

1 Individual size as determinant of sugar responsiveness in 2 ants

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14 15 ABSTRACT

16 Social insects commonly exhibit division of labor in non-reproductive tasks. Task allocation
17 may be related to size, form, and ergonomic differences when workers are anatomically
18 variable. Carpenter ants *Camponotus mus* collecting nectar exhibit a wide forager-size
19 variation, thus raising the question of whether large and minor workers differ in their
20 gustatory responsiveness and specialize, therefore, on different nectar sources. To answer this
21 question, we first established the sucrose concentration at which small and large ants in the
22 laboratory respond appetitively to a sugar solution (sucrose acceptance threshold, SAT) after
23 experiencing a high or a low starvation regime (4- or 1-day carbohydrate deprivation,
24 respectively). Under high starvation, no differences in SATs were found between larger and
25 smaller ants. Under low starvation, both sizes increased their SATs but larger ants had a
26 higher SAT, thus preferring more concentrated solutions while smaller ants responded mostly
27 to more diluted sucrose solutions. In a field assay in which the distribution of larger and

28 smaller ants on sugary food sources was analyzed, small and medium ants were found -in
29 different proportions- at all food sources while larger ants were only found at nectar sources
30 with a higher sugar flow rate, i.e. providing more sugar per unit time. Both field and
31 laboratory assays supported that sugar-related parameters act as determinants of the size
32 distribution of ants among food sources. In addition, interindividual differences in alternative
33 non-sugar related variables may contribute to this distribution, leading thereby to a potential
34 nectar foraging specialization.

35

36 **Key words:** Carpenter ants; Sucrose threshold, Worker size, Nectar foraging.

37

38 **Significance statement**

39 Task specialization is crucial for the ecological success of social insects. Carpenter ants
40 allocate individuals of variable size to foraging, thus raising the question of whether they
41 differ in their food preferences. We determined the sugar concentration at which an appetitive
42 response occurs in small and large carpenter ants, and analyzed their distribution on natural
43 and artificial nectar sources in a field assay. Under low starvation, larger ants responded more
44 than smaller ants to higher sucrose concentrations. Coincidentally, in the field assay, they were
45 mainly present at sources with higher sucrose delivery. This kind of specialization may reflect
46 the fact that larger ants have larger feeding apparatuses, which may confer a better capacity to
47 deal with the higher viscosity of more concentrated nectars and allow collecting more food at
48 nectaries with higher sugar flow-rates. Size specialization may thus increase colony success in
49 the exploitation of variable food sources.

50

51 **INTRODUCTION**

52 Social insects, with their complex colony organization, division of labor, and sophisticated
53 communication systems, provide an ideal model for studying the biological bases of social
54 organization (Wilson 1971; Hölldobler and Wilson 1990; Page and Erber 2002). Among the
55 reasons that explain the ecological and evolutionary success of bees, ants and other social
56 insects, task allocation, i.e. the fact that some individuals specialize in certain tasks,
57 constitutes a key factor as it provides a basis for adjusting responses to a changing
58 environment (Gordon 1996).

59 Task allocation has been explained based on a ‘response threshold theory’ assuming
60 that individuals differ in their sensitivity and therefore responsiveness to different stimulus
61 modalities. The response threshold model (Robinson and Page 1989; Beshers and Fewell
62 2001) proposes that individuals have internal thresholds for responding to task-specific
63 stimuli. The inter-individual variation in response thresholds within a colony determines
64 differences in stimulus sensitivity and responsiveness that result in individuals performing or
65 not a given task (Beshers and Fewell 2001). Response thresholds are influenced by intrinsic
66 (e.g. physiology, age, genetic traits, etc.) and extrinsic factors (e.g. weather, colony stores,
67 etc.), which affect, therefore, the probability of responding to a given stimulus. In honey bees,
68 for instance, nurse bees are more sensitive and responsive to the stimulation provided by
69 larvae than guards so that they specialize as brood tenders (Robinson 1992). In other words,
70 individuals highly sensitive to a given stimulus are good candidates to become specialized in
71 tasks involving such a stimulus (Page and Erber 2002).

72 In social insects such as honey bees and ants, stimulus sensitivity can be evaluated by
73 measuring response thresholds in experimental protocols that have been explicitly conceived
74 to this end. In the honey bee, a first protocol was established using the proboscis extension
75 reflex (PER), the innate appetitive response to sucrose solution delivered to the antennae of a
76 hungry bee (Minnich 1932). The response of a bee to a series of increasing sucrose

77 concentrations allows determining its sucrose response threshold, i.e. the lowest concentration
78 at which a bee start responding with PER to sucrose (Scheiner et al. 2004). Nectar foragers
79 exhibit higher sucrose thresholds (i.e. higher concentration selectivity) than pollen foragers,
80 which display lower concentration selectivity (Page et al. 1998; Pankiw and Page 1999;
81 Pankiw and Page 2001; Scheiner et al. 2003).

82 Developing a protocol to measure sucrose responsiveness in ants was more
83 challenging as this family (Formicidae) includes numerous species, which differ highly in
84 their anatomical features and behavioral responses. While some species respond well to
85 antennal stimulation with sucrose, others do not (Falibene and Josens 2012). Moreover, some
86 species [e.g. *Camponotus aethiops* (Guerrieri and d'Ettorre 2010)] extend their maxilla-labium
87 when their antennae are stimulated with sucrose, similarly to the PER of bees, while other
88 species (e.g. *Camponotus mus*) almost never exhibit such a sustained extension of the glossa.
89 Furthermore, *C. mus* ants usually do not respond to antennal sucrose stimulation, except if
90 their palps are stimulated first (Falibene and Josens 2012). To overcome this problem, it is,
91 therefore, crucial to determine where (antennae or palps) to stimulate with sucrose to elicit an
92 appetitive response. Using this methodology allowed to demonstrate that sucrose thresholds
93 strongly depend on the sugar reserves in *C. mus* ants (Falibene and Josens 2012).

94 In honey bees, where sucrose responsiveness has been extensively studied, numerous
95 studies have shown that it varies with factors such as age, caste, sex (Pankiw and Page 1999),
96 foraging experience, genotype, feeding (Pankiw et al. 2001), season (Scheiner et al. 2003),
97 stress (handling), hormones thresholds, pheromones (Pankiw and Page 2003; Baracchi et al.
98 2017), among others. Yet, the question of whether individual size affects sucrose
99 responsiveness has not been studied, simply because honey bee foragers do not exhibit
100 significant interindividual size variation within a colony. In bumble bee workers, both task
101 allocation and sensory sensitivity are strongly affected by individual body size. Visual

102 sensitivity and image resolution are better in larger than in smaller individuals (Spaethe and
103 Chittka 2003). Olfactory sensitivity is also higher in larger bumble bees owing to higher
104 number and density of olfactory sensilla on their antennae (Spaethe et al. 2007). As far as we
105 know, there are no evidences for size-related differences in the gustatory sensitivity of bumble
106 bees and other social insects. This question is, nevertheless, relevant in the case of ant
107 foragers, which in some species vary significantly in size despite being allocated to the same
108 type of food collection.

109 Here we focus on *C. mus*, a carpenter ant from Argentina, in which forager size varies
110 between 4 and more than 10 mm and weight ranges from 3-4 mg to more than 22 mg. Larger
111 ants can carry larger loads than smaller ants owing to their larger crop (Josens 2002). They
112 can also ingest sugar solutions faster (Josens 2002; Paul and Roces 2003; Davidson et al.
113 2004) based on larger anatomical features involved in the process of sucking (muscle mass,
114 radius of the food canal, etc.). These features render ingestion and foraging motivation of
115 larger ants more susceptible to lower flow rates of nectar than those of smaller ants (Medan
116 and Josens 2005). Thus, food sources with lower sugar flow may have a reduced subjective
117 value for a large ant because of the limitation of food intake they impose. This factor may
118 lead to a specialization of large ants on nectaries with higher sugar flows satisfying their
119 higher food intake rate. Furthermore, large ants would have advantages to cope with nectars
120 that are more viscous. As viscosity relates in an exponential manner to sucrose concentration
121 and is responsible for the resistance of a fluid to circulate within a small-diameter tube [as
122 defined by the Hagen-Poiseuille equation (Sutera and Skalak 1993)], ingestion of nectars that
123 are more concentrated and thus more viscous would be favored in large ants with wider
124 feeding ducts.

125 We thus posit that individual ants could specialize in particular nectar sources
126 according to their size. Larger ants could forage on nectars with higher sucrose concentration,

127 i.e. with higher viscosity, as well as on nectaries with a higher sugar flow, while smaller ants
128 could prioritize less viscous nectars and lower sugar flows (Medan and Josens 2005). To
129 address this hypothesis, we asked if major and minors of *C. mus* differ in their sucrose
130 response thresholds and if differences translate into specializations for particular food sources
131 in foraging activities.

132

133

134 **MATERIAL AND METHODS**

135 Colonies of *Camponotus mus* were placed in an artificial nest consisting of a plastic box
136 (30x50x30 cm) with a plaster bottom covered by an acrylic plate. Colonies were kept in the
137 laboratory under a natural L/D cycle at a nearly constant temperature ($24\pm 1^\circ\text{C}$). Ants could
138 move freely within the nest and had access to a water source. Ants were fed with honey-water
139 and chopped insects (honeybees and cockroaches) between experiments.

140

141 ***Sucrose Acceptance Threshold (SAT)***

142 Individual ants were first classified as minors and majors by observation. This classification
143 was then confirmed by weighing ants chosen in the same way, which yielded clear different
144 values (5.30 ± 0.48 mg and 17.41 ± 0.71 ; mean \pm S.E.; $n = 11$ and $n=10$), thus showing that
145 discriminating minors and majors was straightforward. Nests were set under two regimes of
146 carbohydrate starvation: 4 days (high starvation) or 1 day of starvation (low starvation).

147 To quantify sucrose acceptance thresholds (SATs), ants were individually placed in
148 Eppendorf tubes and anesthetized on ice for about 2–4 min. This allowed harnessing each
149 individual into a micropipette tip (10–100 μl) having its end cut off. Given the size
150 differences between smaller and larger ants, the micropipette tip was cut closer to its end to
151 get a smaller hole for smaller ants, while it was slightly larger for larger ants. In both cases,

152 only the head of the ant protruded through the resulting hole so that only the antennae and
153 mouthparts could move freely.

154 Palps of harnessed ants were touched with a toothpick imbued with 0.3, 1, 3, 10, 30 or
155 50% (w/w) sucrose solution. These concentrations were presented to the ants in ascending
156 order. Before the first sucrose stimulation and between sucrose trials, ants were tested in the
157 same way for their response to water (control). The interstimulus interval varied between 4
158 and 5 min.

159 In all cases, the response was considered positive when the ant exhibited a licking
160 behavior after contacting the solution, and negative when there was no licking behavior after
161 stimulation (Falibene and Josens 2012). All ants were assayed until their first positive
162 response to a given sucrose concentration. Afterwards, they were eliminated. The
163 concentration at which an ant showed licking behavior represents its sucrose acceptance
164 threshold (SAT), which is an indicator of the individual responsiveness to sucrose. Ants that
165 responded to water stimulation immediately before the first positive response to sucrose and
166 ants that did not respond to any sucrose stimulation were discarded from the analyses. The
167 percentage of these non-responding ants was also measured.

168

169 *Pumping Frequencies*

170 Pumping frequency (i.e. number of contractions of the sucking-pump muscles per second)
171 during ingestion depends on colony starvation and reflects the motivational state of individual
172 ants (Falibene and Josens 2008). Higher starvation results in higher pumping frequency, i.e. in
173 higher ingestion rate (Falibene and Josens 2008). Prior recordings did not show an incidence
174 of individual ant size on pumping frequency (Josens 2002; Falibene and Josens 2008). To
175 determine if variations in sucrose responsiveness related to size differences are due to the

176 ants' motivational state, we recorded the pumping frequency of majors and minors from the
177 same nest. This quantification was done for various nests.

178 Pumping frequency was recorded during intake of sucrose solution using a non-
179 invasive method described in previous studies (Josens et al. 2006; Falibene and Josens 2008;
180 Falibene et al. 2009; Falibene and Josens 2012). Briefly, a wooden bridge (2 cm x 7 cm) led
181 to the recording arena, which consisted of a metallic mesh (approximately 2 cm²) covered by
182 a thin layer of conductor gel and a wet filter paper underneath. An Eppendorf tube (0.5 ml)
183 filled with sucrose solution was inserted into the mesh. A drop of solution protruded from the
184 open tube, and one electrode was fixed to the metallic mesh while another was in contact with
185 the solution. When the ant stood on the mesh and contacted the solution with its mouthparts,
186 the circuit closed, which allowed recording the electrical signals generated by the ant during
187 feeding (amplification 210x; band-pass filter 0.4-17 Hz, -3 dB; sampling rate: 200 Hz). The
188 electrical signal observed corresponds to the rhythmic contraction of the pharyngeal pump.
189 No differences in signal quality were found between smaller and larger workers. Pumping
190 frequency (pumps/s) was defined as the highest peak in the periodogram that resulted from
191 the analysis of the entire signal (entire intake). Recordings were stored in a computer using an
192 analogue-to-digital converter (ADC-212, Pico Technology Limited, UK).

193

194 ***Field assays: natural distribution of ant sizes between nectar sources with different sugar***
195 ***flow***

196 We performed field assays in the campus of the Faculty of Exact and Natural Sciences of the
197 University of Buenos Aires (36° 32' 47" S; 58° 26' 20" W). We aimed at determining if larger
198 ants are more likely to forage on nectar sources that are more concentrated and if they
199 prioritize higher sugar flow rates than smaller ants. The latter would prefer nectar sources
200 with lower concentration and sugar flow rate.

201 In the first assay, we chose five plants on which *C. mus* ants foraged for nectar at extra
202 floral nectaries [the plants belonged to the genera *Senna* (1), *Ipomoea* (3), and *Vigna* (1)].
203 Plants were separated from each other by at least 1.5 m. We collected all ants found on the
204 plants and we measured their size under a binocular reticle microscope (Leica MZ 8) after
205 anesthetizing them with cold. Head width (HW: maximum width at the height of the eyes
206 when viewed from above) was used as a proxy of individual size (Josens 2002). Size
207 measurements were conducted blind with respect to the nectar characteristics of the plant on
208 which the ants were collected.

209 We measured the nectar concentration and estimated the nectar flow of each of the
210 five plants using two hollow Styrofoam sphere halves (10 cm in diameter), which allowed to
211 enclose the plant zone with the highest density of extra floral nectaries. The sphere halves
212 were bound together by masking tape, thus creating an hermetic compartment. A wet gauze
213 bandage was fixed on the inner wall of the inferior half sphere to humidify the air inside the
214 compartment and minimize the evaporation of the nectar and its variation in concentration.
215 Six hours after placing the spheres in each plant, the nectar accumulated on the extra floral
216 nectaries was collected with graduated 5 μ l-micropipettes, which allowed quantifying the
217 nectar volume. Nectar concentration was measured with a hand refractometer (A. Krüss
218 Optronic, Germany). Sugar flow was calculated for each plant by considering the
219 concentration and volume of nectar collected after the 6-h period.

220 As the concentration range of nectar found at extra floral nectaries was relatively
221 reduced (from 11 to 19%), we performed an additional assay in which we included an
222 artificial source with a higher sucrose concentration in contact with a plant. We aimed at
223 determining if this source attracted individuals of larger sizes, according to our original
224 hypothesis. To this end, we placed a drop (ca. 0.5 ml) of sucrose solution 20 or 60% (w/w) on
225 a Petri Dish connected to the main plant stem by means of a wooden stick. The drop was

226 replaced when necessary. When at least 10 individuals gathered simultaneously at the food
227 source, we collected them with those ants that were foraging simultaneously at the extra floral
228 nectaries of the plant. They were separated in different glass flasks to estimate their size by
229 measuring head width as described above.

230

231 ***Statistics***

232 In the SAT experiments, the frequencies of smaller and larger ants responding to the different
233 sucrose concentrations under the two starvation regimes were compared using a χ^2 -test. The
234 frequencies of larger and smaller ants not responding to any concentration were compared
235 using the same test.

236 In the pump frequency experiment, both the ant weight and the pumping frequency
237 were analyzed by means of a Kruskal-Wallis test.

238 In the field assays, ant size was categorized as large, medium or small while natural
239 sources were categorized according to their sugar flow rate as low, medium and high (see
240 above for details). Size frequency distribution among categories of sugar flow rate was
241 analyzed by means of a χ^2 -test. The same analysis was performed for the assay in which the
242 distribution of ants of different sizes between natural nectar sources and an artificial source
243 offering 60% sucrose solution was analyzed after pooling the three replicates of this
244 experiment based on their similar tendencies.

245

246 ***Data availability***

247 The datasets during and/or analyzed during the current study available from the
248 corresponding author on reasonable request.

249

250

251

252

253 **RESULTS**

254

255 *Sucrose Acceptance Threshold*

256 Larger and smaller *C. mus* workers under high and low sugar starvation were assessed for
257 their *sucrose acceptance threshold* (SAT), which corresponds to the first concentration at
258 which an ant exhibits a licking response to sucrose. Under high sugar starvation (Fig 1a),
259 minor and majors did not differ in their SAT distribution ($\chi^2 = 5.33$, df:5, $p = 0.38$; NS). Most
260 ants exhibited a SAT at 1% of sucrose concentration although high responsiveness was also
261 observed at 3%. Under low sugar starvation (Fig. 1b), the SAT distributions of majors and
262 minors were biased towards higher sucrose concentrations as expected from their nutritional
263 state. However, both distributions differed significantly ($\chi^2 = 11.95$, df:5, $p = 0.035$) as
264 minors were more biased towards lower concentrations compared majors. Minors responded
265 maximally at 10% while majors responded maximally at 30%. In both cases, low starvation
266 made the ants more selective, responding to higher concentrations, but the SATs differed
267 between ant sizes (Fig. 1b). Overall, the results of this experiment show that starvation of a
268 whole colony is a critical factor for determining SATs but also that size categories differ
269 significantly in their sucrose selectivity under low starvation conditions. Smaller ants respond
270 more to less concentrated solutions while larger ants respond more to solutions that are more
271 concentrated.

272 The number of individuals that did not respond to any sucrose concentration differed
273 between the two starvation regimes and agreed with the previous results. Under a high
274 starvation regime, 17.2 % of the majors (20 of 116 ants) and 5.3% of the minors (6 of 114

275 ants) did not respond to any sugar concentration. These values differed significantly from
276 each other ($\chi^2 = 8.23$, df:1, $p < 0.01$), thus showing that smaller ants were more responsive
277 than larger ants under high starvation. Under a low starvation regime, 54% of the majors (109
278 of 204 ants) and 37% of the minors (75 of 203 ants) showed no response to any sugar
279 concentration, thus showing that minors were again more responsive than majors, also under
280 low starvation ($\chi^2 = 11.16$, df:1, $p < 0.001$).

281

282 *Pumping Frequencies: differences in SAT do not correlate with differences in pumping*
283 *frequency*

284 Motivational states, given by the sugar requirements of a colony or a group, modulate both the
285 pumping frequency (Falibene and Josens 2008) and the SAT (Falibene and Josens 2012) in
286 workers of the same size. Although recordings of pumping frequency yielded similar results
287 for ants of different sizes (Josens 2002; Falibene and Josens 2008), this frequency may vary in
288 a size-dependent manner under certain starvation conditions, similarly to what occurs with the
289 SATs. SAT differences between ants of different size occurring only under a particular
290 starvation conditions could reflect differences in motivational states between majors and
291 minors. We thus aimed at determining if majors and minors experiencing the low-starvation
292 regime differed in their pumping frequency. To this end, we recorded pumping frequencies of
293 majors and minors from the same colony, in six different colonies.

294 In all cases, ants of different sizes exhibited similar pumping frequencies in all
295 colonies studied (Table 1). Frequencies ranged from 3 to 5.5 pumps/s and did not differ
296 between ant sizes within a colony. Considering that pumping frequency varies with foraging
297 motivation, we conclude that majors and minors did not differ in their motivational state.
298 Thus, differences in SAT cannot be ascribed to differences in foraging motivation but may be
299 related with a size-depending specialization.

300

301 *Field assays: natural distribution of ant sizes between nectar sources with different sugar*
302 *flow*

303 In a first assay, we collected ants at five different plants in which we measured nectar
304 characteristics using a sphere (one per plant) enclosing a zone with a high density of extra
305 floral nectaries. The head width of collected ants varied between 1.17 and 1.91 mm, which
306 corresponds to a weight range varying between 4.5 and 19 mg after the head width/weight
307 relationship described for *C. mus* (Josens 2002).

308 In order to better visualize the relationship between ant size and sugar flow, we
309 defined three size categories, small (head width < 1.4 mm), medium (1.4 mm < head width <
310 1.55 mm) and large (head width > 1.55 mm), and three sugar-flow categories, low (sugar flow
311 < 3 $\mu\text{g}/\text{min}$), medium (3 < sugar flow < 4.5 $\mu\text{g}/\text{min}$) and high (sugar flow > 4.5 $\mu\text{g}/\text{min}$), and
312 represented our data accordingly. The distribution of ant sizes varied with the flow rate
313 category ($\chi^2 = 54.4$; df:4; $p < 0.0001$), thus showing that ants of different sizes preferred
314 different sugar flows. Figure 2 shows that larger ants were present at food sources with higher
315 sugar flows and absent at food sources with lower sugar flows. Conversely, smaller ants were
316 majority at the lower sugar flows and minority at the higher sugar flows. Medium ants were
317 found mostly at medium and higher sugar flows. Thus, individual ants specialize in particular
318 nectar sources according to their size.

319 In a second assay, we connected a Petri dish containing a 60% sucrose solution to the
320 main plant stem and collected ants both at this artificial food source and on the plant. We
321 performed three replicates of this assay, using a different plant in each case. In one replicate,
322 the plant stem was connected to two Petri dishes, one with 20% and the other with 60%
323 sucrose solution. Based on their similar tendencies, we pooled the data of the 3 replicates
324 taking into account the proportion of large ants (head width > 1.55 mm) present at the natural

325 sources and those present at the artificial source presenting a 60% sucrose solution ($N_{\text{nat}}= 72$;
326 $N_{60}= 50$) as these two categories were common to all three replicates. The proportion of large
327 ants differed between both food sources. While in the artificial food source presenting a
328 concentrated sucrose solution (60%) the proportion of large ants was 0.30, it was only 0.06 in
329 the natural sources offering more diluted nectar (around 11%~13%; $\chi^2= 13.4$; $df:1$; $p=$
330 0.00025). It is worth mentioning that no extra-large (XL) ants (head width > 2 mm) were
331 found at the natural food sources while 6 XL ants were found at the 60%-sources.

332 To sum up, the proportion of large ants was higher at the artificial food sources with
333 higher sucrose concentration and food *ad libitum*. This, together with the fact that XL ants
334 were only caught at these food sources, confirmed that larger ants forage mainly on nectars
335 that are more concentrated and present higher sugar flows.

336

337

338 **DISCUSSION**

339

340 Our results show that sucrose acceptance thresholds (SATs) vary with the starvation regime
341 and with individual ant size. When the colony was starved, no differences in SATs were
342 found between larger and smaller ants. However, under low starvation, larger ants showed a
343 SAT that was higher than that of small ants. In other words, smaller ants responded more to
344 less concentrated solutions than larger ants, which responded more to solutions that were
345 more concentrated. Under this condition, both groups of ants had higher SATs than those
346 observed under high starvation, thus indicating that ants were more selective in their response
347 to sucrose. This result is consistent with significant changes in SATs observed in foragers of
348 *C. mus* separated from the colony when their individual food reserves varied: starved ants had
349 lower SATs while fed ants had higher SATs (Falibene and Josens 2012). Here we show that

350 SATs also change when the whole colony is submitted to sugar deprivation. However, the
351 most relevant point in the light of the question we raised was the finding that SATs depend on
352 the individual size of an ant, irrespectively of motivational factors. The distribution of SATs
353 between majors and minors and their modulation according to starvation may be one of the
354 key factors for allocating foragers to particular food sources in species that present different
355 forager sizes.

356

357 Our present results are in accordance with the individual behavior of foragers of
358 different sizes when confronted with artificial nectar sources differing in flow rate or viscosity
359 (Medan and Josens 2005). Low nectar flow rates determine that large and medium-size ants,
360 which can drink faster, leave the food source with smaller crop loads. Small ants, on the
361 contrary, which cannot drink faster, stay longer at the food sources and transport relatively
362 larger loads than large ants (Medan and Josens 2005). In addition, only large ants increase
363 their feeding time in the presence of highly viscous food sources (Medan and Josens 2005).
364 Small ants reach similar crop loads under a variety of conditions (i.e. increased viscosity,
365 regulated flow rate or *ad libitum* food sources) while large ants rather focus on highly
366 productive food sources. This difference suggests that small foragers are the most common
367 worker size for nectar gathering, while large ants are only recruited when sources particularly
368 productive. In our field assays, workers with heads ranging from 1.2 to 1.5 mm, i.e. small and
369 medium ants, were present in almost all the sources studied, thus indicating that nectar
370 foraging relies primarily on those sizes. Another remarkable difference in ant size observed in
371 our field assays occurred for the extra-large (XL) individuals (HW>2 mm), which only
372 appeared at sources with high productivity (the artificial ones). Even if these ants were never
373 numerous at our artificial food sources, they could never be observed at the natural sources
374 we studied. This suggests that these XL ants do not normally forage on nectar but they do so

375 when a particularly productive nectar source is discovered. The differences found in SATs
376 (see above) may explain the preferential allocation of large ants to highly concentrated
377 sources. Note, however, that both experiments (laboratory analysis of SAT and field assays on
378 worker distribution among sugary food sources) are not homologous, but analogous.
379 Homology is excluded since the variables considered in these experiments were different
380 (sugar concentration and sugar flow rate, respectively). Yet, they both refer to sugar-related
381 variables.

382 An alternative explanation could be provided by feeding experiments on the
383 monomorphic ant *Lasius niger* (Mailleux et al. 2000). This study showed that these ants must
384 ingest an amount of sucrose solution above a certain threshold before they started recruiting.
385 Larger ants would have larger thresholds (if we assume that the threshold is a percentage of
386 their crop) so that the results of the field study could be explained by the fact that plants with
387 higher flow rates or *ad libitum* feeders can get larger ants over this threshold, thus inducing
388 the larger ants to return to them. Yet, leaving apart that the fact that the size factor – the
389 crucial point of our work – cannot be addressed in *L. niger*, we did not measure the volume
390 transported by ants of different size. This renders difficult to determine if the crop-load
391 critical volume found for *L. niger* recruiting behavior (Mailleux et al. 2000), also applies to *C.*
392 *mus* ants, in particular to larger workers. Moreover, *L. niger* stays longer at food sources with
393 low sugar flow to reach its threshold crop load, while *C. mus* do not necessarily stay longer to
394 attain a threshold volume (Medan and Josens 2005; Falibene et al. 2009). In some situations,
395 they stay longer to reach a similar load, while in other cases they keep the feeding time
396 constant even if it leads to different crop loads (Falibene et al. 2009).

397

398 Physiological and anatomical constraints can account for the feeding specialization
399 according to size found in our work. During nectar-feeding, the fluid is ingested via the food

400 canal so that this process depends on the properties of the nectar and on specific aspects of
401 insect morphometry. Nectar viscosity is the main property affecting fluid dynamics. It
402 increases exponentially with sucrose concentration, thus implying that for higher
403 concentrations, increases in viscosity are higher. In other words, concentrated sucrose
404 solutions with higher viscosity offer more resistance during their circulation in thin ducts. The
405 morphometry of the mouthparts (shape, size, etc.), food canal (diameter, length, etc.) and
406 pharyngeal pump (volume, muscular mass, strength, etc.) of an insect have also a direct
407 incidence on the feeding dynamics (Kingsolver and Daniel 1979). Muscles conforming the
408 sucking pump bring the liquid into the mouth and push it through the esophagus, operating
409 against forces imposed by the narrow food canal. Insect morphometry determines, in
410 consequence, a critical viscosity from which a feeding system is unable to counteract
411 viscosity increases, thus resulting in a slowing-down of fluid intake rate. Theoretically, this
412 critical viscosity is lower for a narrower and/or longer food canal, and for a smaller muscle
413 mass in charge of generating a difference of pressure. Thus, although we did not measure
414 directly the viscosity of our sucrose solutions, taking into account this variable and the ants'
415 morphometric features, the size-related distribution found in our experiments can be better
416 understood: small ants, with their reduced food canals and smaller muscular mass, are unable
417 to reach the high intake-rates achieved by larger ants (Boggs 1988; Josens 2002; Paul and
418 Roces 2003; Davidson et al. 2004; Medan and Josens 2005). According to this, large ants are
419 expected to forage on more viscous nectar sources and on those with higher flow rates.

420

421 Differences at the level of peripheral and central mechanisms of sucrose processing
422 could also confer different sucrose sensitivities to small and large ants. At the peripheral level,
423 populations of sucrose receptors with different concentration tuning are usually found in the
424 antennae and mouthparts of social insects (Haupt 2004). If small ants and large ants were

425 endowed with more receptors tuned to lower and higher sucrose concentrations, respectively,
426 they would be more sensitive and thus respond preferentially to their corresponding
427 concentration range. Additionally, if besides a differential distribution of sucrose receptors
428 with different concentration tuning, larger ants would also have an expansion in their receptor
429 number compared to smaller ants, differences in sucrose sensitivity could be amplified.
430 Differences in sucrose sensitivity could also arise at the level of the central nervous system,
431 where these two groups of ants may differ in their biogenic-amine contents, thus resulting in
432 different levels of arousal and responsiveness to sucrose of different qualities (Scheiner et al.
433 2002; Scheiner et al. 2006). Biogenic-amine contents and ratios are variable in the insect
434 nervous system and may account for differences in food preferences (Benard et al. 2006)).
435 Such variability could also underlie the change in SAT that occurred both for small and large
436 ants under different starvation regimes.

437

438 Size has been shown as a determinant of stimulus sensitivity in few ant species in
439 different behavioral contexts. Small workers of *Atta capiguara* are disproportionately more
440 likely to respond with an alarm reaction when exposed to crushed ant heads along foraging
441 trails, suggesting that their main function is to patrol the trail area (Hughes and Goulson
442 2001). In *Atta sexdens*, minor and major foragers follow trails with higher fidelity than
443 medium ants (Morgan et al. 2006). Small workers of *Atta vollenweideri* are less responsive
444 than large workers to single conspecific or heterospecific trails. It was thus suggested that
445 large workers have a lower threshold than small workers to respond to the trail pheromone
446 and its components (Kleineidam et al. 2007). Similarly, in the polymorphic ant *Pheidole rhea*,
447 the largest workers can detect the trail pheromone at concentrations lower than those detected
448 by minors and soldiers (Gordon et al. 2018). *C. mus* ants might also have a higher threshold to
449 follow the trail pheromone. This difference could also contribute to the allocation of large

450 foragers to more productive sources, considering that more attractive sources usually result in
451 more trail-pheromone deposition (Beckers et al. 1993; Mailleux et al. 2000; Schilman 2011).

452 Which social mechanism(s) could explain the differential distribution of ants with
453 different sizes among food sources of different quality? Besides the SAT difference shown in
454 our study, one could focus on the scouts' recruitment behavior and/or on the interaction
455 patterns between recruiter and recruited-to-be, which are involved in the stimulation to
456 foraging (Gordon 2002; Cassill 2003). In social insects, high-quality sources trigger more
457 frequent and intense recruitment behavior [bees: (Seeley et al. 1991); ants: (Cassill 2003);
458 (Detrain and Pasteels 1991)]. Firstly, as large ants are minoritarian in terms of size within the
459 colony, an increment of recruitment interactions increases the probability to involve them.
460 Secondly, large ants may also have a higher threshold to leave the nest for foraging; they
461 might need more stimulation than smaller ants to be recruited. In this scenario, large foragers
462 would only be recruited after intense interactions (either in number or in strength) occurring
463 when high profitable sources are available. This is the case of the ant *Pheidole pallidula*,
464 where majors have a higher threshold to respond to the recruiting trail and to tactile
465 invitations than minors (Detrain and Pasteels 1991). In another ant species, *Temnothorax*
466 *albipennis*, corpulence predicts better than other factors if an ant initiates foraging upon
467 increased recruitment: leaner ants exhibit lower thresholds for foraging, irrespectively of age
468 (Robinson et al. 2009). Even in some species typically considered as monomorphic, a
469 significant relationship between worker size and task preference has been reported: larger
470 workers perform labor preferentially outside the nest while smaller individuals work inside
471 the nest (Herbers and Cunningham 1983; Westling et al. 2014; Grześ et al. 2016); even more
472 guards are larger than foragers (Grüter et al. 2012).

473

474 The adaptive value of differential size distribution among sources of different quality
475 remains to be determined, as a high variation in threshold or in size does not necessarily imply
476 better colony performance (Jandt and Dornhaus 2014). In bumble bees, the impact of body
477 size on thermoregulation and undertaking was studied using worker removals to restrict
478 threshold or body size differences within the colony. In general, there was no significant
479 effect of this treatment on fanning or undertaking success. Instead, colonies with a narrower
480 range of size variation had more success at undertaking (Jandt and Dornhaus 2014). These
481 authors thus suggested that size variation may be important under conditions not considered in
482 their experiments, for instance during periods of starvation; this interplay may generate
483 complex trade-offs consistent with the notion that within-colony size variation may have
484 evolved to cope with long-term environmental conditions (Jandt and Dornhaus 2014). Such a
485 long-term perspective could be valid for understanding the kind of size distribution –non
486 random and correlated with source productivity– found in our work. This distribution may
487 develop as food sources become stable and more persistent. Size distribution among sources
488 of different quality could occur in parallel to the development of food source fidelity, in *C.*
489 *mus* and other *Camponotus* ants, which present a high fidelity to the nectar sources they visit
490 over several days [*C. sericeiventris*: (Yamamoto and Del-Claro 2008); *C. compressus*:
491 (Palavalli Nettimi and Iyer 2015)] or even weeks [*C. sericeus*: (Mody and Linsenmair 2003),
492 *C. mus*: data not shown)]. A size related distribution could therefore help coping with long-
493 term food source exploitation. Models on collective decision making, which have been
494 applied to various social insects and behavioral contexts, would be useful to provide a first
495 test of this hypothesis.

496

497

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628 **Epigraphs**

629 **Figure 1:** Relative Frequency of Sucrose Acceptance Thresholds (SATs) for small (black
630 bars) and large (white bars) ants depending on sucrose concentration (% w/w), under two
631 sugar regimes: a) high sugar starvation and b) low sugar starvation. When the colony was
632 highly starved, both distributions did not differ ($p = 0.38$) and exhibited maximal frequencies
633 at 1%. However, when the colony was under low starvation, both distributions shifted towards
634 higher values, with maximal frequencies at 10 % for the small ants, and 30 % for the large
635 ants. These distributions differed significantly ($p = 0.035$).

636

637 **Figure 2:**

638 Proportions of ant sizes categorized as Small (white), Medium (gray) and Large (black) for
639 three sugar flow rate categories (low, medium and high) of different nectar sources visited by
640 ant foragers. Small ants represent the highest proportion at the low-sugar-rate sources, and the
641 lowest at the high-sugar-rate sources. Large ants were absent at the sources with low sugar
642 rate, but constituted the 50% of ants present at the sources with high sugar rate ($N_{tot} = 110$).

643

644

645 **Table 1:**

646 Pumping Frequency of large and small ants that belong to the same colony, for six different
647 colonies (1-6). The table shows the weight (mean \pm SE) of large and small ants, the
648 significance of the differences between weights of both sizes (Kruskal-Wallis test, KW), the
649 pumping frequency (pumps/s; mean \pm SE) of large and small ants, and the significance of the
650 differences between pumping frequencies of both sizes (Kruskal-Wallis test).

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Table 1:

| Nest (N) | Small ants weight | Large ants weight | K-W (Weight) | Small ants Pump Freq | Large ants Pump Freq | K-W (P Fr) |
|-----------------|--------------------------|--------------------------|---------------------|-----------------------------|-----------------------------|-------------------|
| 1 (30) | 5.57±0.2 | 14.42±0.7 | p<0.0001 | 3.75±0.1 | 3.67±0.1 | p=0.13 |
| 2 (24) | 5.39 ± 0.2 | 13.84 ± 0.8 | p<0.0001 | 3.3 ± 0.1 | 3.3 ± 0.1 | p=0.64 |
| 3 (19) | 7.39 ± 0.2 | 16.29 ± 0.4 | p=0.0002 | 4.7 ± 0.2 | 4.6 ± 0.1 | p=0.51 |
| 4 (27) | 7.17 ± 0.4 | 15.33 ± 0.9 | p<0.0001 | 5.3 ± 0.2 | 5.4 ± 0.1 | p=0.68 |
| 5 (31) | 6.75 ± 0.3 | 17.22 ± 0.7 | p<0.0001 | 5.4 ± 0.1 | 5.1 ± 0.2 | p=0.39 |
| 6 (31) | 6.67 ± 0.4 | 16.65 ± 0.5 | p<0.0001 | 5.1 ± 0.1 | 5.2 ± 0.1 | p=0.36 |

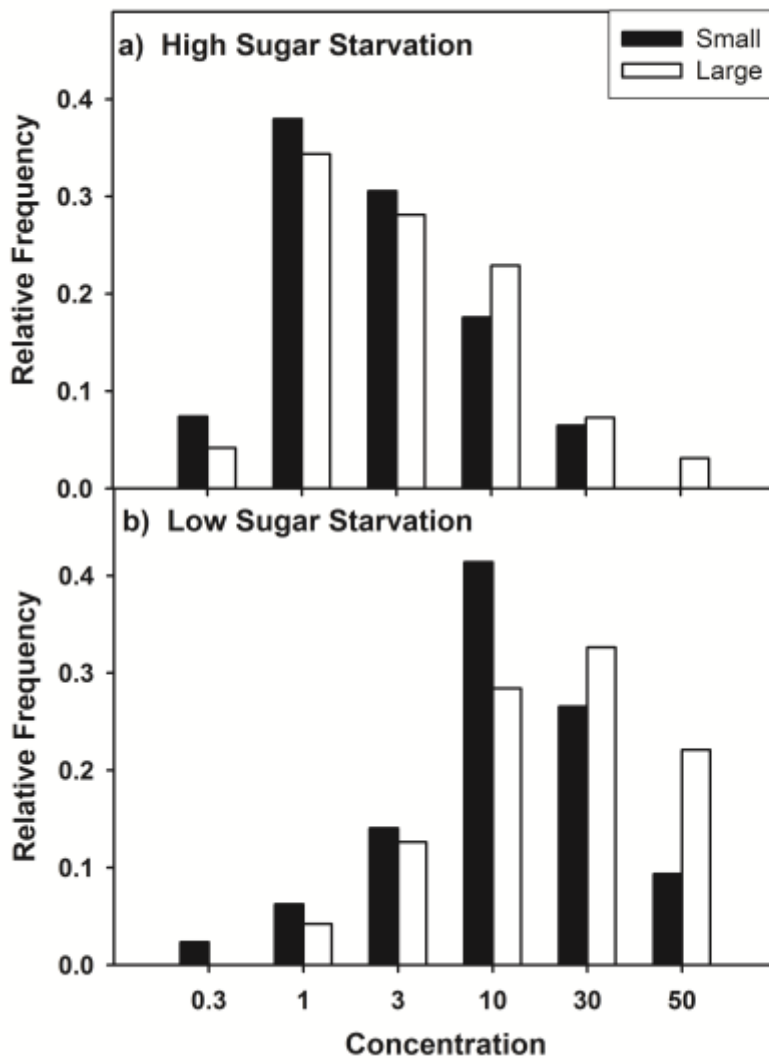
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665 **Table 2:**
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| | | Natural Sources | Artificial Food Sources | |
|--------------------|-------------------|------------------------|--------------------------------|------|
| Replicate 1 | <i>Sources</i> | <i>a</i> | | 60% |
| | <i>N tot</i> | 34 | | 17 |
| | <i>Prop Large</i> | 0.06 | | 0.18 |
| | <i># X-Large</i> | 0 | | 3 |
| Replicate 2 | <i>Sources</i> | <i>b</i> | | 60% |
| | <i>N tot</i> | 18 | | 22 |
| | <i>Prop Large</i> | 0 | | 0.36 |
| | <i># X-Large</i> | 0 | | 1 |
| Replicate 3 | <i>Sources</i> | <i>c</i> | 20% | 60% |
| | <i>N tot</i> | 20 | 15 | 11 |
| | <i>Prop Large</i> | 0.1 | 0.2 | 0.36 |
| | <i># X-Large</i> | 0 | 1 | 2 |

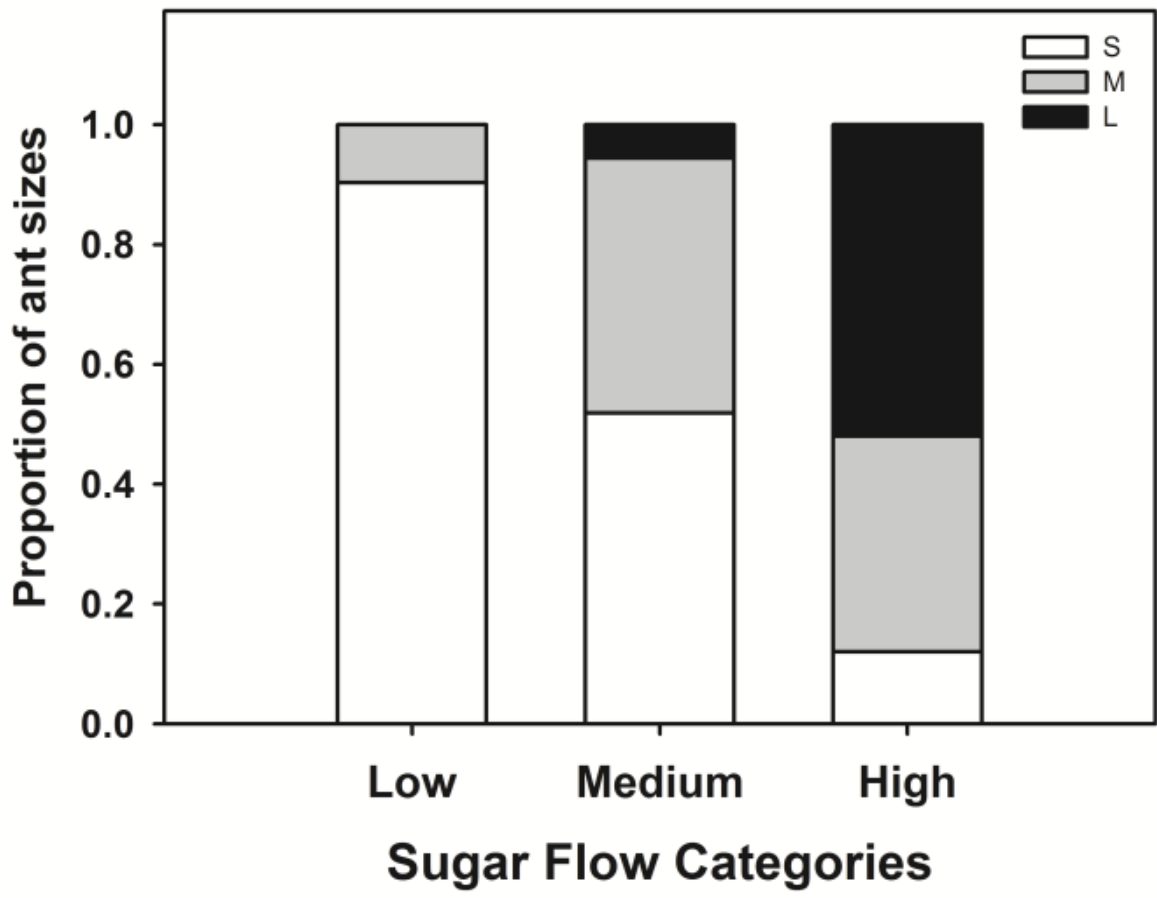
669
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671 Fig. 1



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