

## RESEARCH

# Performance of the Species-Typical Alarm Response in Young Workers of the Ant *Myrmica sabuleti* (Hymenoptera: Formicidae) Is Induced by Interactions with Mature Workers

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**ABSTRACT.** Young workers of the ant *Myrmica sabuleti* (Hymenoptera: Formicidae) Meinert 1861 perceived nestmate alarm pheromone but did not display normal alarm behavior (orientation toward the source of emission, increased running speed). They changed their initial behavior when in the presence of older nestmates exhibiting normal alarm behavior. Four days later, the young ants exhibited an imperfect version of normal alarm behavior. This change of behavior did not occur in young ants, which were not exposed to older ants reacting to alarm pheromone. Queen ants perceived the alarm pheromone and, after a few seconds, moved toward its source. Thus, the ants' ability to sense the alarm pheromone and to identify it as an alarm signal is native, while the adult alarm reaction is acquired over time (= age based polyethism) by young ants. It is possible that the change in behavior observed in young ants could be initiated and/or enhanced (via experience-induced developmental plasticity, learning, and/or other mechanisms) by older ants exhibiting alarm behavior.

**Key Words:** Age polyethism, attraction, angular speed, linear speed, ontogenesis

In eusocial insects, each individual is able to perform many social tasks and can respond to many different sources of information. In ants, workers generally use multiple information sources such as nest odor (Cammaerts and Cammaerts 2000), one or several nest entrance and vicinity odors (Cammaerts and Cammaerts 1999), nestmate odors (Hamidi et al. 2004), a foraging area odor (Cammaerts-Tricot et al. 1977), trail pheromones (Evershed et al. 1981, Hölldobler and Wilson 1990), a recruiting odor (Cammaerts 1978, Hölldobler et al. 2001), visual and odorous cues to help in navigation (Philippides et al. 2011, Cammaerts and Rachidi 2009, Cammaerts et al. 2012b), acoustic signals (Roces and Hölldobler 1996, Casacci et al. 2013), and antennal contacts (Bonavita-Cougourdan and Morel 1984, Czaczkes et al. 2013). Ant workers perform numerous social tasks such as caring for the brood, foraging, collecting food, recruiting nestmates, performing trophallaxis, and nest defence. Young ants, <6 months old, have been observed to be unable to perform such social tasks and to correctly respond to their specific social signals (Cammaerts 2013a).

Previous studies have investigated the ontogenesis of ants' cognitive abilities. It has been shown that, in *Myrmica rubra* (L.) 1758, young workers (not yet fully pigmented, about 4 months old) learn the odor of their nest entrance via imprinting (Cammaerts 2013b), the visual characteristics of their nest entrance via operant conditioning (Cammaerts 2013b), become imprinted with their foraging area odor (Cammaerts 2014), and recognize their trail pheromone and begin to efficiently follow a trail when in the presence of older trail following nestmates (Cammaerts 2013c). Here, I report similar research on the acquisition of the response to alarm pheromone.

Alarm behavior is one of the most spectacular reactions of ants, and many alarm pheromones have been chemically identified (Passera and Aron 2005). Although long studied, alarm behavior and the chemical composition of alarm pheromones are still under investigation (e.g., Amoore et al. 1969, Mizunani et al. 2010). *Myrmica* ants possess an alarm pheromone produced by the workers' mandibular glands. It is essentially a mixture of 3-octanone and 3-octanol (Cammaerts et al. 1981) and it induces a typical alarm reaction consisting of a true

attraction, an increased walking speed (= positive orthokinesis) and a decreased path sinuosity (= negative klinokinesis) (Cammaerts-Tricot 1973). Using *Myrmica sabuleti* Meinert 1861 colonies maintained in the laboratory, I addressed the following questions. 1) Do young ants, <4 months old, recognize their specific alarm pheromone and respond to it in a manner similar to that of older workers (walking toward the emission source and rapidly circling it)? 2) If the response of young workers is different, what causes workers to eventually display a normal alarm response? Do worker ants simply display the normal alarm response as the mature, or is exposure to mature ants displaying normal alarm behavior necessary for young ants to begin displaying normal alarm behavior themselves?

## Materials and Methods

**Collection and Maintenance of Ants.** Three colonies of *M. sabuleti* were used. Two colonies were collected in March at Marchin (Condroz, Belgium) and one was collected in July at Olloy/Viroin (Ardenne, Belgium). Each colony contained one or two queens, brood, and about 500 workers. From March to May, several workers emerged in the two colonies collected from Marchin and were about 4 months old in June, when experiments 1–4 were undertaken. From July to September, callows emerged in the third colony and were about 4 months old at the end of October when a supplementary experiment (5) was performed. Callow ants are lighter in color than older ones and are expected to live for nearly 3 years (Cammaerts 1977). I also performed a sixth experiment on the queens of two previously used colonies and two other smaller ones, collected at Marchin (Condroz, Belgium).

The 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 (42 × 27 × 7 cm), the sides of which were covered with talc to prevent the ants from escaping. The trays served as foraging areas in which the ants were fed with sugar–water provided ad libitum in a small glass tube plugged with cotton, and with cut *Tenebrio molitor* larvae (Linnaeus 1758) provided twice a week on a glass slide. Temperature

was maintained between 18 and 22°C, and humidity at about 80%, with these conditions remaining constant over the course of the study. Lighting was at a constant intensity of 330 lux when caring for the ants (e.g., providing food, renewing nesting tubes) and during testing. During other time periods, the lighting was dimmed to 110 lux. The electromagnetic field had an intensity of 2–3  $\mu\text{W}/\text{m}^2$ .

**Obtaining, Recording, and Analyzing the Ants' Alarm Reaction.** In order to test differently aged workers and queens separately, without disturbing the colonies, 40 individuals per assay were transferred to an experimental tray (28 × 15 × 3.5 cm), the borders of which were covered with talc. A few minutes after the transfer, either a piece of blank white strong paper (1 cm<sup>2</sup>) or a piece of this paper supporting a worker's isolated head was deposited in the middle of the experimental tray. The species alarm pheromone is produced by the workers' mandibular gland (Cammaerts et al. 1981). 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, in terms of the dimensions of the emitting source (the mandibular glands opening) and the quantity of pheromone emitted. When presented with an isolated nest-mate worker head, surrounding ants present the species' typical alarm reaction (Cammaerts-Tricot 1973). The behavior of the individuals located at about 6 cm from the presented stimulus was then observed for 10 min (Fig. 1A and B) and 2 × 30 (30 for assessing the orientation of ants approaching the source of emission, and 30 for assessing the linear and angular speed of ants having approached the source of emission, see below) of their trajectories were recorded and analyzed as follows. Trajectories were recorded manually, using a water-proof marker pen, on a glass slide placed on the top of the experimental tray, set horizontally 3 cm above the area where the tested individuals were moving. A metronome set at 1 s was used as a timer for assessing the total time of each trajectory (not for entering the trajectories in the assessing system, see below). Each trajectory was recorded until the ant reached the stimulus or walked for about 6 cm. All the trajectories were then traced (copied) with a water-proof marker pen onto transparent polyvinyl sheets (Fig. 1C). These sheets could then be affixed to a PC monitor screen. The trajectories were then analyzed using specifically designed software (Cammaerts et al. 2012a and figures therein). Briefly, each trajectory was defined in the software by clicking as many points as needed with the mouse. Then, the total time of the trajectory (assessed using the metronome) was entered, and feature of the trajectory could be measured (orientation, linear speed, angular speed). I have often used this method for studying ants' locomotion (e.g., Cammaerts et al. 2013).

The three variables used to characterize the recorded trajectories were defined as follows:

The orientation ( $\circ$ ) of an animal toward a given point (here an empty piece of paper or an ant's head) is the sum of the angles, measured at each successive point of the registered trajectory, made by the segment "point  $i$  of the trajectory → given point" and the segment "point  $i$  → point  $i + 1$ " divided by the number of measured angles. This variable was measured in angular degrees. When such a variable ( $\circ$ ) equals 0°, the observed animal perfectly orients itself toward the reference point; when  $\circ$  equals 180°, the animal fully avoids the reference point; when  $\circ$  is lower than 90°, the animal has a tendency to orient itself toward the point; when  $\circ$  is larger than 90°, the animal has a tendency to avoid the point.

The linear speed ( $V$ ) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. It was measured in mm/s.

The angular speed ( $S$ ) (i.e., the sinuosity) of an animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by the segment "point  $i$  → point  $i - 1$ " and the segment "point  $i$  → point  $i + 1$ ", divided by the length of the trajectory. This variable was measured in angular degrees/cm.

In total, 30 trajectories were recorded and analyzed so as to quantify the orientation toward the presented stimulus of individuals approaching the stimulus, and 30 other trajectories were recorded and analyzed for assessing the linear and angular speed of individuals having approached the stimulus and then moving in its vicinity. Each distribution of 30 variables were characterized by their median and their quartiles (since they were not Gaussian) (Table 1) and were compared with one another using the nonparametric Chi-square test (Siegel and Castellan 1989, pp. 111–116) as well as the nonparametric Mann–Whitney test (Siegel and Castellan 1989, pp. 128–137). Two distributions were considered statistically different at  $P < 0.05$ . After having been tested, ants were carefully returned to their colony.

**Marking of the Ants.** During experiment 3, old and young ants were assayed together but their trajectories had to be separately recorded. Though old ants are darker than young ones, it was difficult to tell them apart when they were in motion. Thus, old ants were marked with a small spot of blue paint (enamel, Airfix) deposited on the first tergite of the gaster, using an entomology pin.



**Fig. 1.** Some images of the experiments. (A) Foragers reacting to a worker's isolated head: they are very near the head and generally oriented toward the head, they are walking toward the head. (B) Young ants in the presence of a worker's head: they are not very near the head and generally not oriented toward the head; they do not move toward the source of alarm pheromone but rather avoid it. (C) A few young ants' trajectories near a worker's head: the trajectories are not statistically oriented toward the head. (D) A queen in presence of a worker's head: she is oriented toward the head; she is moving toward that source of alarm pheromone.

**Remarks.** Due to the complexity of the experimental protocol, the specifics of each experiment are provided alongside their results (see “Results” section).

## Results

**Experiment 1. Foragers' Alarm Reaction (Table 1, Line 1).** Foragers (with dark pigmentation, at least 18 months old) did not walk toward or avoid a blank piece of paper: their median orientation value did not equal 30–50°, and was not more than 90°. In the vicinity of such a control stimulus, they went on moving as usually. The obtained numerical values of orientation, linear, and angular speed were similar to those usually obtained while making control experiments on *M. sabuleti* (e.g., Cammaerts et al. 1981, 2013). By contrast, they obviously perceived, from a distance of about 5–8 cm, the specific alarm pheromone released from the isolated worker heads during experimental treatments and immediately oriented themselves toward these sources (Fig. 1A): their median orientation value was 38.3° ( $\chi^2 = 13.46$ ,  $df = 2$ ,  $P = 0.001$ ;  $z = 3.80$ ,  $P < 0.001$ ). They returned many times to the source of the alarm pheromone, and circled it, displaying an increase in linear speed ( $\chi^2 = 10.56$ ,  $df = 2$ ,  $P < 0.01$ ;  $z = -2.92$ ,  $P \approx 0.003$ ) and a decrease in angular speed ( $\chi^2 = 18.03$ ,  $df = 2$ ,  $P < 0.001$ ;  $z = 3.23$ ,  $P \approx 0.001$ ). They thus presented the specific alarm reaction of *Mymica* ants (Cammaerts-Tricot 1973).

**Experiment 2. Response of Naïve Young Ants to Their Alarm Pheromone (Table 1, Line 2).** Young ants, which had not been experimentally exposed to alarm pheromone, moved more slowly than their older congeners ( $\chi^2 = 17.5$ ,  $df = 2$ ,  $P < 0.001$ ) and were slightly, nonsignificantly, affected by the presence of a blank paper (median  $O = 94.4^\circ$  vs.  $84.5^\circ$ ;  $\chi^2 = 2.36$ ;  $df = 2$ ;  $P \approx 0.30$ ; NS). They appeared to perceive, from a distance of about 3–6 cm, the specific alarm pheromone released from the isolated worker heads during experimental treatments but did not orient themselves toward such a source (Fig. 1B and C;  $\chi^2 = 1.63$ ,  $df = 2$ ,  $P > 0.30$ , NS; and  $z = -0.47$ , NS). In fact,

young ants' orientation toward an isolated head ( $103^\circ$ ) was higher than that of older ants ( $38.3^\circ$ ) but also higher than older ants' control one ( $84.5^\circ$ ). So, young ants not only did not orient themselves toward an isolated head but also somewhat avoided it. They also neither exhibited increased linear speed (8.1 vs. 8.0 mm/s; NS with the two kinds of test) nor decreased path sinuosity (126 vs.  $139^\circ/\text{cm}$ ; NS with the two kinds of test) as their older congeners did when presented with a source of alarm pheromone. They thus recognize their alarm pheromone, but present a different reaction than that exhibited by older ants.

**Experiment 3. Mixed Marked Old Foragers' and Unmarked Young Ants' Reaction to the Alarm Pheromone (Table 1, Line 3).** In the presence of young nestmates, older foragers continued to perceive a source of alarm pheromone when exposed to an isolated worker head and to react to it by a true attraction, an increase of linear speed, and a slight decrease of angular speed ( $\chi^2 = 22.13$ , 14.04, 11.06, respectively;  $df = 2$ ,  $P < 0.001$ ,  $< 0.001$ ,  $< 0.01$ , respectively;  $z = 4.45$ ,  $-3.78$ , 1.29, respectively;  $P < 0.001$ ,  $< 0.001$ , NS, respectively). Their reaction was even slightly, but not significantly, stronger than that exhibited in the absence of younger nestmates ( $O$ : 36 vs.  $38^\circ$ ;  $V$ : 13.8 vs. 13.0 mm/s; NS).

When young ants perceived a source of alarm pheromone in the presence of older nestmates, they no longer avoided such a source; however, they also did not orient themselves toward it. Indeed, their orientation averaged  $82.5^\circ$ , showing a slight decreasing trend compared with  $103.9^\circ$  ( $\chi^2 = 5.03$ ,  $df = 2$ ,  $0.05 < P < 0.10$ ; NS;  $z = 2.33$ ,  $P = 0.02$ ). However, the orientation values were nonetheless far from values associated with true attraction (30–50°). They moved more rapidly (10.4 vs. 8.1 mm/s;  $\chi^2 = 15.09$ ,  $df = 2$ ,  $P < 0.001$ ;  $z = -3.57$ ,  $P < 0.001$ ), showed hesitations, and displayed a large increase of path sinuosity (200 vs.  $126^\circ/\text{cm}$ ;  $\chi^2 = 20.58$ ,  $df = 2$ ,  $P < 0.001$ ;  $z = -5.06$ ,  $P = 0.0000$ ). Thus, the behavior of young naïve ants in the presence of older ants differed from that of young naïve ants in the absence of older ants, but was yet different from the behavior of older ants. In fact, they

**Table 1. Locomotion of foragers, young ants and queens of the ant *M. sabuleti* in front of a blank piece of paper or an isolated head (= a source of alarm pheromone)**

Workers Experiments ( $N = 30$ )	Orientation angular degrees	Linear speed mm/sec	Angular speed ang.deg./cm
Foragers			
Control	84.5 (54.2–115.8) —	11.4 (10.6–11.9) —	136 (114–157) —
Isolated head	38.3 (30.1–61.1) —	13.0 (12.0–13.4) —	104 (83–113) —
Young ants			
Control	94.4 (77.6–121.8) —	8.0 (7.7–9.7) —	139 (126–161) —
Isolated head	103.9 (88.6–114.3) —	8.1 (7.1–9.6) —	126 (103–144) —
Foragers + young ants			
Foragers	36.0 (27.2–48.8) —	13.8 (12.7–16.9) —	107 (98–124) —
Young ants	82.5 (47.8–101.6) —	10.4 (9.6–11.7) —	200 (190–235) —
Same young ants, later			
Control	95.1 (80.7–107.9) —	8.9 (7.4–9.9) —	173 (133–188) —
Isolated head	45.1 (35.9–51.5) —	10.3 (9.6–11.4) —	141 (118–165) —
Other young ants			
Control	96.6 (54.9–114.2) —	7.6 (6.8–8.8) —	133 (112–173) —
Isolated head, 1 <sup>st</sup> time	102.8 (86.9–122.3) —	7.7 (6.1–8.7) —	140 (119–164) —
Isolated head, 2 <sup>nd</sup> time	98.5 (76.9–131.3) —	8.3 (7.7–9.2) —	139 (111–159) —
Queens			
Control	100.0 (66.7–111.5) —	11.5 (9.8–13.2) —	117 (98–149) —
Isolated head	38.2 (28.7–59.4) —	13.1 (12.5–15.0) —	90 (73–100) —

The ants' locomotion was characterized by their orientation toward the presented stimulus, their linear and angular speed in the vicinity of the stimulus. The table gives the median and quartiles (in brackets) of the distributions of 30 values collected for each variable and experiment. Details of the method and statistical results are given in the text.

ceased avoiding the source of the alarm pheromone. They did not yet present the true attraction and the increase of linear speed commonly exhibited by older ants perceiving alarm pheromone. On the contrary, they appeared to perform pronounced klinokinesis (see “Discussion” section).

The young ants tested were carefully replaced inside their nest tubes at the end of the experiment.

**Experiment 4. “Alarm Response” of Young Ants Previously Tested With Responding Older Foragers (Table 1, Line 4).** Three and a half days after the experiment described above (note that workers remain young for 4–6 months), young ants were seen moving in the colonies’ foraging area, not only in the vicinity of the nest entrances but also further afield. These young ants were those previously tested in the presence of older ones because no emergence occurred in the intervening 3½ d. All of these young ants were removed and retested, as previously, in the experimental tray, in the absence of older congeners, first with a blank paper and then with an older ant’s isolated head. First, the young ants were no longer affected by the presence of a blank piece of paper: their control orientation (95.1°) was similar to that of older workers (84.5°) ( $\chi^2 = 2.2$ ,  $df = 2$ ,  $0.30 < P < 0.50$ ;  $z = -0.51$ , NS). Second, unexpectedly, they oriented themselves toward the presented isolated head (45.1 vs. 95.1°;  $\chi^2 = 17.16$ ,  $df = 2$ ,  $P < 0.001$ ;  $z = 4.02$ ,  $P < 0.001$ ), moved more quickly in its surroundings (10.3 vs. 8.9 mm/s;  $\chi^2 = 12.99$ ,  $df = 2$ ,  $P < 0.01$ ; and  $z = -3.14$ ,  $P \approx 0.001$ ) (though not as quickly as older ants—13.0 mm/s) and somewhat less sinuously (141 vs. 173°/cm;  $\chi^2 = 8.81$ ,  $df = 2$ ,  $P < 0.02$ ; and  $z = 1.62$ ,  $P = 0.10$ ) (though more sinuously than older ants—104°/cm). Thus, they exhibited alarm behavior qualitatively similar to that of older nestmates (but not yet fully quantitatively). These young ants thus seem to have acquired the specific alarm reaction while being in the presence of their older nestmates. However, this experiment did not rule out the possibility that young ants could acquire the species’ alarm reaction simply by being repeatedly exposed to the alarm signal, whether or not they were grouped with older reacting nestmates. The possibility that previous experience with older nestmates is necessary for the acquisition of a mature alarm response was tested by performing a supplementary experiment (5) on naive callows, which were about 4 months old, and belonged to a separate colony (see “Material and Methods”).

**Experiment 5. “Alarm Response” of Young Ants Having Never Met Older Responding Ants (Table 1, Line 5).** In total, 40 callows from the colony collected at Olloy/Viroin were transferred into the experimental tray and tested in the presence of a blank paper, and then a worker’s isolated head. They were then kept in the tray for three days, and provided with water and a red cover. They were retested in front of a worker’s head, all the experiments having been performed blind. First, even if issued from two different localities, young ants of all three colonies studied behaved similarly during initial testing. Second, when tested for the first time, young ants did not orient themselves toward the isolated head (102.8 vs. 96.6°;  $\chi^2 = 2.15$ ,  $df = 2$ ,  $0.30 < P < 0.50$ ; and  $z = -1.67$ , NS), did not move more rapidly (7.7 vs. 7.6 mm/s;  $\chi^2 = 3.27$ ,  $df = 2$ ,  $P \approx 0.20$ ; and  $z = 1.27$ , NS) nor obviously less sinuously (140 vs. 133°/cm;  $\chi^2 = 1.25$ ,  $df = 2$ ,  $P \approx 0.50$ ; and  $z = -2.92$ ,  $P < 0.01$ ) like old ants would have done in the same situation. When tested for the second time, the callow ants behaved like they did during the first experiment: they displayed no true attraction (98.5 vs. 96.6°;  $\chi^2 = 3.28$ ,  $df = 2$ ,  $P \approx 0.20$ ; and  $z = -1.32$ , NS), no increase in walking speed (8.3 vs. 7.6 mm/s;  $\chi^2 = 3.27$ ,  $df = 2$ ,  $P \approx 0.20$ ; and  $z = -0.93$ , NS), and no decrease in path sinuosity (139 vs. 133°/cm;  $\chi^2 = 2.44$ ,  $df = 2$ ,  $P \approx 0.30$ ; and  $z = -0.73$ , NS). They thus did not begin to react like older ants faced with an alarm signal in the absence of older nestmates.

**Experiment 6. Queens’ Response to Their Specific Alarm Pheromone (Table 1, Line 6).** After performing the experiments described above, I asked whether queens (founder queens, the mothers of all the workers and sexuals) could recognize the species’ alarm pheromone and react to it. I tested the queen of four colonies confronted to a

blank piece of paper, and then an old worker’s isolated head exactly as I tested foragers and young ants. All the four queens behaved similarly. For the first few seconds, the queens appeared not to perceive the alarm pheromone. After that, they generally stopped, moved their antennae, turned around, and apparently detected the presented source of alarm pheromone. They oriented themselves toward the presented isolated head (Fig. 1D, 38.2 vs. 100°;  $\chi^2 = 21.93$ ,  $df = 2$ ,  $P < 0.001$ ; and  $z = 3.91$ ,  $P < 0.001$ ), moved more quickly (13.1 vs. 11.5 mm/s;  $\chi^2 = 12.53$ ,  $df = 2$ ,  $P < 0.01$ ; and  $z = -3.26$ ,  $P = 0.001$ ), and moved less sinuously (90 vs. 117°/cm;  $\chi^2 = 9.62$ ,  $df = 2$ ,  $P < 0.01$ ; and  $z = 2.27$ ,  $P < 0.05$ ). They thus recognized the alarm pheromone and apparently reacted to it in a similar manner to old ants. However, as for the statistical significance of the results, it must be noted that the 30 values of orientation, linear speed, and angular speed were obtained by testing four queens (the queen of four different colonies), which reacted several times, and not by using 30 different queens. Thus, the statistical analysis suffered from nonindependence and is intended for illustrative purposes only.

## Discussion

Ants’ cognitive abilities are well documented, involving navigation, recruitment, communication, food exchange, and brood care (Passera and Aron 2005, Cammaerts and Cammaerts 2014). However, there is little information about the ontogenesis of such behaviors. Here, I examined the acquisition of a species’ cognitive ability. Using *M. sabuleti* as a model, I examined how young ants acquire the species’ specific reaction to alarm pheromone. I found that young ants recognize the pheromone (= innate behavior) but react to it in a different manner to older ants: they do not walk toward the alarm signal, and even slightly avoid it, and do not run as quickly as old ants. Their behavior seems adaptive to their young age: by avoiding alarm signals, they may avoid performing risky tasks such as defense or foraging. Such temporal polyethism has previously been reported by other authors, e.g., Hölldobler and Wilson (1990) on Green tree ants, Muscedere et al. (2009) in *Pheidole dentata*, and Vieira et al. (2010) in *Ectatomma vizzottoi*. I also found that young ants (about 4 months old) change their behavior and begin to respond to an alarm signal when in the presence of older responding ants. They may eventually acquire the specific alarm reaction (orientation toward the source of alarm, quickly moving) by themselves, but their acquisition may be more rapid if they encounter older reacting ants. In other words, as a potential explanation of our results, it is possible that alarm behavior acquisition may be initiated and/or enhanced by older responding ants. Such an acquisition via interactions with older reacting congeners may result from different mechanisms. Some form of imitation may occur (Cammaerts and Nemeghaire 2012, Cammaerts 2013d). Some kind of task allocation may exist: individuals are then more likely to perform given behaviors after having encountered congeners performing such behaviors (Gordon and Mehdiabadi 1999). Older ants may emit some primer pheromone initiating the development of alarm response (= experience-dependent developmental plasticity). Older ants may mediate the learning of the alarm response through classical conditioning. Note that, under natural conditions, young ants will almost always perceive the alarm signal for the first time while in presence of older reacting ants, except for the very first young workers emerging in founding colony: how these first workers acquire the species’ behaviors has yet to be investigated. Note also that the visual perception ability of *Myrmica* workers allows them to see congeners moving rapidly toward an alarm signal (Cammaerts and Cammaerts *Biologia* 2014). Whatever the precise mechanism, young ants must be old enough to begin behaving like older congeners. In nature, age-based polyethism and task allocation on one hand, and imitation and subsequent learning (see below) on the other hand, could be nonmutually exclusive mechanisms by which workers develop the ability to respond appropriately to alarm pheromone. During their initial alarm reaction acquisition, young ants seem not to be able to orient

themselves toward the alarm signal using efficient true taxis; they turn left and right, and do not walk directly toward the source of emission. They seem to resort using pronounced kinesis, as evidenced by a high sinuosity of movement. In other words, it is possible that, being yet unable to perform efficient taxis, they performed a pronounced klinokinesis, a simpler behavior which allows them to progressively approach the alarm signal (Fraenkel and Gunn 1961). It is also possible that, after their first "alarm" experience, young ants will continue improving their alarm reaction by some operant conditioning and/or true learning, through individual experience, in the course of exposure to further alarm situations. Indeed, middle-aged ants (12–24 months old) perfectly orient themselves toward an alarm signal, move very quickly, and are ready to behave in a risky manner (Cammaerts-Tricot 1973). The present work also revealed that founder queens recognize the alarm pheromone and move toward its source of emission. Inside the nest, queens seldom have the opportunity to do so. However, the alarm pheromone is a species-specific signal which indicates the presence of specific (perhaps even nestmate) workers. It is thus logical that queens, deprived of their workers, move toward an alarm signal, a specific odor probably emitted by nest-mate workers.

In conclusion, *M. sabuleti* workers' specific response to their alarm pheromone results from innate behavior (the pheromone is natively recognized), age-based polyethism (very young ants avoid the alarm signal source and so potential danger), eventual imitation (young ants imitate older reacting ants) and task allocation (young ants are inclined to behave like encountered older workers), experience-dependant developmental plasticity or classical conditioning mediated by older workers, and, presumably, from improvement acquired through individual experience during exposures to alarm situations.

Young individuals' acquisition of their species' behavioral patterns is well documented. Two aspects of such acquisition are age-based polyethism (already largely examined, Hölldobler and Wilson 1990 and references therein) and task allocation (also somewhat studied, for instance by Gordon and Mehdiabadi 1999). On the other hand, from the numerous studies concerning the ontogenesis of behavior in invertebrates (among others, cuttlefish, spiders, damselflies, ants, stingless bees, bees) and in vertebrates (fishes, birds, squirrels, and monkeys) (Caillere 1974, Jaisson 1975, Livecche 1979, Ferron 1980, Wehner and Ghering 1999, Pompignac-Poisson 2000, Tarnaud 2003, Poirier 2004, Delcourt 2008, Reichle et al. 2013), it can be concluded that an individual's behavior is acquired via genetically stored information, early social experience, and experience gained in the course of the individual's life.

In the present study, performed on ants and their alarm behavior, all these elements seem to exist (see above). However, these elements do not explain the ontogenesis of every behavior. Several cognitive abilities are, at least partly, acquired thanks to imprinting, habituation, and to classical conditioning. In ants, the situation can be summarized as follows. Soliciting of food, as well as trying to leave the nest and re-enter it, is apparently innate behavior (personal observation). Nest odor and brood recognition are acquired via imprinting and/or habituation (Jaisson 1975, Boss and d'Ettorre 2012). Odor of the nest entrance and foraging area odor are "learned" via imprinting (Cammaerts 2013b, 2014). The visual aspect of congeners is "learned" at emergence via imprinting (Cammaerts and Gosset in press). The trail following behavior and the alarm reaction are acquired via innate behavior, imitation and/or task allocation, and operant conditioning and/or true learning (Cammaerts 2013c, 2014). The use of olfactory and visual cues for navigation is acquired thanks to operant conditioning (Schatz et al. 1999, Cammaerts and Rachidi 2009, Cammaerts et al. 2012b, Schultheiss and Cheng 2011, Steck et al. 2011, Wolf et al. 2000, Wystrach et al. 2011).

Our understanding of the ontogenesis of ant behavior would be greatly improved by future studies focusing on the acquired abilities of naïve callow ants, removed from their colonies as pupae, and maintained either in a group or in isolation. Preliminary results suggested that such young ants are unable to collect food, recruit nestmates, and relocate their nest.

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