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Association, in an Ant, of a Quantity of an Element with the Time Period of Its Learning

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Abstract

The workers of the ant *Myrmica sabuleti* detain numerosity abilities, have a notion of the running time, and can acquire operant conditioning. The present work examines if, according to these skills and through conditioning, the workers of this ant can associate a learned quantity of a given element with the time period of its occurrence. We collectively trained such ants from 8 to 19 o'clock to a stand bearing a given quantity of an element and from 20 o'clock to 7 o'clock next day to a stand bearing another quantity of the same element, and we tested them in front of these two amounts at 16 o'clock and 4 o'clock next day. At 16 o'clock, the ants reacted essentially to the amount presented during training from 8 to 19 o'clock, and at 4 o'clock to the amount presented during training from 20 o'clock to 7 o'clock. They thus associated the learned quantity of an element with the period of the day during which this learning occurred. It may be argued that this association simply results from the three cognitive capabilities cited here above, and does not require any other more complex skill. In addition, the ants appeared to have better learned from 20 to 7 o'clock than from 8 to 19 o'clock, i.e., during the time of day corresponding to their period of highest natural activity.

Keywords: quantity assessment, *Myrmica sabuleti*, operant conditioning, periods of activity, running time

1. Introduction

The aim of the present work was to examine if the workers of the ant *Myrmica sabuleti* Meinert, 1861 can associate the sight (the learning) of a quantity of an element with the time period during which this quantity was learned. Here below, we successively briefly report information about the numerosity abilities and the sense of running time of *M. sabuteli* ants, then of other animal species.

The workers of the ant *M. sabuleti* have many cognitive abilities. Among others, they detain numerosity abilities summarized, for the readers' convenience, in Cammaerts & Cammaerts (2020d, 2020e, 2022a). Briefly, *M. sabuleti* workers can add and subtract one visual or olfactory cue to and from other ones when perceiving the results of the operation; they natively possess a left to right oriented number line with a logarithmic mental positioning of the quantities on this line. Through experiences, they acquire a concrete notion of zero. They can acquire numerical symbolisms and use the symbols for adding. They can expect the following element in an increasing or decreasing arithmetic or geometric sequence when being in presence of the sequence. They add two numbers of elements if these elements are identical, and if they are sighted simultaneously, i.e., if they are located from one another at a horizontal distance not exceeding 5 cm and at a vertical distance not exceeding 4 cm, as well as if they are not perceived after a time gap longer than 7 minutes and 45 seconds between them (Cammaerts & Cammaerts, 2019a, 2019b, 2021c, 2021d, 2022c).

Myrmica sabuleti ants have a notion of the running time (Cammaerts, 2010). This was revealed through several experimental works (Cammaerts 2004, 2010, 2013a), and was particularly obvious when demonstrating that these ants can expect the time o'clock of the occurrence of an event on the basis of previous occurrences of this event (Cammaerts & Cammaerts, 2016b). However, young ants do not yet detain this ability (Cammaerts, 2013b; Cammaerts & Cammaerts, 2015c). In addition, the workers of *M. sabuleti* natively detain a circadian rhythm (Cammaerts et al., 2011).

Similar abilities have been found in many vertebrates and invertebrates. A non-exhaustive list of these abilities is here below reported and much more information can be found in the references here above cited.

Numerosity abilities can be ranked in four successive steps: evaluating amounts of elements, counting the number of elements, adding and subtracting elements, acquiring numerical symbolisms. Evaluating amounts of elements has been observed for instance in fishes (Agrillo et al., 2017), rats (Cox & Montrose, 2016), and spiders (Rodriguez et al., 2015). Counting elements has been shown for instance in frogs (Rose, 2018), birds (Pepperberg, 2012) and monkeys (Beran, 2008). Adding and subtracting elements is an ability detained for instance by birds (Rugani et al., 2009) and monkeys (Flombaum et al., 2005). Birds (Xia et al., 2000) and chimpanzees (Biro & Matsuzawa, 2001) can acquire numerical symbolisms. New born chicks were even shown to possess a number line (Rugani et al., 2015). The notion of zero was showed to be acquired by birds (Pepperberg & Gordon, 2005) and chimpanzees (Biro & Matsuzawa, 2001). Learning a sequence of numbers or of other elements has been observed in several animal species (Kershenbaum et al., 2014). Expecting the following element of a sequence was observed e.g., in monkeys (Brannon & Terrace, 2000).

Expectative behavior requires having a notion of the running time. Such a notion has been found to be detained by, e.g., great apes (Osvath & Osvath, 2008). Bees, according to their navigation system, also detain this notion (Eban-Rothschild & Bloch 2012). In addition to their notion of the running time, most of animal species including humans detain an innate circadian rhythm. The trait and properties of such a rhythm are explained for instance in the reviews of Murphy and Campbell (1996) and of Rivkees (2007). These authors focus on mammalians and provide information on the physiological mechanisms which act in accordance with a circadian rhythm. Forager honeybees possess an obvious circadian rhythm ruled by a molecular clock that is more similar to that of mammals than to that of fruit flies (Eban-Rothschild and Bloch 2012). Using anesthesia, an experimental work on the clock time behavior of the honeybees showed that the circadian clock of these insects is ruled by mRNA oscillations of two genes, *cryptochrome-m* and *period* (Cheeseman et al., 2012).

Workers of the ant *M. sabuleti* aged of about 2 years have a notion of the running time and are able to acquire conditioning (as shown in all of our here above cited references). They might thus be able to associate a given quantity of elements with its time period of occurrence. The ability to associate elements with their time period of occurrence could possibly be acquired thanks to conditioning. Indeed, it is nowadays admitted that during a conditioning process, the individuals not only associate the reward with the conditional stimulus, but also associate the reward and the conditional stimulus with several other parameters such as events occurring at the same time, environment characteristics, and time o'clock. The latter parameter is probably the main one memorized (Enquist et al., 2016)

Therefore, for *M. sabuleti* workers, detaining the ability to associate a learned quantity of an element with the time period during which this quantity has been learned is not a meaningless hypothesis. This hypothesis is all the more plausible since these ants can associate visual cues (squares, circles, stars) with the part of the day corresponding to their learning (Cammaerts & Cammaerts, 2022b). We here investigate about the potential ability of the workers of the ant *M. sabuleti* to associate learned quantities of elements with the time period during which these quantities were learned.

2. Materials and Method

2.1 Collection and Maintenance of Ants

We experimented on four colonies of *Myrmica sabuleti*, Meinert 1861 (labelled A, B, C, D) collected in May 2021 from an abandoned quarry located in the Aise valley (Ardenne, Belgium). The colonies lived under stones, and contained about 600 workers, brood and a queen. 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 ants. The tubes were covered with transparent red paper for providing the ants with a low lighting inside their nest. The nest tubes of each colony were deposited in a tray (34 cm x 23 cm x 4 cm) the borders of which having been slightly covered with talc to prevent ants from escaping. These trays served as foraging areas in which food was delivered, namely pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) provided three times per week, and sugar water continuously provided in small cotton-plugged tubes. The lighting of the laboratory was provided by a window (natural daytime light) and by the low artificial lighting (night-time light) of annexed rooms. Artificial lightning equaled *ca* at least 330 lux while testing the ants (during about 10 minutes), and about 110 lux during the other night-time periods. The temperature equaled *ca* 20 ℃, the humidity *ca* 80%, and the electromagnetic field *ca* 2 μWm^2. These conditions were suitable to the used species, *M. sabuleti*. Ants are here often named 'workers' or 'nestmates' or 'congeners' as usually do researchers on social insects.

2.2 Experimental Planning and Design

The figures 1 and 2 help to understand the following subsections.

Two experiments were performed, a first one on colonies A and B, a second one on colonies C and D. Each time, the ants were trained during four successive days and tested eight times over these training days. More precisely, the ants of colonies A and B were trained to 1 black rectangle from 20 o'clock until 7 o'clock and to 3 black rectangles from 8 o'clock until 19 o'clock, the ants being thus deprived of any stimulus from 7 to 8 o'clock and from 19 to 20 o'clock. The ants of colonies C and D were trained to 2 black circles from 8 o'clock until 19 o'clock and to 4 black circles from 20 o'clock until 7 o'clock, these ants being thus deprived, such as those of colonies A and B, of any stimulus from 7 to 8 o'clock and from 19 to 20 o'clock. The 1 and 3 black rectangles as well as the 2 and 4 black circles are described in the following paragraph. This planning allowed managing a time interval of 16 hours between the experiments I and II. Let us add that we choose such kinds of graphical elements because we know that the workers of the ant *M. sabuleti* can discriminate them (Cammaerts, 2008). Over their training, the ants of colonies A, B, C, and D were tested each day at 4 and 16 o'clock (in total, eight times) in front of the two kinds of stimuli used during training. The training and testing protocols as well as the analysis of the recorded data are detailed in the paragraphs below.

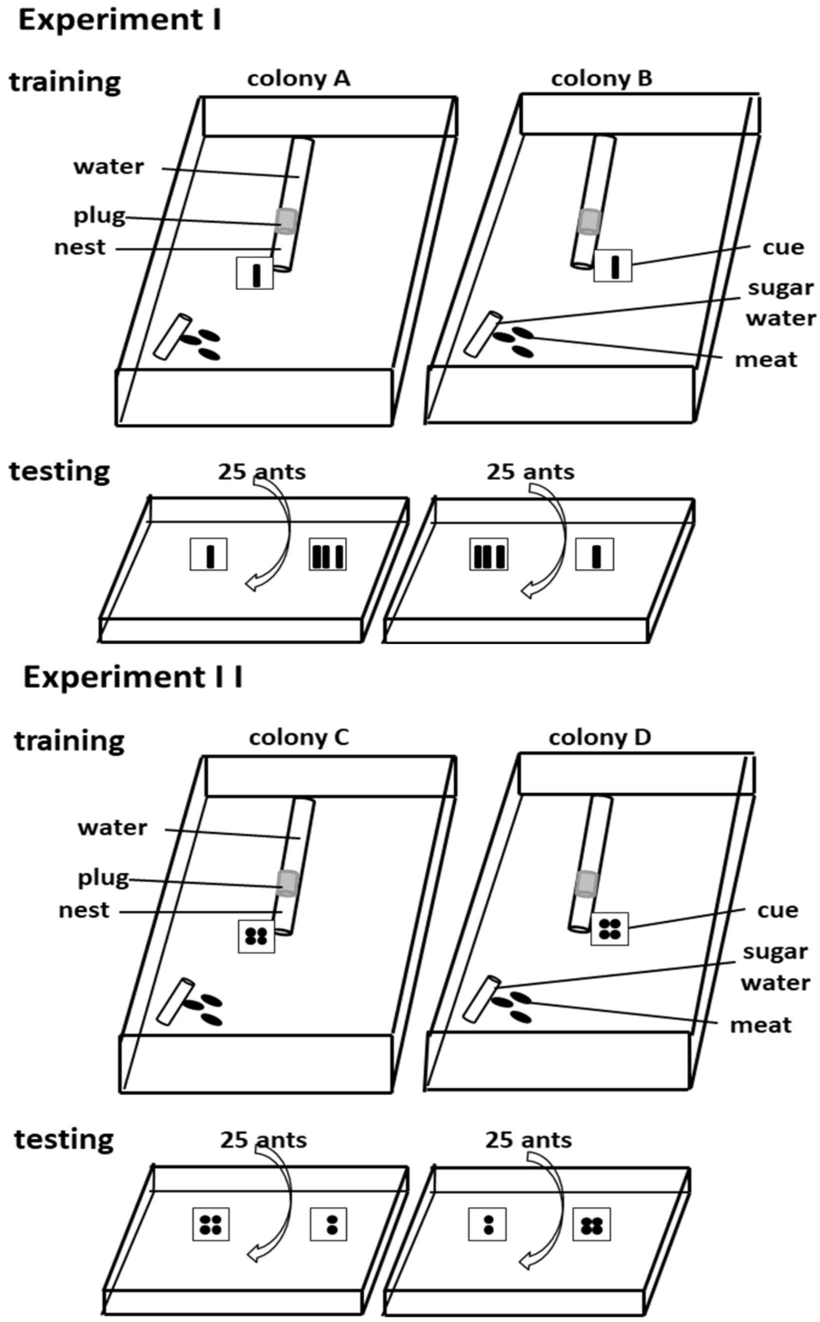

Figure 1. Schematized experimental design and protocol

During four days, the ants were trained to a given number of an element during a defined time period and to another given number of an element during another time period. The ants were tested in front of these two quantities of elements twice each day, at two o'clock times corresponding to the two training time periods, in order to see if they could associate each of the two quantities with the time period during which they were trained to them.

2.3 Cues

The cues presented to the ants were 1 or 3 black rectangles for experiment I (colonies A and B) (1.1 cm x 0.25 cm) as well as 2 or 4 black circles for experiment II (colonies C and D) (diameter: 0.45 cm). These cues were drawn using Microsoft Word® software inside a white square (2 cm x 2 cm), these squares being then printed and cut. Using extra transparent sticky paper, each square was tied on the front face of a stand constructed in strong white paper (Steinbach®, Malmedy, Belgium, 250g/m^2), presenting a vertical part (2 cm x 2 cm; on which the cue was tied), as well as a duly folded horizontal part [2 x (1 cm x 0.5 cm)] for ensuring the vertical maintenance of the stand. The cues were tied on the stands three days before starting the experiments in order to avoid any remaining odor. The cues and stands used to test the ants were identical to those used for training them, but were new, never used.

2.4 Experimental Protocol

The ants were trained in their foraging area to each cue during its allocated time period of presence, the cue being set close to the nest entrance which served as a reward, on the left of the entrance for colonies A and C, and on its right for colonies B and D. The ants underwent so operant conditioning to the cue. Colonies A and B (experiment I) were provided with 1 black rectangle from 20 o'clock to 7 o'clock and with 3 black rectangles from 8 o'clock to 19 o'clock, while colonies C and D (experiment II) were provided with 2 black circles from 8 o'clock to 19 o'clock and with 4 black circles from 20 o'clock to 7 o'clock. This difference between experiments I and II aimed to avoid any possible link between the time period and the size (smaller or larger) of the presented amounts of elements. The two cues were presented during four successive days according to the above cited planning, which includes a gap of one hour between the presentations of each of the two cues. A gap of sixteen hours existed between the last test made during Experiment I and the first cue deposit made for Experiment II.

Over these four days, the ants were tested at 4 and 16 o'clock, thus eight times in total, each time in a separate tray, each colony having its own tray devoted to testing. Each tray measured 21 cm x 15 cm x 7 cm, had its borders slightly covered with talc, and contained the two kinds of cues to which the ants have been conditioned. These two quantities (the smaller and the larger) of elements were randomly set on the left or the right in the tray. To make a test on a colony, 25 ants of this colony were transferred into their tray devoted to testing, and half a minute later the number of visits made by the ants when approaching each of the two presented cues at a distance less than 2 cm were counted 20 times over 10 minutes. After that, the ants were returned to their foraging area. For each test, each colony and each kind of cue, the sum of the 20 counts was established. These sums, i.e. the total numbers of ants sighted near each cue during each test on each two colonies of an Experiment are given in Table 1.

Adding the corresponding sums obtained for each test made on the two colonies used for an Experiment allowed calculating the proportions of ants having visited each two presented cues (i.e., the ants' conditioning scores). These proportions are given in the text. The variability of the numbers of ants sighted during testing near the stands bearing the quantities they saw during the time of the day corresponding or not to the time of the day of their learning is shown in Figure 3.

There was no statistical difference between the conditional scores of the ants of colonies A and B, nor between those of the ants of colonies C and D, regarding the correspondence between the time of the test and the daily period of the training (Mann-Whitney tests). The scores of colonies A + B (experiment I) and of C + D (experiment II) were thus pooled for further statistical analyses.

During the first testing session in each of these two experiments, the ants were faced to a cue to which they had already been trained and to another cue which was new for them. This first testing session was thus not taken into account in the following statistical analysis. The effect of the predictors 'training period' (day-time or night-time), 'time of testing' (4 or 16 o'clock) and 'colony' (A, B, C or D, 'colony' set as a block effect) and of the interaction between training and testing time on the workers' response (i.e., the total number of sighted workers near each cue) was analyzed by a GLMM regression based on the 'car' package in R software (function glm, formula: 'total number of workers ~ training period*time of testing + colony'). Likelihood-ratio tests were made for each predictor. The link function was chosen by comparing the median, mean and variance of the worker's responses in each case of a training period corresponding to a time of testing. All 8 cases, each containing 6 to 8 response values depending on whether the first counting session could or could not be taken into account, showed a variance very different from the mean which, in turn, was similar to the median, what means that a Gaussian family link function appeared to be the best choice.

3. Results

3.1 Experiment I

Numerical and statistical results are given in Table 1, photos are shown in Figure 2, and a graphical synthesis of the variability of the results can be seen in Figure 3.

Table 1 shows that the ants of colonies A and B very significantly associated the learned quantities of elements and the time period of the day during which they were presented to them. Their responses at 4 or 16 o'clock were strongly correlated to the time periods 20 to 7 or 8 to 19 o'clock (P = 2.095E-9).

The mean response of the ants of colonies A and B to one black rectangle to which they were trained during the night and tested at night (at 4 o'clock) equaled 89.26%, and their mean response to 3 of these rectangles to which they were trained during the day and tested at day (at 16 o'clock) equaled 86.11%. Trained at night and tested at day, their mean response equaled 13.97% while when trained during the day and tested at night, their mean response equaled 10.74%.

Figure 3 shows that, when tested, the number of visits made by the ants to a cue (a quantity) at a time of the day corresponding to that of its learning was higher and did not overlap with the number of visits they made to a cue (a quantity) at a time of the day not corresponding to that of its learning.

Table 1. Results of two experiments made for knowing if ants can associate through conditioning a given number of elements with the time period during which it was learned. *: the learning period corresponding to this test having not yet taken place, the first counting session was not taken into account in the statistical analysis

Experiment I.	N °of ants of colonies A and B trained during		Predictor's effect on the workers' response
Days 1 to 4,	20h to 7h	and 8h to 19h	
	and sighted in front of		(GLM, likelihood ratio test)
Testing time	1 element	3 elements	
1, 4h	35 and 40	13* and 1*	Training period:
16h	8 and 5	31 and 46	$\chi^2 = 0.030$; df = 1; P = 0.8634
2, 4h	34 and 20	4 and 3	Time of testing:
16h	7 and 8	36 and 53	$\chi^2 = 0.155$; df = 1; P = 0.6935
3, 4h	112 and 29	14 and 2	Colony:
16h	6 and 5	22 and 49	$\chi^2 = 0.821$; df = 1; P = 0.3648
4, 4h	45 and 40	2 and 3	Interaction, training and test time:
16h	9 and 4	49 and 33	$\chi^2 = 35.884$; df = 1; P = 2.095E-09
Experiment II.	N °of ants of colonies C and D trained during		Predictor's effect on the workers' response
Days 1 to 4,	8h to 19h	and 20h to 7h	
	and sighted in front of		(GLM, likelihood ratio test)
Testing time	2 elements	4 elements	
1, 16h	54 and 53	14* and 5*	Training period:
4h	3 and 5	49 and 38	$\chi^2 = 8.088$; df = 1; P = 0.0045
2, 16h	57 and 48	10 and 7	Time of testing:
4h	11 and 0	58 and 42	$\chi^2 = 0.398$; df = 1; P = 0.5280
3, 16h	27 and 32	1 and 9	Colony:
4h	3 and 3	36 and 42	$\chi^2 = 0.002$; df = 1; P = 0.9661
4, 16h	33 and 27	5 and 7	Interaction, training and test time:
4h	4 and 4	26 and 61	$\chi^2 = 108,101$; df = 1; P < 2.2E-16

Experiment I: when the number of elements they saw was 1 (presented from 20 to 7 o'clock) or 3 (presented from 8 to 19 o'clock), the ants went essentially to 1 element at 4 o'clock and to 3 elements at 16 o'clock. They have thus associated each learned amount with its time period of occurrence. Experiment II: when 2 elements were presented on a stand from 8 to 19 o'clock, and 4 from 20 to 7 o'clock, the ants mostly reacted to 2 elements at 16 o'clock and to 4 elements at 4 o'clock. They have thus also associated the two learned quantities with their time period of occurrence.

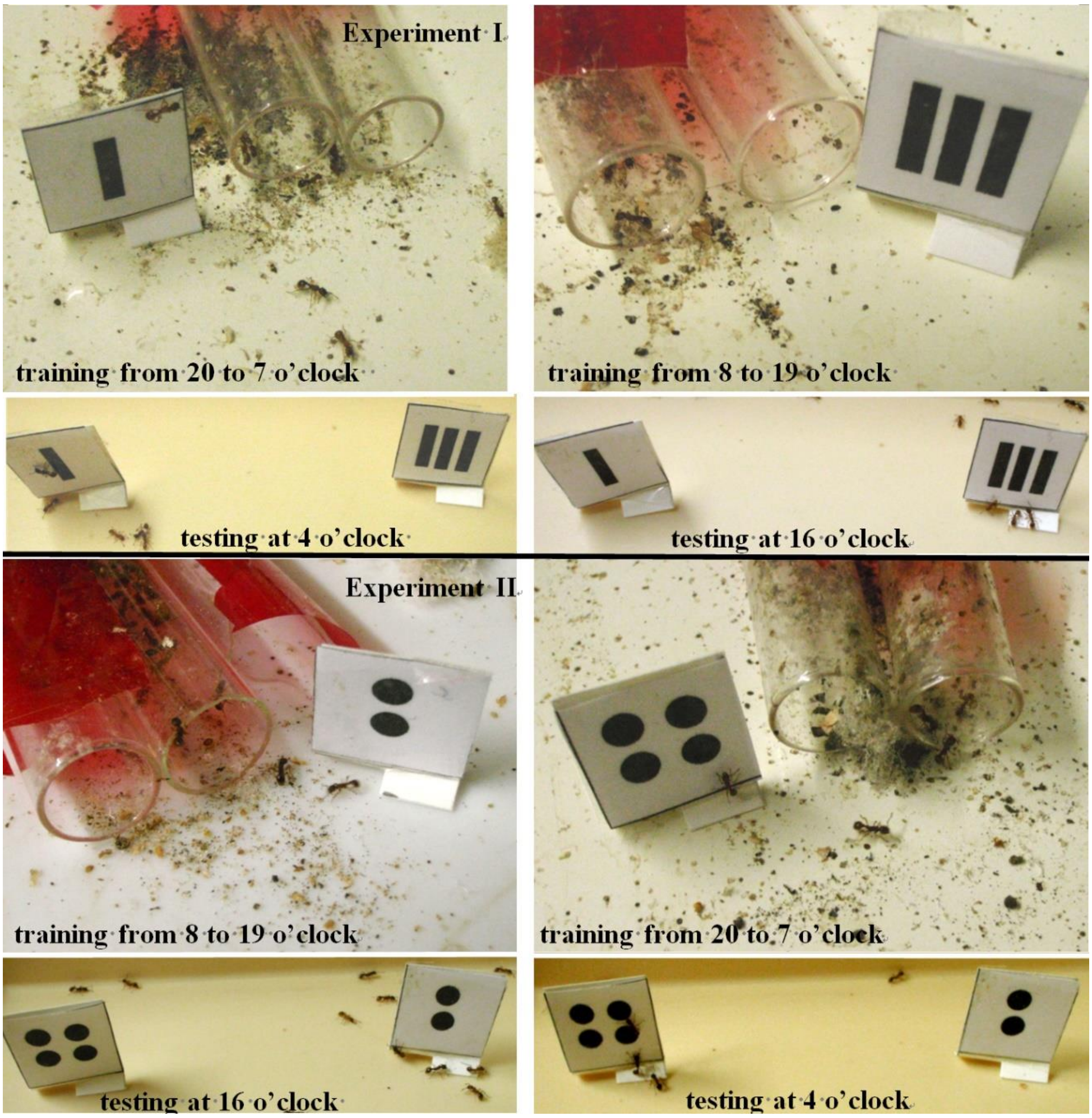

Figure 2. Some views of the experiments

For Experiments I and II, the upper photos show the ants' training and the lower photos show the ants' testing. Experiment I: trained to 1 black rectangle from 20 to 7 o'clock, the ants responded mostly to that cue when tested at 4 o'clock (i.e. during the time period of their training to 1 rectangle); trained to 3 black rectangles from 8 to 19 o'clock, the ants responded mostly to that cue when tested at 16 o'clock (i.e. during the time period of their training to 3 rectangles). Experiment II: in the same way, trained to 2 black circles from 8 to 19 o'clock, the ants responded essentially to that cue when tested at 16 o'clock (i.e. during the time period of their training to 2 circles), and trained to 4 black circles from 20 to 7 o'clock, they responded essentially to that cue when tested at 4 o'clock (i.e. during the time period of their training to 4 circles). The ants associated thus the correct numbers of elements with the time periods during which they were conditioned to them.

3.2 Experiment II

Numerical and statistical results are given in Table 1, photos are shown in Figure 2, and a graphical synthesis of the variability of the results can be seen in Figure 3.

Table 1 shows that the ants of colonies C and D also duly associated the learned quantities of elements with the time periods of the day during which they were trained to them. A strong correlation again existed between the ants' responses at 16 or 4 o'clock and the time periods 8 to 19 or 20 to 7 o'clock ($P < 2.2E\text{-}16$). In this experiment, the time of day when the training took place had some influence on the ants' response (P = 0.005), but not much compared to the relation between training period and test time.

The mean response of the ants of colonies C and D to 2 black circles, to which they were trained during the day and tested at day, i.e., 16 o'clock, equaled 84.96%, and their mean response to 4 of these circles, to which they were trained during the night and tested at night, i.e., 4 o'clock, equaled 91.53%. Trained during the day and tested by night, their mean response equaled 8.47% while when trained during the night and tested by day, their mean response equaled 15.04%.

Figure 3 shows again that, when tested, the number of visits made by the ants to a cue (a quantity) at a time of the day corresponding to that of its learning was higher and did not overlap with the number of visits they made to a cue (a quantity) at a time of the day not corresponding to that of its learning.

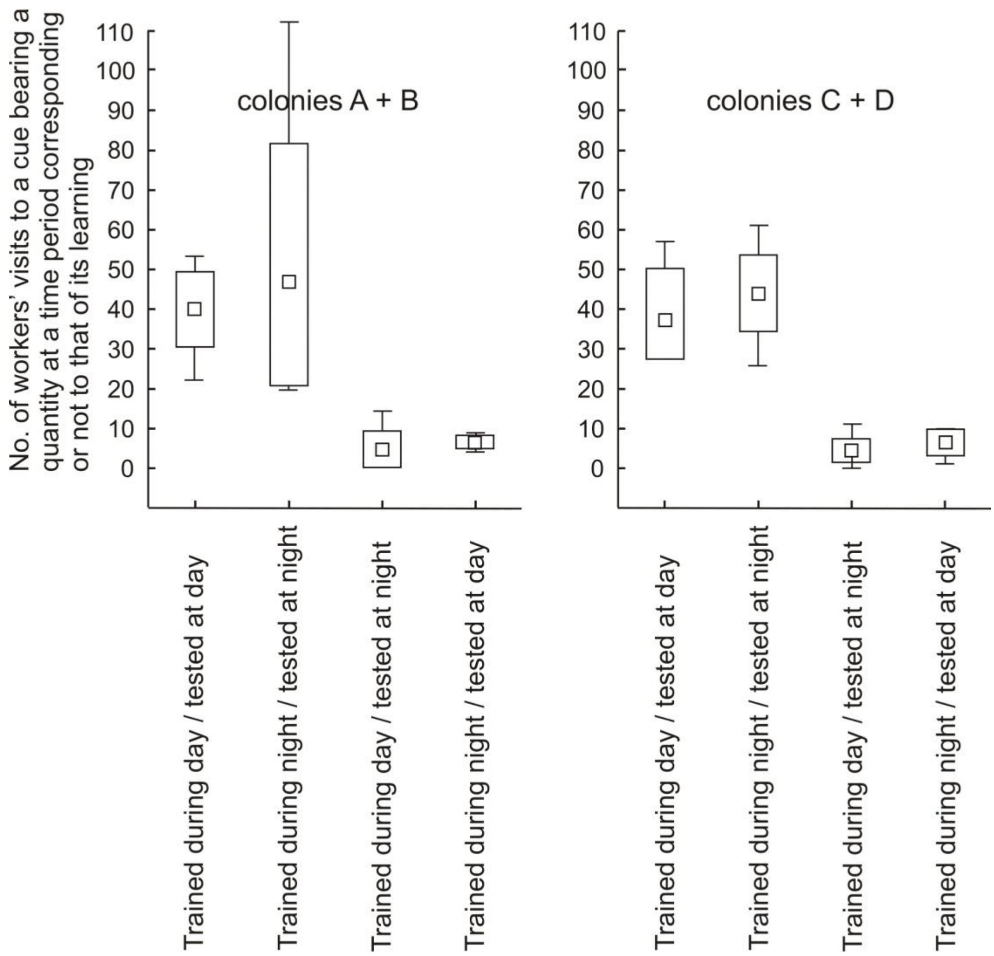

Figure 3. Graphical summary of the ants' association between a quantity of an element and the time period of the day during which this quantity was learned

The ants' responses were assessed by their numbers of visits to each cue (a stand bearing a quantity) at a 'day time' (16 o'clock) and a 'night time' (4 o'clock) corresponding or not to the time during which the ants learned the cue (the quantity). The box-plots show the mean, 95% confidence interval and extremes of the numbers of visits to the learned quantities of elements. The first testing session was not taken into account since the learning of one of the two quantities had not yet begun. The ants reacted each time more to the quantity expected to be present at a time of the day corresponding to its learning.

3.3 Comparison of Experiments I and II

The results of the two experiments were in agreement with each other though the size of the presented quantities and the time period of their presentation were inverted. The association between the learned elements and the time period of their perception by the ants does not depend on the size or the quantity learned.

The ants' percentages of responses at 4 o'clock to the elements presented during training from 20 to 7 o'clock (1 element was then presented on a stand during Experiment I, and 4 elements during Experiment II) were somewhat higher than those recorded at 16 o'clock to the amounts presented from 8 to 19 o'clock during training (3 elements were then presented on a stand during Experiment I, and 2 elements during Experiment II). This difference between the ants' conditioning scores in function of the time of the day, although not particularly marked (the mean of the ants' proportions of responses at the time of the day corresponding to that of their learning was 91.49% (84.06 – 100, with $\sigma = 4.44$) when tested during the night and 85.56% (78.05 – 96.43, with $\sigma = 5.33$) when tested during the day), was nevertheless statistically significant. Indeed, a Wilcoxon test made between twelve pairs (three pairs per colony because the first counting session could not be taken into account) of scores (percentages of visits in favor of the cues corresponding to the time of the day of their learning) gives $P = 0.028$ for $T = 11$. Furthermore, several days after the end of the experiments, thus not in presence of any element, the ants that were moving a few centimeters at the surrounding of their nest entrance were in each colony punctually counted (i.e., during at most a few seconds), each hour from 0 to 6 o'clock and each hour from 12 to 19 o'clock. Thus, a total of 32 counts (8 counts x 4 colonies) were made during the night and of 32 counts (8 counts x 4 colonies) during the afternoon. The mean number of ants observed there during the night was 8.49 (extremes: 3 – 20) and that during the afternoon 1.41 (extremes: 0 – 4). Assuming that one hour between each count was sufficient for avoiding counting the same ants, a Mann-Whitney U test was performed, and this showed that these two series of numbers differed highly significantly (Z, adjusted = 6.764; $P < 10E\text{-}6$). The ants have thus best learned at the time of the day when they walked the more around the cues set at their nest entrance.

4. Discussion

We have here shown that, through conditioning, the workers of *M. sabuleti* could associate a sighted quantity of an element with its time period of occurrence. Let us precise that the graphical elements presented to the ants during each choice test had the same shape, color and size. The only difference between those presented during the two training time periods was their number and their totalized surface. Thus, the ants may have associated quantities with time periods. Note that the inside of the ants' nest was maintained under low lighting thanks to red transparent paper, that foragers navigated all over their foraging area for short time periods as in nature, and that the choice of the concerned periods of the day does not allow us to conclude anything about the existence of a possible association between cue learning and the circadian rhythm of the workers, such a study being not the aim of the present research.

It was also not the purpose of the present work to know whether, when ants associate quantities with the time of day of their learning, their reaction depends (as in an operation) or does not depend (as in a count) on confounding variables such as the shape, color, or size of the items presented.

It is debatable whether the workers' response was due to a preference toward the cue they saw most recently and an aversion to the cue they saw least recently, which is perceived as a novelty (neophobia). Their correct choice of a new element in an arithmetic or geometric sequence (Cammaerts & Cammaerts, 2021a, 2021b) shows that this should not be the case. In addition, experiments about potential neophobia were conducted in the course of our work on ants' association of odors with the time period of the day during which these odors were learned. The results of these experiments allowed concluding that ants obviously and statistically do not display neophobia (Cammaerts & Cammaerts, submitted)

Associating a cue (a visual cue, an odor, or a given quantity of elements) with its time period of occurrence may simply result from conditioning acquisition. Indeed, as stated in the Introduction section, during a conditioning process, several parameters, with among others the running time, play a role, and are memorized together with the conditional stimulus and the reward. In the here performed experiments, the reward was the nest entrance, and on the basis of the high conditioning scores obtained, it can be admitted that, for ants, their nest entrance is a valuable reward.

This ability to associate through conditioning elements with their time periods of occurrence may not be detained by young ants. Indeed, ants 1 to 2 years old must yet acquire the notion of the running time (Cammaerts, 2013b; Cammaerts & Cammaerts, 2015c). The associating skill here revealed in foraging ants certainly exists in other animals, including humans who can associate learned amounts of items with their time period of occurrence. Such an association can be useful along the everyday life. The individuals would know that, during a given time period, they can look for a given amount of items and not for another one. They thus would earn time and save working.

In the laboratory, the workers of *M. sabuleti* were observed to be more active during several time periods between 20 and 7 o'clock than during the 8 to 19 o'clock period. This difference in the ants' daily activity is in accordance with their better learning during the nocturnal period of the day than during the diurnal period. In other words, the ants seemed to better learn while being active. Let us point out that a link between learning and periods of greater daily activity is also observed in humans (Montagner, 2008).

To conclude, we here revealed one more skill detained by ants thanks to their ability of acquiring conditioning, to their perception of the running time and to their capability of distinguishing quantities of elements: the association of sighted quantities with the time of day of their learning. Associating cues to a particular time of day could help ants in their daily tasks.

Conflict of interest

We affirm having no conflict of interest concerning the topic here investigated.

References

Agrillo, C., Miletto Petrazzini, M. E., & Bisazza, A. (2017). Numerical abilities in fish: a methodological review. *Behavioral Processes, 141*(Pt 2), 161-171. https://doi.org/10.1016/j.beproc.2017.02.001

Beran, M. J. (2008). Monkeys (*Macaca mulatta* and *Cebus apella*) track, enumerate, and compare multiple sets of moving items. *Journal of Experimental Psychology: animal behavior processes, 34*(1), 63-74. https://doi.org/10.1037/0097-7403.34.1.63

Biro, D., & Matsuzawa, T. (2001). Use of numerical symbols by the chimpanzee (*Pan troglodytes)*: cardinals, ordinals, and the introduction of zero. *Animal Cognition, 4*(3-4), 193-199. https://doi.org/10.1007/s100710100086

Brannon, E. M., & Terrace, H. S. (2000). Representation of the numerosities 1 - 9 by Rhesus Macaques (*Macaca mulatta*). *Journal of Experimental Psychology: Animal Behavior Processes, 26*(1), 31-49. https://doi.org/10.1037/0097-7403.26.1.31

Cammaerts, M-C. (2004). Classical conditioning, temporal learning and spatial learning in the ant *Myrmica sabuleti. Biologia, 59*, 243-256. https://citeseerx.ist.psu.edu › viewdoc › download

Cammaerts, M.-C. (2008). Visual discrimination of cues differing as for their number of elements, their shape or their orientation, by the ant *Myrmica sabuleti*. *Biologia*, *63*(6), 1169-1180. https://doi.org/10.2478/s11756-008-0172-2

Cammaerts, M.-C. (2010). Estimation of elapsed time by ants. *Bulletin de la Société Royale Belge d'Entomologie*, *146*, 189-195. https://biblio.naturalsciences.be › arti...

Cammaerts, M.-C. (2013a). Spatio-temporal learning in three *Myrmica* species (Hymenoptera, Formicidae). *Bulletin de la Société Royale Belge d'Entomologie*, *49*, 131-138. https://biblio.naturalsciences.be › srbe-147-2011-1

Cammaerts, M.-C. (2013b). Age dependent spatio-temporal learning in the ant *Myrmica sabuleti* (Hymenoptera, Formicidae). *Bulletin de la Société Royale Belge d'Entomologie*, *149*, 205-212. https://biblio.naturalsciences.be › srbe-147-2011-1

Cammaerts, M.-C., & Cammaerts, R. (2015c). Expectative behavior can be acquired by ants in the course of their life. *Trends in Entomology, 11*, 73-83.

Cammaerts, M.-C., & Cammaerts, R. (2016b). Ants can expect the time of an event on basis of previous experiences. *ISRN Entomology*, Article ID 9473128. 9 pages. https://doi.org/10.1155/2016/9473128

Cammaerts M.-C., & Cammaerts, R. (2019a). Ants' capability of adding numbers of identical elements. *International Journal of Biology, 11*(3), 25-36. https://doi.org/10.5539/ijb.v11n3p25

Cammaerts M.-C., & Cammaerts, R. (2019b). Ants fail to add numbers of same elements seen consecutively. *International Journal of Biology, 11*(3), 37-48. https://doi.org/10.5539/ijb.v11n3p37

Cammaerts, M.-C., & Cammaerts, R. (2020d). Ants' numerosity ability defined in nine studies. *Journal of Biology and Life Sciences, 11* (1), 121-142. https://doi.org/10.5296/jbls.v11i1.16278

Cammaerts, M.-C., & Cammaerts, R. (2020e). Summary of seven more studies on numerosity abilities in an ant, four of them relating to human competence. *Journal of Biology and Life Sciences, 11*(2), 296-326. https://doi.org/10.5296/jbls.v11i2.17892

Cammaerts, M.-C., & Cammaerts, R. (2021a). Ants can anticipate and correctly increment the last quantity of a learned arithmetic sequence. *International Journal of Biology, 13*(1), 16-25. https://doi.org/10.5539/ijb.v13n1p16

Cammaerts, M.-C., & Cammaerts, R. (2021b). Ants can expect the size of the next element in a geometric sequence of increasing or decreasing shapes, only if this sequence is present. *International Journal of Biology, 13*(2), 37-48. https://doi.org/10.5539/ijb.v13n2p37

Cammaerts M.-C. & Cammaerts R. (2021c). Critical distance between two identical visual cues allowing their mental addition by an ant. *Trends in Entomology*, *17*, 43-56.

237 Cammaerts M.-C. & Cammaerts R. (2021d). Critical vertical distance between two visual cues for allowing ants to mentally add them. *Trends in Entomology*, *17*, 77-80

Cammaerts, M.-C., & Cammaerts, R. (2022a). A synthesis of six recent studies on numerosity abilities in an ant. *Journal of Biology and Life Sciences*, *13*(1), 1-23. https://doi.org/10.5296/jbls.v13i1.19346

Cammaerts, M.-C., & Cammaerts R. (2022b). Association between visual cues and time of day in an ant. *Journal of Ethology.* https://doi.org/10.1007/s10164-022-00751-4

Cammaerts M.-C., & Cammaerts R. (2022c). Maximum time interval between two visual cues that still allows an ant to add them up. *Trends in Entomology,* in press.

Cammaerts, M.-C., Rachidi, Z., & Cammaerts, D (2011) Collective operant conditioning and circadian rhythms in the ant *Myrmica sabuleti* (Hymenoptera, Formicidae). *Bulletin of the Belgian Entomological Royal Society, 147*, 142-154. Retrieved from https://biblio.naturalsciences.be>srbe-147-2011

Cheeseman, J. F., Winnebeck, E. C., Millar, C. D., Kirkland, L. S., Sleigh, J., Goodwin, M., … Warman, G. R. (2012) General anesthesia alters time perception by phase shifting the circadian clock. *PNAS, 109*(18), 7061-7066. https://doi.org/10.1073/pnas.1201734109

Cox, L., & Montrose, V. T. (2016). Quantity discrimination in domestic rats, *Rattus norvegicus*. *Animals*, *6*, 46. https://doi.org/10.3390/ani6080046

Eban-Rothschild, A., & Bloch, G. (2012) Circadian Rhythms and Sleep in Honey Bees. In: Galizia C, Eisenhardt D, Giurfa M. (eds) Honeybee Neurobiology and Behavior. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-2099-2_3

Cox, L., & Montrose, V. T. (2016). Quantity discrimination in domestic rats, *Rattus norvegicus*. *Animals*, *6*, 46. https://doi.org/10.3390/ani6080046

Eban-Rothschild, A., & Bloch, G. (2012). Circadian Rhythms and Sleep in Honey Bees. In: Galizia C, Eisenhardt D, Giurfa M. (eds) Honeybee Neurobiology and Behavior. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-2099-2_3

Enquist, M., Lind, J., & Ghirlanda, S. (2016). The power of associative learning and the ontogeny of optimal behaviour. *Royal Society of Open Science, 3,* 160734. http://dx.doi.org/10.1098/rsos.160734

Flombaum, J. I., Junge, J. A., & Hauser, M. D. (2005). Rhesus monkeys (*Macaca mulatta)* spontaneously compute addition operations over large numbers. *Cognition, 97*, 315-325. https://doi.org/10.1016/j.cognition.2004.09.004

Kershenbaum, A., Blumstein, D. T., Roch, M. A., Akcay, C., Backus, G., Bee, M. A., & al. (2014). Acoustic sequences in non-human animals. *Biological Reviews, 92,* 13-52. https://doi.org/10.1111/brv.12160

Montagner, H. (2008). Les temps, les rythmes et la sécurité affective de l'enfant, fondements obligés de l'aménagement du temps scolaire. *Cahiers Pédagogiques.* https://www.cahiers-pedagogiques.com›les-temps-les-r...

Murphy, P. J., & Campbell, S. S. (1996) Physiology of the circadian system in animals and humans. *Journal of Clinical Neurophysiology, 13*(1), 2-16. https://doi.org/10.1097/00004691-199601000-00002

Osvath, M., & Osvath, H. (2008). Chimpanzee (*Pan troglodytes*) and orangutan (*Pongo abelii*) forethought: Self-control and pre-experience in the face of future tool use. *Animal Cognition, 11*, 661-674. https://doi.org/10.1007/s10071-008-0157-0

Pepperberg, I. M. (2012). Further evidence for addition and numerical competence by a Grey parrot (*Psittacus erithacus*). *Animal Cognition, 15*(4), 711-717. https://doi.org/10.1007/s10071-012-0470-5

Pepperberg, I. M., & Gordon, J. D. (2005). Number comprehension by a grey parrot (*Psittacus erithacus*), including a zero-like concept. *Journal of Comparative Psychology, 119*(2), 197–209. https://doi.org/10.1037/0735-7036.119.2.197

Rivkees, S. A. (2007). The development of circadian rhythms: from animals to humans. *Sleep Medicine Clinics, 2*(3), 331-341. https://doi.org/10.1016/j.jsmc.2007.05.010

Rodriguez, R. L., Briceno, R. D., Briceno-Aguilar, E., & Höbel, G. (2015). *Nephila clavipes* spiders (Araneae: Nephilidae) keep track of captured prey counts: testing for a sense of numerosity in an orb-weaver. *Animal Cognition, 18*(1), 307-314. https://doi.org/10.1007/s10071-014-0801-9

Rose, G. J. (2018). The numerical abilities of anurans and their neural correlates: insights from neuroethological studies of acoustic communication. *Proceedings of the Royal Society: B,* 373. http://doi.org/10.1098/rstb.2016.0512

Rugani, R., Fontanari, L., Simoni, E., Regolin, L., & Vallortigara, G. (2009). Arithmetic in newborn chicks. *Proceedings of the Royal Society: B, 276*, 2451-2460. http://doi.org/10.1098/rspb.2009.0044

Rugani, R., Vallortigara, G., Priftis, K., & Regolin, L. (2015). Number-space mapping in the newborn chick resembles humans' mental number line. *Science, 347*(6221), 534–536. https://doi.org/10.1126/science.aaa1379

Xia, L., Sieman, M., & Delius, J. D. (2000). Matching of numerical symbols with number of responses by pigeons. *Animal Cognition*, *3*, 35–43. https://doi.org/10.1007/s100710050048

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