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Critical distance between two identical visual cues allowing their mental addition by an ant

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ABSTRACT

Workers of the ant *Myrmica sabuleti* are known to mentally add visual cues when they are similar (have the same shape, color and size) and are simultaneously perceived. We here aimed to define the latter condition, i.e. what is the largest topographical distance between two signals that still allows ants to add them up? Ants belonging to four colonies were trained and tested over 72 hours with two cues placed on either side of their nest entrance and separated by 3.5 to 6.5 cm from each other. These ants mentally summed the cues separated by less than 5 cm from each other and not those separated by more than 5 cm. The critical distance is thus 5 cm for *M. sabuleti*. An additional experiment using two different kinds of cues presented at the same time, each kind of cue differently distant from the other, confirmed this critical distance measurement. This result leads to some ecological and physiological considerations, as well as to the need for further investigation.

KEYWORDS: *Myrmica sabuleti*, numerosity ability, operant conditioning, summation, visual perception.

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The present work was done after their retirement.

ABBREVIATIONS

cm: centimeter, d: distance, h: hours, n^{os}: numbers, %: proportion, percentage, *vs*: *versus*.

INTRODUCTION

The workers of the ant *Myrmica sabuleti* Meinert, 1861 mentally add visual cues when these cues are identical and simultaneously seen [1, 2, 3]. Identical cues are cues having the same shape, size and color, but whose location may vary. They are simultaneously viewed when they are located 'near' one another, for instance at a distance of 2 cm from each other. This distance can be larger but still needs to be defined. The aim of the present paper is thus to define the maximum distance between two identical cues in order for the ants to be still able to mentally add them. Before describing our methods and results, we recall what is nowadays known about the animals' ability in adding numbers of elements, including that of *M. sabuleti*.

The ability of adding elements has been investigated in several vertebrate and invertebrate animals. Before acquiring this ability, the animals should be able to evaluate the amount of elements. Distinguishing different amounts of elements without counting but ranking them on a scale is a rather common ability in the animal kingdom. This has been observed, among others, in fishes, amphibians, birds and mammals and even in insects [e.g. 4-13]. Counting elements and obtaining a precise notion of their amount is an ability observed, for instance, in male frogs, birds and monkeys [e.g. 14-17]. Going a step further and being able to add or subtract numbers of elements

is a skill shown in some birds, monkeys and apes [e.g. 18-25]. Honeybees have been proved to detain all these abilities, including adding and subtracting elements, even when having learned to do so using a color code [26].

Concerning the ant *M. sabuleti*, it has been found that its workers can add two sighted numbers of 1, 2 or 3 elements, i.e. could preferentially react to the sum of these elements (i.e. amounting 2 to 5 elements) instead of to the initial numbers or to a wrong sum [1], but only if these elements were seen simultaneously [2]. Moreover, these ants appear to add elements only if they are identical, at least in their shape, color or size [3]. However, although seeing elements 'simultaneously' is precisely defined in terms of time (i.e. at the same time), it has not yet been topographically defined.

It can be guessed that, when the elements are seen aside each other, the ants will mentally add them, and when the elements are located far from one another, the ants will not mentally perceive them as a sum of elements but simply as separated elements. Defining the critical physical distance beyond which two identical elements are no longer mentally added is the aim of the present work. This kind of investigation has not yet been made in other animal species.

MATERIALS AND METHODS

Collection and maintenance of the ants

The experiments were conducted on four ant colonies (labeled A, B, C, and D) collected in May 2021 from an abandoned quarry located in the Aise valley (Belgium, Ardenne). Each colony contained *ca* 500 workers, a queen and brood. They were maintained in the laboratory in one to two glass tubes half-filled with water, a cotton plug separating the ants from the water section. The nest tubes of each colony were laid in a tray (34 cm x 23 cm x 4 cm) which served as a foraging area in which a cotton-plugged tube containing sugared water was permanently provided, while pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were provided three times per week. The ambient lighting equaled *ca* 330 lux, the temperature *ca* 20 °C, the humidity *ca* 80%, and the electromagnetic field *ca* 2 μWm2. These conditions of ants' maintenance were suitable to the species used in this study. We here often use the words 'workers' or 'nestmates' instead of 'ants' as do researchers on social insects.

Experimental planning

This planning is summarized in Table 1. Six experiments were performed: I, III, V on colonies A and B, and II, IV, VI on colonies C and D, with the experiments I and II, III and IV, and V and VI performed at the same time. During each of these six experiments, the ants were trained for 72 hours in their foraging area using two identical visual cues set one on the left, the other on the right of the nest entrance, with a blank cue set far from any reward (i.e. far from the nest entrance and the food sites). The distance between the two presented cues equaled 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 cm for Experiments I to VI, respectively. The trained ants were tested six times over 72 hours in front of three stands, one stand bearing the kind of cue used for training, another one bearing two of these cues duly juxtaposed, and a third stand bearing nothing. A resting time period of 24 hours was allowed between the experiments I + II and III + IV, as well as between the experiments III + IV and V + VI.

Cues presented to the ants

These cues are schematically shown in Figure 1. Each cue was drawn in a square (2 cm x 2 cm) using Microsoft Word® software. They were individually cut and tied with extra transparent sticky paper on the front face of a stand two to three days before the experiments to allow the vanishing of any odor. Each stand was made of Steinbach® strong white paper (Malmedy, Belgium, 250g/m^2). It had a vertical part (2 cm x 2 cm) and was maintained vertically thanks to a duly folded horizontal part [2 x (1 cm x 0.5 cm)]. The cues presented to the ants were a black square, a black circle, a black triangle, a black vertical rectangle (▌), a black horizontal rectangle (▬), and a black star, the dimensions of which are given in Table 1, for the Experiments I, II, III, IV, V, and VI, respectively. In other words, colonies A and B were successively experimented using a black square, a black triangle and a black horizontal rectangle (▬), while colonies C and D were successively experimented using a black circle, a black vertical rectangle (▌) and a black star. The cues used for testing were identical to those used for training, but newly built ones.

Table 1. Experimental planning. This table may help in understanding the processes planned for finding the maximum distance between two visual cues beyond which the ants no longer mentally juxtapose them.

Experiment – distance between cues	Colonies	Cues and dimensions	Duration of the two experiments
I – 3.5 cm II – 4.0 cm	A B C D	Square, side = 5 mm Circle, diameter = 6 mm	72 hours
III – 4.5 cm IV – 5.0 cm	A B C D	Triangle, height = 7 mm, base = 6 mm Rectangle, height = 6.5 mm, width = 3.5 mm	72 hours
V – 5.5 cm VI – 6.0 cm	A B C D	Rectangle, height = 3.5 mm, width = 6.5 mm Star, span = 7.5 mm	72 hours

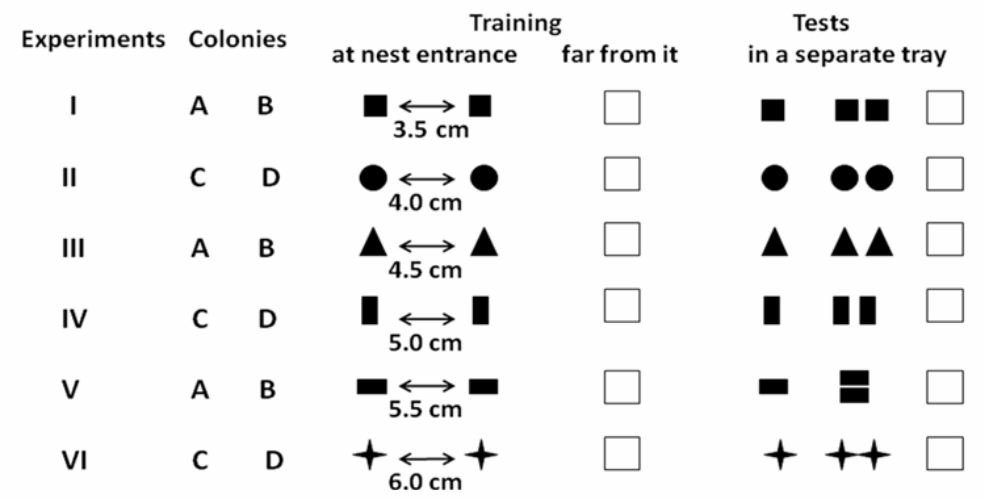

Figure 1. Visual cues used for defining the largest distance between them beyond which the ants no longer mentally juxtapose them. Each cue was presented on a stand and the ants were trained as well as tested as schematized in Figure 2 and as visible in Figure 3. Details can be found in the text, section 'Materials and Methods'.

Experimental design and protocol

The design is schematized in Figures 2 and 3. It represents that of Experiment I, but is similar for the six experiments. Each experiment (I to VI) was made on two colonies, A and B or C and D. To conduct an experiment on a colony, at a given time, two identical cues were set one on the left and the other on the right of the nest entrance, and a blank stand was set far from any reward (Figure 2, upper part). The two identical cues set at the nest entrance were distant by 3.5, 4.0, 4.5, 5.0, 5.5 or 6.0 cm from one another for the experiments I, II, III, IV, V or VI, respectively. The ants' training lasted 72 hours during which the distance between the two presented cues must remain unchanged. This was achieved thanks to a device schematized in Figure 3. The two stands, each bearing the same cue, were tied to a piece of white paper and then deposited at the ants' nest entrance. The two stands made an angle of 45° with the axis of the nest tube, while also making an angle of 90° between them. Between the location of each cue and the corresponding extremity of the white paper, the distance equaled about 0.5 cm + $2^{1/2}$ cm = 1.91 cm.

Over the 72 training hours, the trained ants were tested successively after 7, 24, 31, 48, 55, and 72 training hours in a separate tray (21 cm x 15 cm x 7 cm) into which three stands had been deposited, one bearing the kind of cue used during training, a second one bearing two such juxtaposed cues (i.e. set at 0.2 cm from each other, = added), and a third blank stand (Figure 2, lower part). For carrying out a

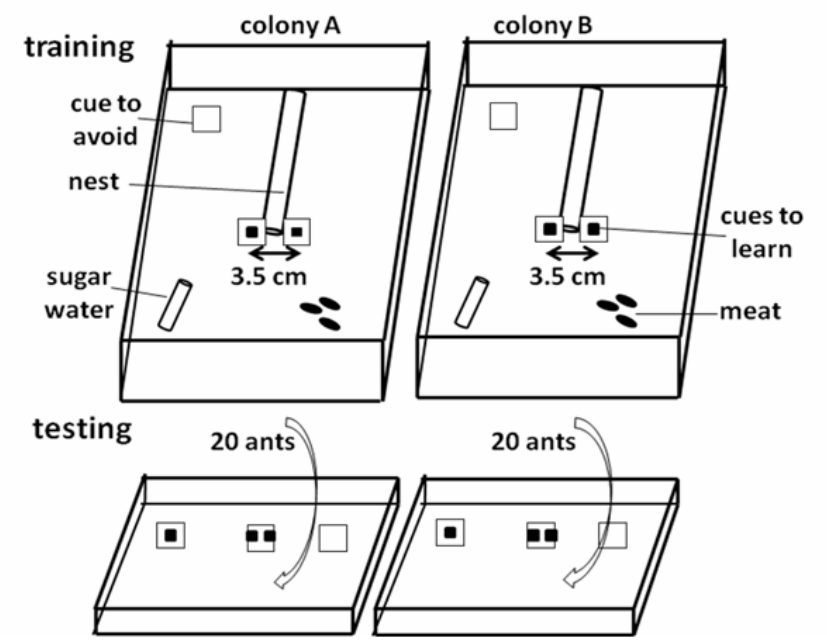

Figure 2. Experimental design used for defining the largest distance between two visual cues beyond which the ants no longer mentally add them. The ants were trained in their foraging area using two cues set at a distance of 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 cm from each other according to the experiment (upper schema). They were tested over time in front of one of these cues, of the two cues juxtaposed (i.e. added) and in front of a blank cue (lower schema). More ant visits to the juxtaposed cues signified that the ants mentally added them; more ant visits to one cue signified that they no longer added them and that the distance between the two cues presented during training was too large for doing so. The different cues are schematized in Figure 1, and Figure 3 shows how they were set at the nest entrance. The experimental planning is given in Table 1, and photos of the experiments are shown in Figure 4.

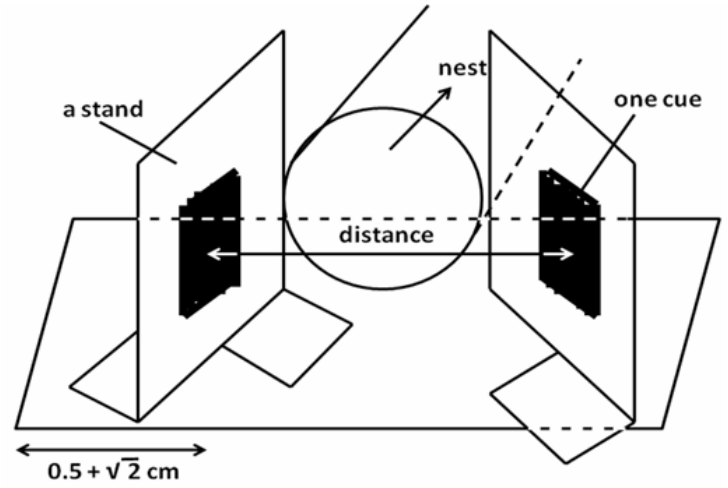

Figure 3. Experimental design for obtaining a given distance between two cues set at the nest entrance, which stayed unchanged over 72 experimental hours. The two stands bearing the cue were tied to a piece of white paper (upon which the nest entrance was laying), at a distance of 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 cm from each other according to the experiment (I to VI). The cues are shown in Figure 1 and the entire experimental design in Figure 2.

test on a colony, 20 ants were transported to the middle of their tray devoted to testing. The ants saw the three stands, moved in the tray, and went towards the stand of their choice, staying there for about 2 to 20 seconds. Half a minute after the ants had been set in their tray devoted to test, those present at less than 2 cm from each cue were punctually counted every 30 seconds over 10 minutes. The number of counts made for each colony equaled 20 and thus for each experiment it equaled 40. For each presented stand, the sum of the 20 counts made for each colony at each of the six testing sessions was established and are given in Table 2. The sum of the 40 counts made for the two colonies was also calculated which allowed establishing the proportion of ants that visited each of the three presented stands. These proportions are given in the subsection 'Results'. In addition, the counts obtained for each colony and every training time period were added and used for statistical analysis (Table 2, right column, see below). After each test, the 20 ants were released into the foraging area of their own colony.

Statistical analysis of the results

The goodness-of-fit χ^2 test was used to compare (1) the number of ant visits to the three stands, i.e. that bearing one cue, that bearing the juxtaposed cues and the blank stand, (2) the number of visits to the stand bearing one cue and that bearing the two juxtaposed cues with the number of ant visits expected if ants randomly (equally) visited each stand [27]. Also, the six successive numbers of ant visits to the stand bearing one cue were compared to the six successive numbers of visits to the stand bearing the two juxtaposed cues using the non-parametric test of Wilcoxon [27]. The statistical results are given in Table 2, and summarized and commented in the text.

Supplementary experiment

On the basis of the obtained results, a supplementary experiment was planned. It consisted in training and testing the ants of colonies A and B by presenting them at the same time the kind of cues previously presented to colonies C and D and set at a distance of 4 cm and of 6 cm from each other as well as in training and testing the ants of colonies C and D by presenting them at the same time the kind of cues previously presented to colonies A and B and set at a distance of 3.5 cm and of 5.5 cm from each other. The two kinds of distant cues presented here could be located near each other: they were different (had a different shape) and should thus not be mentally added by the ants [3]. The methods, protocol, mathematical and statistical analysis were identical to those of Experiments I to VI.

RESULTS

Presentation of the results, partial recall

For Experiments I to VI, numerical and statistical results are given in Table 2, two photos of each experiment (I to VI) are shown in Figure 4, and the proportions of the ants' responses are graphically presented in Figure 5. For the supplementary experiment, numerical and statistical results are given in Table 3, and the cues, the design, two photos, and a graphical summary of the results are shown in Figure 6.

Below, for each experiment, we successively report the proportions of ant visits over the 72 training hours to the stand bearing a single cue, to the stand bearing the two juxtaposed (added) cues and, for experiments I to IV, to a blank stand. Then, for the entirety of the time period, we report the result of the statistical comparison between the ants' responses to the three or four presented stands, and between their responses to a single cue and the two juxtaposed ones, and finally we conclude and give the overall proportions of the ants' responses to the three or four kinds of cues.

Experiment I, on colonies A and B, using squares set at 3.5 cm from each other during training

The proportions of ants' visits to the stand bearing one cue were 11.69%, 10.22%, 9.35%, 22.58%, 18.60% and 21.43% over the 72 training hours; those to the stand bearing the two juxtaposed cues were 60.39%, 70.07%, 84.11%, 59.98%, 69.18%, and 66.52% during the same time period; and those to the blank stand equaled 27.92%, 19.71%, 6.54%, 18.43%, 12.21% and 12.05%. The numbers of ant visits to the three stands statistically differed from those expected if ants had randomly gone to each of them ($P < 0.001$). Moreover, the numbers of ant visits to the stand bearing one cue and to that bearing the two juxtaposed cues also statistically differed from those resulting from a random choice by the ants ($P < 0.001$) and the six successively

Table 2. Ants' responses (number of visits) to one cue and to two of such juxtaposed cues, after having been trained using two of these cues separated by a given distance, this allowing to know the maximum distance between two cues that enables ants to mentally add them.

Experiment, cue, time (h)	**Colony, distance between cues (d) n° for 1 cue, added cues, blank cue**						**After summing both colonies: statistics on the three sums; on the two first sums, and again on the latter**
I, square	colony A		d = 3.5 cm		colony B		goodness-of-fit χ^2 test; 171, 675, 165 vs random n^{os}:
7	12	61	24	6	32	19	$\chi^2 = 508.55$, df = 2, P < 0.001
24	5	31	18	9	65	9	
31	8	32	7	2	58	0	goodness-of-fit χ^2 test; 171, 675 vs random n^{os}:
48	19	51	18	30	77	22	$\chi^2 = 300.25$, df = 1, P < 0.001
55	18	44	7	14	75	14	
72	21	93	13	27	56	14	Wilcoxon test; 18, 14, 10, 49, 32, 48 vs 93, 94,
Σ of visits	83	312	87	88	363	78	90, 128, 119, 149: N = 6, T = +21, P = 0.016
II, circle	colony C		d = 4.0 cm		colony D		goodness-of-fit χ^2 test; 156, 582, 86 vs random n^{os}:
7	18	58	25	6	36	6	$\chi^2 = 524.68$, df = 2, P < 0.001
24	21	65	4	9	35	6	
31	6	52	5	11	44	7	goodness-of-fit χ^2 test; 156, 562 vs random n^{os}:
48	32	75	12	10	32	5	$\chi^2 = 266.68$, df = 1, P < 0.001
55	24	67	2	1	37	0	
72	5	48	12	13	33	2	Wilcoxon test; 24, 30, 17, 42, 25, 18 vs 94, 100,
Σ of visits	106	365	60	50	217	26	96, 107, 104, 81: N = 6, T = +21, P = 0.016
III, triangle	colony A		d = 4.5 cm		colony B		goodness-of-fit χ^2 test; 229, 527, 30 vs random n^{os}:
7	42	74	4	15	59	8	$\chi^2 = 477.63$, df = 2, P < 0.001
24	15	42	6	17	54	4	
31	20	22	3	40	44	2	goodness-of-fit χ^2 test; 229, 527 vs random n^{os}:
48	8	36	0	12	33	0	$\chi^2 = 117.46$, df = 1, P < 0.001
55	15	35	0	23	51	0	
72	9	34	2	13	43	1	Wilcoxon test; 57, 32, 60, 20, 38, 43 vs 133, 96,
Σ visits	109	243	15	120	284	15	66, 69, 86, 56: N = 6, T = +21, P = 0.016
IV, ▌ rectangle	colony C		d = 5.0 cm		colony D		goodness-of-fit χ^2 test; 389, 391, 19 vs random n^{os}:
7	28	61	6	12	32	2	$\chi^2 = 344.58$, df = 2, P < 0.01
24	23	47	1	21	29	1	
31	28	52	2	25	25	0	goodness-of-fit χ^2 test; 389, 391 vs random n^{os}:
48	40	18	0	40	19	0	$\chi^2 = 0.005$, df = 1, 0.98 < P < 0.99
55	43	30	4	32	21	2	
72	65	41	1	32	16	0	Wilcoxon test; 40, 70, 80, 58, 73, 106 vs 93, 50,
Σ of visits	227	249	14	162	142	5	50, 59, 53, 48: N = 6, T = +6, -15, P = 0.219
V, ▬ rectangle	colony A		d = 5.5 cm		colony B		goodness-of-fit χ^2 test; 590, 325, 68 vs random n^{os}:
7	54	35	4	90	40	6	$\chi^2 = 415.78$, df = 2, P < 0.001
24	26	64	5	71	24	14	
31	57	20	7	44	32	6	goodness-of-fit χ^2 test; 590, 325 vs random n^{os}:
48	45	15	5	46	24	3	$\chi^2 = 72.02$, df = 1, P < 0.001
55	23	22	5	2	8	6	
72	48	29	6	44	18	1	Wilcoxon test; 144, 07, 101, 91, 55, 92 vs 75, 88,
Σ of visits	263	185	32	327	140	36	52, 39, 30, 47: N = 6, T = -21, P = 0.016
VI, star	colony C		d = 6.0 cm		colony D		goodness-of-fit χ^2 test; 625, 182, 36 vs random n^{os}:
7	60	22	5	49	17	1	$\chi^2 = 669.61$, df = 2, P < 0.001
24	109	25	2	40	25	3	
31	46	16	9	36	7	11	goodness-of-fit χ^2 test; 625, 182 vs random n^{os}:
48	74	13	2	30	18	0	$\chi^2 = 251.18$, df = 1, P < 0.001
55	56	8	0	35	8	0	
72	40	9	1	50	14	2	Wilcoxon test; 109, 149, 82, 104, 91, 90 vs 39, 50,
Σ of visits	385	93	19	240	89	17	23, 31, 16, 23: N = 6, T = -21, P = 0.016

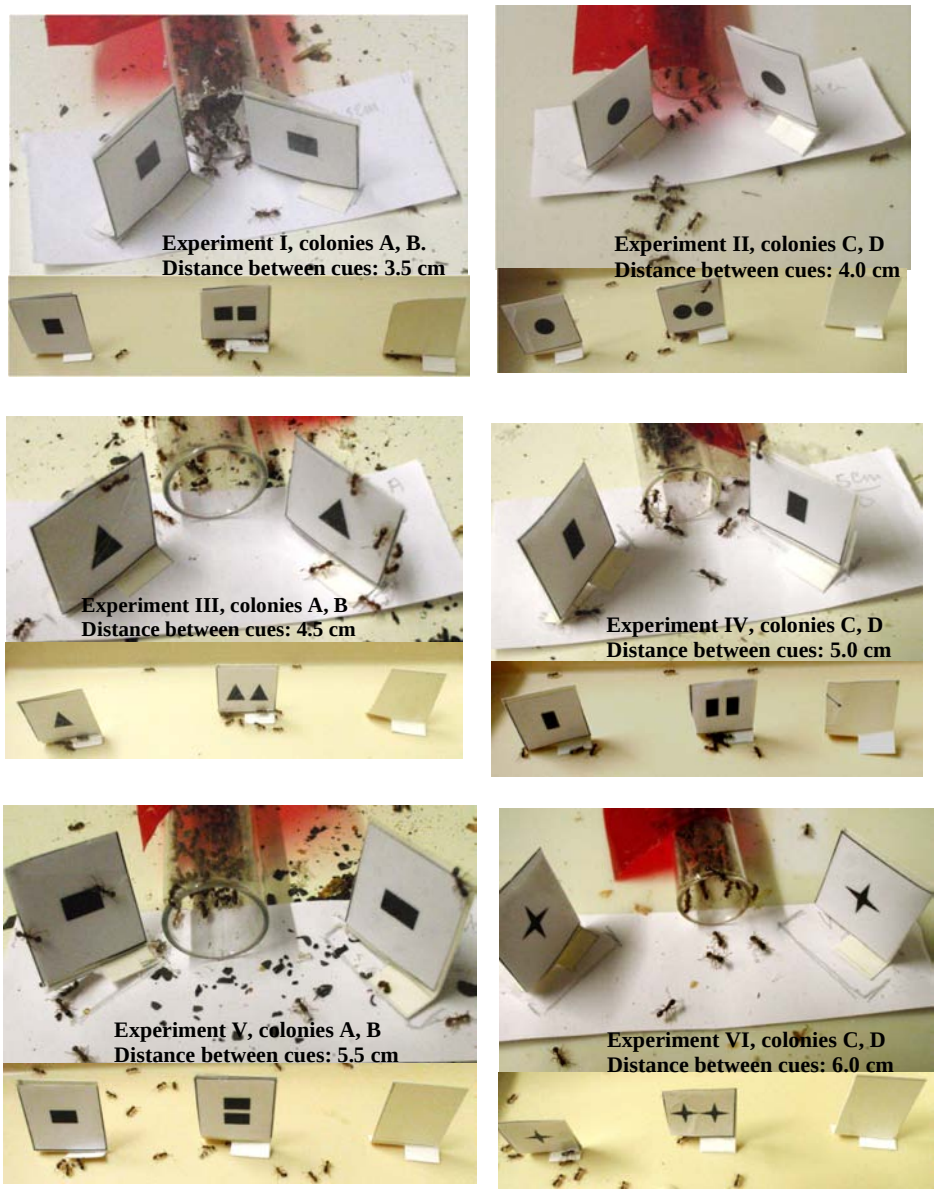

Figure 4. Some views of the experiments carried out to define the maximum distance between two visual cues in order for ants to mentally juxtapose them, i.e. beyond which distance they no longer add them. In experiments I, II and III, the cues were set at a distance of 3.5 cm, 4.0 cm and 4.5 cm, respectively: the ants then reacted mostly to the juxtaposed cues. In experiment IV, the cues were set at a distance of 5.0 cm: the ants reacted equally to one cue and to the juxtaposed cues. In experiments V and VI, the cues were set at a distance of 5.5 cm and 6.0 cm, respectively: the ants reacted mostly to one cue. Consequently, the ants stopped adding two similar presented cues when they were set at a distance of more than 5.0 cm. The critical distance we aimed to define thus equals 5.0 cm.

obtained numbers for the stand with one cue and for the stand with the two cues all along the experiment statistically differed (P = 0.016) with the ants' obvious preference for the stand bearing the juxtaposed cues. It could thus be concluded that the ants mentally juxtaposed (added) the two cues presented during training, when the two cues were set at a distance of 3.5 cm from each other. Using the results of the six performed tests, it appeared that the ants' choice of one cue equaled 16.91%, of the juxtaposed cues equaled 66.76%, and of the blank cue equaled 16.32% (Figure 5).

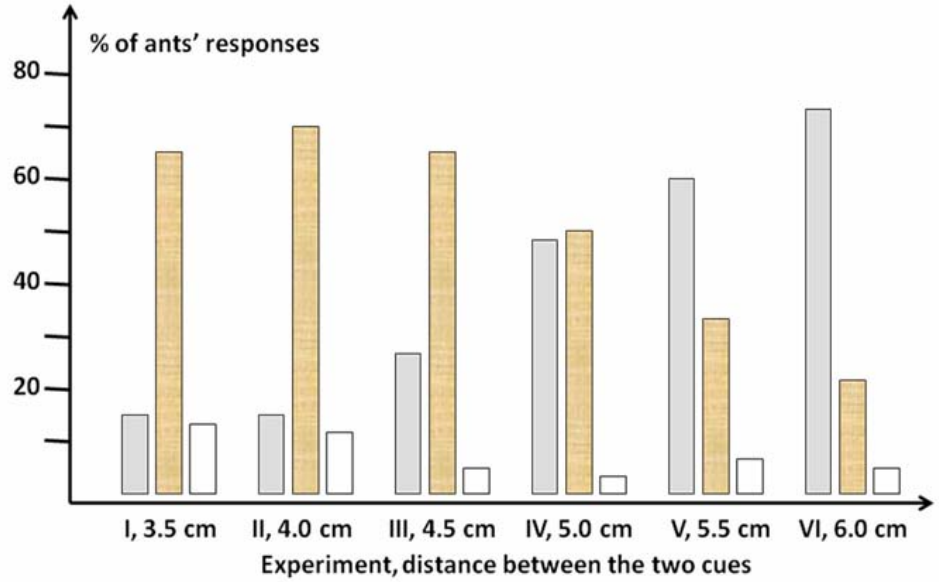

Figure 5. Graphical summary of the results of the Experiments I to VI. The sums of the ants' responses to one cue (blue-gray color), two added cues (brown color) and to a blank cue (white) are given, in percentages, for two cues set at a distance of 3.5 to 6.0 cm from each other according to the experiment. The ants responded to the juxtaposed cues when these cues were set at a distance of less than 5 cm from each other; they responded mostly to the single cue when the cues were set at a distance of more than 5 cm from each other. The maximum distance between two identical cues for ants to mentally add them thus equals 5 cm. Beyond this distance, the ants react to each separated cue. The cues and the experimental design are shown in Figures 1, 2, 3; numerical and statistical results are given in Table 1. Details can be found in the text.

Experiment II, on colonies C and D, using circles set at 4.0 cm from each other during training

Over the 72 training hours, during the six performed tests, the proportions of ants sighted near the stand bearing one cue equaled 16.11%, 21.43%, 13.6%, 25.30%, 19.08% and 15.93%, those of ants sighed near the stand bearing the juxtaposed cues equaled 63.09%, 71.43%, 76.80%, 64.46%, 79.39% and 71.58%, and those relative to the blank stand were 20.81%, 7.14%, 9.60%, 10.24%, 1.53% and 12.39%. Statistically, the numbers of ants sighted in front of each presented stand differed from those resulting from a random movement of the ants ($P < 0.001$). In addition, the numbers of ants sighted in the vicinity of the stand bearing one cue and of that bearing the two juxtaposed cues also statistically differed from those expected if ants randomly went to each of these two stands ($P < 0.001$), and moreover the six successive counts of ants in front of one or the other of these two stands differed ($P = 0.016$) with a clear preference of the ants for the stand bearing the juxtaposed cues. We could thus conclude that when identical visual cues were set at a distance of 4.0 cm from each other, the ants mentally added them. Taking into account the six successively performed tests, the proportions of ants sighted in front of one cue, the juxtaposed cues and the blank cue equaled 18.93%, 70.63%, and 10.44% respectively (Figure 5).

Experiment III, on colonies A and B, using triangles set at 4.5 cm from each other during training

The frequencies of the ants' visits to the stand bearing one cue equaled 28.22%, 23.19%, 45.80%, 22.47%, 30.64% and 21.57% all along the 72 training hours; the frequencies of the visits to the stand bearing the two juxtaposed cues equaled 65.84%, 69.56%, 50.38%, 77.53%, 69.35% and 75.49% all along the same time period; and of the visits to the blank stand equaled 5.94%, 7.25%, 3.82%, 0.00%, 0.00% and 2.94% during that time. The numbers of ants' visits to the three stands statistically differed from those expected if ants had randomly gone to each

Table 3. Ants' responses (number of visits) to one cue and to two such cues juxtaposed on a stand while being trained at the same time to two cues set at a distance of 4 cm and two cues set at a distance of 6 cm from one another (colonies A, B) or to two cues set at a distance of 3.5 cm and two cues set at a distance of 5.5 cm from one another (colonies C, D). The tested ants mostly reacted to the juxtaposed cues when these cues were separated by a distance of 4 or 3.5 cm during training and to the single cue when the cues were set at 6 or 5.5 cm apart during training. Thus, the ants mentally added cues located near each other, and perceived as separate cues located far from each other, the critical distance for leading to one or the other of these two behaviors being 5 cm.

Time (h)	**Colony, cue, distance between cues**							
	A, circle: 4 cm		A, star: 6 cm		B, circle: 4 cm		B, star: 6 cm	
	one cue	added cues	one cue	added cues	one cue	added cues	one cue	added cues
7	4	35	57	8	1	24	22	6
24	2	19	38	3	7	25	47	2
31	0	35	47	8	7	49	22	0
48	6	32	40	0	13	43	45	1
55	2	35	39	3	2	36	37	6
72	2	20	30	3	7	42	63	3
Σ of visits	16	206	251	25	37	219	215	18
Statistics	Sum of two colonies; 53, 425, 487, 43 *versus* 252, 252, 252, 252 : $\chi^2 = 708.35$, df = 2, P < 0.001 4 cm, one cue *vs* two juxtaposed; 53, 425 *versus* 239, 239 : $\chi^2 = 289.50$, df = 1, P < 0.001 5, 9, 7, 19, 4, 9 *versus* 59, 44, 84, 75, 71, 62 : N = 6, T = +21, P = 0.016 6 cm, one cue *vs* two juxtaposed; 487, 43 *versus* 265, 265 : $\chi^2 = 371.96$, df = 1, P < 0.001 79, 85, 69, 85, 76, 93 *versus* 14, 5, 8, 1, 9, 6 : N = 6, T =-21, P = 0.016							
	C, square: 3.5 cm		C, rectangle: 5.5 cm		D, square: 3.5 cm		D, rectangle: 5.5 cm	
	one cue	added cues	one cue	added cues	one cue	added cues	one cue	added cues
7	5	57	33	7	2	19	18	1
24	4	27	51	7	3	33	29	0
31	3	22	36	4	0	24	36	2
48	1	23	65	0	2	31	24	0
55	0	27	32	6	2	29	34	0
72	3	34	26	3	1	25	33	0
Σ of visits	16	190	243	27	10	161	174	3
Statistics	Sum of two colonies; 26, 351, 417, 30 *versus* 206, 206, 206, 206 : $\chi^2 = 625.83$, df = 2, P < 0.001 3.5 cm, one cue *vs* two juxtaposed; 26, 351 *versus* 188.5, 188.5 : $\chi^2 = 280.16$, df = 1, P < 0.001 7, 7, 3, 3, 2, 4 *versus* 76, 60, 46, 54, 56, 59 : N = 6, T = +21, P = 0.016 5.5 cm, one cue *vs* two juxtaposed; 417, 30 *versus* 223.5, 223.5 : $\chi^2 = 335.04$, df = 1, P < 0.001 51, 80, 72, 89, 66, 59 *versus* 8, 7, 6, 0, 6, 3 ; N = 6, T =-21, P = 0.016							

of them (P < 0.001). Moreover, the numbers of ants' visits to the stand bearing one cue and to that bearing the two juxtaposed cues also statistically differed from those resulting from a random choice by the ants (P < 0.001) and the six successive numbers obtained in front of either of these two stands all along the experiment statistically differed (P = 0.016) with the ants' obvious preference for the stand bearing the juxtaposed cues. It could thus be concluded that the ants mentally juxtaposed (added) the two cues presented during training, these two cues being set at a distance of 4.5 cm from each other. Using the results of the six performed tests, it appeared that the ants' choice of one cue equaled 29.13%, of the juxtaposed cues equaled 67.05%, and of the blank cue equaled 3.82% (Figure 5).

Experiment IV, on colonies C and D, using vertical rectangles (▌) set at 5.0 cm from each other during training

In the course of the six tests made while training the ants, the amounts of ants counted for the stand bearing one cue equaled 28.37%, 36.06%, 40.15%,

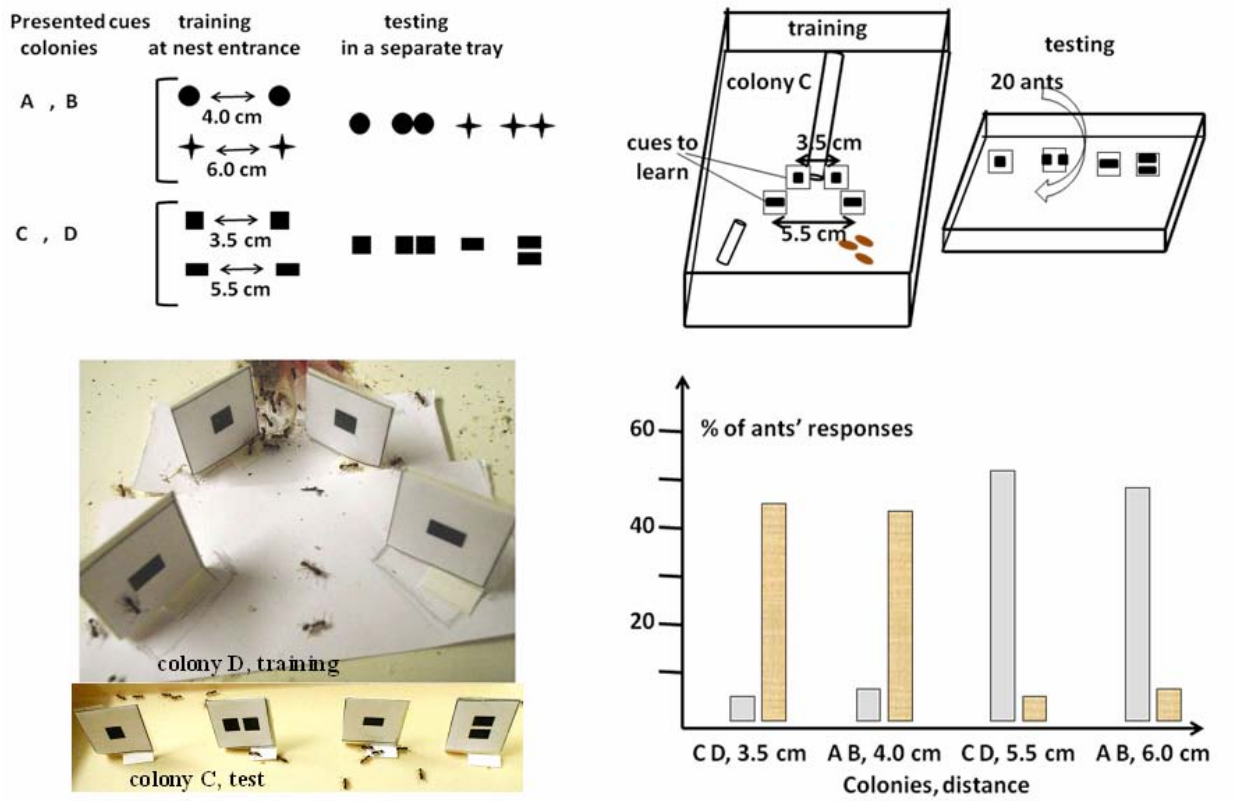

Figure 6. Supplementary experiment: upper part: cues and experimental design, lower part: two photos of the experiment and a graphical summary of the results (blue-gray color: response to one cue; brown color: response to juxtaposed cues). Numerical and statistical results are given in Table 2. Trained at the same time with two cues placed 4 cm apart and two other cues placed 6 cm apart, as well as, in another trial, with two cues placed 3.5 cm apart and two other cues placed 5.5 cm apart, the ants responded the most to the juxtaposition of these same cues when, presented in isolation during training, they had been located at 4 and 3.5 cm from each other and responded the most to the isolated cues when they had been located at 6 and 5.5 cm from each other. This confirmed that the distance between similar cues below which the ants react by mentally adding the cues and beyond which they react as they were sighted separately equals 5 cm.

68.38%, 56.82% and 62.58%, for the stand bearing the juxtaposed cues equaled 65.96%, 62.29%, 58.33%, 31.62%, 38.64% and 36.77%, and for the blank stand they equaled 5.67%, 1.64%, 1.52%, 0.00%, 4.54% and 0.65%. Statistically, the numbers of ants sighed in front of each presented stand differed from those resulting from a random movement of the ants ($P < 0.001$). On the contrary, the numbers of ants sighted in the vicinity of the stand bearing one cue and of that bearing the two juxtaposed cues did not statistically differ from those expected if ants randomly went to each of these two stands ($0.98 < P < 0.99$). Moreover, the six successive numbers of ants in front of one or the other of these two stands also did not statistically differ ($P = 0.219$), which revealed a lack of ants' preference for the stand bearing either one or the added cues. We could thus conclude that when identical visual cues were distant of 5.0 cm from each other, the ants reacted equally to the stand bearing one cue and to that bearing the two added cues; they thus neither preferably add or not add the cues. Taking into account the six successively performed tests, the mean proportions of ants sighted in front of one cue, of the juxtaposed cues and of the blank cue equaled 48.68%, 48.94%, and 2.38%, what confirms the ants' equal choice of separated and added cues.

Experiment V, on colonies A and B, using horizontal rectangles (▬) set at 5.5 cm from each other during training

The proportions of ants counted in the vicinity of the stand bearing one cue equaled 62.89%,

47.55%, 60.84%, 68.42%, 57.29% and 63.01% in the course of the 72 training hours; those of the stand bearing the two juxtaposed cues equaled 32.75%, 43.14%, 31.32%, 29.32%, 31.25% and 32.19% in the course of the same time period; and those of the blank stand equaled 4.36%, 9.31%, 7.83%, 2.25%, 11.46% and 4.79%. The numbers of ants counted in the vicinity of the three stands statistically differed from those expected if ants had randomly gone to each of them ($P < 0.001$). Moreover, the numbers of ants counted near the stand bearing one cue and near that bearing the two juxtaposed cues also statistically differed from those resulting from a random walking of the ants ($P < 0.001$). Also, the six numbers obtained successively in front of one or the other of these two stands over the entire experiment statistically differed ($P = 0.016$) with some preference for the stand bearing the one cue. It could thus be concluded that the ants poorly mentally juxtaposed (added) the two cues presented at a distance of 5.5 cm from each other, and reacted more to a single cue. On the basis of the results of the six performed tests, it could be established that the ants' choice of one cue equaled 60.02%, of the juxtaposed cues equaled 33.06%, and of the blank cue equaled 6.92% (Figure 5).

Experiment VI, on colonies C and D, using stars set at 6.0 cm from each other during training

The proportions of ants' visits to the stand bearing one cue reached 70.78%, 73.04%, 65.60%, 75.91%, 85.05% and 77.58% over the 72 training hours; those to the stand bearing the two juxtaposed cues reached 25.32%, 24.15%, 18.40%, 22.63%, 14.95% and 19.83% over the same time period; and those to the blank stand reached 3.89%, 2.45%, 16.00%, 1.46%, 0.00% and 2.58%. The numbers of ants' visits to the three stands statistically differed from those resulting from a random choice ($P < 0.001$). In addition, the numbers of ants' visits to the stand bearing one cue and to that bearing the two juxtaposed cues also statistically differed from those expected if ants randomly went towards these two stands ($P < 0.001$). Also, the six numbers obtained successively for one or the other of these two stands during the whole experiment statistically differed ($P = 0.016$) with the ants' obvious preference for the stand bearing one cue. It could thus be deduced that the ants did not mentally juxtapose (add) the two cues presented during training at a distance of 6 cm between them but acted as perceiving them independently from each other. The results of the six performed tests allowed in calculating that the ants' global choice of one cue equaled 74.14%, of the juxtaposed cues equaled 21.59%, and of the blank cue equaled 4.27% (Figure 5).

Supplementary experiment, on colonies A and B using circles set at a distance of 4 cm and stars set at a distance of 6 cm, and on colonies C and D using squares set at a distance of 3.5 cm and rectangles set at a distance of 5.5 cm

Concerning the experiment made on colonies A and B, the proportions of ants' visits, over the 72 training hours, to one circle equaled 3.18%, 6.29%, 4.16%, 10.55%, 2.50% and 5.29%, to two juxtaposed circles equaled 37.58%, 30.77%, 50.00%, 41.66%, 44,37% and 36.47%, as well as to one star equaled 50.32%, 59.44%, 41.07%, 47.22%, 47.50% and 54.70%, and to two juxtaposed stars equaled 8.92%, 3.49%, 4.76%, 0.55%, 5.62% and 3.53%. The number of ants' visits to the four presented cues statistically differed from those expected if ants randomly went to these cues ($P < 0.001$). Also, the number of visits to one circle and to two juxtaposed circles statistically differed from random numbers ($P < 0.001$), and the six successively obtained numbers for these two cues statistically differed ($P = 0.016$) with the ants' obvious preference for the two juxtaposed circles. In the same way, the number of visits to one star and two juxtaposed stars statistically differed from random numbers ($P < 0.001$), and the six successively obtained numbers for these two cues statistically differed ($P = 0.016$) with the ants' obvious preference for the one star. This allowed concluding that the ants mentally added the circles set at a distance of 4.0 cm from each other during training, and did not add stars set at a distance of 6.0 cm from each other during training. Using the results of the six performed tests, the overall proportions of the ants' visits to one circle appeared to be 5.26%, to two juxtaposed circles 40.14%, to one star 50.04% and to two juxtaposed stars 4.26% (Figure 6, lower right part).

As for the experiment conducted on colonies C and D, the proportions of ants sighted over the 72 training hours in front of one square equaled

4.93%, 4.54%, 2.36%, 2.05%, 1.54% and 3.20%, in front of two juxtaposed squares equaled 53.52%, 38.96%, 36.22%, 36.99%, 43.07% and 47.20%, in front of one rectangle equaled 35.91%, 51.95%, 56.69%, 60.96%, 50.77% and 47.20%, as well as in front of two juxtaposed rectangles equaled 5.63%, 4.54%, 4.72%, 0.00%, 4.61% and 2.40%. The number of ants sighted in front of the four presented cues statistically differed from those expected if ants randomly visited these four cues ($P < 0.001$). Moreover, the numbers of ants counted in front of one square and two juxtaposed squares statistically differed from expected random numbers ($P < 0.001$), and the six successively obtained numbers for these two cues also statistically differed ($P = 0.016$) with an obvious larger number of visits to the two juxtaposed squares. Similarly, the number of ants counted in front of one rectangle and two juxtaposed rectangles statistically differed from expected random numbers ($P < 0.001$), and the six successively obtained numbers for these two cues statistically differed ($P = 0.016$) with obviously more ants' visits to one rectangle. This led to the conclusion that the ants mentally juxtaposed the squares set at a distance of 3.5 cm from each other during training, and reacted as having distinctively perceived the rectangles set at a distance of 6.0 cm from each other during training. Using the results of the six conducted tests, the overall proportions of ants counted in front of one square equaled 3.15%, in front of the two juxtaposed squares equaled 42.60%, in front of one rectangle equaled 50.60% and in front of two juxtaposed rectangles equaled 3.64% (Figure 6, lower right part).

Note that, during this experiment, the ants altogether scored somewhat better for one cue (50.04% and 50.58%) than for two juxtaposed cues (40.14% and 42.66%).

DISCUSSION

Knowing that the workers of the ant *M. sabuleti* mentally add identical cues seen simultaneously but not consecutively, we tried to define what is the largest distance between two identical cues in order for the ants to be able to add them (i.e. to react more to their juxtaposition than to a single cue) and the distance beyond which the ants no longer add the two cues. Working on four colonies and performing six experiments and a supplementary one, we demonstrated that this critical distance (the horizontal distance: see below) equals 5 cm.

The variability of the ants' conditioning scores observed here is in the range of those generally observed during operant conditioning [e.g. 28, 29]. We should also note that in the present work, the ants scored better for a single cue (Experiment VI: mean = 74.14%; supplementary experiment: mean = 50.04 and 50.58%) than for juxtaposed cues (Experiments I, II and III: mean = 66.76%, 70.63% and 67.05%; supplementary experiment: mean = 40.14% and 42.66%). This suggests that it is easier for an ant to memorize a single cue than to mentally add two cues and to memorize the result of the addition.

Mentally adding two cues located close to each other and not doing so for cues located far from one another is an ants' behavior brought to the fore in the present work. It allows acquiring a correct and simple representation of the environment and may thus be advantageous to ants for optimally navigating, i.e. for quickly and easily reaching food sites or the nest. There may be individual variability in the critical distance between cues for ants adding them, this distance probably depending on the size of the eyes and the visual perception performance, but a greater variability is likely to be found between ant species.

The ants' behavior quantified here shows that these insects mentally sum cues when they see them sufficiently close to each other, i.e. as if they form a single cue. This behavior does not require perspective vision, even if *M. sabuleti* workers possess this kind of vision thanks to a differential sensitivity to visible and UV light of distinct specialized ommatidia [30].

A critical distance below which visual cues can be mentally added should also exist for cues located above each other (vertically instead of horizontally located) and it can be presumed that this vertical distance is somewhat smaller than the horizontal one since the ants are more sensitive to a change of orientation of a horizontal segment than to a change of orientation of a vertical segment [31]. Defining the critical vertical distance between two cues allowing ants to mentally juxtapose them will be investigated in a subsequent work.

When the cues are sounds which should be counted, compared and added, they cannot be presented at the same time but only one after the other, and in this case, the critical distance between the cues is a temporal limit. A temporal distance, empirically set up without defining a limit distance, has often been used when working on honeybees' numerosity abilities [e.g. 32]. Indeed, investigation on addition and subtraction in bees concerned elements whose presentation is separated by some period of time [26].

CONCLUSION

Let us conclude by stating that, for *M. sabuleti* ants, in order to be able to mentally add visual cues, these cues must be of the same kind, be perceived at the same time and spatially close enough from each other, the critical distance between two identical horizontally positioned cues being 5 cm. The notion of 'adding simultaneously sighted cues' used in preceding works [1, 3] indeed corresponded to a distance between cues not exceeding 5 cm.

CONFLICT OF INTEREST STATEMENT

We affirm having no conflict of interest with regard to the physiological and ethological topic of the present work.

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