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Research article

Queen and colony odour in the multiple nest ant species, *Cataglyphis iberica* (Hymenoptera, Formicidae)

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Summary

We investigated the queen's effect on the cuticular hydrocarbon profile of workers in the monogynous and polydomous species *Cataglyphis iberica*. Within each of the three colonies tested, workers were separated for three months in queenright and queenless groups. After regrouping, nestmate recognition remained unchanged but the duration of antennal interactions between workers previously separated increased relative to controls. Separated groups presented slightly divergent cuticular hydrocarbon profiles which may induce the longer antennations. A quantitative analysis of major cuticular hydrocarbons showed that the total amount per unit of cuticular surface area remains similar between the two castes; but queens had higher quantities of n-alkanes than workers. The lack of a queen did not affect the workers' cuticular hydrocarbon profile in queenless groups. Indeed, the profile of queenless workers remained significantly different from the queen profile as did that of queenright workers. These results show that queens are not at the origin of the hydrocarbons' colonial profile. Two recognition processes seem to coexist within *C. iberica* colonies: nestmate discrimination based on the colonial odour which includes all nestmate workers, and a queen caste-specific odour. In a polydomous species such as *C. iberica*, the queen does not seem to contribute more than any other nestmate to the colonial odour, which probably derives from worker cues, confirming the existence of a "Gestalt" colonial odour.

Introduction

The recognition of nestmates is widespread among animals and often involves specific odours (Fletcher and Michener, 1987). In social insects, this phenomenon is mediated by specific compounds or "discriminators" recognisable by all nestmates (Hölldobler and Michener, 1980), allowing colonies to maintain their integrity against intruders.

Previous studies have investigated the composition of these substances and all suggested that cuticular hydrocarbons are involved in nestmate discrimination (see review by Lenoir et al., 1998; Vander Meer and Morel, 1998 for a critical review). Recently, Lahav and Hefetz (pers. comm.) proved by experimental assays

in *Cataglyphis niger*, using purified hydrocarbon extracts, the involvement of cuticular hydrocarbons in nestmate recognition. This was also verified in two termite species (Takahashi and Gassa, 1995). Moreover, cuticular hydrocarbons are exchanged between nestmates through trophallaxis, mutual licking, and passive contact (Soroker et al., 1994, 1995; Vienne et al., 1995). These exchanges lead to a "Gestalt" chemical signature (Crozier and Dix, 1979) which constitutes the reference odour used for acceptance or rejection of any encountered individual.

The relative contribution of queen and workers in the formation of the colonial "Gestalt" odour is not well established. Some studies showed or suggested that the queen constitutes the major source of colonial recognition cues in some ant species while other studies attribute a low importance to the queen in providing such cues (see Hölldobler and Wilson, 1990 and review by Lenoir et al., 1998).

In ants, the queen is attractive to the workers (Stumper, 1956; Jouvenaz et al., 1974; Vander Meer et al., 1980; Cammaerts, 1985; Keller and Passera, 1989; Berton et al., 1991; Cariou-Etienne and Passera, 1993). However, this attraction is sometimes linked with colonial odour and closure of societies (e.g., Keller and Passera, 1989). Workers attraction to their queen is generally tied in social insects to a pheromonal control over reproduction (Winston, 1987; Hölldobler and Wilson, 1990) and does not involve labelling of workers with queen-produced recognition cues. In *Apis mellifera* for example, the royal pheromone generating the worker retinue around the queen inhibits the workers' ovarian development (Velthuis and van Es, 1964). However, the colonial odour responsible for the closure of societies derives from chemical cues provided by workers and wax comb (Breed, 1983; Breed et al., 1995). The queen pheromone also seems to exist in ants. Its presence was clearly suggested in the Argentine ant *Linepithema humile* (= *Iridomyrmex humilis*) (Vargo and Passera, 1991) and in the fire ant *Solenopsis invicta* (Vargo and Laurel, 1994), but it is not identified yet. In ants, the queen's impact on colonial odour has been mainly demonstrated in species exhibiting a high dimorphism between queen and workers (e.g., *Camponotus* spp.). However, in species with a low caste dimorphism, this effect does not exist and the colonial odour is produced by worker cues (e.g., *Cataglyphis niger*, *Leptothorax curvispinosus*, *Pseudomyrmex ferruginea*, *Rhytidoponera confusa*). This suggests a link between the degree of queen-worker dimorphism and the involvement of the queen in the formation of the colonial odour (Lenoir et al., 1998).

In the present work, we tested this hypothesis in *Cataglyphis iberica* with regard to its low queen-worker dimorphism and to its diffuse colony structure. *Cataglyphis iberica* is a monogynous and polydomous ant species which has very closed societies (Dahbi et al., 1996a). We analysed the consequences of the absence of a queen during group separation on intracolonial recognition and on the cuticular hydrocarbon profile of workers. We first looked for differences in cuticular hydrocarbon profiles between workers and the queen and further analysed divergence between workers' profiles as a consequence of their separation. Finally, we investigated the degree to which the presence of the queen in only one of the separated groups influenced this divergence by analysing the similarities between queen and worker profiles. It was also interesting to determine if the queen has a major role in the establishment of the colonial odour in this species in a way that could explain the higher frequency

of adult transports connecting satellite nests observed at the end of hibernation, transports essentially directed towards the queenright nest (Cerdá et al., 1994).

Material and methods

Three queenright colonies, A, B and C, each including about 300 to 400 workers and brood were collected during the summer of 1994 in Bellaterra (Barcelona area, Spain). Each colony originally comprised two nests joined together after excavation and maintained in the laboratory under standard conditions (25 °C ± 2 °C, relative humidity of 50 % and a photoperiod of 10:14 D/L).

Each colony was randomly divided into two equal groups (150 to 200 workers): one queenright (Q^+) and one queenless (Q^-). Separation lasted for three months during which all groups were fed identically with mealworms and a honey/apple mixture. Brood was excluded from the study by removing it before splitting the colonies and by taking eggs from queenright groups during the separation period.

Behavioural study of interindividual recognition

We conducted dyadic encounters of 10 min between Q^+ and Q^- workers (test dyads Q^+/Q^-), at the beginning of group separation and three months later. For each colony five replicates were conducted and compared to control encounters (5 dyads Q^+/Q^+ and 5 dyads Q^-/Q^-) at the same periods. Encounters were conducted in a circular plastic box (8 cm in diameter), cleaned with alcohol before each test. Tested workers were changed after each encounter to avoid any familiarisation effect or odour transfer. Observed interactions mainly consisted in mutual antennations. We therefore recorded in each encounter, the frequency and duration of antennal exchanges between the dyadic partners using an event-recorder.

Study of cuticular hydrocarbon profiles

We analysed the cuticular hydrocarbon profile of five workers randomly chosen from each colony before group separation. In order to study profile divergence during the three months of separation, we also analysed Q^+ and Q^- profiles after their separation. The cuticular extracts of these workers as well as those of queens were individually analysed using gas chromatography. We used acetone as solvent since the extracts are identical to those obtained with pentane in *Cataglyphis* species. Each analysed ant was immersed in 1 ml of acetone for 10 min. The extract was then dried under nitrogen and diluted with 30 µl of acetone. Two microliters of the diluted extract were injected into a DELSI 300 chromatograph using a capillary column (Chrompack CPSIL 5 WCOT, long: 25 m, diameter: 0.22 mm) with a programmed temperature ranging from 100 °C to 280 °C at 3 °C/min. The surface of the different peaks of the cuticular spectrum were provided by an ENICA 21 integrator. The complete analysis of postpharyngeal gland secretion in *C. iberica*,

which much extracted cuticular lipids (authors, pers. comm.), resulted in the identification of only hydrocarbons (Dahbi et al., 1996a; Dahbi et al., 1996b).

We calculated the mean of cuticular surface area (CSA) for the two castes, workers (n = 10) and queens (n = 4). For each individual, the total surface expressed in mm^2 was deduced by calculating and summing the surfaces of head, thorax and abdomen. Surface calculation was computed after fitting the real shape of each ant body part by an ellipsoid. Calculations were carried out using Mathematica software.

We used octadecane (nC_{18}) as external standard to calculate the respective quantities of the 24 major peaks of the spectrum using the following formula:

$$Pn = 15 * [Sn * (P_{(es)}/S_{(es)})]/S_m$$

where Pn is the weight of the peak n in ng/mm^2 of CSA; 15 is the dilution factor; Sn is the integration surface of the peak n; $P_{(es)}$ is the weight of the external standard (C_{18}) in ng; $S_{(es)}$ is the integration surface of the external standard; and S_m is the mean of CSA in mm^2.

According to these quantities, expressed in ng/mm^2 of CSA, we studied quantitative differences in the cuticular hydrocarbons between Q^+ and Q^- workers and determined which hydrocarbons changed quantitatively during separation. We also analysed the quantitative divergence of these compounds between worker and queen castes in order to determine caste-specific compounds.

We performed a Factorial Analysis of Correspondences (Addad software) to analyse the evolution of the colonial profile during the three months. We compared Q^+, Q^- and queen profiles to highlight divergence due to separation and caste. For this analysis, we used the relative proportions of the same 24 major peaks of the cuticular spectrum.

In order to quantify the similarities in cuticular profiles, we calculated amalgamation distances, i.e., the geometrical distances between individual profiles in a multidimensional space. These distances were calculated within and between Q^+ and Q^- groups, and between queens and Q^+ or Q^- groups.

Results

Behavioural study of interindividual recognition

The separation of nestmates for three months never induced aggression when workers were reunited. Recognition ability between nestmates was maintained and interactions were limited to mutual antennations. However, worker separation induced longer mutual antennations between workers previously separated (Q^+ vs Q^-) than between workers of the same group (Table 1). This behavioural modification was similar for the three colonies tested (1.59 s ± 0.40 s, 1.64 s ± 0.25 s and 1.30 s ± 0.50 s, for colonies A, B and C, respectively) (mean ± SD) (Mann-Whitney U-test, $P > 0.05$). Within the same type of dyads, the duration of antennal investigations after separation increased in the dyads Q^+ vs Q^- but surprisingly significantly decreased in control encounters (Q^+ vs Q^+ and Q^- vs Q^-). Moreover, the number of

Table 1. Mean durations (in sec) (±SD) of a single antennal investigation and mean frequencies (±SD) of antennal interactions during a dyadic encounter of 10 min. Q^+ and Q^- correspond to workers of queenright and queenless groups, respectively. For each of the two periods (beginning and end of separation), significant differences are shown by different letters (ANOVA, Newman Keuls test, $P<0.05$). For each type of dyad, the values recorded at the beginning of separation and three months later were compared using the Student t-test ($P<0.05$)

	Control dyads		Test dyads
	Q^+ vs Q^+	Q^- vs Q^-	Q^+ vs Q^-
Duration			
Beginning of separation	1.2 ± 0.6 (a)	1.1 ± 0.5 (a)	1.0 ± 0.3 (a)
Three months later	0.6 ± 0.2 (a)	0.8 ± 0.3 (a)	1.5 ± 0.4 (b)
P (Student t-test)	0.0009	0.0349	0.0006
Frequencies			
Beginning of separation	30.3 ± 6.1 (a)	30.4 ± 5.7 (a)	32.4 ± 7.7 (a)
Three months later	29.0 ± 11.6 (a)	30.1 ± 8.0 (a)	28.5 ± 6.5 (a)
P (Student t-test)	NS	NS	NS

antennations remained unchanged and separation did not lead to an increase in antennal interaction frequencies which were stable over all dyads and periods (around 30 interactions per 10 min) (Table 1).

Study of cuticular hydrocarbon profiles

Table 2 shows the quantities (ng/mm^2 of CSA) of the 24 major hydrocarbons of the cuticular spectrum in Q^+ and Q^- groups and in queens. Separation leaded to a slight quantitative variation between Q^+ and Q^- groups. This variation affected only two dimethylnonacosane (11, 13 and 7, 17 $DiMeC_{29}$). Queen and workers displayed the same hydrocarbons, but their profiles were quantitatively quite different, mostly due to n-alkanes. A significantly higher quantities of heptacosane (nC_{27}), octacosane (nC_{28}), nonacosane (nC_{29}) and x, y dimethylnonacosane ($DiMeC_{29}$) was found in the queen profile. In contrast, the worker profile had greater quantities of 11+13 methylheptacosane (MeC_{27}). Nevertheless, the total amount per mm^2 of CSA of all 24 hydrocarbons was statistically similar in both castes despite the obvious difference in n-alkanes.

The comparison of cuticular hydrocarbon profiles by Factorial Analysis of Correspondences performed on relative proportions (Fig. 1) shows significant changes occurring during the three months of separation. For each of the three colonies, Q^+ and Q^- profiles differed clearly from the initial profile (prior to separation). In addition, queen profiles (indicated by the letter Q on Fig. 1) were clearly distinguishable from those of their respective workers. This queen-worker distinction was effective along Factor 2 and was essentially due to n-alkanes (C_{27}, C_{28} and C_{29}) which highly contributed to the variance on this factor (average variance of 44 % for the three colonies). Indeed, the relative abundance of these compounds in the spectrum was higher in queens (27.15 % ± 3.46 %) (mean ± SD) than in workers

Table 2. Chemical identity of the 24 major cuticular hydrocarbons of *Cataglyphis iberica* workers and queens (Me and DiMe correspond to methyl and dimethyl, respectively). Values indicate, for each compound, the mean weight (ng/mm^2 of insect cuticular surface area) (± SD) for a 10 min extraction with acetone (t indicates the products in traces). Values are calculated for Q^+ workers (n = 15), Q^- workers (n = 15) and queens (n = 3) and asterisks mean that differences are significant for each compound between Q^+ and Q^- workers or between all workers and queens

Chemical determination	Group Q^+	Group Q^-	Queens	Mann-Whitney U-test	
				Q^+ vs Q^-	Workers vs queens
n-Alkanes					
C_{27}	0.12 ± 0.13	0.05 ± 0.07	0.68 ± 0.51	–	**
C_{28}	0.21 ± 0.20	0.17 ± 0.13	0.81 ± 0.24	–	**
C_{29}	4.66 ± 3.94	2.77 ± 1.79	8.26 ± 2.16	–	*
C_{31}	1.73 ± 1.44	1.07 ± 0.60	2.04 ± 0.82	–	–
Sub-total	*6.72 ± 5.64*	*4.05 ± 2.50*	*11.79 ± 3.52*	–	*
Monomethylalkanes					
11+13Me-C_{27}	0.36 ± 0.32	0.30 ± 0.34	t	–	*
13+15Me-C_{29}	2.38 ± 0.87	2.30 ± 1.28	1.51 ± 0.73	–	–
9Me-C_{29}	0.36 ± 0.15	0.32 ± 0.14	0.31 ± 0.12	–	–
7Me-C_{29}	0.28 ± 0.14	0.32 ± 0.19	0.34 ± 0.16	–	–
5Me-C_{29}	1.42 ± 0.88	1.24 ± 0.49	1.85 ± 0.52	–	–
3Me-C_{29}	3.33 ± 2.29	2.58 ± 0.95	4.50 ± 1.47	–	–
4Me-C_{30}	1.13 ± 0.61	1.21 ± 0.62	1.23 ± 0.34	–	–
15Me-C_{31}	2.17 ± 0.70	2.18 ± 1.19	1.40 ± 0.80	–	–
9Me-C_{31}	0.66 ± 0.34	0.64 ± 0.35	0.69 ± 0.12	–	–
7Me-C_{31}	0.72 ± 0.39	0.63 ± 0.25	0.69 ± 0.25	–	–
5Me-C_{31}	0.67 ± 0.53	0.46 ± 0.20	0.64 ± 0.40	–	–
Sub-total	*13.49 ± 6.00*	*12.19 ± 4.82*	*13.16 ± 4.38*	–	–
Dimethylalkanes					
11, 13DiMe-C_{29}	0.36 ± 0.41	0.81 ± 0.66	1.10 ± 0.44	*	–
7, 17DiMe-C_{29}	0.25 ± 0.27	0.54 ± 0.44	0.73 ± 0.30	*	–
5, 9DiMe-C_{29}	0.49 ± 0.23	0.55 ± 0.35	0.60 ± 0.12	–	–
x, yDiMe-C_{29}	0.31 ± 0.36	0.14 ± 0.24	0.67 ± 0.26	–	*
3, 9DiMe-C_{29}	1.14 ± 0.53	1.35 ± 0.78	1.61 ± 0.44	–	–
11, 15DiMe-C_{31}	3.67 ± 2.17	4.08 ± 3.02	3.67 ± 1.40	–	–
4, 22DiMe-C_{32}	1.08 ± 0.53	1.22 ± 0.74	1.20 ± 0.30	–	–
x, 15DiMe-C_{32}	0.64 ± 0.28	0.68 ± 0.41	0.58 ± 0.20	–	–
13, 17DiMe-C_{33}	1.03 ± 0.46	1.10 ± 0.75	1.14 ± 0.26	–	–
Sub-total	*8.97 ± 4.69*	*10.47 ± 7.06*	*11.30 ± 3.64*	–	–
Total amount	29.18 ± 15.0	26.71 ± 12.1	36.26 ± 10.7	–	–

* $P < 0.05$, ** $P < 0.01$.

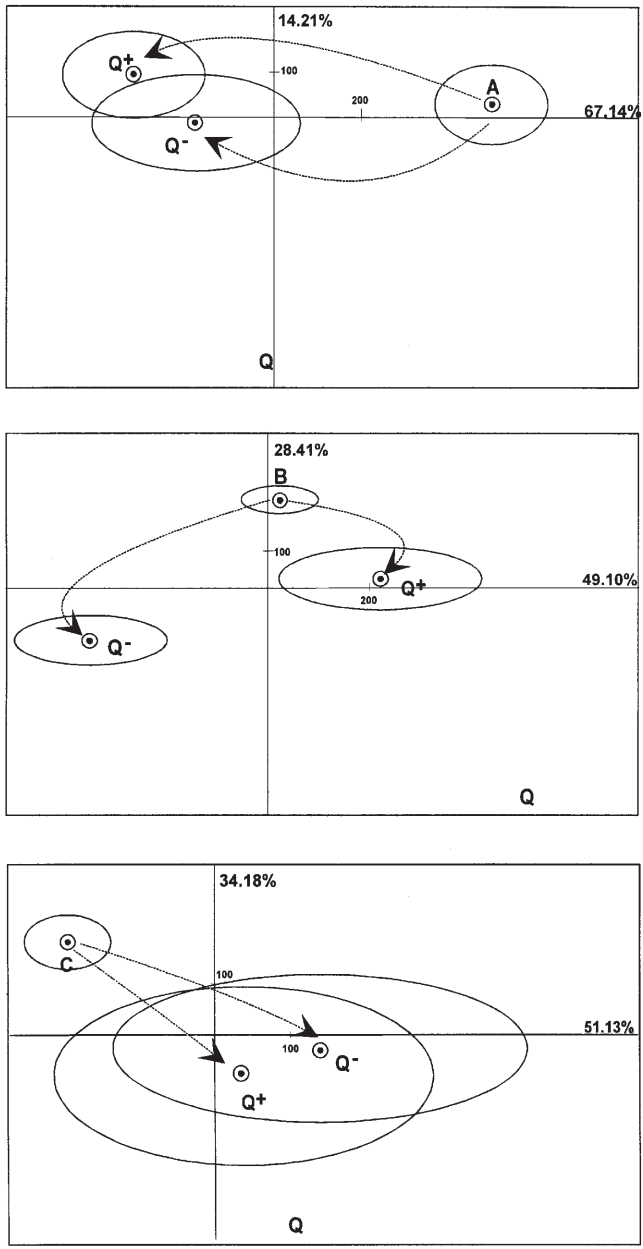

Figure 1. Factorial Analysis of Correspondences carried out for each of the three colonies (A, B and C) of *Cataglyphis iberica*. Each colony is represented by the cuticular profile of workers before separation (n = 5) and cuticular profiles of Q^+ (n = 5) and Q^- (n = 5) individuals three months after separation. The queen profile is indicated by the letter Q. Each worker group is represented by the mean point of its individual profiles, and the ellipses correspond to standard deviations of the individual co-ordinates in the bidimensional space. The ellipses visually represent the level of homogeneity of individual profiles within groups and the divergence of profiles between groups

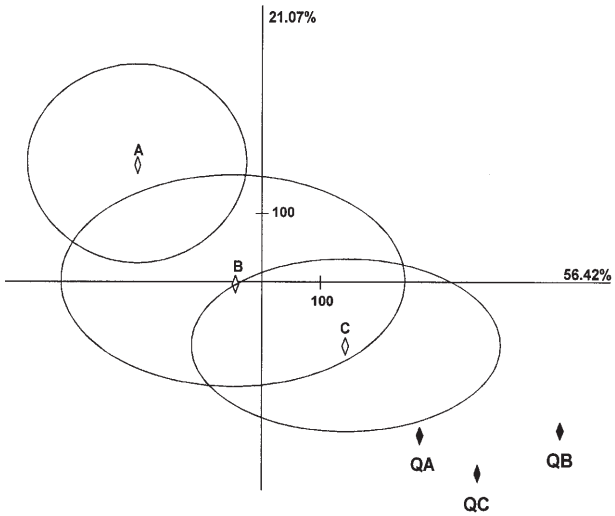

Figure 2. Factorial Analysis of Correspondences carried out on the individuals of the three colonies (Q^+ and Q^- together) (n = 10 for each colony) and their respective queens QA, QB and QC (for example: QA represents the queen of colony A)

(13.72 % ± 6.82 %) (Mann-Whitney U-test, $P < 0.05$) with nonacosane (C_{29}) being the most significant compound (23.06 % ± 2.40 % and 12.81 % ± 6.37 % for queens and workers, respectively) (Mann-Whitney U-test, $P < 0.05$).

Amalgamation distances show that profile similarities within Q^+ and Q^- groups were very close (9.31 ± 4.85 and 9.72 ± 4.75, respectively). The lack of a queen did not induce profile heterogeneity within the queenless groups compared to queen-right groups (Student t-test, $P > 0.05$). However, Q^+ and Q^- profiles diverged slightly during the separation period, but the level of divergence vary between the three colonies. The distances which separate the worker profiles of groups compared to those separating the profiles within each group is significantly higher in colony B (15.43 ± 6.66) (Student t-test, $P < 0.05$). The same tendency appeared for the other colonies, specially in colony A, as it is shown in Figure 1, but was not statistically significant ($P = 0.24$). This is probably due to the high heterogeneity observed within groups, and the relatively short period of group separation (3 months).

Figure 2 shows that the profile of each queen was closer to that of the other queens than to its own worker profile. This apparent lack of colonial specificity of the queen profile clearly appears in the amalgamation distances calculated between the profiles of the queens and those calculated between the profiles of the queens and their respective workers. Distances between queens (6.17 ± 1.13) were significantly lower than queen/Q^+ distances and queen/Q^- distances (14.03 ± 5.69 and 16.82 ± 6.35, respectively) (ANOVA, Newman Keuls, $P < 0.05$). In addition, distances separating the queen profile from the Q^+ and Q^- profiles were similar (ANOVA, $P > 0.05$). These results suggest that the lack of a queen had no dramatic consequences on the queenless workers' profile. Each of the three queens presented a characteristic profile, quite different from that of its own offspring.

Discussion

The behavioural and chemical consequences of worker separation support our previous observations on *C. iberica* (Dahbi and Lenoir, 1998). Separation induced slight behavioural changes without suppressing nestmate recognition in this species. When we reunited the groups, we only observed longer mutual antennations between previously separated workers without aggressive reactions. This extension in antennal duration was probably due to the slight divergence observed in the cuticular hydrocarbon profiles of the workers during separation. According to Bonavita-Cougourdan et al. (1987), each worker memorises specific proportions of hydrocarbons allowing her to detect and reject any intruder displaying divergent proportions. Profile divergence in our results was not so obvious to induce aggression between queenright and queenless nestmates, but was enough for workers to detect individuals from the other group. A similar result was observed by Provost (1989) in *Leptothorax lichtensteini*. This author showed that the confrontation between two groups of workers previously separated for four months comes with an increase in mutual antennations prior to the total fusion of the groups. Furthermore, when changing the chemical signature of *Camponotus vagus* workers by topical application of 9-(Z) tricosene on their cuticle, Meskali et al. (1995) observed more antennations towards treated workers without eliciting aggression.

Some studies have clearly demonstrated dynamic changes in cuticular hydrocarbon profiles over time (Vander Meer et al., 1989; Provost et al., 1993). In our experiments, the cuticular hydrocarbon profile of *C. iberica* workers changed in the two groups over the three months of separation, but group profiles did not change in the same manner, diverging slightly during separation. This difference suggests a transfer of cues between workers, leading to a characteristic group "Gestalt" (Crozier and Dix, 1979), and resulting in a unilateral change within each group. However, hydrocarbon profile divergence was more important when group separation lasted 12 months (Dahbi and Lenoir, 1998). In the present study, the 3 months group separation may not have been so long to induce a significant divergence of group profiles. Even when the profile differences between the groups were not significant, the behavioural modifications appeared. It suggests that ants are capable to detect a slight hydrocarbon profile divergence, but also suggests that cuticular hydrocarbons may not be the unique compounds mediating nestmate recognition. Indeed, other chemical classes are probably involved in the recognition process as it was previously suggested by Franks et al. (1990) and discussed by Vander Meer and Morel (1998).

Do queens affect the homogeneity of worker profiles and are they involved in the profile divergence between queenright and queenless groups? Amalgamation distances show that the homogeneity of cuticular hydrocarbon profiles was the same in both queenright and queenless groups. Therefore, the absence of the queen did not induce a heterogeneity of worker profiles between queenless nestmates. The situation is different in *Formica* sp. where queenless workers lost their chemical homogeneity 10 days after being orphaned. This homogeneity was recovered as soon as the queen was reintroduced into the queenless group (Yamaoka, 1990). In our queenright groups, if a transfer of compounds had occurred between queens and workers during separation, considering that the queen profile had higher quan-

tities of n-alkanes, the Q^- workers would have displayed lower quantities of these compounds relatively to their Q^+ nestmates, which was not observed. We previously demonstrated in *C. iberica* that after separation, trophallactic exchanges, which are the principle vehicle for chemical compounds transfer (Soroker et al., 1994), were much more frequent between worker nestmates previously separated (Dahbi et al., pers. comm.). But only infrequent exchanges occurred between workers and their queen. This could explain at least partly the lack of convergence between queen and worker profiles. A similar quantitative divergence in the cuticular hydrocarbon profile between the two castes was reported by Butts et al. (1995) on the European hornet *Vespa crabro*. These authors showed that the profile of the queen can be distinguishable from that of the workers by its higher proportion of n-alkanes, mainly pentacosane (nC_{25}). In *Polistes dominulus*, the profiles of reproductive females are clearly differentiated from those of their offspring mainly due to n-alkanes (Bonavita-Cougourdan et al., 1991).

The queen's contribution to the colonial odour has been demonstrated in several ant species. In some *Camponotus*, the queen is the main source of the colonial odour (Carlin and Hölldobler, 1983, 1986). In *Leptothorax lichtensteini*, Provost (1989) showed that nestmate recognition remained effective between separated workers only when the queen was placed alternatively in the different groups during the period of their separation. After splitting a colony of *Myrmecia tarsata* into two halves (queenright and queenless) for four months, Haskins and Haskins (1950) observed antagonistic interactions between workers when they were reunited. However, opposite results were obtained by Wallis (1962) in *Formica fusca*, leading this author to conclude that the queen has no effect on nestmate recognition. If the queen's effect on colonial odour in *C. iberica* affects the physiognomy of the cuticular hydrocarbon spectrum of the workers, the profile of Q^+ workers and that of the queen would be much more similar. However, queens exhibited a specific profile clearly different from the hydrocarbon "Gestalt" of its offspring. The queen hydrocarbon profiles of the three colonies studied were closer to each other than to profiles of their respective workers. If cuticular hydrocarbons are involved in the colonial odour, we suggest that *C. iberica* queens are not responsible for the generation of the colonial "Gestalt" odour. Queens display their own "royal" profile which is recognised by all nestmate and non-nestmate workers as it was shown in *Cataglyphis cursor* by Berton et al. (1991). However, adoption of alien conspecific queens in *C. iberica* colonies is unsuccessful (Authors, personal observation, and also observed in *C. cursor* by Berton et al. (1991)). These authors suggested that *C. cursor* queens produce pheromones which attract workers and other pheromones which mediate their discrimination by their workers. Vienne et al. (1998) also hypothesised this double recognition system using mixed colonies. In light of our results, we suggest the coexistence of two recognition processes within *C. iberica* colonies: one involves the "Gestalt" odour common to all sister workers and allows them to discriminate intruders; the other involves the memorisation of the queen's caste-specific odour. This queen odour is attractive to workers irrespective of the colonial origin of the queen (Berton et al., 1991), and the high quantities of n-alkanes in the queen profile makes them good candidates for this caste discrimination. However, a subtle discrimination mechanism allows workers to discriminate the odour of their mother queen and to reject foreign queens. This discrimination

could occur during neoimaginal learning since callow workers are always located at the proximity of their queen inside the nest.

We recently demonstrated that mutual transports between satellite nests at the end of hibernation in *C. iberica* are involved in odour homogenisation among all colony members and that these transports are essentially focused on the queenright nest (Dahbi et al., 1997). We thus hypothesised that the *C. iberica* queen could play a role in the mechanisms of cue exchange involved in the establishment of the colonial odour, as is the case in *Camponotus* spp. (Carlin and Hölldobler, 1983, 1986). The present results do not corroborate this hypothesis and suggest that transports concentrated on the queenright nest are not tied to the "Gestalt" odour of workers but could be a consequence of the higher number of workers in the queenright nest or linked to the control of young workers' reproduction which necessitates, from time to time, their transport to the queenright nest. Moreover, in contrast with *Camponotus* spp., queen weight in *C. iberica* is only two to three times higher than that of workers. It seems therefore doubtful, in a species with multiple nest colonies, each containing several hundred workers (Cerdá and Retana, 1992; Cerdá et al., 1994), to conceive that the queen could be the source of the colonial odour. In addition, the separation of workers into queenless groups for a long time duration does not affect their recognition ability when they are reunited (Dahbi and Lenoir, 1998). Furthermore, the total quantity of hydrocarbons per unit of CSA in *C. iberica* is the same in workers and queens. These results, combined with those reported by Soroker et al. (1996) on *C. niger* which show a higher rate of cuticular hydrocarbon production in workers and flux exchanges of these compounds mainly directed between workers, indicate that the queen may be outside the "Gestalt" odour system, at least the hydrocarbon "Gestalt", and is not the main source of chemical cues involved in nestmate recognition in *Cataglyphis* species.

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