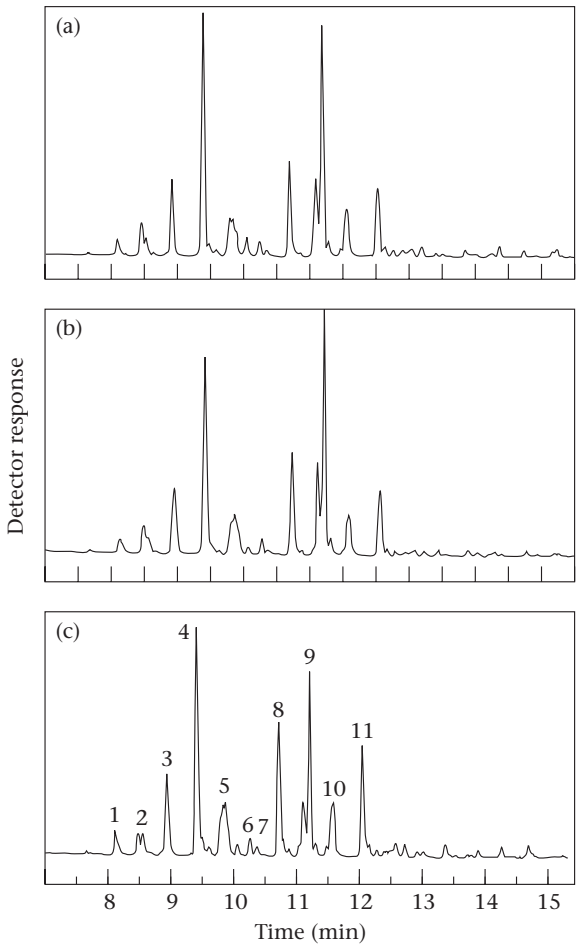

Figure 1. Representative gas chromatograms of hydrocarbons found on the cuticles of (a) alate, (b) newly mated and (c) established *F. gnava* queens. The peak numbers in (c) correspond to those in Table 3.

Table 2. Paired *t* test values comparing offspring number at each developmental stage in nests of newly mated *F. gnava* queens 15 days prior to attack with offspring number on day of attack

Developmental stage compared	Mean difference	t_2	P
Eggs	−0.667	−0.105	0.930
Larvae	7.667	2.95	0.098
Pupae	−1.330	−0.76	0.530
Workers	−5.000	−2.4	0.140

Offspring of queen NMFG#25−98 were not included in analyses because all but one worker escaped before test dates.

DISCUSSION

The presence of, or encounters with, nonreproductive offspring did not stimulate *P. breviceps* queens to attack newly mated *F. gnava* queens. Fatal attacks on lone, established *F. gnava* queens and on all newly mated *F. gnava* gynes tested 204 days after mating, however, strongly suggest that gyne chemistry changes with maturity, and

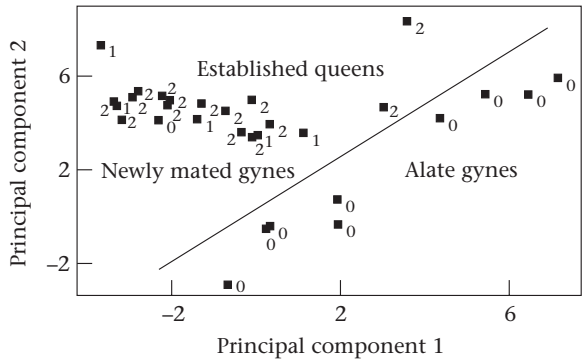

Figure 2. Principal component map of cuticular hydrocarbons from alate (0), newly mated (1) and established (2) *F. gnava* queens. Based on the first and second principal components, which accounted for 57% of the total cumulative variance, newly mated and established *F. gnava* queens clustered together, suggesting no effective differences between profiles; their separation from alate *F. gnava* queens indicates significant differences between profiles.

Table 3. Compound structures of peaks in hydrocarbon profiles of alate, newly mated and established *F. gnava* queens

Peak number	Carbon number	Structures	Kovat Index
1	25	11-;12-MeC_{24}	2425
2	25	2-MeC_{24}	2462
3	26	2,10-; 2,12-; 2,14-DiMeC_{24}	2492
4	26	9-; 11-; 13-MeC_{25}	2528
5	27	9,13-; 11,15-DiMeC_{25}	2561
6	27	5,11-; 5,13-; 5,15-DiMeC_{25}	2580
7	27	2,10-; 2,12-; 2,14-DiMeC_{25}	2595
8	27	10-; 11-; 12-; 13-MeC_{26}	2625
9	27	2-MeC_{26}	2669
10	28	2,12-; 2,14-; 2,16-DiMeC_{26}	2697
11	28	9-; 11-; 13-MeC_{27}	2734

that this change may induce aggression in *P. breviceps* queens. The qualitative, peak relative proportion and quantitative similarity of cuticular hydrocarbon profiles of newly mated and established *F. gnava* queens indicate that cuticular hydrocarbons were probably not the cue to attack. We find these results surprising, considering that ovarian development (Röseler et al. 1980; Ross & Gamboa 1982; Turillazzi et al. 1982) and cuticular hydrocarbon production (Adams et al. 1984; Blomquist et al. 1984; Trabalon et al. 1990; Schal et al. 1991) are under hormonal control in several insect taxa, and that cuticular hydrocarbon profiles are associated with the degree of ovarian development in several ant species (e.g. Monnin et al. 1998; Peeters et al. 1999; Liebig et al. 2000).

The stimulus that released aggression in *P. breviceps* queens appeared to be all or none: there were no signs of aggression at least 191 days before the day on which aggression was observed; then the attacks were full blown. The releaser stimulus thus appears to develop over time and may also be a product of host queen maturation as defined by ovarian development. Although we did not take typical measures of ovarian development, it is likely that the ovaries of newly mated gynes were developed and functional long before we observed aggression, because 19 ± 3 ($\bar{X} \pm$ SD) workers had been produced by the gynes. Perhaps there is a threshold of ovarian development that newly mated queens must exceed for the corresponding cue to emerge. Our sampling of host queen chemistry was limited to hydrocarbons for practical reasons, as well as because hydrocarbons (1) make up a significant proportion of cuticular lipids, (2) have been implicated in mediating nestmate recognition, and (3) produce a kairomonal effect in other insect taxa (Lockey 1980, 1988; Blomquist & Dillworth 1985). Thus, to attribute the production of aggressive reactions specifically to polar chemicals or even other nonpolar chemicals requires further investigation. None the less, most investigations into the chemistry of social insects have been restricted to hydrocarbons. It is not farfetched that in these instances the differences in hydrocarbon profiles do not provide the entire story, and that with more intense sampling, investigations will reveal at least a seminal contribution of other compounds to nestmate recognition and the like (e.g. Breed 1998; Vander Meer & Morel 1998).

Why Not Target Newly Mated *F. gnava* Queens?

From an ultimate evolutionary standpoint, a newly mated *P. breviceps* queen might avoid attacking a newly mated *F. gnava* gyne because she lacks workers. Without workers, a young gyne will die within 10–15 days after mating in the laboratory (C. Johnson, personal observation), and probably sooner in the field. Typically but not always, newly mated *P. breviceps* queens locate established *Formica* queens by following raiding nestmates to a *Formica* nest (Topoff & Greenberg 1988; Topoff et al. 1988). A newly mated *P. breviceps* queen thus may encounter an *F. gnava* queen that has not yet founded a colony. If such an encounter were to take place and result in attack, a newly mated *P. breviceps* queen would have expended substantial energy without gaining a worker force, possibly even handicapping a future attempt at usurping an *F. gnava* nest. By being able to discriminate between queens with and without colonies, a *P. breviceps* queen can avoid attacking host queens that are unable to supply at least a minimal workforce. By day 204 in our experiment, newly mated *F. gnava* queens had produced, on average, 19 workers. This number may be sufficient to support a newly mated queen; however, we did not test for this. This point may be irrelevant, because at this stage of testing it was midwinter, and invasions by new *P. breviceps* queens would not take place until summer when an incipient nest would undoubtedly be larger.

From a proximate evolutionary standpoint, a *P. breviceps* gyne may not target a newly mated *F. gnava* gyne because the latter lacks the chemicals necessary for a slave-maker queen to usurp a host nest. Upon attacking a host queen, the *Polyergus* queen acquires chemicals from the host queen that result in her being accepted by host workers when invading a nest (Errard & D'Ettorre 1998; Johnson et al. 2001). Attacking a chemically wrong queen

might result in the slave-maker queen being treated by newly enslaved workers as merely a subordinate or a newly mated queen, whose position in the colony may be tenuous, preventing the slave-maker queen from establishing herself as the colony reproductive. If true, this would also suggest that the cuticular hydrocarbons of the species involved represent only some of the chemicals that may be involved in queen–worker recognition. The cuticular hydrocarbon profiles of newly mated and established *F. gnava* queens did not differ in our study and are the same profiles that were acquired by the *P. breviceps* queen during attack (Johnson et al. 2001). Therefore, if hydrocarbon transfer alone was the critical event in the adoption of the parasite queen, then those hydrocarbons could come from newly mated or established *F. gnava* queens. Conversely, the cuticular hydrocarbon profile of a newly mated *F. gnava* queen might be adequate to secure adoption, but the cue that induces aggressive behaviour in *P. breviceps* queens may be lacking.

Why Do *F. gnava* Queens Differ?

Our results show that *P. breviceps* queens have evolved the ability to detect differences between newly mated and established queens of *F. gnava*, and to respond in a manner that is likely to increase a *Polyergus* queen's chances of establishing a colony. This, however, raises an additional question. Why do newly mated *F. gnava* queens differ from established *F. gnava* queens at all? We propose two, not mutually exclusive, hypotheses. Female reproductives, which contribute most of the viable eggs in a colony, maintain a chemical relationship with nonreproductive members that is distinct from typical nestmate recognition (Hölldobler & Wilson 1983; Heinze et al. 1992; Chen & Vinson 1999). Reproductive queens in polygyne nests that draw most worker attention towards themselves (or at least bring nonreproductives closer relative to other individuals) may annex greater reproductive success, because their chance of being fed and groomed is greater. In several ant species, the alpha queen stands apart from others with respect to her cuticular hydrocarbon profile and tends to produce more offspring (e.g. Monnin et al. 1998; Peeters et al. 1999; Liebig et al. 2000; Cuvillier-Hot et al. 2001). Newly mated *F. gnava* queens may simply differ from established queens because they are still subordinate in a hierarchical polygynous colony.

That nests of *F. gnava* are either monogynous or polygynous suggests that some queens establish nests independently and others mate intranidally or seek adoption after mating flights. For new gynes to form or maintain associations with residents of an established nest, the tendency of colony members to reject individuals that pose a threat to fitness must be attenuated. Maintaining an innocuous signal might lessen a newly mated gyne's chances of being rejected or ejected from a colony.

The success of *P. breviceps* rests on their specialized ability to exploit the foraging, feeding and brood care behaviours of their *Formica* host species at all stages of the parasite life cycle. During colony founding, a new *P. breviceps* needs to find a host nest and to convert host worker hostility into queen care in order to bring in new generations of slave-makers. As our understanding of *Polyergus* increases, it becomes more apparent that the adaptations needed to accomplish these tasks are abundant and finely tuned. The ability to redirect worker behaviour favourably towards the slave-maker queen requires several processes that seem to be linked. First, merely ridding a host colony of its queen is insufficient for immediate usurpation. Aggressive attacks by host workers on an invading slave-maker gyne seem to transform into grooming behaviour only if the slave-maker gyne has attacked the resident queen (Zaayer 1967; Topoff et al. 1988, 1990; Topoff & Zimmerli 1993; Mori et al. 1995). This, along with the dramatic changes in *P. breviceps* gyne cuticular chemistry that are specific to the species of host queen killed (Johnson et al. 2001), strongly suggests that cuticular camouflage is crucial for the conversion of host worker behaviour and that camouflage is at least initially accomplished only via attacks on the host queen. Second, our results indicate that it may not be sufficient to attack just any reproductive female. Instead, an attacked gyne needs to have at least a small colony of workers and may also need to have the cuticular signature that allows the slave-maker to take over the nest.

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