The Acquisition of Cognitive Abilities by Ants:

A Study on Three *Myrmica* Species

*(Hymenoptera, Formicidae)*

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Abstract

It has been showed in the ant *Myrmica sabuleti* that freshly emerged ants partly recognize their congeners’ odor. We here demonstrate that this knowledge is acquired due to a process of habituation and not by operant conditioning. We previously demonstrated that, just at their emergence, ants become imprinted to their nestmates’ visual characteristics. We previously elucidated the ontogenesis of five other ants’ cognitive abilities: young ants become imprinted to the odor of their nest entrances when approaching them; later on they learn the visual appearance of such entrances by operant conditioning; they become imprinted to their foraging area odor when moving on it for the first time; they learn to follow their trail pheromone and to react to their species-specific alarm pheromone in the presence of older workers showing these abilities. Knowing that at least two cognitive abilities are acquired in the presence of older ants, the question remains as for the learning of these abilities by the first emerged ants in a newly founded colony. The present work aimed to answer this question. We showed that the founder queens of a newly founded colony went out of their nest and walked to the food sites during a few days, accompanied by their daughters. When these firstly emerged workers were thereafter tested in front of a source of alarm pheromone they correctly responded to the pheromone. The knowledge of some important ants’ cognitive abilities might thus be transmitted to the first emerged workers by the founder queens. We also showed that males having moved for two
days on their colony foraging area know the specific alarm reaction. Sexuals detain thus some specific cognitive abilities. It may be presumed that future queens learn, like the workers, several specific cognitive abilities, while living in their native colony, before swarming away from it and founding a new one. The other ants’ cognitive abilities are acquired via conditioning during about 2/3 of the ants’ life. The ontogenesis of ants’ cognitive capabilities is thus on the way to be elucidated, at least for the species *M. sabuleti*.

**Keywords:** alarm reaction, callow ants, founder queens, kin recognition, larvae

**Introduction**

Ants are among the most evolved invertebrates, living in highly organized societies, and detaining numerous cognitive abilities. The ants’ behavior is highly complex. Their colonies present a strong division of labor, an age-based polyethism and social regulation [1]. Ants perfectly recognize the members of their colony from those of alien ones [2, 3]. They care for their brood and build sophisticated nests. They have a lot of glands emitting numerous compounds having a behavioral function [4]. They chemically colony specifically mark the inside of their nest, their nest entrances, and their nest surroundings, as well as species specifically [REM pour le trait d’union: species-specific et species specifically] their foraging area [5]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [5]. They are able to navigate using memorized visual and olfactory cues [6, 7, 8]. Moreover, they can learn the exact time, the exact place and even both the exact time and place when and where food is available [9, 10]. They efficiently recruit nestmates where, when and as long as it is necessary [11]. Finally, they provide their area with cemeteries [12]. But, at their emergence, callow ants do not detain all these cognitive abilities [13]. However, it has been shown that just emerged ants already, though partly, know their congeners’ odor. They acquire this knowledge during their larval life and are at their emergence, quickly and forever, imprinted to nestmates’ visual appearance, at least that of the front part of their head [14]. Here, we try to find how larvae acquire the knowledge of their nestmates’ odor. It may be through habituation or by means of operant conditioning. The present work aimed to fill this gap by finding which of these behavioral processes are involved.

Then, having collected a newly founded colony in the wild, we observed the founder queens’ behavior, and we tested the first emerged callow ants of this colony in front of their specific alarm pheromone. We made similar tests with males obtained in an already settled experimental colony.

We so filled two other gaps of the study of the ants’ cognitive abilities ontogenesis. Finally we summarize the present and previous results, i.e. how young ants acquire their kin recognition capability, and the knowledge of their nest entrances [15], of their foraging area odor [16], their trail following behavior...
[17] and alarm reaction [18]. All this knowledge represents an important step in the understanding of the ontogenesis of ants’ cognitive abilities.

Material and Methods

Collection and maintenance of ants

The newly performed experiments were made on four large colonies of *Myrmica ruginodis* Nylander 1846, collected in summer 2013 in the Aise Valley (Ardenne, Belgium), nesting under stones or in wood at the borders of forest, under branches, where the sky was partly visible. The four colonies contained queens, abundant brood, and 500 – 1,000 workers. A complementary observation was performed on a newly founded colony of *Myrmica rubra* Linnaeus 1758, collected in September 2014, at Haine-Saint-Paul (Hainaut, Belgium), on a coal mining heap named ‘Chef Lieu’. Another observation was made on a colony of *Myrmica sabuleti* Meinert 1861, containing brood, queens, and about 1,000 workers, collected in summer 2013 in the Aise Valley, under a stone, on a field distant from the forest and covered with small plants, often odorous, and young trees.

Experiments previously made and reported in the references cited in the ‘Introduction’ have been performed on colonies of *M. sabuleti*, *M. rubra* and *M. ruginodis* collected in spring and autumn 2010, 2011 and 2012 in the Aise Valley, on three different fields. All the used colonies were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, with a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (34 cm x 23 cm x 4 cm), which internal sides were slightly covered with talc to prevent the ants from escaping. These trays served as foraging areas, food being delivered into them. The ants were fed with sugar-water provided *ad libitum* in a small glass tube plugged with cotton, and with pieces *Tenebrio molitor* larvae (Linnaeus 1758) provided twice a week on a glass slide. Temperature was maintained between 18°C and 22°C with a relative humidity of circa 80% all over the course of the study. Lighting had a constant intensity of 330 lux while caring for the ants, training and testing them. During other time periods, lighting was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 µW/m². All the members of a colony are here named nestmates, as commonly done by researchers on social hymenoptera. The ants one to three years old are named workers or foragers; those newly or recently emerged are named callows.

Experimental apparatus and methods

Caring for larvae and giving them the opportunity to acquire knowledge of thyme odor

In November 2013, the four experimental colonies of *M. ruginodis* contained some hundred larvae (Fig. 1A). They received each two days two cut larvae of *T. molitor*. From 20 November 2013 until 30 May 2014 (callows emerged on the 31th of May), pieces of thyme were added, twice a week, inside
the nest tubes for two of the nests (nests 1 and 2) and, during the same time period, on the foraging area, closely beside the pieces of meat food (T. molitor) for the two other nests (nests 3 and 4) (Fig. 1B, C). Let us recall that larvae essentially receive food containing proteins (meat food) while workers mostly take food containing carbohydrates. Each day, we checked if thyme was duly located, i.e. near the workers present in the nest tubes of nests 1 and 2, as well as besides or even on the meat food of nests 3 and 4. In this way, larvae of nests 1 and 2 lived, until their emergence, in an environment and among nestmates smelling thyme, while larvae of nests 3 and 4, lived, until their emergence, in an environment and among nestmates with only the nest and the members of the colony odor, but they were fed with meat food smelling thyme. Consequently, callows emerging from larvae of nests 1 and 2 might have been habituated to thyme odor, while callows emerging from larvae of nests 3 and 4 might have associated thyme odor to the food they received from the foragers and thus might have been conditioned to thyme odor.

**Testing callows emerged from differently cared larvae**

Callow ants emerged in each four colonies of *M. ruginodis* at the end of May 2014. They could be removed from the nest, cautiously, and without imperiling their live, at the beginning of June 2014. For conducting an experiment on a colony, 20 callow ants (removed in two successive batches of 10 ants so as to not maintain delicate callows during a too long time period out of their colony) were removed from their nest and set to wait in a polyacetate glass which border had been slightly covered with talc. The callow ants of each colony were then tested, each at a time, in a Y apparatus. After a callow was tested, it was temporarily transferred into another polyacetate cup, until the 10 ants of a first batch were tested and before a second batch of 10 callows was in turn removed from the colony in order to be tested. This prevented to test twice the same ant. The callows of the first batch were then placed back in their colony, and after having been tested, each at a time, those of the second batch were also brought back into their nest.

Each colony had its own Y-shaped apparatus and the four experiments were conducted in two days. The Y apparatus (Fig. 1 E, F, G) were made of strong white paper according to the description and figure given in [19] and set in a small tray (30 cm x 15 cm x 4 cm), aside from the experimental colony’s tray. The Y apparatus had their own bottom and their sides were slightly covered with talc to prevent the ants from escaping. In the Y-apparatus, the ants deposited no trail since they were not rewarded. However, they might have utilized other chemical secretions as traces. As a precaution, the floor of each Y-apparatus was changed between each test. One branch of each Y-apparatus was provided with pieces of thyme. Half of the tests were conducted with thyme in the left branch, and the other half in the right branch of the Y maze, and this was randomly done.

Each ant was transferred into the entry branch of the Y-maze, a few cm from the choice point, and observed until it turned either to the left or to the right in the Y-maze, which choice was recorded. Only the first choice of the ant was recor-
ded and this only when the ant was entirely in a branch of the Y maze, i.e. beyond a thin pencil drawn line indicating the entrance of the branch. The numbers of ants which turned towards the branch containing thyme, or went to the empty branch of the Y were recorded. The total number of tested ants being 20 for each colony, 20 + 20 callow ants of colonies into which thyme had been set, and 20 + 20 callow ants of colonies which have been fed with meat food smelling thyme, were tested (Tab. 1). The results obtained for nests 1 and 2, as well as for nests 3 and 4 were compared to those expected if ants randomly navigated the maze, using the non parametric goodness of fit χ² test [20, p. 45-51].

Assessment of callow ants’ and males’ orientation to a source of alarm pheromone

An isolated worker’s head, with widely open mandibles, is an ideal experimental source of alarm pheromone identical to that produced by an alarmed worker, as regards the dimensions of the emitting source (the mandibular glands opening) and the quantity of pheromone emitted. When in presence of an isolated nestmate worker head, surrounding foragers show the species typical alarm reaction (true orientation towards the source of emission and increase of linear speed. [21]). The behavior of callow ants just emerged in a newly founded M. rubra colony collected in the field and of that of males emerged in an experimental M. sabuleti colony, was observed for about 5 and 10 minutes respectively, in presence of such a head isolated from a worker of the same species (Fig. 1 D). Each time, 20 trajectories were recorded and analyzed as follows. Trajectories were manually recorded using a water-proof marker pen, on a glass slide horizontally placed 3 cm above the experimental tray area, where the tested individuals were moving. A metronome set at 1 second was used as a timer for assessing the total time of each trajectory. Each trajectory was recorded until the ant reached the stimulus or walked for about 6 cm. All the trajectories were then copied with a water-proof marker pen onto transparent polyvinyl sheets. These sheets could then be affixed to a PC monitor screen and remained in place due to their own static electricity charge. The trajectories were then analyzed using specifically designed software [22], each trajectory being entered in the software by clicking as many points as wanted with the mouse (for instance, 20 points in a trajectory length of 5 cm) and by entering then the location of the presented worker’s head. After that, the total time of the trajectory was entered, and the software allowed calculating the individuals’ orientation towards the presented stimulus, this variable being defined as follows. The orientation (O) of an animal towards a given point (here a small blank piece of paper used as a control or an ant’s head) is the sum of the angles, measured at each successive point of the recorded trajectory, made by each segment ‘point i of the trajectory - given point’ and each segment ‘point i - point i + 1’, divided by the number of measured angles. This variable was here measured in angular degrees. When such a variable (O) equals 0°, the observed animal perfectly orients itself towards the given (source) point; when it equals 180°, the animal fully avoids the source point; when O is lower than 90°, the animal has a tendency to orient itself towards
the source point and when it is larger than 90°, the animal has a tendency to avoid the source. Each distribution of 20 variables was characterized by its median and quartiles (since being not Gaussian) and the distributions could be compared to one another using the non-parametric $\chi^2$ test [20, p 111-116]. Two distributions were considered as statistically different when $P < 0.05$.

**Results**

**Larvae’s ‘learning’ (Tab. 1)**

The aim of this experiment was to find how callow ants could acquire the knowledge of odors, i.e. those, at least partly, of their nestmates. Callow ants emerging in nests into which pieces of thyme had been continuously placed obviously choose the branch of the Y apparatus containing pieces of thyme (Fig. 1 G). They scarcely hesitated when being tested, their choice being highly significant ($\chi^2 = 25.6$, df = 1, $P < 0.001$). On the contrary, callow ants emerged in nests into which no thyme had been placed, but in the foraging area of which thyme was continuously present beside the meat food, obviously preferred the way without thyme, and even seemed to avoid thyme odor (Fig. 1 F). They seldom hesitated and generally promptly went to the way free of thyme, this choice being highly significant ($\chi^2 = 19.6$, df = 1, $P < 0.001$). So, only ants emerged from larvae having lived in an environment provided with thyme odor knew that odor (i.e. have learned it) and even searched it, while those emerged from larvae having perceived thyme odor only when receiving food (serving as a reward) did not know that odor (i.e. have not memorized it) and even avoided it.

**Figure 1:** Some views of the experiments. A: An experimental colony, containing numerous larvae. B: Inside of a nest provided with pieces of thyme (arrows). C: Meat food, on the foraging area, beside pieces of thyme (arrows). D: The first emerged ants in a newly founded colony reacting to an isolated worker’s head (arrow) as a source of alarm pheromone. E: An ant, tested in a Y apparatus and walking from the entry branch, is on the way of making its choice between the left empty branch and the right branch containing thyme. F: The ant chose the empty branch. G: An ant chose the branch containing thyme. The arrows superposed on the Y apparatus indicate the entry branch and the choice.
Table 1. Response of 40 callow ants to thyme odor, this odor having been present during their larval life, either inside the nest among nestmates (Fig. 1B, nests 1, 2) or located on the foraging area, at close proximity of the meat food (Fig. 1C, nests 3, 4). The callow ants were tested, one by one, in Y mazes (Fig. 1E, F, G). Callow ants of nests 1 and 2 chose the thyme odor (they have been habituated to it), and those of nests 3 and 4 avoided it (they did not retain some association between thyme and the rewarding food, and so were not conditioned to that odor). Larvae ‘learn’ thus their nestmates’ (and nest) odor via habituation.

<table>
<thead>
<tr>
<th>Tested nests; location of thyme during the larval life</th>
<th>N° of callows choosing the branch free of thyme</th>
<th>N° of callows choosing the branch provided with thyme</th>
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<tr>
<td>nests 1 and 2; thyme inside the nest</td>
<td>4</td>
<td>36</td>
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<tr>
<td>nests 3 and 4; thyme on the meat food</td>
<td>34</td>
<td>6</td>
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Experiment on the first callow ants emerged in a newly founded colony.

Workers of about one year old efficiently learn at least two cognitive abilities – the trail following behavior and the alarm reaction – while being and after having been in presence of older workers presenting these abilities [17, 18]. This leads to the question: how the first workers emerging in a newly founded colony acquire these cognitive abilities? It might be presumed that founder queens transmit this knowledge to their first worker offspring after having acquired it during their previous living in their native colony, like the workers of a colony established since a long time.

If founder queens can acquire some specific cognitive abilities while living in their native colony, it is essential, for the survival of the newly founded colony, that they transmit this knowledge to their firstly emerged workers. A newly founded *M. rubra* colony containing four founder queens and about 60 firstly emerged workers was placed in an artificial nest deposited in a tray serving as foraging area. We firstly carefully observed the behavior of the founder queens and of the newly emerged workers.

One or two of the founder queens were often seen moving outside the nest, on the foraging area, going to food sites, staying there some times, then coming back inside the nest. While presenting these behaviors, the queens were accompanied by three to eight newly emerged workers. Such queens’ walking outside the nest could be observed during about one week. After that, queens nearly always stayed inside the nest tube while a few newly emerged workers from time to time went on moving on the foraging area.
After that, we tested the callow ants, on their foraging area, in presence of a blank paper, and then of a *M. rubra* worker’s isolated head. The callows did not orient towards a blank paper, they never came repeatedly towards it, and soon returned to their nest. Indeed, the median and quartiles of their orientation values towards the paper equaled 80.9 (64.4 – 96.5) angular degrees, what correspond to random movement. On the contrary, tested against an isolated *M. rubra* worker’s head, they obviously moved towards it and came back many times towards this stimulus (Fig. 1 D). The median and quartiles of their orientation values towards the alarm signal equaled 42.6 (37.5 – 60.1) angular degrees, what shows a quite good tendency to orient towards the source and was statistically lower than the values obtained using a blank paper \((\chi^2 = 16.03, \text{df} = 2, P < 0.001)\). Each tested callow reacted as described above during 3 – 5 min, then rather quickly re-entered its nest. It was thus evident that the firstly emerged workers of a newly founded colony knew the alarm pheromone and adequately responded to it, though yet only for a short time.

**Experiment on males**

A few males appeared in September in a *M. sabuleti* colony, and moved on the foraging area during two days before flying away or dying. On the second day, we could isolate 14 males in a small tray, and experiment on them for a short time. We had no time enough for observing them in front of a blank paper but we succeeded in testing them in front of an isolated worker’s head, a source of the specific alarm pheromone. The males had to be placed in a small tray, apart their colony, because when an isolated head is presented on a foraging area, it is immediately surrounded by numerous workers, what kept the males away from the source of pheromone. Twenty males’ trajectories were recorded and analyzed in order to assess their orientation towards the presented alarm signal. The obtained median and quartiles were 42.0 \((20.0 – 59.6)\) angular degrees, what revealed a true positive orthokinesis towards the source of alarm pheromone, statistically identical to the usual orientation of workers towards a congener’s head (i.e. 38.3 \((30.1 – 61.1)\), [18]). Males have thus acquired, in the course of two days the knowledge of the alarm pheromone and the response to it, possibly like young workers do ([18], see the discussion section).

**Conclusion and Discussion**

First, the present work fills a gap in our investigation on the ant cognitive abilities ontogenesis: that of the larvae ‘learning’. It was known that just emerged callow ants recognize, though yet imperfectly, their congeners’ odor [14]. Here, we have shown that callows know that odor via habituation occurring during their larval life, and not by operant conditioning. This is in agreement with the findings of other authors [e.g. 23] and leads to two questions or comments. 1) Why have newly emerged workers not yet fully developed kin recognition and what is the advantage of this incomplete knowledge? The likely answer is that the sensory organs of larvae are by far not as efficient as those of imagoes, the larvae being
eyeless and devoid of antennae and legs. Also, an incomplete kin recognition may allow the learning of it to be regularly updated in the course of a continuous changing kin odor. Kin odor effectively changes according to several factors, such as the age of the individuals, the food consumed, and the environment. 2) During metamorphosis, it is commonly known that most if not all organs are newly formed; it is thus surprising that emerged ants retain elements ‘learned’ when they were still at larval stage. However, in their study of the development and the evolution of the mushroom bodies in larvae, nymphs and adult workers of *Camponotus floridanus*, Gronenberg et al. [24] have shown that these cerebral structures largely subsist throughout the successive changes of the nymphs and enlarge during the imago’s life, due to the ants’ experience.

Secondly, to complete the knowledge of the ontogenesis of ant cognitive abilities, two other points were here examined. Several cognitive abilities are ‘learned’ with the help of older workers [17, 18], what leads to wonder how the first workers firstly emerged in a newly founded colony acquire the knowledge of these cognitive abilities. It could be presumed that future founder queens (winged females) ‘learn’ by means of the same ethological processes as workers several cognitive abilities in their native colony before swarming and founding a new colony (first step). Then, to ensure the survival of their foundation, the founder queens would have to transfer their acquired information to their firstly emerged workers (second step). The founder queens of a newly founded colony were here observed moving on the foraging area, towards food sites, accompanied by a few of the firstly emerged workers. Later on, queens no longer foraged. Moreover, these first emerged workers of a newly founded colony recognized their specific alarm pheromone and adequately responded to it. This suggests that the second step is realistic.

In the present work, males were proved to correctly respond to the specific alarm pheromone. However, it has not been examined how they acquire this knowledge. They may detain it innately or have learned it like young workers, by the way of imitation and learning. Anyway, the present experience shows that sexuals may acquire several cognitive abilities before swarming away from their native colony. Indeed, queens respond to their species alarm and trail pheromone [17, 18], having thus acquired these knowledge before founding their new colony. The first step suggested here above may thus occur.

So, larvae acquire the knowledge of their nestmates’ odor (and probably also of their nest) by way of habituation. It is the reason why, at their emergence, they recognize that odor, though yet not fully [14]. At their emergence, callow ants become rapidly imprinted with visual characteristics of their congeners [14]. In fact, callow ants need help from congeners for a successful emergence, and just after that, they need to be fed by trophallaxis for survival (same reference as above). So, in laboratory lit up nests, callow ants see nestmates, at least their front part, while emerging or just after, and become then imprinted with what they see.
Information on *Myrmica* species’ visual perception is given later on in this section.

In the course of former studies on the ontogenesis of ants’ cognitive abilities, the following facts were demonstrated. Young workers (i.e. a few months old) acquire the knowledge of the odor of their nest entrances by the way of imprinting when approaching for the first time the entrances. Later on, they learn the visual aspect of the entrances, via operant conditioning, when coming back into their nest after their first outside trips (at least 3 - 4 trips) [15]. Workers about one year old (when many older workers are present in the colony) acquire the knowledge of their specific area marking odor by imprinting (in a very short time period and forever) when coming on this area for the first time [16]. Later on, the ants acquire the specific trail following behavior [17] as well as the specific alarm reaction [18] in the presence of older ants, acquiring such reactions, partly by imitation and partly by true learning or Skinnerian conditioning [17, 18].

These facts can be completed by the following ones. Ants a few months aged cannot acquire conditioning [25]. When they are one to two years old, they do so perfectly and highly memorize and they can even acquire spatio-temporal knowledge [10]. During that time period, they memorize useful cues in order to navigate, collect food, go to the cemeteries, and recruit congenerants. They preferentially learn what requires less work, and need sugar food for learning [25]. When aged two to three years old, they scarcely learn, no longer memorize and are less incline to re-enter their nest (same reference as before).

As a conclusion, it can be stated that individuals successively go across time periods of habituation, imprinting, imitation, Skinnerian conditioning, complex conditioning, memorization, with some overlap between these periods. And finally, they progressively lose some of these capabilities. Ants appear thus to be as sophisticated as are highly evolved species, as for their cognitive abilities and their ‘learning’. They are highly evolved at a physiological and an ethological point of view, and live in highly organized colonies (see the introduction section). However, they might be considered as poorly evolved on the basis of their visual perception, but it has been shown that even ants with a poor vision, such as *Myrmica* species, detain a visual perception sufficient for performing complex tasks and recognizing plenty of cues. They distinguish the number of cue elements, the dimensions and orientation of cues as well as forms, colors and perspective, and adapt their vision to the light intensity. *Myrmica ruginodis* can even perceive and might be able to use celestial cues [26, 27, 28, 29, 30, 31].

We have not studied the ontogenesis of a last cognitive ability, but it has already been examined by Morel [32]. Young callow ants cannot perform trophallaxis [13, 25]. They might ‘learn’ the correct antennal movements while receiving food from older workers. This learning should be thoroughly analyzed using colored liquid food while observing trophallaxis under a stereomicroscope.

As a matter of fact, ants are excellent biological models for studying, among others, the ontogenesis of individuals’ capabilities.
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