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Trophallaxis Mediates Uniformity of Colony Odor in *Cataglyphis iberica* Ants (Hymenoptera, Formicidae)

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We studied the effect of nestmate separation on trophallaxis in the polydomous ant Cataglyphis iberica. *After dividing three colonies into two equivalent subgroups, one queenright and one queenless, we quantified the frequency of trophallaxis within each subgroup, between the workers from the two subgroups ("mixed" trophallaxis), and trophallaxis involving the queen. Observations of trophallaxis were conducted over four periods of time: for 2 weeks before the separation of the two subgroups, 8 weeks during separation, immediately after reunification, and 3 weeks following reunification. Subgroups were identically fed on the eve of each day of observation. Group separation induced an increase in "mixed" frequencies of trophallaxis just after reunification, after which trophallaxis returned to the initial level observed before separation. Previous results showed that group separation in* C. iberica *induces hydrocarbon profile divergence and that reunification restores this chemical modification. The current results seem to indicate that increased trophallaxis permits a uniform odor to be reestablished among previously separated ants. Trophallaxis involving the queen is infrequent and does not seem to be crucial in the process of odor exchange. Our data confirm that trophallaxis plays a key role in establishing the "Gestalt" colony odor, particularly among naturally separated satellite nests in a polydomous species like* C. iberica.

KEY WORDS: trophallaxis; "Gestalt" colony odor; Formicidae; polydomy.

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INTRODUCTION

In ants, nestmate recognition is usually achieved through exchange of chemical signals which are generally considered to be mainly hydrocarbons (Vander Meer and Morel, 1998; Lahav *et al.*, 1999; Lenoir *et al.*, 1999), resulting in a mixture of odors or "Gestalt" (Crozier and Dix, 1979). The existence of such exchanges has been proven in several species of ants using radioisotopes (Soroker *et al.*, 1994, 1995, 1998; Vienne *et al.*, 1995; Meskali *et al.*, 1995). Trophallaxis, whose role has long been considered exclusively trophic, is widely involved in the process of odor exchange. During trophallaxis, the hydrocarbons stored in the postpharyngeal gland are exchanged independent of the alimentary flow (Soroker *et al.*, 1994). Allogrooming is also used to exchange odors, but at least in formicine ants, trophallaxis is the major mode of establishing the Gestalt.

In the monogynous and polydomous species *Cataglyphis iberica*, colonial odor seems to be established according to the Gestalt model (Dahbi and Lenoir, 1998a) and separation of nests over a long period of time, including hibernation, raises the problem of maintaining this Gestalt because the colonies of this species have well-developed nestmate recognition (Dahbi *et al.*, 1996). Two results confirm indirectly the Gestalt nature of colony odor in this species. It was observed (1) that separation of nests induces a divergence in hydrocarbon profiles (Dahbi and Lenoir, 1998a) and (2) that transports of workers between different nests permit the redistribution over satellite nests of young adults whose profiles differ from those of mature workers and, consequently, that these transports seem to be necessary for the maintenance of the collective colonial odor (Dahbi *et al.*, 1997). We hypothesized that when nestmates are naturally or artificially separated, the nestmate recognition cues will tend to segregate and that homogenization during reunification will be achieved by trophallaxis. In this study we tested the prediction that the trophallaxis rate between ants that have been separated and then regrouped would be higher than the normal intragroup trophallaxis.

MATERIALS AND METHODS

Three queenright colonies of *C. iberica* were used in this study. They were collected in Bellaterra (near Barcelona, Spain) and kept in the laboratory in artificial nests at a temperature of 25 ± 2°C, 50% humidity, and a photoperiod of 10D : 14L. The ants were provided with a honey–apple mixture and mealworms twice a week on the eve of observation days.

From each of the three colonies, an experimental group of 240 workers (20 to 30% of each colony), including the queen, was created. Each group was subdivided randomly into two equal havles (α and β), and workers from each half were marked with a same-color dot for easier identification. The α and β subgroups were kept together for a week for acclimatization prior to onset of

the experiment. Previous observations have shown that such marking does not affect the ants' recognition behavior.

The experiment was composed of four periods of observations, as follows.

Period A: A 2-week preseparation (control) period in which the two subgroups, α and β, were kept as a single group including the queen.

Period B: 8 weeks of separation during which the two subgroups, α and β, were placed in two separate identical nests. Subgroup α was queenright and subgroup β was queenless.

Period C: The first 2 days following reunification of the two subgroups in a neutral nest (identical in shape to that used in period A).

Period D: The 3 weeks following period C.

During periods A, B, and D, observations were conducted twice a week for 30 min in the morning (from 0900) and 30 min in the afternoon (from 1300). All colonies were observed on the same day. For period C we noted the frequency of trophallaxis over 6 h—4 h in the morning (from 0800) and 2 h in the afternoon (from 1300)—and for 2 h the following day—1 h in the morning (from 1000) and 1 h in the afternoon (1300).

During each observation period we recorded the number of trophallactic events but not their duration. Since the ants were fed on the eve of each day of observation, they were not in a low nutritional state and performed mainly short trophallactic exchanges. We distinguished between trophallaxis performed between workers belonging to the same subgroup (intrasubgroup) and that between workers belonging to different subgroups ("mixed" trophallaxis). In addition, we recorded all trophallactic events involving the queen.

When "mixed" trophallaxis were observed, we distinguished between "donor" and "receiver" ants based on behavioral sequences (antennal movements, the opening of the mandibles) described earlier (Lenoir and Jaisson, 1982; Bonavita-Cougourdan, 1983; Hölldobler and Wilson, 1990).

Results are presented as the mean frequencies of trophallaxis observed per hour (±SE), and the statistical comparisons were conducted using ANOVA (Duncan test), Student's t test, and Wilcoxon matched-pairs test as appropriate. As no significant differences were observed among the three colonies (ANOVA, $P > 0.05$), the results were pooled.

RESULTS

Trophallaxis between workers seemed to be a relatively frequent behavior in the tested *C. iberica*, with an average frequency of spontaneous trophallaxis of 80.4 ± 9.3 events per hour (for a total of 240 workers) (Fig. 1; all trophallactic

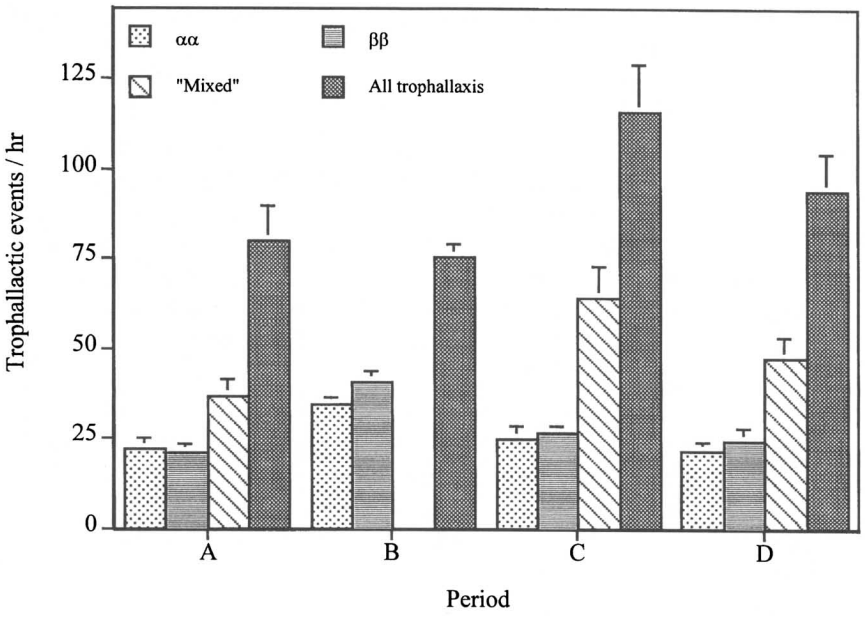

Fig. 1. Average frequencies of trophallaxis per hour (±SE) between *Cataglyphis iberica* workers observed for each type of encounter during the four observation periods.

exchanges, period A; $n = 24$), representing 0.67 ± 0.08 trophallaxis performed by each worker per hour (considering the two ants for each trophallaxis event). Trophallaxis was uniformly performed throughout the active period of the day, without any apparent differences between the morning and the afternoon sessions (90.5 ± 15.3 and 70.3 ± 10.3 for the morning and afternoon, respectively; Wilcoxon matched-paired test, $P > 0.05$).

The number of worker–worker trophallactic events per hour under the different social conditions is depicted in Fig. 1. Intrasubgroup trophallaxis (i.e., trophallaxis observed between α–α or β–β workers) remained unchanged when the ants were kept as a single group, i.e., during the preseparation period (A) and during the reunification periods (C and D, respectively), but was higher during separation period B (ANOVA, Duncan test, $P < 0.05$). We attribute this increase to the higher probability for a worker α or β to interact within its own subgroup. This increase was more pronounced in the queenless groups (trophallaxis β–β; Student's t test, $P = 0.036$) and was present uniformly throughout the 8 weeks of separation. However, the total frequency of trophallaxis during this period was not different from that in period A. In contrast, the total frequency of trophallaxis increased significantly following the reunification of nestmates (period C) in comparison to the presepartion and separation periods (80.4 ± 9.3 and $75.7 \pm$

3.7 for periods A and B, respectively, vs 116.2 ± 13.1 for period C; ANOVA, Duncan test, $P < 0.05$). This was due to a significant increase in the "mixed" trophallaxis rates (64.3 ± 8.5 in period C vs 37.3 ± 4.2 for period A; ANOVA, Duncan test, $P < 0.05$) but not in intragroup trophallaxis, which remained similar to that observed in the control period A. Period D lasted for 3 weeks following reunification, during which the ants exhibited intermediate levels of mixed trophallaxis (47.4 ± 5.4). These were lower, but not significantly so, than during period C and higher, but not significantly so, than during period A. These differences were also due to the levels of mixed trophallaxis, because the intragroup trophallaxis remained unchanged.

Because workers within a subgroup were color marked, it was possible to distinguish "donors" (classified as the ant with open mandibles) from "receivers" (closed mandibles). The results indicate an asymmetry in mixed trophallaxis during period C. The increased trophallaxis during period C is linked to a higher frequencies of α–β trophallaxis (α donor and β recipient) compared to these in period A (39.3 ± 6.0 vs 17.9 ± 2.2 for periods C and A, respectively; ANOVA, Duncan test, $P < 0.05$). The β–α frequencies, however, remained unchanged compared to those in period A (24.95 ± 2.8 vs 19.3 ± 2.35; ANOVA, Duncan test, $P > 0.05$). In fact, throughout the experiment the frequencies of trophallaxis α–α, β–β, and β–α were similar; only trophallaxis α–β during period C clearly demonstrated a higher frequency (ANOVA, Duncan test, $P < 0.05$).

Trophallaxis involving the queen was rare compared to worker–worker trophallaxis. In all these cases the queen was never a "donor" (Table I). However, when we calculated the frequency of worker–worker trophallaxis per worker it did not differ from that of queen–worker trophallaxis (0.67 vs 0.75 for period A and 0.97 vs 0.92 for period C) and was significantly lower during

Table I. Average Frequencies of Trophallaxis per Hour (±SE) Involving the Queen in *Cataglyphis iberica* During the Four Observation Periods[a]

Type of encounter	Period A	Period B	Period C	Period D
α-queen	0.42 ± 0.27 (a)	1.94 ± 0.31 (b)	0.58 ± 0.22 (a)	1.17 ± 0.34 (a,b)
β-queen	0.33 ± 0.20 (a)	—	0.33 ± 0.13 (a)	2.00 ± 0.55 (b)
Comparison α-Q/β-Q	NS		NS	NS
All trophallaxis with the queen	0.75 ± 0.38 (a)	1.94 ± 0.31 (b)	0.92 ± 0.28 (a)	3.17 ± 0.68 (b)
Mean trophallaxis per worker (worker–worker)	0.67 ± 0.08 (a)	0.63 ± 0.03 (a)	0.97 ± 0.11 (b)	0.79 ± 0.08 (a,b)

[a]Within each line, statistical differences are shown by different letters (ANOVA, Duncan test, $P < 0.05$). Comparisons of α-queen and β-queen trophallaxis frequencies for each period were performed by a Student t test ($P < 0.05$). The last line indicates, for comparison, the mean number of worker–worker trophallaxis per worker.

periods B (0.63 vs 1.94) and D (0.79 vs 3.17) (Student's t test, $P < 0.05$). In addition, α-queen and β-queen trophallaxis did not differ through the A, C, and D periods.

DISCUSSION

In polydomous and closed ant colonies like those of *C. iberica*, the problem arises of maintaining the "Gestalt" odor through long periods of separation, including hibernation. In such situations nestmate recognition cues will tend to segregate and we predicted that this would lead to efforts to rehomogenize. As demonstrated by Soroker *et al.* (1994), trophallaxis concerns more than just food transfer and is also involved in the process of odor homogenization. We thus predicted that an elevated amount of trophallaxis would be stimulated by disparate signals. Our results confirm that an elevated amount of trophallaxis between the α and the β ants ("mixed" trophallaxis) did indeed occur when the ants were reunited.

It was observed previously that separating *C. iberica* nestmates affects their PPG hydrocarbon profile and leads to a profile divergence inducing longer antennations in dyadic encounters. Reunification reverses this trend (Dahbi and Lenoir, 1998a). The high frequency of trophallaxis occurring after experimental separation (period C) suggests that this behavior contributes to the elaboration of the Gestalt odor in this species. However, no aggression appeared after reunification, indicating that soliciting and giving trophallaxis are not appeasement behaviors as was described in *Ponera coarctata* (Liebig *et al.*, 1997).

The increase in the mixed trophallaxis frequency takes place immediately after reunification and decreases during the following weeks. This result suggests that homogenization by means of trophallaxis might be accomplished during the hours following reunification of nestmates and that behavioral expression is directly linked to the chemical divergence within the group. However, this increase cannot be due to a nutritional homogenization among separated workers, as the experiment was designed to exclude this effect. Social isolation of *Camponotus fellah* workers leads to a similar result: during reunification, the duration of trophallaxis increases as the isolation period augments, even though the ants are fed normally (Boulay *et al.*, 1999). Using artificially mixed colonies of *Manica rubida* and *Formica selysi*, Vienne *et al.* (1995) demonstrated that the transfer of hydrocarbons between individuals occurred mainly during the first 24 h following homospecific or allospecific dyadic encounters.

The supernumerary trophallactic events observed during reunification concerns only α–β interactions. This asymmetry cannot be linked to the involvement of the queen in the colony odor in this species as shown by Dahbi and Lenoir (1998b). It could, however, be linked to the dispersion of a queen signal(s) by α workers to their β sisters that may have deteriorated while kept under queenless

conditions. Such a distribution of queen pheromone by "messenger" workers was reported in *Apis mellifera* (Naumann *et al.*, 1993) but never in ants.

During interindividual encounters, *C. iberica* workers react aggressively (allocolonial) or amicably (homocolonial) without behavioral gradation in their response (Dahbi *et al.*, 1996). The increase in trophallaxis frequency between separated nestmates shows that workers are able to discriminate a weak odor divergence and then preferentially orient their interactions. The nestmate discrimination in *C. iberica* colonies may be based on a threshold corresponding to a precise value of the discrepancy between the template and the odor encountered. Any value above the threshold elicits full aggression (between colonies), while for values below the threshold, the response is amicable but the behavior is manifested with longer antennations (within colonies). We hypothesize that the magnitude of odor divergence between separated workers remains below the tolerance threshold. When reunified, previously separated nestmates perceive the slight odor differences through intensive mutual antennations and manifest more interactions, especially trophallaxis (present results) or adult transport (Dahbi *et al.*, 1997) which results in a uniform colony odor. A similar homogenization process was observed previously in other ant species (e.g., *Myrmica rubra*), in which isolated workers were more frequently licked by their nestmates when returned to the colony (De Vroey and Pasteels, 1978). In *Leptothorax lichtensteini*, previously separated groups remerge slowly after an intensive period of mutual antennations (Provost, 1989). This author concluded a Gestalt process of colony odor in this species. This conclusion can now be confirmed in the light of the newly acquired knowledge of Gestalt colony odor.

Such a flexible recognition process may be adaptive for polydomous colonies of *C. iberica* since satellite nests, each containing several hundred individuals, are completely separated during the 5-month hibernation period (Cerdá *et al.*, 1994; Dahbi *et al.*, 1997). At the end of hibernation, acceptance between nestmates belonging to different nests still remains possible and intensive trophallactic exchanges may be how a uniform odor is reestablished throughout all colony members.

During the experiments the queen received rather than offered. Queen–worker trophallaxis was elevated during periods B (separation) and D (after reunification), which could be due to variations in queen physiology. In the control period, queen–worker trophallaxis did not differ from worker–worker trophallaxis. The β workers that were separated from the queen did not demonstrate specific trophallaxis with their queen just after reunification. We conclude that in *C. iberica* the queen does not differ from the workers with respect to the distribution of colony recognition cues and that she does not constitute the origin of the Gestalt. This is congruent with previous data on this genus [Berton *et al.* (1991) for *C. cursor*, Dahbi and Lenoir (1998b) for *C. iberica*, Lahav *et al.* (1998) for *C. niger*].

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