

## Olfactory memory established during trophallaxis affects food search behaviour in ants

Yael Provecho and Roxana Josens\*

Grupo de Estudio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II, C1428EHA Buenos Aires, Argentina

\*Author for correspondence (roxy@bg.fcen.uba.ar)

Accepted 14 July 2009

### SUMMARY

***Camponotus mus* ants can associate sucrose and odour at the source during successive foraging cycles and use this memory to locate the nectar in the absence of other cues. These ants perform conspicuous trophallactic behaviour during recruitment while foraging for nectar. In this work, we studied whether *Camponotus mus* ants are able to establish this odour–sucrose association in the social context of trophallaxis and we evaluated this memory in another context previously experienced by the ant, as a nectar source. After a single trophallaxis of a scented solution, the receiver ant was tested in a Y-maze without any reward, where two scents were presented: in one arm, the solution scent and in the other, a new scent. Ants consistently chose the arm with the solution scent and stayed longer therein. Trophallaxis duration had no effect on the arm choice or with the time spent in each arm. Workers are able to associate an odour (conditioned stimulus) with the sucrose (unconditioned stimulus) they receive through a social interaction and use this memory as choice criteria during food searching.**

Key words: trophallaxis, olfactory memory, ant, *Camponotus mus*.

### INTRODUCTION

Many social insects perform group or cooperative foraging. In this kind of behaviour, the organization of the group activities is based on the decisions made by individuals in response to stimuli from the environment and the local information obtained from nestmates (Wilson, 1971; Hölldobler and Wilson, 1990; Seeley, 1995; Breed et al., 1996; Mailleux et al., 2000; Gordon, 2007). A variety of communication channels are involved in recruiting other individuals: tactile, chemical, vibrational, conspicuous displays, such as a bee dance, or mouth-to-mouth food exchange (trophallaxis), among others. All of them are modulated by the resource properties and the colony's needs of that resource (Cammaerts and Cammaerts, 1980; Waddington, 1982; Traniello, 1983; Beckers et al., 1992; Roces and Nuñez, 1993; Farina, 1996; Mailleux et al., 2000; Sanders and Gordon, 2002; Portha et al., 2002; Cassil, 2003; Goyret and Farina, 2003; Le Breton and Fourcassié, 2004; McCabe et al., 2006). Such interactions allow exchange of information among workers; for example, unemployed foragers may know about available sources and active foragers about alternative sources or the current colony requirements (Hölldobler and Wilson, 1990; Nuñez, 1982; Seeley, 1989; Farina, 2000; Gil and Farina, 2002).

Since ants are central place foragers (i.e. they transport the resource to the nest) the nest is the place where not only all the resources are centred but also where all the information about the available sources converges. Most nectivorous ants take the nectar in their crop to the nest where it is unloaded through trophallaxis to receiver nestmates. In addition, trophallaxis plays an important role in nestmate recognition and in maintaining the colony integration (Soroker et al., 1995; Boulay et al., 1999). Trophallaxis also affects the subsequent behaviour of donors and receivers during recruitment, which suggests that both update information during the interaction. The reservoir level may affect the response threshold

of workers at the nest, in such a way that the same stimulus can either trigger, or not, a receiver's activation (Cassil, 2003). Likewise, the donor can either return immediately to the source or not, depending on the receivers' response (Cassil, 2003).

In the last few years, many learning-related behaviours have been studied in the context of trophallactic interaction, particularly in bees (Farina et al., 2005; Farina et al., 2007; Gil and De Marco, 2005; Martinez and Farina, 2008). The acquisition of these memories is considered as social learning, defined as individual learning that takes place in a social context (Heyes, 1993; Brown and Laland, 2003; Leadbeater and Chittka, 2007).

In spite of the known olfactory nature of many aspects of their biology and although the use of odour cues in an appetitive context of food search is well known, ants have not been deeply studied in relation to learning of odours not belonging to the colony itself. For instance, when actively oriented to or searching for food, the forager walks against the wind through the odour plume until it finds the food [leaf-cutting ants (Littleddyke and Cherrett, 1978); *Cataglyphis* (Wolf and Wenner, 2000; Wolf and Wenner, 2005)]. Although the use of olfactory cues in such orientation is clear, it does not necessarily involve a learning process, as it could be explained as a mere odour preference or attractiveness.

Recently, it has been demonstrated that nectivorous ants can establish an associative memory between sucrose (unconditioned stimulus, US) and odour (conditioned stimulus, CS) at the source during successive foraging cycles, by means of an individual learning paradigm (Dupuy et al., 2006) which leads to long-lasting memory (Josens et al., 2009). In a similar way, during foraging, ants are able to establish memories with other different sensory modalities, such as spatiotemporal (Schatz et al., 1994), visual cues for routing or navigation (Collet et al., 1992; Durier et al., 2003; Cammaerts and Lambert, 2008), source quality (Schilman and Roces, 2003) and thermal radiation (Kleineidam et al., 2007).

Odour learning during recruitment has only been shown for leaf-cutting foragers (even when the exposure to stimuli was not controlled) that learn the odour of the solid food brought to the nest by the scout and then use this information in food choice once at the source (Roces, 1990). Cut leaves are known to emit volatile compounds induced by herbivory (reviewed by Dicke et al., 1998; van Poecke and Dicke, 2004), so they provide clear cues for leaf-cutting foragers. Moreover, insects that forage on flowers have highly developed associative cognition abilities in relation with olfactory cues [in honeybees (Bitterman et al., 1983); in bumble bees (Laloi et al., 1999); in stingless bees (McCabe et al., 2007)]. In the case of nectivorous ants, which mostly obtain nectar from extrafloral nectaries and homopteran honeydew, nectar odour cues are less evident than those produced by flowers.

In this work, we studied whether the nectivorous ant *Camponotus mus* was able to establish an association between odour and nectar in the social context of trophallaxis. Thus, the contingency of both stimuli (unconditioned stimulus (US): sugar and conditioned stimulus (CS): odour) occurred during that interaction, which is commonly associated with recruitment in this species. Receiver ants were tested one at a time immediately after the trophallaxis, in a context previously experienced as a nectar source: a Y-maze that presented two non-rewarded scents – the solution scent and a new one. We also analyzed whether the duration of the trophallaxis affected the subsequent behaviour once at the maze.

The food for nectivorous ants is available (1) at extra floral nectaries localised in many different parts of a plant, depending on the species, (2) in fewer cases, at floral nectaries, (3) by attending aphids that could be on leaves or green stems, and (4) at any part of the plant, when the food is other insects. Thus, it may be helpful to identify and localise such plants chemically, using any odour. Therefore, for this study we used plant scents. Other field investigations of odour learning in ants, in fact, worked directly with fresh stem sections from specific plants or cut leaves (Helmy and Jansen, 2003).

## MATERIALS AND METHODS

### Insects

Two queenright colonies of *Camponotus mus* Roger, one from Buenos Aires city and the other from General Rodriguez, Buenos Aires Province (34°41'S, 58°57'W), obtained during 2005, were used. They were kept for 2 years in laboratory conditions. Briefly, each colony was kept inside glass boxes at a room temperature of 25±2°C and under a natural long day regime. The ants had free access to a water source and were daily fed with honey water and twice a week with freshly killed cockroaches and honeybees. During the experiments, we limited the entry of honey water to the nest to enhance the appetitive motivation of experimental foragers to look for sucrose solutions. Experiments were performed only from October 2005 to May 2006 and from October 2006 to May 2007, which is the period of greatest activity of wild colonies in Buenos Aires city.

### Experimental design and procedure

#### Source-recognition phase

The first phase was to allow ants to become familiar with looking for sugar solution at a Y-maze. One at a time, powder-marked ants were allowed to walk from a vertical stick placed on the nest onto a piece of cardboard, which was then placed at the entrance to the Y-maze. The entrance channel (8 cm long) of the maze led to two arms (6 cm long each) separated by 90 deg. The maze was placed on a rectangular base from which it could be removed to be cleaned.

The floor of the maze was covered with filter paper, which was replaced after each visit of an ant to the maze to avoid the use of pheromone trails. Before the entrance to the arms, a decision area remained delimited in which, for every visit, a drop of sucrose solution 30% w/w (0.3 µl) was offered on a small plastic square (0.5 cm×0.5 cm) centrally positioned.

As the walls of the maze were painted with fluon to prevent ants from escaping, the only exit from the maze was the piece of cardboard placed at the entrance. Once on it, the ant was driven back to the nest. The whole procedure was repeated once for each experimental ant, which is enough for the ant to become familiar with both the procedure and the Y-maze as a nectar source (for details, see Dupuy et al., 2006).

#### Familiarization phase

At the nest, after unloading the solution to its nestmates, the ant returned to the vertical stick to go back to the source. From this point, both the marked ant and a recruited one were gently put in an experimental arena (an acrylic container 5 cm in diameter with 3 cm high fluon-painted walls and a base covered with plaster). Each pair of ants was left in the arena for 90 min in order to become familiar with the experimental environment. After that, the unmarked worker was placed in a feeding site (a 3 cm×5 cm acrylic plate) where a drop (0.4 cm in diameter) of 30% (w/w) sucrose solution aromatized with a floral scent was offered (0.05 µl of scent per 1 ml of sugar solution).

#### Recording phase

After drinking from the solution, the ant was returned to the experimental arena with its partner. Once in the arena, the trophallactic duration of the first contact was recorded, and in if there was no contact this recording was finished after 10 min. After the first trophallaxis, the receiver ant (the experimental marked ant) was placed in a new Y-maze (Fig. 1). This new maze was similar to that previously described but without fluon on the walls, and contained the solution scent in one arm and a new scent in the other arm. Each scent was in a 10-ml micropipette tip with the point sealed, containing 0.1 ml of scent on a small piece of filter paper. Each tip was inserted in a hole in the base, specially created for this purpose, and close to the entrance of each arm (Fig. 1). An air stream filtered by active charcoal and humidified by water was driven from the back wall of each arm by means of an air pump through plastic tubes. The mild air flow allowed the odours to be driven in a laminar flow towards the decision area of the maze.

For these recordings as well as for the first phase, a white semi-cylindrical cardboard wall (60 cm high and approximately 40 cm in diameter) was placed around the maze. This wall prevented the ants from using external visual cues to guide their choices. We made sure that illumination coming from artificial lamps and laboratory windows was homogeneous and symmetrical (with respect to the left and right arms of the maze). An air extractor was situated approximately 38 cm above the maze to eliminate the escaping odours from the maze throughout the experiment. As in the source-recognition phase, after every visit to the maze, the filter paper was replaced. In the same way, the maze, base and feeding arena were systematically cleaned with alcohol and dried with hot air provided by a hair dryer after each ant visit to avoid pheromone marks or odour contamination. We also carefully eliminated all possible alcohol traces that could affect the ant's choice. The tips containing the scents were renewed either every hour during the experiment or if the ant walked on one of them.

It is worth mentioning that no reinforcers were provided in the maze during memory evaluation. In each test, the ant found two

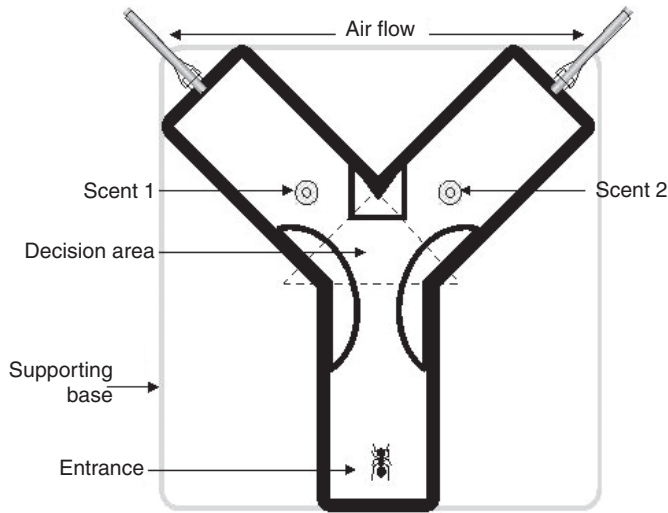


Fig. 1. Top view of the Y-maze used to evaluate ants in an olfactory discrimination task. Each ant was transported to the entrance of the maze after a single trophallaxis. The ant moved towards the decision area, delimited by the dashed lines in the figure. The airflow ensured a laminar flow of the scents towards the decision area, where the ant had to choose between the two scents. The entrance and the arms of the maze were 8 and 6 cm long, respectively. The arms were separated by 90 deg. The maze could be partially covered/uncovered by a removable glass plate (10×15 cm) that left the entrance channel free. The floor of the maze was covered with a piece of filter paper, which was replaced with a clean one after each visit of an ant to avoid the use of pheromonal traces.

scents at the decision area. We recorded the arm chosen first (a correct first choice was the arm with the solution scent or an incorrect first choice was the arm with the new scent) and the time spent in each arm, for up to 2 min. After this time, the experimental ant and its partner were removed from the set-up and from the colony.

When no trophallaxis occurred during the initial 10 min, the experimental ant was evaluated in the Y-maze in a way similar to those that did perform a single trophallactic contact. The variables measured in non-trophallaxis cases were the first choice and the time spent in each arm for the first minute at the maze.

#### Experimental series

We used two pairs of commercial scents (Pfeiffer-Gerhards, Germany): (1) rose–tea tree and (2) sandalwood–cypress. For each pair of scents, two experimental groups of ants were analyzed, for one group one scent of the pair was added to the sugar solution and for the other group the other scent of the pair. The two groups were presented with both scents of the pair at the Y-maze. In other words, a group of ants that received sucrose solution aromatized with rose (rose group) during the trophallaxis, found rose and tea tree in the decision area of the maze. The other group of ants received solution aromatized with tea tree (tea tree group) during the trophallaxis and also found rose and tea tree scents in the decision area of the maze. The same happened for the second pair of scents (sandalwood group and cypress group). For all groups, even when each ant was evaluated only once, the position of each scent (the right and the left arms) was changed for each test to avoid any position bias.

Scents and their concentrations were chosen after preliminary evaluation of ants' responses to and drinking of 30% aromatized

sucrose solutions. As some scents and some high scent concentrations were rejected, we chose those that were totally accepted. When each scent pairs was evaluated in the Y-maze, no differences were found in the ant's preferences.

#### Statistical analysis

We first tested whether there were differences between the groups fed with each scent of the pair for every variable analyzed. When no differences were detected, both groups were pooled. For the first choice, a  $2 \times 2$  non-parametric test was performed to compare both groups, and when no differences were found, a  $G$ -test was done to analyze whether the chosen scent (solution scent *versus* new scent) differed from the theoretical level of 50%. The time spent in each arm of the maze, was tested with a repeated measures ANOVA (factor group: group of ants fed with each scent of the pair, two levels; repeated factor, chosen scent: solution scent or new scent, two levels).

To compare the test performances of the two scent pairs, we used a  $2 \times 2$   $\chi^2$ -test for the first choice and a  $t$ -test for the relative time in the correct arm.

We also evaluated the relationship between the trophallaxis duration and the proportion of the time spent in the arm containing the solution scent with respect to the time spent in both arms by means of a Spearman correlation. In all cases we considered an  $\alpha$  of 5% to be significant.

As there were few cases where no trophallactic contact was established, we compared performances for both pairs of scents in order to pool all the data and perform the statistical analysis, in the same way as described above for both variables: the first arm choice (i.e. with the solution scent or the new scent) and the time spent in each arm.

#### RESULTS

Most of the ant pairs evaluated established a trophallactic contact. Even when some had been brief, they were also considered. However, for both pairs of smells there were cases without contact between mouthparts in the 10 min pre-stipulated. Nevertheless, these insects were evaluated in the Y-maze. Cases with and without trophallaxis are presented, separately.

#### With mouth-to-mouth contact

##### Rose *versus* tea tree

Firstly we evaluated whether the ants fed with the rose-scented solution and those with the tea-tree-scented solution showed similar profiles in the responses to the correct arm (i.e. the one containing the solution scent). There were no significant differences between groups in the first choice of the arm with the known scent (two-tailed exact Fisher, d.f.=1,  $P=0.45$ ). Therefore, both groups were pooled so as to analyze possible differences between arms.

Once the ant reached the decision area, it found both a known (solution scent) and an unknown smell. A larger proportion of the ants chose the correct arm than chose the incorrect arm ( $G=18.34$ , d.f.=1,  $P<0.005$ ; Fig. 2A). The ants fed with rose chose the arm that contained rose, whereas ants fed with tea tree chose mostly the arm with the tea tree scent.

Regarding the time spent in each arm during the first 2 minutes at the maze, ants also showed the same preference to the correct arm (Fig. 2B). There were no significant differences between the groups that were fed with a solution scented with rose or tea tree. This means that, independently of which scent was at the solution, ants stayed longer in the arm containing the known scent (repeated

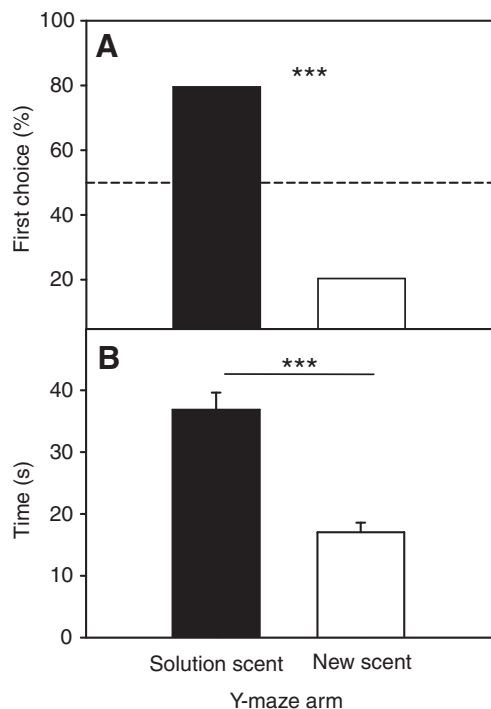


Fig. 2. Orientation of ants in the Y-maze. A pair of ants was isolated together and then one of them was fed with an aromatized sucrose solution – rose or tea tree scent – and then returned to its partner. (A) After a single trophallaxis, the receiver ant was evaluated as to its first choice of direction at a known Y-maze, in which one arm contained the solution scent. The dashed line at 50% indicates random choice between the arms. (B) Time (means $\pm$ s.e.m.) spent in each Y-maze arm (\*\* $P$ <0.01; \*\*\* $P$ <0.001).

measures ANOVA: group  $\times$  arm interaction,  $F_{1,47}=0.02$ ,  $P=0.88$ ; group,  $F_{1,47}=3.07$ ,  $P=0.09$ ; arm,  $F_{1,47}=30.87$ ,  $P<0.0001$ ).

#### Sandalwood versus cypress

For this pair of scents, two groups of ants were also compared. For one of them, the solution scent was sandalwood, and for the other cypress. There were no significant differences between groups in the first choice of the correct arm (two-tailed test Fisher exact: d.f.=1,  $P=1$ ). Therefore, both groups were pooled. When comparing the choice between the correct and the incorrect arm, ants mostly preferred the arm containing the known scent (goodness of fit test:  $G=7.83$ , d.f.=1,  $P<0.01$ ; Fig. 3A). This means that when the scent was sandalwood, ants chose more frequently the arm containing sandalwood. Whereas, when the solution scent was cypress, they mostly chose the arm containing this scent, independently of whether it was placed in the right or left arm.

Regarding the time spent in each arm, both groups showed the same tendency to stay longer in the arm with the solution scent. However, there were differences in the performance between both scent groups (repeated measures ANOVA: scent  $\times$  arm interaction  $F_{1,32}=4.9$ ,  $P=0.03$ ), consequently, they were analyzed separately. The time in the arm with the solution scent was significantly longer for the sandalwood group, whereas the cypress group spent only slightly longer in the arm with the solution scent (repeated measures ANOVA, simple effects for arms; sandalwood:  $F_{1,32}=26.0$ ,  $P<0.005$ ; cypress:  $F_{1,32}=3.84$ ,  $P>0.1$ ; Fig. 3B).

The comparison of the tests without reinforcements between the scent pairs (rose–tea tree and sandalwood–cypress) showed that C.

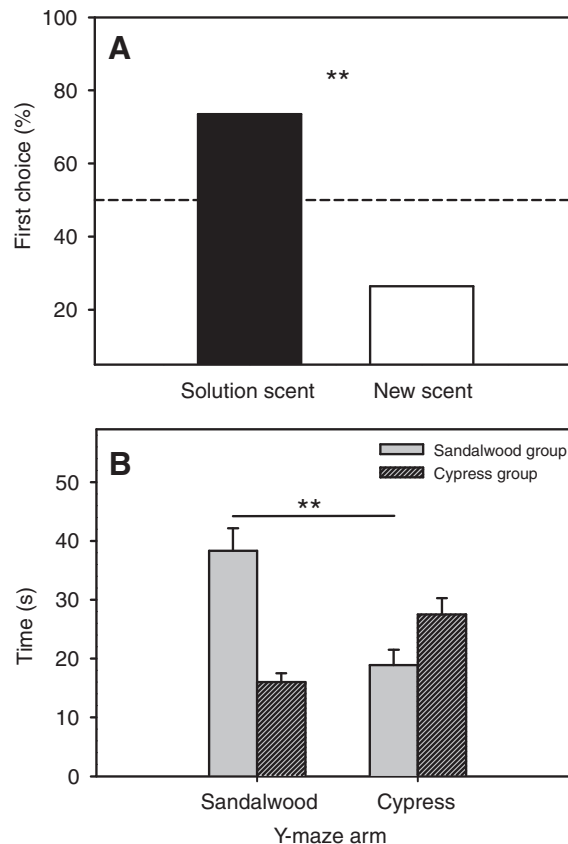


Fig. 3. Orientation of ants in the Y-maze after a single trophallaxis when the receiver tasted an aromatized solution with sandalwood or cypress scent (for further details see Fig. 2 legend). (A) First choice of arm of the Y-maze, in which one arm contained the solution scent. The dashed line at 50% indicates random choice between the arm with the solution scent and that with the new scent. (B) Time (means $\pm$ s.e.m.) spent in each Y-maze arm during the 2 min recorded (\*\* $P$ <0.01).

*mus* ants learned to discriminate odours no matter which pair was considered, as the performance for the first choice was the same for both scent pairs ( $2 \times 2$   $\chi^2=0.19$ ;  $P=0.6$ . NS; Fig. 2A, Fig. 3A).

#### Trophallaxis time

There was a considerable variability in the trophallaxis duration at the arena in our experimental device. For the pooled groups of rose–tea tree, the trophallaxis lasted  $36.3 \pm 19.5$  s (minimum: 3 s; maximum: 156 s) and for the pooled groups of sandalwood–cypress, trophallaxis time reached  $40.85 \pm 18.4$  s (minimum: 2 s; maximum: 108 s). Therefore, to observe whether the trophallaxis duration affected the first choice at the Y-maze, we arbitrarily considered as short trophallaxes those that lasted up to 20 s, and long trophallaxes those that lasted more than that. For the rose–tea-tree pooled group, both short and long trophallaxes resulted in the same proportion of correct first choices: 79% in each case. The same was observed for the sandalwood–cypress pooled group, in which short trophallaxes and long trophallaxes were followed by 80% and 72% of correct choices, respectively.

To analyze a possible relationship between trophallaxis duration and the recording of the times in the Y-maze, we considered the percentage of the time spent in the correct arm with respect to the time in both arms. For example, if an ant spent 1 minute in the correct arm and 1 minute in the entrance of the maze, we considered that

it spent the 100% of its time in the correct arm. The relationship between the trophallaxis duration and the percentage of time in the correct arm was analyzed for each scent pair. There was no relationship between these variables either for rose–tea tree (Spearman correlation:  $N=47$ ;  $P=0.62$ , NS) or for sandalwood–cypress (Spearman correlation:  $N=34$ ;  $P=0.8$ , NS). Therefore, the trophallaxis duration does not seem to affect the time an ant spent in each arm of the Y-maze.

#### Without mouth-to-mouth contact

There were some pairs of ants that did not perform a trophallactic contact during the 10-minute recording. Nevertheless, the potential receiver ant of the couple, which had no contact with the solution, was evaluated at the Y-maze in the same way as the ones that made trophallaxis. When the ant entered either arm of the Y-maze, containing the solution scent or the one containing the new scent, we recorded first choice and time in each arm. All groups of ants showed the same tendency in the results, but some of them had a few cases of non-trophallactic contact. Therefore, as there were no differences between scent pairs ( $2 \times 2 \chi^2=0.02$ ; d.f.=1,  $P>0.5$ , NS), we pooled all the scent groups to increase the sample size in order to obtain a reasonable sample to perform a statistical analysis.

Unexpectedly, the ants that had no contact with the solution chose the arm with the new scent (goodness of fit test,  $G=5.75$ ,  $P<0.05$ ; Fig. 4A). In addition, the time spent in each of both arms during the first minute at the maze reinforces this result. The ants stayed

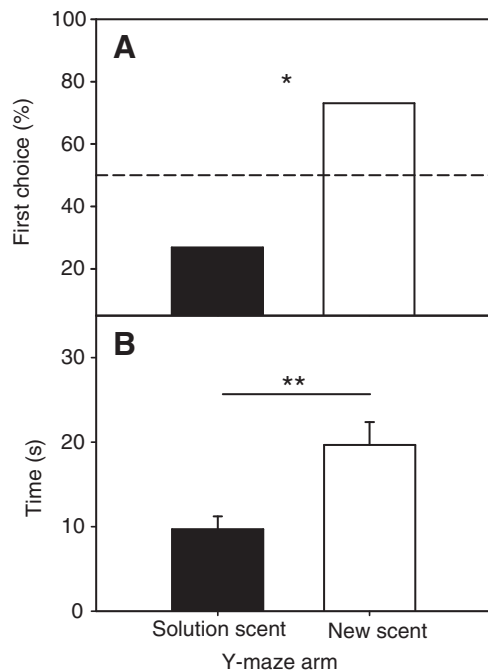


Fig. 4. Orientation of ants in the Y-maze, for the cases without mouth-to-mouth contact, i.e. ants that had not tasted the scented solution before ( $N=26$ ). A pair of ants were isolated for 90 min, and then one of them was fed with a scented sucrose solution and then returned with its partner. The pair of ants remained together for 10 min but did not establish any trophallaxis. The unfed ant was evaluated at the Y-maze. One arm contained the solution scent (solution drunk by its partner) and the other arm contained a new scent. (A) First choice of arm of the Y-maze, in which one arm contained the solution scent. The dashed line at 50% indicates random choice between the arms. (B) Time (means $\pm$ s.e.m.) spent in each arm during the first minute at the maze (\*\* $P<0.01$ ).

significantly less time in the arm that presented the solution scent (repeated measured ANOVA:  $F_{1,25}=8.7$ ,  $P=0.007$ ; Fig. 4B).

#### DISCUSSION

Until recently, the lack of suitable protocols to study olfactory learning in ants limited the analysis about learning of odours not belonging to the ant colony. Therefore, in spite of the olfactory nature of ants, little is known about the ability to establish olfactory memories in these insects. The Y-maze with two different odours – one in each arm – used with one ant at a time proved to be an appropriate design to study olfactory learning. Apart from the capacity of acquisition during successive foraging cycles at the nectar source (Dupuy et al., 2006), the present work demonstrated that the nectivorous ant *Camponotus mus* is also able to learn an odour presented in the solution received during a single trophallaxis and that they use this memory to locate the food once at the source.

At the Y-maze, ants were able to discriminate between the solution scent and an unknown scent that represented a neutral stimulus, both presented without any reinforcement. The use of this memory was reflected not only in the first choice of the arm with the solution scent, but also in the longer time spent in it looking for the solution in the first 2 minutes at the maze. Although the first choice was significant for all scents used, time in the correct arm was higher for all the four scents, being significant only for three and marginally significant for the fourth. Increasing sample size would also probably lead to statistical significance.

Although conditioning during recruitment has been shown in leaf-cutting ants (Roces, 1990; Roces, 1994), whether the association is established in a social context of nestmate interaction or as a result of a simple encounter with the scented food has not been evaluated. Hence, the present work is the first study on ants, in which the exposure to the contingency of both stimuli (US-CS) is controlled and quantified, as we allowed a one-to-one contact and only one trophallaxis and we also measured the time that the experimental ant was in contact with the solution.

The acquisition of associative memories through a social interaction has already been shown or suggested in other social insects [honeybees (Farina et al., 2005; Farina et al., 2007; Gil and De Marco, 2005; Grüter et al., 2006); stingless bees (McCabe and Farina, 2009)], but its functional value in the context of food searching has not been proved before. In bees, the memories established during trophallaxis have been evaluated through a classical paradigm based on the proboscis extension reflex (PER) (Takeda, 1961; Bitterman et al., 1983). However, even though there have been numerous studies on this topic in bees, none of them have fully demonstrated that only one trophallactic interaction under controlled conditions – without other stimuli – can make a bee choose that odour when landing at a food source. Our results also show, for the first time in an insect, that an associative learning mediated by a single social interaction affects the later behaviour when searching for food. In other words, this is a case of social learning that led a free-walking insect to use the memory acquired as a choice criterion in a food-searching context.

The fact that ants without trophallaxis consistently chose the arm with the new scent proved that the smell was perceived in the experimental arena.

Although groups with and without trophallaxis showed an opposite result, they experienced exactly the same protocol, with the only difference that the latter did not taste the solution. Therefore, the evaluation of the ants without trophallaxis serves as a suitable control, and also confirms effectively that there is an association between scent (CS) and reward (US) in the individuals that did taste

the solution. In summary, the same odour could either be chosen when there was a previous contact or not be chosen when there was no contact.

A possible explanation for the unexpected non-trophallactic result is that it was not a memory process, but a rejection of a scent by some individuals. This rejection would lead these individuals to not establish a trophallaxis and to not choose the rejected scent once at the Y-maze. However, in our preliminary evaluation of scents to be used in experiments, no evidence of this kind of inter-individual variability in the acceptance of scents was shown. When a scent or its concentration caused rejection, responses were similar – to a lesser or greater degree – among all individuals. In fact, the choice of scents and their concentration was based on the acceptance by all the ants. Hence, we believe that a memory process might be involved in the non-trophallactic results. The question that arises is what kind of memory underlies the operant nature in this case with the experimental design used. We can suggest that ants chose the new scent searching for nectar, considering that the experimental ant had previously experienced the Y-maze as a foraging site and that the other scent did not drive the individual to nectar in the previous context. We can also suggest that ants could have avoided the known scent, perhaps because it could have been associated with the isolated container in the familiarization phase as a negative context or stimulus (US). If the latter proposal was confirmed in future investigations, it might become the basis of an operant paradigm for olfactory avoidance in ants. This odour-avoidance behaviour after aversive conditioning has been observed in fruit flies (Aceves-Pina and Quinn, 1979) and recently in honeybees (Carcaud et al., 2009).

It is noteworthy that in *Camponotus mus* ants, memory acquisition in a social context of trophallaxis required only one exposure to the odour-reward contingency. However, in a context of individual foraging, these ants need up to 12 visits to the food source to establish the same association (Dupuy et al., 2006). It might thus be inferred that in a context of individual foraging probably other cues, such as visual and/or spatial, have greater hierarchy, whereas in the social context of recruitment during the one-to-one trophallaxis, olfactory cues probably become more relevant. This fact might indicate that for some social insects, social learning seems to lead to a faster and more robust way of learning than the one acquired in an individual context. Whether the duration of both memories acquired in individual and social contexts are comparable remains to be further studied.

The duration of the trophallaxis established under the conditions used in this work was between 2 and 156 s. Despite this variation in the length of trophallaxis, neither the first choice nor the time spent in each arm in the first 2 minutes at the Y-maze was affected. Thus, trophallaxis as long as 2 or 3 s allowed receivers to choose the correct arm and stay longer therein. Therefore, odour learning is not affected by trophallaxis duration, which probably means that an effective ingestion is not necessary and the mere taste of a scented sugar solution by mouth parts might be sufficient to establish the association in this social context. In bees, it has been observed that using the PER protocol they are also able to establish olfactory associations, without ingesting a measurable amount of solution during the trophallaxis (Bitterman et al., 1983) and that there is no relationship between trophallaxis duration and acquisition ability (Gil and De Marco, 2005) (reviewed by Leadbeater and Chittka, 2007).

Our experiments in *Camponotus* ants showed that olfactory memories are particularly resistant to context changes; memories were established in the experimental arena and evaluated in the Y-

maze. Context changes in the framework of studies on olfactory learning and retention are a useful procedure. The design used in this work represents a considerable advantage in the experimental protocol to study olfactory memories in ants since it is shorter, easier and less sensitive to manipulation than the one used previously throughout successive foraging cycles (Dupuy et al., 2006). Simple and robust protocols are indispensable to continue with odour-learning studies in ants.

According to our findings, during recruitment, trophallaxis would allow an ant in the nest to access information related to an unknown source recently visited by a nestmate. For ant species that follow pheromonal trails, these trails lead a recruited forager to the proximity of the nectar source. Once there, where the pheromonal mark is spread, the odour memory acquired during the trophallactic interaction would be evoked in this new context and used in the subsequent search behaviour to improve the fine localization of the nectar.

Probably, foragers use this olfactory social information to identify unfamiliar food sources, but prioritise other source cues (such as landmarks, spatial memory, visual, etc) and their individual information once they have visited and experienced the source.

We thank the two anonymous referees for comments to improve the manuscript. We also thank Andres Arenas for his helpful suggestions and Walter Farina for his valuable comments on a previous version of the manuscript. This work was supported by ANPCYT-FONCYT, PICT 2006-1155.

## REFERENCES

- Aceves-Pina, E. O. and Quinn, W. G. (1979). Learning in normal and mutant *Drosophila* larvae. *Science* **206**, 93-96.
- Beckers, R., Deneubourg, J. L. and Goss, S. (1992). Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). *Insectes Soc.* **39**, 59-72.
- Bitterman, M. E., Menzel, R., Fietz, A. and Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybee (*Apis mellifera*). *J. Comp. Psychol.* **97**, 107-119.
- Boulay, R., Hefetz, A., Soroker, S. and Lenoir, A. (1999). *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Anim. Behav.* **59**, 1127-1133.
- Breed, M. D., Bowden, R. M., Garry, M. F. and Weicker, A. L. (1996). Giving-up time variation in response to differences in nectar volume and concentration in the giant tropical ant, *Paraponera clavata* (Hymenoptera: Formicidae). *J. Insect Behav.* **9**, 659-672.
- Brown, C. and Laland, K. N. (2003). Social learning in fishes: a review. *Fish Fish.* **4**, 280-288.
- Cammaerts, M. C. and Cammaerts, R. (1980). Food recruitment strategies of the ants *Myrmica sabuleti* and *Myrmica ruginodis*. *Behav. Proc.* **5**, 251-270.
- Cammaerts, M. C. and Lambert, A. (2008). Maze negotiation by a myrmicine ant (Hymenoptera: Formicidae). *Myrmecological News* **12**, 41-49.
- Carcaud, J., Roussel, E., Giurfa, M. and Sandoz, J. C. (2009). Odour aversion after olfactory conditioning of the sting extension reflex in honeybees. *J. Exp. Biol.* **212**, 620-626.
- Cassil, D. (2003). Rules of supply and demand regulate recruitment to food in ant society. *Behav. Ecol. Sociobiol.* **54**, 441-450.
- Collet, T. S., Dillmann, E., Ginger, A. and Wehner, R. (1992). Visual landmarks and route following in desert ants. *J. Comp. Physiol. A* **170**, 435-442.
- Dicke, M., Takabayashi, J., Posthumus, M. A., Schutte, C. and Krips, O. E. (1998). Plant-phytoseiid interactions mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. *Exp. Appl. Acarol.* **22**, 311-333.
- Dupuy, F., Sandoz, J. C., Giurfa, M. and Josens, R. (2006). Individual olfactory learning in *Camponotus* ants. *Anim. Behav.* **72**, 1081-1091.
- Durier, V., Graham, P. and Collett, T. S. (2003). Snapshot memories and landmark guidance in wood ants. *Curr. Biol.* **13**, 1614-1618.
- Farina, W. M. (1996). Food-exchange by foragers in the hive: a means of communication among honey bees? *Behav. Ecol. Sociobiol.* **38**, 59-64.
- Farina, W. M. (2000). The interplay between dancing and trophallactic behavior in the honey bees *Apis mellifera*. *J. Comp. Physiol. A* **186**, 239-245.
- Farina, W. M., Gruter, C. and Diaz, P. C. (2005). Social learning of floral odours inside the honeybee hive. *Proc. Biol. Sci.* **272**, 1923-1928.
- Farina, W. M., Grüter, C., Acosta, L. and McCabe, S. (2007). Honeybees learn floral odors while receiving nectar from foragers within the hive. *Naturwissenschaften* **94**, 55-60.
- Gil, M. and Farina, W. M. (2002). Foraging reactivation in the honeybee *Apis mellifera* L.: factors affecting the return to a known nectar source. *Naturwissenschaften* **89**, 322-325.
- Gil, M. and De Marco, R. J. (2005). Olfactory learning by means of trophallaxis in *Apis mellifera*. *J. Exp. Biol.* **208**, 671-680.
- Gordon, D. M. (2007). Control without hierarchy. *Nature* **446**, 143.

- Goyret, J. and Farina, W. M.** (2003). Descriptive study of antennation during trophallactic unloading contacts in honeybees *Apis mellifera carnica*. *Insectes Soc.* **50**, 274-276.
- Grüter, C., Acosta, L. E. and Farina, W. M.** (2006). Propagation of olfactory information within the honeybee hive. *Behav. Ecol. Sociobiol.* **60**, 707-715.
- Helmy, O. and Jander, R.** (2003). Topochemical learning in black carpenter ants (*Camponotus pennsylvanicus*). *Insectes Soc.* **50**, 32-37.
- Heyes, C. M.** (1993). Anecdotes, training, trapping and triangulating: do animals attribute mental states? *Anim. Behav.* **46**, 177-188.
- Hölldobler, B. and Wilson, E. O.** (1990). *The Ants*. Cambridge, MA: Harvard University Press.
- Josens, R., Eschbach, C. and Giurfa, M.** (2009). Differential conditioning and long-term olfactory memory in individual *Camponotus fellah* ants. *J. Exp. Biol.* **212**, 1904-1911.
- Kleineidam, C. J., Ruchty, M., Casero-Montes, Z. A. and Roces, F.** (2007). Thermal radiation as a learned orientation cue in leaf-cutting ants (*Atta vollenweideri*). *J. Insect Physiol.* **53**, 478-487.
- Laloi, D., Sandoz, J. C., Picard-Nizou, A. L. and Pham-Delègue, M. H.** (1999). Olfactory conditioning of the proboscis extension reflex in the bumble bee *Bombus terrestris*. *Ann. Soc. Entomol. Fr.* **35**, 154-158.
- Le Breton, J. and Fourcassié, V.** (2004). Information transfer during recruitment in the ant *Lasius niger* L. (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **55**, 242-250.
- Leadbeater, E. and Chittka, L.** (2007). Social learning in insects: from minianute brains to consensus building. *Curr. Biol.* **17**, R703-R713.
- Littleddyke, M. J. and Cherrett, M.** (1978). Olfactory response of the leaf-cutting ant *Atta cephalotes* (L) and *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae) in the laboratory. *Bull. Entomol. Res.* **68**, 273-282.
- Mailleux, A. C., Detrain, C. and Deneubourg, J. L.** (2000). How do the ants assess food volume? *Anim. Behav.* **59**, 1061-1069.
- Martinez, A. and Farina, W. M.** (2008). Honeybees modify gustatory responsiveness after receiving nectar from foragers within the hive. *Behav. Ecol. Sociobiol.* **62**, 529-535.
- McCabe, S. I. and Farina, W. M.** (2009). Odor information transfer in the stingless bee *Melipona quadrifasciata*: effect of in-hive experiences on classical conditioning of proboscis extension. *J. Comp. Physiol. A* **195**, 113-122.
- McCabe, S., Farina, W. M. and Josens, R. B.** (2006). Antennation of nectar-receivers encodes colony needs and food-source profitability in the ant *Camponotus mus*. *Insectes Soc.* **53**, 356-361.
- McCabe, S. I., Hartfelder, K., Santana, W. C. and Farina, W. M.** (2007). Odor discrimination in classical conditioning of proboscis extension in two stingless bee species in comparison to Africanized honeybees. *J. Comp. Physiol. A* **193**, 1089-1099.
- Núñez, J. A.** (1982). Honeybees foraging strategies at a food source in relation to its distance from the hive and the rate of sugar flow. *J. Apic. Res.* **21**, 139-150.
- Porta, S., Deneubourg, J. L. and Detrain, C.** (2002). Self-organized asymmetries in ant foraging: a functional response to food type and colony needs. *Behav. Ecol.* **13**, 776-781.
- Roces, F.** (1990). Olfactory conditioning during the recruitment process in a leaf-cutting ant. *Oecologia* **83**, 261-262.
- Roces, F.** (1994). Odour learning and decision-marking during food collection in the leaf-cutting ant *Acromyrmex lundii*. *Insectes Soc.* **41**, 235-239.
- Roces, F. and Núñez, J. A.** (1993). Information about food quality influence load-size selection in recruited leaf-cutting ants. *Anim. Behav.* **45**, 135-143.
- Sanders, N. J. and Gordon, D. M.** (2002). Resources and the flexible allocation of work in the desert ant, *Aphaenogaster cockerelli*. *Insectes Soc.* **49**, 371-379.
- Schatz, B., Beugnon, G. and Lachaud, J. P.** (1994). Time-place learning by an invertebrate, the ant *Ectatomma ruidum* Roger. *Anim. Behav.* **48**, 236-238.
- Schilman, P. E. and Roces, F.** (2003). Assessment of nectar flow rate and memory for patch quality in the ant *Camponotus rufipes*. *Anim. Behav.* **66**, 687-693.
- Seeley, T. D.** (1989). Social foraging in honey bee: how nectar foragers assess their colony's nutritional status. *Behav. Ecol. Sociobiol.* **11**, 287-293.
- Seeley, T. D.** (1995). *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Cambridge, MA: Harvard University Press.
- Soroker, V., Vienneand, C. and Hefetz, A.** (1995). Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae). *J. Chem. Ecol.* **21**, 365-378.
- Takeda, K.** (1961). Classical conditioned response in the honey bee. *J. Insect Physiol.* **6**, 168-179.
- Traniello, J. F. A.** (1983). Social organization and foraging success in *Lasius neoniger* (Hymenoptera: Formicidae): behavioral and ecological aspects of recruitment communication. *Oecologia* **59**, 94-100.
- van Poecke, R. M. P. and Dicke, M.** (2004). Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biol.* **6**, 387-401.
- Waddington, K. D.** (1982). Money bee foraging profitability and round dance correlates. *J. Comp. Physiol.* **148**, 297-301.
- Wilson, E. O.** (1971). *The Insect Societies*. Cambridge, MA: Harvard University Press.
- Wolf, H. and Wehner, R.** (2000). Pinpoint food source: olfactory and anemotactic orientation in desert ant, *Cataglyphis fortis*. *J. Exp. Biol.* **203**, 857-868.
- Wolf, H. and Wehner, R.** (2005). Desert ants compensate for navigation uncertainty. *J. Exp. Biol.* **283**, 4223-4230.