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# 1 **Quantitative cognition in carpenter ants**

2 Patrizia d'Ettorre<sup>1,2</sup>, Patricia Meunier<sup>1</sup>, Pietro Simonelli<sup>1</sup>, Josep Call<sup>3</sup>

3 <sup>1</sup> Laboratory of Experimental and Comparative Ethology, University of Sorbonne Paris Nord,  
4 99 avenue J.-B. Clément, 93430 Villetaneuse, France

5 <sup>2</sup> Institut Universitaire de France (IUF)

6 <sup>3</sup> School of Psychology and Neuroscience, University of St Andrew, St Mary's Quad, South  
7 Street, St Andrews, Fife, KY16 9JP, Scotland, United Kingdom.

8 ***Corresponding Author:*** Patrizia d'Ettorre

9 Phone: +33 1 4940 3196, e-mail: d-ettorre@univ-paris13.fr

10 ***Keywords:*** *Camponotus*, spontaneous quantitative discrimination, sequential landmarks,  
11 training, social insects

## 12 **Abstract**

13 Processing information about quantities allows animals to make optimal decisions during many  
14 natural contexts, such as foraging, territorial defense, offspring care, mate choice and intra-  
15 sexual competition. Compared to the wealth of information available in vertebrates, much less  
16 is known in invertebrates, even though the processing of quantities is equally relevant for both  
17 taxa. Here, we used two separate ecologically relevant tasks (brood pile preference and  
18 landmark-guided foraging) to investigate two dimensions of quantitative cognition in carpenter  
19 ants: spontaneous quantitative judgements and trained use of sequential landmarks. Individual  
20 ants spontaneously discriminated between two piles of dummy cocoons both when the choice  
21 involved smaller (1 vs. 2, 3, 4) and larger numerical contrasts (2 vs. 4, 6, 8). Ants used both  
22 chemical and visual/tactile cues and their performance was dependent on the numerical ratio.  
23 In the second task, ants preferentially searched near the trained landmark (out of five identical  
24 ones) despite alterations in its position, suggesting that they used ordinal information about its  
25 location when searching for food. In this experiment, ants showed a limit at four since their  
26 performance drastically decreased when they were trained to the 5<sup>th</sup> landmark. We showed that  
27 carpenter ants use both relative quantity and relative position to make efficient decisions. Our  
28 study contributes to the scant body of knowledge available on quantitative cognition in  
29 invertebrate species.

## 30 **Significance Statement**

31 There is ample experimental evidence that non-human animals are able to process quantitative  
32 information, in particular mammals, some birds and fish, and that this ability is useful in a  
33 variety of ecologically relevant situations. Yet, information about quantitative cognition in  
34 invertebrates is very scarce. We show that individual ants are able to use both relative quantity  
35 and relative position of items. Nurse ants spontaneously discriminated between two brood piles  
36 of different size and foragers were able to learn to identify a landmark associated to food on the  
37 basis of its position in a series of identical ones. These results suggest that ants might use  
38 quantitative information to make optimal choices in their natural environment although the  
39 precise mechanisms underlying this ability should be further investigated.

40

## 41 **Introduction**

42 Many animals have evolved the ability to discriminate between two different quantities, i.e. to  
43 make relative judgments of magnitude differences (more versus less), because this has a  
44 significant adaptive value in several ecologically relevant contexts, such as antipredator  
45 behavior, social interactions and foraging. For instance, the preference of guppies and other fish  
46 to join the larger shoal of conspecifics (Agrillo 2008; Bisazza et al. 2010) is an efficient  
47 antipredator strategy given that being in a larger group decreases an individual's chances of  
48 being predated upon (dilution effect, Dehn 1990). Similarly, treefrogs facing a dichotomous  
49 choice prefer a patch with more abundant or tall grass than one with shorter or less abundant  
50 grass since this type of microhabitat offers greater protection and more resources (Lucon-  
51 Xiccato et al. 2018). As predicted by game theory (Maynard Smith and Parker 1976), the  
52 willingness to engage in contests should relate to numerical advantage in social species.  
53 Therefore, it is advantageous for lions, chimpanzees and spotted hyaenas to perform relative  
54 numerical assessment in order to adjust their agonistic behavior to the number of potential  
55 opponents (McComb et al. 1994; Wilson et al. 2001; Benson-Amram et al. 2011). In the context  
56 of foraging, being able to spontaneously select the higher amount of food is clearly beneficial.  
57 For instance, birds and fish can discriminate the number or size of food items and prefer the  
58 larger food quantity (e.g., crows: Bogale et al. 2014; guppies: Lucon-Xiccato et al. 2015). In  
59 laboratory experiments, orangutans and baboons showed the capacity to select the larger  
60 quantity of food items (Call 2000; Barnard et al. 2013) and semi-free ranging rhesus monkeys  
61 discriminated between different quantities of apple pieces (Hauser et al., 2000). Several other  
62 vertebrate species have been shown to prefer large over small quantities of food, including  
63 domestic dogs and coyotes (Ward and Smuts 2007; Baker et al. 2011), horses (Uller and Lewis  
64 2009) and elephants (Plotnik et al. 2019).

65 Another ability that can be advantageous for animals is keeping track of the serial  
66 position of targets in the context of repeated foraging trips to valuable food sources. Several  
67 species can identify a landmark associated with food in a series of identical landmarks based  
68 on its relative position. For instance, rats could easily learn to select a target box containing  
69 food based on its position in an array of several identical boxes (up to the 12<sup>th</sup> position is a  
70 sequence of 18 boxes) and remembered this information for more than 1 year (Davis and  
71 Bradford 1986; Suzuki and Kobayashi 2000). Young domestic chicks were able to learn to  
72 identify a target and peck for food on the basis of its serial position in a sequence of 10 targets  
73 (Rugani et al. 2007). More recently, similar results were obtained with fish. Guppies easily  
74 learned to identify the 3<sup>rd</sup> feeder in a row of 8 identical feeders, but made more errors when

75 they were trained to a feeder in the 5<sup>th</sup> position, suggesting that 5 is close to their numerical  
76 threshold (Miletto Petrazzini et al. 2015). Several primate species, including rhesus and  
77 capuchin monkeys were able to order stimuli containing one, two, three, or four items in  
78 ascending order and generalized the learned rule to novel stimuli outside of the trained range  
79 (Brannon and Terrace 1998; Judge et al. 2005).

80         These are only some of the many documented examples showing that quantitative  
81 cognition is widespread in non-human animals and helps individuals make adaptive decisions  
82 in a wide range of ecological contexts. Compared to the wealth of information available in  
83 vertebrates (see Agrillo and Bisazza 2018; Nieder 2018; Rugani 2018 for reviews), less is  
84 known about quantitative cognition in invertebrates, with the exception of honey bees (see Pahl  
85 et al. 2013; Skorupski et al. 2018; Giurfa 2019 for reviews). Worker honey bees can be trained  
86 to use a sequence of identical landmarks, with the limit of four, to find a food source. This was  
87 first shown in field experiments (Chittka and Geiger 1995) and then confirmed in controlled  
88 laboratory experiments (Dacke and Srinivasan 2008) in which the use of other cues, for  
89 instance, the distance from the starting point of the fly tunnel, was ruled out. Honey bees can  
90 use the number of items on a stimulus to perform a match-to-sample task (Gross et al. 2009)  
91 and, among other numerical skills (Giurfa 2019), individual honey bees appear to be able to  
92 represent zero (an empty set) as the low end of a series of positive numbers (Howard et al.  
93 2018). Recently, honey bees have been tested for spontaneous quantity discrimination of  
94 artificial flowers and appear to make the difference only between one versus more (1 vs 3, 1 vs  
95 4, 1 vs 12; Howard et al. 2020).

96         Information about the quantitative abilities of other insects is relatively scant and it is  
97 unclear whether other insects can cope with various aspects of quantitative information.  
98 Mealworm beetles spontaneously discriminate chemical cues reflecting different numbers of  
99 females (Carazo et al. 2009), and are also able to keep track of up to 4 rival males encountered  
100 sequentially in the context of sperm competition and mate guarding (Carazo et al. 2012).  
101 Crickets tested in a dichotomous choice between different sets of geometrical shapes  
102 representing potential shelters spontaneously chose those with the larger numerosity, with the  
103 limit at 3–4 items (Gatto and Carlesso 2019). These crickets make use of continuous variables  
104 of objects, such as surface area, convex hull, rather than counting the number of items in each  
105 set. Animals might be able to use both pure number and continuous quantities, and possibly be  
106 more efficient when both cues are available, as in mosquitofish (Agrillo et al. 2011).

107         Ants have evolved the most advanced social organization and show remarkable

108 learning, memory and recognition of identity (d’Ettorre 2013; Perez et al. 2015; Piqueret et al.  
109 2019), but their quantitative skills have been less explored than those of honey bees. Similar to  
110 chimpanzees, lions and hyaenas, workers of the ant species *Formica xerophila* appear to assess  
111 the number of potential competitors and are more aggressive when they perceive themselves as  
112 part of a large group (Tanner 2006). Workers of the Japanese ant, *Myrmecina nipponica* are  
113 able to quantify the number of nestmates present in a new nest site during a relocation process  
114 (Cronin 2014). By conditioning entire colonies of *Myrmica sabuleti* in the laboratory,  
115 Cammaerts (2008) showed that ant workers can distinguish figures containing different  
116 numbers of the same element (e.g. 1 vs 2 and 2 vs 3 black circles) by using continuous variables,  
117 and appear to react to the basic notion of zero, i.e. the absence of visual or olfactory cues is  
118 rewarded (Cammaerts and Cammaerts 2019). Individual founding queens of the ant *Lasius*  
119 *niger* adjust their egg laying to the quantity of brood present in the nest. Queens produced fewer  
120 eggs when their colony contained ample brood, suggesting that they could estimate the total  
121 amount of brood present (Holman et al. 2010). Studies in red wood ants suggested that workers  
122 possess numerical competence and symbolic communication (Reznikova and Ryabko 1996;  
123 2011). Ant scouts were trained in different variants of a “counting maze”, equipped with several  
124 different branches, to find food on a particular branch. When returning to the nest, a scout  
125 typically engaged in antennal contact with foragers and the duration of this antennation was  
126 proportional to the number of branches to be passed on the way to reach the food. The foragers  
127 could often find the location of the food without the scout. As noted by Reznikova (2008), this  
128 is not direct evidence that a scout memorized the number of branches, since the ant could pass  
129 information about the distance to the food (see also Pahl et al. 2013). Indeed, ants can measure  
130 distance very accurately, as shown in desert ants that use an internal odometer to count steps  
131 (Wittlinger et al. 2006).

132 We investigated two aspects of the quantitative abilities of carpenter ants, *Camponotus*  
133 *aethiops*: spontaneous quantitative judgements and trained use of sequential landmarks. We  
134 investigated each of these two aspects in two separate tasks reflecting the ecology of the ants.  
135 The first task was a brood pile preference task. We presented nurse ants with two options  
136 containing different numbers of cocoons (dummies made of pieces of filter paper impregnated  
137 with chemical extract of natural cocoons). We expected that ants would select the option with  
138 the largest quantity of cocoons. The second task was a landmark-guided foraging task. Ants  
139 moved along a straight corridor aligned with five landmarks. After they learned that food could  
140 be found next to one of the landmarks (e.g., the second from the entrance), we changed the  
141 relative position of the landmarks to investigate, during an unrewarded test, whether ants

142 encoded the position of the food in terms of the distance travelled or the relative position of the  
143 landmark. We expected that, if ants used the order of appearance of the landmarks, they would  
144 search near the location of the landmark whose ordinal position matched the original position  
145 of the food.

## 146 **Material and Methods**

147 **Study organism.** Queenright colonies of *Camponotus aethiops* were collected in 2016 at  
148 Pompertuzat (Midi-Pyrénées, France, latitude 43.5°, longitude 1.516667°) and kept in the  
149 laboratory (25°C, light-dark cycle = 12:12, 40% humidity) in artificial nests composed of two  
150 plastic boxes connected by a plastic hose. One of the boxes (25.5 × 18 × 9 cm) was covered by  
151 cardboard and contained a plaster floor to form the nest; the other box (27.5 × 28 × 8 cm),  
152 exposed to light constituted the foraging arena. The inner sides of the two boxes were coated  
153 with Fluon© to prevent ants from escaping. Ants were fed twice a week with a mixture of  
154 apples and honey and frozen crickets, water was provided ad libitum.

155 **Spontaneous quantitative discrimination.** In a circular arena (12 cm diameter) with clean  
156 filter paper on the floor, we placed dummy cocoons made of filter paper (1 cm<sup>2</sup> rolled as a  
157 cylinder) inside two circular zones (4 cm diameter defined with a light pencil trait) equidistant  
158 from the starting point of the ant, which was a plastic cylinder (2.2 cm diameter), Fig. S1. The  
159 position right/left of the different stimuli (i.e., groups of different number of cocoons) was  
160 alternated in different replicates.

161 Chemical extracts of real ant cocoons were prepared by immersing one freshly frozen  
162 cocoon in 200 µl of pentane (HPLC grade, Sigma-Aldrich, France) for 10 minutes. Each  
163 dummy cocoon was perfumed with 20 µl of chemical extract (1/10 cocoon equivalent). In any  
164 given trial, each dummy cocoon was perfumed with a chemical extract from a different cocoon  
165 issued from the same colony as the tested ant. Three numerical ratios were presented: 0.50, 0.33  
166 and 0.25, for smaller (1 vs. 2, 1 vs. 3, 1 vs. 4) and larger numerical contrasts (2 vs. 4, 2 vs. 6, 2  
167 vs. 8). For each of the six combinations, the number of tested ants was  $n = 15$ .

168 The tested ant was a nurse collected from inside the nest. The ant was placed in the  
169 plastic cylinder and allowed to acclimatize for one minute, then the cylinder was gently lifted  
170 and the ant was free to explore the arena for 5 minutes, during which the time spent in contact  
171 with the dummy cocoons in the two different zones was recorded with the help of a behavioral  
172 transcription software (Ethoc version 1.2, CNRS Research Centre on Animal Cognition,  
173 Toulouse). Each ant was tested only once.

174 **Cues used for discrimination.** To investigate the importance of chemical cues compared to  
175 visual/tactile cues, we tested ants in the same apparatus as above but this time either the number  
176 of cocoons in each pile or the total amount of chemical extract was constant. Ants were either  
177 confronted to the same number of dummy cocoons perfumed with different amounts of the  
178 same chemical extract (1 cocoon without chemical extract *vs.* 1 cocoon with 20  $\mu$ l of chemical  
179 extract,  $n = 12$ ; 1 cocoon with 10  $\mu$ l of extract *vs.* 1 cocoon with 20  $\mu$ l of extract,  $n = 15$ ) or to  
180 different number of cocoons perfumed with the same total amount of chemical extract (1  
181 cocoon with 20  $\mu$ l extract *vs.* 2 cocoons with 10  $\mu$ l extract each,  $n = 15$ ; 2 cocoons with 20  $\mu$ l  
182 extract each *vs.* 4 cocoons with 10  $\mu$ l extract each,  $n = 15$ ). Each ant was tested only once.

183 **Statistical analyses.** Statistical analyses were performed with R (R Core Team 2019). The time  
184 spent in contact with the two stimuli (cocoon piles) was compared using the Paired Samples  
185 Wilcoxon test for all combinations separately. To check if ants inspected the cocoons  
186 sequentially, and therefore spent more time with the pile containing a higher number of  
187 cocoons, we recorded the behaviour of the ants after the first inspection of both piles in the 2  
188 versus 6 trials. For this, in each test we waited that the ant inspected one pile, then the other  
189 pile, and only after these first inspections we recorded the time spent antennating each pile and  
190 the occurrences of contact (Fig. S3). For the effect of numerical ratio, data were analyzed with  
191 a linear model in which the percentage of time spent investigating the larger cocoon pile was  
192 the dependent variable and ratio (0.50, 0.33, 0.25) was inserted as a factor. P-values were  
193 extracted by permutation test (1000 permutations, R package *pgirmess* (Giraudoux 2016)). If  
194 the effect of the factor ratio was significant, post-hoc comparisons were performed with the  
195 same model but a reduced dataset.

196 **Sequential landmark test.** A rectangular plexiglass corridor (length 50 cm, width 5 cm) with  
197 clean filter paper on the floor was equipped with 5 landmarks consisting of cardboard stripes  
198 (length 9 cm, width 1.6 cm) covered with grey adhesive tape. When placed on the floor, the  
199 stripes climbed 2 cm on both side walls as shown in Fig. 3a. The walls were covered with  
200 Fluon© so that the ants could not climb and all around the corridor there were cardboard walls  
201 to prevent the use of visual cues outside the corridor. During the training phase, the reward (2  
202  $\mu$ l of sugar solution 30% w/w) was placed right after the first, second, third, fourth or fifth  
203 landmark depending on the experimental group of ants. A separate group of ants was  
204 individually trained for each landmark reward position (Landmark 1:  $n = 15$ ; L2:  $n = 15$ ;  
205 L3:  $n = 17$ ; L4:  $n = 14$ ; L5:  $n = 15$ ). For each ant, the training phase consisted of 8 consecutive  
206 training trials. Within each trial, the inter-landmark distance was held constant, but across trials  
207 the distance varied (Fig. 2a, Fig. S2, Table S1). In this way, the ants could not use any spatial

208 information to locate the reward (e.g., distance from the starting point) because even if the  
209 reward was always placed after the same landmark, its position in the arena varied at every  
210 training trial.

211 An ant marked with a small dot of paint was collected from the foraging area of its  
212 colony, placed in a plastic cylinder (2.2 cm diameter) at one end of the arena (Fig. 2a) and  
213 allowed to acclimatize for one minute. After the removal of the cylinder the forager ant was  
214 free to explore the arena. Once the reward found, the ant was allowed to drink the sugar solution  
215 and then was placed for 3 minutes back to the foraging area of its colony where it usually  
216 engaged in trophallaxis (mouth to mouth exchange of liquid food) with nestmates. During this  
217 time the filter paper at the floor of the experimental arena was changed and the stripes  
218 constituting the landmarks cleaned to avoid the use of any possible chemical cue left by the ant.  
219 The position of the landmarks was changed and then the next training trial for the same ant  
220 started. For each training trial, the time needed to the ant to find the reward was recorded. At  
221 the end of the 8 training trials, the ant was placed back to its colony for 10 minutes and then  
222 tested in the same arena (after cleaning). The test was unrewarded and lasted two minutes. The  
223 landmarks were irregularly spaced and their configuration was new compared to the training  
224 trials (Fig. 2a and Fig. S2). Each test was videotaped. Each ant was used only for one training  
225 sequence and one unrewarded test.

226 Video recordings of the tests were analyzed with EthoVision 8.5 tracking software  
227 (Noldus). The arena was virtually divided in 50 zones of equal size (1 cm) and the time spent  
228 by the ant in each zone was calculated.

229 **Statistical analyses.** To analyze the dynamics of time needed to find the reward during the 8  
230 successive training trials, we used linear mixed models (LMM, R package *nlme* (Pinheiro et al.  
231 2016)) with time as dependent variable and trial as factor, individual identity was entered as  
232 random factor to account for repeated measures. P values were calculated by 1000 permutations  
233 ( $\alpha$  level = 0.05). For the analysis of the unrewarded test, we considered three contiguous zones  
234 around each landmark and calculated the 'Counting Performance Index' (CPI), as was done for  
235 an experiment with honey bees (Dacke and Srinivasan 2008), which is the ratio of time spent  
236 searching around the trained landmark to the average of the time spent searching at the other  
237 landmarks. A CPI higher than 1 indicates preference for the trained landmark, this was tested  
238 using the One-Sample Wilcoxon test for each group of ants trained to a different landmark (L1  
239 to L5). To compare the time spent searching by the tested ants in the zone of the trained



240 landmark to the time spent searching in the zone where the reward was positioned at the last  
241 training trial we used the Paired Samples Wilcoxon test.

## 242 **Results**

### 243 *Spontaneous quantitative discrimination*

244 Individual ants tested in a neutral arena were able to make relative quantity judgments between  
245 two finite sets (piles) containing different amounts of dummy cocoons. Ants spent more time  
246 inspecting a group of 2, 3 or 4 cocoons than inspecting a single cocoon (1 vs. 2:  $z = 2.613$ ,  $p =$   
247  $0.009$ ,  $n = 15$ ; 1 vs. 3:  $z = 3.107$ ,  $p = 0.002$ ,  $n = 15$ ; 1 vs. 4:  $z = 3.351$ ,  $p = 0.001$ ,  $n = 15$ ; Fig.  
248 1a), and they spent more time investigating a group of 4, 6 or 8 cocoons than investigating a  
249 group of 2 cocoons (2 vs. 4:  $z = 3.237$ ,  $p = 0.001$ ,  $n = 15$ ; 2 vs. 6:  $z = 3.408$ ,  $p = 0.001$ ,  $n = 15$ ;  
250 2 vs. 8:  $z = 3.408$ ,  $p = 0.001$ ,  $n = 15$ ; Fig. 1a). Overall, investigation time was significantly  
251 influenced by numerical ratio, discrimination increased when the numerosity ratio was smaller  
252 (Fig. 1b, overall: LM,  $p = 0.05$ ; post-hoc tests: ratio 0.5 vs ratio 0.33,  $p = 0.16$ ; 0.5 vs 0.25,  $p =$   
253  $0.03$ ; 0.33 vs 0.25,  $p = 0.29$ ).

254 To investigate the importance of chemical cues relative to other visual/tactile cues, we tested  
255 the ants with the same number of dummy cocoons in each pile (1 vs. 1) but different amount of  
256 chemical extract (natural odor of cocoons). In all cases, ants spent significantly more time  
257 investigating the cocoon with the higher amount of chemical extract (no extract vs. 20  $\mu$ l  
258 extract:  $z = 3.059$ ,  $p = 0.002$ ,  $n = 12$ ; 10  $\mu$ l vs. 20  $\mu$ l extract:  $z = 1.988$ ,  $p = 0.047$ ,  $n = 15$ ; Fig.  
259 1c, top panel).

260 We then tested ants with different numbers of cocoons but the same total amount of  
261 chemical extract in each pile. Ants spent significantly more time with the higher number of  
262 cocoons (1 cocoon with 20  $\mu$ l extract vs. 2 with 10  $\mu$ l extract:  $z = 2.300$ ,  $p = 0.021$ ,  $n = 15$ ; 2  
263 with 20  $\mu$ l vs. 4 with 10  $\mu$ l:  $z = 2.385$ ,  $p = 0.017$ ,  $n = 15$ ; Fig. 1c, bottom panel), indicating that  
264 ants use not only chemical cues but also visual and/or tactile cues for quantity discrimination.

265 One could hypothesize that ants would contact each pile one time and inspect each cocoon  
266 sequentially, which would lead to the observed longer time spent in contact with the pile  
267 containing the higher number of cocoons. This is very unlikely since the test lasted 5 minutes  
268 and each ant returned several times to each pile of cocoons. Typically, ants inspected both  
269 cocoon piles within the first 30 seconds, and then moved from one pile to the other. In the 2  
270 versus 6 trials, after the first inspection of both piles, the duration and occurrence of contact  
271 were significantly higher for the pile containing 6 cocoons than for the pile containing 2  
272 cocoons (duration: 6 cocoons  $37.42 \pm 4.54$  s (mean $\pm$ SE); 2 cocoons  $8.58 \pm 1.75$  s;  $z = 3.06$ ,

273  $p = 0.002$ ; occurrence: 6 cocoons  $9.33 \pm 1.36$ ; 2 cocoons  $3.92 \pm 0.47$ ;  $z = 2.82$ ,  $p = 0.0047$ ; Fig.  
274 S3), indicating that the ants are more attracted by the pile with more cocoons after they have  
275 assessed both piles. On the other hand, the time spent by the ants investigating the cocoons  
276 increased with the area occupied by the cocoons on the soil of the arena (Linear Model,  $t =$   
277  $12.33$ ,  $p = 0.001$ ; Fig. S4).

### 278 **Trained sequential landmark test**

279 Ants were trained to find a food reward in a rectangular arena equipped with 5 landmarks, the  
280 reward was placed just after one of the landmarks (Fig. 2a). The ant could not use any spatial  
281 information to locate the reward (e.g., distance from the starting point) because even if the  
282 reward was always placed after the same landmark, its position in the arena varied at every  
283 training trial (example for reward at landmark 3: Fig. 2a; for all other landmarks: Fig. S2).  
284 Along the training trials, in all five configurations (reward positioned after landmark 1, 2, 3, 4  
285 or 5) ants progressively reduced the time needed to reach the reward, as revealed by the  
286 significant effect of trials on the time to find the reward (LMM, effect of trial  $p < 0.001$  in all  
287 cases, Fig. 2b), which indicates that the ants learned the rule.

288 During the subsequent unrewarded test, the landmarks were irregularly spaced and their  
289 configuration was new compared to the training trials (Fig. 2a, Fig. S2). We calculated a  
290 ‘Counting Performance Index’ (CPI) (Dacke and Srinivasan 2008), the ratio of the searching  
291 time in three zones around the trained landmark, to the average of the searching time around  
292 the other landmarks (CPI = 1 represents uniform searching).

293 In all cases, the CPI of ants was higher than 1 (Fig. 3a, Supplementary Video 1 and Video 2)  
294 and it was significantly different from 1 for ants trained to landmark 1 ( $z = 1.978$ ,  $p = 0.048$ ,  $n$   
295  $= 15$ ), for ants trained to landmark 2 ( $z = 2.499$ ,  $p = 0.012$ ,  $n = 15$ ) and for ants trained to  
296 landmark 3 ( $z = 3.955$ ,  $p < 0.0001$ ,  $n = 17$ ). It was not significantly different from uniform  
297 searching for ants trained at landmark 4 ( $z = 1.759$ ,  $p = 0.078$ ,  $n = 14$ ) and particularly for ants  
298 trained at landmark 5 ( $z = 1.202$ ,  $p = 0.23$ ,  $n = 15$ ).

299 We also compared the time spent searching in the zone where the reward was supposed  
300 to be under the hypothesis that the ants used the sequence of landmarks as cue (i.e., right after  
301 the trained landmark), to the searching time in the zone where the reward was found during the  
302 last training trial. This analysis shows that ants spent significantly more time searching at the  
303 trained landmark (Fig. 3b, L1:  $z = 2.613$ ,  $p = 0.009$ ; L2:  $z = 2.698$ ,  $p = 0.007$ ; L3:  $z = 2.84$ ,  $p =$   
304  $0.004$ ; L4:  $z = 2.574$ ,  $p = 0.01$ ; L5:  $z = 3.09$ ,  $p = 0.002$ ), indicating that the ants did not use the  
305 last memorized distance from the starting point to locate the reward.

306 **Discussion**

307 The ability to perform relative numerosity judgements is widespread throughout the animal  
308 kingdom but most studies concern vertebrates. Using a free choice test, we found that nurse  
309 carpenter ants discriminated the larger of two quantities of cocoons. This spontaneous behavior  
310 is likely similar to the behavior expressed in natural conditions in which a larger pile of cocoons  
311 inside the nest would require more attention and care than a smaller pile. Ants' discrimination  
312 of small sets of items (1 vs 2, 1 vs 3 and 1 vs 4) is consistent with much of the literature on  
313 invertebrates which places a limit of four items for successful discrimination. For instance, male  
314 beetles could discriminate between odors representing 1 vs 3 and 1 vs 4 females (Carazo et al.  
315 2009) and crickets between shapes representing shelters when given the choice between 1 vs 3,  
316 1 vs 4, 2 vs 3 and 2 vs 4 (Gatto and Carlesso 2019). However, ants also discriminated between  
317 larger quantities of cocoons (2 vs 4, 2 vs 6, 2 vs 8), contrarily to honeybees, which  
318 spontaneously discriminated 1 vs 12, 1 vs 4, and 1 vs 3 artificial flowers but did not make a  
319 difference between 2 vs more (Howard et al. 2020). As the authors suggest, preferring multiple  
320 flowers to one flower may be useful for honeybees, while differentiating between patches  
321 containing multiple flowers may not necessary increase foraging efficiency. For nurse ants  
322 tending cocoons it will always be useful to discriminate between quantities. When grouping  
323 and moving cocoon piles across different nest chambers, it is more advantageous to pick up  
324 two cocoons to add them to a pile of four, than doing the opposite.

325 The accuracy of numerosity discrimination typically improves with increasing numerical  
326 distance between two values (distance effect: 1 vs 4 is easier than 1 vs 3) and decreases with  
327 absolute magnitude at a given numerical distance (magnitude effect: 1 vs 4 is easier than 11 vs  
328 14) (Moyer and Landauer 1967). The combination of these magnitude and distance effects gives  
329 rise to a discrimination performance that is ratio-dependent with an accuracy set by Weber's  
330 Law (Jordan and Brannon 2006), which states that the smallest noticeable difference is a  
331 constant proportion of the stimulus magnitude. Indeed, the male beetles cited above could not  
332 discriminate 1 vs 2 and 2 vs 4 females (ratio 0.5), although they successfully discriminated a  
333 numerical ratio equal to 0.33 (1 vs 3) and 0.25 (1 vs 4). Ants were successful at discriminating  
334 all tested ratio (0.5, 0.33 and 0.25), but their overall performance improved when the numerical  
335 ratio was smaller. Ratio-dependent discrimination is very common and it is typically stronger  
336 for numerosities larger than four (e.g., Agrillo et al. 2012). This ratio effect may support the  
337 existence of an approximate number system. Several cognitive systems have been proposed to  
338 explain quantity discrimination. Two of the best well-known core number systems are the  
339 object file system (OFS) and the analogical magnitude system (AMS) (Feigenson et al. 2004).

340 Whereas OFS allows individuals to conduct precise operations with small quantities (between  
341 1 and 4 items), the AMS allows individuals to process large quantities (virtually no upper limit)  
342 but only returns approximate calculations (approximate number system) and it is constrained  
343 by a ratio distance. The AMS appears to be widespread among vertebrates (Cacchione et al.  
344 2014; Beran 2017). Future studies should expand the number of quantities tested to obtain a  
345 more robust estimation of the ratio effect in carpenter ants to more clearly elucidate the system  
346 underlying their spontaneous quantity judgments.

347         Our data show that chemical information was a significant cue used by ants to  
348 perform relative quantitative discrimination. It is not surprising that insects use chemical cues  
349 in similar tasks (Carazo et al. 2009; Thomas and Simmons 2009) but also some vertebrate  
350 species do, such as meadow voles (Ferkin et al. 2005) and elephants (Plotnik et al. 2019). While  
351 it is possible to consider discrete objects in the visual modality –in which most of the  
352 experiments have been carried out- it is improbable that the odors of objects could be perceived  
353 as separate units. However, relative numerosity judgements do not require animals to know the  
354 precise numerical value in order to make a correct choice, as occurs in crickets which can  
355 estimate quantities without relying on numerical information (Gatto and Carlesso 2019).  
356 Animals might discriminate between quantity via a representation of the number of objects (true  
357 number processing) or by relying on continuous variables that covary with numerosity (e.g.,  
358 cumulative surface area, cumulative stimulus intensity) and it is not always simple to  
359 distinguish between these alternatives because animals might be able to use both (e.g. fish,  
360 Agrillo et al. 2011). We present compelling data showing that visual (and/or tactile) stimuli and  
361 olfactory stimuli are both important for ants when they quantify, suggesting that they use  
362 different sources of information to make their choice. The inspection time increased  
363 significantly with the area occupied by the cocoons, suggesting that ants might assess size/area  
364 information and/or true numerical information. We can safely exclude the ants spent more time  
365 with the larger pile due to sequential investigation of the stimuli (as occurs in bumblebees,  
366 MaBouDi et al. 2020), because our ants explored both cocoons piles first and returned several  
367 times to both piles (Figure S3). Further experiments should clarify whether ants rely on genuine  
368 numerical information rather than, more generally, quantitative information to discriminate  
369 stimuli.

370         In the second task, foraging ants learned to search for food near a landmark that changed  
371 its absolute position over time while maintaining its relative position to four other identical  
372 landmarks. During the unrewarded test, ants continued to search near the trained landmark in  
373 the absence of food and despite a further change in its absolute position in relation to previous

374 trials (including the last one). Ants could not use any spatial information, such as distance from  
375 the starting point, to locate the reward. Ants' search accuracy was best explained by the relative  
376 position of the landmark (i.e., the order in which it was encountered upon entering the arena)  
377 rather than the distance travelled from the starting point. Indeed, they did not stop searching at  
378 the last baited location prior to the test. This suggests that ants can count the number of  
379 landmarks that they encounter on the way to a food source. Our findings are comparable to  
380 those found in honey bees in analogous tasks (Chittka and Geiger 1995; Dacke and Srinivasan  
381 2008). Similar to honey bees and guppies (Dacke and Srinivasan 2008; Miletto Petrazzini et al.  
382 2015), our ants show a limit at four since their performance in the unrewarded test decreased  
383 drastically when trained to forage at landmark 5, while rats appear to have a much higher upper  
384 limit in analogous experimental conditions (Davis and Bradford 1986; Suzuki and Kobayashi  
385 2000).

386 In conclusion, our study contributes to the scant body of knowledge available on  
387 individual quantitative cognition in insects besides bees. Using two ecologically relevant tasks,  
388 we showed that carpenter ants used relative quantity and relative position to make efficient  
389 decisions. Our results suggest that ants might use two types of relative information about  
390 quantities, numerosness and ordinality, although further studies are necessary to precisely  
391 elucidate which kind of cues are used by ants and what is the underlying system allowing these  
392 cognitive performances and whether they can use numerical information after controlling for  
393 stimulus size or area which are confounded in quantitative judgments.

394 **Acknowledgements.** We are grateful to Paul Devienne for technical assistance and for the ant  
395 picture in figure 3. Thanks to Heiko Rödel and Jean-Luc Durand for advice about the statistics.  
396 Many thanks to Angelo Bisazza for helpful comments. P.dE. is funded by 'Institut Universitaire  
397 de France (IUF)'.

398 **Author Contributions.** P.dE. and J.C. conceived the experiments. P.dE. and P.M. developed  
399 the methodology. Experimental data were taken by P.S. for spontaneous quantity  
400 discrimination (cocoon experiment) and by P.M. for use of ordinal information (landmark  
401 experiment). Data analysis was performed by P.dE. The paper was written by P.dE. and  
402 J.C., with input from all authors.

403 **Competing interests.** The authors declare no competing interests.

404 **Data availability:** The data used in this study are available at the Open Science Framework  
405 ([https://osf.io/m2k3b/?view\\_only=73691a2fe7114712b40ebedbb6bd1da8](https://osf.io/m2k3b/?view_only=73691a2fe7114712b40ebedbb6bd1da8)).

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558 **Figure captions**

559

560 **Figure 1. Spontaneous quantitative discrimination.** Time (seconds) spent by the ants  
561 inspecting different amounts of dummy cocoons, each perfumed with 20  $\mu$ l of chemical extract  
562 of natural cocoons,  $n = 15$  in all cases (a). Proportion of time spent near the larger cocoon pile  
563 against the numerical ratio (b). Time (seconds) spent inspecting two zones with one cocoon  
564 each, but a different amount of chemical extract (top,  $n = 12$  left,  $n = 15$  right). Time spent  
565 inspecting piles of different numbers of cocoons, but with the same total amount of chemical  
566 extract (bottom,  $n = 15$  in both cases) (c). Box plots show the median, 25<sup>th</sup>/75<sup>th</sup> percentiles,  
567 whiskers indicate 5<sup>th</sup>/95<sup>th</sup> percentiles. Asterisks indicate significant differences: \*  $p < 0.05$ , \*\*  
568  $p < 0.01$ , \*\*\*  $p < 0.001$ .

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570

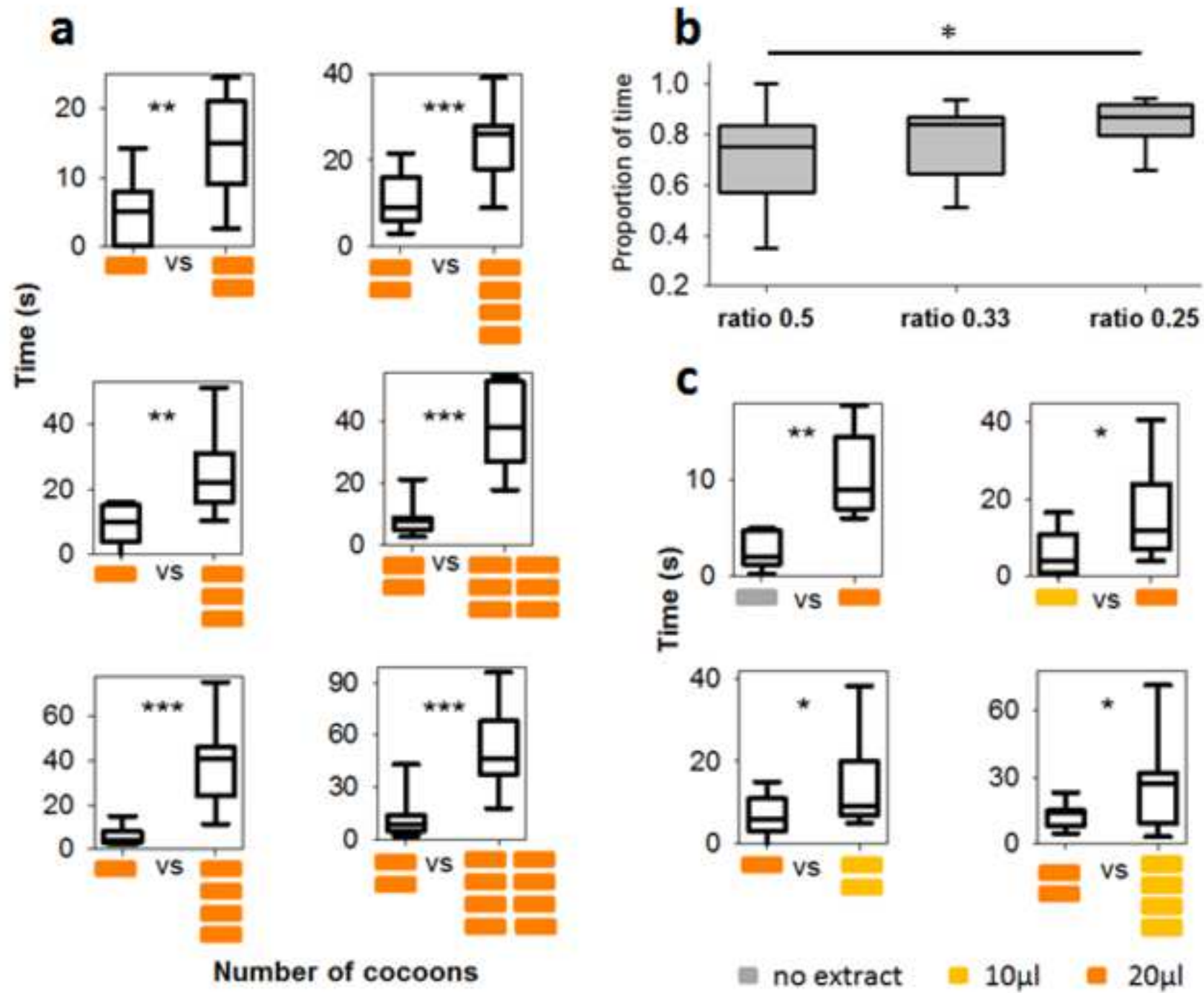
571 **Figure 2. Sequential landmark training.** Picture of the rectangular arena with the ant starting  
572 point represented by the cylinder (left); position of the five landmarks over the eight training  
573 trials for the group of ants trained to find the reward after landmark 3 (the reward, a drop of  
574 sugar solution, is represented by an asterisk); unrewarded test for ants trained to landmark 3  
575 (right): the landmarks are in a totally new configuration and are spaced at irregular intervals (a)  
576 See also Fig. S2 and Table S1. Learning curves showing that ants decrease the reward searching  
577 time along the 8 training trials (LMM,  $p < 0.001$  in all cases; L1: ants trained to find the reward  
578 at landmark 1,  $n = 15$ ; L2,  $n = 15$ ; L3,  $n = 17$ ; L4,  $n = 14$ ; L5,  $n = 15$ ). The picture shows an ant  
579 drinking the sugar reward, Photo by Paul Devienne (b).

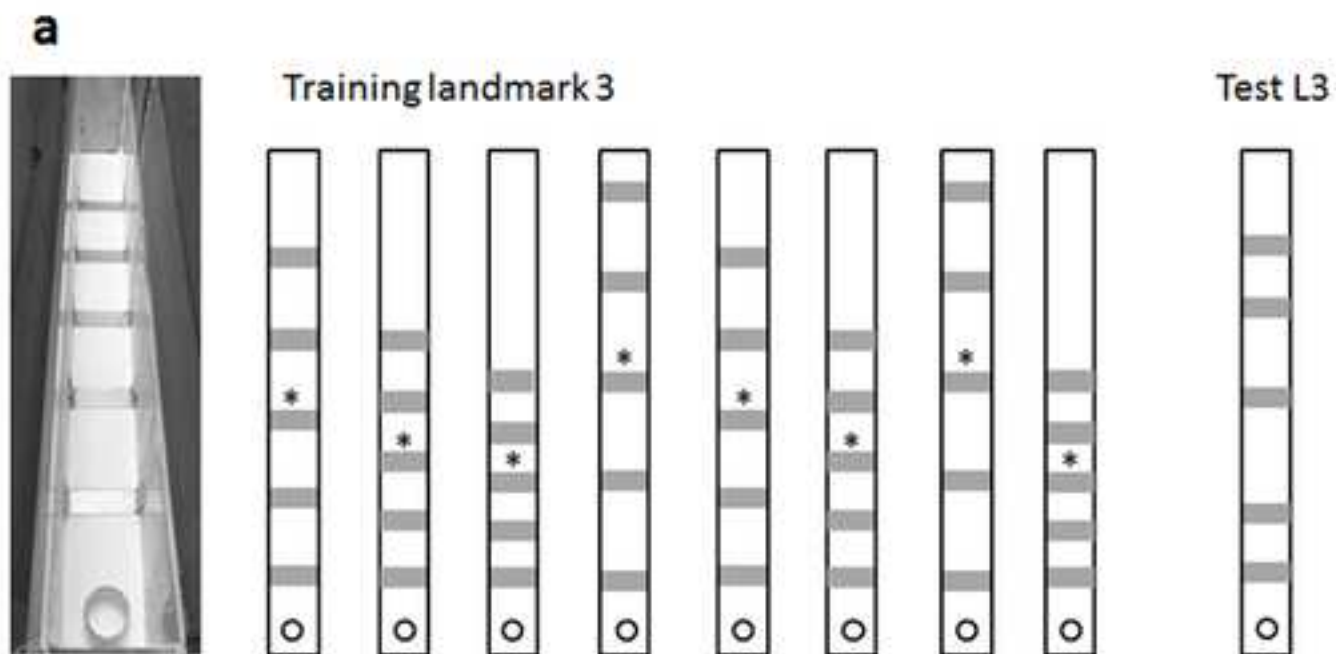
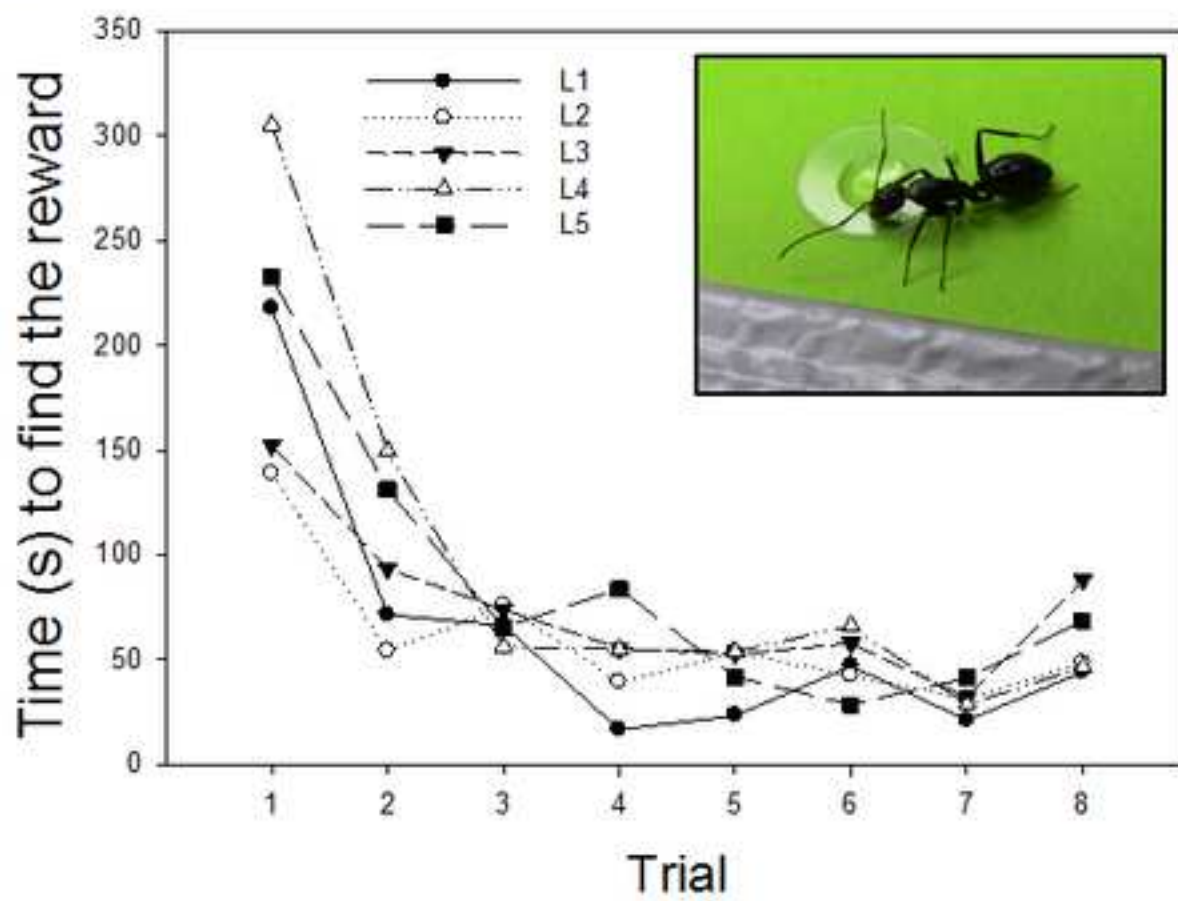
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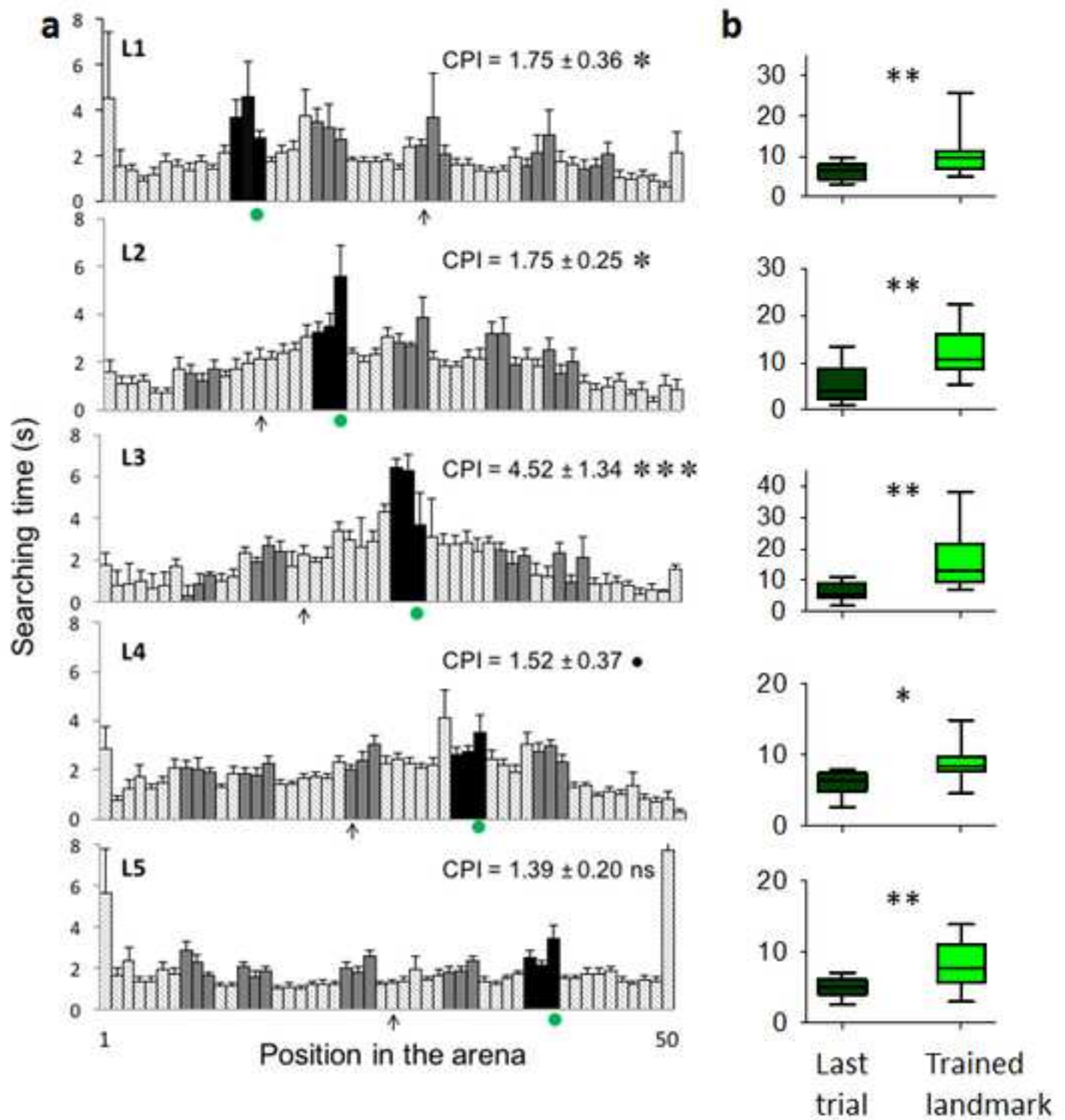
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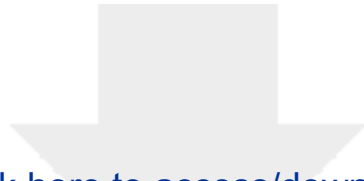
582 **Figure 3. Use of sequential landmark information.** Distribution of time spent searching for  
583 the reward by ants that were tested without reward after being trained for reward at landmark 1  
584 (L1), landmark 2 (L2), landmark 3 (L3), landmark 4 (L4) and landmark 5 (L5). The arena was  
585 divided in 50 equal zones of 1 cm each. The zones used to calculate the ‘counting performance  
586 index’, CPI (mean  $\pm$  SE), i.e., the ratio of time spent around the trained landmark to the average  
587 of the time spent searching at the other landmarks (Dacke and Srinivasan 2008) are highlighted:  
588 dark grey for the neutral landmarks, black for the trained landmark. Asterisks indicate  
589 significant differences (One-sample Wilcoxon test), L4:  $p = 0.078$ . The green dots indicate the  
590 position where the reward was expected to be (at the trained landmark) and the arrows show  
591 the position of the reward at the last training trial, just prior to the test (a). In all cases ants spent  
592 more time searching for the reward at the trained landmark (green), than at the position where  
593 the rewarded was located during the last training trial (Wilcoxon test) (b). Box plots show the  
594 median, 25<sup>th</sup> /75<sup>th</sup> percentiles, whiskers indicate 5<sup>th</sup>/95<sup>th</sup> percentiles. Asterisks indicate  
595 significant differences: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

596



**b**

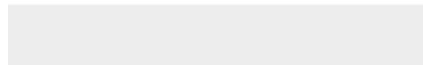




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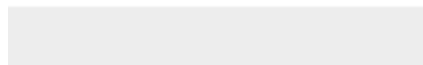




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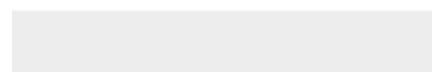
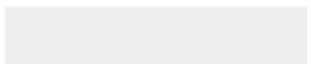
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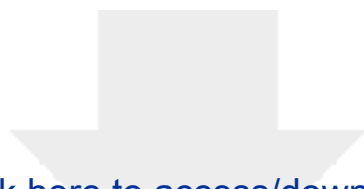






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