

## Olfactory and visual operant conditioning in the ant *Myrmica rubra* (Hymenoptera: Formicidae)

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### Abstract

*Myrmica rubra* Linnaeus, 1758 foragers rely on olfactory and visual cues to a similar degree for navigation. To explain so, we examined the first and the second acquisition and loss of olfactory and visual collective operant conditioning by workers of this species under as natural as possible conditions. The ants successively acquired and lost, then again acquired and lost conditioning for olfactory cues on one hand and for visual ones on the other hand in different given periods of time and reach different given scores as follows: for olfactory cues, 76% in 10 hrs, 8% in 8-9 hrs, 85% in 4 hrs, 10% in 18 hrs; for visual cues, 80% in 12 hrs, 10% in 20-21 hrs, 83% in 9-10 hrs, 15% in 30-32 hrs. Thus, the ants' olfactory learning was more rapid than their visual one but their final score and memory were stronger for visual than for olfactory conditioning. On the other hand, the difference between the first and the second acquisitions of conditioning was more pronounced for olfactory than for visual learning. Consequently, *M. rubra* workers' olfactory and visual learning are ultimately of equivalent efficiency, what explains the species' navigation system.

**Keywords:** learning, memory, orientation.

### Introduction

Ants forage around relying on their species - and/or colony - specific trail pheromone and marking chemicals. They also use visual cues according to a snapshot or a sketch map model, and, to a lesser extent, odours they encounter, as well as some other egocentric cues (PASSERA & ARON, 2005). Ants therefore memorize certain cues, at least briefly, on their outward journey in order to retrace their steps upon returning to the nest.

The ants' system of orientation have intensively been studied; they largely varied with the species and their environment (CAMMAERTS, 2012a, references therein). We examined the navigation system of three *Myrmica* species. *Myrmica sabuleti* Meinert, 1861 foragers essentially use odours to navigate but soon stop responding to obsolete olfactory cues while they continue following incorrect visual ones (CAMMAERTS & RACHIDI, 2009). These ants can be conditioned to olfactory and visual cues (CAMMAERTS *et al.*, 2011). They better learn olfactory than visual cues, but their olfactory memory is shorter than their visual one. *Myrmica sabuleti* workers' olfactory and

visual learning abilities thus explain why this species uses such a navigation system. *M. ruginodis* Nylander, 1846 workers rely mainly on visual cues from above, using odours only in darkness or in the absence of visual stimuli (CAMMAERTS *et al.*, 2012). Their olfactory and visual learning have recently been examined; surprisingly, these ants never memorized an odour, but demonstrated a long-lasting visual memory (CAMMAERTS & NEMEGHAIRE, 2012). Once more, foragers' learning characteristics explain why these ants use such a navigation system. *Myrmica rubra* Linnaeus, 1758 similarly relies on olfactory and visual cues to navigate (CAMMAERTS, 2012a). Its ability to learn olfactory and visual information remains unknown: how does it learn these cues, do workers of this species demonstrate an olfactory and/or a visual memory, do they have a short- or long-term olfactory or visual memory? Such information would explain why the species has such a navigation system.

Since the present aim was to explain what occurs in nature, an as natural as possible method of conditioning – e.g. a collective operant approach – was used. In such an approach, the animals are free moving, live in

their colony and come to the training apparatus as they wish. This is not a common experimental method. Learning has already been widely studied in many animals, including bees and ants. However, experiments are generally performed on individual animals (JOSENS *et al.*, 2009), which may even be restrained (GUERRIERI *et al.*, 2007). Moreover, the studies often dealt with simple conditioning (MACQUART *et al.*, 2008), olfactory conditioning only (DUPUY *et al.*, 2006), or classical conditioning (CAMMAERTS, 2004a) and/or first conditioning only (HARRIS *et al.*, 2005; NARENDRA *et al.*, 2007). Briefly, the animals are generally trained and tested in unnatural conditions; their learning appears ‘artificial’ and only partially examined. To solve the present problem, it is necessary to submit ant colonies to full conditioning like in nature. Consequently, we here tempted to define the first and the second acquisition and loss of olfactory and visual conditioning (if this could occur) by foragers of four colonies of *M. rubra* maintained as naturally as possible in the laboratory, this being performed according to a collective operant method. This will allow explaining the navigation system of *M. rubra*, drawing comparisons with two other *Myrmica* species, and pointing out generalities about learning in animals.

## Material and methods

### Collection and maintenance of ants

The experiments were performed on four experimental colonies derived from large colonies collected in the Aise valley (Ardenne, Belgium) on open grassland. Each experimental colony was demographically identical: they contained a queen, about 400 workers and brood. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (42 cm x 27 cm x 7 cm), the sides of which were covered with talc. The trays served as foraging areas; food was placed in them, and the ants were trained on the floor of the trays (Fig. 1 A, B; Fig. 2 A, B).

Temperature was maintained at  $20^{\circ} \pm 2^{\circ}$  C, humidity at about 80%, this remaining constant over the course of the experimentation. The lighting had a constant intensity of 330 lux when caring for the ants (e.g. providing food,

renewing nesting tubes), training the ants and testing them.

Sugar-water was provided *ad libitum* in a small glass tube plugged with cotton, and chopped cockroach was served twice a week on a glass-slide. Sugar-water served as a reward during training (Fig. 1 A, B; Fig. 2 A, B).

### Ants’ training and testing

The ants were collectively trained in their tray and were individually tested in another small tray (22.5 cm x 16 cm) containing an appropriate experimental apparatus. Each colony had its own tray and testing apparatus (Figs. 1A, 2A).

They were collectively **olfactory trained** by depositing pieces of onion on the right and the left of the opening of the tube containing the sugar-water, and by depositing pieces of thyme on another place of the ants’ tray, far from any food (Fig. 1 A, B). The onion, associated with reward, was considered as being the ‘correct’ choice (the one the ants should make when tested, for giving the correct response). The pieces of thyme, onion and the tubes containing the sugar-water were relocated once or twice each day, but never periodically (e.g. never after lapses of time of 12h), and the reward was replenished if necessary. This was done to avoid the establishment of a trail (CAMMAERTS & CAMMAERTS, 1980) and to prevent the acquisition of spatial and/or temporal learning by the ants (CAMMAERTS, 2004a).

The ants were collectively **visually trained** using an experimental apparatus consisting of green or yellow gazebo-like frames constructed of strong paper (Canson®; Fig. 2 A, B). The colors had been previously analyzed to determine their wavelength reflection (CAMMAERTS, 2007b; CAMMAERTS & CAMMAERTS, 2009). The cubes were constructed according to the instructions given in CAMMAERTS & NEMEGHAIRE (2012) and were held together with sellotape®. Ants could see the cubes and easily go underneath (Fig. 2 B). The green-colored apparatus was set over the opening of the tube filled with sugar-water. The yellow-colored apparatus was set at another place in the ants’ tray far from any food (Fig. 2 A). The green apparatus, associated with reward, was considered as being the ‘correct’ choice (the one the ants should make when tested for giving the correct response). Each cube was relocated once or twice each day, but never periodically (e.g. never after lapses of time of 12h or 24h)

and the reward was then renewed if necessary. This was done for the same reason as that here above given (olfactory training).

Ants were individually **tested** in a Y-apparatus made of strong white paper (Fig. 1 C, Fig. 2 C). The apparatus had its own bottom and its sides were covered with talc to prevent the ants from escaping. Its construction has schematically been explained in CAMMAERTS *et al.* (2011) and CAMMAERTS & NÉMEGHAIRE (2012). In the Y-apparatus, the ants deposited no trail since they were not rewarded. However, they may lay down other chemical secretions. Consequently, the floor of each Y-apparatus was changed between tests. For control experiments, the Y-apparatus was left empty. For test experiments, the Y-apparatus was provided with either two large pieces of onion in one of its branches (one piece on each side of this branch) and several small pieces of thyme in the other branch (a few ones on each side of that branch; Fig. 1 C) or with a green cube in one branch and a yellow one in the other branch, with a piece of same squared paper set on the cubes (Fig. 2 C). Onion and thyme, as well as the green and the yellow cubes, were randomly set in one or the other branch of the Y-apparatus for each tested colony and each test experiment.

### Assessment of the ants' response

To conduct an experiment on a colony, 10 foragers – randomly chosen among the foragers population of that colony – were transferred, one at a time, to the area at the entrance of the Y-apparatus. Each ant was observed until it turned either left or right in the Y-apparatus, and its choice was recorded. We recorded only the first choice of the ant and only when the ant had crossed the line pencil drawn on the floor marking the entrance to a branch (Fig. 1 C, Fig. 2 C). Afterward, the tested ant was removed and transferred into a polyacetate cup (Fig. 1 A, Fig. 2 A), whose rim was covered with talc, until 10 individuals had been tested, to avoid testing twice the same ant in the course of one experiment.

Ants' response was quantified during the control and test experiments. For each experiment performed on one colony, the number of ants turning left or right in the Y-apparatus (control) as well as going towards the onion or the green cube (i.e. giving the 'correct' response) or toward the thyme or the yellow cube (i.e. giving the 'wrong' response)

(test) were recorded. The number of ants which correctly responded per colony was so assessed. Then, the total number of correct responses, among the 40 obtained from the four used colonies, was established. This allowed calculating the mean conditioning score (= the percentage of correct responses) for the four colonies.

The total number of correct responses obtained for all colonies was statistically analyzed using the non-parametric  $\chi^2$  test, comparing the observed values to those expected under the null hypothesis that ants navigate the Y-apparatus at random. Doing so avoided to make correction consequently to numerous successive assessments. Responses were considered not significant when  $P > 0.05$  (SIEGEL & CASTELLAN, 1989).

### Planning of the experimentation

The first period of conditioning began when the onion or the green cube, (and the corresponding reward) and the thyme or the yellow cube were placed in the colony's tray. From then on, the stimuli were relocated on multiple occasions and the time was continuously recorded. The ants were tested, as related above, at given times until their score no longer increased. This sequence of events constituted the first conditioning period. The pieces of onion and of thyme or the colored cubes were then removed from the ants' tray and the ants were tested again, the time being once more carefully recorded. This constituted the first loss of the conditioning. When the ants' score no longer decreased, a second period of conditioning was undertaken in the same manner as the first, the ants being tested and the time recorded. This was the ants' second conditioning. When the ants' score no longer increased, onion and thyme or the colored cubes were removed from the ants' tray, and the ants tested for a fourth time, the duration being again recorded. This constituted the ants' second loss of conditioning.

## Results

### Control experiment

Before conditioning, the foragers of each colony had no preference at all for one or the other branch of the Y-apparatus (Figs 3, 4, control). In the study of *M. rubra*'s navigation system (CAMMAERTS, 2012), foragers also did

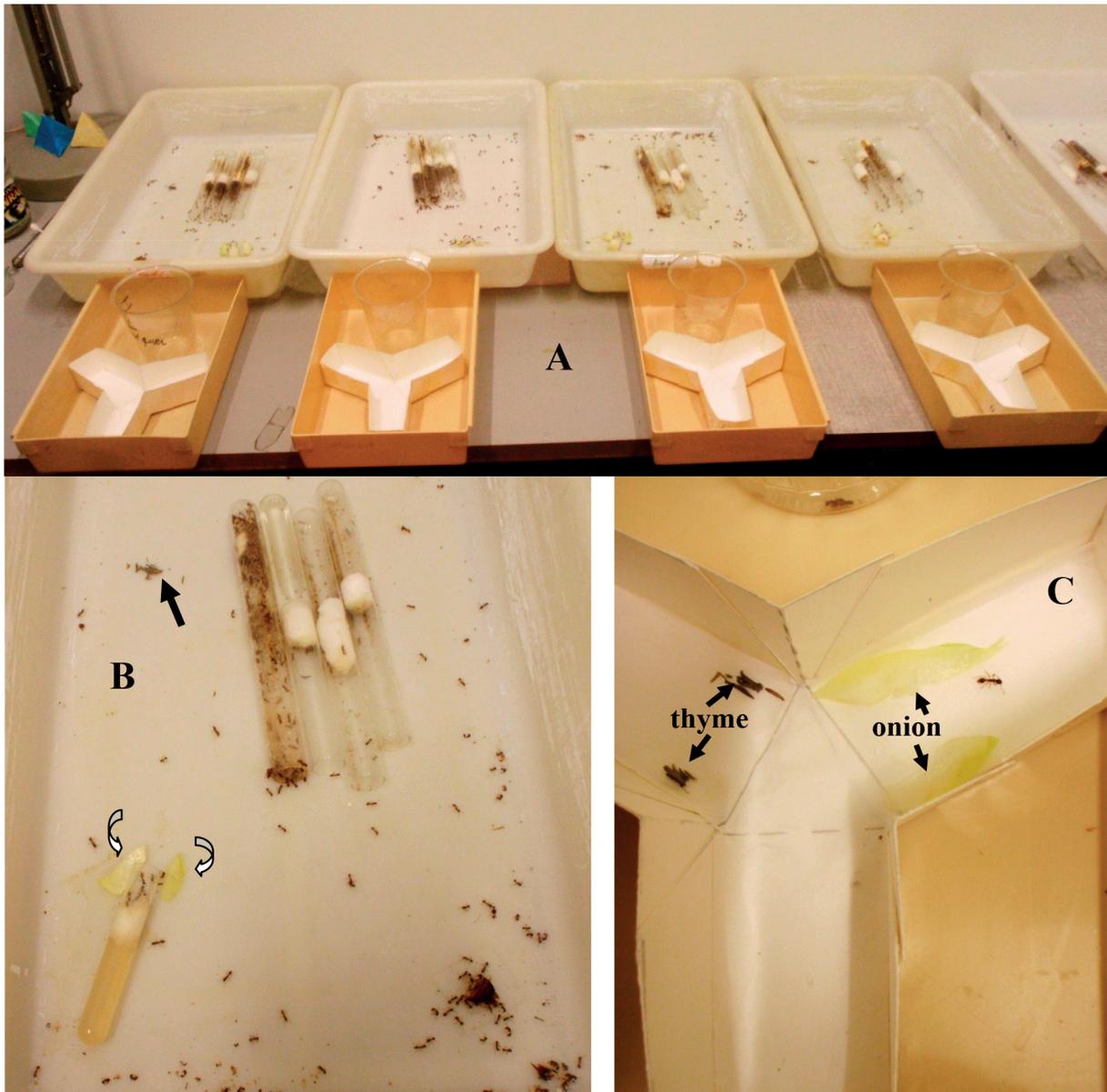


Figure 1. Experimental design to study *Myrmica rubra* foragers' olfactory conditioning. **A:** the four experimental colonies and, in front of them, their testing apparatus. **B:** the ants were collectively trained on their foraging area: pieces of onion were set next to a source of sugar water (curved arrows); pieces of thyme were set elsewhere (straight arrow). **C:** the ants were individually tested in a Y-apparatus provided with onion in one branch and thyme in the other.

not initially orient themselves towards either onion or thyme, or a green or a yellow cube. The following observations of ant preferences for one vs. another cue are thus valid, pointing out true ants' conditioning.

### Olfactory conditioning

#### First acquisition (Fig. 3, first acquisition)

*Myrmica rubra* foragers acquired a statistically significant olfactory conditioning after having been trained for 9 to 10 hrs. Thereafter, their score increased from 75% (after 10 to 25 hrs;  $P < 0.05$ ) to 77.5% (after 30 to 36

hrs;  $P < 0.02$ ). These ants can thus be olfactory conditioned in a few hours and their mean conditioning score then equal 76%.

#### First loss (Fig. 3, first loss)

The foragers of *M. rubra* kept their olfactory conditioning, and even exhibited a higher score, for at least 7 hrs after the removal of the cues. They acquired a statistically non-significant score (65%;  $\chi^2 = 1.28$ ;  $df = 1$ ) after having found, during 10 hrs, their sugared food no longer associated to the previously learned odour. They continued to slowly lose their olfactory conditioning, but nevertheless

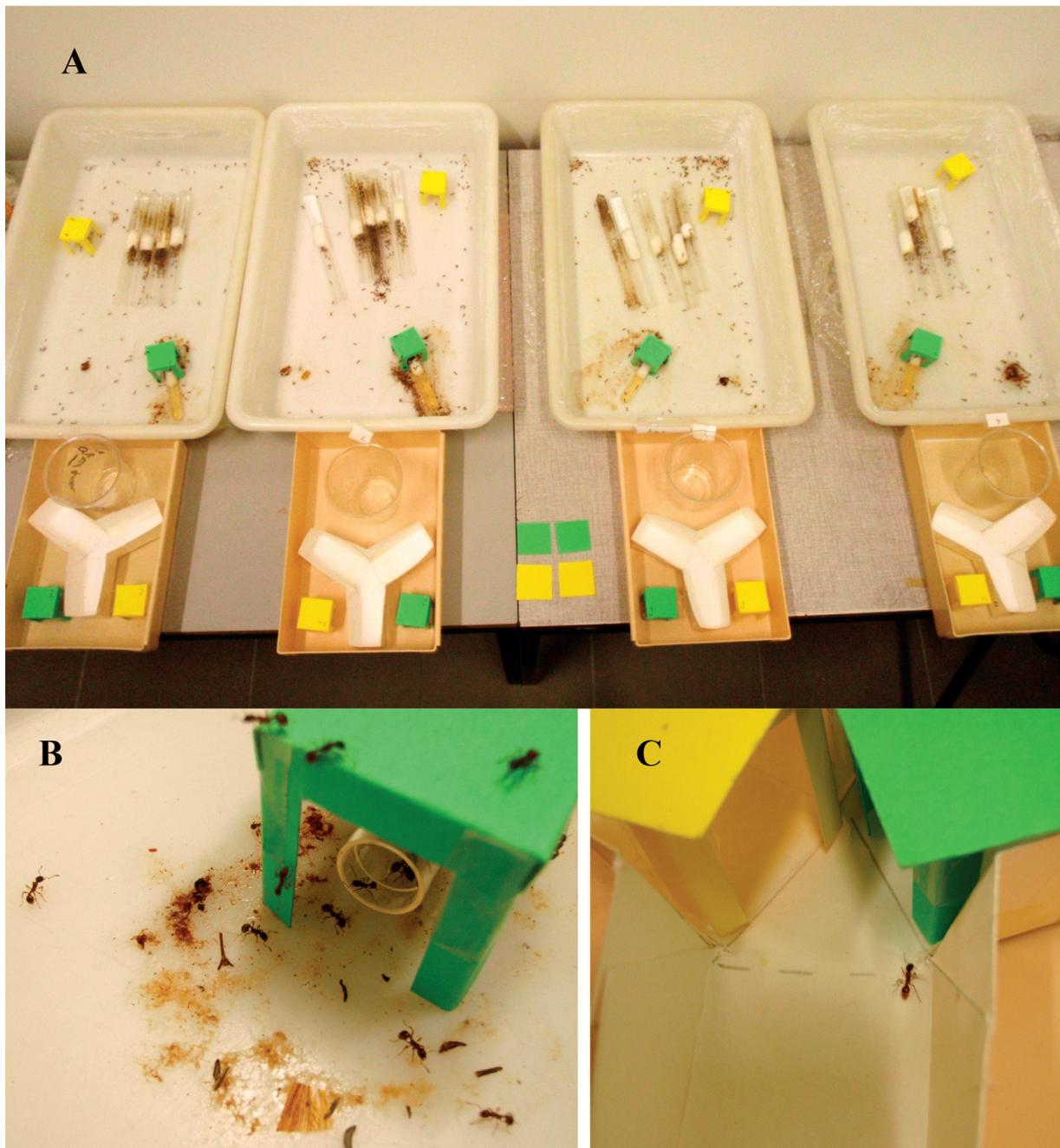


Figure 2. Experimental design to study *Myrmica rubra* foragers' visual conditioning. **A**: same legend as for Fig. 1 **A**. **B**: same legend as for Fig. 1 **B** except that a green cube was set over the sugar water and a yellow cube elsewhere. **C**: same legend as for Fig. 1 **C** except that the apparatus was provided with a green cube in one branch and a yellow cube in the other.

demonstrated the rather high but non-significant score of 72.5% ( $\chi^2 = 3.37$ ;  $df = 1$ ) 36 hrs after training had ceased. During these experiments, the ants hesitated for several minutes before moving to one or the other branch of the Y-apparatus. Finally, after 53 hrs without training, the ants moved nearly at random through the Y-apparatus. Their conditioning score equaled 57.5% ( $\chi^2 = 0.20$ ;  $df = 1$ ), which although not statistically significant was still not

entirely identical to the initial control score (50%).

*Myrmica rubra* workers may thus have a medium-term olfactory memory. If this is the case, they ought to acquire a second conditioning in a somewhat shorter time than the first one (perhaps reaching a better score), and ought to lose that second conditioning in a somewhat longer time than the first one (perhaps retaining some more). The following experiments examine these possibilities.

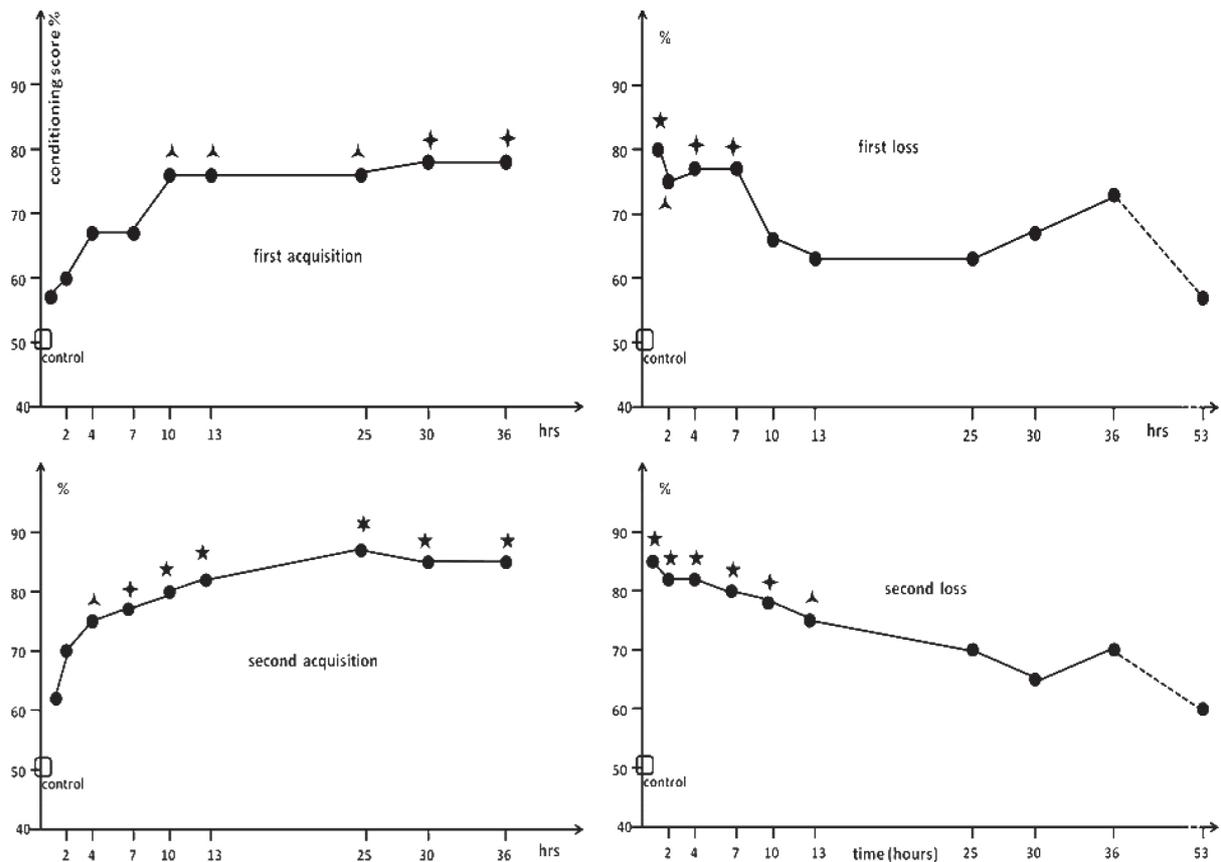


Figure 3. Kinetics of the first and second acquisition and loss of an olfactory conditioning by *Myrmica rubra* foragers. The ants were continuously collectively trained while living in their colony and were individually tested in the course of time in a Y apparatus. Time is given along the x-axis, the ants' conditioning score along the y-axis. The ants acquired their first conditioning in 10 hrs, reaching a mean score of 76%, which was lost in 8-9 hrs and ended with a score of 57.5%. The ants acquired their second conditioning in 4 hrs, reached then a mean score of 85%, lost it in 18 hrs and ended with a score of 60%. They have thus a medium-term olfactory memory but their score increases in the course of successive conditioning periods. Results of non-parametric  $\chi^2$  tests between the obtained values and the randomly expected ones: ▲ =  $P < 0.05$ ; ✦ =  $P < 0.02$ ; ★ =  $P < 0.01$ ; ★ =  $P < 0.001$ .

#### Second acquisition (Fig. 3, second acquisition)

When trained a second time, *M. rubra* foragers acquired the statistically significant score of 75% in 4 hrs ( $\chi^2 = 4.32$ ;  $df = 1$ ) and achieved, after 7 hrs of training, a mean score of 77.5% ( $\chi^2 = 5.41$ ;  $df = 1$ ), a value not demonstrated until 30 hrs of training during the first conditioning. Moreover, these ants reached a higher score at the end of their second conditioning than after their first one (82.5%, 87.5%, 85%, 85%;  $\chi^2 = 8.05, 11.4, 9.63, 9.63$  respectively, for 13, 25, 30 and 36 hrs of conditioning respectively). Their average final score equaled 85%. Their olfactory memory is therefore very probably medium-term.

#### Second loss (Fig. 3, second loss)

*Myrmica rubra* foragers retained conditioning from their second training for more than 13 hrs (at 13 hrs: 75%;  $\chi^2 = 4.32$ ;  $df = 1$ ) and

lost it after about 18 hrs (at 25 hrs: 70%;  $\chi^2 = 2.55$ ;  $df = 1$ ). This is twice the time observed during the loss of the first conditioning. These ants had thus not entirely forgotten what they had learnt during the first conditioning. They then exhibited the rather high, quite significant scores of 70%, 65% and 70% ( $\chi^2 = 2.55, 1.28, 2.55$  respectively;  $df = 1$ ) 25, 30 and 36 hrs respectively after the training stopped. After 53 hrs, they achieved a score of 60% ( $\chi^2 = 0.45$ ;  $df = 1$ ), which whilst non-significant was yet larger than that demonstrated at the same time during the first loss of conditioning.

#### Visual conditioning

##### First acquisition (Fig. 4, first acquisition)

Submitted to collective visual operant conditioning, *M. rubra* foragers responded to the cue only after 13 hrs of training (score = 77.5;  $\chi^2 = 5.41$ ;  $df = 1$ ;  $P < 0.02$ ). After 36 hrs, the

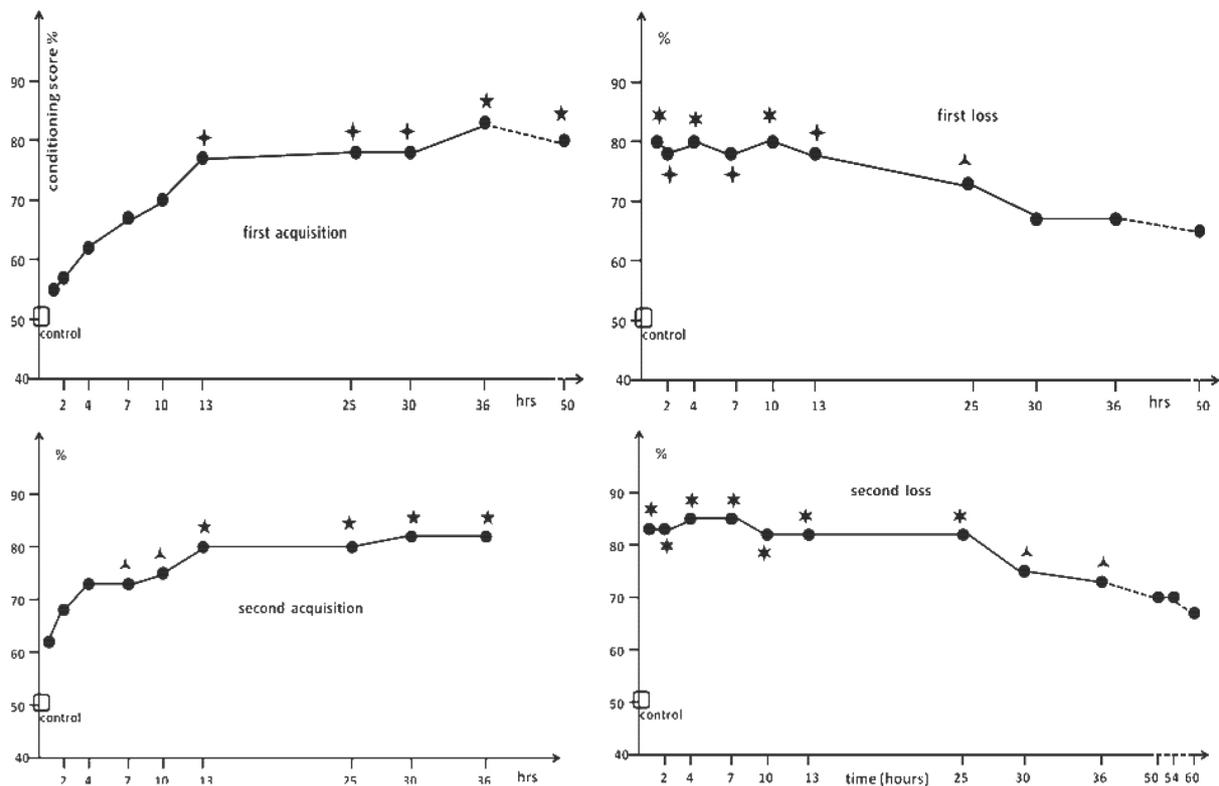


Figure 4. Same legend as for Fig. 3 except that the ants were visually conditioned. They acquired their first conditioning in 12hrs, reached a mean score of 80%, lost it in 20-21 hrs and ended with a score of 62.5%. They acquired a second conditioning in 9-10 hrs, reached a mean score of 82.5%, lost this in 30-32 hrs and ended with a permanent score of 65%. They have so a long term visual memory. Results of non-parametric  $\chi^2$  tests as for Fig. 1.

mean conditioning score equaled 82.5% ( $\chi^2 = 8.05$ ;  $df = 1$ ;  $P < 0.01$ ). It then decreased to the stable, statistically significant value of 80.0% ( $\chi^2 = 6.64$ ;  $df = 1$ ;  $P < 0.01$ ).

#### First loss (Fig. 4, first loss)

When the visual cues were removed, the foragers continued to respond correctly for 13 hrs (at that time: 77.5%;  $\chi^2 = 5.54$ ;  $df = 1$ ;  $P < 0.02$ ). After that, the ants' mean conditioning score decreased to 65% 50 hrs after training ceased ( $\chi^2 = 1.28$ ;  $df = 1$ ;  $P < 0.30$ ). After 80 hrs (not shown in Fig. 4), that score remained at the stable, non-significant value of 62.5% ( $\chi^2 = 0.81$ ;  $df = 1$ ;  $P < 0.50$ ). The ants thus retained 12.5% of a first conditioning. They may so have a rather long lasting visual memory. The same reasoning as that made for olfactory conditioning can be applied for the ants' second acquisition and loss of visual conditioning.

#### Second acquisition (Fig. 4, second acquisition)

After having reached the non-significant conditioning score of 62.5%, the foragers stayed without training for 16 hrs. Then, the visual cues were again set into the trays. The foragers

demonstrated the non-significant score of 72.5% after 7 hrs ( $\chi^2 = 3.37$ ;  $df = 1$ ;  $P < 0.10$ ) and the significant one of 75% after 10 hrs ( $\chi^2 = 4.32$ ;  $df = 1$ ;  $P < 0.05$ ). They were thus conditioned again after 9 hrs, a shorter time than that observed for the first conditioning. The ants' score went on increasing to the stable value of 82.5% after 30 hrs ( $\chi^2 = 8.05$ ;  $df = 1$ ;  $P < 0.01$ ) which is a higher value than that observed at the end of the first conditioning.

#### Second loss (Fig. 4, second loss)

The visual cues were kept in place overnight and removed the following day at 10:00. Surprisingly the foragers continued to respond correctly to the cues for 30 hrs, at which time they demonstrated a mean conditioning score of 75% ( $\chi^2 = 4.32$ ;  $df = 1$ ;  $P < 0.05$ ). After 36 hrs without training, the ants presented a nearly significant score of 72.5% ( $\chi^2 = 3.32$ ;  $df = 1$ ;  $P \approx 0.05$ ). *Myrmica rubra* foragers so retained the visual conditioning they acquired for a second time for 35 hrs, which was a longer time than that observed at the end of the ants' loss of a first conditioning. After that, the ants' mean conditioning score slowly decreased until

reaching the non-significant value of 65% after 106 hrs which remained so at 124 hrs and 150 hrs (the later assessments are not shown on Fig. 4). *Myrmica rubra* workers so retained 15% of a second visual learning period, exhibiting thus a rather long-lasting visual memory.

### Conclusion

The present work defines the kinetics of the first and second acquisition and loss of olfactory and visual conditioning in the ant *M. rubra*.

**Similarities** appeared between the kinetics of the two conditionings:

- each demonstrated a latency period;
- the second acquisition was always more rapid than the first one, had a shorter latency period and stabilized at a higher conditioning score;
- the second loss was always slower than the first one, had a longer latency period and ended at a higher final conditioning score, suggesting that more information had been retained.

Olfactory and visual conditionings demonstrated **differences**:

- the acquisition of an olfactory conditioning was more rapid than that of a visual one;
- the difference between the first and the second conditioning periods (the fact that the second conditioning leads to a higher overall score than the first) was more pronounced for olfactory conditioning than for visual conditioning. After several successive olfactory conditioning periods, the ants' memory ought to be of high quality;
- visual conditioning leads to a higher final score and a stronger memory than olfactory conditioning.

**Finally**, for *M. rubra*, the efficiency of successive olfactory and visual conditioning periods will be equivalent. This explains the similar uses of visual and olfactory cues by the foragers of this species to navigate.

### Discussion

While studying the navigation system of *Myrmica sabuleti*, *M. ruginodis* and *M. rubra*, we demonstrated that these ants were actually (*sensu stricto*) olfactory conditioned since they correctly reacted to aqueous extracts of onion

and thyme (CAMMAERTS & RACHIDI, 2009; CAMMAERTS *et al.*, 2012, CAMMAERTS, 2012a).

For each three studied species, olfactory and visual conditionings differed in their acquisition. This reflects the fact that the neurological processes underlying these two kinds of conditioning differ. The steps, sequences and histological implications occurring during vision (and visual memory) on one hand and olfaction (and olfactory memory) on the other hand are not identical. Examination of the neurological processes occurring in an ant's brain (and in the neural lobes controlling the eyes and the antennae) would be of interest. Studies similar to those performed by Prof. M. Giurfa and colleagues should be undertaken (examples of such works: EHMER & GRONENBERG, 2004; GIURFA, 2007; HOURCADE *et al.*, 2010).

Let us compare the present findings on *M. rubra* to those previously obtained for *M. sabuleti* and *M. ruginodis*.

*Myrmica sabuleti* foragers demonstrate very efficient olfactory conditioning, with no latency period and a high final conditioning score, but have a short-term olfactory memory. These ants present visual conditioning of intermediate quality but have a rather long-lasting visual memory (CAMMAERTS *et al.*, 2011). They primarily use odours to navigate but soon stop responding to obsolete cues while continuing to follow incorrect visual cues (CAMMAERTS & RACHIDI, 2009). *M. ruginodis* foragers acquire olfactory conditioning only in darkness and have no olfactory memory while they demonstrate a long-lasting visual memory (CAMMAERTS & NÉMEGHAIRE, 2012). These ants only use visual cues from above to navigate (CAMMAERTS *et al.*, 2012). *Myrmica rubra* foragers can be conditioned to olfactory and visually stimuli with equal efficiency (present work), and navigate using, as best as they can, odours and visual cues (CAMMAERTS, 2012a). A concordance exists thus between foragers' conditioning abilities and navigation system for the three studied species.

These conditioning abilities and navigation systems are also in agreement with the three species' visual perception capabilities and subtended angle of vision. Indeed, *M. sabuleti* workers cannot discriminate different shapes or hollow forms (although they can perceive colors, UV and perspective) (CAMMAERTS 2007a,b, 2008); *M. ruginodis* workers distinguish forms and even different patterns of small luminous

points (CAMMAERTS, 2012b); *M. rubra* distinguish filled shapes but not hollow forms (CAMMAERTS, in press). These three species' eyes morphology (RACHIDI *et al.*, 2008) is also in agreement with their visual perception, visual conditioning abilities and navigation system. It would be interesting to examine the sensory organs located on the funiculae extremity of these ants' antennae, a preliminary study having revealed larger numbers of sensory sensillae in *M. sabuleti* workers.

Recent experiments performed on *M. sabuleti* showed that isolated ants, each one maintained alone in an artificial nest, individually visually or olfactory conditioned according to an operant method, present the same kinetics of acquisition and loss of conditioning, reached the same conditioning scores and kept the same remembering of learned elements than the entire colony, except that they appeared to be somewhat more performing (CAMMAERTS, submitted). Conclusions drawn from collective conditioning, as done in the present work, are therefore valuable not only at a collective but also at an individual level.

Our studies on ants' conditioning are here below compared to those of other researchers, albeit cautiously since the experimental methods are not always identical. Twenty years ago, ROCES (1990) demonstrated olfactory conditioning in a leaf-cutting ant during its recruitment process. This was a first step in the research on ants' learning and the ants were observed in near-natural circumstances. Very recently, SAVERSCHEK *et al.* (2010) once more demonstrated and detailed the olfactory conditioning leaf-cutting ants can acquire. In the mean time, we obtained classical conditioning, spatial and temporal learning, spatial conditioning and operant conditioning in *Myrmica* ants, and inferred some characteristics of these ants' learning abilities (CAMMAERTS, 2004a,b,c). Thereafter, DUPUY *et al.* (2006) succeeded in demonstrating individual olfactory learning in *Camponotus* ants. Later on, GUERRIERI & D'ETTORE (2010) performed classical conditioning on *Camponotus aethiops* workers, the ants' response being the maxilla-labium extension. In these two latter studies, the animals were restrained ('harnessed' in the words of the authors), and thus trained and tested in an artificial situation. An olfactory memory of 1 hour was detected. Such a short-term memory in comparison with what we

obtained for *M. rubra* may be due to species differences, but also to the non-natural experimental conditions. However, some long-term olfactory memory has been postulated in *Camponotus fellah* ants and was proved to be stabilized via protein synthesis (JOSENS *et al.*, 2011). Recently, BOS *et al.* (2010) went a step further in the knowledge of olfactory learning by the ant *Camponotus aethiops*: the ants' chemical recognition depends on the context. Finally, these works allow to advance a hypothetical generality about ants' (or any animals') learning processes. The animals can obviously be conditioned to different cues, in accordance with their perception abilities, and their memory depends on their learning experience and process. Slow learning generally leads to strong retention while very quick learning leads to quick forgetting. A more or less long-term memory may result from cytological and biochemical changes in specialized parts of the brain, including the synthesis of proteins. So, ants can 'learn' several cues they then use to perform tasks and their learning differs according to the method of learning, the cues to learn and the species. Learning changes the ants' behaviour, brain cytology and nervous cells biochemistry. A recent interesting work, tangential to the present subject, is that of VAN WILGENBURG *et al.* (2011) who showed that social insects can learn and discriminate cuticular hydrocarbons, which is crucial for their social life. A work on course reveals that some learning abilities are not innate in ants but acquired in their first months of life.

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