

Visual cue generalisation and spatial conditioning in the ant *Myrmica sabuleti*

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Since classical conditioning and spatial learning are possible in the ant *Myrmica sabuleti*, we aimed to investigate the possibility of spatial conditioning in this ant species. First we demonstrated that workers can distinguish between different cues. Second we used these cues as conditioned stimuli (CS) to try to obtain spatial conditioning. Reinforcing experiments were performed according to three methods: (i) repeatedly alternating presentations of two distinct CS, each one associated with an unconditioned stimulus (UCS) located at a precise place; (ii) repeatedly alternating presentations of two CS, each one associated with a UCS located at a precise place and a “non-reward” at the opposite place; (iii) successive presentations of two CS, each one associated with a UCS located at a precise place. The last method was repeated so that the order of presentation of each CS was alternated. Spatial conditioning was obtained with each of the three methods: the proportion of spatially conditioned ants was 43% (first method), 35% (second method) and 45% (third method). With the first method, spatial conditioning could be shown only on the basis of this proportion. With the second method, spatial conditioning was suggested on the basis of the numbers of ants responding to the CS. With the third method, spatial conditioning was obvious on the basis of the numbers of responding ants. The ants’ conditioning and spatial conditioning extinguished in three (first method), four (second method) and five (third method) experiments of extinction. The third method showed that conditioning to the first CS presented was always stronger than that to the second CS used. In short, (i) spatial conditioning exists in *Myrmica sabuleti*, which might explain the ants’ use of cues for orienting themselves; (ii) a first conditioning is stronger than the following which might explain the ants’ route fidelity.

Key words: ant, *Myrmica sabuleti*, spatial conditioning, insect orientation.

Introduction

Orientation due to landmarks, navigation using celestial information and maze-learning are known to exist in many ant species (HÖLLDOBLER & WILSON, 1990). It has been shown many times that ants are able to use landmarks (e.g. VOWLES,

1965; WEHNER & RABER, 1979; HENQUEL & ABDI, 1981; ROSENGREN et al., 1986; ARON et al., 1988; COLLETT et al., 1992). However, it has not been shown that ants can be spatially conditioned to a landmark and in this way associate a cue to one way (or one direction or one place) to go. Consequently, we aimed to investigate the abil-

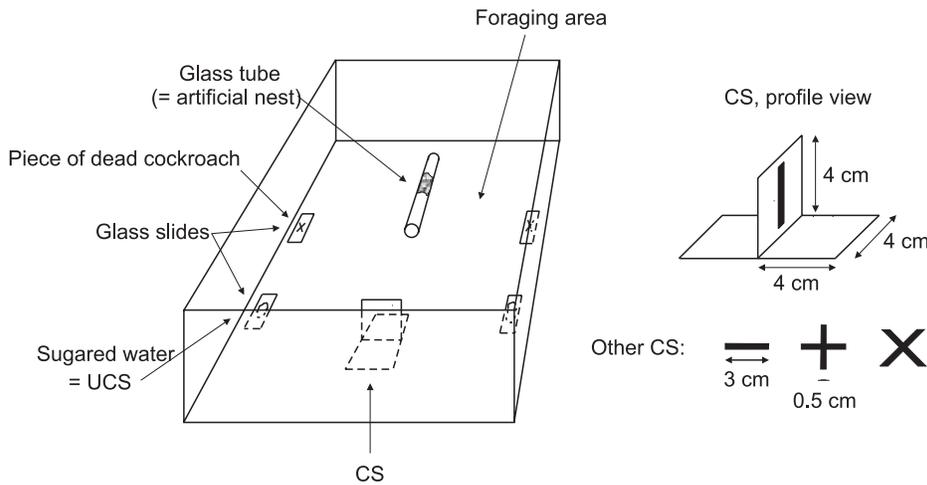


Fig. 1. Experimental nest, design, and stimuli used. CS – conditioned stimulus; UCS – unconditioned stimulus.

ity of an ant to acquire a simple (i.e. classical) spatial conditioning with two conditioned stimuli. For this study, we choose the ant species *Myrmica sabuleti* Meinert, 1860 because experiments demonstrated first that, in this ant species, classical conditioning is possible (if the conditioned stimulus is presented either a few minutes before, just before or at the same time as the unconditioned stimulus) (CAMMAERTS, 2001a) and secondly, that spatial learning exists (CAMMAERTS, 2001b). Thus, the aim of the present work is to investigate if it is possible to condition workers of *M. sabuleti* to two distinct conditioned stimuli: one of them will be associated with an unconditioned stimulus presented at a precise place, while the other one will be associated with the same kind of unconditioned stimulus but presented at another place. The ants would be spatially conditioned if, after several reinforcing experiments, they individually went to the correct places in the presence of each of the two conditioned stimuli used and in the absence of the unconditioned stimulus. Prior to the study, it was established if *M. sabuleti* workers can distinguish between two different stimuli (first part of the present work), and later, a detailed analysis of this ant species' visual perception will be published.

Material and methods

Collection and maintenance of ants

Two large natural colonies of *M. sabuleti* were collected from Pont-St. Vincent (48°36'03" N, 06°05'00" E) (Dept. Meurthe-et-Moselle, France) and each of them

was divided in eight laboratory colonies. These laboratory colonies contained one queen, brood and two hundred and fifty workers. They were kept in glass-tubes, half-filled with water, a cotton-plug separating the ants from the water reservoir. Each artificial nest was placed in a polyethylene tray (42 × 28 × 7 cm) serving as foraging area, where food was provided. Between the experiments, this food consisted of dead cockroaches and sugared water provided *ad libitum*. During the experiments, no sugared water was given, and only half a cockroach was offered once a week, placed on two glass-slides situated on the left and the right side of the artificial nest (Fig. 1).

Stimuli used

The unconditioned stimulus (UCS) consisted of a droplet of sugared water (a saturated water solution of brown sugar, diluted twice), deposited on a glass-slide placed, for the preliminary study, in front of the conditioned stimulus (13 cm in front of the nest entrance) and placed, for the spatial conditioning study, either on the left or right side of the nest entrance (depending on the conditioned stimulus presented), at 18 cm from that entrance as shown in Fig. 1.

The conditioned stimuli (CS) consisted of either a vertical line, a horizontal line, a "+" sign or a "x" sign, each line being 3 cm long and 0.5 cm wide, drawn with Indian ink on one of the two faces of the vertical part of a white sheet of paper (80 g m⁻²; total surface = 16 × 4 cm) which had been folded in the form of a reverse T. Each CS was placed at a distance of 18 cm in front of the nest entrance. Fig. 1 shows the CS and the position on the foraging area. When two stimuli were used on one colony, these two stimuli were either the vertical or the horizontal lines, or the "+" and the "x" signs: they thus had the same area of black surface and only differed in their shape.

Table 1. Common legend to Figs 2–5 and Tables 2–6.

Nests used	Study of generalisation of cues	Study of spatial conditioning	
		CS with UCS on the left side	CS with UCS on the right side
A			—
B	—	—	
C	+	+	×
D	×	×	+

Control numbers – numbers of ants coming onto the glass-slides during a two minutes period, in the absence of the UCS and of the CS; Experimental numbers – numbers of ants coming onto the glass-slides during a two minutes period, in the absence of the UCS, but in the presence of a stimulus or of the CS.

$L_W^{w,nw,y}$ $R_Y^{y,ny,w}$: L – number of ants coming onto the glass-slide placed on the left side of the nest entrance during a two minutes period; R – number of ants coming onto the glass-slide placed on the right side of the nest entrance during a two minutes period; w – number of ants marked with white colour and coming onto the glass-slide; nw – number of ants newly marked in white (that is marked during the counting in process); W – total number of ants marked in white since the beginning of the experiments of extinction; y – number of ants marked with yellow colour and coming onto the glass-slide; ny – number of ants newly marked in yellow (that is marked during the counting in process); Y – total number of ants marked in yellow since the beginning of the experiments of extinction. CS → l – presentation of the CS previously associated with the UCS placed on the left side of the nest entrance; CS → r – presentation of the CS previously associated with the UCS placed on the right side of the nest entrance. S – spatial conditioning = proportion of ants “spatially conditioned”, that is going to the two correct sides ($S = (w/W + y/Y)/2$).

Planning of the experiments

Four experimental colonies, originating from the same large natural colony, were used for one experiment. More precisely, the study of cue generalisation was performed on four experimental colonies and each of the three methods used for performing the spatial conditioning study were made on four different experimental colonies. Although these experimental colonies differed between the successive studies made, they were similarly named.

In the cue generalisation study, we attempted to condition a colony (named A) to the vertical line, another one (named B) to the horizontal line, a third one (C) to the “+” sign, and a fourth one (D) to the “x” sign.

In the spatial conditioning study, one colony received separately, at different times, and according to a sequence that is explained later, two distinct CS. The colony named “nest A” received the “vertical line” CS, associated with the UCS placed on the left side of the nest entrance and the “horizontal line” CS, associated with the UCS placed on the right side. The colony named “nest B” received the “horizontal line” CS, associated with the UCS placed on the left side of the nest entrance and the “vertical line” CS, associated with the UCS placed on the right side. The colony named “nest C” received the “sign +” CS, associated with the UCS placed on the left side of the nest entrance and the “sign x” CS, associated with the UCS placed on the right side. The colony named “nest D” received the “sign x” CS, associated with the UCS placed on the left side of the nest entrance and the “sign +” CS, associated with the UCS placed on the right side. This experimental set-up is summarized in Table 1, together with other information.

Experimental procedure

Part one: Ants’ visual cue generalisation

Training phase. Each experimental colony was trained with only one CS. To do this, a total of 12 reinforcing experiments were performed, two experiments every 24 h, successively at different times of the day (for example Day 1, 4:00 p.m.; Day 2, 1:00 a.m.; Day 2, 5:00 p.m.; Day 3, 2:30 a.m.; Day 3, 3:00 p.m.; Day 3, 12:00 p.m.; Day 4, 4:30 p.m.) in order to avoid temporal learning (such learning being possible in *M. sabuleti*, CAMMAERTS, 2001b). In order to perform a reinforcing experiment, first a CS was presented for 5 min to the experimental colony. Then, with the CS still located on the foraging area, the UCS was presented, for 15 min, just in front of the CS, to the colony being tested. Then, the CS and one minute later the UCS were taken away from the colony being tested. During the 15 min of the UCS presentation, at the end of each experimental minute, the ants present around (at a distance of 0 to 3 mm from, i.e. ants eating or having eaten or about to eat) the UCS were counted and the mean of the counts was established.

Test phase. In the absence of any UCS and CS, we counted the ants that arrived during two minutes onto a glass-slide located at the place where the UCS had been offered during the reinforcing experiments. The numbers obtained represented the control numbers. Then, the same counts were made in the presence of a CS distinct from the one used during the reinforcing experiments. The comparison between these numbers and the control numbers demonstrated the ants’ ability to discriminate between the two CS used. Following this, we counted the ants arriving, during two minutes, onto the glass-slide, in the presence of the CS used during the reinforcing experiments. The com-

parison between the numbers obtained here and the control ones evaluated the extent of the ants' conditioning. In fact the last counts were performed during a first set of extinction experiments. The experiments had to be carried out in this order since generalisation of cues must be studied before performing an experiment of extinction relative to the CS used during the reinforcing experiments.

Part Two: Ants' spatial conditioning

Training phase. Each experimental colony was trained with two distinct CS, one being associated with the UCS placed on the left side of the nest entrance, the other being associated with the UCS placed on the right side of the entrance. Twelve reinforcing experiments were made on each colony, for each CS and each reinforcing experiment was performed exactly as those made for studying the ants' generalization of cues. Three kinds of spatial conditioning methods were used. They differed in their sequence of presentation of the two CS.

– 1st method: the two CS were presented 12 times alternately (that is one CS, then the other one, then the first one and so on)

– 2nd method: the two CS were presented 12 times alternately, but while the UCS was offered at the correct side, a droplet of an acetic acid solution (8%) was placed at the wrong site. We observed that, when confronted with an acetic acid solution (8%), the ants did not drink it, but went away calmly from it, then avoided it. They were neither attracted nor repulsed by the solution; they did not change their speed of locomotion or their sinuosity of movement.

– 3rd method, first time: the two CS were presented 12 times successively, that is 12 times the CS associated with the UCS on the left side, then 12 times the CS associated with the UCS on the right side of the nest entrance. A reinforcing experiment relative to the first CS was performed after the 6th, 9th and 12th presentation of the second CS used.

– 3rd method, second time: the two CS were presented 12 times successively but this time, the CS associated with the UCS on the right side was presented first and the other second.

Each time, we counted the ants "surrounding" (generally eating) the UCS at the end of each of the 15 min of the UCS presentation, and calculated the mean of the counts.

It should be pointed out that the trail pheromone of *M. sabuleti* consists of 3-ethyl-2,5-dimethylpyrazine and is quite volatile (EVERSHED et al., 1981; CAMMAERTS et al., 1993). Therefore, even if the ants deposited a trail during one CS and UCS presentation, this trail would have vanished and become ineffective before the subsequent CS and UCS presentation and, a fortiori, before quantifying the ants conditioning and spatial conditioning (by performing the experimental procedure described below).

Test phase. After having performed the spatial conditioning reinforcing experiments, we counted the ants arriving during two minutes onto the glass-slides

placed on the left and on the right side of the nest entrance in the absence of any CS and UCS. The numbers obtained were the control ones. Then we counted the ants arriving during two minutes onto the glass-slides, in the presence of the CS previously associated with the UCS placed on the left side of the nest entrance, but in the absence of the UCS. After that, the same counts were made in the presence of the CS previously associated with the UCS placed on the right side of the nest entrance. The numbers obtained due to these two last counts could be compared to the control numbers in order to appreciate the ants' acquired conditioning. The successive presentations of the two CS constituted an extinction experiment. It was repeated until the complete extinction of the ants' response, a period of one hour elapsing between successive experiments.

During these extinction experiments, the ants were marked. While presenting the CS previously associated with the UCS placed on the left side of the nest entrance, the ants arriving during two minutes onto the glass-slide there located were marked with a droplet of white colour. While presenting the CS previously associated with the UCS placed on the right side of the nest entrance, the ants arriving during two minutes onto the correct right place were marked yellow. Marking was carried out by gently depositing a minute droplet of Humbrol® or Revell® enamel on the dorsal face of the ant's thorax or gaster, with the extremity of a n° 2 size entomological pin, disturbing them minimally.

While counting the ants arriving during two minutes onto a glass-slide, we separately counted those marked in white and yellow, respectively, and the newly marked ants (i.e. the ants marked during the counting process). The ants' spatial conditioning was assessed by the variable "S" which is the mean between (i) the number of ants marked white arriving on the glass-slide placed on the right side of the nest entrance (w) divided by the total number of the white marked ants of the colony while counting took place (W) (w/W represents the proportion of ants having gone onto the correct left side, then also moving onto the correct right side) and (ii) the number of ants marked yellow arriving on the glass-slide placed on the left side of the nest entrance (y) divided by the total number of the ants of the colony marked yellow when the current counting was performed (Y) (y/Y represents the proportion of ants having gone to the correct right side, then moving onto the correct left side). So, $S = (w/W + y/Y)/2$, varying from 0 to 1 (Tabs 3–6).

Statistical analysis

Test numbers obtained during each extinction experiment, for each colony and each correct site were compared to the corresponding control numbers using the non parametric test of Wilcoxon (SIEGEL & CASTELLAN, 1988). The values of N , T and P are given in the text. Results were considered non significant when $P > 0.06$ (indicated as ns) because some tests were made on only four experimental values, and 0.06 is the smaller possible value of P with $N = 4$.

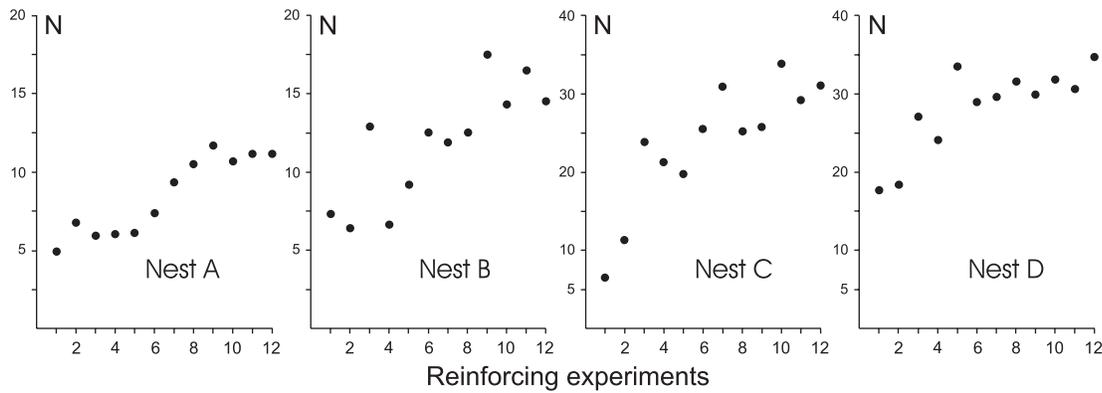


Fig. 2. Mean numbers of ants eating (N) during every 12 reinforcing experiments (study of generalisation of cues).

Results

Results are illustrated and numerically summarized in Figs 2–5 and Tables 2–6. The figures and tables have a similar legend, given in Table 1.

Study of the ants' generalisation of cues

The mean numbers of ants surrounding the UCS during each reinforcing experiment are given in Fig. 2. The differences between the four tested nests are due to differences in physiological state. An increase in the number of ants surrounding the UCS does not prove (but suggests) the acquisition by the ants of some conditioning. After the training phase, the numbers of ants responding during two minutes in the absence of the CS and the UCS (which are the control numbers) were very small (Tab. 2, line 1). Those obtained in the presence of a CS never previously presented to the ants were also small and not statistically different from the control numbers (Tab. 2, line 2; $N = 3$, $T = 4.5$, $0.250 < P < 0.375$; ns). In contrast, the numbers of ants responding to the CS used during the reinforcing experiments were statistically greater than the control numbers (Tab. 2, line 3, $N = 4$, $T = 10$, $P = 0.06$). The ants therefore discriminated the vertical line from the horizontal one, and the “+” sign from the “x” one. Since the ants could discriminate between two distinct CS, these two stimuli were used for trying to obtain spatial conditioning.

Spatial conditioning study

First method

The mean numbers of ants present near the UCS during the reinforcing experiments slightly increased during the course of these 12 experiments

Table 2. Results of the study of the ants' generalisation of cues.

Tested nests	A	B	C	D
Control numbers	0	2	5	9
Stimuli then presented	—		×	+
Experimental numbers	0	4	4	10
CS then presented		—	+	×
Experimental numbers	7	19	14	25

for each of the two CS presented to each of the four experimental colonies (Fig. 3), but as discussed later, this observation did not prove ants' conditioning.

The results of the test phase relative to each of the four colonies were very similar (Tab. 3). The ants' conditioning to the two CS was obvious during the first experiments of extinction: the numbers of ants arriving on the glass-slides during two minutes differed statistically from the control numbers ($N = 8$, $T = 36$, $P = 0.0039$). During the second extinction experiments, these numbers, although smaller, still differed statistically from the control ones ($N = 8$, $T = 36$, $P = 0.0039$). Then, when the CS were presented a third time, the ants' conditioning appeared to be extinguished, the experimental numbers no longer differing from the control ones ($N = 6$, $T = 18$, $P = 0.0781$; ns).

The ants' spatial conditioning could not be detected on the basis of the numbers of ants arriving during two minutes onto each of the two glass-slides. However, some ants' spatial conditioning could be revealed by “S”, that is on the basis

• : CS → UCS on left side
 ○ : CS → UCS on right side

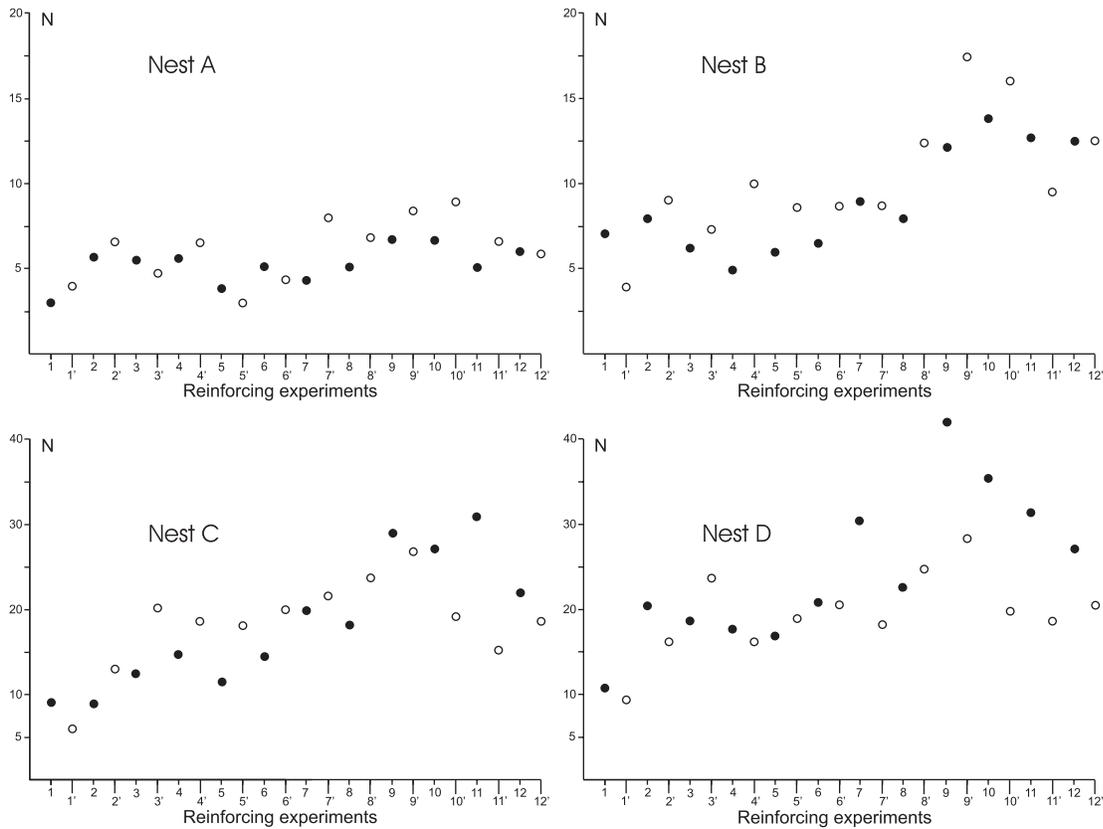


Fig. 3. First method of spatial conditioning. Mean numbers (N) of ants eating during 12 reinforcing experiments with a CS and the UCS on the left side of the nest entrance performed in alternation with 12 experiments with another CS and the UCS on the right side of the entrance.

Table 3. Results of the first method of spatial conditioning (legend in Tab. 1).

Tested nests	A		B		C		D	
Variables	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$
Control numbers	1	1	1	0	4	6	6	5
1 st exp. Extinction								
CS → l	$6_6^{0,6,0}$	6	$5_5^{0,5,0}$	4	$11_{11}^{0,11,0}$	12	$14_{14}^{0,14,0}$	13
CS → r	5	$8_5^{0,5,3}$	4	$9_{7,2}^{0,7,2}$	6	$14_{8,6}^{0,8,6}$	11	$14_{10}^{0,10,4}$
<i>S</i>		0.500		0.400		0.545		0.285
2 nd exp. Extinction								
CS → l	$3_9^{0,3,0}$	2	$3_8^{0,3,0}$	3	$6_{15}^{0,4,2}$	7	$9_{21}^{0,7,2}$	8
CS → r	2	$4_6^{0,1,3}$	3	$6_{10}^{0,3,3}$	5	$9_{13}^{0,5,4}$	8	$8_{16}^{0,6,2}$
<i>S</i>		0.166		0.188		0.258		0.148
3 rd exp. Extinction								
CS → l	$1_9^{0,0,1}$	1	$2_9^{1,1,0}$	0	$5_{19}^{1,4,0}$	5	$7_{24}^{2,3,2}$	5
CS → r	1	$2_6^{2,0,0}$	2	$2_{11}^{0,1,1}$	4	$5_{15}^{0,2,3}$	6	$5_{19}^{1,3,1}$
<i>S</i>		0.083		0.056		0.078		0.083

- : CS → UCS on left side + 8% acetic acid on right side
- : CS → UCS on right side + 8% acetic acid on left side

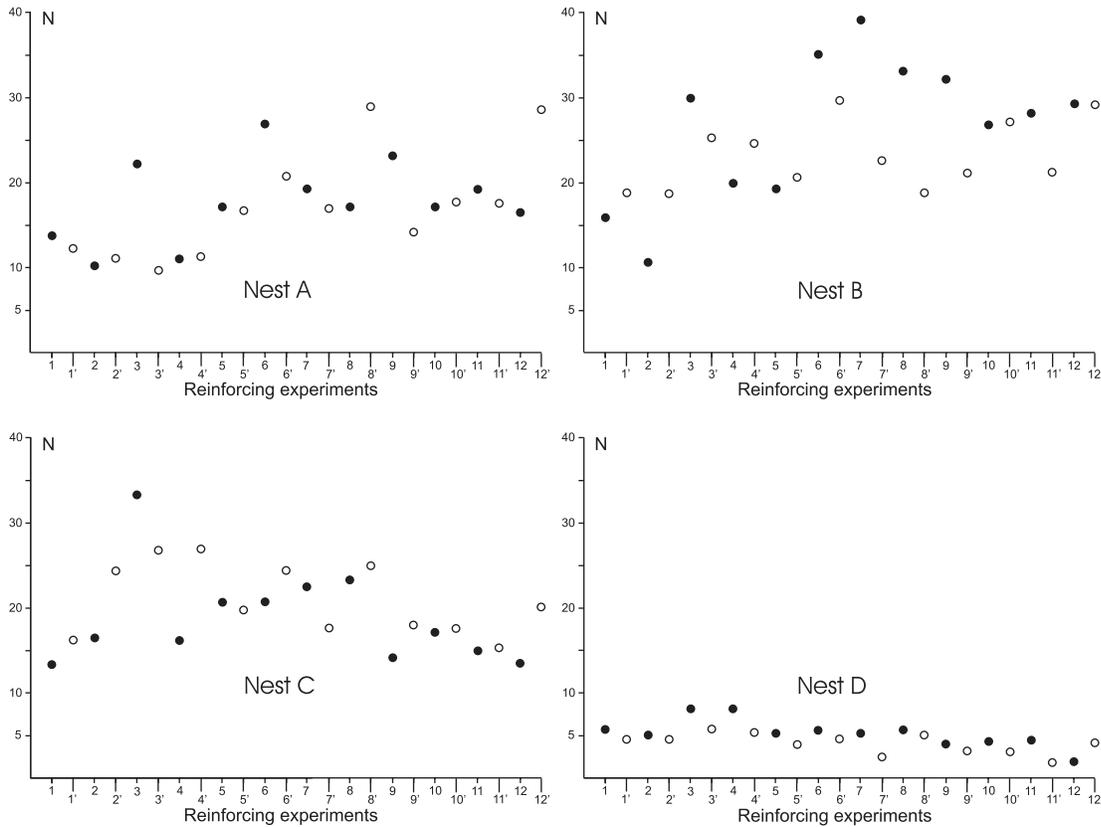


Fig. 4. Second method of spatial conditioning. Mean numbers (N) of ants eating during 12 reinforcing experiments made with a CS, the UCS being on the left side of the nest entrance and a 8% acetic acid water solution on the right side of the entrance, in alternation with 12 experiments made with another CS, the UCS being on the right side of the entrance, and the acetic solution on the left side.

of the proportion of ants using the two correct sites. Indeed, during the first extinction experiments, this proportion varied from 28.5 to 50.0% (mean = 43%). It decreased to values varying from 25.8 to 14.8% (mean = 19%) during the second extinction experiments, and became weaker in the course of the third experiments of extinction, varying then from 5.6 to 8.3% (mean = 7.5%).

Second method

In the course of the 12 experiments performed on nests A and B, the mean numbers of ants surrounding the UCS slightly increased. They did not so in the course of the 12 experiments performed on nests C and D, and this occurred for the two CS presented to each colony (Fig. 4). These dif-

ferences are due to differences in the physiological state of the colonies and allow no prediction about the acquisition by the ants of any conditioning.

As for the ants' conditioning and spatial conditioning obtained, the results, numerically summarised in Table 4, were very similar between the four experimental colonies.

The ants' conditioning to the two CS was obvious during the first three experiments of extinction (for each of these experiments, $N = 8$, $T = 36$, $P = 0.0039$). It extinguished during the fourth set of experiments ($N = 5$, $T = 9$, $P = 0.4063$).

The ants' spatial conditioning could slightly be detected on the basis of the numbers of ants arriving on the two glass-slides since the numbers of ants going onto the "correct" sites were larger

Table 4. Results of the second method of spatial conditioning (legend in Tab. 1).

Tested nests	A		B		C		D	
Variables	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$
Control numbers	5	5	7	7	10	9	0	0
1 st exp. Extinction								
CS → l	23 ^{0,23,0} ₂₃	12	17 ^{0,17,0} ₁₇	14	24 ^{0,24,0} ₂₄	14	2 ^{0,2,0} ₂	0
CS → r	10	19 ^{0,11,8} ₁₁	11	15 ^{0,10,5} ₁₀	16	20 ^{0,14,6} ₁₄	0	4 ^{0,3,1} ₃
S		0.348		0.294		0.250		0.500
2 nd exp. Extinction								
CS → l	13 ^{3,5,5} ₂₈	8	11 ^{2,5,4} ₂₂	10	16 ^{2,9,5} ₃₃	13	3 ^{1,1,1} ₃	0
CS → r	9	16 ^{5,5,6} ₁₆	9	12 ^{2,5,5} ₁₅	12	16 ^{2,8,6} ₂₂	1	3 ^{0,3,0} ₆
S		0.334		0.314		0.269		0.167
3 rd exp. Extinction								
CS → l	13 ^{7,2,4} ₃₀	7	10 ^{3,3,4} ₂₅	8	15 ^{5,5,5} ₃₈	13	2 ^{1,0,1} ₃	1
CS → r	6	12 ^{5,3,4} ₁₉	7	8 ^{3,3,2} ₁₈	12	14 ^{4,5,5} ₂₇	1	1 ^{1,0,0} ₆
S		0.192		0.173		0.179		0.083
4 th exp. Extinction								
CS → l	6 ^{6,0,0} ₃₀	5	6 ^{5,0,1} ₂₅	4	9 ^{4,3,2} ₄₁	5	1 ^{1,0,0} ₃	0
CS → r	5	5 ^{3,0,2} ₁₉	7	7 ^{6,0,1} ₁₈	6	9 ^{7,0,2} ₂₇	0	1 ^{1,0,0} ₆
S		0.033		0.068		0.061		0.000

than those of ants going onto the “wrong” sites. But these latter numbers were still larger than the control numbers ($P = 0.06$ for the three first extinction experiments).

The ants’ spatial conditioning was clearly revealed by the proportion of ants going onto the two “correct” sites (S): this proportion varied from 25 to 50% (mean = 34.8%) in the course of the first set of extinction experiments, from 16.7 to 33.4% (mean = 27%) in the course of the second set of extinction experiments, and from 8 to 19.2% (mean = 16%) in the course of the third set of extinction experiments. During the fourth set of experiments, the proportion became very weak (mean = 4%; extremes 0–6.8%). Thus the extinction of the ants’ spatial conditioning took longer than that obtained using the first method employed. However, the initial value (34.8%) was not larger than the initial value (43%) observed after having performed the first method. Thus, by presenting ants with a “non-reward” (acetic acid) on the “wrong” side, the proportion of conditioned ants was not increased (on the contrary), but the conditioned ants kept their conditioning a little longer (until four instead of three extinction experiments).

Third method, first time

The mean numbers of ants present around the

UCS during the 12 presentations of the first CS increased during the course of these 12 presentations (Fig. 5, top four graphs). During the first set of presentations of the second CS, the mean numbers of ants around the UCS were small. Then, for nests A, B and D but not for nest C, they increased during the course of the 12 presentations of this second CS, but not to such an extent as in the course of the 12 presentations of the first CS (Fig. 5, top four graphs). During the reinforcing experiments relative to the first CS, performed in the course of the 12 presentations of the second CS, the mean numbers of ants obtained were greater than those observed during the initial presentation of the first CS, but were fewer than those obtained during the twelfth of the 12 first presentations of this first CS (Fig. 5, top four graphs). All these increases and differences result from physiological modifications as well as from conditioning acquisition, and nothing can be deduced from such observations.

As for the ants’ conditioning and spatial conditioning, the results, numerically given in Table 5, were very similar for the four colonies used. The ants’ conditioning was obvious: the numbers of ants arriving on the “correct” glass-slides were statistically larger than the control ones until the fifth set of extinction experiments (1st and 2nd experiments: $N = 8$, $T = 36$, $P = 0.0039$; 3rd experiment:

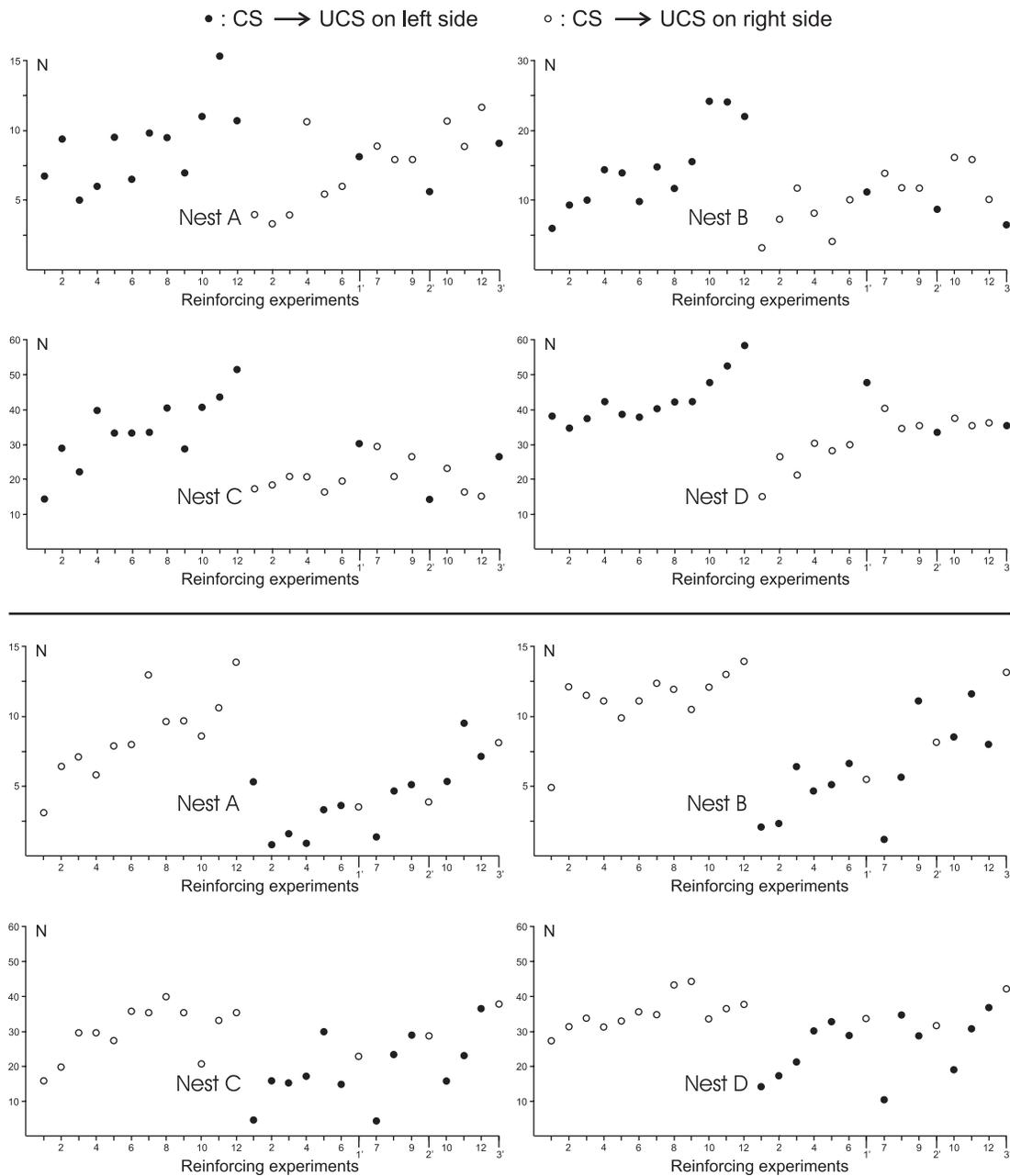


Fig. 5. Third method of spatial conditioning. Mean numbers (N) of ants eating during 12 successive reinforcing experiments with a CS and the UCS on one side of the nest entrance, followed by 12 experiments with another CS and the UCS at the opposite side. Three reinforcements (1', 2' and 3') with the UCS placed on the first side of the entrance (and the corresponding CS) were made during the session of reinforcements with the UCS placed at the second side (and the corresponding CS). Top four graphs: the UCS being placed at first at the left side of the entrance. Lower 4 graphs: same nests, but the UCS being placed at first at the right side of the entrance.

$N = 7$, $T = 28$, $P = 0.0078$; 4th experiment: $N = 21$, $P = 0.1484$; ns). The ants' spatial conditioning 7, $T = 25$, $P = 0.039$; 5th experiment: $N = 7$, $T =$ could already be seen on the basis of the numbers

Table 5. Results of the third method of spatial conditioning, first time (legend in Tab. 1).

Tested nests	A		B		C		D	
Variables	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$
Control numbers	0	4	0	6	0	2	4	4
1 st exp. Extinction								
CS → l	8 ^{0,8,0} ₈	2	9 ^{0,9,0} ₉	2	13 ^{0,13,0} ₁₃	3	14 ^{0,14,0} ₁₄	3
CS → r	2	10 ^{0,7,3} ₇	3	11 ^{0,7,4} ₇	4	11 ^{0,6,5} ₆	5	10 ^{0,5,5} ₅
S		0.375		0.444		0.385		0.357
2 nd exp. Extinction								
CS → l	8 ^{4,2,2} ₁₀	4	9 ^{2,4,3} ₁₃	4	7 ^{2,3,2} ₁₆	3	14 ^{3,6,5} ₂₀	1
CS → r	2	8 ^{3,2,3} ₉	1	10 ^{3,2,5} ₉	3	8 ^{2,3,3} ₉	3	9 ^{1,4,3} ₉
S		0.293		0.407		0.260		0.575
3 rd exp. Extinction								
CS → l	8 ^{3,3,2} ₁₃	2	7 ^{3,2,2} ₁₅	2	7 ^{3,2,2} ₁₈	3	8 ^{2,4,2} ₂₄	3
CS → r	3	6 ^{2,0,4} ₉	1	6 ^{2,2,2} ₁₁	2	7 ^{2,2,3} ₁₁	2	6 ^{1,4,1} ₁₃
S		0.265		0.177		0.194		0.132
4 th exp. Extinction								
CS → l	7 ^{1,2,2} ₁₅	4	5 ^{3,1,1} ₁₆	1	5 ^{2,1,2} ₁₉	2	6 ^{2,2,2} ₂₆	2
CS → r	2	3 ^{1,1,1} ₁₀	2	5 ^{2,0,3} ₁₁	2	6 ^{3,0,3} ₁₁	3	4 ^{1,1,2} ₁₄
S		0.145		0.138		0.170		0.115
5 th exp. Extinction								
CS → l	3 ^{1,1,1} ₁₆	1	2 ^{2,0,0} ₁₆	1	5 ^{4,0,1} ₁₉	2	6 ^{2,2,2} ₂₈	2
CS → r	1	3 ^{2,0,1} ₁₀	0	2 ^{0,0,2} ₁₁	3	4 ^{2,2,0} ₁₃	3	4 ^{1,1,2} ₁₅
S		0.081		0.063		0.046		0.107

of ants on the glass-slides: the numbers relative to the “correct” sides were larger than those relative to the “wrong” sides, and in this case, these latter numbers were not statistically different from the control ones (ns for the five extinction experiments).

According to the proportion of ants going to the two “correct” sites, the ants’ spatial conditioning was obvious. Five sets of extinction experiments were needed to reduce this proportion to a weak one. During the first set of extinction experiments, this proportion varied from 35.7 to 44.4% (mean = 39%). Its mean value was 38% (extremes 26–57.5%) during the second set of extinction experiments, 19% (extremes 13.2–26.5%) during the third one, and 14% (extremes 11.5–17%) during the fourth one. It varied from 4.6 to 10.7% (mean = 7%) during the fifth set of extinction experiments.

Although more experiments were necessary to extinguish the ants’ conditioning and spatial conditioning, the initial proportion of spatially conditioned ants obtained due to the third method employed (= 39%) was not larger than that ob-

tained by using the first or the second method (43%, 35%).

During all five sets of extinction experiments, spatial conditioning relative to the left side (the first performed) was stronger than the spatial conditioning relative to the right side (the second performed). Table 5 shows the numbers of ants found on the correct left side and those on the correct right side compared to the corresponding control numbers (e.g. for nest A: left: successively 8, 8, 8, 7 and 3 *vs* 0; right: successively 10, 8, 6, 3 and 3 *vs* 4). The Wilcoxon tests applied separately to the correct left and right scores indicated that for the correct left scores $P = 0.06$ for the 5 extinction experiments, while for the correct right scores, $P = 0.06$ for the 1st and the 2nd extinction experiments, $P = 0.125$ for the 3rd one, $P = 0.625$ for the 4th one and $P = 0.375$ for the 5th one. It may be that some unknown element, impossible to verify had been present on the left side of the experimental colonies and had helped the ants to acquire a spatial conditioning for this side. Thus, it is not possible to deduce if the stronger spatial conditioning obtained was the one relative to

the left side or that performed first. To resolve the problem, an identical experiment of spatial conditioning was performed on the same experimental colonies, identically positioned in the laboratory, with an unchanged environment, but by presenting first the CS associated with the UCS placed on the right side of the nest entrance, and second, the CS associated with the UCS placed on the left side (see below).

Third method, second time

For nests A, B and C, the mean numbers of ants surrounding the sugared water increased during the course of the 12 reinforcing experiments relative to the first CS used (Fig. 5, lower four graphs). The mean numbers obtained during the 12 presentations of the second CS also increased during these presentations, but generally by a smaller amount than the mean numbers observed during the presentations of the first CS, and generally with a decrease after the reinforcing experiments relative to the first CS, performed after the sixth and the ninth presentations of the second CS (Fig. 5, lower four graphs). In general, the mean numbers of ants obtained during the three reinforcing experiments relative to the first CS used and made in the course of the 12 presentations of the second CS were smaller than the mean numbers obtained during the last of the 12 first presentations of this first CS but were larger than the mean numbers observed during the first reinforcing experiments relative to the second CS. The mean numbers of ants surrounding the UCS generally increased in the course of the three reinforcing experiments relative to the first CS and made in the course of the 12 presentations of the second CS (Fig. 5, lower four graphs). But as previously stated and discussed later, all these increases allow no prediction of the ants' acquisition of any conditioning to be made.

Table 6 shows that the ants' conditioning and spatial conditioning were very similar to those obtained by using the first session of the third method employed and were similar for the four colonies tested. The ants' conditioning was obvious up to the fourth set of extinction experiments (1st, 2nd, 3rd experiments: $N = 8$, $T = 36$, $P = 0.0039$). It was still detectable during the fourth set of extinction experiments ($N = 7$, $T = 28$, $P = 0.0078$) and tended to be non significant during the fifth set of extinction experiments ($N = 7$, $T = 23$, $P = 0.0781$; ns). The ants' spatial conditioning was already evident on the basis of the numbers of ants arriving during two minutes on the two glass-slides: the numbers of ants going to the "correct"

sides were larger than those of ants going to the "wrong" sides and these last numbers did not differ from the control values (ns for the 5 extinction experiments).

On the basis of the proportion of ants going onto the two "correct" sites, the ants' spatial conditioning was evident during four successive experiments of extinction, requiring five experiments in order to be extinguished (similar to the first session of the third method of spatial conditioning). Indeed, during the first set of extinction experiments, the proportion of spatially conditioned ants varied from 40 to 75% (mean = 50%), during the second set of experiments, from 20.2 to 37.5% (mean = 26%), during the third one, from 10.8 to 27.1% (mean = 19%) and during the fourth one, from 4.5 to 12.1% (mean = 9%). During the fifth one, this proportion was very weak and non significant (mean = 3%; extremes 0–6.3%).

Although five experiments were necessary to extinguish the ants' spatial conditioning, the initial value observed (50%) was not much larger than that observed after having performed the first method of spatial conditioning (43%), for which only three experiments of extinction had to be made.

Expressed as the proportion of ants spatially conditioned, the results relative to the first and the second execution of the third method employed were so similar that they were pooled. Consequently, the proportion of ants spatially conditioned obtained due to the two replications of the third method employed was 45%, 32%, 19%, 12% and 5% respectively during the five successive experiments of extinction.

The number of ants going to the "correct" right sides was this time greater than the number of ants going to the "correct" left sides. Table 6 shows the numbers of ants on the correct right and the correct left sites in relation to the corresponding control numbers (e.g. for nest A: right: successively 8, 8, 5, 3, 3 *vs* 1; left: successively 4, 7, 4, 2, 1 *vs* 1). Wilcoxon tests carried out separately on the right and the left "correct" numbers lead to the conclusion that for the correct right side, $P = 0.06$ for the 5 extinction experiments while for the correct left side, $P = 0.06$ for the 3 first extinction experiments, $P = 0.125$ for the 4th one and $P = 0.375$ for the 5th one. During the first session of the third method, the largest numbers were those of ants going to the "correct" left sides. Therefore, it can be deduced that it was neither the left side, nor the right one of the nest entrance which gave a better spatial conditioning, but that it was the first spatial conditioning performed that gave the

Table 6. Results of the third method of spatial conditioning, second time (legend in Tab. 1).

Tested nests	A		B		C		D	
Variables	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$	$L_W^{w,nw,y}$	$R_Y^{y,ny,w}$
Control numbers	1	1	1	0	5	3	4	5
1 st exp. Extinction								
CS → l	$4_4^{0,4,0}$	1	$5_5^{0,5,0}$	1	$11_{11}^{0,11,0}$	4	$10_{10}^{0,10,0}$	4
CS → r	0	$8_5^{0,5,3}$	1	$8_4^{2,4,2}$	3	$17_{12}^{0,12,5}$	4	$17_{13}^{0,13,4}$
S		0.750		0.400		0.455		0.400
2 nd exp. Extinction								
CS → l	$7_7^{3,3,1}$	1	$4_6^{2,1,1}$	1	$8_{16}^{0,5,3}$	3	$9_{16}^{1,6,2}$	5
CS → r	1	$8_8^{1,3,2}$	1	$7_6^{2,2,3}$	3	$11_{18}^{2,6,3}$	5	$13_{18}^{4,5,4}$
S		0.243		0.375		0.219		0.202
3 rd exp. Extinction								
CS → l	$4_8^{1,1,2}$	0	$3_8^{0,2,1}$	2	$7_{19}^{2,3,2}$	3	$7_{19}^{2,3,2}$	4
CS → r	3	$5_9^{2,1,2}$	1	$7_7^{3,1,3}$	2	$8_{22}^{2,4,2}$	3	$11_{23}^{3,5,3}$
S		0.250		0.271		0.108		0.135
4 th exp. Extinction								
CS → l	$2_8^{1,0,1}$	0	$3_{10}^{0,2,1}$	1	$3_{19}^{2,0,1}$	3	$4_{22}^{1,3,0}$	3
CS → r	1	$3_9^{2,0,1}$	0	$4_8^{2,1,1}$	1	$8_{27}^{1,5,2}$	2	$8_{26}^{3,3,2}$
S		0.118		0.121		0.075		0.045
5 th exp. Extinction								
CS → l	$1_8^{1,0,0}$	0	$2_{10}^{2,0,0}$	1	$4_{22}^{0,3,1}$	1	$3_{24}^{1,2,0}$	2
CS → r	0	$3_9^{2,0,1}$	0	$2_8^{2,0,0}$	1	$5_{27}^{4,0,1}$	3	$6_{26}^{5,0,1}$
S		0.063		0.000		0.041		0.021

best scores, the second spatial conditioning leading to lower scores.

Discussion and conclusion

In brief, the present work shows that spatial conditioning can be obtained in the ant *M. sabuleti* and that the proportion of spatially conditioned ants is about 40%, depending on the method used for performing the reinforcing experiments. Three methods were used, leading to slightly different results. Of course, during the training phases, a usual turnover of foragers occurred, which prevented spatial conditioning from reaching very high scores. Our work also shows that ants are able to discriminate between a vertical and horizontal line, as well as between a “+” and “x” sign. This was shown in the first study reported here and was confirmed later while performing the third method of spatial conditioning: indeed, following the 12 presentations of the first CS, only a few ants surrounded the UCS in the presence of the second CS used.

Before summarizing the results, it should be noted that during each training period, the num-

bers of ants surrounding the UCS were initially low. These numbers sometimes increased during the course of the successive reinforcing experiments. Likewise, during the control experiments and after having performed the required experiments of extinction, the number of ants going onto the correct places was low again. All these observations show that the numbers of responding ants (i.e. going onto the correct places) cannot be ascribed to rapid recruitment or to disturbance caused by the CS presentation.

On the other hand, the increase in the number of ants counted around the UCS during the training phase could be due to an increase in the colony’s foraging activity and/or recruitment rate (since the colony was undergoing a period of starvation). These (not always observed) increases in the numbers of ants eating did not prove that the ants were undergoing conditioning. Only a following test phase can reveal the existence of such conditioning.

During each training phase, we performed 12 reinforcing experiments because previously, while studying ants’ classical conditioning (CAM-

MAERTS, 2001a), we experimentally found that such a number of reinforcing experiments permitted obvious classical conditioning to be observed.

Let us now summarize the slightly different results obtained thanks to the three used methods of spatial conditioning. By alternately presenting two distinct CS, each one 12 times and associated with a UCS located at a precise place, (i) the ants' conditioning and spatial conditioning extinguished in three experiments of extinction, (ii) the ants' spatial conditioning could only be seen on the basis of the proportion of spatially conditioned ants (43%). By alternately presenting two distinct CS, each one 12 times and associated with a UCS located at a precise place as well as with a "non-reward" at the opposite place, (i) the ants' conditioning and spatial conditioning extinguished in 4 experiments of extinction, (ii) the ants' spatial conditioning could already be assumed on the basis of the numbers of ants responding to the CS and the proportion of spatially conditioned ants was 35%. By presenting successively two CS, each one 12 times, with a further three presentations of the first CS after the 6th, the 9th and the 12th presentations of the second CS, (i) the ants' conditioning and spatial conditioning extinguished in five experiments of extinction, (ii) their spatial conditioning was already obvious on the basis of the numbers of responding ants, and the proportion of spatially conditioned ants was 45%.

The third method also revealed that the ants' conditioning to a first CS perceived was always stronger than that to a second CS presented. This leads us to think that the route fidelity of ants, present in many species, e.g. *Dinoponera gigantea* (Perty, 1833) (FOURCASSIÉ et al., 1999) may be partly due to the ants' difficulty to associate more than one stimulus to a reward. To confirm this hypothesis, it would be necessary to try spatial conditioning with three or more distinct CS. Conversely, method 2 and 3 should be combined to see if better scores of spatial conditioning could be obtained.

Many ethological studies on insects suggest that spatial conditioning is possible in these invertebrates. Indeed, many insects can associate elements of their environment with the presence of food, and sometimes of different food items. For instance, cockroaches seem to learn the locations of specific food resources and associate particular locations with particular food resources (DURIER & RIVAULT, 2001). An experiment carried out on bumblebees by FAURIA et al. (2001) demonstrated that these insects were able to bind the same pattern elements into several configurable

units. Therefore, bumblebees might be able to acquire spatial conditioning. In honeybees, associative learning is a fast and robust process (HAMMER & MENZEL, 1995). Generally, the CS used is chemical, but the problem of discrimination and generalisation of visual cues has also largely been considered (e.g. PHAM-DELEGUE et al., 1993; SANDOZ et al., 2001), sometimes at a high level (e.g. SANDOZ & MENZEL, 2001; DEISIG et al., 2001). This research suggests that spatial conditioning is possible in these Hymenoptera. With respect to ants, foragers searching for food must logically associate several terrestrial cues with the nest and/or food sites. Indeed, much experimental work suggest that ants perceive cues and use them for orienting themselves (towards their nest, for instance), even if the species possess a trail pheromone (VOWLES, 1965; HENQUELL & ABDI, 1981; ROSENGREN & FORTELLIUS, 1986; ARON et al., 1988, COLLETT et al., 1992). VOWLES (1965), for instance, clearly showed that *Formica rufa* L., 1761 workers could use visual patterns for orienting themselves in a maze. COLLETT et al. (1992) experimentally showed that *Cataglyphis spp.* foragers use landmarks for piloting and proposed that these ants would learn whether to pass to the left or to the right of a particular landmark. These studies clearly show that ants use visual cues but direct proof of ants' spatial classical (and not operant) conditioning did not previously exist. It was thus useful to investigate the problem as we did in the present work. Our results demonstrate that *Myrmica sabuleti* foragers can acquire such a spatial conditioning.

A further step would be to obtain spatial conditioning to two or three CS in an ant, and to present these CS in another context, for instance along a complex route to food resources. Such a study would allow us to assess the ants' memory and transfer of information. Similar studies have been done on ants, e.g. by VOWLES (1965), COLLETT et al. (1992) and CHAMERON et al. (1998) as well as on honeybees (SANDOZ et al., 1995; SANDOZ et al., 2000), even at a high physiological level (MENZEL, 1999, 2001). However, before doing so, we considered it necessary to detail *M. sabuleti* foragers' discrimination and generalisation of cues, to precise their distance of vision and analyze other characteristics of their visual perception. This work, presented in a poster (CAMMAERTS, 2002) will be published soon in detail and the results compared to those obtained by other authors (VOSS, 1967; WEHNER, 1981).

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First records of *Pemphigus fuscicornis* (Homoptera, Pemphigidae) from Slovakia

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Sugar beet aphid, *Pemphigus fuscicornis* (Koch, 1857) (Homoptera, Pemphigidae), is an important pest of sugar beet in E Europe (ČAMPRAĀ et al., 2003). It is known from Poland (HEIE, 1980), Hungary, Bulgaria, Romania, Serbia, Croatia (ČAMPRAĀ et al., 2003), Greece (IOANIDIS, 1996), Ukraine and Russia (PISNYA & FEDORENKO, 1988). Distribution in W Europe is little known (HEIE, 1980). Accounts are available only for Germany (BOSCH & DUDA, 1994), Denmark, Sweden (HEIE, 1980) and Finland (ALBRECHT et al., 1996). Except Europe, there are records from Asia: Georgia, Armenia, Kazakhstan (ČAMPRAĀ et al., 2003) and Iran (SHADMEHRI et al., 2001). Until now, no information has been available about *P. fuscicornis* in Slovakia, but its occurrence was expected.

The species is similar to *P. bursarius*, but greyish green, not whitish yellow (HEIE, 1980). The rear portion of the body produces a mass of white waxy material. The aphids are most readily seen in the white mold-like material that is found on the infested roots and in the surrounding soil. The aphid is associated with fibrous roots rather than the main root. Although HEIE (1980) mentioned the primary host is unknown, BOSCH & DUDA (1994) noted that winged adults migrate from roots of secondary hosts (Chenopodiaceae)

to the cottonwood trees (*Populus* sp.) that are primary hosts, and lay their eggs there. On the other hand, anholocyclic populations often persist all year on secondary hosts (ČAMPRAĀ et al., 2003).

P. fuscicornis lives on roots of *Matricaria* sp., *Tripleurospermum* sp. (ALBRECHT et al., 1996) and wild and cultivated species from the family Chenopodiaceae, including sugar beet *Beta vulgaris* prov. *altissima* (Doell) (= *Bva*) (PISNYA, 1986). We recorded lambsquarters *Chenopodium* sp. (= *Ch.*), oraches *Atriplex* sp., sugar beet and red beet *B. vulgaris* prov. *conditiva* (Alef.) (= *Bvc*) as host plants in Slovakia. The results of this study show that *P. fuscicornis* is usual insect in SW Slovakia with high potential to become an important pest of sugar beet.

Material examined. SW Slovakia, wingless and winged (w) females of *P. fuscicornis* were found during July, September and October at 31 sites/host plant for each site is mentioned: **Branč** (48°13' N, 18°09' E, 7774 = grid reference number of the Databank of the Fauna of Slovakia), 137 m a.s.l., 26.IX.2003, 61 ♀♀, 11(w) ♀♀/*Bva*; *Ch.*; **Čeladice** (48°20' N, 18°15' E, 7675), 170 m a.s.l., 25.IX.2003, 33 ♀♀, 35(w) ♀♀/*Bva*; *Bvc*; *Ch.*; **Dolný Štál** (47°56' N, 17°43' E, 8072), 112 m a.s.l., 23.IX.2003, 38 ♀♀/*Bva*; *Ch.*; **Domadice**

(48°11' N, 18°47' E, 7878), 168 m a.s.l., 3.X.2003, 44 ♀♀, 2(w) ♀♀/Ch.; **Hontianska Vrbica** (48°08' N, 18°43' E, 7878), 173 m a.s.l., 3.X.2003, 52 ♀♀, 1(w) ♀♀/Ch.; **Hontianske Moravce** (48°11' N, 18°51' E, 7879), 159 m a.s.l., 3.X.2003, 83 ♀♀, 2(w) ♀♀/Ch.; **Hosťová** (48°20' N, 18°13' E, 7675), 198 m a.s.l., 25.IX.2003, 56 ♀♀, 5(w) ♀♀/Ch.; **Hurbanovo** (47°52' N, 18°12' E, 8175), 115 m a.s.l., 11.X.2003, 48 ♀♀, 18(w) ♀♀/Ch.; **Jelka** (48°09' N, 17°31' E, 7871), 123 m a.s.l., 13.X.2003, 35 ♀♀, 16(w) ♀♀/Ch.; **Kostolné Kračany** (47°59' N, 17°35' E, 8071), 117 m a.s.l., 23.IX.2003, 24 ♀♀/Bva; Ch.; **Lipová**, časť Ondrochov (48°08' N, 18°11' E, 7875), 126 m a.s.l., 26.IX.2003, 25 ♀♀/Ch.; **Maňa** (48°09' N, 18°17' E, 7875), 131 m a.s.l., 11.X.2003, 9 ♀♀, 2(w) ♀♀/Ch.; **Nemčiňany** (48°18' N, 18°28' E, 7676), 212 m a.s.l., 2.X.2003, 28 ♀♀, 36(w) ♀♀/Ch.; **Nevidzany** (48°17' N, 18°23' E, 7776), 181 m a.s.l., 2.X.2003, 49 ♀♀, 2(w) ♀♀/Ch.; **Nová Dedina** (48°17' N, 18°40' E, 7777), 190 m a.s.l., 3.X.2003, 37 ♀♀, 39(w) ♀♀/Ch.; **Kostolné Kračany**, časť Pinkové Kračany (47°58' N, 17°35' E, 8071), 119 m a.s.l., 23.X.2003, 31 ♀♀/Bva; Ch.; **Pribeta** (47°54' N, 18°19' E, 8075), 135 m a.s.l., 11.X.2003, 39 ♀♀, 3(w) ♀♀/Ch.; **Santovka** (48°09' N, 18°46' E, 7878), 162 m a.s.l., 3.X.2003, 45 ♀♀/Ch.; **Sokolce** (47°51' N, 17°50' E, 8172), 112 m a.s.l., 23.IX.2003, 19 ♀♀, 7(w) ♀♀/Bva; Ch.; **Tehla** (48°11' N, 18°23' E, 7876), 180 m a.s.l., 1.X.2003, 65 ♀♀, 3(w) ♀♀/Ch.; **Tlmače** (48°17' N, 18°32' E, 7777), 220 m a.s.l., 2.X.2003, 47 ♀♀/Ch.; **Tomášikovo** (48°05' N, 17°42' E, 7972), 118 m a.s.l., 23.IX.2003, 4 ♀♀/Bva; **Tôň** (47°48' N, 17°50' E, 8173), 112 m a.s.l., 23.IX.2003, 60 ♀♀/Ch.; **Trávnica** (48°09' N, 18°20' E, 7876), 130 m a.s.l., 26.IX.2003, 79 ♀♀/Ch.; **Veľké Chyndice** (48°17' N, 18°18' E, 7775), 190 m a.s.l., 12.VII.2003, 53 ♀♀/Ch.; **Veľký Cetín** (48°13' N, 18°12' E, 7875), 130 m a.s.l., 6.X.2003, 28 ♀♀/Ch.; **Vlkanovo** (47°57' N, 18°14' E, 8075), 130 m a.s.l., 11.X.2003, 22 ♀♀, 2(w) ♀♀/Ch.; **Vráble** (48°15' N, 18°19' E, 7775), 142 m a.s.l., 23.X.2003, 22 ♀♀, 1(w) ♀♀/*Atriplex* sp., Ch.; **Záhorská Ves** (48°22' N, 16°51' E, 7667), 149 m a.s.l., 12.X.2003, 6 ♀♀/Ch.; **Zlaté Klasy** (48°07' N, 17°25' E, 7870), 124 m a.s.l.,

13.X.2003, 25 ♀♀, 5(w) ♀♀/Ch.; **Zohor** (48°15' N, 16°58' E, 7667), 146 m a.s.l., 12.X.2003, 74 ♀♀, 2(w) ♀♀/Ch. Leg. P. Tóth, M. Tóthová et J. Tancik; det. et. coll. J. Holman et P. Tóth.

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