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ARE ANTS (HYMENOPTERA, FORMICIDAE) CAPABLE OF SELF RECOGNITION?

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ABSTRACT

In front of a mirror, and consequently of their reflection view, ants behaved otherwise than when in front of nestmates seen through a glass. Seeing nestmates through a glass, ants behaved as usual, i.e. without taking close notice of them. In front of a mirror, they rapidly moved their head and antennae, to the right and the left, touched the mirror, went away from it and stopped, cleaning then sometimes their legs and antennae. As long as they could not see themselves in a mirror, ants with a blue dot painted on their clypeus did not try to remove it. Set in front of a mirror, ants with such a blue dot on their clypeus tried to clean themselves, while ants with a brown painted dot – of the same color as that of their cuticle – on their clypeus and ants with a blue dot on their occiput did not clean themselves. Very young ants did not present such behavior. Contrary to the other kinds of marking, a blue dot on the clypeus induced aggressiveness in nestmates. The front part of the head is thus an essential species specific character for leading to acceptance. Although further experiments are required, preferentially on ants and social hymenoptera with an excellent visual perception, our observations suggest that some ants can recognize themselves when confronted with their reflection view, this potential ability not necessary implicating some self awareness.

Keywords: Aggressiveness, Ethology, Mirror experiments, *Myrmica*, Self awareness.

INTRODUCTION

Ants are among the most social of insects and even of animals. The members of their colonies are able to accomplish many complex tasks. They can perfectly distinguish members of their own colony from those of alien ones [1,2], and they display, among others, the following abilities (references given after their enumeration). They can distinguish the different odors of their nest, their nest entrances, the surroundings of their nest, and their foraging area. In general, they also use alarm, trail and recruiting pheromones. They can memorize visual and/or olfactory cues for navigating – this ability allowing them to forage far from the nest, to find their way back to the nest and re-enter it. They can learn the exact time, the exact place, as well as even the exact time and place at which a given food source is available. They can recruit congeners when, where, and for as long as it is necessary to do so. They can find a better nest site and relocate the entire colony there in a remarkably short time. They clean the inside of their nest and transport corpses to distant cemeteries. Ants' colonies also display a rigorous age polyethism, with workers caring for the brood, others guarding the entrances, and older individuals foraging. All of these ants' capabilities are described and analyzed in an abundant literature [among others: 3, 4 with references therein, 5, 6, 7, 8]. These cognitive abilities are progressively 'learned' by way of habituation, imprinting, and operant conditioning, by the larvae, the callows (newly emerged workers) and the young workers [9-13]. Finally, being eusocial insects, the social organization of ants is at least as sophisticated as that of some social birds, dolphins, and monkeys. For the three latter vertebrates, self recognition experiments have already been carried out [birds: 14; dolphins: 15, 16; monkeys: 17] with mixed and/or eventual positive results. We were curious to attempt similar experiments on ants, using the species *Myrmica sabuleti* Meinert (1861), *Myrmica rubra* Linnaeus (1758) and *Myrmica ruginodis* Nylander (1846) as models.

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Our research on ants, spanning a period of 45 years, has concentrated mainly on these three species. Among other experiments, we have examined these species' visual perception. Compared with other ant species, the visual perception of the above three species is of medium quality. *Myrmica sabuleti* workers have an all round vision, but whereas they can distinguish cues made of different numbers of elements, as well as differently oriented cues, they have difficulty in discriminating shapes. They discriminate colors from gray and different colors from one another [18, 19, 20]. *Myrmica rubra* foragers have a visual perception of higher quality than that of *M. sabuleti*: they can discriminate filled shapes from one another, but have difficulty in discriminating between hollow forms [21]. *Myrmica ruginodis* foragers have a better visual perception than *M. rubra*: they can distinguish filled shapes as well as hollow forms from one another. They can also see transparent cues on a black background and even discriminate different patterns composed of small luminous points on a black ceiling [22]. This ability should allow them to perceive the stars in the sky, and details of the canopy, both perceptual cues they could use in foraging. These visual perception abilities have been shown to correspond to the three species' eye morphology [23] and subtended angle of vision [24], as well as with their navigation system: *M. sabuleti* uses essentially odors, *M. rubra* similarly odors and visual cues, and *M. ruginodis* exclusively visual cues for navigating [25, 26, 8]. While the visual perception ability of the three ant species is not of the highest quality, it appears sufficiently refined to allow them distinguishing different visual characteristics of their nestmates [13]. Therefore, our attempt to examine the possibility of self recognition ability in ants was not entirely absurd.

As the ant species here used very well perceived the colors [19] and could, for instance, 'learn' that their nest entrances are marked in blue color [9], we used colored spots for experimenting on the subject.

The present experimentation brought unexpected results. They led to wonder if ants might be able to recognize themselves in mirrors, and if this ability might confer on them some degree of self awareness.

Experimental planning

For studying the ants' potential ability of self recognition, we performed the following observations and assessments.

• We set ants in front of a mirror, as well as in front of congeners seen through a glass, and compared their behavior and their locomotion in the two situations.

• We marked ants in blue on their clypeus and comparatively examined their behavior 1) when set alone in a loggia in which they could not see their reflection view, 2) when set alone in front of a mirror, 3) when set with congeners in front of a mirror.

• We examined in the same manner the behavior of callows (very young workers) marked in blue on their clypeus and set alone in front of a mirror.

• We observed ants either marked in brown on their clypeus, or marked in blue on their occiput, and set in front of a mirror.

• We observed and assessed the aggressiveness induced by the different markings.

MATERIAL AND METHODS

Collection and maintenance of ants

We opted to make the experiments on three *Myrmica* species for approaching as best as possible the difficult tackled subject. We used colonies of *M. sabuleti*, *M. rubra* and *M. ruginodis* collected in an old quarry of the Aise valley (Ardenne, Belgium), in July 2013. The colonies of *M. sabuleti* were located in a field invaded by small plants, most of them being odorous, those of *M. rubra* were nesting on a grass land, at about 100 meters from the forest, and the colonies of *M. ruginodis* inhabited the borders of the forest, under branches, where the sky was partly visible. The ants were often nesting under stones. Each collected colony contained one to four queens, brood (larvae, nymphs) and about 500 workers. The colonies were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (34 cm x 23 cm x 3 cm), the sides of which were covered with talc to prevent ants from escaping. These trays served as foraging areas, food being delivered in them. The ants were fed *ad libitum* with sugar-water provided in a small glass tube plugged with cotton, and with pieces of *Tenebrio molitor* larvae three times a week. Temperature was maintained at 20° ± 2° C, and relative humidity at about 80%, this remaining constant over the course of the experimentation. The lighting had a constant intensity of 330 lux when caring for the ants (e.g. providing food, renewing nesting tubes) and testing them; during the other time periods, the lighting was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 3 – 5 $\mu W/m^2$. 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'.

Setting observed ants

The ants were observed being set in trays either in front of a mirror, or separated from congeners by a glass, or set alone in a loggia, or being on their foraging area.

In front of a mirror

A mirror (13 cm x 8 cm) was tied vertically along a border of a tray (30 cm x 20 cm x 6 cm), the borders of which having been slightly covered with talc. A piece of very strong white paper (Steinbach ®) of appropriate dimensions was tied in front of the mirror to help the ants walking to the mirror and scotch tape was glued on each side of the mirror to prevent the ants from going behind the mirror. For experimenting, one or six ants were gently set on the strong white paper tied in front of the mirror and were then observed, each one, during six minutes. The mirror was carefully cleaned between each experiment and the white paper changed.

Separated from congeners by a glass

A glass sheet (15 cm x 15 cm) was tied vertically in the middle of a tray (30 cm x 15 cm x 5 cm), the borders of which having been slightly covered with talc, so that the tray area was divided into two similar parts into which twenty ants of a colony could be placed and observed during six minutes. The glass was perfectly cleaned between each experiment.

In a loggia

Ants were individually placed in a loggia (5.5 cm x 6 cm x 4 cm), made of strong white paper lightly covered with talc, so in places where they could not see their reflection view.

On their foraging area

For each species, 18 foragers were removed from their colony; six ones were only slightly touched with an entomological pin, six ones were marked in blue on their occiput, and six ones were marked in blue on their clypeus (the technical process is described here below). These three kinds of six manipulated ants were, one by one, set back on their foraging area, near nestmates the potential aggressiveness of which was then recorded during six minutes.

Marking the ants

For marking ants with a small spot of blue or brown paint (enamel, Airfix ®) deposited on the ant's clypeus or occiput, the ant was maintained in an adequate position, under a stereomicroscope, using appropriate pliers with one hand, and a very small spot of paint was made on its clypeus or occiput, using a very thin entomology pin held in the other hand. The ant was maintained during a few minutes in a polyacetate glass (base: diam. = 3.3 cm; top: diam. = 5 cm; height: 5.2 cm) before being tested.

Observation and assessment of ants' behavior

According to the experiment performed and with the aim of examining the ants' self recognition capability, the ants' unusual behavior, linear and angular speed, cleaning behavior and aggressiveness were observed and/or quantified.

Unusual behavior

Ants set in front of a mirror appeared often touching it, sometimes with their mouth parts. This unusual behavior was assessed while observing the ants for 6 minutes, by the mean number of times one ant touched the mirror, and by the number of ants, among ten ones, touching the mirror with their mouth parts (Table 2). These numbers were statistically compared to those obtained for ants seeing congeners through a glass, using the non parametric Mann-Whitney U test [27].

Linear and angular speed

For the ants set in front of a mirror, and for those seeing nestmates through a glass, 20 trajectories were manually recorded on a glass covering the tray. A metronome set at 1 second was used as a timer for assessing the total time of each trajectory (not for entering the trajectories in the assessing system, see below). All the trajectories recorded on the glass were copied with a water-proof marker pen onto transparent polyvinyl sheets which could be stuck onto a PC monitor screen and remained in place due to their own static electricity charge. They were then analyzed using software developed by Cammaerts et al. [28]. Briefly, each trajectory was entered in the software by clicking on it as many points as wanted (for instance 20 points in a trajectory length of 5 cm) with the mouse and by entering the total time of the trajectory (assessed using the metronome). The software was then asked to calculate two variables defined as follows:

The linear speed (V) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. It was here 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 each segment 'point i to point i – 1' and the following segment 'point i to point i + 1', divided by the length of the trajectory. This variable was here measured in angular degrees/cm.

Each distribution of 20 variables was characterized by its median and quartiles (since not Gaussian) (Table 1) and the distributions were compared to one another using the non-parametric χ^2 test [27]. Two distributions were considered statistically different at $P < 0.05$.

Cleaning behavior

According to the experiment performed, control or marked ants of each species were observed, one by one or six at the same time, during six minutes, and their cleaning behavior assessed by the numbers of times they tried to clean themselves (= their own head) or their reflection view (= the mirror, just at the place their head is visible) (Tables 2, 3).

Aggressiveness

For each species, six foragers taken from their colonies, were differently treated, and then set back, one by one, on their foraging area near nestmates (see above: 'on their foraging area'). The behavior of nestmates to these treated ants was observed for six minutes, and their aggressive levels were defined using the following different behavioral patterns. Level 0: none of the nestmates showed any aggressive reaction; level 1: nestmates inspected the treated ant with their antenna and/or opened their mandibles; level 2: nestmates gripped, pulled, or stung the treated ant. For each species and each kind of treated ants, the observations made on the six used ants were pooled, the sum assessing the level of aggressiveness induced by the ants' treatment (Table 4). The interactions between pairs of foragers, both not treated, were also recorded to obtain control data. The pooled aggression scores obtained with manipulated ants were statistically compared with the control data using the non parametric χ^2 test [27].

RESULTS

Ants' behavior in front of a mirror and in front of congeners seen through a glass

Description

In front of a mirror (Fig. 1A), a forager behaved unusually. It climbed on the mirror. It moved very slowly; it turned its head to the right and to the left, many times, and moved its antennae very quickly, otherwise than in front of a congener; it often touched its reflection view in the mirror, and sometimes the mirror with its mouth parts. All this generally lasted several minutes. The ant then moved away from the mirror and either came back towards it and behaved again as here described, or stopped at one to five cm from the mirror, staying motionless or cleaning their antennae and legs, what can be a displacement behavior. All these behavior were common for each tested ants, for each three species.

Ants set in front of nestmates seen through a glass (Fig. 1B) behaved totally differently. They did not try to climb on the glass, and moved as usual i.e. not slowly and not sinuously. They moved their head and antennae as they usually do. They only seldom touched the glass, and never with their mouth parts. Sometimes, they tried to go under the glass. When they went away from the glass, they did not stop and never or very seldom cleaned their legs and antennae.

Quantification

Given the observed behaviors, we assessed the ants' linear and angular speeds (n = 20) and recorded their contacts with the mirror or the glass (n = 10). The numerical results were similar for each of the three used species (Table 1).

The linear speed of ants moving in front and on a mirror was very lower than that of ants moving near a glass (*M. sabuleti*: $\chi^2 = 40$, df = 1, P < 0.001; *M. rubra*: $\chi^2 = 40$, df = 1, P < 0.001; *M. ruginodis*: $\chi^2 = 36.19$, df = 1, P < 0.001) while the ants' sinuosity was very larger when they walked in front and on a mirror than when they walked near a glass (*M. sabuleti*: $\chi^2 = 40$, df = 1, P < 0.001; *M. rubra*: $\chi^2 = 36.19$, df = 1, P < 0.001; *M. ruginodis*: $\chi^2 = 29.56$, df = 1, P < 0.001).

The mean number of times an ant touched the mirror with its antennae or forelegs was more than 20, 15 and 13 times larger than the mean number obtained when experimenting with a glass, for *M. sabuleti*, *M. rubra* and *M. ruginodis* respectively (the Mann-Whitney U test gave identical highly significant results for the three species: Z = 3.73, P = 0.00001). Finally, all the ants tested in front of a mirror touched, at least once, the mirror with their mouth parts, while none of the ants seeing nestmates through a glass touched the glass with their mouth parts. The latter result required no statistical analysis.

Table 1. Some behavioral traits of workers in front of their reflection view in a mirror and in front of congeners seen through a glass. For each species and each condition, 10 workers were tested, and, each time, 20 trajectories were recorded. The ants' walking speed (linear speed) and sinuosity (angular speed) were assessed using software, and statistically compared using the non parametric χ^2 test. The table gives the median [and the quartiles] of these two variables. The mean number of times the ants touched the mirror or the glass with their antennae or forelegs (n° of contacts with mirror) as well as the number of ants (among 10) which touched, at least once, the mirror or the glass with their mouth parts (ants making mouth contact) were established. The first counts were compared using the non parametric Mann-Whitney U test (P = level of probability); the second ones required no statistical analysis.

Species	variables	seeing themselves	seeing congeners	P
M. sabuleti	linear speed (mm/sec)	3.3 [2.7-3.7]	11.2 [10.0-12.7]	<0.001
	angular speed (ang.deg./cm)	404 [345-474]	159 [153-184]	<0.001
	n° of contacts with mirror	mean: 21.3	mean: 0.8	=0.00001
	ants making mouth contact	10/10	0/10	–
M. rubra	linear speed (mm/sec)	3.7 [3.3-4.4]	12.0 [11.0-12.8]	<0.001
	angular speed (ang.deg./cm)	332 [291-382]	154 [118-168]	<0.001
	n° of contacts with mirror	mean: 15.8	mean: 0.8	=0.00001
	ants making mouth contact	10/10	0/10	–

M. ruginodis	linear speed (mm/sec)	4.5 [4.1-5.5]	10.5 [9.7-12.6]	<0.001
	angular speed (ang.deg./cm)	279 [237-315]	138 [119-174]	<0.001
	n° of contacts with mirror	mean: 13.0	mean: 0.6	=0.00001
	ants making mouth contact	10/10	0/10	–

On the basis of these observations, it may only be said that, in front of their reflection view, ants might perceive something different than while seeing nestmates through a glass. Indeed, unusual behavior in front of a mirror is often presented by animals [29]. Consequently, a further experiment had to be performed. For this purpose, a small colored paint spot was put on ants so that the marked individuals could not see this marking and were not perturbed by it, and that the paint spot could be seen on the ants' reflection in a mirror. When such marked ants were, one by one, set in front of a mirror, three kinds of behavior were expected to occur: either, the ant may try to remove the painted dot from its body, making for instance obvious legs movement (this should be in favor of some self recognition by the ant), or the ant may never try to remove the dot from its body, making no appropriate movement (this should be in favor of an absence of self recognition), or the ant may touch the mirror and try to remove the reflection of the dot (this should signify that the ant saw a congener in the mirror).

Behavior of control ants, and of ants with a blue spot on their clypeus and set in front of a mirror

Description (Fig. 1 C, D)

Before the experimentation, six unmarked ants of each species were observed each one during six minutes, on their foraging area: they never cleaned their clypeus. Then, ants marked in blue on their clypeus were individually set in a small tray and observed: they never tempted to clean themselves. When such marked ants were set, one by one, in front of a mirror, they never tried to clean their reflection view in the mirror, but, after a few seconds, they rubbed their clypeus with a foreleg (Fig. 1 C), and/or touched it with an antenna. They behaved in this manner either while staying vertically on the mirror surface or, more often, after having withdrawn a little away from the mirror and staying horizontally on the bottom of the tray. After having moved in the latter horizontal position, the ants generally moved again towards the mirror, repeating their attempt in cleaning their clypeus, but never trying to clean their reflection view.

Table 2. Number of times workers cleaned their clypeus or their reflection view in a mirror, during 6 minutes. In brackets are the number of times cleaning occurred away from the mirror after the ants had seen themselves in it. The workers were either both unmarked and observed on their foraging area (= control 1), or marked with a blue dot on their clypeus and placed apart in a tray (= control 2), or marked with such a dot and individually placed in front of a mirror (test). Obviously, the ants never tried to clean their reflection view, but cleaned only themselves and this only after having been in front of a mirror.

Tested species	N° of times ants cleaned themselves			N° of times ants tried to clean their view in the mirror
M. sabuleti	control 1	n = 6	0	–
	control 2	n = 6	0	–
	test	n = 12	4 (1)	0
			5 (3)	0
			3 (2)	0
			3 (2)	0
			2 (0)	0
			4 (3)	0
			0 (0)	0
			3 (3)	0
			4 (4)	0
			1 (0)	0
			3 (3)	0
			3 (3)	0
M. rubra	control 1	n = 6	0	–
	control 2	n = 6	0	–
	test	n = 5	3 (2)	0
			1 (1)	0
			5 (2)	0
			6 (2)	0
			6 (4)	0

M. ruginodis	control 1	n = 6	0	–
	control 2	n = 6	0	–
	test	n = 7	9 (8)	0
			5 (3)	0
			4 (2)	0
			5 (3)	0
			6 (2)	0
			2 (1)	0
			4 (2)	0

Quantification (Table 2)

Unmarked workers, as well as ants marked on their clypeus but having not the possibility to see their reflection view, never cleaned their clypeus (= control observations). On the contrary, ants marked in blue on their clypeus, and set in front of a mirror, did so, according to the following quantified observations. Note that the final number of available correctly marked ants may differ between the three used species, and that each marked ant was observed for six minutes. Twelve *M. sabuleti* workers could be correctly marked, in blue, on their clypeus. In total, these ants cleaned themselves 35 times, doing so 24 times at a short distance from the mirror, and never tried to clean their reflection seen in the mirror. Five *M. rubra* workers were correctly marked. These ants cleaned themselves in total 21 times, doing so 11 times while being a little away from the mirror. They never tried to clean their view in the mirror. As for *M. ruginodis*, 7 workers could be correctly marked. They cleaned themselves 35 times in total, doing so 21 times at a short distance from the mirror, and never tried to clean their reflection in the mirror.

On the basis of these observations, it may be presumed that self recognition might exist in ants. A supplementary experiment, similar to the previous one, was made for further examining this presumption.

Behavior of ants with a blue spot on their clypeus, set in a control situation, then set in groups in front of a mirror

For each species, six ants were marked in blue on their clypeus, and then isolated, each one, in a strong white paper loggia. Each isolated marked ant was observed for six minutes and its possible cleaning behavior was recorded. The six marked ants were then set, all together, in front of a mirror and observed for six minutes. The cleaning movements of these ants were again recorded, and the behavior of encountering ants examined.

For each species, the isolated ants marked on their clypeus never cleaned, or tried to clean, themselves during the six experimental minutes. Once placed together in front of the mirror, each marked ant tried to clean its clypeus with its legs, at least one time, always only after having walked in front or on the mirror, and either while being still on the mirror or after having moved a few cm away from it. These results are not presented in a table since they simply consist in zero cleaning for ants isolated in strong white paper loggias and 18/18 cleanings for the same ants then set in groups in front of a mirror. Moreover, when 2 or 3 ants marked on their clypeus encountered, they touched each other with their antennae and gripped each other with their mandibles, thus presenting some aggressive behavior. No death occurred in the course of the six experimental minutes. No precise quantification was here performed because it could not be stated if marked ants became aggressive or if opponent ants were aggressive towards marked ones. It should also be recall that away from their nest area, ants are less aggressive than when staying on their own area [3]. Another experiment, with ants on their own foraging area, was later on conducted for examining this aggressive behavior (see below, *Nestmates' aggressiveness towards differently treated ants*).

The observations here presented corroborating the previous ones, ants' self recognition may thus now be more realistically presumed. Such a potential ability may not be detained by very young ants, i.e. by recently and/or newly emerged callow ants, since it is known that young ants cannot perform most of the older workers' tasks [6] but progressively learn their cognitive abilities in the course of their life [9, 10, 11, 12, 13]. So, experiments identical to those related above were made on newly, and on recently emerged ants of the three studied species.

Behavior of callows with a blue spot on their clypeus and set in front of a mirror

Each marked callow was observed for 6 minutes. Newly emerged ants, a few days old (a total of n = 8), did not clean themselves when set in front of a mirror, but ants a few weeks old (a total of n = 22), somewhat did so, though rather imperfectly and not during the first three minutes of their presence in front of the mirror (Table 3). The ants aged a few weeks presented the unusual behavior observed in much older workers (foragers): movement of the head, quick movement of the antennae, stop, and sometimes a probable displacement behavior, the cleaning of legs and antennae.

The assumption that foragers might be able of self recognition, in front of their reflection in a mirror, became thus more obvious in the course of our experimentation. However, another checking experiment was made, on foragers of the three species, which consisted in testing, in front of a mirror, ants marked on their clypeus with a brown spot, i.e. a dot of the same color as that of the cuticle.

Table 3. Total numbers of times a few days or a few weeks old callow ants, marked with a blue dot on their clypeus, and set in front of a mirror, cleaned themselves (in brackets, the numbers of times this occurred away from the mirror after the ants had seen themselves in the mirror) or their reflection in the mirror. The observations were made either during six minutes or separately during the first three and the last three minutes of that time period. No ant cleaned its reflection view; the ants only tried to clean themselves.

***Species* age and sample**		**Time periods**	**Total n° of times ants cleaned themselves**	**Total n° of times ants cleaned their reflection**
M. sabuleti				
a few weeks old	**10**	0 – 3 min	0	0
		4 – 6 min	27 (17)	0
M. rubra				
a few days old	**4**	0 – 6 min	0	0
a few weeks old	**6**	0 – 3 min	0	0
		4 – 6 min	16 (6)	0
M. ruginodis				
a few days old	**4**	0 – 6 min	0	0
a few weeks old	**6**	0 – 3 min	0	0
		4 – 6 min	8 (0)	0

Behavior of ants with a brown spot on their clypeus and set in front of a mirror

Ten foragers of each species were marked with a brown spot on their clypeus. They were, one by one, set in front of a mirror, and observed for six minutes (n = 10 for each species, t = 6 min). They presented the unusual behavior of foragers being confronted with their reflection in a mirror (movement of head, quick movement of antennae, moving away from the mirror, stop, legs and antennae cleaning) (Fig. 1E). They never cleaned themselves, neither when moving on the mirror, nor when being away from it. They only cleaned their antennae and their mouth parts, probably as a displacement behavior. Only one *M. rubra* worker tried to clean itself (i.e. it rubbed an anterior leg on the dot) after having been confronted with its reflection: this ant was darker than the other ones, with the dot appearing light brown on the dark cuticle. No table is provided for these results.

The assumption of self recognition by ants confronted with their reflection view became so more probable. A last checking experiment, related below, was made.

Behavior of ants with a blue spot on their occiput and set in front of a mirror

Six foragers of each species were marked in blue on their occiput and set, one by one, in front of a mirror. They were observed for six minutes (n = 6 for each species, t = 6 min). They behaved identically for each species. They moved in front and on the mirror in an unusual manner, exactly as did non marked ants in the same situation: they quickly moved their antennae, moved their head to the right and to the left, went away from the mirror, stopped, cleaned their antennae (a putative displacement behavior) and went back to the mirror (Fig. 1F). The most important observation was that the 18 tested ants behaved similarly as for one behavior: they never tried to clean their blue marking which was not visible on their reflection in the mirror.

Nestmates' aggressiveness towards differently treated ants

The control situation was that of not touched ants. Eighteen encounters between ants on their own foraging area were observed. During these meetings, zero aggressive behavior occurred. The observations related below were statistically compared to this control situation.

For each species, the six workers, which have been only touched with an entomological pin, but were not marked, moved quickly, erratically, when set back on their foraging area, and never aggressed congeners nor were aggressed by nestmates (18 non aggressive meetings and 0 aggressive one, Table 4, $\chi^2 = 0$, df = 1, NS). The six ants of each species marked in blue on their occiput moved quickly when set back in their foraging area. They induced, in their congeners, antennal contacts and mandibles openings, but never strong aggression. More precisely, 11 cases of aggressiveness level 0, 7 cases of aggressiveness level 1 and zero case of level 2 were observed (Table 4, $\chi^2 = 8.69$, df = 1, $0.001 < P < 0.01$). For each species, the six ants marked in blue on their clypeus were soon gripped, maintained motionless, pulled, and seldom induced some assays of stinging. In fact, no case of aggressiveness level 0, 7 cases of level 1 and 11 cases of level 2 were observed (Table 4, $\chi^2 = 36$, df = 1, $P < 0.001$). The aggressed marked ants may have emitted pheromones from their mandibular glands (they widely opened their mandibles, a behavior allowing them to emit their mandibular secretion, which reduces the workers' aggressiveness), and possibly also from their metapleural glands (this secretion also reduces the workers' aggressiveness [30]). Some of these marked ants could be progressively accepted, but five among the 18 marked ants on their clypeus were killed by their nestmates.

Table 4. Ants' aggressiveness towards differently treated congeners. Six workers of each species were used for each treatment. Three levels of aggressiveness were considered: 0 = no aggressive behavior; 1 = antennal contacts, mandible openings; 2 = gripping, pulling, stinging. Results were statistically compared to what occurred for totally untreated ants (= control) using the non parametric χ^2 test. **P** = level of probability; **NS**: non significance for P = 0.05.

Ants' treatment	Aggressiveness level	*M. sabuleti*	*M. rubra*	*M. ruginodis*
Totally untreated = control	0	6	6	6
	1	0	0	0
	2	0	0	0
Manipulated without marking **NS**	0	6	6	6
	1	0	0	0
	2	0	0	0
Marked in blue on the occiput **P < 0.01**	0	3	4	4
	1	3	2	2
	2	0	0	0
Marked in blue on the clypeus**P < 0.001**	0	0	0	0
	1	3	3	1
	2	3	3	5

A complementary observation

In the course of previous experiments made on ants [for instance 26, 25, 7, 8], it was observed that workers or queens removed from their colony for experimental purposes, then returned into it after a few minutes or hours, were inspected by congeners for a few seconds and then accepted as members of the colony. During the present work, the ants marked in blue on their clypeus, and set back in their colony once experimented, were surrounded by a few congeners, and either vigorously cleaned and/or strongly attacked (griped, pulled, stung), several of them having even been killed. On the contrary, acceptance occurred for the ants marked in brown on their clypeus, and for ants marked in blue on their occiput.

Note that ants marked on the first tergite of their gaster (a usual method for marking ants) are accepted by congeners [31, 32, 5, 10]. All these observations show that, with a blue spot on the upper middle part of their fore head, and only with such a marking, the ants no longer present the correct visual species specific configuration. Ants seeing such a dot on their head are expected to have the survival behavior of trying to clean themselves in order to remove the alien mark. Ants set in front of a mirror appeared to present that behavior. They might see the image in the mirror as themselves, and not see it as a nestmate.

DISCUSSION AND CONCLUSION

Ants perfectly recognize the members of their colony and are aggressive towards any alien individual [1, 2]. They did so using visual and olfactory cues. The latter cues, consisting essentially of individuals' cuticular lipids, are probably the most important [33, 34]. But the species' and nestmates' visual characteristics also matter for an efficient kin recognition [13]. From [13], it appears that callow ants know since their emergence the specific and colonial odors of their nestmates, the knowledge of these odors being thus acquired during the larval live. At their emergence they are imprinted with the visual characteristics of their nestmates. Let us recall that the three *Myrmica* species here considered have a visual perception of medium quality which should nevertheless allow them to distinguish at least the front part of their nestmates, and that they very well distinguish the colors, being for instance able to learn that their nest entrances have been marked in blue (references in the Introduction section). So, ants can visually recognize their nestmates – at least their front part – even if, as a matter of fact, odors are more important for the ants' kin recognition. The ontogenesis of ants' kin recognition [13] resembles, at least as for the visual specific characteristics, that of highly evolved vertebrates [e.g. 35]. In a few vertebrates, self recognition was investigated and estimated to be possible [birds: 36; elephants: 37; dolphins: 16, 15; monkeys: 38] but this presumption leads to discussion [39, p. 323]. In general, self recognition is associated with self awareness, and sometimes with consciousness (though this is not always the case). As a matter of fact, the entire subject is really difficult to apprehend [40, 41, 42, 43, 44, 45]. Here, we only wondered if self recognition (not awareness) ability should be possible in ants. Investigation on the subject has only poorly been undertaken in invertebrates, and even in social insects [see here below: 46, 47] though kin recognition in the latter is largely documented [48, 49, 50, 1, 51, 52, 53, 2, 54, 55]. On the other hand, ants detain numerous cognitive abilities; they can, for instance, learn to do specific tasks at specific times, memorize several visual and olfactory elements, use cues for navigating, chose the more adequate food, find a better nest site, …[4]. As for several of their physiological, ecological and reproductive aspects, ant colonies are as organized and complex as are societies of vertebrates [56]. Ants' brain is highly developed and may sophistically function in order to associate events, memorize elements, and solve problems [57, 58, 59, 60]. It was thus not unreasonable to wonder if ants might detain some self recognition capability and to tempt experimenting on that sensitive subject.

The present work provided the eight following results.

- In front of their reflection view in a mirror, ants behaved and moved unusually, not as in front of nestmates seen through a glass.
- Ants marked with a blue dot on their clypeus (this changed their species specific appearance), and set in front of a mirror, tried to clean themselves, to remove this alien spot from their head, but never tried to clean their reflection view.
- As long as they had not the possibility to see the marking on their clypeus (thus, when being not in front of a mirror), marked ants did not clean themselves. They did so when being or after having been in front of a mirror, even if being in a group. In the last situation, being away from their own area, nestmates displayed some but not strong aggressiveness towards other nestmates marked in blue on the clypeus.
- The very young ants (which cannot yet perform most of the social tasks) did not present such older ants' behavior.
- If foragers had a brown, thus not really visible, spot on their clypeus, they did not try to remove it.
- Ants marked in blue on their occiput (the ant bearing such a colored spot cannot see it when being in front of a mirror) never tried to clean themselves.
- Only ants marked in blue on their clypeus induced strong aggressiveness in nestmates staying on their foraging area. This means that such marked ants were no longer considered as members of the colony. In other words, the sight of a blue dot on the clypeus largely affected the recognition of the individual as a nestmate, and this lack of recognizing the individual's specific visual characteristics lead to non acceptance. The ants marked by paint dots not visible by nestmates, or not affecting the individual specific aspect of their fore head, were accepted.

The problem of self recognition in ants was here approached by several experiments, and different experimental protocols. The samples used were large enough for ethological studies (generally conducted on small samples), and non parametric statistics was used for analyzing the numerical results. Moreover, the entire work was performed on three *Myrmica* species, what allowed widening the observations.

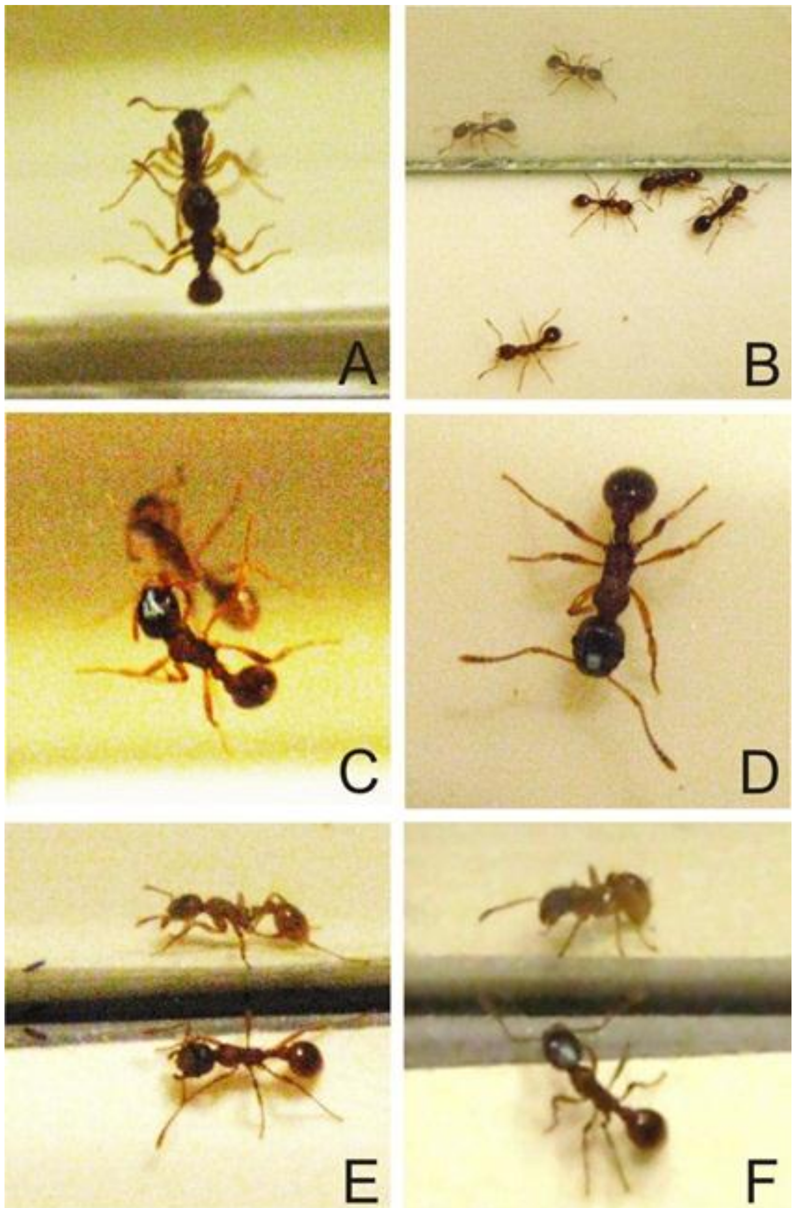
Very young ants are unable to perform most of the social tasks [6] and learn them progressively, though sometimes in a very short time (a few seconds, for instance), essentially at the beginning of their life [13, 9, 10, 11, 12]. It is thus not surprising that very young callows did not present signs of self recognition and that lesser young callows delayed in presenting such signs.

In fine, it can be deduced that foragers marked in blue on their clypeus clean themselves after they could see the dot on their reflection view in a mirror. Let us recall that the three ant species tested have a sufficiently efficient visual perception for seeing at least such a blue marking on the clypeus, and that, according to the aggressiveness induced by such a marking, the clypeus appeared to be a basic visual characteristic for the *Myrmica* ants. In other words, a colored marking on the clypeus largely affects the ants' recognition by nestmates, imperiling their acceptance and survival in their colony. It is logical that ants try to clean themselves if they see such a strange marking on their head, and do not try to remove such a marking if they cannot see it (i.e. if the marking is either a brown spot on the clypeus or a blue one on the occiput).

If ants see in a mirror a blue marking on their clypeus, they see themselves, and might thus recognize themselves. But this deduction is only a presumption. Indeed, an identical investigation has been performed by Tibbetts on wasps. After having analyzed the wasps' visual signals of individual identity and some of their complex social behavior [61, 62], Tibbetts and co-authors examined the question of individual recognition in these hymenoptera [46, 47]. According to these and some other researchers (references in the last cited paper), the experiments with mirrors cannot lead to a non ambiguous conclusion about self recognition capability. Briefly, if an animal detains self recognition ability, it will recognize itself in a mirror and will try to clean the alien colored spot it bears. The inverse is not always true: if an animal clean itself in front of a mirror, it might do so without recognizing itself. So, on the basis that ants conspicuously marked on their clypeus clean themselves while ants marked otherwise do not, both only after having been in front of a mirror, it can be presumed (but not yet asserted) that, for the *Myrmica* species presently tested, and for individuals of a given age, self recognition is not impossible. Of course, further experiments are wanted on ants having a better visual perception than the three *Myrmica* species here studied, which have only 109 – 169 ommatidia per eye, for instance, on *Cataglyphis cursor* or especially on *Gigantiops destructor*, the most visually impressive ant species, with about 3,000 ommatidia per eye. Experimentation should also be extended to bees.

Self recognition is not synonymous of self awareness, and the 'sense of self' may be more or less

Figure 1. Some views of the experiments. **A**: a *Myrmica sabuleti* worker climbing on a mirror and rapidly moving its antennae. **B**: *Myrmica sabuleti* workers seeing congeners through a glass and behaving as usual. **C**: a *M. ruginodis* worker with a blue spot on its clypeus, on a mirror and trying to remove the spot with an anterior leg. **D**: a *M. ruginodis* worker motionless at a few cm of distance from the mirror, after having been confronted with its reflection. **E**: a worker with a brown dot on its clypeus and set in front of a mirror: it did not clean itself. **F**: a worker marked in blue on its occiput and set in front of a mirror (the dot was not visible in the mirror): it did not clean itself.



sophisticated. Even if our results suggest a certain degree of self recognition in ants, they do not explain how ants take and use such information, how then functions the underlying cognitive processes, and if ants detain some self awareness. For many animals, such an assumption is not unanimous [39, 17]; for ants, we are conscious that it might even be less plausible. Here, we only showed that the assumption of some self recognition by ants, in front of their reflection, is not unrealistic.

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