

Original article

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Abraham HefetzNestmate recognition in the ant *Cataglyphis niger*: do queens matter?

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Abstract This study compares two basic models for the origin and maintenance of colony gestalt odor in the polygynous ant species *Cataglyphis niger*. In the first model, queens are centers of de novo biosynthesis and distribution of recognition odors ("queen-centered" model); in the second, colony odors are primarily synthesized and distributed by workers ("worker-centered" model). We tested the behavioral patterns that are predicted from each model, and verified by biochemical means the distributional directionality of these signals. Encounters between nestmates originating from split colonies were as amicable as between nestmates from non-split colonies; queenless ants were as aggressive as their queenright nestmates, and both were equally aggressed by alien ants. These results indicate that queens have little impact on the recognition system of this species, and lend credence to the worker-centered model. The queen-centered model predicts that unique queen substances should be produced in appreciable quantities and that, in this respect, queens should be more metabolically active than workers. Analysis of the chemical composition of postpharyngeal glands (PPGs) or cuticular extracts of queens and workers revealed high similarity. Quantitatively, queens possessed significantly greater amounts of hydrocarbons in the PPG than workers, but the amount on the thoracic epicuticle was the same. Queens, however, possess a lower hydrocarbon biosynthesis capability than workers. The biochemical evidence thus refutes the queen-centered model and supports a worker-centered model. To elucidate the directionality of cue distribution, we investigated ex-

change of hydrocarbons between the castes in dyadic or group encounters in which selective participants were prelabeled. Queens tended to receive more and give less PPG content, whereas transfer to the epicuticle was low and similar in all encounters, as predicted from the worker-centered hypothesis. In the group encounters, workers transferred, in most cases, more hydrocarbons to the queen than to a worker. This slight preference for the queen is presumably amplified in a whole colony and can explain their copious PPG content. We hypothesize that preferential transfer to the queen may reflect selection to maintain her individual odor as close to the average colony odor as possible.

Key words Nestmate recognition · Colony odor · Postpharyngeal gland · Hydrocarbons · *Cataglyphis*

Introduction

Models for nestmate recognition in social insects predict that in large colonies the recognition signal is a blend of individual signals to give a unified colony odor, a "gestalt" (Crozier and Dix 1979; Crozier 1987). Recent experimental evidence has shown that hydrocarbons, postulated as recognition cues in ants, are exchanged between nestmates, providing a mechanism for creating the gestalt odor (Soroker et al. 1994, 1995a; Meskali et al. 1995; Vienne et al. 1995). Odor sharing reduces individual worker variability within the nest, explaining the observed similarity in cuticular hydrocarbons between members of the same nest, and it also accentuates the genetically based intercolonial odor differences (Bonavita-Cougourdan et al. 1987; Vander Meer et al. 1989; Henderson et al. 1990; Nowbahari et al. 1990; Provost et al. 1993). It was further demonstrated that the postpharyngeal gland (PPG) in several formicine and myrmecine species acts as a "gestalt organ" serving as a reservoir from which the ants refresh their own cuticular hydrocarbons and exchange them with nestmates (So-

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rocker et al. 1995a; Vienne et al. 1995). The use of a glandular reservoir for storing recognition cues is adaptive since it enables the incorporation and admixing of genetically based worker or queen discriminators, as well as odors that originate from the diet or the environment. These can be rapidly distributed in the nest through various social actions (i.e., trophallaxis and allogrooming) regardless of whether they originate from a single or several contributors.

There are no general rules specifying the relative importance of queen- and worker-derived nestmate recognition cues, but two models seem to emerge from the published literature. One hypothesis is that the queen is a dominant factor in determining the nature of the label ("queen-centered" model). The alternative hypothesis stresses the prevalence of worker-derived cues, and places them at the distributional center of colony odor ("worker-centered" model). The experimental evidence supporting or refuting these alternative models is scant, making generalizations as yet impossible. Using heterospecific and homospecific cross-fostering in several species of *Camponotus*, it was demonstrated that the queen has a decisive role in the formation of colony odor and that her own label masks any innate label that workers may possess (Carlin and Hölldobler 1983, 1986, 1987). At least in large colonies, fecund queens were much more effective than infertile ones in transferring their label to workers. *Leptothorax lichtensteini* workers separated into two groups for 120 days will not reunite unless the queen is transferred between the two groups during the period of separation (Provost 1989). Recently, comparative chemical analysis of cuticular hydrocarbon profiles of artificially created colonies of *Messor barbarus* with one, two, or three queens revealed differences that were interpreted as queen contribution (Provost et al. 1994). All these investigations support the queen-centered model.

In contrast, experiments with *Rhytidoponera confusa* have shown that workers that were separated from their mother colony and housed with an alien queen were treated upon their return by their original nestmates in the same manner as the latter treated control nestmates. The resident workers were nevertheless highly aggressive towards non-nestmate non-related workers (Crosland 1990). Likewise, *L. curvispinosus* workers kept in isolation from the pupal stage were readily accepted by their parent colonies when reintroduced as adults (Stuart 1987). These studies indicate that worker discriminators contribute more than queen discriminators to colony odor, lending credence to the worker-centered model.

The different results may reflect different social structures (e.g., monogyny as opposed to polygyny; populated vs. unpopulated nest), or may reflect disparate evolutionary trends. All these examples utilize either bio-behavioral or chemical experiments, but lack a direct biochemical/behavior approach. Our recent development of a method for following the biosynthesis and transfer of hydrocarbons in the polygynous species

Cataglyphis niger enabled a more direct assessment of the relative contributions of the queen and workers to the colony odor, using the hydrocarbon constituents of the PPG as a model for recognition cues.

The objective of this study was to test the predictions stemming from these two basic models for the origin and maintenance of colony gestalt odors. The queen-centered model states that queens are the origin of de novo biosynthesis of recognition odors that are subsequently distributed among the workers. The predictions from this model are that the queen probably biosynthesizes de novo queen-specific cues in appreciable quantities, and is more active metabolically, establishing her as the "center of the gestalt," and that queenless workers will change or lose these cues compared to their queenright nestmates, resulting in augmented aggression between the two groups. The worker-centered model states that colony odors are primarily synthesized and distributed by workers. The predictions from this model are that there is no center of the gestalt, that mutual aggression between nestmates from split colonies will not develop, and that the queen(s) were selected to develop mechanisms placing them "in the center of the gestalt" as opposed to "being the center of the gestalt" as predicted from the alternative model.

We have investigated these alternative models in the polygynous species *C. niger* (1) by testing whether aggression between nestmate workers developed after splitting colonies into queenright and queenless groups, (2) by qualitative and quantitative comparisons of the hydrocarbon composition of the PPG secretions between queens and workers and assessment of the extent of their de novo biosynthesis, and (3) by investigating the exchange of PPG hydrocarbons between queens and workers to clarify the direction in which recognition cues flow through the colony.

Methods

Collection and maintenance of the ant colonies

Colonies of *C. niger* were collected around Tel-Aviv, Israel, between October 1993 and February 1995, and included the brood, queens, and workers. In the laboratory, the ants were transferred to artificial nests, the nest population size was estimated, and the number of queens determined. The nests were placed in a rearing room under a controlled temperature of $28 \pm 2^\circ\text{C}$ and a photoperiod of 14L:10D. The ants were provided with an identical diet of sugar water and minced insects three times a week. Newly emerged female alates were color marked within 48 h of emergence.

Behavioral assay

Behavioral assays to assess the effect of the queen on the aggressive behavior of workers were conducted with randomly selected ants from queenright or queenless colonies (two parts of the same colony) that were challenged either with a nestmate or with alien ants from a queenright colony. Queenless colonies comprising at least 200 workers were created by isolating worker ants from three unrelated polygynous colonies (4–14 queens) for at least 5 months. All

alien workers originated from the same queenright colony. Each alien ant was randomly tested twice: once against a worker from a queenright colony and once against a worker from the respective queenless fragment. Encounters between nestmates served as a control. All the encounters were dyadic (two workers per encounter) and were conducted in a neutral arena (9 cm diameter, lined with a filter paper that was changed after each test). Before each test, workers were acclimatized to the arena for 2 min by placing them on the arena surface confined in separate glass tubes. The test began by removing the tubes, and the behavior of both ants was recorded for 2 min. All encounters were videotaped. The behavior of one of the ants was monitored during the actual test using a computerized event recorder, while the behavior of the second ant was monitored in the same manner by viewing the videotape. Aggression was scored using the following behavioral acts: 0, antennation; 1, mandibular threat; 2, short biting with jumping; 3, biting; 4, spraying of formic acid. The aggression index was calculated as follows:

$$\frac{\sum_{i=1}^n Ali * ti}{T}$$

where *Ali* and *ti* are the aggression score and duration of each act respectively, and *T* is the total interaction time defined as the sum of times in which the ants were in physical contact.

Chemical analysis

Quantification of hydrocarbons in the PPG secretion and epicuticular extracts of queens and workers was performed by gas chromatography (on-column injector) using an SE 54 capillary column temperature-programmed from 60 °C to 100 °C at 20 °C/min then from 100 °C to 270 °C at 5 °C/min. Tricosane, 750 ng per sample, was added as an internal standard. The quantities of each compound were assessed by peak integration. The identity of the peaks was ascertained by gas chromatography/mass spectrometry (VGM 250Q, EI).

Measurements of body size and reproductive status

Head width and thorax width and length were measured under a dissecting microscope using a scaled ocular. Head width was determined as the distance between the two compound eyes. Thorax surface area was estimated by assuming that it is an approximation of a symmetric ellipsoid that revolves around its large axis, using the implicit formula:

$$S = 4\pi ab \int_0^1 \sqrt{1 + \left(\frac{a^2}{b^2} - 1\right)u^2} du$$

where *a* is the small half axis, *b* is the large half axis, and *u* is a surface unit.

The ants were weighed with a Sartorius 1518 MP8 analytical balance to an accuracy of 0.1 mg. Ovarian development was assessed by scoring the number of chorionated oocytes and measuring the length of the largest oocyte.

Biosynthesis of hydrocarbons in queens and workers – in vivo studies

Hydrocarbon biosynthesis in workers and queens was measured as follows. Either 1 µCi [1-¹⁴C]sodium acetate (56 mCi/mmol, NEN, Boston, Mass.) or [1-¹⁴C]sodium propionate (51 mCi/mmol, NEN) was dissolved in 0.5 µl medium (according to Katase and Chino, 1984) and injected into the hemolymph through the abdominal intersegmental membrane. Twenty-four hours postinjection, the ants were sacrificed by freezing, dissected, and the amount of labeled hydrocarbons in various body parts was determined. PPGs

and ovaries were extracted for 24 h in 100 µl pentane. Epicuticular hydrocarbons were extracted by immersing thoracic carcasses in 400 µl of pentane for 5 min. All extracts were subjected to thin-layer chromatography as previously described (Soroker et al. 1994). Radioactivity of the hydrocarbon fraction was detected by exposure on an image plate for 22 h using a Phosphor-Imager Autoradiography System (Fuji BAS 100 analyzer).

Hydrocarbon transfer between workers and queens

Donor ants were injected with [1-¹⁴C]sodium acetate or sodium propionate as described above and after 24 h incubation (26 °C) each was presented with a prestarved (for 24 h) nestmate (recipient) in a petri dish (9 cm diameter). All encounters were stopped 24 h later by freezing the ants, and both donor and recipient were monitored for the presence of labeled hydrocarbons in the PPG and on the epicuticle as described above. The magnitude of labeled-hydrocarbon transfer to the various recipients was calculated individually for each pair. This was expressed as the percentage of labeled hydrocarbons present in the recipient's tissue (i.e., PPG or epicuticle) relative to the total labeled hydrocarbons found in the donor and recipient (PPG + epicuticle) taken together.

To assess whether there is a preference in transfer of hydrocarbons to queens or workers, eight donor ants were injected with [1-¹⁴C]sodium acetate as described above and after 24 h of incubation (26 °C) they were presented with two recipients: a worker and a queen, in a plastic box (9 cm diameter and 8 cm height). All encounters were stopped 5 days later by freezing the ants, and levels of labeled hydrocarbons in the PPG and on the epicuticle in the donors and recipients were measured as described above. Transfer to the worker or the queen was assessed for each group separately as described above. The transfer experiments were accompanied by behavioral observations during the first 6 h of each dyadic encounter. The behavior of both ants was recorded every 5 min and classified in five categories: trophallaxis, self-grooming, allogrooming, physical contact (antennal and body contact), and no contact.

Results

Effect of the queen on aggressive behavior of the workers

To evaluate the impact of the queen on either the magnitude of worker aggression, or the distinctiveness of the label, we conducted encounters between queenright and queenless nestmates, as well as between these workers and queenright alien ants (Table 1). Ants that were separated from their mother colony and kept as a queenless group were neither aggressive towards nor aggressed by nestmates from the parent queenright colony. The hypothesis that queenless workers are less aggressive than their queenright nestmates was tested by evaluating the aggression of these two types of workers towards alien ants. The results of these tests showed that there were no differences in the magnitude of aggressive behavior exhibited by the resident workers, irrespective of their nest of origin. In the converse experiment, alien workers were as aggressive toward queenright workers as toward their nestmates reared under queenless conditions.

Table 1 Aggression between resident ants reared under queenright (QR) or queenless (QL) conditions and alien ants from a queenright colony in *Cataglyphis niger*

Type of encounter	Rearing conditions of resident ants	Index of aggression (mean \pm SE)	<i>P</i> ^a
Resident vs. alien	Queenright	0.82 \pm 0.11	0.72
	Queenless	0.82 \pm 0.11	
Alien vs. resident	Queenright	0.78 \pm 0.11	0.85
	Queenless	0.79 \pm 0.11	
Resident QR vs. nestmate QL ^b	Queenright	0.08 \pm 0.03	0.48
Resident QL vs. nestmate QR	Queenless	0.11 \pm 0.04	

^aWilcoxon matched-pair test

^bAnts from the same mother nest after at least 5 months of separation

Quantitative amounts of hydrocarbons

Analyses of PPG and epicuticular extracts by combined gas chromatography/mass spectrometry showed that their composition was not qualitatively different from that published previously (Soroker et al. 1995b). Queens and workers differed only slightly in the relative proportions of some components, but there was a significant quantitative difference in PPG hydrocarbons between the two castes (Table 2). Queens possessed three times more secretion than workers, despite the fact that their head size, assessed by measuring the distance between the compound eyes, was comparable to that of workers. In contrast, the amount of hydrocarbons on the thorax surface was the same for queens and workers.

De novo biosynthesis of hydrocarbons in queens and workers

The larger amounts of PPG hydrocarbons found in queens may have been due to higher synthesis rates or to more extensive accumulation from exogenous sources. These possibilities were tested by following the biosynthesis of hydrocarbons in queens as compared to queenright and queenless workers.

As depicted in Fig. 1, the amount of newly synthesized hydrocarbons in queen PPGs was 20 times lower than in either type of worker (1174 \pm 238 dpm/ant in queens vs. 28 634 \pm 4361 and 18 994 \pm 3947 dpm/ant in queenright and queenless workers, respectively). There was also a significant difference in hydrocarbon biosynthesis between the two types of workers: queenright workers exhibited higher rates than queenless

workers. Although the amount of labeled hydrocarbon on the queen's epicuticle was three times that found in the PPG, it was still lower than that measured in workers (4571 \pm 599 dpm/ant in queens vs. 17 415 \pm 2386 and 7779 \pm 994 dpm/ant in queenright and queenless workers, respectively). If we consider the difference in thorax surface, these differences are accentuated (queen = 160 dmp/mm²; queenright worker = 1350 dmp/mm²; queenless worker = 603 dmp/mm²). Queens and workers also differed in the distribution of labeled hydrocarbons between the PPG and epicuticle (Fig. 1, inset). Relative to epicuticle, queen PPGs contained only about 30% of the labeled hydrocarbons, whereas workers of both types had about twice the quantity of labeled hydrocarbons in their glands.

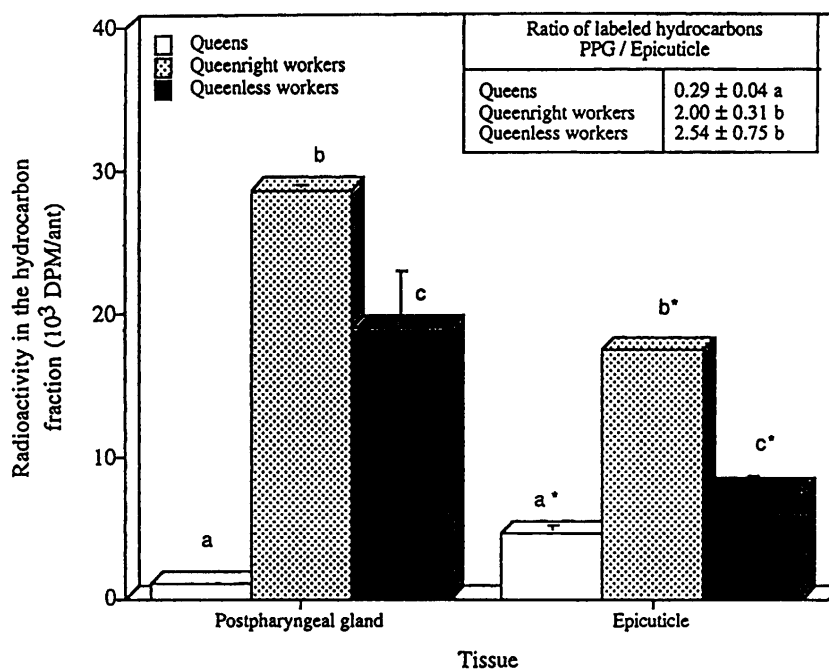
Thus, there is an apparent discrepancy between the quantitative chemical and biosynthetic data. Queens possessed three times more hydrocarbons in their PPGs than workers, but had considerably lower amounts of newly synthesized hydrocarbons. One explanation for this discrepancy is that the radiolabeled precursor in queens is incorporated into hydrocarbons of other tissues. Queens and queenless workers, for example, had a significantly larger number of chorionated eggs than queenright workers (1.46 \pm 0.6, 1.2 \pm 0.3, and 0.09 \pm 0.05, respectively; ANOVA, *P* < 0.05; Table 2). Since the chorionated egg surface is covered with hydrocarbons (Kawooya et al. 1988), we examined the levels of labeled hydrocarbons in the ovaries of the different groups. The amount of labeled hydrocarbons in queen ovaries was 546 \pm 171.9 dpm/ant, which comprised only 8.2% of total newly synthesized hydrocarbons, and 92 \pm 43.2 dpm/ant and 109 \pm 37.6 dpm/ant,

Table 2 Morphological measurements of *C. niger* workers and queens, and quantities of hydrocarbons (mean \pm SE) in the postpharyngeal gland (PPG) secretion and on the epicuticle of

queens, queenright workers and queenless workers (different letters indicate groups that are significantly different at *P* < 0.05; *t*-test or ANOVA, followed by Fisher's protected least significant difference

Parameters	Queens (<i>n</i>)	Queenright worker (<i>n</i>)	Queenless workers (<i>n</i>)
Head width (mm)	3.00 \pm 0.0 (28)	3.00 \pm 0.0 (47)	3.00 \pm 0.0 (45)
Thoracic surface (mm ²)	28.6 \pm 4.4 a (20)	12.9 \pm 2.6 b (20)	—
Body weight (mg)	43 \pm 1.1 a (28)	37 \pm 0.8 b (47)	36 \pm 1.0 b (45)
Maximal egg size (mm)	0.7 \pm 0.04 a (28)	0.26 \pm 0.05 b (47)	0.7 \pm 0.06 a (45)
Number of chorionated eggs	1.46 \pm 0.63 a (28)	0.09 \pm 0.05 b (47)	1.2 \pm 0.26 c (45)
Amount of hydrocarbons per PPG (μg)	157 \pm 33 a (10)	52 \pm 6.8 b (10)	—
Amount of cuticular hydrocarbons on the thorax (μg/mm ²)	0.90 \pm 0.25 a (10)	1.0 \pm 0.2 a (10)	—

Fig. 1 Biosynthesis and distribution of hydrocarbons in queens, queenright workers, and queenless workers of *Cataglyphis niger* (mean \pm SE). The table inserted in the figure depicts the ratio of occurrence of newly synthesized hydrocarbons between the postpharyngeal gland (PPG) and the epicuticle. Different letters indicate groups that are significantly different (Mann-Whitney *U*-test; $P < 0.05$)



amounting to 0.7% and 0.96% in the queenless and queenright workers, respectively.

We then tested the hypothesis that young alate queens synthesize hydrocarbons to the same extent as workers, but due to little turnover or intracolony transfer, these hydrocarbons accumulate in the mature queen's PPG. Figure 2 illustrates the biosynthesis of hydrocarbons in alate virgin queens as a function of age. These queens synthesized progressively more hydrocarbons as they aged (Kendall correlation coefficient $z = 3.29$, $P = 0.01$ for PPG; $z = 3.02$, $P = 0.0026$ for the cuticle) reaching the highest amounts in the oldest virgin queens tested (100–120 days old). The amount of labeled hydrocarbons in the PPG of the

oldest virgin queens was not significantly different from that in functional queens, but functional queens possessed significantly higher amounts of hydrocarbons on their cuticle ($P = 0.001$; Mann-Whitney *U*-test). Compared to workers, however, both virgin and functional queens showed poor synthetic abilities (Fig. 2 inset).

Hydrocarbon transfer between queens and workers

The next hypothesis we tested was that the larger amounts of hydrocarbons present in queens is a result of intensive acquisition from workers by trophallaxis. We conducted a series of dyadic encounters between a

Fig. 2 The effect of age on de novo hydrocarbon biosynthesis in virgin queens and functional queens (F. Q.) of *C. niger* (mean \pm SE). For comparison, the rates of synthesis by workers, alates (100–120 days old), and queens are presented in the inset. Different letters indicate groups that are significantly different (Mann-Whitney *U*-test; $P = 0.0001$)

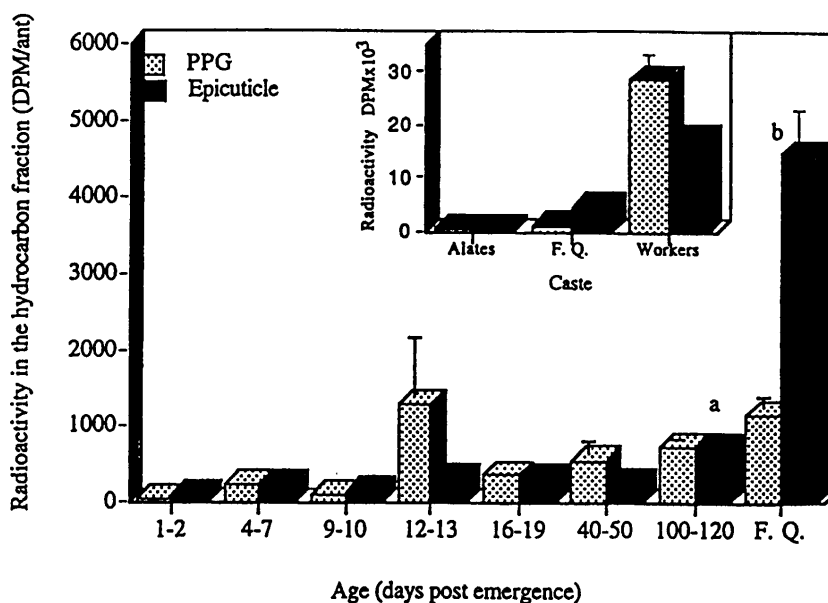
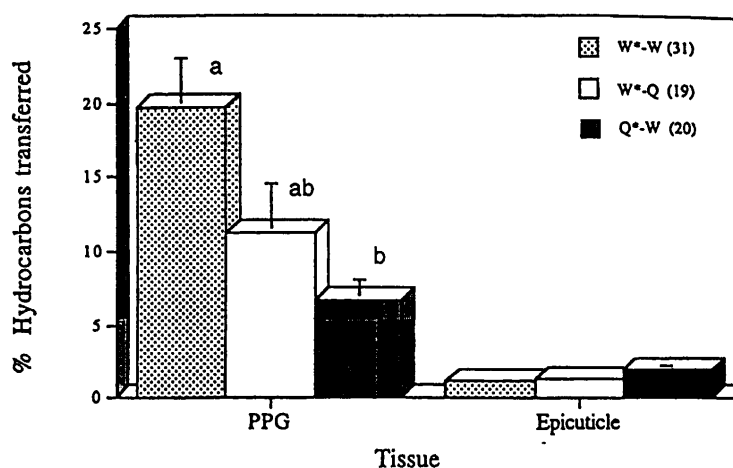


Fig. 3 Hydrocarbon transfer between queens (Q) and workers (W) in dyadic encounters (mean \pm SE). An asterisk designates the ant injected with the radioactive precursor (donor). Different letters indicate groups that are significantly different ($P < 0.05$; ANOVA followed by Fisher's protected least significant difference for arcsin-square-root-transformed data)



worker and radiolabeled queen, between a queen and a radiolabeled worker, and between a radiolabeled worker and its nestmate. Transfer of newly synthesized hydrocarbons into the recipient PPG and cuticle was observed in all encounters. In all cases, significantly more material was transferred to the PPG (Fig. 3). A similar amount of material was transferred to the epicuticle among the three groups, but transfer to the PPGs was not equal (Fig. 3). The transfer to the gland during the worker-worker dyadic encounters was significantly higher than from queens to workers ($P < 0.05$; ANOVA, followed by Fisher's protected least significant difference for arcsin-square-root-transformed data). Transfer from workers to queens was intermediate and not significantly different from transfer between workers or between queens and workers. Behavioral observations undertaken during these experiments showed that trophallaxis frequency was similar in all the experimental groups, but queens performed significantly less allogrooming than workers (0.45 ± 0.17 vs. 2.45 ± 0.32 , for queens and workers, respectively; $P = 0.001$, Mann-Whitney U -test).

Given that the queens received more hydrocarbons than they gave, we tested in a choice experiment whether queens receive preferentially more hydrocarbons than workers. Encounters between eight prelabeled nestmate workers, one unlabeled queen and one unlabeled worker conducted for 5 days (Fig. 4), showed a slight preferential transfer to queens. This, however, was not significant (12.9 ± 4.82 and 8.78 ± 2.04 mean \pm SE for transfer to the queen and to the worker, respectively; $P = 0.37$, Wilcoxon signed-rank test) because of high variation between experiments in the magnitude of transfer to the queens. Nevertheless, in 73% ($n = 11$) of the groups, transfer to the queen's PPG was 1.15–7.44 times higher than to the respective worker. If we take only these cases, the preference to the queen is significant (15.97 ± 6.36 and 6.80 ± 1.78 mean \pm SE for transfer to the queen and to the worker, respectively; $P = 0.01$, Wilcoxon signed-rank test). In contrast, transfer of hydrocarbons to the epicuticle of queens and workers was similar.

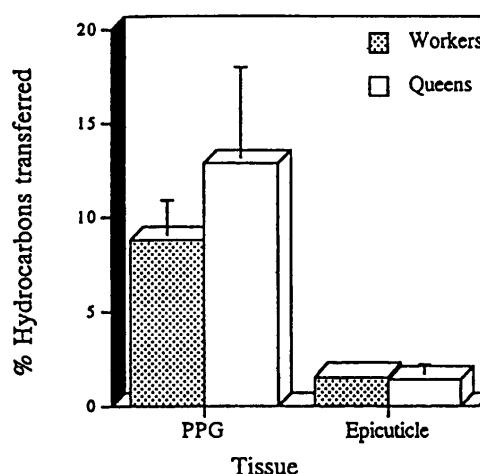


Fig. 4 Transfer of hydrocarbons from eight prelabeled workers to their queen or a nestmate worker (mean \pm SE)

Discussion

When comparing the queen-centered versus worker-centered models for the origin and maintenance of colony gestalt odors, we must consider the nature as well as within-colony dispersal of the appropriate cues. For example, the queen-centered model predicts that queen-specific cues must be synthesized in large quantities. The behavioral and chemical data regarding nestmate recognition systems in ants indicate that the signal is manifested by each nest member over its entire body surface. Thus, even if the signal constitutes a monomolecular layer, each individual must carry and update a substantial amount of material during its lifetime. Moreover, several independent studies have shown that the pattern of cuticular lipids changes with time (Vander Meer et al. 1989; Provost et al. 1993), increasing the need for an updated homogenization of the colony odor. Queen-specific discriminators, to operate properly, must therefore be produced and transferred in large quantities to workers, and these in turn must spread the cues onto

their body surface and at the same time transfer some of the material to other nestmates. This necessitates the evolution of a formidable biosynthetic machinery in the queens in order to supply enough secretion to provide for the many thousand workers in the nest. This situation is strikingly different from the production and dissemination of other putative queen pheromones. There is no need here for worker display of the signal. The retinue workers that acquire the pheromone from the queen are affected by mere exposure, and apparently transmit it further without any additional self-use. This, for example, is the case with the honeybee queen mandibular pheromone (Juska et al. 1981; Nauman et al. 1991). Even in this case, the amount of the pheromone required is rather large, and indeed the queen honeybee produces substantial mandibular gland secretion (Winston and Slessor 1992). Another possibility is that the queen admixes with the PPG secretion small amounts of queen-specific compounds that turn the signal nest specific. The bulk of hydrocarbons present in worker PPG act in this case as a carrier for disseminating such queen-specific substances within the colony. While this mechanism may be applicable to the dissemination of other non-volatile queen-derived pheromones (for example, admixing mandibular queen pheromone with the PPG hydrocarbons is plausible since both open to the mouth cavity), it seems unlikely that discriminators operate in such a way. The necessity to produce large amounts of these discriminators still holds. At least in the case of the polygynous *C. niger*, we could not detect any appreciable amounts of queen-specific compounds either in the PPG secretion or in cuticular washes. Likewise, caste-specific substances were not found in the few comparative analyses of cuticular hydrocarbons between queens and workers of other ant species. Only qualitative differences, assessed mostly from discriminant analyses or similar statistical tests, were found and these can be readily explained by the worker-centered model.

The behavioral evidence presented here refutes the queen-centered hypothesis. The expected augmentation in aggressive behavior between nestmates from split colonies, the outcome of the loss of queen-derived cues in the queenless group, was not apparent. Queenless workers that had been separated for at least 5 months were neither aggressive toward nor aggressed by their queenright nestmates. Although we cannot exclude the possibility that a queen-derived cue persists on the workers, it seems unlikely. The rate of synthesis of PPG secretion and the rate of secretion exchange among workers will probably dilute the queen-specific compounds below the threshold of detection after 5 months of separation. It is still possible that in the absence of the queen (no queen-derived discriminators), workers rely on worker-derived cues. It is hard to postulate, however, selective pressures for developing a dual recognition system. Unless colonies lose their queen frequently, there should be no lack of queen-derived recognition cues and therefore no need for a worker-derived "backup recognition system" until new queens are reared. There is no

evidence, however, of frequent queen turnover in ants. In any case, it is clear that the discriminator profiles of the two groups of ants did not diverge enough to be recognized as alien. We further tested the validity of another possible explanation for the lack of aggression in the above experiments, i.e., that the queen modulates the aggressive behavior of the workers in a way that is not directly linked to the recognition system. In our behavioral tests, queenless workers of *C. niger* kept for 5 months did not behave differently from their queenright nestmates in aggression toward alien ants. Similarly, alien ants did not behave differently towards either group. These data indicate that the queen in this species neither modulates the aggressive behavior of workers nor contributes a dominant odor/recognition cue to the colony.

From the behavior of the ants it can be concluded that the *C. niger* system is similar to that of *R. confusa* (Crosland 1990) and *L. curvispinosus* (Stuart 1987), but contrasts that of *Camponotus* (Carlin and Hölldobler 1983, 1986, 1987), *Myrmica* (Brian 1986), and *L. lichtensteini* (Provost 1989). These differences may reflect species specificity and/or may be associated with differences in colony structure such as monogyny versus polygyny. In monogynous colonies, the queen is thought to have a more decisive role in shaping the nestmate recognition system than in polygynous colonies (Hölldobler and Michener 1980). Polygyny in *C. niger* (unpublished field observations) may explain the minor role of the queen in establishing the colony odor. The number of queens, however, is not always a good predictor of queen contribution to colony odor. In the facultative polygynous and polydomous species, *L. ambiguus*, queen number did not affect nestmate recognition abilities of either monogynous, polygynous, or queenless colonies (Stuart 1991). In the polygynous species *Formica podzolica*, queen-derived cues are much more important than in the monogynous species *F. argentea* (Bennett 1989).

The above conclusions are based on behavioral observations during experimental manipulations. These can provide only indirect and circumstantial evidence on the relative contributions of the castes to the composition of colony odor, since behavior can be affected by multiple ecological and biological constraints. In this study we have taken a more direct biochemical approach to studying the contribution of the queen to colony odor.

As discussed above, one prediction from the queen-centered hypothesis is the production and dissemination of queen-specific recognition chemicals in rather large quantities. The quantitative superiority in the amount of material in the PPG of queens seemed at first to support the possibility that the queen is the main contributor to colony odor. However, the biosynthesis studies refute this hypothesis. The poor hydrocarbon biosynthesis exhibited by queens compared to workers indicates that the large amount of material in their PPGs must be sequestered from external sources. Moreover, the

ontogeny studies preclude the possibility that the larger amounts of hydrocarbons found in queen PPGs are a consequence of synthesis early in adulthood and low turnover thereafter. Similarly, the low levels of labeled hydrocarbons in other tissues that may have produced hydrocarbons, such as ovaries that contain chorionated eggs, preclude the possibility that hydrocarbon biosynthesis in queens is diverted to other tissues. Thus, the biochemical data strengthen our hypothesis that in *C. niger* the direct contribution of the queen to the composition of a colony odor is minor, refuting the queen-centered hypothesis.

Why then do queens possess more secretion in their PPGs and what is the origin of this secretion? To elucidate this point, we assessed the magnitude of hydrocarbon exchange between the different castes in the colony. Exchange of PPG secretion between colony members has been reported for several ant species (Soroker et al. 1994, 1995a; Meskali et al. 1995; Vienne et al. 1995). It was further demonstrated in two different species that the glandular secretion modulates the aggressive behavior of the workers, suggesting that it contains recognition cues (Soroker et al. 1994; Hefetz et al. 1996). The PPG secretion can therefore serve as a model system for the transmission and spread of recognition cues within the colony. We estimate that copious secretion in queen PPGs is the consequence of differential exchanges between queens and workers. One possibility is that more workers participate in hydrocarbon exchange with queens than with other workers; the other is that during the exchange process, queens take more than they give. A combination of the two is also conceivable. During the dyadic encounters we have shown that queens are likely to receive more secretion to their PPG than they are inclined to give away. Transfer to the epicuticle, on the other hand, was low and similar in all groups. This suggests that of the known modes of transfer, trophallaxis was the main route by which the preferential transfer from workers to queens took place. Thus, in every bout of trophallaxis between a worker and the queen, there is a higher probability that the queen will gain more secretion than she gives away, and the identity of the secretion in the queen's PPG becomes progressively more worker-like (or for that matter "gestalt-like"). This process must be regulated by behavioral and/or biochemical means, since it is evident that the queen cannot indefinitely receive more than she transfers back. The dyadic experiments also showed that the exchange between workers is much higher than between queen and workers. Taken together, these results are in agreement with the worker-centered model.

Although the group encounters were not clearly conclusive, they further revealed that given the choice there was a slight bias for transfer to queens. The lack of significance in these experiments evolved, in our opinion, from the high variability between the cases. This variation was probably caused by the procedure of randomly selecting queens and workers from each nest. *C. niger* is a polygynous species and therefore a colony can be

composed of workers from different matriline. It is possible that in certain groups, workers from a certain matriline were indifferent and did not exchange secretions with a queen that was not their mother (or may have shown nepotism if the queen was their mother). Moreover, it is reasonable to assume that a certain degree of polyethism exists, and therefore not all the selected workers equally attended the queens. Either or both of these phenomena could explain the high variability in the observed transfer. A preference in transfer to the queen was detected in 8 of the 11 groups tested, suggesting that despite the lack of statistical significance, this trend seems to be correct.

Although the worker-centered model predicts that worker-worker exchanges will not be different from worker-queen exchanges, such differential transfer does not negate the hypothesis. The accumulation of worker-originated hydrocarbons in the queen's PPG puts it in the center of the gestalt, thus facilitating the mixing of the recognition cues of individual workers. By redirecting a blend of recognition cues to colony members, homogeneity of colony odor can be achieved more effectively. A mechanism placing the queen as a "distribution center" may be adequate in small colonies and can explain the observations of Yamaoka and Kubo (1990) on the effect of queens on hydrocarbon uniformity in *Formica* sp. workers. However, we do not think that it operates in large, mature polygynous colonies such as those of *C. niger* in which the rate of transfer among workers is high enough to account for a uniform distribution of the signal within the colony. Additional selection for preferential queen acquisition of worker-originated signals is the adaptive value of having a secretion that is the closest to the average colony odor than in any other member of the colony. Carrying an averaged colony odor reduces the probability of misidentification of the resident queens by the workers, preventing consequent aggression. The ability of workers to distinguish between familiar and unfamiliar queens has been previously reported in several ant species: *Iridomyrmex humilis* (Keller and Passera 1989), *Solenopsis invicta* (Fletcher and Blum 1983) *C. cursor* (Berton et al. 1991), and *R. confusa* (Crosland 1990). Since queens depend on worker attendance it is imperative that they be as close to the colony gestalt as possible. Any possible stress exerted by workers on the queen may result in decreased fecundity, which is maladaptive, especially in a highly polygynous society. The constant acquisition of secretion from workers and the exchange between the PPG and the cuticular surface ensures that the queen has an updated colony odor.

Queens of *C. niger* may also have an indirect, but significant, effect on the turnover of colony label. In the absence of the queen, hydrocarbon biosynthesis in workers is lower than under queenright conditions. Although we cannot exclude the possibility that this reduction in hydrocarbon biosynthesis reflects a general depression in worker metabolism under queenless conditions, we hypothesize that the presence of the queen

actively stimulates, among other things, the production of recognition cues in workers. Enhancing the rate of cue turnover is especially adaptive in polygynous species, but not exclusively so, because when coupled with high exchange rates it reduces the chemical variability that stems from the genetic variability within the colony. The presence of the queen can be mediated via a queen-specific "recognition cues biosynthesis enhancing pheromone," or via a general queen signal (Keller and Nonacs 1993). This hypothesis fits well with observations on *L. lichtensteini*, in which changes in cuticular hydrocarbon profiles over a 1-year period were accelerated in the presence of the queen (Provost et al. 1993).

In conclusion, behavioral and biochemical data using hydrocarbons as a cue model and the PPG as a model distribution organ suggest that in *C. niger*, the nestmate recognition system follows a worker-centered model. The contribution of the queen to the colony gestalt odor is at best minor, since she donates only a small portion of her secretion. While it is hard, in view of the few species studied so far, to state whether this is a general system for ants, or is specific to their polygynous species, it emphasizes the significance of a direct biochemical approach in testing the mechanisms underlying social behavior.

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References

- Bennett B (1989) Nestmate recognition systems in a monogynous-polygynous species pair of ants (Hymenoptera: Formicidae). I. Worker and queen derived cues. *Sociobiology* 16:121-139
- Berton F, Lenoir A, Nowbahari E, Barreau S (1991) Ontogeny of queen attraction to workers in the ant *Cataglyphis cursor* (Hymenoptera: Formicidae). *Insects Soc* 38:293-305
- Bonavita-Cougourdan A, Clément JL, Lange C (1987) Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus* Scop. *J Entomol Sci* 22:1-10
- Brian MV (1986) Bonding between workers and queens in the ant genus *Myrmica*. *Anim Behav* 34:1135-1145
- Carlin NF, Hölldobler B (1983) Nestmate and kin recognition in interspecific mixed colonies of ants. *Science* 222:1027-1029
- Carlin NF, Hölldobler B (1986) The kin recognition of carpenter ants (*Camponotus* spp.). I. Hierarchical cues in small colonies. *Behav Ecol Sociobiol* 19:123-134
- Carlin NF, Hölldobler B (1987) The kin recognition system of carpenter ants (*Camponotus* spp.). II. Larger colonies. *Behav Ecol Sociobiol* 20:209-217
- Crosland MWJ (1990) The influence of the queen, colony size and worker ovarian development on nestmate recognition in the ant *Rhytidoponera confusa*. *Anim Behav* 39:413-425
- Crozier RH (1987) Genetic aspects of kin recognition: concepts, models, and synthesis. In: Fletcher DJC, Michener CD (eds) *Kin recognition in animals*. Wiley, New York, pp 55-73
- Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odour in social Hymenoptera. *Behav Ecol Sociobiol* 4:217-224
- Fletcher DJC, Blum M (1983) Regulation of queen number by workers in colonies of social insects. *Science* 219:312-314
- Hefetz A, Errard C, Chambris A, Le Negrate A (1996) Postpharyngeal gland secretion as a modifier of aggressive behavior in myrmicine ant *Manica rubida*. *J Insect Behav* 9:709-717
- Henderson G, Andersen JF, Philips JK, Jeanne RL (1990) Internest aggression and identification of possible nestmate discrimination pheromones in polygynous ant *Formica montana*. *J Chem Ecol* 16:2217-2228
- Hölldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: Markl H (ed) *Evolution of social behavior: hypotheses and empirical tests*. Chemie, Weinheim, pp 35-58
- Juska A, Seeley TD, Velthuis HHW (1981) How honeybee queen attendants become ordinary workers. *J Insect Physiol* 27:515-519
- Katase H, Chino H (1984) Transport of hydrocarbons by hemolymph lipophorin in *Locusta migratoria*. *Insect Biochem* 14:1-6
- Kawooya JK, Osir EO, Law JH (1988) Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. *J Biol Chem* 263:8740-8747
- Keller L, Nonacs P (1993) The role of queen pheromones in social insects: queen control or queen signal? *Anim Behav* 45:787-794
- Keller L, Passera L (1989) Influence of the number of queens on nestmate recognition and attractiveness of queens to workers in the Argentine ant, *Iridomyrex humilis* (Mayr). *Anim Behav* 37:733-740
- Meskali M, Bonavita-Cougourdan A, Provost E, Bagnères A-G, Dusticier G, Clément J-L (1995) Mechanism underlying cuticular hydrocarbon homogeneity in the ant *Camponotus vagus* (Scop.) (Hymenoptera: Formicidae): Role of postpharyngeal glands. *J Chem Ecol* 21:1127-1147
- Naumann K, Winston ML, Slessor KN, Prestwich GD, Webster FX (1991) Production and transmission of honey bee queen (*Apis mellifera* L.) mandibular gland pheromone. *Behav Ecol Sociobiol* 29:321-331
- Nowbahari E, Lenoir A, Clément J-L, Lange C, Bagnères A-G, Joulie C (1990) Individual, geographical and experimental variation of cuticular hydrocarbons of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): their use in nest and subspecies recognition. *Biochem Syst Ecol* 18:163-73
- Provost E (1989) Social environment factors influencing mutual recognition of individuals in the ant *Leptothorax lichtensteini* Bondr. *Behav Proc* 18:35-59
- Provost E, Riviere G, Roux N, Morgan ED, Bagnères A-G (1993) Change in the chemical signature of the ant *Leptothorax lichtensteini* Bondroit with time. *Insect Biochem Mol Biol* 23:945-957
- Provost E, Riviere G, Roux M, Bagnères A-G, Clément J-L (1994) Cuticular hydrocarbons whereby *Messor barbarus* ant workers putatively discriminate between monogynous and polygynous colonies. Are workers labeled by queens? *J Chem Ecol* 20:2985-3003
- Soroker V, Vienne C, Nowbahari E, Hefetz A (1994) The postpharyngeal gland as a "Gestalt" organ for nestmate recognition in the ant *Cataglyphis niger*. *Naturwissenschaften* 81:510-513
- Soroker V, Vienne C, Hefetz A (1995a) Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger*. (Hymenoptera Formicidae). *J Chem Ecol* 21:365-378
- Soroker V, Hefetz A, Cojocaru M, Billen J, Franke S, Francke W (1995b) Structural and chemical ontogeny of the postpharyngeal gland in the desert ant *Cataglyphis niger*. *Physiol Entomol* 20:323-329
- Stuart RJ (1987) Transient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. *Behav Ecol Sociobiol* 21:229-235
- Stuart RJ (1991) Nestmate recognition in leptothoracine ants: testing for effects of queen number, colony size and species of intruder. *Anim Behav* 42:277-284

- Vander Meer RK, Saliwanchik D, Lavine B (1989) Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*. Implications for nestmate recognition. *J Chem Ecol* 15:2115-2125
- Vienne C, Soroker V, Hefetz A (1995) Congruency of hydrocarbon pattern in heterospecific groups of ants: transfer and/or bio-synthesis? *Insects Soc* 42:267-277
- Winston ML, Slessor KN (1992) The essence of royalty: honey bee queen pheromone. *Am Sci* 80:374-385
- Yamaoka R, Kubo H (1990) Identity of cuticular hydrocarbon profile among workers of the ant which is maintained by

presence of the queen would be the nestmate recognition chemical cue. In: Veeresh GK, Mallik B, Viraktamath CA (eds) *Proceedings of the 11th international congress: social insects and the environment*, Bangalore, Oxford's IBH Publishing Co Pvt Ltd. New Delhi. pp 406-407

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