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Ants Can Associate a Symbol with a Number of Elements Through Conditioning

Marie-Claire Cammaerts[1] & Roger Cammaerts[2]

[1] Independent researcher, retired from the Biology of Organisms Department, University of Brussels, Belgium

[2] Independent researcher, retired from the Natural and Agricultural Environmental Studies Department (DEMNA) of the Walloon Region, Belgium

Correspondence: Marie-Claire Cammaerts, independent researcher, 27, Square du Castel Fleuri, 1170 Bruxelles, Belgium. Tel: 32-2-673-4969. E-mail: mccammaerts@gmail.com

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Abstract

On the basis of the known numerosity abilities of the ant *Myrmica sabuleti*, it was checked if workers of this species could associate a symbol with a number of elements through conditioning, an ability rarely encountered in invertebrates and never mentioned in ants. Working each time on two colonies, we observed that these ants could associate a particular shape used as a symbol with 1, 2, 3 or 4 displayed dots. This acquired association was only slightly affected by a change in shape, color or size of the sighed elements. This elementary association of a symbol with a number by an ant has also been observed in bees, while vertebrates such as birds and monkeys can bring this ability at a more complex level.

Keywords: *Myrmica sabuleti*, Numbers Discrimination, Operant Conditioning, Symbolism

1. Introduction

Workers of the ant *Myrmica sabuleti* Meinert, 1861 can add and subtract an element from a number of elements when they see the result of this operation during training (Cammaerts & Cammaerts, 2019 b, c, e) and can acquire the notion of zero through experiences as well as locate this zero at its due numerical place (Cammaerts & Cammaerts, 2019a,d, 2020b). These ants have natively a left to right mental number line on which they non-linearly represent quantities 1 to 7 (non-symbolic elements such as dots) (Cammaerts & Cammaerts, 2019f, 2020a, b). Abilities of this kind have been found in other invertebrates. The best documented studies concern honeybees which were shown to also add and subtract under conditioning, and to have the notion of zero (Howard, Avarguès-Weber, Garcia, Greentree, & Dyer, 2018, 2019a). Even some spiders can estimate the amount of their prey (in a Nephilid: Rodriguez, Briceno, Briceno-Aguilar, & Höbel, 2015; in a Salticid: Cross & Jackson, 2017) and males of the mealworm beetle, *Tenebrio molitor*, can estimate the number of mating competitors (Carazo, Fernandez-Perea, & Font, 2012). Numerosity ability has been more studied in non-human vertebrates, for instance in fishes (Agrillo, Dadda, Serena, & Bisazza, 2008, 2009; Agrillo, Miletto Petruzzini, & Bisazza, 2017), pigeons (Brannon, Wusthoff, Gallistel & Gibbon, 2001), parrots (Pepperberg, 2006a), robin birds (Garland & Low, 2014), and in monkeys (Beran, 2008).

An invertebrate, the honeybee, has been shown to master numerical symbolism. It can be learned to associate a symbol such as a given color with an addition or a subtraction to be made (Howard et al., 2019a) or to associate symbols such as shapes with a given number of elements (2 or 3 dots) (Howard, Avarguès-Weber, Garcia, Greentree, & Dyer, 2019b). As expected, vertebrates were more tested as for this ability. Pigeons can associate visual symbols and up to 4 elements (Xia, Siemann, & Delius, 2000) and a parrot (*Psittacus erithacus*) could be learned to associate vocal and visual symbols with up to at least 6 elements (Pepperberg, 2006a, b). Chimpanzees (*Pan troglodytes*) can associate Arabic numerals with 1 up to 7 or 9 elements (Murofushi, 1997; Matsuzawa, 1985; Beran, 2004) including the symbol 0 for "nothing" (Biro & Matsuzawa, 2001) and make summation using Arabic numerals 0 to 4 (Boysen & Berntson, 1989). Arabic numerals 0 to 9 could also be associated with their corresponding number of food items by rhesus monkeys (*Macaca mulatta*: Washburn & Rumbauch, 1991), squirrel monkeys (*Saimiri sciureus*: Olthof, Iden, & Roberts, 1997), and capuchin monkeys (*Cebus apella*: Beran et al., 2008).

We here intended to learn ants to associate a specific symbol with 1 to 4 elements (dots), experimenting with each number of elements on two *M. sabuleti* ant colonies.

2. Material and Methods

2.1 Collection and Maintenance of Ants

The experiments were conducted on eight colonies of *M. sabuleti* (labeled A to H) collected in September 2019 in an abandoned quarry located at Olloy/Viroin (Ardenne, Belgium). These colonies nested in grass and under stones; they contained about 500 workers, a queen and brood. Each colony was maintained in the laboratory in one to two glass tubes half filled with water, with a cotton plug separating the ants from the water. The nest tubes of each colony were deposited in a tray (30cm x 15cm x 5cm) serving as foraging area. In this tray, pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were deposited three times per week on a glass piece, and sugar water was permanently provided in a cotton plugged tube. The ambient temperature was *ca* 20°C, the humidity *ca* 80%, the lighting 330 lux while working on ants, and the electromagnetism 2μWm^2.

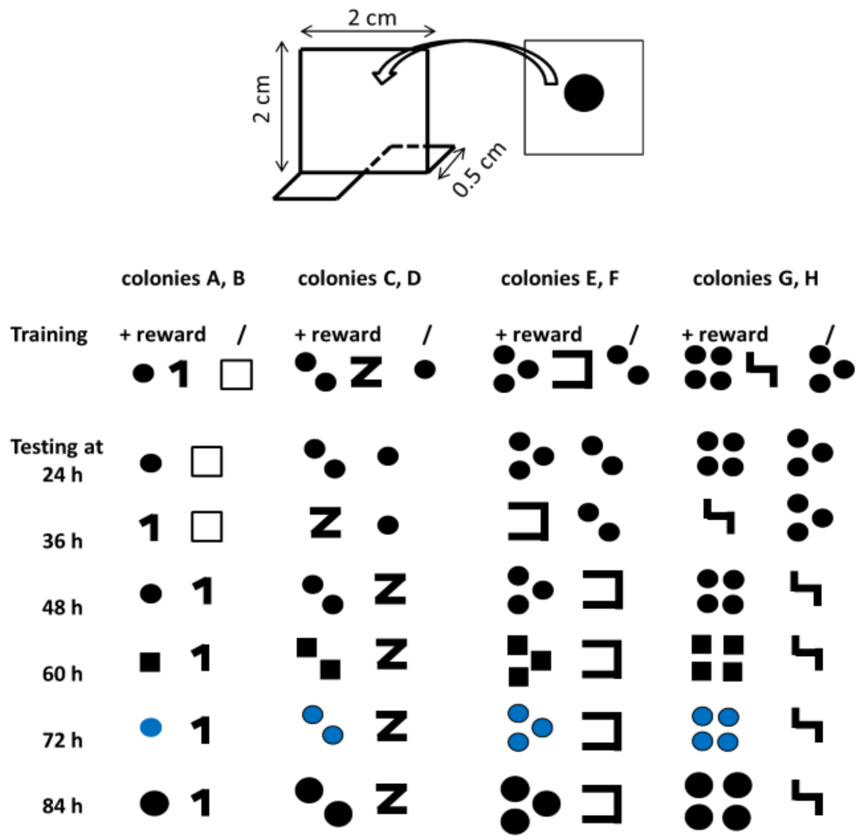

Figure 1. Cues (numbers of elements, symbols, changes of shape, color or size of the elements) used for examining if ants could associate symbols with small numbers of elements

Foraging ants of two colonies were trained to a number (1 or 2 or 3 or 4) of elements and a corresponding symbol (the 'correct cues') set near a reward while another number of elements (the 'wrong' cue) was set far from the reward (/ = no reward). They were tested in front of the 'correct' and the 'wrong' number of elements or in front of the symbol. Furthermore, testing was also made with the 'correct' number of elements having had its shape, color or size changed. The experimental protocol is schematized in Figure 2, numerical results are given in Tables 1, 2, 3, 4 and photos of the experiments are shown in Figures 3, 4, 5, 6.

2.2 Experimental Planning

The colonies A and B were used to examine if ants can associate the symbol "1" with one element (a black circle). In the same way, the colonies C and D, E and F, G and H were used to examine if ants can associate another particular symbol with 2, 3, or 4 elements. Each time six experiments were performed to check if the ants have memorized the number of elements (1, 2, 3, or 4), have memorized the correspondence of the particular symbol with that number, have associated the learned number with its symbol, and if this association was independent of – or slightly dependent on – the shape, the color or the size of the elements representing the number (1, 2, 3 or 4).

2.3 Cues Presented to the Ants

The cues are schematized in Figure 1.

The elements representing the numbers 1 to 4 were black circles (diameter = 0.5cm). Each one was drawn in a square (2cm x 2cm) using Word® software. The squares containing the represented numbers were printed and cut. Each square was then tied on the vertical front face of a stand (2cm x 2cm) made of strong white paper (Steinbach®), and maintained vertically thanks to a horizontal part [2 x (1cm x 0.5cm)] duly folded.

Each colony was provided with a given number of elements and its corresponding symbol near its food as well as, far from food, with the same number of elements minus 1. The ants of each colony were tested six times in front of two different cues as detailed below.

Colonies A and B were provided with a black circle and its corresponding symbol near their food, and, far from food, with a white paper (i.e. 0 (zero) element). In the same way, colonies C and D, E and F, G and H were provided near their food with 2, 3, and 4 black circles respectively and with a symbol corresponding to the number of circles perceived; they were also provided respectively with 1, 2, and 3 black circles far from food.

During the six tests, foraging ants of the eight colonies were confronted successively, after 24 training hours, to the number of elements set near food and that set far from food, after 36 hours, to the symbol set near the food and the number of elements set far from food, after 48 hours, to the number of elements and its symbol set near food, then after 60 hours, to the symbol and the number of elements set near food, but these elements having another shape, after 72 hours, to the symbol and the number of elements set near food, but these elements having another color, and after 84 hours, to the symbol and the number of elements set near food, but these elements having another size. Of course, the cues (numbers of elements, symbols) used during testing were not those used during training, but were new, never used ones. Note that the workers of the ant *M. sabuleti* can distinguish all the colors, even under low light intensities and are, among others, very sensitive to blue (Cammaerts, 2007; Cammaerts M.-C. & Cammaerts D., 2009). These ants have also a rather long-lasting visual memory (Cammaerts M.-C., Rachidi, & Cammaerts, D., 2011).

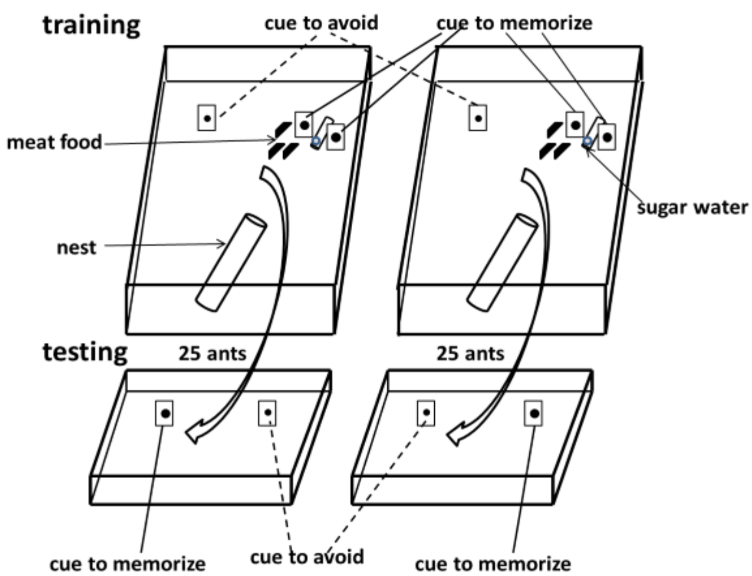

Figure 2. Experimental design schematized for helping understanding the protocol of the experiments

The foraging ants of two colonies were trained on their foraging area (upper schema) to two cues to memorize, i.e. a number of elements and a corresponding symbol. They were tested in separate trays (lower schema) for checking if they could associate the presented number of elements and its corresponding symbol. The cues are schematized in Figure 1; photos are shown in Figures 3, 4, 5, 6, and results are given in Tables 1, 2, 3, 4.

2.4 Training and Testing

This is illustrated in Figure 2.

Training. The ants were trained on their foraging area, where a given number of elements and its corresponding symbol (the two 'correct' cues) were set on stands aside the food (the reward) with their position relative to the sugar water tube differing for each of the two colonies trained to the same number of elements (see the photos of the experiments in Figures 3 to 6). Moreover, the same number of elements minus one (the 'wrong' cue) was always set far from food. The entire training period was 84 hours. During that time, the ants present all around the three presented cues were counted 16 times over the 84 hours (i.e. twice each day for each colony), and the mean of these counts was established. The latter information, provided in the text only, is given for showing that the number of ants present during training around the cues was sufficient to allow them associating the cues with the reward.

Testing. The foraging ants were tested after 24, 36, 48, 60, 72 and 84 elapsed training hours, each time in front of a pair of different cues set at about 8 cm from one another in a separate tray (21cm x 15cm x 7cm), the borders of which having been slightly covered with talc to prevent escaping. These two cues are schematized in Figure 1 and enumerated in sub section 2.3. To make a test, 25 foragers (a number enabling to easily count the ants according to the size of the tray devoted to testing) were transferred at once from their foraging area to the tray devoted to testing and deposited in front of the middle space between the two cues. These ants move continuously and freely in their new space, staying only a few seconds at the same place. The same ants could thus go to the different cues or elsewhere. The numbers of those approaching each kind of cue at a distance less than 2 cm were punctually counted 20 times (at each 30 seconds) in the course of 10 experimental minutes. The twenty numbers chronologically obtained for each cue were summed by four to obtain five groups of numbers. The five pairs of groups of numbers so obtained for the two cues were compared to one another using the non-parametric matched-pairs signed-ranks test of Wilcoxon, reading the critical P value in Siegel & Castellan's (1988) Table H for small sample sizes. Note that the counts for each two colonies trained and tested to the same cues could be added because these colonies reacted in the same way (see the % of responses given in Tables 1 – 4). These counts also allowed calculating the proportion of responses to each cue (see Tables 1 to 4). Half of the tests were made with the 'correct' cue set on the left, and half of the tests with the 'correct' cue set on the right. After each test, the ants were transferred again into their nest, being deposited near their nest entrance.

3. Results

We separately relate the results relative to the quantities 1, 2, 3 and 4. Each time, we successively comment the results corresponding to the ants training, to the ants' response to the 'correct' number *vs* the 'wrong' one, to the symbol *vs* the 'wrong' number, to the 'correct' number *vs* the symbol, and after that, to the symbol *vs* the 'correct' number, the latter being then represented by elements with another shape, color or size than those used during training.

Table 1. Ants' learning of a symbol corresponding to number 1

Cues presented to the ants during the tests	N° of ants of colonies A and B sighted near one and the other cue (mean % for each cue)	Number of ants counted near each of the two cues, chronologically allocated in five groups	P value for the paired groups of numbers
1 black circle; a blank paper	51 and 59 (72.4%); 13 and 29 (27.6%)	20,26,23,24,17; 6,11,12,8,5	0.031
symbol for 1; a blank paper	45 and 43 (74.6%); 12 and 18 (25.4%)	13,16,18,19,22; 3,6,5,6,10	0.031
1 black circle; symbol for 1	29 and 34 (48.1%); 30 and 38 (51.9%)	15,14,13,10,11; 15,14,14,12,13	0.125
1 black square; symbol for 1	30 and 34 (46.7%); 35 and 38 (53.3%)	14,15,13,8,14; 15,15,12,13,18	0.157
1 blue circle; symbol for 1	24 and 21 (44.1%); 30 and 27 (55.9%)	6,10,10,8,11; 10,11,15,11,10	0.079
1 larger circle; symbol for 1	32 and 33 (47.4%); 36 and 36 (52.6%)	13,15,15,12,10; 14,17,19,14,8	0.223

Trained to 1 black circle and a symbol for 1 set near the food, the tested ants reacted more to these two cues than to a blank paper set far from food, and equally to these two cues even if the element representing the number 1 had a different shape, color, or size. They thus duly associated the symbol for 1 with its corresponding number of elements. Details are given in the text and photos are shown in Figure 3.

Table 2. Ants' learning of a symbol corresponding to number 2

Cues presented to the ants during the tests	N° of ants of colonies C and D sighted near one and the other cue (mean % for each cue)	Number of ants counted near each of the two cues, chronologically allocated in five groups	P value for the paired groups of numbers
2 black circles; 1 black circle	41 and 37 (72.9%); 14 and 15 (27.1%)	17,13,15,13,20; 6,4,7,6,6	0.031
symbol for 2; 1 black circle	48 and 42 (65.7%); 27 and 20 (34.3%)	14,19,16,19,22; 7,9,9,12,10	0.031
2 black circles; symbol for 2	24 and 34 (48.7%); 24 and 37 (51.3%)	13,13,10,10,12; 12,13,13,8,15	0.313
2 black squares; symbol for 2	41 and 28 (45.4%); 49 and 34 (54.6%)	11,15,13,14,16; 12,14,20,18,19	0.079
2 blue circles; symbol for 2	34 and 30 (48.9%); 35 and 32 (51.1%)	16,14,13,10,11; 16,16,10,10,15	0.375
2 larger circles; symbol for 2	40 and 31 (46.4%); 46 and 36 (53.6%)	11,13,15,16,16; 15,15,21,14,17	0.125

Trained to 2 black circles and a symbol for 2 set near the food, the tested ants reacted more to these two cues than to 1 black circle set far from food, and equally to these two cues even if the elements representing the number 2 had a different shape, color, or size. They thus duly associated the symbol for 2 with its corresponding number of elements. Details are given in the text and photos are shown in Figure 4.

Table 3. Ants' learning of a symbol corresponding to number 3

Cues presented to the ants during the tests	N° of ants of colonies E and F sighted near one and the other cue (mean % for each cue)	Number of ants counted near each of the two cues, chronologically allocated in five groups	P value for the paired groups of numbers
3 black circles; 2 black circles	46 and 42 (69.3%); 25 and 14 (30.7%)	15,19,21,17,16; 5,5,12,9,8	0.031
symbol for 3; 2 black circles	46 and 43 (70.1%); 22 and 16 (29.9%)	14,20,18,18,19; 5,10,8,8,7	0.031
3 black circles; symbol for 3	44 and 32 (48.7%); 47 and 33 (51.3%)	14,14,12,14,22; 15,14,15,14,22	(NS)
3 black squares; symbol for 3	31 and 41 (47.1%); 38 and 43 (52.9%)	10,11,14,17,20; 11,11,17,20,22	0.063
3 blue circles; symbol for 3	51 and 40 (47.2%); 58 and 44 (52.8%)	16,16,21,19,19; 19,20,23,25,15	0.188
3 larger circles; symbol for 3	40 and 32 (48.3%); 38 and 39 (51.7%)	12,13,14,19,14; 12,14,16,19,16	0.125

Trained to 3 black circles and a symbol for 3 set near the food, the tested ants reacted more to these two cues than to 2 black circles set far from food, and equally to these two cues even if the elements representing the number 3 had a different shape, color, or size. They thus duly associated the symbol for 3 with its corresponding number of elements. Details are given in the text and photos are shown in Figure 5.

Table 4. Ants' learning of a symbol corresponding to number 4

Cues presented to the ants during the tests	N° of ants of colonies G and H sighted near one and the other cue (mean % for each cue)	Number of ants counted near each of the two cues, chronologically allocated in five groups	P value for the paired groups of numbers
4 black circles; 3 black circles	36 and 49 (67.5%); 20 and 21 (32.5%)	16,20,16,17,16; 5,11,6,9,10	0.031
symbol for 4; 3 black circles	38 and 36 (74.7%); 11 and 14 (25.3%)	19,15,13,14,13; 4,5,7,3,6	0.031
4 black circles; symbol for 4	45 and 32 (49.4%); 48 and 31 (50.6%)	9,18,16,17,17; 9,20,20,13,17	0.500
4 black squares; symbol for 4	30 and 32 (45.9%); 35 and 38 (54.1%)	12,15,12,9,14; 10,19,16,14,14	0.125
4 blue circles; symbol for 4	28 and 35 (44.4%); 34 and 45 (55.6%)	13,16,12,12,10; 17,20,15,12,15	0.063
4 larger circles; symbol for 4	25 and 38 (46.3%); 33 and 40 (53.7%)	9,13,14,14,13; 12,15,15,14,17	0.063

Trained to 4 black circles and a symbol for 4 set near the food, the tested ants reacted more to these two cues than to 3 black circles set far from food, and equally to these two cues even if the elements representing the number 4 had a different shape, color, or size. They thus duly associated the symbol for 4 with its corresponding number of elements. Details are given in the text and photos are shown in Figure 6.

3.1 Learning a Symbol Corresponding to 1 Element

Numerical results are given in Table 1 and photos are shown in Figure 3.

During their training, the ants of colonies A and B were meanly 9.5 in moving or staying near the three presented cues; they thus could be efficiently trained to the sighted cues.

Tested in front of the black circle and the blank paper, the ants presented a significant conditioning score (i.e. a proportion of correct responses) of 72.4% (P = 0.031). In front of a symbol corresponding to number 1 and a blank paper, the ants similarly presented a significant conditioning score (74.6%, P = 0.031). Faced with a black circle and the symbol for 1, the ants went equally to the two cues (48.1% and 51.9%; P = 0.125). Consequently, the ants acquired similar conditioning to a black circle and to the symbol for 1.

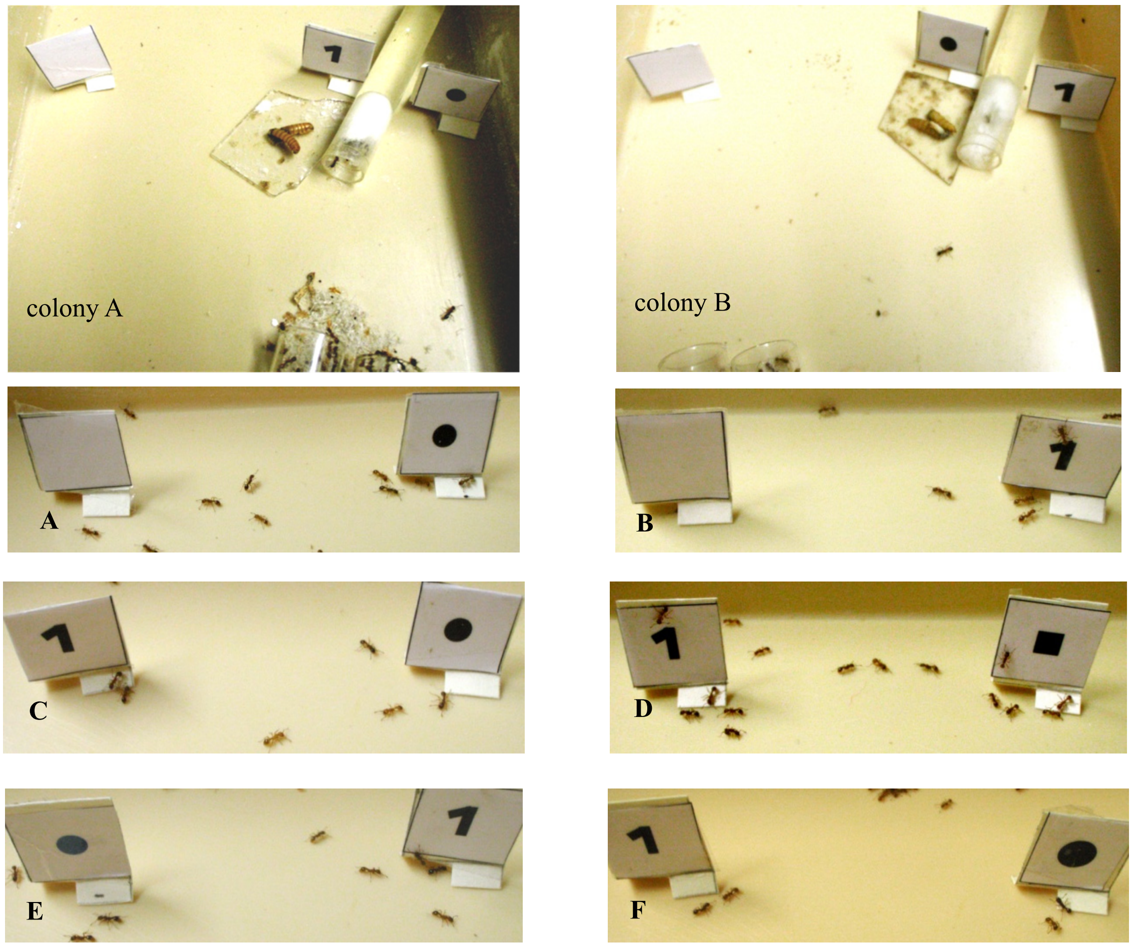

Figure 3. Photos of the experiments made on colonies A and B for learning to the ants a symbol corresponding to 1 element

The ants duly reacted more to 1 black circle and its symbol than to a blank paper (A, B), equally to the 1 element and its symbol (C), and nearly equally to the symbol "1" and the 1 element with a different shape (D), color (E) or size (F). The ants have thus associated the symbol "1" with the number 1.

Did they keep such equal response when the element has another shape, color or size? Faced with a black square and the symbol for 1, 46.7% of the ants went to the black square and 53.3% to the symbol, the difference being not significant (P = 0.157). Faced with a blue circle and the symbol for 1, the tested ants reacted slightly more to the symbol for 1 (55.9%) than to the blue circle (44.1%), but this difference was not significant (P = 0.079). Tested in presence of a black circle larger than that provided during training and the symbol for 1, the ants went statistically equally to the two cues (P = 0.223). The somewhat lower, though not significant, responses to the black square, the blue circle and the larger circle as compared to the symbol could be accounted for the fact that the ants never saw such cues during their training while they continuously saw the symbol "1". On the basis of the three here above related experiments, we can conclude that the ants' learning of a symbol corresponding to the numerosity 1 was in a broad extent independent of the characteristics (shape, color, size) of the element representing the number 1, changes in these characteristics having had only a slight influence. It should be noted that in a previous experiment (Cammaerts & Cammaerts, 2020d), the counting ability of ants was also, though not significantly, affected by changes in the features of the learned elements.

3.2 Learning a Symbol Corresponding to 2 Elements

Numerical results are given in Table 2 and photos are shown in Figure 4.

During their training, meanly 14.0 ants of colonies C and D were present at any time all around the three presented cues, i.e. two black circles and its corresponding symbol "Z" (the correct cues) and a black circle (the wrong cue). The ants had thus the opportunity to be well trained to the sighted cues.

Faced with 2 black circles and 1 black circle, the tested ants went essentially to the stand bearing two circles (72.9% of the ants; P = 0.031). In front of the "Z" symbol for 2 and 1 black circle, the ants went significantly more to the former cue than to the latter (65.7% vs 34.3%; P = 0.031). When tested faced with 2 black circles and the symbol for 2, the ants went equally to the two cues (48.7% and 51.3%; P = 0.313). The ants acquired thus similar conditioning to the 2 black circles and to the "Z" symbol for 2, associating the symbol for 2 with the 2 black circles.

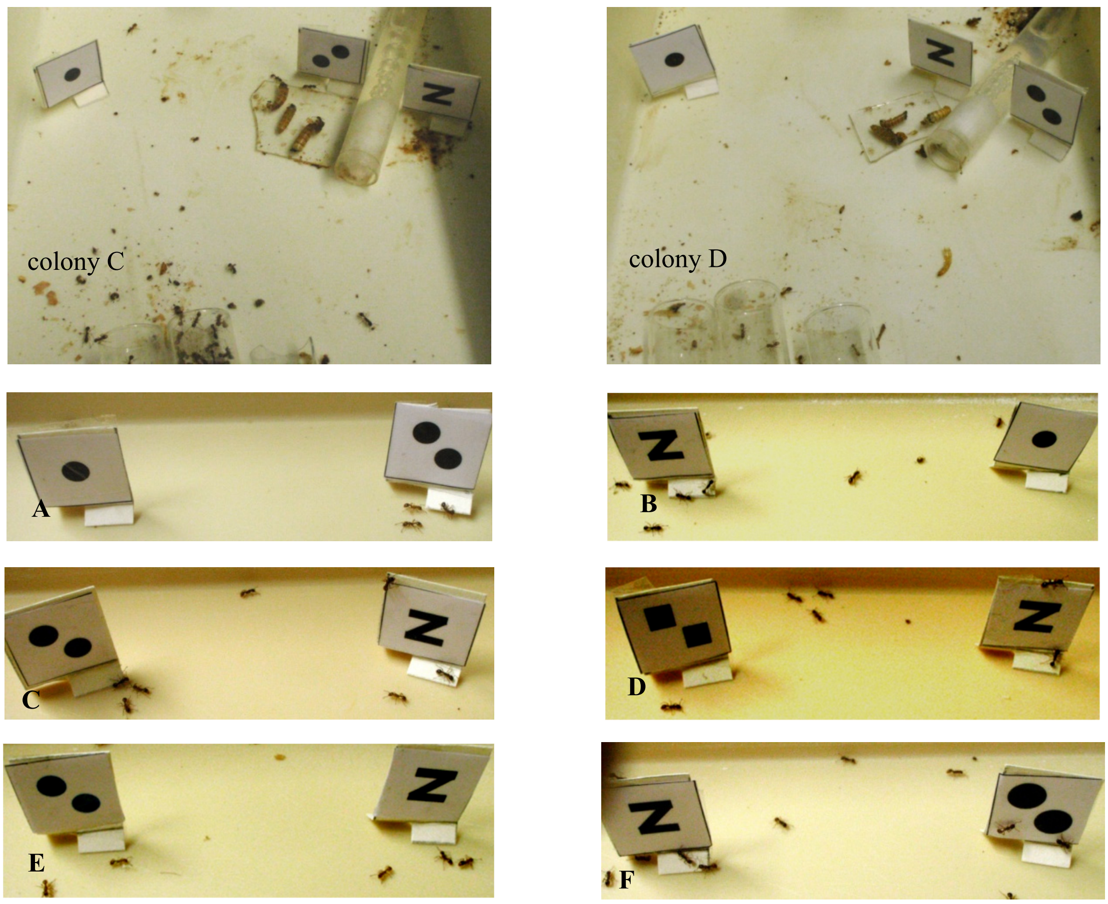

Figure 4. Photos of the experiments made on colonies C and D for learning to the ants a symbol corresponding to 2 elements

The ants duly reacted more to 2 elements and its symbol than to a black circle (A, B), equally to the 2 elements and its symbol (C), and nearly equally to the symbol and the corresponding number of elements, the later having a different shape (D), color (E) or size (F). The ants have thus associated a symbol for 2 with the number 2.

Did they maintain this association when the two circles have another shape, color or size? Confronted with 2 black squares and the "Z" symbol for 2, the ants went nearly equally to the two cues, but somewhat preferred the symbol (54.6% of the ants), although this difference was not significant (P = 0.079). Faced with 2 blue circles and the symbol for 2, the ants went similarly to the two cues (P = 0.375). Tested in front of 2 circles larger than those used for training and the symbol for 2, the ants reacted nearly equally to the two cues, going only slightly but not significantly more to the symbol (53.6% of the ants; P = 0.125). Thus, the ants somewhat less responded to the 2 elements which have been changed than to their symbol. This can be accounted for the fact that these changed elements somewhat differed from those they saw during training. The ants' association between a symbol and two elements was thus only slightly influenced by the appearance of the elements. Such an effect was also found in another experimental work (Cammaerts & Cammaerts, 2020d). The above related experiments show that the ants acquired numerosity symbolism for the amount 2 through conditioning.

3.3 Learning a Symbol Corresponding to Number 3

Numerical results are given in Table 3 and photos are shown in Figure 5.

During their training, meanly 14.0 ants of colonies E and F were continuously sighted in the vicinity of the three presented cues, i.e. three black circles and a symbol for this number (the correct cues) as well as two black circles (the wrong cue). They had thus the opportunity to be well-trained.

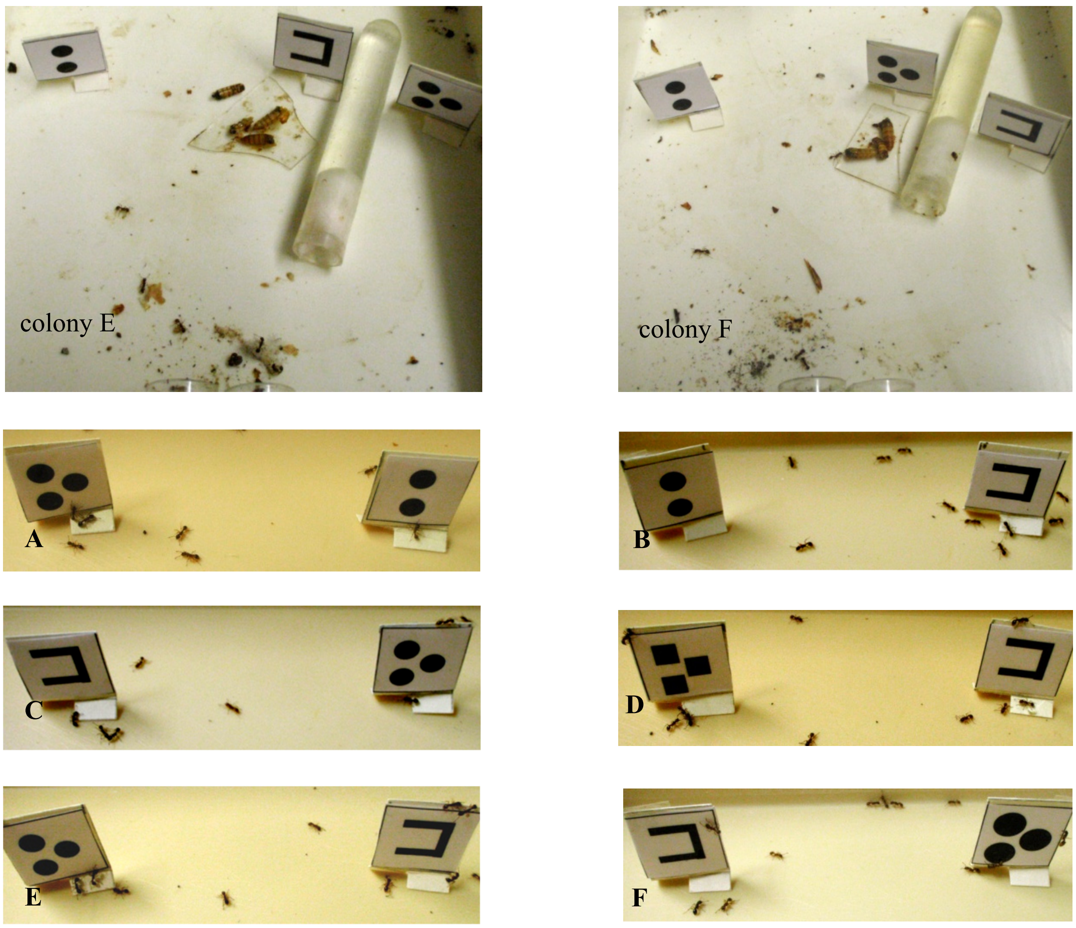

Figure 5. Photos of the experiments made on colonies E and F for learning to the ants a symbol corresponding to the number 3

The ants duly reacted more to the number 3 and its symbol than to 2 black circles (A, B), equally to the number 3 and its symbol (C), and nearly equally to the symbol for 3 and to 3 elements which had a different shape (D), color (E) or size (F). The ants have thus associated the symbol for 3 with 3 elements.

Tested faced with 3 black circles and 2 black circles, the ants went significantly more to the 3 black circles (69.7% of the ants; P = 0.031). Confronted to the symbol for 3 and 2 black circles, the ants also reacted essentially to the symbol (70.1%; P = 0.031). Faced with 3 black circles and the symbol for 3, the ants went statistically equally to the two cues (NS). The ants have thus associated through conditioning the symbol for 3 with the presence of 3 elements. Did this association subsist when the 3 elements had another appearance than that they presented during training?

For answering the later question, the ants were confronted to the symbol for 3 and to the 3 elements with their shape, color or size changed. Faced with 3 black squares and the symbol for 3, the ants went nearly equally to the two cues though, at the limit of significance (P = 0.063), somewhat more to the symbol (52.9%). Tested in front of 3 blue circles and the symbol for 3, the ants nearly equally approached the two cues, but also somewhat more the symbol (52.8%), the difference between their two choices being however not significant (P = 0.188). In front of 3 black circles but larger than those presented during training and the symbol for 3, the tested ants moved nearly equally to these two cues (with 51.7% going to the symbol), the difference being also not significant (P = 0.125). The somewhat lesser number of ants going to the 3 squares, the 3 blue circles and the 3 larger circles could result from the difference in similarity between these cues and those seen during training (a same effect was previously shown in Cammaerts & Cammaerts, 2020d). These described experiments allow concluding that the ants' association of the symbol for 3 with three elements was statistically independent of the appearance of these elements. The ants acquired thus, through conditioning, some numerosity symbolism for the amount 3.

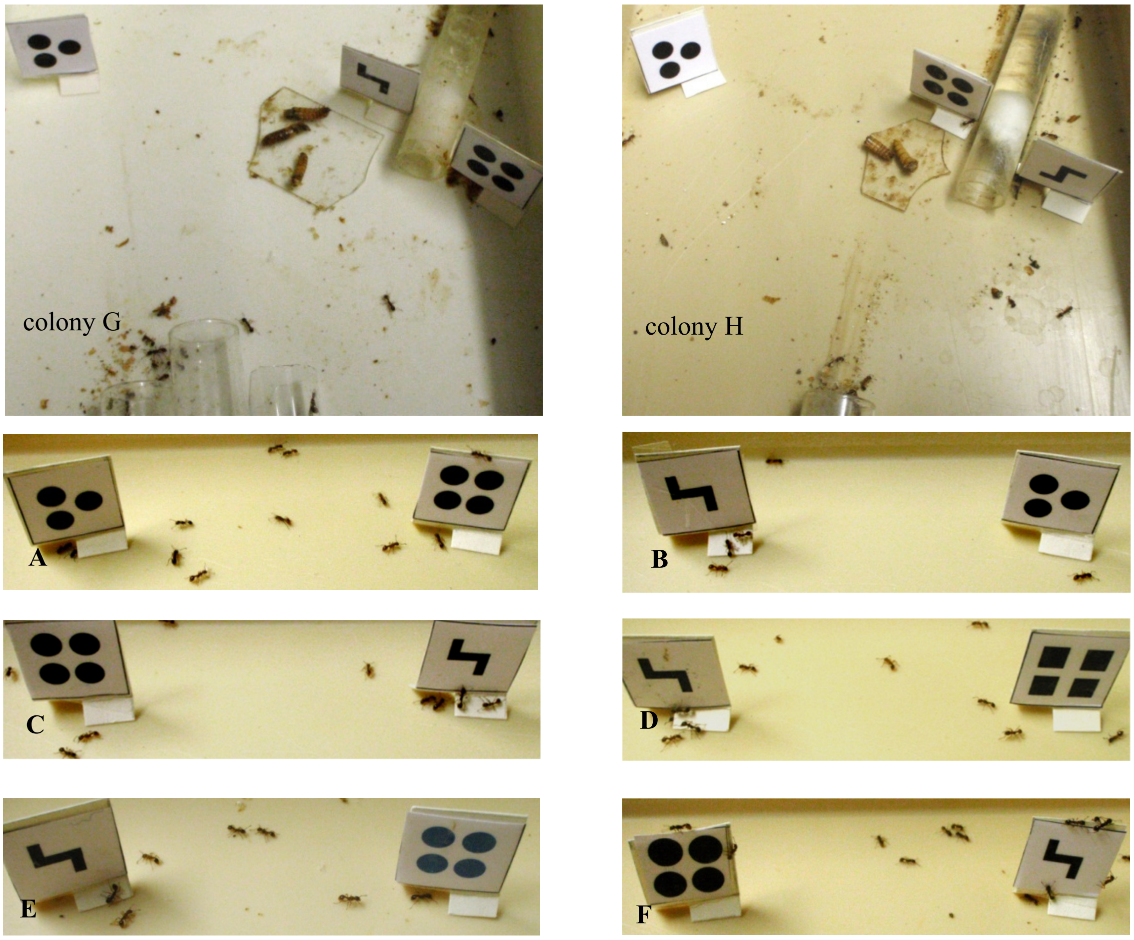

Figure 6. Photos of the experiments made on colonies G and H for learning to the ants a symbol corresponding to the number 4

The ants duly reacted more to the number 4 and its symbol than to 3 black circles (A, B), equally to the number 4 and its symbol (C), and nearly equally to the symbol representing 4 and the number 4, the elements of which had a different shape (D), color (E) or size (F). The ants have thus associated the symbol for 4 with the number 4.

3.4 Learning a Symbol Corresponding to Number 4

Numerical results are given in Table 4 and photos are shown in Figure 6.

While being trained, meanly 13.0 ants of colonies G and H could be seen at any time all around the three presented cues, i.e. 4 black circles and a symbol for this number (= the correct cues), and 3 black circles (the wrong cue), what was sufficient for being duly trained.

Tested in front of 4 and 3 black circles, the ants went statistically mostly to the 4 black circles (67.5% of the ants; P = 0.031). Faced with the symbol for 4 and 3 black circles, the ants went far more to the symbol than to the circles (74.7% of the ants), a statistically significant difference (P = 0.031). Such a high difference could be explained by the fact that the chosen symbol for 4 might be easier to distinguish from 3 black circles than 4 black circles from 3 black circles. Confronted to 4 black circles and the symbol for 4, the ants approached equally these two cues (P = 0.500). Consequently, under conditioning, the ants could associate the symbol for 4 with 4 elements (4 black circles). Did they keep this association when the 4 elements looked differently than while provided during training?

To answer this last question, the trained ants were tested in front of the symbol for 4 and of the 4 elements which this time differed by their shape, color or size from those used for training. Confronted to 4 black squares and to the symbol for 4, the ants went a little more to the symbol (54.1% of the ants), though not significantly (P = 0.125). Faced with 4 blue circles and the symbol for 4, the ants went somewhat more to the symbol (55.6% of the ants), the difference between these two choices being at the limit of significance (P = 0.063). When tested in front of 4 black circles larger than those presented during training and the symbol for 4, the ants went also slightly more to the symbol, a difference also at the limit of significance (P = 0.063). The ants' somewhat lower response to the 4 squares, the 4 blue circles and the 4 larger circles could be accounted for a difference of appearance between the cues seen during training and those seen during testing (as previously shown for such a difference in Cammaerts & Cammaerts, 2020d). These three last reported experiments showed that the ants' association of the symbol for 4 and four elements was only slightly influenced by the shape, color and size of these elements. It can be admitted that thanks to conditioning, the ants can associate a symbol with the quantity 4.

4. Discussion – Conclusion

Based on the numerosity abilities observed in the ant *M. sabuleti*, and according to findings made on other animal species, especially a few invertebrates (see the Introduction section), we presumed that the workers of this ant could acquire some numerosity symbolism, at least for small numbers, i.e. 1 to 4, and we undertook operant conditioning experiments for checking this presumption. Indeed, we observed that these workers could associate a given symbol with 1 to 4 sighted elements, almost independently of their shape, color and size. This experimental work calls for some remarks.

In this work we observed, as previously (Cammaerts & Cammaerts, 2020d), some slight impact on the ants' counting ability (i.e. recognition of the number of elements) when changes in shape, color, or size of the elements to be counted were made between training and testing. Each of these slight impacts was here revealed thanks to only one experiment while previously they have been demonstrated thanks to eight experiments, each assessed by a test, each test made at four different times. Also, the experimental conditions differed between the present work and the previous one. It could thus be only observed that the appearance of the elements to count had a slight effect on the ants' counting accuracy. While foraging, the ants may take into account the numbers as well as the shape, color, size and position of the sighted elements. It has to be measured in further experiments to which extent changing the characteristics of the elements to count can affect the accuracy of their counting and their association with a symbol.

Six successive tests were performed on each colony for examining the degree of the ants' association between a number of elements and a symbol. Each test somewhat disturbed the ants for about one hour. As the ants' brood was in the course of its spring development, we tried to limit the number of experiments on each colony. This is why we learned only one symbol to each colony. Of course, it should be of interest to study if the ants could learn up to four distinct symbols, either the one after the other, in the course of four successive training and testing sessions, or at the same time by setting, during training, each number of elements and its corresponding symbol aside a reward. In the course of such a study, changes of shape, color and size of the elements to learn would not be performed.

Let us now compare our results to those obtained by other researchers on other animals. Honeybees could acquire numerical symbolism though this ability had a limit. Trained to associate either two quantities of a number of elements (2, 3) with their corresponding symbols, or in the opposite way, these symbols with the quantities, they correctly responded when asked to repeat the learned association even if the color, shape or configuration of the numerosity were changed. However, if they had learned only one of these directional associations, they were unable to correctly respond to the reverse association (Howard et al., 2019b). Pigeons could associate symbols to numbers: they correctly pecked a number of times on a symbol representing that number (Xia et al., 2000). In a first experiment, nine pigeons could allocate 1, 2, 3, or 4 pecks to a corresponding symbol. This recalls what we performed on ants. A second experiment examined the maximum of 'number to symbol' associations the pigeons could acquire, an experimental work we did not perform: six pigeons could learn five associations and four pigeons could learn six associations. A grey parrot could count 1 to 6 items and even construct cardinal meaning of 7 and 8, associating these numbers to vocal and Arabic number symbols (Pepperberg & Carey, 2012), what is exceptional compared to the competence of until now studied animals. Chimpanzees can learn to label Arabic numerals to numerosity and to order them (Murofushi, 1997), this ability being retained during 6 months and even during 3.25 years, but becoming of lower quality, with more errors, over time (Beran, 2004). Rhesus monkeys could also match 1 to 4 elements with their corresponding Arabic numerals, the association between numerical values and shapes being mainly managed in the prefrontal cortex neurons (Diester & Nieder, 2007).

The results presently obtained on *M. sabuleti* workers show that ants can associate numerical categories with shapes as symbols, even if color, shape or size of the elements representing the numbers somewhat changed. We have not yet tempted to learn several symbols to the ants of a same colony, nor to learn them to use symbols (i.e. to substitute them to numbers) in simple numerical operations, what is a required step for proving symbolic number representation, as explained by Howard et al. (2019b) in their work on honeybees.

Let us add that among all the animal species, no one has been shown to have spontaneously created a numerical symbolism. Only the human species did so (Howard et al., 2019b).

In conclusion, we showed that ants can easily and rapidly acquire a single numerical symbolism when learned to do so under operant conditioning. We still have to know if they could acquire multiple numerical symbolisms, i.e. associate different numbers of elements with different corresponding symbols, and acquire a true symbolic number representation.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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