Colony insularity through queen control on worker social motivation in ants

Raphaël Boulay[1*], Tamar Katzav-Gozansky[1], Robert K. Vander Meer[2] and Abraham Hefetz[1]

[1]*Department of Zoology, Tel Aviv University, Ramat Aviv, 69978 Tel Aviv, Israel*
[2]*USDA/ARS, CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA*

We investigated the relative contribution of the queen and workers to colony nestmate recognition cues and on colony insularity in the Carpenter ant *Camponotus fellah*. Workers were either individually isolated, preventing contact with both queen and workers (colonial deprived, CD), kept in queenless groups, allowing only worker–worker interactions (queen deprived, QD) or in queenright (QR) groups. Two weeks post-separation QD and QR workers were amicable towards each other but both rejected their CD nestmates, which suggests that the queen does not measurably influence the colony recognition cues. By contrast, aggression between QD and QR workers from the same original colony was apparent only after six months of separation. This clearly demonstrates the power of the Gestalt and indicates that the queen is not a dominant contributor to the nestmate recognition cues in this species. Aggression between nestmates was correlated with a greater hydrocarbon (HC) profile divergence for CD than for QD and QR workers, supporting the importance of worker–worker interactions in maintaining the colony Gestalt odour. While the queen does not significantly influence nestmate recognition cues, she does influence colony insularity since within 3 days QD (queenless for six months) workers from different colony origins merged to form a single queenless colony. By contrast, the corresponding QR colonies maintained their territoriality and did not merge. The originally divergent cuticular and postpharyngeal gland HC profiles became congruent following the merger. Therefore, while workers supply and blend the recognition signal, the queen affects worker–worker interaction by reducing social motivation and tolerance of alien conspecifics.

Keywords: nestmate recognition; queen; social motivation; trophallaxis; hydrocarbons; colony merging

1. INTRODUCTION

It has long been hypothesized that social animals possess a social drive, and that separation from the group induces physiological and behavioural modifications comparable to those resulting from the deprivation of food or drink (Wheeler 1928). Although the neurobiological bases of motivation as well as its behavioural expression are species specific, the 'social hunger', which may have evolved independently, constitutes underlying common features in that individuals depend on the group to improve their survival and reproduction. Social motivation was mainly investigated in vertebrates where it can be expressed by increasing play behaviour or social preference induced by the deprivation of partners (Ikemoto & Panksepp 1992; Varlinskaya *et al.* 1999). However, social motivation may also govern the behaviour of social invertebrates, as exemplified by ants, in the form of the increased demand of social care (particularly trophallaxis) following social deprivation. For instance, social isolation in the Carpenter ant *Camponotus fellah* increases the frequency of trophallaxis with nestmates and non-nestmates, irrespective of satiety (Boulay *et al.* 1999; Boulay & Lenoir 2001). This isolation-induced increase in trophallaxis events is suppressed by octopamine (Boulay *et al.* 2000a), a biogenic amine known to modulate motivational behaviour (Adamo *et al.* 1995; Robinson *et al.* 1999). However, these

results are complicated because the influence of the queen and/or the workers were not differentiated; thus the absence of the queen may be sufficient to elicit the effect. For instance, in a few *Camponotus* species, and in *Solenopsis invicta*, the removal of the queen decreases worker aggressiveness and stimulates trophallaxis and allogrooming even with alien conspecifics (Hölldobler 1962; Benois 1972; Vander Meer & Alonso 2002).

The induction of congenial social interactions between non-nestmate conspecifics is surprising because queen-right (QR) colonies of these species are normally closed to conspecific non-nestmates, a characteristic of many social insects. Prevention of outside intrusions is brought about through a nestmate recognition system by which the ants are able to differentiate quantitative (conspecific) and qualitative (heterospecific) nestmate recognition discriminators, after comparing them with their own template. Because one ant is unlikely to have numerous templates corresponding to the chemical phenotype of each of its nestmates, it was proposed that colony members exchange recognition cues so that each one carries and recognizes a single mean recognition signal or Gestalt odour (Crozier & Dix 1979; Crozier 1987; Vander Meer & Morel 1998). Using hydrocarbons (HCs) as a model system for recognition cues, inter-individual HC exchange by trophallaxis and allogrooming were further demonstrated in several species including *C. fellah* (Soroker *et al.* 1994; Meskali *et al.* 1995; Soroker *et al.* 1998; Lenoir *et al.* 2001b). The postpharyngeal gland (PPG), located in front of the head, is a reservoir that can be used by the ants to

*Author and address for correspondence: Estación Biológica de Doñana, CSIC, Apdo. 1056, 41013 Sevilla, Spain (boulay@ebd.csic.es).

Proc. R. Soc. Lond. B (2003) **270**, 971–977
DOI 10.1098/rspb.2002.2325

971

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admix external (received from nestmates) and endogenous recognition cues to build a Gestalt odour. Both cuticular and PPG HC composition are dynamic and change with time, so that separated groups tend to form new odour profiles that progressively diverge from each other (Vander Meer *et al.* 1989; Lahav *et al.* 2001). Likewise, individually isolated ants exhibit, over time, more variable cuticular profiles than workers kept in the mother colony (Boulay *et al.* 2000*b*; Lenoir *et al.* 2001*a*). The likely explanation is that in the absence of exchange with other workers each individual loses its former colony HC profile owing to a combination of compound turnover and that newly synthesized cues are only self-derived. The result is that each individual changes independently of the others and progressively deviates from the Gestalt colony profile.

Creating a uniform colony odour does not necessarily imply that workers and queen(s) contribute equally to the colony label. In *Camponotus* spp. the queen was postulated to be the major contributor of colony discriminators (Carlin & Hölldobler 1986, 1987) while in other species a central role of the queen was not found (e.g. *L. curvispinosus* (Stuart 1987), *Rhytidoponera confusa* (Crosland 1990), *Cataglyphis iberica* (Dahbi & Lenoir 1998) and *C. niger* (Lahav *et al.* 1998)). This may indicate the evolution of multiple nestmate recognition systems with differing hierarchies of cue source importance. Irrespective of the recognition cue source it is clear that a mechanism to create a uniform colony odour is required, such as that mentioned here involving the PPG.

Nestmate recognition might be affected either by: (i) changing the colony label; (ii) altering the motivational state of the workers; or (iii) altering the worker's ability to detect subtle conspecific discriminator differences (Vander Meer & Alonso 2002).

We evaluate the relative contribution of the queen in the production of recognition cues (experiment 1) and her effect on worker aggressiveness and social motivation (measured by the trophallaxis rate) during interactions with non-nestmates (experiment 2) in the Carpenter ant *C. fellah*. Unpublished field observations (R. Boulay and A. Hefetz) indicate that queens individually found colonies (monogyny) although oligogyny cannot be excluded. Adult colonies are large and polydomous with apparently overlapping territories, which may enhance contacts between alien queenless nests. Workers were either experimentally kept under queen-deprived (QD) or colony-deprived (CD, equivalent to social isolation) conditions. We hypothesized that if queen-derived cues dominate over worker discriminators, QD workers will be rapidly aggressed by their QR nestmates but QD workers will not aggress their CD nestmates. If worker-derived cues dominate we would expect to quickly observe aggressive interactions for both QR and QD toward, but not between, QR and QD nestmates. If the queen has a general effect on worker social motivation, an increased demand for trophallaxis in QD workers is expected and QD colonies may be more permissive of conspecific alien ants than QR colonies. Finally, if the queen affects worker social motivation, increasing demands for trophallaxis in both CD and QD workers are expected, whereas if worker–worker interactions satisfy this demand, CD and QD should differ in this respect.

2. MATERIAL AND METHODS

(a) *Queen and colony deprivation effects on interactions between nestmates*

Sixteen large *C. fellah* colonies (more than 500 workers) were reared from newly mated queens collected in Tel Aviv (Israel) after nuptial flights. They were maintained in a controlled temperature ($29 \pm 2\,^\circ\text{C}$) room in artificial nests, with an identical diet of dead mealworms plus 20% w/v sugar water. Workers (about 50) from each colony were CD by keeping them individually in Petri dishes ($3\,\text{cm} \times 0.5\,\text{cm}$), on a sugar water (20% w/v) diet only. The remaining workers from each colony were divided equally into QD and QR parts with the same quantity of brood and reared under the same conditions as stock colonies. Bioassays for measuring aggressive behaviour between CD, QD and QR ants were conducted 1, 7, 14, 21, 28 and 63 days (two months) after separation. Aggressive behaviour between workers from the QD and QR groups was also tested three and six months after separation. Bioassays were performed in a circular arena ($9\,\text{cm} \times 7\,\text{cm}$ diameter and height, respectively) containing 50 workers (resident ants) into which a marked ant (a dot of paint on the thorax (Unipaint; Mitsubishi Pencil Co., Ltd, Japan)) was introduced (intruder ant). Resident ants were QR or QD workers. Intruder ants were QR, QD or CD. The resident workers were placed in the arena for 30–60 min before each test to allow acclimation. Observations were conducted at 3 and 40 min after introduction of the intruder. During each session, the number of resident ants and their behaviour toward the introduced ant was recorded 20 times, every 10 s. Recorded behaviours were amicable social behaviour (trophallaxis and grooming) and aggressive behaviour (gaster flexion, threats with open mandibles and bites). After the second session, intruders were sacrificed and the resident ants were returned to their original group.

Chemical analyses of PPG and cuticular HCs from QR, QD and CD workers were done on day 21 post-separation. Cuticular and PPG sample preparation and peak identification by gas chromatography mass spectrometry were achieved by a standard method described by Lahav *et al.* (2001). Quantification of PPG and cuticular HCs was done by gas chromatography (Varian CP 3800; FID detector) equipped with DB-1 fused silica column temperature programmed from 150 to 300 °C at 5 °C min^{-1}. Pentadecane was added to each sample as an internal standard. Of the identified peaks, only those that were reliably and reproducibly quantified were used for the statistical analyses (28 and 17 peaks for PPG and cuticular HCs, respectively; see electronic Appendix A, available on The Royal Society's Publications Web site, for the description). Heavily tailing peaks and peaks below 1% or that did not appear in some of the chromatograms owing to low concentration of the sample were not considered.

(b) *The effect of the queen on between non-nestmate interactions and the merging of alien colonies*

To test tolerance between workers from alien QD or QR groups six months post-separation, seven QD–QD and four QR–QR nest-pairs were connected via a common neutral foraging arena ($30\,\text{cm} \times 30\,\text{cm}$). The nest entrance of each colony was opened at the onset of the experiment so the ants could move freely between nests by crossing the common arena. Workers were distinctly marked with a colour dot on the abdomen to identify their nest of origin, and their behaviour in the common arena as well as within each nest was recorded. The number of aggressive acts between alien ants, and trophallaxis
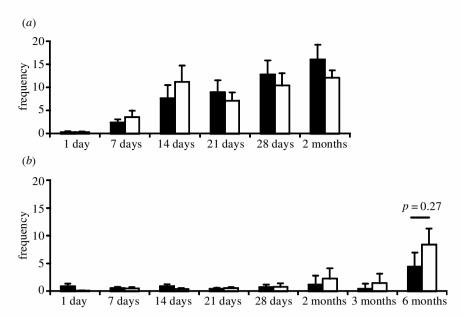
Figure 1. Mean (± s.e.) frequency of aggression between nestmate ants at different intervals post-separation. The black and white bars represent the behaviour of QD and QR resident ants, respectively, towards CD workers (*a*) and towards workers of the complementary group (*b*). A total of 564 encounters were done. (*a*) ANOVA: resident/introduced pair effect: $F_{1,168} = 0.18$, $p = 0.668$; time effect: $F_{5,168} = 9.68$, $p < 0.001$; interaction: $F_{5,168} = 0.68$, $p = 0.638$. (*b*) ANOVA: resident/introduced pair effect: $F_{1,212} = 3.11$, $p = 0.079$; time effect: $F_{7,212} = 7.74$, $p < 0.001$; interaction: $F_{7,212} = 1.28$, $p = 0.260$.

between alien ants and/or nestmates, was recorded 50 times over 3 days with at least 30 min intervals between recordings. For each QD–QD and each QR–QR pair 10 ants were randomly collected from each nest on the day before and after the merging phase for chemical analyses of their PPG content following the method described above (§ 2a).

(c) Statistical analysis

In the first experiment, aggressive and amicable scores were obtained by averaging the frequency of these behaviours during the first and the second session of each encounter. Three independent two-way ANOVAs were then run on these scores, each considering resident/introduced pair as first factor and time as second factor (first ANOVA: QD–CD versus QR–CD pairs crossed with time: 1 day to two months; second ANOVA: QD–QR versus QR–QD crossed with time: 1 day to six months; third ANOVA: QD–QD versus QR–QR crossed with time: 1 day to two months). In the second experiment the numbers of ants in each nest-pair were not equal. We therefore expressed both trophallaxis and aggressive interactions as relative frequencies (the absolute frequency divided by the total number of workers in the respective pair before the onset of experiment multiplied by 100). Relative frequencies were compared using two-way ANOVA: first factor, treatment of the colonies (QR and QD); second factor, day after unification (1, 2 and 3). Specific effects were tested with the Newman–Keuls *post hoc* test.

Cuticular and PPG HC profiles were analysed with forward stepwise discriminant analyses. Squared Mahalanobis distances (SMds) between centroids were calculated to estimate the level of between-group discrimination.

3. RESULTS AND DISCUSSION

(a) Queen and colony deprivation effects on the interactions between nestmates

The central role of the queen in the production of the colony recognition label predicts that because of queen

label fade-out after separation from the queen, QD workers will be aggressed by QR workers. However, the level of aggression between QR and QD workers was very low, even after three months of separation; aggression between workers became significant only after six months post-separation (figure 1*b*). The factor resident/intruder pair did not affect significantly the level of aggression between QR and QD ants although after six months of separation QR residents aggressed more QD intruders than vice versa (figure 1*b*; Newman–Keuls, $p = 0.027$). In control encounters (QD–QD and QR–QR) the level of aggression was null or very low (data not shown) and not significantly affected by the factor resident/introduced pair nor by the factor time (ANOVA: $F_{1,168} = 0.16$, $p = 0.686$ and $F_{5,168} = 0.93$, $p = 0.670$, respectively). In contrast to the above, during the first two months post-separation, aggressive interactions were observed almost only when CD workers were introduced into either QR or QD groups (figure 1; statistical significances are given in the legend). From day 14 of isolation onwards, CD workers were strongly aggressed irrespective of the resident ants they had encountered (figure 1*a*). Aggression after 28 days even culminated in the death of 3 and 5 out of 16 introduced CD ants by QR and QD resident ants, respectively.

A queen-centred nestmate recognition signal should fade out with time when the ants become queenless. However, QD workers were recognized as nestmates by QR workers even after three months, which tends to refute this hypothesis because the workers are unlikely to retain queen-derived cues for such a long time owing to compound turnover. For the same reason, the special condition of colony deprivation may induce faster signal divergence than queen deprivation if the workers have a major role, but not if queen-produced cues dominate. The fact that aggression of CD workers was independent of the presence of the queen indicates that both QR and QD, but not CD, ants maintained a common colony recog-

Table 1. Distances between groups created by discriminant analysis based on the relative amounts of selected HCs.
(Ants were separated for 21 days. For each colony, the first line shows SMds between the centroids of the CD, QD and QR groups for the cuticle and the PPG, respectively. The second line shows Wilk's lambda, F and p values for each discriminant function analysis.)

colony	cuticle			PPG		
	CD–QD	CD–QR	QD–QR	CD–QD	CD–QR	QD–QR
25	2090.75	2268.18	11.75	227.37	257.31	6.59
	$\lambda = 0.001$; $F_{10,16} = 52.5$; $p < 0.0001$			$\lambda = 0.082$; $F_{12,60} = 4.15$; $p < 0.0001$		
18	241.25	216.34	73.47	249.86	210.46	40.61
	$\lambda = 0.016$; $F_{22,30} = 32.7$; $p < 0.0001$			$\lambda = 0.001$; $F_{26,60} = 125.6$; $p < 0.0001$		
10	14.97	12.12	11.83	63.96	41.88	35.99
	$\lambda = 0.098$; $F_{20,76} = 8.3$; $p < 0.0001$			$\lambda = 0.025$; $F_{38,82} = 11.4$; $p < 0.0001$		
39	27.51	20.99	5.58	103.69	113.60	5.97
	$\lambda = 0.089$; $F_{14,35} = 5.36$; $p < 0.0001$			$\lambda = 0.102$; $F_{38,82} = 5.82$; $p < 0.0001$		

nition signal, providing additional evidence for a minor contribution of the queen in the production of the colony signal in *C. fellah*. The analyses of cuticular and PPG HCs, our model system for recognition cues, support this hypothesis. Discriminant analyses revealed that separation of CD, QD and QR ants for 21 days was sufficient to achieve between-group significant discriminations for both cuticular and PPG HC profiles (table 1). However, the SMds between the centroids of the CD groups and both QR and QD groups were higher (colonies 10 and 39) or much higher (colonies 25 and 18) than between the centroids of the QR and QD groups. This indicates a sharp dissimilarity induced by colony deprivation irrespective of the presence of the queen and is congruent with the significant increase of aggressive interactions in both QR–CD and QD–CD encounters at that time interval while aggression interactions in QR–QD and QD–QR were still low.

The fact that HC profiles of workers kept in separated groups diverged slowly is not surprising and was shown in the polydomous ant *Cataglyphis iberica* and the desert ant *Cataglyphis niger* (Dahbi & Lenoir 1998; Lahav *et al.* 2001). It is hypothesized that in grouped ants random changes in individually based recognition cues are averaged into a group Gestalt through social interactions (grooming and trophallaxis) as opposed to the isolated ants where only the individual recognition cues can be expressed. This explains why the time-dependent shift in the HC profile is faster in individually housed ants (CD) than in grouped ants (QD or QR) and why CD intruders were aggressed by QR and QD residents after only two weeks. Thus, in *C. fellah* both behavioural and chemical evidence points to a worker-derived rather than a queen-derived nestmate recognition signal.

However, analysis of the amicable interactions (trophallaxis) in the experiment described in the previous paragraph revealed that removal of the queen did affect worker–worker interactions. Amicable interactions were initiated more frequently by QD than by QR residents irrespective of whether the introduced workers were CD, QD or QR (figure 2*a–c*; statistical significances are given in the legend). Solicitation of amicable interactions was highest when QD and CD workers were encountered. This is consistent with previous findings that colony deprivation stimulates trophallaxis to a great extent (Boulay *et al.*

1999). As the time of separation increased, the amicable interactions tended to decrease and were replaced by overt aggression (only at six months for QR–QD). This was more pronounced in encounters involving CD-introduced ants and to a lesser extent in encounters involving workers from the complementary group.

(b) *Queen deprivation effects on the merging of alien colonies*

The results of our second experiment further demonstrate that queen deprivation affects colony insularity (territoriality). As soon as two nests were connected to a common foraging arena workers from both nests, whether QD–QD or QR–QR, started to explore it. On the first day the alien ants interacted primarily in the common arena, and the number of aggressive interactions was not significantly different between alien QD–QD and alien QR–QR workers (figure 3*a*). By contrast, on day 3 of the experiment, QD workers became significantly less aggressive towards the QD alien workers and aggression was replaced by an increasing amount of trophallaxis between non-nestmates (figure 3*b*). After 24 hours from the onset of the merger experiment some QD workers were observed entering the alien nest without being injured, allowing sizeable exchange of workers between nests. The end result was that in all QD pairs, both colonies merged to a single colony. By contrast, QR colonies were mutually aggressive and maintained their own territorial boundaries by closing the foraging arena entrance with nesting material. Trophallaxis between nestmates was high irrespective of the colonial type throughout the experiment but they were significantly more frequent in the QD nests than in the QR nests (figure 3*c*), confirming the stimulating effect of queen deprivation on trophallaxis.

Increased motivation for trophallaxis between QD alien ants allowed an extensive exchange of nestmate recognition cues that can be represented by HCs. The PPG HC profiles from representative ants of both QD nests (originated from colonies 35 and 38, respectively, in figure 4*a*) before the onset of the experiment were clearly distinct (SMd = 642.10) but clustered together and overlapped each other to a large extent (SMd = 98.88) after unification. By contrast, the profiles of QR workers remained distinct before and after the experiment (figure 4*b*; SMd = 551.86 and SMd = 931.65, respectively). Thus,
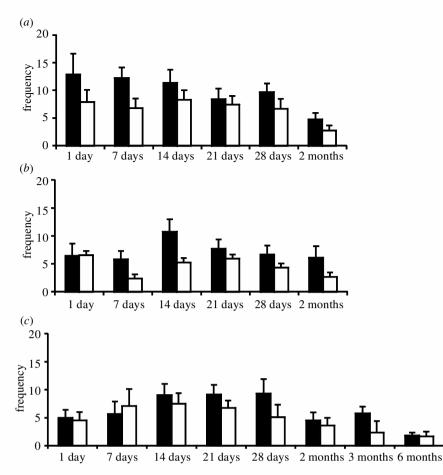
Figure 2. Mean (± s.e.) frequency of amicable interactions (trophallaxis plus grooming) between nestmate ants at different intervals post-separation. Black and white bars represent the behaviour of QD and QR resident ants, respectively, towards CD workers (*a*), towards workers of the same group (*b*) and towards workers of the complementary group (*c*). (*a*) ANOVA: resident/introduced pair effect: $F_{1,168} = 7.34$, $p = 0.007$; time effect: $F_{5,168} = 2.71$, $p = 0.021$; interaction: $F_{5,168} = 0.28$, $p = 0.882$. (*b*) ANOVA: resident/introduced pair effect: $F_{1,168} = 6.74$, $p = 0.010$; time effect: $F_{5,168} = 1.66$, $p = 0.145$; interaction: $F_{5,168} = 0.56$, $p = 0.720$. (*c*) ANOVA: resident/introduced pair effect: $F_{1,168} = 4.06$, $p = 0.045$; time effect: $F_{5,168} = 2.40$, $p = 0.022$; interaction: $F_{5,168} = 0.49$, $p = 0.837$.

workers out of the influence of their queen are not as closed a society as QR colonies and engage in unusual non-nestmate interactions promoting trophallaxis and grooming and thereby speeding up the merging of the alien colonies.

4. CONCLUSION

In *C. fellah*, recognition cues seem to be worker derived, but the queen clearly controls colony insularity by affecting worker social motivation and aggressiveness. The first conclusion is based on the fact that QD ants are not aggressed by their QR nestmates until several months of separation. By contrast, individually isolated nestmates were recognized as different within two weeks of separation. Since queen-derived recognition cues are expected to be evenly distributed to all colony members, the CD and QD workers start the experiment equal in terms of queen influence. That CD workers, but not QD workers, are quickly recognized as different by QR workers further supports the lack of the queen's influence on the recognition cues in this species. The second conclusion stems from the ease with which alien QD colony fragments merged as opposed to complete insularity kept by their respective QR fragments. The mechanism of the queen's

influence is not clear, but we suggest that it operates by augmenting worker social motivation (needed for trophallaxis) in the absence of the queen while reducing the cohesion of the colony. The decrease in aggression of queenless colonies may be adaptive in that it may enable workers to adopt new queens on the one hand and/or to increase survival through mergers to create mega-colonies on the other hand. In monogynous colonies of *S. invicta*, the decrease in conspecific aggression under queenless situations facilitates the adoption of a new queen (Vander Meer & Alonso 2002). This phenomenon might be more general and apply to *C. fellah* as well. Despite the absence of direct evidence of replacement of the queen in this particular species, adoption of unrelated queens was observed in the laboratory in *C. ligniperdus* and *C. herculaneus* (Hölldobler 1962) and was proposed as a mechanism leading to oligogyny in *C. ligniperdus* (Gadau *et al.* 1998). In *C. fellah*, virgin females disperse from the natal nest during nuptial flights (R. Boulay and A. Hefetz, unpublished data) but their adoption in parental or alien nests after mating cannot be excluded.

As postulated by Reeve (1989), social insects might be more permissive toward aliens when the fitness cost induced by the acceptance of non-nestmates is reduced. Queenless groups may even benefit from merging to larger
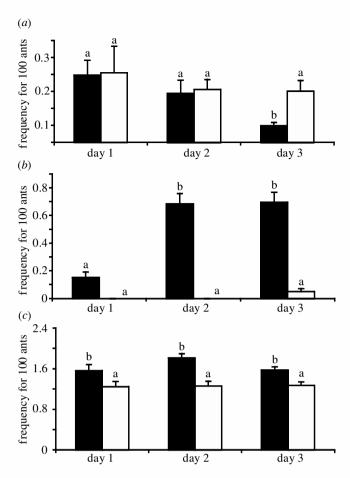
Figure 3. Mean (± s.e.) relative frequencies of aggressive behaviours between non-nestmates (*a*) and trophallaxis between non-nestmates (*b*) and nestmates (*c*) during a 3-day merger experiment. Black and white bars represent QD–QD and QR–QR alien colonies, respectively. Different letters denote significant differences (two-way ANOVA, Newman–Keuls *post hoc* test). (*a*) ANOVA: treatment of the colonies: $F_{1,544} = 0.27$, $p = 0.60$; day: $F_{2,544} = 4.79$, $p = 0.008$; interaction: $F_{2,544} = 2.27$, $p = 0.1$. (*b*) ANOVA: treatment of the colonies: $F_{1,544} = 69.24$, $p < 0.0001$; day: $F_{2,544} = 8.16$, $p = 0.0003$; interaction: $F_{2,544} = 6.72$, $p = 0.001$. (*c*) ANOVA: colony treatment effect: $F_{1,544} = 22.69$, $p < 0.0001$; day: $F_{2,544} = 1.3$, $p = 0.27$; interaction: $F_{2,544} = 1.3$, $p = 0.27$.
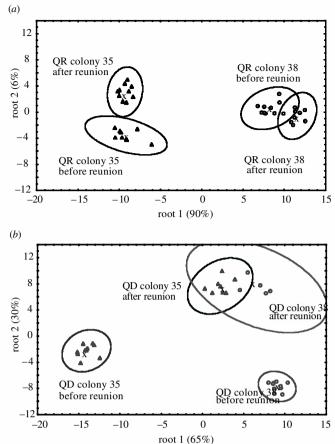

Figure 4. Two first roots of discriminant analyses conducted using the relative amounts of 28 major HC peaks extracted from the PPG of workers from two QR colonies (*a*) (labelled 35 and 38) and their respective QD fragments (*b*). Workers were sampled before or after the two colonies were given the opportunity to merge. The centroid of each group is represented by an x at the centre of the 95% confidence ellipses. (*a*) Wilk's lambda: < 0.001, $F_{51,51} = 38.78$, $p < 0.001$. (*b*) Wilk's lambda: < 0.001, $F_{66,24} = 13.01$, $p < 0.001$.

groups because large colony size protects it better from predation and parasitism (Herbers 1993). Such mergers may give the colonies the edge needed for successfully rearing young larvae to sexuals, the high cost of which may be prohibitive to a small colony. In *C. fellah* queenless workers were observed to have developed ovaries (R. Boulay, unpublished data). The viability of these oocytes is not known but the possibility to lay male eggs might also elevate worker social motivation in order to use alien forces for male brood rearing. Hence, alien acceptance probability might be higher as workers' expectation to live long enough to ensure brood rearing decreases, explaining why queenless nest merging is not likely to be a short-term process. Colony orphaning may be a rare event judging from laboratory observations that indicate particularly long queen lifespan in highly evolved ants (Hölldobler & Wilson 1990; Keller 1998). Nevertheless, in large polydomous species like *C. fellah* contacts between neighbouring alien nests out of queen influence and that share large por-

tions of their territories might be underestimated owing to the lack of field data. This phenomenon could constitute an evolutionary force, in reducing colony insularity in the absence of the queen.

Thus, in *C. fellah* nestmate recognition may be governed by both workers and the queen. The former supply the recognition cues, but the response to these cues depends on the presence of the queen. Her presence reduces social motivation and promotes colony insularity (territoriality), whereas when absent the workers are more likely to accept alien workers. Finally, these results underline the importance of the concept of social motivation to describe invertebrate behavioural flexibility under changing social environment.

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