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When Associating Numbers of Elements With Their Time Period of Occurrence, the Ants Take Account of the Characteristics of the Elements

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Abstract

After having shown that the workers of the ant *Myrmica sabuleti* can associate amounts of elements with their time periods of occurrence and knowing that these ants do not take into account the characteristics of elements when counting but take them into account when adding the elements, we wondered if, when associating amounts with their time periods of occurrence, these ants take or do not take account of the characteristics of the elements. Working on six colonies and using three kinds of visual cues during training and these three cues modified as for their size (small, large), color (blue, yellow) or shape (triangle, star) during testing, we revealed that, when associating amounts of elements with their time periods of occurrence, the ants take into account the characteristics of the elements. We checked if, without changing the elements characteristics, the ants effectively associated the perceived amounts (1 – 3, 2 – 4, 3 -5) of elements (squares, blue circles, triangles) with their time periods of occurrence (8 – 19 o'clock, 20 – 7 o'clock), and they did. We also made a complementary experiment on newly collected colonies using a slightly different protocol, and we obtained identical results which leaded to the same conclusion. So, the present work confirmed our previous results and solved the last asked question on the subject.

Keywords： episodic-like memory, *Myrmica sabuleti*, numerosity ability, time perception, visual perception

1. Introduction

The present work follows a series of studies on the ants' numerosity abilities, and more precisely, it is devoted to answer a question which arose at the end of one of these studies. More precisely, ants can count numbers of elements, and when doing so, they do not take into account the characteristics (size, color, shape) of the elements (Cammaerts & Cammaerts, 2020 a). They can also add numbers of elements (Cammaerts & Cammaerts, 2019 a), but for doing so, the elements must be identical as for all their characteristics (Cammaerts & Cammaerts, 2021 a), must be seen simultaneously (Cammaerts & Cammaerts, 2019 a, b), and consequently must be located at no more than a horizontal distance of 5 cm (Cammaerts & Cammaerts, 2021 b) and a vertical distance of 4 cm (Cammaerts & Cammaerts, 2021 c), as well as be seen with an interval gap not exceeding 7 ¾ minutes (Cammaerts & Cammaerts, 2022 c). The ants can also associate a number of elements with its time period of occurrence (Cammaerts & Cammaerts, 2022 b), but we do not know if, when doing so, they take or do not take into account the characteristics of the elements, i.e., their size, color and shape. In the present work, we tried to answer this question. Before relating our work, we report some information about similar skills in other animal species.

Counting amounts of elements is a skill detained by many animal species, such as fishes (Agrillo, Dadda, Serena & Bisazza, 2009), amphibians (Rose, 2017), robins (Garland & Low, 2014), corves (Bogale, Kanata, Mioko & Sugita, 2011), parrots (Pepperberg, 2006), newborn chicks (Rugani, McCrink, deHevia, Vallortigara & Rogolin, 2016), rodents (Reznikova, Panteleeva & Vorobyeva, 2019), and monkeys (Hauser, Carey & Hauser, 2000) as well as bees (Howard, Avargues-Weber, Garcia, Greentree & Dyer, 2019). The more evolved ability consisting in adding numbers of elements has been experimentally proved to be or presumed to be detained by, among others, spiders (Rodriguez, Briceno, Briceno-Aguilar & Höbel, 2015), bees (same reference as above), amphibians (Hoag, 2003), birds (Peperberg, 2006; Rugani, Fontanari, Simoni, Regolin & Vallortigara, 2009; Garland & Low, 2014), rats (Church & Meck, 1984), and monkeys (Beran, 2008; Flombaum, Junge & Hauser, 2005; Woodruff & Premack, 1981; Beran, Perdue & Evans, 2015).

Humans, who have a precise notion of the passing time, commonly associate experienced events with the time period during which they experienced these events, and this obvious skill allows humans optimizing their different activities. Such an ability has not been largely shown in animals. Nevertheless, monkeys were proved, at a physiological level, to memorize the contingency of visual cues associated with a reward and a given time context (Eradath et al, 2015). In fact, associating learning (conditioning) concerns not only the conditional and the unconditional stimuli but also other environmental parameters prevailing at the training time, such as some events, the localization, the time period (Mollet and Miller, 2014). This concern is labeled 'Temporal Coding Hypothesis' (Chandram & Thorwart, 2021). The latter cited parameter (the time) appears to be the most important one (Enquist et al, 2016; Cooper, 1991; Savastan & Miller, 1998; Balsam & Gallistel, 2009). This accounted for the ants' association between cues and the time at which they perceived them.

Coming back to the present work, we previously demonstrated that the workers of the ant *M. sabuleti* can associate visual cues, odors, and quantities with the time period during which these elements were perceived (Cammaerts & Cammaerts, 2022 a, submitted, 2022 b). At the end of the latter cited work, we reported that we trained and tested the ants using elements identical as for all their characteristics, and that it is still required to define if, while making such a temporal association, the ants take or do not take into account the characteristics of the elements. Resolving this problem is the aim of the present work: the elements used to train and to test the ants differed by their size, color, shape, or filling. We also checked if, in presence of identical elements, the ants associated these elements with their time period of occurrence.

2. Materials and Methods

They were very similar to those used in a previous work on the subject (Cammaerts & Cammaerts, 2022) since we aimed to complete this previous work by answering a not yet investigated question, i.e., when associating numbers of elements with their time of occurrence, do the ants take or not take into account the characteristics of these elements? Self-plagiarism could thus not be avoided.

2.1 Collection and Maintenance of Ants

The experiments were performed on six colonies of *M. sabuleti* (labelled A, B, C, D, E and F) collected in May 2021 in the Aise valley (Ardenne, Belgium), as well as on two similar colonies collected in September 2022 at the same site. They ants were living under stones and in grass, in an abandoned quarry. The colonies contained *ca* 550 workers, 1 to 2 queens, eggs, larvae and nymphs. In the laboratory, each colony was maintained in one to three glass tubes half-filled with water, a cotton plug separating the water from the part devoted to the ants. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm), the borders of which being covered with talc to prevent ants from escaping. The bottom of these trays was the ants' foraging areas in which food was delivered. This food consisted of pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) delivered three times per week, and of a cotton-plugged tube filled with sugared water (~ 15% of sugar) permanently set. The lighting varied between 330 and 110 lux, the ambient temperature constantly equaled 20-21 °C, the humidity 80%, and the electromagnetic field 2 µWm2. These conditions are suitable for *M. sabuleti*.

2.2 Experimental Planning

Three experiments were performed, each one on two colonies respectively labeled A and B, C and D, E and F. Each time, the ants of these two colonies were trained during three days to an amount of an element from 8 o'clock until 19 o'clock and to another amount of the same element from 20 o'clock until 7 o'clock. More precisely, ants of colonies A and B were trained to 1 small black square since 8 o'clock until 19 o'clock and to 3 small black squares since 20 o'clock until 7 o'clock; ants of colonies C and D were trained to 2 blue circles since 8 o'clock until 19 o'clock and to 4 blue circles since 20 o'clock until 7 o'clock; and ants of colonies E and F were trained to 3 black triangles since 8 o'clock until 19 o'clock and to 5 black triangles since 20 o'clock until 7 o'clock. During their training, the ants of the six colonies (3 groups of 2 colonies) were daily tested at 16 and 4 o'clock (thus 6 times in total) face to the two same amounts of cues presented for training them, but these cues differed by one of their characteristics. Precisely, ants of colonies A and B were tested in front of 1 and 3 large squares, the ants of colonies B and C in front of 2 and 4 yellow circles, and ants of colonies E and F in front of 3 and 5 stars. In addition, at the day following each of the three performed experiments, a check test was conducted using this time cues identical (but new, never used) to those used for training the ants. The experimental design, as well as the protocols used to train and to test the ants are detailed in the following paragraph; the construction and the dimensions of the different cues are defined in the still following paragraph. Figures 1 and 2 allow understanding the planning and the protocol, and visualizing the design; photos are shown in Figure 3.

Thereafter, a complementary experiment was conducted on two newly collected colonies labelled G and H using a slightly different protocol. This protocol, the used cues and some photos are presented in Figure 4. The ants were trained for three days to 2 empty circles since 20 o'clock to 7 o'clock, and to 4 such circles since 8 o'clock to 19 o'clock. They were tested at 3.45 o'clock as well as at 15.45 o'clock in front of 2 and 4 empty circles, then, five minutes later, in front of 2 and 4 black circles, this during three consecutive days (total number of tests = 6 on two colonies). The design, protocol and used cues are detailed in the two following subsections.

2.3 Design and Experimental Protocol

The design is schematically represented in Figure 1; the protocol of the first three experiments is summarized in Figure 1, the protocol of the complementary experiment is summarized in Figure 4.

During three days, the ants underwent, in their tray, visual operant conditioning to every adequate visual cue during the temporal laps of time devoted to their presentation, by setting the cue near the nest entrance which served as a reward. For colonies A, C, E, and G the cue was deposited on the left of the nest entrance, and for colonies B, D, F, and H the cue was deposited on the right of this entrance.

During these three training days, testing the ants was performed, at *ca* 4 and *ca* 16 o'clock, in a separate tray (21 cm x 15 cm x 7 cm) the borders of which having been slightly covered with talc, and into which two cues had been deposited. These two cues were similar to those used during training as for their amounts of elements. During Experiments I, II, and III, the elements differed by one of their characteristics. For colonies A and B, the size of the elements differed (small cues during training, large ones during testing); for colonies C and D, the color of the elements differed (blue during training, yellow during testing); for colonies E and F, the shape differed (triangle during training, star during testing). After that, a check test was made in the same way except that the presented cues were this time identical to those used to train the ants (i.e., same size, color and shape). Concerning the complementary experiment, the ants were tested in the same way but twice at 3.45 as well as at 15.45 o'clock, first in front of the cues used to train them kept unchanged (i.e., empty circles), then secondly in front of these cues filled (i.e., black circles). Each time, the protocol was the following one. In the tray, the two amounts of elements (1 and 3, 2 and 4, 3 and 5; 2 and 4) were *ca* the same number of times deposited either on the left or on the right. To make a test on a colony, 25 foragers were transported into the tray devoted to their testing, and thirteen seconds later, the ants which went close to each presented cue (i.e., at less than 2 cm from the cue) were counted twenty times during ten minutes. After the test, the ants were transferred into their tray, near their nest entrance. The mathematical and statistical analysis of the recorded data was identical for the three first and the complementary experiments, and is explained in a following paragraph.

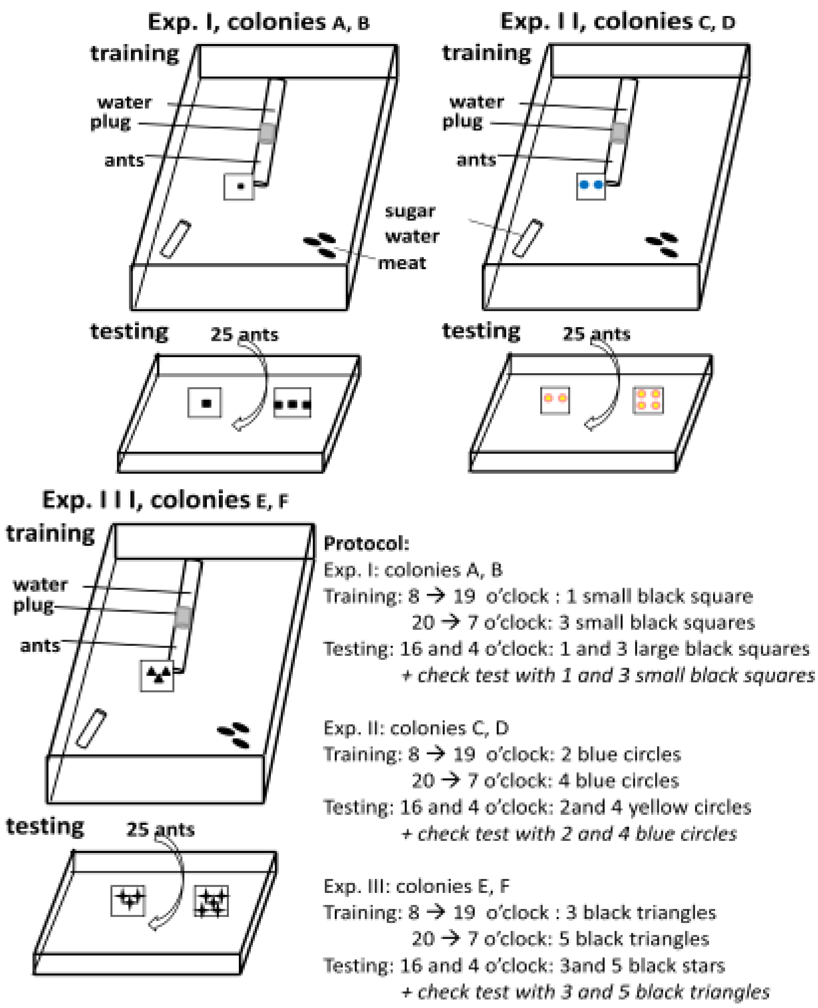


Figure 1. Experimental design (Exp I, II, III) and protocol (right lower part of the figure) used to know if ants take or do not take into account the characteristics of visual cues when associating numbers of these cues with their time periods of occurrence. The used visual cues are shown in Figure 2 and photos can be seen in Figure 3

2.4 Cues

The cues used during Experiments I, II, and III are schematized in Figure 2, those used during the complementary experiment are schematized in Figure 4.

Let us recall that the cues presented to the ants were 1 and 3 black small or large rectangles for colonies A and B (Experiment I), 2 and 4 blue or yellow circles for colonies C and D (Experiment II), 3 and 5 triangles or stars for colonies E and F (Experiment III), 2 and 4 empty or black circles for colonies G and H (complementary experiment). The dimensions of these cues are given in Figures 2 and 4. Using Microsoft Word® software, a square (2 cm x 2 cm) was drawn for each cue, and the latter was drawn inside of this empty square. All the squares containing each one a given cue were printed, and each of them was then separately cut. After that, each square containing each one a given cue was tied on a stand thanks to extra transparent sticky paper. Each stand was constructed in Steinbach® white paper, had a front vertical part (2 cm x 2 cm bearing the cue) as well as a horizontal part [2 x (1 cm x 0.5 cm)] which duly folded ensured its vertical position. The construction of the stands and their tied cues were performed 2-3 days before their use to avoid the remaining of any odor.

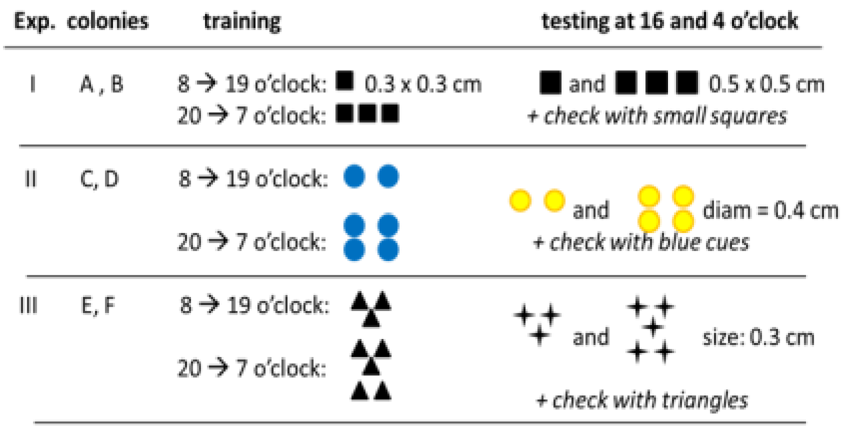


Figure 2. Cues used, during Experiment I, II, and III, to train and to test ants for knowing if they take into account the characteristics (size, color, shape) of the elements when associating numbers of these elements with their time period of occurrence. Figure 1 and Table 1 allow understanding the entire process

2.5 Mathematical and Statistical Analysis

For each test, colony and kind of cue, the 20 recorded counts were added. The obtained sums are given in Tables 1 and 2. For each test, the numbers of ants recorded for each two colonies for each kind of cue were correspondingly added, on the basis of what the proportions of ants which have gone near each presented cue could be established. These proportions are given in the text.

Two statistical analyses of the recorded data were conducted. For each kind of cue presented during testing, the numbers of ants counted for the two colonies were correspondingly added, and the 20 obtained sums were chronologically added by four, what provided 5 sums for each cue, which were then compared to one another using the non-parametric test of Wilcoxon (Siegel & Castellan, 1989; given N, T, P as defined by these authors). Also, for each kind of cue, the numbers of times 0, 1, 2, 3 etc… ants of the two experimented colonies were sighted near the cue were established, and the distributions of these numbers of time obtained for each kind of cue were compared to one another using the non-parametric χ^2 test (Siegel & Castellan, 1989).

3. Results

3.1 Experiment I

The proportions of ants of the two colonies having approached 1 *versus* 3 black squares were at 16 and 4 o'clock respectively: at day 1: 48.61% *vs* 51.39% and 52.05% *vs* 47.95%, at day 2: 49.28% *vs* 50.72% and 50.0% *vs* 50.0%, at day 3: 53.95% *vs* 46.05% and 50.60% *vs* 49.40%. For each of these six tests, the numbers of ants counted on one hand near 1 black square and on the other hand near 3 such squares did not statistically differ (Table 1, upper part). Thus, the ants did not associate each two presented numbers of elements with their time period of occurrence when the size of the elements presented during testing differed from that of the elements used during training. During the check test (made with cues identical to those used during training), the proportions of ants having visited 1 *versus* 3 black squares were, at 16 and 4 o'clock respectively, 76.39% *vs* 23.61% and 18.67%% *vs* 81.33% %. This time, the numbers of ants sighted near each two cues statistically differed. The ants thus duly associated the 1 black square with its temporal period of occurrence (8 – 19 o'clock) and the 3 black squares with its own time period of occurrence (20 – 7 o'clock). Consequently, when associating the numbers 1 and 3 of black squares with their respective time period of occurrence, the ants took into account the size of the squares, i.e., they did not associate the numbers of larger squares with the temporal periods of occurrence of the same numbers of small squares. The responses of the ants of the two colonies were in agreement with each other, what increased the validity of the results. Photos are shown in Figure 3, upper part.

3.2 Experiment II

The ants of the two colonies went, at 16 and 4 o'clock, near the 2 *versus* the 4 yellow presented circles, with the following proportions, at day 1: 52.27% *vs* 47.73% and 55.20% *vs* 44.78%, at day 2: 48.81% *vs* 51.19% and 52?46% *vs* 47.54%, at day 3: 50.95% *vs* 49.05% and 54.29% *vs* 45.71% (Table 1, middle part). They ants have not associated the numbers of yellow elements with the time period of occurrence of the numbers of blue elements. They have thus taken into account the color of the presented elements for making such an association. The ants behaved otherwise during the check test made with 2 and 4 blue circles fully similar to those presented during training. During the check test made at 16 and 4 o'clock, the ants of the two colonies responded to 2 *versus* 4 blue circles with the respective proportions of 88.8% *vs* 11.2% and 10.13% *vs* 89.87%. The ants have thus associated the two blue circles with the temporal period 8 to 19 o'clock and the four blue circles with the time period 20 to 7 o'clock, i.e., with their respective period of occurrence. Consequently, when associating numbers of elements with their time period of occurrence, the ants took into account the color of these elements; they did not similarly associate same numbers of differently colored elements. Photos of this Experiment II are shown in Figure 3, middle part.

Table 1. Numerical and statistical results of tests made, at 16 and 4 o'clock, on ants of two colonies trained to a smaller number of elements from 8 to 19 o'clock and to a larger number of these elements from 20 to 7 o'clock. The cues used for testing the ants during three days differed from those used during training by their size (Exp. I), color (Exp. II) or shape (Exp. III); they were similar during the check test. The table gives the numbers of ants having approached the smaller *versus* the larger amounts of elements (column 3), as well as the results of non-parametric Wilcoxon and χ^2 tests comparing the ants' responses to one and the other of these two amounts (column 4)

Experiment, colonies amount, difference	**days, time o'clock**	**N° of ants of the two colonies counted near the smaller *vs* the larger amounts**		**Wilcoxon**			**χ^2 tests**		
				N	**T**	**P**	**χ^2**	**df**	**P**
Exp. I,	1 16	21 *vs* 19	14 *vs* 18	4	5.5	0.501	0.33	2	<0.40
colonies A and B	4	17 *vs* 18	21 *vs* 17	5	7.5	>0.50	2.31	2	<0.50
1 *versus* 3	2 16	18 *vs* 17	16 *vs* 18	4	5.5	0.502	0.082	2	<0.80
difference: size	4	20 *vs* 21	18 *vs* 17	4	6	0.438	3.86	2	<0.20
	3 16	17 *vs* 15	24 *vs* 20	3	NS		3.21	2	~0.20
	4	22 *vs* 24	20 *vs* 17	4	6	0.438	1.40	2	<0.30
	check 16	24 *vs* 7	31 *vs* 10	5	15	0.031	31.2	2	<0.001
	4	6 *vs* 29	8 *vs* 32	5	15	0.031	45.78	2	<0.001
Exp. II,	1 16	27 *vs* 18	19 *vs* 24	4	8	0.188	0.95	2	<0.70
colonies C and D	4	17 *vs* 16	20 *vs* 14	3	NS		4.01	2	<0.20
2 *versus* 4	2 16	23 *vs* 25	18 *vs* 18	3	NS		1.35	2	<0.70
difference: color	4	15 *vs* 18	17 *vs* 11	3	NS		0.57	2	<0.80
	3 16	13 *vs* 8	14 *vs* 18	5	7.5	NS	0.21	1	<0.70
	4	18 *vs* 20	20 *vs* 12	2	NS		1.82	2	<0.50
	check 16	46 *vs* 6	65 *vs* 8	5	15	0.031	63.2	2	<0.001
	4	6 *vs* 36	2 *vs* 35	5	15	0.031	58.24	2	<0.001
Exp. III,	1 16	26 *vs* 22	20 *vs* 18	3	NS		1.63	2	<0.50
colonies E and F	4	16 *vs* 11	19 *vs* 20	4	8.5	0.157	2.81	1	<0.10
3 *versus* 5	2 16	25 *vs* 19	26 *vs* 37	3	NS		2.71	2	<0.30
difference: shape	4	20 *vs* 23	18 *vs* 16	3	NS		0.71	2	~0.70
	3 16	22 *vs* 21	28 *vs* 27	1	NS		0.24	2	<0.90
	4	18 *vs* 14	14 *vs* 14	4	8	0.188	0.25	1	<0.70
	check 16	56 *vs* 9	40 *vs* 10	5	15	0.031	48.24	2	<0.001
	4	11 *vs* 45	6 *vs* 37	5	15	0.031	40.73	2	<0.001

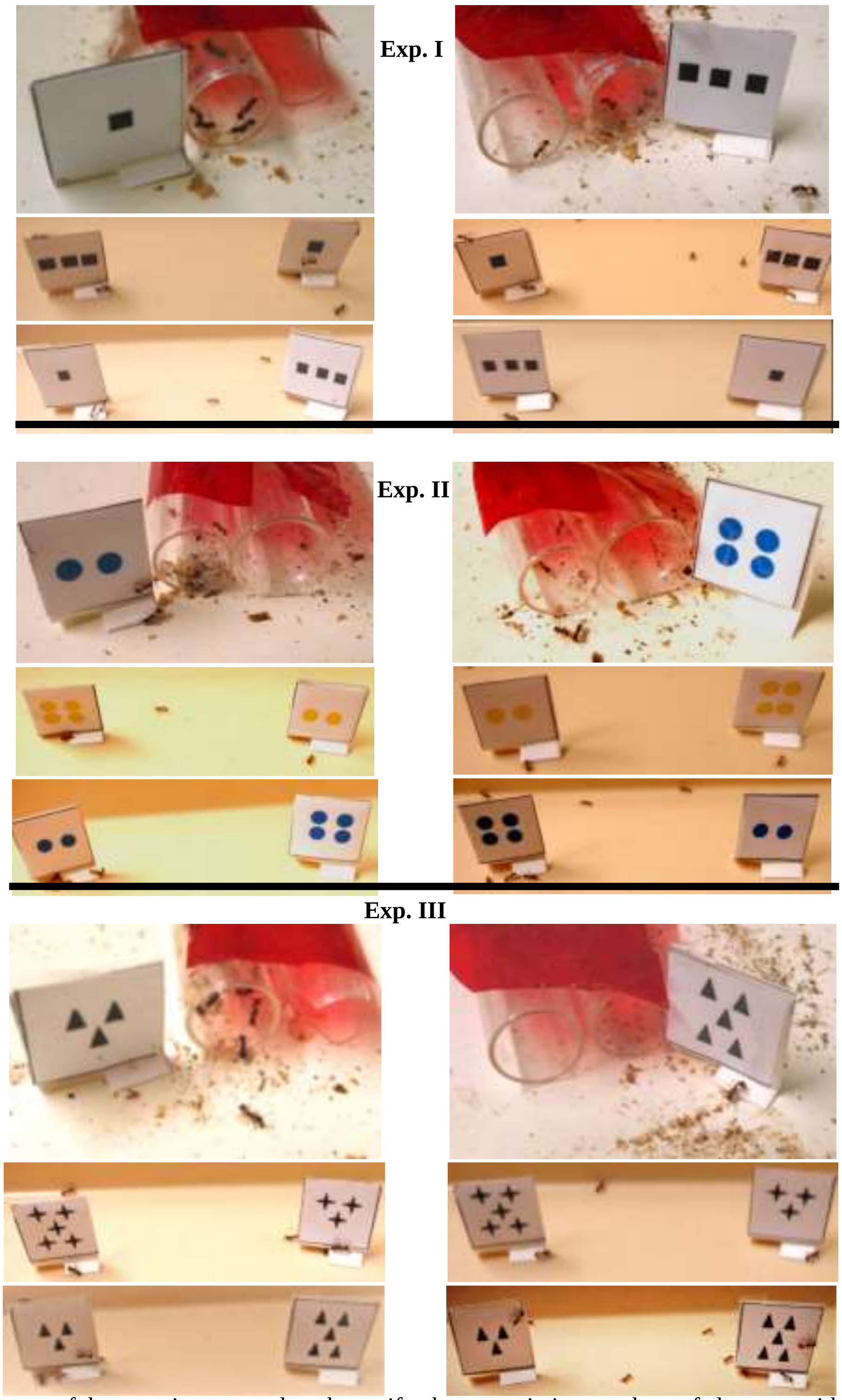


Figure 3. Some photos of the experiments made to know if, when associating numbers of elements with their time period of occurrence, the ants take into account the characteristics of the elements. For experiments I, II, and III, the upper part shows the training of the ants, the middle part shows the testing of the ants, and the lower part shows the check test made without changing the characteristics of the elements. Each time, the left photos concern the time period 8-19 o'clock, the right ones the time period 20-7 o'clock. When the size (Exp. I), or the color (Exp. II), or the shape (Exp. III) of the elements were changed, the ants equally responded to the two numbers whatever the time o'clock of the test; when none of these characteristics was changed, the ants responded more, at 16 and 4 o'clock, to the cues perceived during the time period including each of these two times o'clock. Thus, when associating 'numbers of elements and time', the ants took into account the characteristics of the elements

3.3 Experiment III

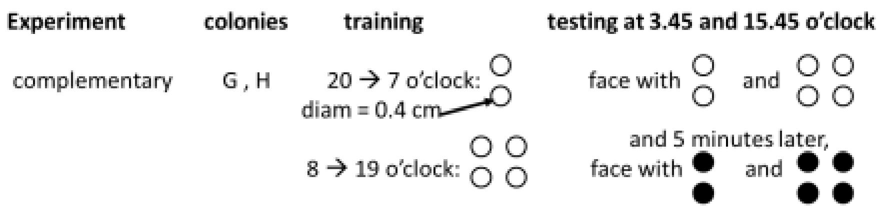
When tested, the workers of colonies E and F approached the 3 *versus* the 5 presented stars, at 16 and 4 o'clock respectively with the following proportions: at day 1: 53.49% *vs* 46.51% and 53.03% *vs* 46.97% (with % for 3 > % for 5, thus the inverse of what should be), at day 2: 47.66% *vs* 52.34 (with % for 3 < % for 5, so the inverse of what should be) and 49.35% *vs* 50.65%, at day 3: 51.02% *vs* 48.98% and 53.33% *vs* 46.67% (with % for 3 > % for 5, the inverse of what should be) (Table 1, lower part). Each time, the difference between the ants' response to one and the other presented cues was not significant. Thus, the ants did not associate the 3 and the 5 stars with the temporal periods of the 3 and the 5 triangles occurrence. They have taken into account the shape of the perceived visual cues for associating them with their respective time period of occurrence. During the check test, when the tested ants were in front of 3 and 5 black triangles, they approached these two cues, at 16 and 4 o'clock with respectively 83.48% *vs* 16.52% and 17.17% *vs* 82.83%. They have thus duly associated each number (3, 5) of triangles with its temporal period of occurrence (8 – 19 o'clock, 20 – 7 o'clock). Therefore, when making such an association, the ants took into account the shape of the perceived elements, i.e., they did not associated elements having another shape. Some photos of this experiment are shown in Figure 3, lower part.

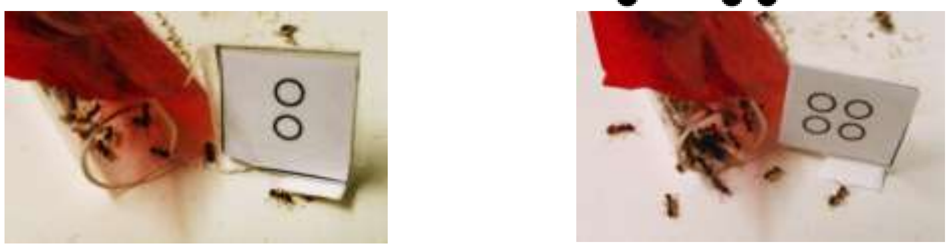
Complementary experiment
Colonies G, H during 3 days

Training: 20 o'clock → 7 o'clock: 2 empty circles
8 o'clock → 19 o' clock: 4 empty circles

Testing: at 3.45 o'clock: in front of 2 and 4 empty circles
at 4.05 o'clock: in front of 2 and 4 back circles

at 15.45 o'clock: in front of 2 and 4 empty circles
at 16.05 à'clock: in front of 2 and 4 black circles





20 – 7 o' clock

8 – 19 o' clock

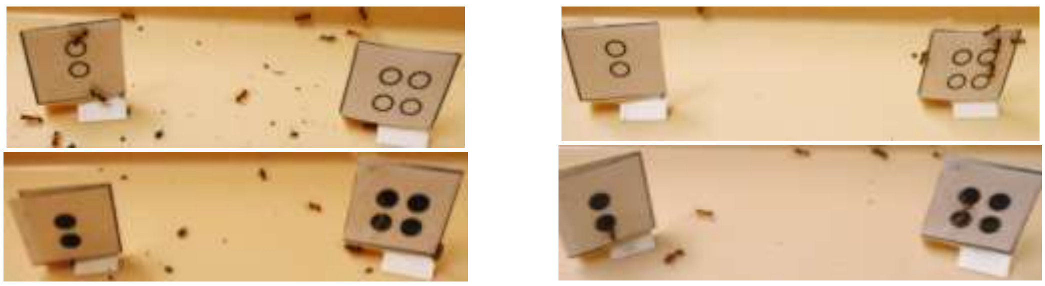


Figure 4. Protocol (upper part), cues used (middle part) and photos (lower part) of the complementary experiment. Numerical and statistical results are given in Table 2. The ants were trained to 2 empty circles from 20 to 7 o'clock and to 4 ones from 8 to 19 o'clock (upper photos), and tested (lower photos) at 3.45 (left photos) and 15.45 (right photos) o'clock face to 2 and 4 empty circles, then face to 2 and 4 black circles. Each time, they responded to the number learned during the corresponding time period only if the circles were empty. They thus associate cues with their time period of occurrence taking into account the filling (the aspect) of these cues

Table 2. Numerical and statistical results of tests made, at 3.45 and 15.45 o'clock, on ants of colonies G and H trained to 2 empty circles from 20 to 7 o'clock and to 4 such circles from 8 to 19 o'clock. The circles presented during testing were firstly identical to those used for training and were secondly black circles (see Figure 4). For each testing time, the table gives the numbers of ants having gone near each two amounts of circles (column 3), as well as the results of non-parametric Wilcoxon (N, T, P defined as in Siegel and Castellan (1989)) and χ^2 tests comparing the ants' responses to 2 and to 4 circles (column 4). The ants essentially responded to the number corresponding to its time period of learning only when the circles were empty (i.e., as during training). NB: day 1: in front of black circles, the ants essentially responded to the 'wrong' number of circles

Experiment, colonies amount, difference	**days, time o'clock**	**N° of ants of colonies G and H counted near 2 *vs* 4 empty and black circles**			**Wilcoxon**			**χ^2 tests**		
					N	**T**	**P**	**χ^2**	**df**	**P**
Complementary exp.	1 3.45	empty:	40 *vs* 13	41 *vs* 8	5	15	0.031	63.85	2	<0.001
		black:	29 *vs* 35	8 *vs* 16	4	10	0.063	15.86	2	<0.001
colonies G and H	15.45	empty:	4 *vs* 46	7 *vs* 49	5	15	0.031	53.82	2	<0.001
2 *versus* 4		black:	22 *vs* 24	44 *vs* 41	4	6	0.438	0.21	1	<0.70
Firstly: no difference (empty circles)	2 3.45	empty:	33 *vs* 7	73 *vs* 8	5	15	0.031	51.15	1	<0.001
		black:	11 *vs* 14	13 *vs* 21	4	7	0.313	2.14	1	<0.30
Secondly: filled black circles	15.45	empty:	7 *vs* 29	16 *vs* 87	5	15	0.031	48.46	2	<0.001
		black:	30 *vs* 24	24 *vs* 25	5	11	0.219	2.20	2	<0.30
	3 3.45	empty:	44 *vs* 8	32 *vs* 5	5	15	0.031	56.64	2	<0.001
		black:	18 *vs* 30	14 *vs* 12	4	9	0.125	3.20	2	<0.30
	15.45	empty:	2 *vs* 27	3 *vs* 79	5	15	0.031	62.22	1	<0.001
		black:	19 *vs* 16	44 *vs* 34	3	NS		2.65	1	<0.20

3.4 Complement Experiment

The tested ants of colonies G and H approached the 2 *versus* the 4 presented circles when these circles were empty then black, at 3.45 and 15.45 o'clock with the following proportions: at day 1: 79.41% *vs* 20.59% then 42.69% *vs* 57.30%, and 10.38% *vs* 89.62% then 50.38% and 49.62%; at day 2: 87.60% *vs* 12.40% then 40.00% *vs* 60.00%, and 16.55% *vs* 83.85% then 52.43% *vs* 47.57%; at day 3: 85.39% *vs* 14.61% then 43.24% *vs* 56.76%, and 4.50% *vs* 95.50% then 55.75% *vs* 44.25%. At day 3, the ants' response to 4 empty circles at 15.45 o'clock was spectacular. The ants have thus 1) duly responded to the number of circles seen during the corresponding training time periods (they have associated each amount with their respective time of occurrence), but 2) they did not do so when the circles were black instead of empty. The ants have thus taken into account the aspects of the circles for associating their amount with their time period of perception. These two observations, based on the numbers of counted ants, were statistically significant (Table 2), and confirmed what Exp. I, II, and III revealed.

4. Discussion

At the end of our previous work on the subject, we wondered if, when associating numbers of elements with their time period of occurrence, the ants take into account the characteristics (size, color, shape) of the elements. The present work answered this question. Three conducted experiments effectively showed that the ants did not associate with their temporal period of occurrence same numbers of elements differing by their size, color or shape. This is schematically presented in Figure 5. A complementary experiment using a slightly different protocol confirmed this conclusion. Such a result is logical: adding two numbers of elements is, in fact, making an association between two numbers (like when associating numbers with their time period of occurrence), and when doing so, the ants take into account the characteristics of the elements (Cammaerts &Cammaerts, 2021 a).

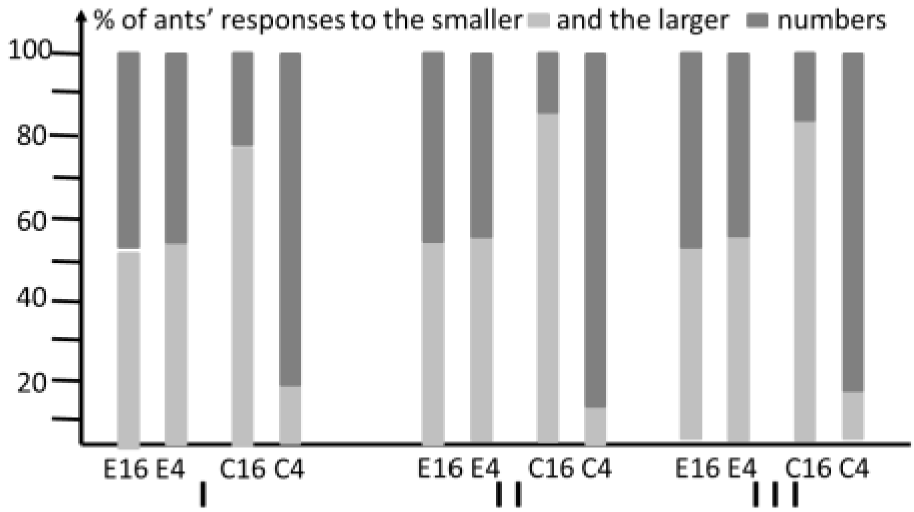


Figure 5. Graphical summary of three experiments (I, II, III) made to know if, when associating numbers of elements with their time period of occurrence, the ants take into account the characteristics (I: the size, II: the color, III: the shape) of the elements. The ants were trained from 8 to 19 o'clock to a small number of elements, and from 20 to 7 o'clock to a larger number. They were tested at 16 and 4 o'clock in front of these two numbers of elements, the latter having another size, color or shape (E16 E4): they did not make the association. They were then similarly tested in front of elements identical to those seen during training (C16 C4): they made the association (= they responded essentially to the numbers perceived during the corresponding time period)

During the present work, for each three first performed experiments, we made a check test consisting in presenting, to the tested ants, numbers of elements this time identical to those used for training, and the ant duly associated these numbers of identical elements with their respective time period of occurrence. During the complementary experiment, we also tested the ants in front of amounts of elements identical to those used during training (i.e., empty elements), (but this time just before testing them in front of modified elements, i.e., filled black elements), and the ants once more duly associated each amount with its temporal period of occurrence. The present work is thus in agreements with our previous ones (Cammaerts & Cammaerts, 2022 a, submitted, 2022 b).

The results of the check tests were excellent, somewhat better than the previously obtained ones. This could be explained by the fact that no test was here carried out during the three training days (as they have been done during the previous works), and a test is similar to an experience of extinction. Indeed, during a test, the conditional stimulus is presented without the unconditional one, and this is the procedure of an extinction experiment (Pearce, 2008).

For associating numbers of elements of exactly the same aspects with their time periods of occurrence requires precisely memorizing past events, knowing where they occurred, and localizing them in time. Therefore, the precise here confirmed association have the three criteria of an episodic-like memory: being able to remember which, where and when events occur. Several animal species have been proved to have such a memory and to use it for adequately acting in the future (Zentall, 2005). Concerning *M. sabuleti*, other experimental works prove that these workers detain some episodic-like memory. Trained to a kind of cue associated with meat and to another kind of cue associated with sugar water, when deprived of one or the other of these two foods, they duly significantly reacted to the cue associated with the lacking food (Cammaerts & Cammaerts, 2018). Our findings of these ants' ability to associate visual cues, odors and amounts of elements with their respective temporal periods of occurrence also prove their possession of some episodic-like memory (Cammaerts & Cammaerts, 2022 a, submitted, 2022 b).

For associating any experienced events with time, a notion of passing time is required. *Myrmica sabuleti* workers 2-3 years old have this notion, but not the younger ones. Indeed, workers 2 to 3 years old, but not young ants can acquire spatial-temporal learning (Cammaerts, 2013 a, b). In the same way, contrary to old ants, young ones cannot expect the time of the following food delivery (Cammaerts & Cammaerts, 2015). It might be presumed that the young ants (the callows) acquire the notion of time through a maturation process.

Being able to associate perceived precise numbers of given elements with their time period of occurrence is a skill useful over the daily life of any animals including humans and ants. Concerning the ants, if they have associated time periods with the presence of given numbers of visual cues, odors or any other elements they can perceive, then they can adapt their foraging activity as well as their nest relocation in order to maximize their food collection, minimize the presence of predators, avoid the temporal periods during which the environmental conditions are uncomfortable. The usefulness of time perception and every cognitive ability linked to such a perception have been detailed by Ng, Garcia, Dyer and Stuart-Fox (2021) and underlined by Vasconcelos, de Carvalho and Machado, A. (2017).

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Conflict of interest

We certify we have no conflict of interest at all as for the subject we examined in the present work.

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