

Expectative behavior can be acquired by ants in the course of their life

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ABSTRACT

Expectative behavior (acting as expecting the occurrence of an event) has seldom been studied in animals. Here we examined if workers of the ant species *Myrmica sabuleti* present this ability by studying their expectation for food location, and if young ants (about one year old) already present this expectative behavior. We found that, after two food shift training, old workers present expectative behavior as they moved onto the subsequent potential food location, irrespective of whether the food was shifted along a linear or a circular line. On the contrary, young ants searched for food essentially on the previously experienced food location, thus presenting no expectative behavior. It could be concluded that *M. sabuleti* has the ability of acting with expectation, and that such ability is not innate but acquired in the course of life.

KEYWORDS: anticipation, behavior, foraging, foresight, *Myrmica sabuleti*

INTRODUCTION

In nature, food location seldom remains unchanged. Generally, it may vary temporally (for instance, for flower nectar) or spatially (for instance, for potential prey), sometimes periodically (example: prey available at early night), or shifts farther or more aside in the course of the food consumption (such as fruits, seeds, grasses and crops). Animals

display foraging behavior as well as behavioral adaptation to the potential food localization. They can present temporal anticipation. For instance, bees, dogs and cats can react to the presence of food some time before its effective presence [1]. Animals can also acquire spatio-temporal learning, i.e. they can learn to arrive at the exact place where food will be available, generally at the optimal time. For example, using feeders, Laca [2] experimentally showed that steers (*Bos taurus* x *B. indicus*) avoid areas where they learned that no food is available and present long-term spatial memory for returning to previous food-loaded locations. Ksiksi and Laca [3] moreover demonstrated that steers remembered food locations for at least 48 days. Working with feeders in a semi-natural environment experiment, Winter and Stick [4] showed that nectar-feeding bats (*Glossophaga soricina*) learn to avoid depleted food locations and are able to memorize at least 40 behavior actions for efficiently finding food. More commonly, everyone can observe such ability in crows, cats and foxes, among others. Expectative behavior is more seldom pointed out, even though many evolved animal species might present it. Nevertheless, it was shown in monkeys, at a neuronal level, that expecting a reward enhances the learning performance [5] and that expecting an event can enhance receptive fields in the cerebral cortex, and consequently the animal performance [6]. A kind of expectation has been observed in bumblebees too. These insects check the quality of the flower nectar (they take some nectar from one flower) before foraging on a new patch of

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flowers and go on foraging only if the harvest of nectar is expected to be higher than a given quantity, below which their energy intake would not be optimal [7, 8].

As for ants, they possess the four ethological abilities required for presenting expectative behavior: knowing well the areas where food may be available, having rather long-lasting memory, being rather provident and presenting some anticipative behavior. These abilities have been observed in the course of our studies on ants as well as by many other researchers on these insects. Ants duly mark their foraging area [9, 10] and memorize visual as well as olfactory cues for navigation [11, 12, 13]. They have a rather long-lasting visual memory [14, 15, 16]. Some species stock up seed provisions; others have workers permanently devoted to stock honey in their gaster [17]. Ants can also react before the occurrence of an event [18].

Furthermore, we have recently demonstrated that the ant *Myrmica ruginodis* Nylander 1846 can present expectative behavior concerning food location when this food is shifted, step by step, farther or nearer to the nest, as well as more to the left or more to the right of the nest entrance. Indeed, the workers of this ant species search for food around the subsequent potential food location [19]. For doing so, the *M. ruginodis* workers may have used their excellent visual perception faculties. Indeed, they can even distinguish patterns of small luminous dots located above them on a black background [20]. During that experimental work, we did not focus on the age of the reacting ants, but we estimated that they were not very young. We may now wonder if an ant species with a poor vision can also present such an expectative behavior and if young ants that are not yet experienced could exhibit some expectation.

The aim of the present work was to complete our previous study on *M. ruginodis*. It thus deals with expectative behavior concerning food location in a species having a rather poor visual perception, *Myrmica sabuleti* Meinert 1861, and examines this potential behavioral ability in the youngest and the oldest workers of the colony separately. We are already familiar with the eye morphology of the *M. sabuleti* worker [21], its visual perception and subtended angle of vision [22, 23] and its

navigation system which primarily uses olfactory cues [11].

MATERIALS AND METHODS

Collection and maintenance of ants

The experiments were done on two colonies of *M. sabuleti*, one collected from an abandoned quarry located at Treignes (Ardenne, Belgium) and the other one from an abandoned coal-mining heap (Terril de Ferrand), located at Audregnies (Hainaut, Belgium). The colonies contained one or two queens, about 500 workers and brood. They were maintained in the laboratory in artificial nests made of two glass tubes (length: 16 cm, diameter: 1.5 cm) 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 4 cm), the internal sides of which were slightly covered with talc to prevent ants from escaping. These trays served as foraging areas as food was delivered in them. Before the experiment, the ants were fed with sugar-water provided *ad libitum* in a small glass tube plugged with cotton, and with pieces of *Tenebrio molitor* (Linnaeus 1758) larvae served twice a week on a glass slide. However, during the experiments, the ants were exclusively fed with an aqueous solution (75% of water) of sugared concentrated milk (Nestlé®) served in two small tubes (length = 2.5 cm, diameter = 0.8 cm) plugged with cotton. Temperature was maintained between 18 °C and 22 °C with a relative humidity of about 80% throughout the course of the study. The lighting had a constant intensity of 330 lux while caring for the ants and testing them. During other time periods, lighting was adjusted to 110 lux. The ambient electromagnetic field had an intensity of 2-3 $\mu\text{W}/\text{m}^2$. All the members of the colonies are herein named nestmates, as commonly done by researchers on social hymenoptera.

Experimental design

Two days before the experiment, each colony was divided into two, one made up of the youngest workers and the other made up of the oldest ones, with the brood and queens equally distributed as far as possible between these two 'sub' colonies (Figure 2a, b). The two differently aged workers were distinguished on the basis of their color (the youngest being paler than the oldest), their

position (the youngest being inside the nest or at the most near the entrance of the nests and the oldest being at the entrance or outside) and their activity (the youngest resting or taking care of the brood and the oldest moving, transporting corpses, foraging, or guarding the entrance). Each differently aged group of ants of each initial colony was set in a new glass tube (length = 11.5 cm, diameter = 1.5 cm) half filled with water with a cotton plug separating the ants from the water. Each of these four tubes was set in a new tray (identical to the previous ones), the borders of which were covered with talc. The food delivered after the first (control) counting consisted of sugared milk delivered in small glass tubes as explained above. This experimental design is schematized in figure 1 and can be partly seen in figure 2c-e. The nest tubes were located orthogonally against the middle of one of the small borders of the tray, providing a sufficiently large experimental area in front of the nest entrances. The tray of the ants as well as the nest tubes were never moved during the entire experimental work; all the surrounding visual cues stayed unchanged and the

nest entrances were always located at the same place. In the area in front of the nest tubes, the exact ten (2 x 5: see below) positions at which the food (sugared milk) will be successively given during the experiment were indicated by a small black circle (diameter = 2 cm) drawn lightly with a pencil. Five successive positions, labeled A to E were located along a linear segment; five other successive positions, labeled F to J were located along the half-circumference of a circle. The linear segment started from the nest entrance, had the same orientation as the nest tube, and the successive positions of the food were at 2, 6, 10, 14 and 18 cm from the entrance. As for the arc of circumference, the circumference had a diameter of 16 cm, its center was the nest entrance, and the successive positions of the food measured from the nest entrance were at angles of 30° and 60° (at the right of the entrance), 90° (in front of the entrance), 120° and 150° (at the left of the entrance). The aqueous solution of sugared milk was refreshed every two days, i.e. at the time of counting the ants and relocating the food (see explanation below in the 'Experimental protocol' section).

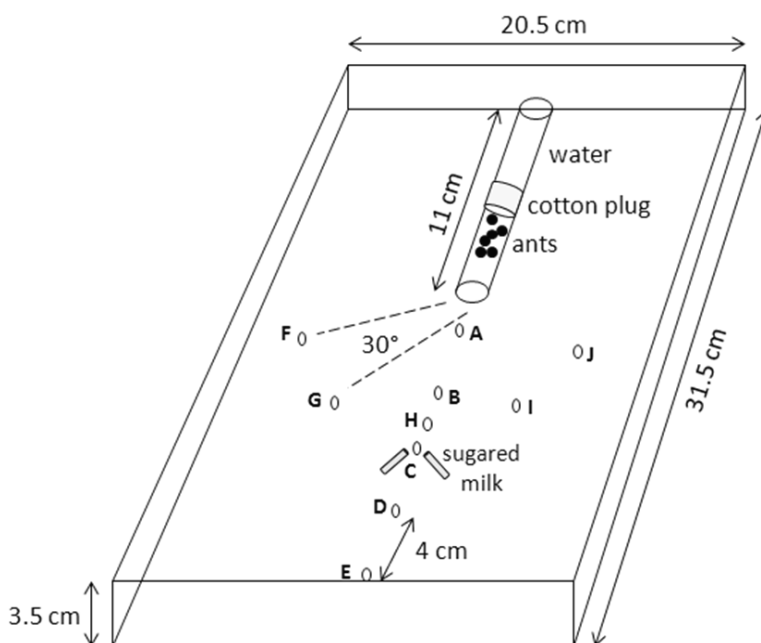


Figure 1. Experimental design. Food (sugared milk) was successively, step by step, located at places A, B, C, D, E, D, C, B, A and then at places F, G, H, I, J, I, H, G, F. After two training shifts, the oldest ants arrived on the future food location before food was delivered on it, while the youngest ants went on foraging in the vicinity of their nest entrance and/or around the food location where food had previously been delivered.

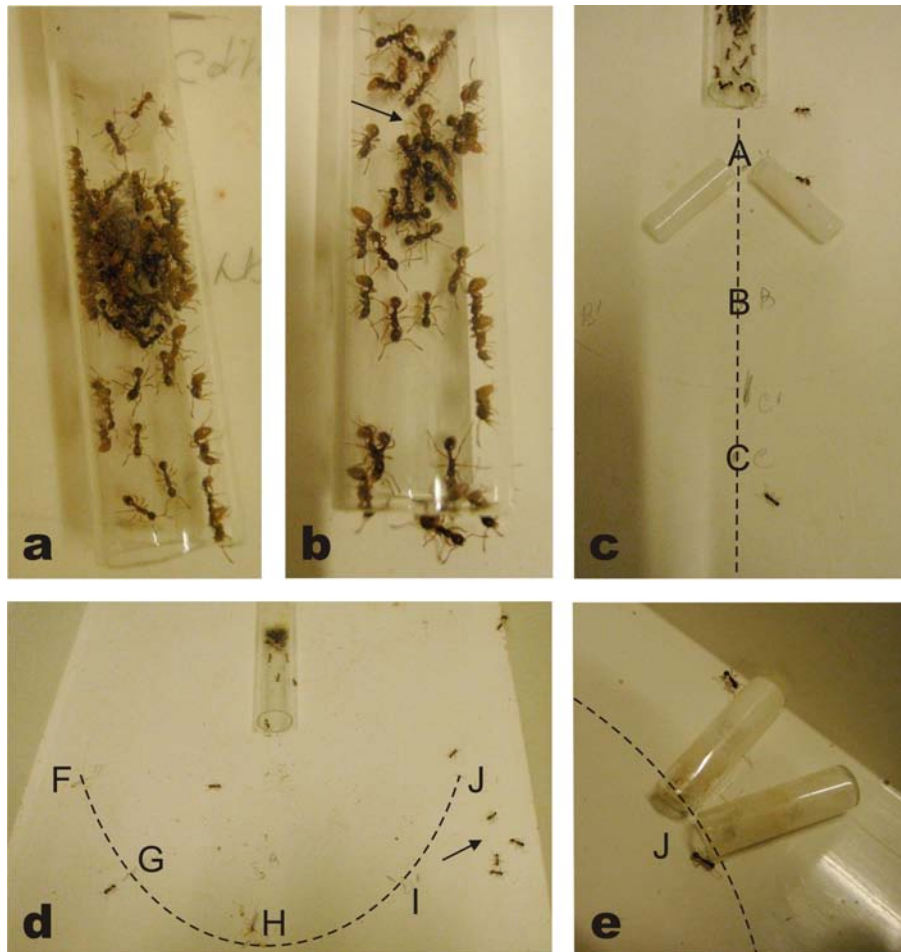


Figure 2. Some photographs of the experiments. **a:** subsample with young ants of colony 1. **b:** subsample with older ants of colony 1; the arrow indicates a queen. **c:** food delivered at location A. **d:** some of the oldest ants of colony 1 already foraging (arrow) in the direction of potential food site J, just after food was removed from location I and before it was set at location J. **e:** food just set at location J after having been removed from location I: the old ants quickly found the food; this event followed the one shown in picture **d**. The dashed lines, drawn on the photos, indicate the linear or circular shifting of the food from one location to another.

The ants could efficiently consume the sugared milk for about one and a half days. After that time period, the cotton plug began to dry and the ants were consequently ready to forage.

Experimental protocol

The protocol was identical for each fragment of the two colonies and each kind of successive relocations of food. It was performed on the same days, at the same times for each fragment of the two colonies. Attention was paid to make the successive handlings (explained below) at different times (at 13 hrs, 14½ hrs, 13½ hrs, 15 hrs, 14 hrs

and so on) to avoid temporal learning. Let us recall that the handlings were made at a time when ants began to be deprived of food and consequently began to forage.

On day 1, a preliminary counting was made (control): the ants present inside a circle ($R = 2$ cm) centered on each food site were counted. After that, food was given at place A. After two days (on day 3), food was removed. Three minutes later, ants of the four fragments of the colonies were counted at each food location 10 times during 10 minutes, and then food was relocated at place B. After two days (on day 5), food was removed; three minutes

later ants were counted exactly as before, and the food was relocated at place C. After two more days (on day 7), food was again removed, ants counted, and food relocated at place D. Again, after two days (on day 9), food was removed, ants counted and food relocated at place E. Then, after two more days (on day 11), food was removed, ants counted, and food was given again at place D. The same handling was performed on days 13, 15 and 17, with food being relocated respectively

at places C, B and A, and the ants being counted 10 times at each food site before such relocations. After three days, exactly the same handlings were performed (same days, same counting, same relocations) but the successive food locations were F, G, H, I, J, I, H, G and F. The scores, separately obtained for each fragment of each colony and relative to the linear and to the circular food displacements, are given in tables 1 and 2, respectively. For each kind of food displacement,

Table 1. Number of youngest and oldest ants of two colonies counted at five potential food places, A to E, linearly located, after having removed food from a location and before setting it at the subsequent one. Experimental details and statistical results are given in the text. In brief, the youngest ants essentially foraged near their nest tube and around the just experienced food location, while the oldest ants progressively foraged mostly around the potential food location expected in the future.

Day, handling	Young workers						Old workers					
	colony	food at: A	B	C	D	E	colony	food at: A	B	C	D	E
1, no food (control), counting, then food set at location A	1	4	0	4	0	0	1	6	2	2	1	1
	2	3	4	1	1	1	2	2	3	3	2	2
	Σ	7	4	5	1	1	Σ	8	5	5	3	3
3, food removed from A, counting, then food set at location B	1	10	2	0	0	0	1	19	7	0	0	0
	2	12	2	0	0	0	2	13	12	5	2	0
	Σ	(22)	4	0	0	0	Σ	(32)	19	5	2	0
5, food removed from B, counting, then food set at location C	1	4	10	0	0	0	1	4	11	12	1	0
	2	4	8	0	0	0	2	4	8	15	1	0
	Σ	8	(18)	0	0	0	Σ	8	(19)	27	2	0
7, food removed from C, counting, then food set at location D	1	10	9	1	0	0	1	0	1	3	8	2
	2	12	9	3	1	0	2	0	0	0	9	8
	Σ	(22)	18	4	1	0	Σ	0	1	3	(17)	10
9, food removed from D counting, then food set at location E	1	10	5	0	0	0	1	0	2	7	7	21
	2	0	1	1	6	1	2	2	1	3	4	13
	Σ	(10)	6	1	6	1	Σ	2	3	10	11	(34)
11, food removed from E, counting, then food set at location D	1	7	7	3	1	1	1	0	0	1	16	18
	2	18	7	2	0	0	2	0	0	0	14	22
	Σ	(25)	14	5	1	1	Σ	0	0	1	(30)	40
13, food removed from D, counting, then food set at location C	1	10	8	2	0	0	1	0	0	7	10	6
	2	6	8	0	0	0	2	0	0	3	7	5
	Σ	(16)	16	2	0	0	Σ	0	0	(10)	17	11
15, food removed from C, counting, then food set at location B	1	6	3	3	5	4	1	1	11	5	4	4
	2	13	2	0	0	0	2	4	14	2	2	0
	Σ	(19)	5	3	0	0	Σ	5	(25)	7	6	4
17, food removed from B, counting, then food set at location A	1	3	5	4	2	0	1	21	7	1	1	1
	2	7	9	2	1	0	2	15	4	1	0	0
	Σ	(10)	14	6	3	0	Σ	(36)	11	2	1	1

Table 2. Same legend as that of table 1 except that the five potential food locations, F to J, were located along an arc of circumference (see figure 1). In brief, the youngest ants essentially foraged around the just experienced food location, and the oldest ants progressively foraged mostly around the subsequent potential one.

Day, handling	Young workers					Old workers						
	colony	food at: F	G	H	I	J	colony	food at: F	G	H	I	J
1, no food (control), counting, then food set at location F	1	2	3	3	2	3	1	5	4	3	3	6
	2	2	4	6	2	1	2	3	5	6	3	1
	Σ	4	7	9	4	4	Σ	8	9	9	6	7
3, food removed from F, counting, then food set at location G	1	6	1	2	3	3	1	8	11	10	0	0
	2	19	3	4	1	0	2	9	9	9	1	0
	Σ	(25)	4	6	4	3	Σ	(17)	(20)	(19)	1	0
5, food removed from G, counting, then food set at location H	1	5	16	0	0	0	1	0	5	7	1	0
	2	7	9	1	0	0	2	0	2	11	0	0
	Σ	(12)	(25)	1	0	0	Σ	0	7	(18)	1	0
7, food removed from H, counting, then food set at location I	1	0	4	11	0	0	1	0	0	3	8	1
	2	0	2	9	0	0	2	0	1	3	20	1
	Σ	0	6	(20)	0	0	Σ	0	1	6	(28)	0
9, food removed from I, counting, then food set at location J	1	0	0	5	11	1	1	0	0	1	4	17
	2	0	0	6	10	0	2	0	0	0	5	11
	Σ	0	6	(11)	(21)	1	Σ	0	0	1	9	(28)
11, food removed from J, counting, then food set at location I	1	0	0	0	0	9	1	0	1	1	16	15
	2	0	0	0	3	14	2	0	0	0	12	12
	Σ	0	0	0	3	(23)	Σ	0	1	1	(28)	(27)
13, food removed from I, counting, then food set at location H	1	0	0	4	11	9	1	0	1	17	3	0
	2	0	0	0	15	3	2	0	1	22	4	0
	Σ	0	0	4	(26)	(12)	Σ	0	1	(39)	7	0
15, food removed from H, counting, then food set at location G	1	0	0	18	4	1	1	0	13	5	3	0
	2	0	5	11	1	0	2	0	10	3	0	0
	Σ	0	5	(29)	5	1	Σ	0	(23)	8	3	0
17, food removed from G, counting, then food set at location F	1	8	11	8	1	0	1	12	2	1	1	0
	2	2	11	4	1	0	2	15	8	2	0	0
	Σ	(10)	(22)	(12)	2	0	Σ	(27)	(10)	3	1	0

the sums of the scores obtained for the two colonies, for the youngest and for the oldest ants, were compared to one another using the non parametric χ^2 test [23] in order to examine if the foraging of ants (i.e. their distribution among the five potential food locations) varied in the course of the successive food relocations and differed between the youngest and the oldest ants. The sums of these scores obtained just before starting one or the other food displacement were compared to the numbers expected if ants randomly foraged among the five potential food locations, using the

non parametric goodness of fit χ^2 test [24]. The difference between two distributions was considered statistically significant when the P value obtained was lower than 0.05. When P was higher than 0.05, the difference was considered as non significant, what is indicated by 'NS'.

RESULTS

Linear food displacement (Table 1)

Before giving food to the ants, we observed that the youngest ants stayed nearer to the nest entrance than the oldest ones, and control counts

revealed that they foraged less (18 ants vs 24), but this difference in foraging behavior was not significant ($\chi^2 = 0.20$, $df = 1$, $0.50 < P < 0.70$). The two differently aged ants, but mainly the oldest, foraged statistically randomly over the five potential food locations (youngest ants: $\chi^2 = 7.53$, $df = 4$, $P > 0.10$, NS; oldest ants: $\chi^2 = 3.51$, $df = 4$, NS). Food was then delivered at location A. When food was retrieved from A (day 3), the youngest and the oldest ants essentially foraged around location A (youngest ants: $\chi^2 = 8.48$, $df = 1$, $P < 0.01$; oldest ants: $\chi^2 = 9.19$, $df = 1$, $P < 0.01$). However, the oldest ants foraged in the vicinity of location B also, the difference of foraging behavior between the youngest and the oldest ants being non significant: $\chi^2 = 3.50$, $df = 1$, NS.

When food was removed from location B, and before it was set at location C (day 5), the youngest ants essentially foraged around locations A and B, i.e. those they had previously experienced. Their foraging distribution statistically differed from their initial one ($\chi^2 = 14.82$, $df = 2$, $P < 0.001$) and the previous one ($\chi^2 = 15.44$, $df = 2$, $P < 0.001$). As for the oldest ants, they foraged somewhat around location B and essentially around location C, i.e. the future one. Their foraging distribution differed a little from their initial one ($\chi^2 = 10.97$, $df = 2$, $P < 0.01$) and largely from their previous one ($\chi^2 = 27.74$, $df = 2$, $P < 0.001$). The foraging difference between the youngest ants and the oldest ones was this time highly significant ($\chi^2 = 20.85$, $df = 1$, $P < 0.001$). When food was removed from C (day 7), and just before it was set at D, the youngest ants foraged around A and B, and moved a little around C. Their foraging distribution did not differ from their previous one ($\chi^2 = 3.02$, $df = 1$, NS). On the contrary, the oldest ants essentially foraged around location D, and their foraging distribution differed from their previous one ($\chi^2 = 41.29$, $df = 2$, $P < 0.001$). The difference in foraging between the two differently aged ants was evidently highly significant ($\chi^2 = 55.20$, $df = 2$, $P < 0.001$). A similar difference was observed when food was removed from D and just before it was set at E (day 9) ($\chi^2 = 34.86$, $df = 2$, $P < 0.001$). In fact, the youngest ants then foraged mostly near their nest entrance i.e. around A, and around the previous food location (i.e. D) while the oldest ants foraged in the vicinity of the previous and the future food locations (i.e. D and E). Their foraging

distribution consequently differed from their previous one, but for another reason (youngest: $\chi^2 = 12.95$, $df = 2$, $P \sim 0.001$; oldest: $\chi^2 = 34.86$, $df = 2$, $P < 0.001$).

Between the tests, while food was not shifted, the numbers of foraging ants varied between 0 and 2 for the youngest workers, and between 2 and 6 for the oldest ones. On day 11, when food was removed from E, and before it was set at D, the youngest ants foraged essentially around locations A and B, and their foraging distribution did not differ from their previous one ($\chi^2 = 3.17$, $df = 2$, NS). The oldest ants foraged essentially around E and, to a lesser extent, around D. Since previously they foraged essentially around E, and to a lesser extent around D and C, their present and previous foraging distributions statistically differed: $\chi^2 = 20.90$, $df = 2$, $P < 0.001$. The same events occurred on day 13 when food was retrieved from D, and then set at C. The youngest ants foraged essentially around A and B, and their foraging distribution did not differ from their previous one ($\chi^2 = 3.01$, $df = 2$, NS). The oldest ants foraged essentially around D, E and C, the future food locations, and their foraging distribution differed from their previous one: $\chi^2 = 23.00$, $df = 2$, $P < 0.001$. During the subsequent food shift to B (day 15), the youngest ants still foraged essentially near their nest entrance, and their foraging distribution was similar to their previous one: $\chi^2 = 3.33$, $df = 2$, NS. For the oldest ants, the events were more pronounced; they foraged essentially around B, and somewhat around A and C. Their foraging distribution statistically differed from their previous one: $\chi^2 = 38.45$, $df = 2$, $P < 0.001$. Finally, on day 17, food was retrieved from B, and then set at A. The youngest ants moved essentially around locations A and B, a little more around the latter one, i.e. the previous food location. Their foraging distribution slightly differed from their previous one ($\chi^2 = 10.41$, $df = 2$, $P < 0.01$) but this is not due to expectation as they foraged more in the vicinity of B and less around A, the future food location. All happened as if young ants foraged essentially around the food location they had just experienced (and not the future potential location), and this when the location was not far from the entrance. When the food location was far from the nest (for example, at site C and even more at sites D and E), the young ants seldom

went onto it. On the contrary, the oldest ants obviously and essentially foraged around food location A, i.e. the food location that could be expected on the basis of the previous ones. Their foraging distribution statistically differed highly from their previous one ($\chi^2 = 36.79$, $df = 2$, $P < 0.001$). All happened as if, after two food shifts, the oldest ants could guess the subsequent food location after food was retrieved from a given location. The circles drawn on table 1 illustrate the foraging response of the youngest and of the oldest ants to the successive food location shifts. The expectative behavior presented by the oldest ants was somewhat more rapid when food was set step by step farther from the nest, a fact commented in the 'Discussion' section.

The youngest ants might have presented no expectation during the above experiment because food was progressively relocated farther from the tube nest, and these ants did not forage far from their nest entrance. To verify the role of distance from the nest on expectation in the youngest ants, during the next experiment, food was always located at the same distance from the nest tube, but was circularly shifted to the left and then to the right of the nest entrance.

Circular food displacement (Table 2)

Before starting the experiment, while no food was delivered, both the young and the old ants foraged randomly among the five potential food sites located along the half-circumference of a circle (youngest: $\chi^2 = 3.76$, $df = 4$, NS; oldest: $\chi^2 = 0.87$, $df = 4$, NS). More old ants foraged than the young ones (39 vs 28), but their foraging distribution among the five potential food locations did not statistically differ ($\chi^2 = 1.03$, $df = 4$, NS). When food was removed from location F (day 3), and before it was set at location G, the youngest ants essentially foraged around F, the location they had just experienced. Their foraging distribution statistically differed from their previous one ($\chi^2 = 14.22$, $df = 2$, $P < 0.001$). The oldest ants foraged around location F, the location they had just experienced, and G and H, the future potential ones. Their foraging distribution statistically differed from their previous one ($\chi^2 = 18.64$, $df = 3$, $P < 0.001$). The foraging distributions of the youngest and the oldest ants statistically differed: $\chi^2 = 23.53$, $df = 2$, $P < 0.001$.

When food was removed from location G (day 5), and before it was set at location H, the young ants essentially foraged around location G, the one they had just experienced. Their foraging distribution differed from their previous one, when they foraged essentially around F ($\chi^2 = 29.73$, $df = 2$, $P < 0.001$). During the same food location shifting, the oldest ants essentially foraged around location H, and a little around G. Their foraging distribution slightly differed from their previous one which consisted of foraging essentially around G, but also around F and H ($\chi^2 = 13.51$, $df = 2$, $P = 0.001$). The foraging distributions of the youngest and the oldest ants largely differed: $\chi^2 = 37.59$, $df = 2$, $P < 0.001$. After having removed food from location H and before setting it at location I (day 7), the youngest ants essentially foraged around location H, the one previously experienced. Their foraging distribution statistically differed from their previous one since the most visited location was then G ($\chi^2 = 30.58$, $df = 2$, $P < 0.001$). During the same food shifting, the oldest ants foraged essentially around location I, i.e. the subsequent expected one. The foraging distribution of the oldest ants statistically differed from their previous one which was essentially around location H ($\chi^2 = 36.49$, $df = 2$, $P < 0.001$). The foraging distributions of the youngest and the oldest ants observed after having removed food from H differed statistically: $\chi^2 = 40.77$, $df = 2$, $P < 0.001$. On day 9, when food was removed from location I and before it was set at J, the youngest ants foraged essentially around I and somewhat around H, the locations they previously experienced. Their foraging distribution statistically differed from their previous one: $\chi^2 = 30.19$, $df = 2$, $P < 0.001$. Contrary to what occurred in the course of the linear food shifting, the foraging distribution of the youngest ants varied according to the successive food locations because food was now always located at the same distance from the nest and was shifted only towards the left. As for the oldest ants, they foraged essentially around location J, the future expected site (Figure 2d). Their foraging distribution statistically differed from their previous one: $\chi^2 = 36.82$, $df = 2$, $P < 0.001$. Of course, the foraging distributions of the youngest and the oldest ants statistically differed: $\chi^2 = 38.17$, $df = 2$, $P < 0.001$.

Food was then relocated, step by step, more to the right. During the first shifting (J to I, on day 11),

the youngest ants foraged essentially around J, the location where they just previously received food, while before that, they essentially foraged around I (see above). Consequently, their foraging distribution differed from their previous one ($\chi^2 = 46.69$, $df = 2$, $P < 0.001$). The oldest ants foraged around J and I, the previous and subsequent expected locations, and their foraging distribution differed thus only slightly from their previous one ($\chi^2 = 7.07$, $df = 2$, $0.02 < P < 0.05$). The foraging distributions of the youngest and the oldest ants were somewhat statistically different: $\chi^2 = 12.60$, $df = 2$, $0.001 < P < 0.01$. After food was removed from location I and before it was set at H (day 13), the youngest ants foraged essentially around I, where they just previously received food, and their foraging distribution differed thus from that of day 11 ($\chi^2 = 23.16$, $df = 1$, $P < 0.001$). The oldest ants foraged mostly around location H and their foraging distribution, this time, highly differed from their previous one ($\chi^2 = 59.69$, $df = 1$, $P < 0.001$). The foraging distributions of the youngest and the oldest ants were this time clearly different ($\chi^2 = 50.90$, $df = 1$, $P < 0.001$). The same events occurred when food was removed from location H and then set at G (day 15). The youngest ants foraged essentially around the just before experienced location H and their foraging distribution differed from their previous one ($\chi^2 = 47.43$, $df = 2$, $P < 0.001$). The oldest ants foraged essentially around the future expected location G and their foraging distribution differed from their previous one ($\chi^2 = 42.86$, $df = 2$, $P < 0.001$). The foraging distributions of the youngest and the oldest ants of course differed: $\chi^2 = 27.09$, $df = 2$, $P < 0.001$. During the last food shifting (day 17, $G \rightarrow F$), the youngest ants foraged essentially around location G, though ants of colony I also somewhat foraged around the adjacent locations F and H, this latter point being commented in the 'Discussion' section. The foraging distribution of the youngest ants differed from their previous one ($\chi^2 = 28.42$, $df = 2$, $P < 0.001$). The oldest ants, during that time, foraged essentially around location F and their foraging distribution differed from their previous one ($\chi^2 = 34.83$, $df = 2$, $P < 0.001$). The foraging distributions of the youngest and the oldest ants statistically differed on day 17 ($\chi^2 = 17.77$, $df = 2$, $P < 0.001$) though to a lesser extent than during the two previous food relocations on days 13 and 15.

DISCUSSION

Having the ability to expect the occurrence of an event, to act according to a future potential situation is certainly advantageous for any animal species. For doing so, animals must know their environment well, memorize experienced events, be rather prevalent and be able to anticipate (see the 'Introduction' section for details and examples). We have previously demonstrated that an ant species with a good visual perception such as *Myrmica ruginodis* presents some expectative behavior, more precisely that workers of this ant species can move onto a predictable future food location, on the basis of previous food locations [19]. In the present work, we examined if a species of the same genus, but with a poor visual perception, i.e. *M. sabuleti*, also has this ability, and if this ability is only presented by the oldest workers or is already exhibited by the young individuals, i.e. is acquired or not in the course of life.

We found that *M. sabuleti* workers effectively could move onto a predictable future potential food location after about (see below for details) two food shift training, the food shifts being either linear or circular, and that only the oldest workers could present this expectative behavior which is thus acquired in the course of life, thanks to maturity and probably through experiences.

We also observed that, when food was shifted step by step farther from the nest, the oldest ants presented expectative behavior just after two food shift training. When food was shifted nearer to the nest, the oldest ants presented moderate expectative behavior after two shift training, but presented an obvious expectative behavior after three food shift training. In other words, expectation was somewhat more quickly exhibited when food was relocated farther from the nest than nearer to it, which corresponds to a more usual situation in the wild.

Young ants foraged not far from the nest. Consequently, during the present linear food relocation, they seldom went onto the far locations C, D and E. During the circular food relocation, they also had the tendency to forage not far from the nest, but the five food locations being at the same distance from the nest, they successively went to the sites where they previously received food.

Expectation is not linked exclusively or essentially to the visual perception of the individuals. It depends

on several physiological traits such as memorizing experienced events, visual perception, olfactory perception, odometry, time lapse evaluation, navigation capability, as well as probably on some other physiological traits and ethological capabilities. For being able to satisfactorily expect a precise event (in the present studied case, the potential location of a future food delivery), about two such events had to be experienced. Moreover, for being able to present expectation after having experienced about two similar events, the individuals had to be old enough, i.e. to be more than one year old, having thus acquired enough maturity. The 'expectation' ability examined here was thus acquired rather late in the course of life, probably during the second-third of the worker's life, but this may depend on the colony demography and on the facts experienced by the individuals. This statement is corroborated by the following observation. During the last experimental food shift performed here (day 17, circular shift), some of the youngest ants of colony 1 (but not of colony 2) unexpectedly foraged around the future food location, i.e. location F. By doing so, they presented some expectative behavior. In fact, in the course of the 2 x 17 experimental days (= 34 days), these workers became somewhat older. As a *Myrmica* ant lives about 3 years while a human lives about 90 years, one day of such an ant's life corresponds to 30 days (= 1 month) of a human's life. During the present experimental work, the ants lived 34 days, which corresponds to 34 months (= about 3 years) for humans. The young ants belonged to a sub colony initially deprived of its old ants and due to social regulation they soon acquired abilities usually presented by old ants.

At their emergence, ants already, though imperfectly, know the odor of their nestmates and of the inside of their nest [25]. They have learned these odors already while being in the larval stage [26]. At their emergence, which is mechanically achieved with the help of nestmates, the callows are imprinted with the appearance of the frontal part of the head of these nestmates [25]. They then progressively learn the odor of their nest entrance, and when they go out of their nest for the first or the first two times, they learn the visual aspect of this entrance [27]. Once they reach their foraging area, they are imprinted to its odor, in a very

short time [28]. Later on, they learn the trail-following behavior [29] and the alarm reaction [30] in the presence of older nestmates. All this is summarized in [26]. The present work shows that, probably in the course of their second year of life (*Myrmica* workers live about for three years [31]), ants such as *M. sabuleti*, progressively, through experiences, acquire some expectative behavior. This behavioral ability should be acquired rather late in the course of life because (as stated in the 'Introduction' section and recalled at the beginning of the present 'Discussion' section) it requires four other capabilities that very young individuals not yet have. Indeed, young ants not yet have a precise and complete knowledge of their foraging area (in the present work, they foraged only in the surroundings of the nest entrance), are probably not yet efficiently prevalent (for instance, young ants scarcely collect food [17]), and cannot precisely anticipate the occurrence of an event [18]. They seem however to have a good memory; in the present work they went to the previously experienced food location. This good memory should allow young individuals to acquire several abilities, among others, that are required for being able to expect an event and act consequently.

Expectative behavior results from complex learning such as operant conditioning and spatial pattern learning [32].

Even though some ant species can expect future food locations, expectation might be rather limited concerning other potential social or environmental events (such as the death of queens, the emergence of males or females, changes in ecological parameters and so on). Anyway, the expectation capability of ants, even if limited, is really advantageous. In the present work, after the oldest ants could expect the future food location, they were on the food within a few seconds after its delivery (Figure 2e), this being more rapid than when food suddenly appears at an unpredictable place.

It should be of interest to look for expectation in other ant species (either more or less evolved than those of the genus *Myrmica*), as well as in other social insects such as bees and wasps, and also in vertebrates such as birds, rats, dolphins and monkeys, for instance. This should constitute a new trend in ethology, as well as in entomology.

CONCLUSION

Workers of the ant species *Myrmica sabuleti*, when about two years old, could acquire, in the course of successive food shifts, the ability to guess at which subsequent place food will be available. The young workers did not present this expectative behavior. This complex and advantageous ethological trait is thus acquired by the workers of this species, during the course of their life, through experiences.

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CONFLICT OF INTEREST STATEMENT

We affirm having no conflict of interest concerning the subject examined here.

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