

Why do workers of *Myrmica ruginodis* (Hymenoptera, Formicidae) navigate by relying mainly on their vision?

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Abstract

In semi-natural conditions, *Myrmica ruginodis* foragers were submitted to collective differential operant visual then olfactory conditioning and were individually tested in the course of time. They acquired visual conditioning in 60h, reached a score of 82% after 72h and kept then a permanent score of 74%. When training ceased, they lost their learning in 2 days and kept thereafter a permanent score of 61%. If trained for a second time, these ants were again visually conditioned in 36h and kept then a permanent score of 77%. When training once more ceased, they never entirely lost their visual conditioning but kept 75% of it. *M. ruginodis* workers have thus a very long term visual memory. At 300-500 lux, these ants never acquired olfactory conditioning. In near-darkness, they were quickly conditioned but forgot what they had learnt as soon as training ceased. When trained in near-darkness a second time, they acquired olfactory conditioning at the same speed and reached the same score as when trained for the first time. When the second training period stopped, they once more rapidly and entirely forgot what they had learnt. So, these ants recognized olfactory cues only in darkness and have almost no olfactory memory. Such observations explain the species' navigation system: these ants primarily use visual cues located above them to navigate, relying on odours only in darkness or in the absence of visual cues.

Keywords: conditioning; learning, memory; operant conditioning.

Introduction

While foraging, ants rely on their trail and area marking pheromones as well as on visual cues (according to a snapshot and/or a sketch map model), to a lesser extent on odours present in the environment and on some other egocentric elements (PASSERA & ARON, 2005). The ant *Myrmica ruginodis* NYLANDER 1846 was shown to use primarily visual cues from above for navigation and to use odours only in the absence of visual cues (CAMMAERTS *et al.*, 2012). Why do these ants rely so heavily on visual cues? The kinetics of *M. ruginodis* foragers' acquisition and loss of visual and olfactory conditioning may answer this question. Visual and olfactory conditionings occur continuously and repeatedly in nature. This means that, on field, ants are collectively differentially conditioned essentially according to an operant method. In our study of the *M. ruginodis* navigation system, we experimented in such conditions and went on using the same experimental conditions in the present work.

Learning has been widely studied in insects and, among others, in ants. However, the animals are generally trained and tested in poorly natural conditions. Research has concentrated on individual conditioning (f.i. JOSENS *et al.*, 2009), restrained ants (GUERRIERI *et al.*, 2007), simple conditioning (MACQUART *et al.*, 2008), only olfactory conditioning (DUPUY *et al.*, 2006), classical conditioning (CAMMAERTS, 2004a) and/or only first conditioning (HARRIS *et al.*, 2005; NARENDRA *et al.*, 2007).

We aimed to examine the first acquisition and loss, as well as the second acquisition and loss of a collective differential visual and olfactory operant conditioning in foragers of *M. ruginodis* maintained in the laboratory in semi-natural conditions. The ants were collectively trained as naturally as possible, were then subjected to a period of no training followed by a second period of training and thereafter a second period of no training, their conditioning score being sequentially assessed in the course of these

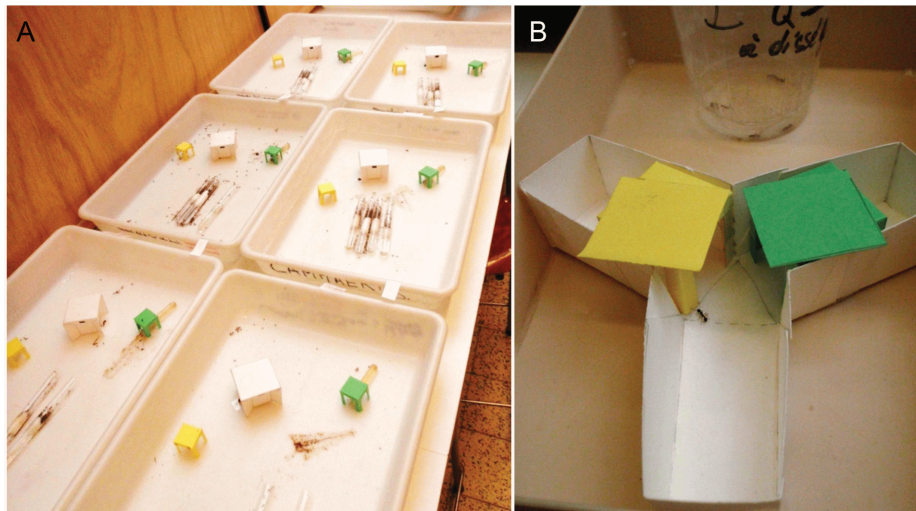


Fig.1. Experimental design and apparatus. **A:** the six colonies of *Myrmica ruginodis* collectively trained to find their food under a green cube (set above a source of sugared water) vs. a yellow cube. **B:** testing system: a Y apparatus, set in a small tray, in which the ants had to choose between a green and a yellow cube, both of which being identical to those encountered during training. Ten ants of each colony were tested, one by one, in this apparatus; they gave a response when crossing the pencil drawn line which signified the beginning of a branch. The ants' choices were recorded and the mean conditioning score so assessed.

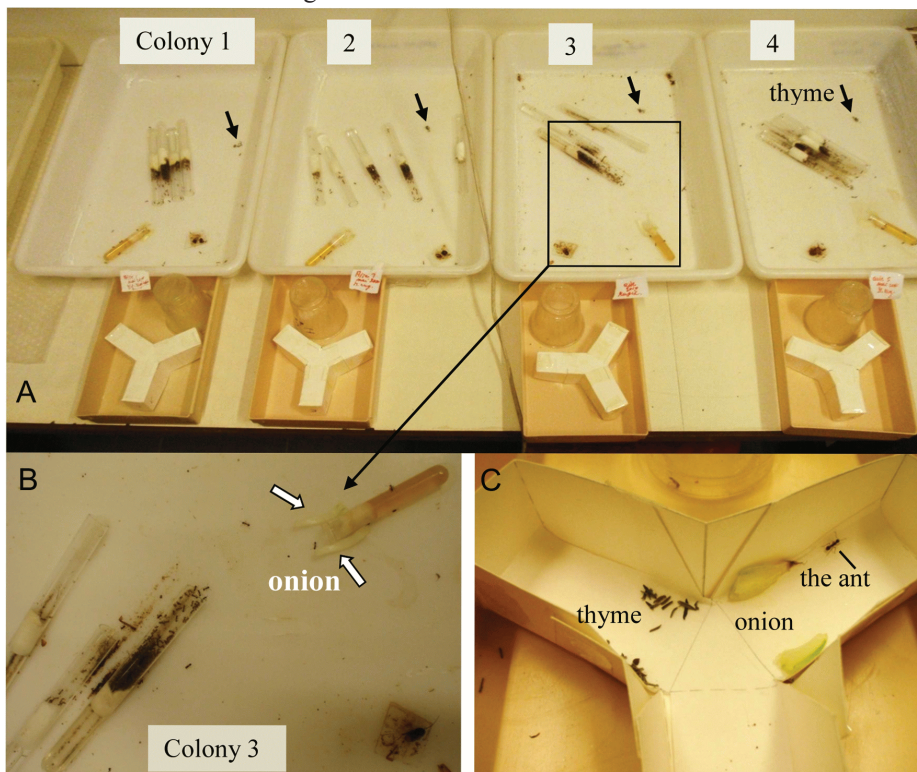


Fig. 2. Experimental design and apparatus. **A:** the four experimented colonies and, in front of their tray, their testing apparatus, set in a small tray. The black arrows indicate the pieces of thyme set in the ants' tray. **B:** training system: pieces of onion (indicated by two white arrows) were set on the left and the right of a tube containing sugared water. **C:** testing system: a Y maze, deposited into a small tray, with pieces of onion on the sides of one branch and pieces of thyme on the sides of the other branch. Ten ants of each colony were tested, their response recorded and their mean score assessed as detailed in the text.

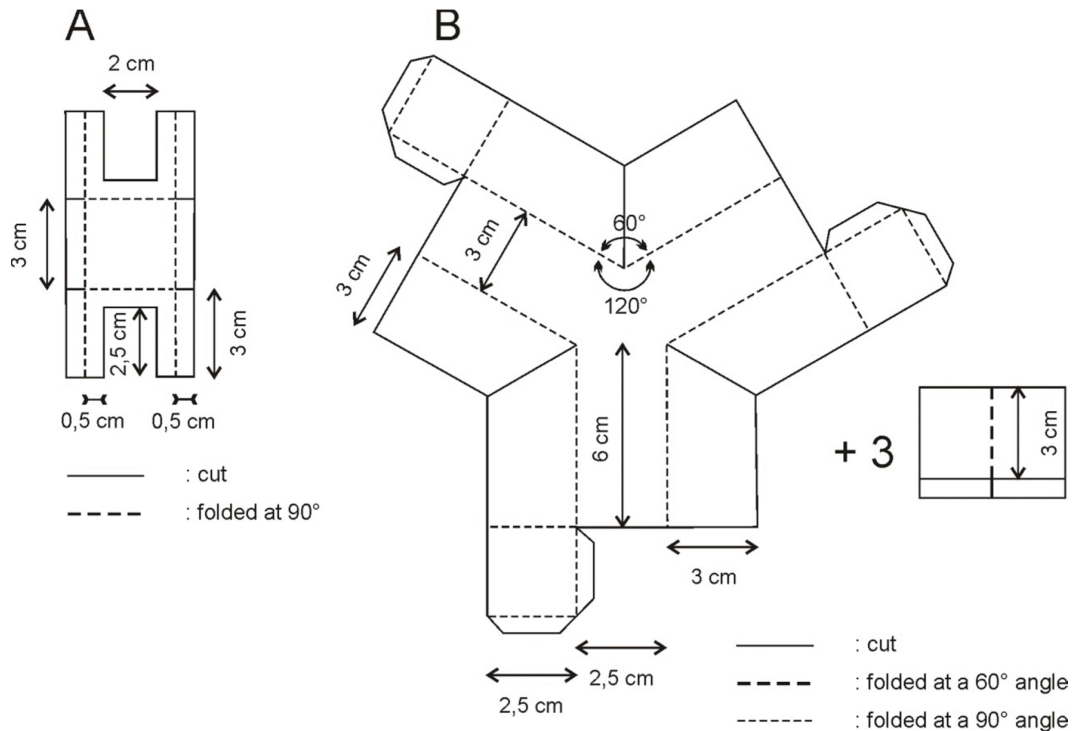


Fig. 3. Instructions for the construction of the experimental apparatus used in the present work: **A**: cube of green or yellow strong paper, used to collectively train foragers, the green apparatus being associated with a reward. **B**: Y-apparatus of white strong paper, used to individually test ants. The three pieces of paper (right of the figure) allowed closing the Y-maze at each of its three angles. During the test, either a green or a yellow cube, covered with a piece of identically colored paper (Fig. 1 B) or pieces of onion or of thyme (Fig. 2 C), were set at the beginning of a branch of the Y-apparatus. More information is given in the text.

experiments. Finally, the results were examined to explain the species' travelling system; comparisons were made with the species *M. sabuleti* Meinert 1861 partly similarly studied (CAMMAERTS *et al.*, 2011) and general conclusions were drawn concerning animal conditioning.

Material and Methods

Collection and maintenance of ants

The experiments were conducted on six (for the visual conditioning) or on four (for the olfactory conditioning) experimental colonies derived from natural populations collected in the Aise valley (Ardenne, Belgium). These colonies were demographically similar, each containing a queen, brood and about 500 workers. They were maintained in the laboratory in artificial nests made of two to four glass tubes half-filled with water. A cotton-plug separated the ants from the water. The glass tubes were deposited in trays (47 x 32 x 7 cm), the sides of which were covered with talc. The trays served as foraging areas: food was placed and ants were trained in

the trays (Figs 1A, 2A).

Temperature was maintained at $20^{\circ} \pm 2^{\circ}$ C. Humidity was about 80% and remained constant over the course of an experiment. The lighting had an intensity of 300 - 500 lux while caring for the ants (e.g. providing food, renewing nesting tubes), training and testing them. Training was also performed in near-darkness (< 5 lux). Chopped cockroach was served twice a week on a glass-slide. Sugar-water was offered *ad libitum* in a small glass tube plugged with cotton. During training, this sugar-water was provided to the ants such as it served as a reward (see 'training') (Figs 1A, 2A, B).

Experimental apparatus; ants' training (= conditioning) and testing

The ants were collectively trained on their foraging area using a simple apparatus, each colony having its own training apparatus (Figs 1A, 2A, B). They were tested individually and sequentially in a test apparatus, with a separate test apparatus for each colony (Figs 1B, 2A, C).

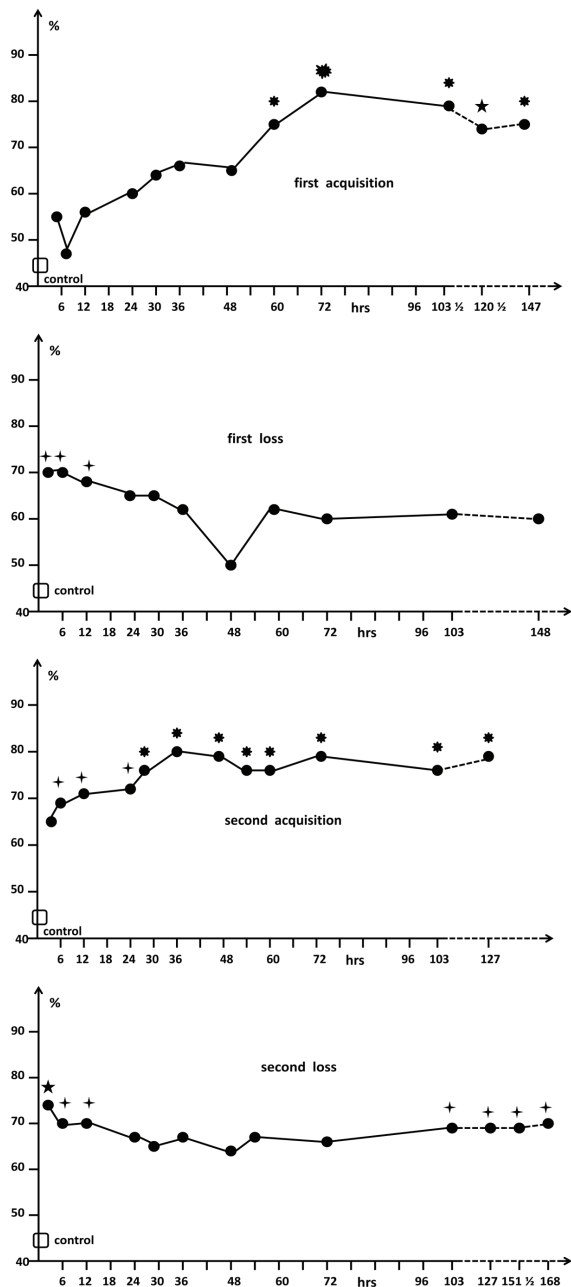


Fig. 4. Kinetics of the first and second acquisition and loss of a collective visual operant conditioning in *Myrmica ruginodis* foragers. The ants of six colonies were collectively trained and 120 (control) or 60 (test) of them were individually tested in the course of time in a Y-maze. Their mean conditioning scores are given on the y-axis, time being on the x-axis. Results of non-parametric χ^2 tests between the obtained values and the randomly expected ones are schematically given: NS: no symbol; + = $P < 0.05$; ★ = $P < 0.02$; * = $P < 0.01$; * = $P < 0.001$. The species obviously presents a strong visual memory.

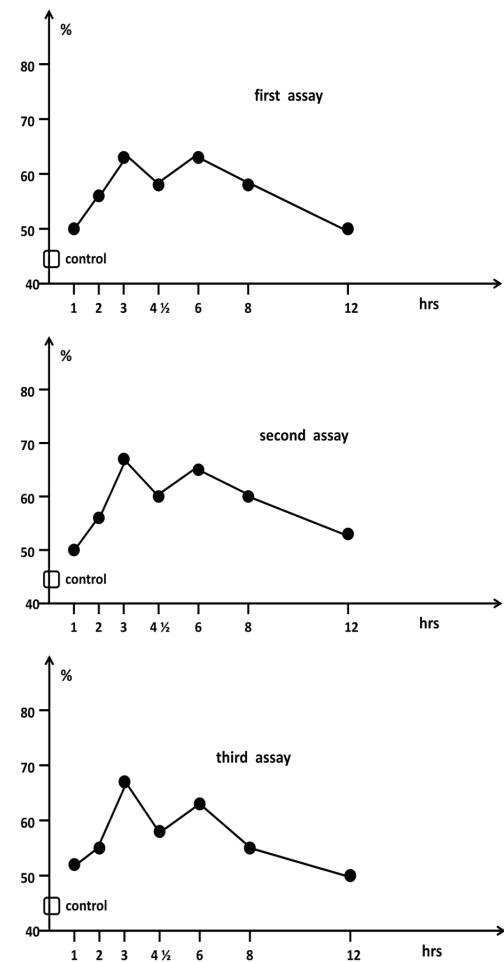


Fig.5. Kinetics of the conditioning score of *Myrmica ruginodis* foragers olfactory trained at diurnal light intensity (300 - 500 lux). Times are given on the x-axis, the conditioning scores on the y-axis. For each three assays, some tendency of conditioning occurred but conditioning obviously failed.

Training

Ants of six experimental colonies were collectively visually trained using an experimental apparatus consisting of two kinds of cubes, a green cube and a yellow one both constructed of strong paper (Canson®). The colors were previously analyzed to determine their wavelength reflection (CAMMAERTS, 2007; CAMMAERTS & CAMMAERTS, 2009). The cubes were constructed according to the instructions given in Fig. 3A, and were held together with cello tape®. The ceiling of each cube was filled contrary to the four vertical faces. Ants could so easily go underneath the cubes. The experimental apparatus were placed in the ants' tray so that they could easily be seen by the foragers. The green-colored apparatus of each

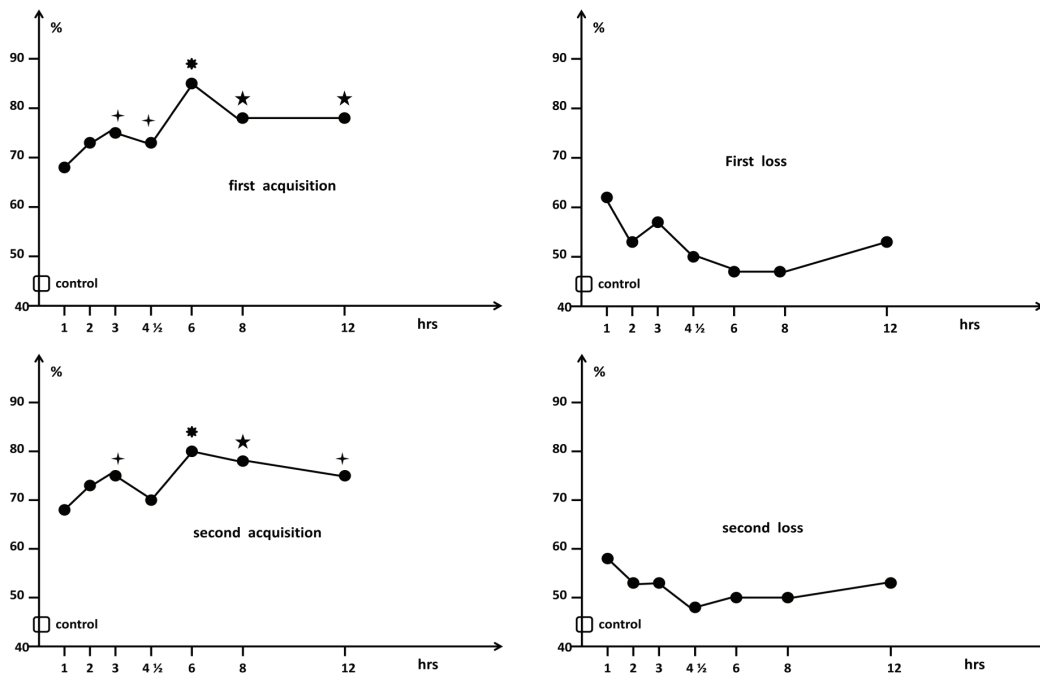


Fig. 6. Kinetics of the first and second acquisition and loss of a collective olfactory operant conditioning in *Myrmica ruginodis* foragers trained in darkness. Training and testing were conducted as summarized in Fig. 4. Time is given on the x-axis, the ants' score along the y-axis. Results of χ^2 tests are schematized as in Fig. 4. Conditioning succeeded but the second one did not lead to a better score than the first one; the first and the second loss of conditioning were rapid and entire. So, no olfactory memory could be detected in this ant species.

colony was set over the opening of the cotton plugged tube filled with sugared water. The yellow-colored apparatus was set at another location in the ants' foraging area and was not associated to a reward (Fig. 1A). The green apparatus was considered as being the 'correct' choice. Each cube was relocated once or twice each day, but never periodically (such as each 12hrs or 24hrs) and the reward was then renewed 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).

To olfactory condition the ants of four experimental colonies, pieces of onion were deposited on the right and on the left of the opening of the tube containing the sugar-water provided to the ants. Pieces of thyme were deposited on another part of the ants' tray, far from any food (Fig. 2 A, B). The ants were so continuously trained (= operant conditioning), going to the experimental apparatus whenever they wanted. The onion was considered as being the 'correct' choice. The pieces of thyme as well as those of onion and the tubes containing the sugar-water were relocated once or twice each

day, but never periodically. The cues and the reward were then renewed if necessary.

Testing

Ants were individually tested in a Y-shaped apparatus constructed of strong white paper according to the instructions given in Fig. 3 B. The apparatus had its own bottom and the sides were covered with talc to prevent the ants from escaping. In the Y-apparatus, the ants deposited no trail since they were not rewarded. However, it is possible that they utilized other chemical secretions as traces. As a precaution, 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 either with a green cube in one branch and a yellow cube in the other branch (Fig. 1B) or with pieces of onion in one branch and pieces of thyme in the other branch (Fig. 2C). A piece of green or yellow paper (3 x 3 cm) was placed on the correspondingly colored cube (Fig. 1B). The cubes as well as the pieces of plants were randomly set in one or the other branch of the Y-apparatus for each tested colony and each test experiment.

To conduct a test on a colony, 10 foragers of

that colony - randomly chosen among the 10 – 20 foragers of that colony - were transferred one by one to the area at the entrance of the Y-apparatus. Each transferred ant was observed until it turned either to the left or to the right in the Y-tube, and its choice was recorded. Only the first choice of the ant was recorded and this only when the ant was entirely under the cube. Afterward, the ant was removed and transferred to a polyacetate cup, in which the rim was covered with talc. To conduct the control, 20 or 10 ants of each colony were similarly tested in an empty Y-apparatus.

Assessment of the ants' response

Ants' response was quantified during the control and test experiments. For each of the experiments performed on a colony, the number of ants was recorded which turned left or right in the Y-apparatus (control) and which approached the green cube or the pieces of onion (= the 'correct' one) or the yellow cube or the pieces of thyme (= the 'wrong' one) (test). The mean number of ants which correctly responded per colony was so assessed. Finally, a total of 60 (visual conditioning) or 40 (olfactory conditioning) responses were obtained from the experiments performed on the six or the four colonies, respectively. The mean score for all the colonies could so be established as well as the percentage of correct responses for the tested ant population.

Of the 60 or 40 ants tested, the number of correct responses was statistically analyzed using the non-parametric χ^2 test applied to the experimentally obtained values and the theoretical expectation of identical values for the two branches of the Y maze. No correction must so be applied to our successive assessments. Each time, responses were considered as being not significant when $P > 0.05$ (SIEGEL & CASTELLAN, 1989).

Train of the experiments

Note that, for the ants' olfactory conditioning, experiments were carried out at either 300-500 lux or near darkness. At 300-500 lux, ants experienced only one conditioning period whilst two in near darkness as well as when experiencing visual conditioning. The protocol was otherwise identical for visual and olfactory conditioning as well as for light and dark experiments.

The first (or the only one) period of

conditioning of the ants began when either the colored cubes or the pieces of plants and the corresponding reward were placed in the colony's tray. From then on, the cubes and the plants were relocated on multiple occasions, as explained above, and the length of time was continuously recorded. The ants were tested, as related above, at given times until their score no longer increased. This sequence of events constitutes the first (or the only one) conditioning. The training cubes or the pieces of plants were then removed from the ants' tray and the ants were again tested with the time being once more carefully registered. This forms the first loss of the conditioning. When the ants' score clearly no longer decreased, a second period of conditioning was undertaken in the same manner as the first, and once again the ants were tested and the time recorded. This was the ants' second conditioning. When the ants' score no longer increased, the training cubes or the pieces of plants were removed from the ants' tray. The ants were then sequentially tested for a fourth time and the duration recorded. This was the ants' second loss of conditioning.

Results

Control experiment

In an empty Y maze, *M. ruginodis* foragers of each of the six (visual conditioning) or four (olfactory conditioning) colonies showed no preference for one or the other branch of the apparatus (Figs 4, 5, 6 control).

Visual conditioning (Fig. 4)

First acquisition of conditioning

During visual training for the first time, foragers underwent a latency period of about 24hrs. Afterward they acquired conditioning and attained the significant mean score of 75% after 60hrs ($\chi^2 = 6.97$) and that of 81.7% after 72hrs ($\chi^2 = 12.0$). Unexpectedly, these ants' visual conditioning thereafter decreased and equaled a permanent value of about 74% after about 147hrs ($\chi^2 = 6.97$).

First loss of conditioning

When training ceased, the ants underwent a latency period of about 12hrs (at 12 hrs: 68.3%; $\chi^2 = 3.45$). Thereafter they progressively lost their learning exhibiting a score of 50% after 48hrs. After that, their score somewhat increased and reached the permanent value of 60.8% after 75hrs ($\chi^2 = 0.84$).

Second acquisition of conditioning

During visual training for a second time, the ants underwent a latency period of 6hrs, attaining then a significant score of 68.3% ($\chi^2 = 3.45$) and reaching that of 80% after 36hrs ($\chi^2 = 10.59$). Thereafter, the ants' conditioning became somewhat less pronounced, the ants' score equaling 77.5% after 75hrs and remaining then unvaried ($\chi^2 = 9.28$).

Second loss of conditioning

When the second conditioning ceased, the ants underwent a latency period of 24hrs. They then slowly and incompletely lost their conditioning, attaining a score of 63.3% after 48hrs ($\chi^2 = 1.55$). Thereafter, they recovered: their score increased to 70% ($\chi^2 = 4.2$) and presented the unexpectedly high values of 71.7% and 75% after 217½hrs and 336hrs respectively ($\chi^2 = 5.04$ and 6.97 respectively; values not shown on Fig. 4).

Summary of the four events

The observed entire phenomena of the first and second acquisition and loss of a visual conditioning by *M. ruginodis* workers can be summarized as follows.

Before ants could differentially learn visual cues, they first underwent a latency period of about one day. They then began to acquire learning over the next few days. Shortly thereafter, they momentarily reached an optimum of visual conditioning, after which the conditioning started to slightly decrease. It reached a certain value and increased no more although training continued. When trained no further, at first they retained their learning for half a day. They then apparently lost it in two days and retained no memory at all. After that they somewhat recovered, retaining some visual conditioning for a long time. When these ants were trained again with the same visual cues, at first they underwent a short latency period. They then quickly acquired conditioning and reached an optimum of visual conditioning in one and an half days. They failed to maintain the maximum score which slightly decreased and remained unvaried although training continued. When the ants were again no longer trained, they retained their learning for at least a day. They then apparently lost conditioning and appeared less conditioned after two days. After that, they recovered. Their visual conditioning slowly 'came back' without training. After 14 days, for instance, it was stronger than before training ceased.

Olfactory conditioning

The tests were performed at the same time o'clock each day (11:00, 12:00, 13:00, 14:30, 16:00, 18:00 and 22:00). Whatever the event on course, the ants' score was always higher at 13:00 and lower at 14:30 (Figs 5 and 6). This may reflect some circadian rhythm related to the ants' olfaction or response ability.

Experiments at 300 – 500 lux (Fig. 5)

At this light intensity, foragers never associated an olfactory element and a reward. For about 5 to 6 hours, the ants exhibited some tendency for acquiring such an association, presenting then the no significant mean scores of 60.0, 62.5 and 62.5 % during the three assays of conditioning. Thereafter, the ants no longer responded to the olfactory elements they should have learned. This unexpected observation was valuable since made three times with similar results. Moreover, after the first and the second attempt of olfactory conditioning, the ants retained nothing from their previous experiences. They continued to behave as if they had never been conditioned.

Experiments at 0 – 5 lux (Fig. 6)

While studying *M. ruginodis* foragers' navigation system (CAMMAERTS *et al.*, 2012), we conducted olfactory conditioning continuously providing the ants with sugar-water located 4 cm on the right of onion and 4 cm on the left of thyme. The ants obviously became conditioned. They might have acquired conditioning during the night, at low light intensity. We consequently attempted a similar trial, in the present work. We tried to obtain olfactory conditioning on a naïve colony, starting the conditioning at 22:00 on one day, in the darkness, and testing 10 of these ants the following day at 10:00, at light intensity. Eight of these 10 ants gave the correct response. These ants had thus acquired olfactory conditioning. We deduced that *M. ruginodis* foragers could acquire such conditioning only at very low light intensity.

Consequently, we again conducted training and testing manipulations on the four previously used colonies (which were still as having never been conditioned) but, this time, the ants' training entirely occurred in darkness while testing was, of course, performed in another lighted room.

First acquisition of conditioning

The ants immediately acquired some olfactory conditioning without a latency period. This

became statistically significant after 3 hrs (75%; $\chi^2 = 4.32$), reached the maximum value of 85% after 6 hrs ($\chi^2 = 9.63$) and then kept the lower, permanent, statistically significant value of 77.5% ($\chi^2 = 5.41$).

First loss of conditioning

The olfactory cues were kept on the ants' tray near their sugar-water during the night and were removed the following day at 10:00. The ants rapidly lost their conditioning. After one hour, the ants' score equaled the non-significant value of 62.5% ($\chi^2 = 0.81$) and after 4.5 hrs, a value identical to the control one. It remained then at that value.

Second acquisition of conditioning

The ants were not trained from 23:00 until 10:00 the following day, the olfactory cues being then set in their positions. Again, the ants immediately acquired olfactory conditioning and reached a significant score after 3 hrs (75%; $\chi^2 = 4.32$). They achieved their conditioning after 6 hrs just like during the first conditioning (80%; $\chi^2 = 6.64$) and this maximum score was not larger than that acquired in the course of the first conditioning (80% vs. 85%). That score thereafter decreased until reaching the permanent significant value of 75% ($\chi^2 = 4.32$), a value somewhat lower than that obtained after the first conditioning.

Second loss of conditioning

As before, the olfactory cues were kept in place during the night and removed the following day at 10:00. Once more, the ants immediately lost their conditioning, achieving the non-significant score of 57.5% after one hour ($\chi^2 = 0.20$) and a score identical to the control one after 4.5 hrs. The ants then permanently kept this non significant conditioning score.

Summary of the events

At a light intensity usually occurring during the day, *M. ruginodis* foragers did not associate an odour with the presence of food. They quickly did so in the darkness but only while the odour surrounded the food. When the odour signal disappeared, *M. ruginodis* foragers rapidly ceased to respond to the odour at all. When the odour signal existed again in darkness, these ants once more quickly learned it, but no better than during their first attempt. When the odour vanished a second time, the ants again very quickly and entirely forgot it. *M. ruginodis* foragers present thus practically no olfactory memory.

Discussion

Our results reveal that successive learning and forgetting of visual elements increase the ants' memorization of these elements. So, *M. ruginodis* foragers in the field can acquire a long-term visual memory for several encountered elements. On the contrary, we concluded that *M. ruginodis* foragers have nearly no olfactory memory. Indeed, first, these ants entirely lost their conditioning as soon as training ceases and secondly, when trained a second time, they acquire a conditioning of the same quality as that resulting from their first conditioning and not a better one. They thus remember nothing from olfactory conditioning experiences.

We affirm that the olfactory conditioning here conducted was effectively an olfactory and not a visual one because, at 300 – 500 lux, the ants saw the olfactory elements and did not acquired conditioning and because, in a previous work (CAMMAERTS *et al.*, 2012), we obtained ants' conditioning to aqueous extracts of the olfactory elements.

The fact that *M. ruginodis* foragers can acquire a strong visual conditioning but only a very limited olfactory one, the latter furthermore occurring only in darkness and only while training persists, is in agreement with the navigation system of the species. Indeed, these foragers mainly use visual cues located above, relying on odours only in the absence of visual cues or in darkness (CAMMAERTS *et al.*, 2012). In fact, these ants got a lot of information from their vision and therefore nearly do not use their olfactory learning abilities. They may learn only very few odours, permanently perceived and located in dark places, for instance odours present at the nest site (odours of leaves, soil, small plants and so on). A follow-up study (CAMMAERTS, unpublished data) showed that the species can use cues located in the sky and the canopy. The present work explains how the ants can memorize such cues and use them for a long time as well as why they do not do so with olfactory cues. This recalls the association between odours and nest exhibited by the ant *Cataglyphis fortis*, which, otherwise, uses its vision to navigate (STECK *et al.*, 2009). We often observed nest relocation in the species *M. ruginodis*; they nest on the edges of forests, under branches and not elsewhere; thus, from time to time, according to the canopy evolution,

they relocate their nest. When this occurs, these ants should soon forget the odours associated with their old nest site.

The first acquisition and loss of visual operant conditioning was analyzed in the ant *M. sabuleti* (CAMMAERTS *et al.*, 2011). Kinetically the events are similar in the two species, *M. sabuleti* and *M. ruginodis*. This is the reason why we cautiously made general statements regarding insect visual conditioning. The acquisition of visual conditioning may be successively achieved when there is a latency period, a precise increase, a maximum value, a slight decrease, and stabilization. The second acquisition differs from the first by a shorter latency period, a quicker increase, a higher final score. The loss of visual conditioning may be successively achieved when there is a latency period, a precise decrease, a minimum value, a slight increase, and stabilization. The second loss differs from the first by a longer latency period, a slower decrease, and a higher final score. Thereafter, we examined the visual and the olfactory conditioning of *M. rubra* workers (CAMMAERTS, in press). The results of the latter study corroborated the above emitted ideas.

During the ants' visual training, we observed that some ants remained in the nest and never approached the green cube. We tested such ants in Y apparatus and pointed out so a hitherto never observed fact: these 'untrained' ants correctly responded. When their congeners were being trained, they could see the green cube as well as congeners going to and away from it. *Myrmica ruginodis* workers have indeed a very good visual perception (CAMMAERTS, unpublished data). This kind of conditioning (by imitation), known in mammals (DAMASIO & MEYER, 2008) and in some birds (in keas for instance, AUERSPERG *et al.*, 2011), has not yet been described in ants. In a following not yet published work performed on *M. sabuleti*, we studied this kind of 'learning' and its consequence.

During each of the seven conducted olfactory kinetics, the ants exhibited a lower score at 14:30, i.e. in the early afternoon. This pattern may be due to some circadian rhythm affecting the ants' olfaction. The ants had been living in the laboratory for two years, in a room without windows, or had been born in the laboratory. If their olfaction is of lower quality in the early afternoon, this might result from an inherent circadian rhythm which obviously resembles

those (one affecting the ants' vision, another affecting the ants' olfaction) exhibited by *M. sabuleti* foragers born in the laboratory from queens maintained for two years in a room without windows (CAMMAERTS *et al.*, 2011).

The fact that an ant cannot acquire olfactory conditioning under lighting condition has never been related. On the contrary, olfactory learning has often been obtained in ants (ROCES, 1990; BECKERS *et al.*, 1994; DUPUY *et al.*, 2006; JOSENS *et al.*, 2009; GUERRIERI & D'ETTORE, 2010). Such a conditioning has also been demonstrated and analyzed under a new point of view by SAVERSCHEK *et al.* (2010) in leaf-cutting ants and by BOS *et al.* (2010) in the ant *Camponotus aethiops*.

At diurnal light intensity, *M. ruginodis* foragers tended, for a few hours, to correctly respond to an odour associated with a reward. They did not achieve significant conditioning, and perhaps consequently, forgot what they had learned. In other words, to be successful, conditioning or learning might have to reach a given threshold value in a given lapse of time, otherwise it fails to occur. A similar phenomenon was observed while studying operant conditioning in *M. sabuleti* as for the extinction (actual extinction and not loss as in the present work) of such a conditioning (CAMMAERTS, 2004b). If the extinction experiments ceased before the ants' score reached a sufficiently low value, the ants recovered and appeared to be still conditioned.

In darkness, *M. ruginodis* foragers very quickly acquired olfactory conditioning, but demonstrated no olfactory memory. At high light intensity, these ants very slowly acquired visual conditioning after a latency period, and then demonstrated a very long lasting visual memory (present work). *M. sabuleti* foragers quickly learned odours and showed a short lasting olfactory memory, while they slowly learned visual cues with a latency period, and demonstrated a long term visual memory (CAMMAERTS *et al.*, 2011). Briefly, in each of our studies on ants' conditioning, we always observed that the more rapid the acquisition of conditioning, the less long term the acquired conditioning. We may thus presume that a strong conditioning and a long lasting memory often result from a slow acquisition of the conditioning with an initial latency period. Our next work on *M. rubra* workers' visual and olfactory conditioning is also in favor of such a

hypothesis (CAMMAERTS, in press).

Associative learning induces changes at the synaptic level in the brain and increases the quantity of membrane receptors involved in the associated reaction (DORÉ & MERCIER, 1992). A lengthy latency period at the beginning of a visual conditioning might be due to the participation of a large number of connected neurons for the establishment of such learning (GIURFA & MENZEL, 1997). Some neurological mechanisms occurring during memorization were studied by Giurfa and colleagues (GIURFA, 2007; HOURCADE *et al.*, 2010). Such studies complement ethological investigations such as our own.

Conclusion

The current work revealed that *M. ruginodis* foragers are able to associate a visual cue to the presence of food and permanently keep their learning as soon as after two acquisition experiences. This work also pointed out that *M. ruginodis* foragers are unable to associate food and olfactory elements at usual light intensity and do so in the darkness but only while the odour surrounds the food; in other words, these ants have nearly no olfactory memory. These results explain why this species' foragers navigate relying mainly on visual cues, neglecting odours except in the absence of visual cues.

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