

# 1 **Ants dynamically adjust liquid foraging strategies in response to**

## 2 **biophysical constraints**

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10

### 11 **Abstract**

12 Ant foragers need to provide food to the rest of the colony, which often requires food transport over  
13 long distances. Foraging for liquid is especially challenging because it is difficult to transport and  
14 share. Many social insects store liquid food inside the crop to transport it to the nest, and then  
15 regurgitate this fluid to distribute it to nestmates through a behaviour called trophallaxis. Some ants  
16 instead transport fluids with a riskier behaviour –holding a drop of liquid between the mandibles  
17 through surface tension– after which the ant shares this droplet with nestmates without ingestion or  
18 regurgitation in a behaviour called pseudotrophallaxis. Here, we hypothesized that ants optimise their  
19 liquid-collection approach depending on food quality and biophysical properties. Working with a  
20 ponerine ant that uses both trophallaxis and pseudotrophallaxis, we investigated why each liquid-  
21 collection behaviour might be favored under different conditions by measuring handling time and  
22 liquid viscosity and reaction to food quality (i.e., sugar concentration and viscosity) using a viscosity  
23 additive. We found that ants could collect more liquid food per unit time by mandibular grabbing than  
24 by drinking. At high viscosities, which in nature correspond to high sugar concentrations, ants  
25 switched their liquid collection method to mandibular grabbing in response to viscosity, and not to  
26 sweetness. In addition, mandibular grabbing of liquid food allowed ants to carry more sugar per unit  
27 time than drinking. Our results demonstrate that ants change not only their feeding preference but also  
28 their transport and sharing methods according to viscosity—a proxy for sugar concentration in nature—  
29 optimising the mass of sugar returned to the nest over time.

30

31 keywords: mandibular pseudotrophallaxis, social bucket, *Diacamma cf. indicum*, liquid  
32 transportation, optimal foraging theory, biomechanics

### 33 **Introduction**

34 Efficient foraging is crucial for animals to survive, grow and reproduce. Organisms need to balance  
35 energy spent with energy gained [1,2]. Optimal foraging theory assumes that animals' foraging  
36 decision making has evolved to the point that the fitness of individuals has been maximised. Foraging  
37 provides energy to survive and reproduce; however, it has the cost of exposing the individual to being  
38 preyed upon by other animals and it costs energy, for example, due to the time spent exploiting,  
39 processing and transporting food.

40         Animals have a wide variety of foraging strategies. Ants are central-place foragers with  
41 diverse diets ranging from complete herbivory to complete predation [3–6]. Morphological and  
42 phylogenetic evidence suggests that the ancestral ant was a predator, and transitions to herbivory  
43 occurred several times in predatory lineages [6–8]. Plant-based food sources, such as plant nectar [9]  
44 or honeydew excreted by sapsucking Hemiptera and scale insects [10–12], are rich in carbohydrates.  
45 In many species and especially in ecologically dominant ant lineages, these sugary liquids are ants'  
46 main source of energy [13]. Additionally, liquid resources, such as honeydew or nectar, are less  
47 ephemeral relative to insect prey, and incur fewer risks for ant foragers relative to hunting. Thus, the  
48 use of plant-based food sources may lead to lower foraging time and less risk for foragers per calorie  
49 returned to the nest.

50         Transportation of liquid food is a foraging challenge. Many ants and bees transport liquid  
51 stored inside their crop, where it cannot easily be lost or stolen [14] during transport. Foragers  
52 regurgitate this fluid to distribute it to nestmates through a behaviour called trophallaxis. Many liquid-  
53 feeding ants have acquired morphological adaptations for trophallaxis. The ant crop is separated from  
54 their midgut by a variably developed proventriculus, which allows the crop to store a large amount of  
55 liquid in some species [15]. The structure of proventriculus varies considerably across taxa [16–18],  
56 and liquid-feeding ants often have a more elaborate proventriculus. The gaster and crop also need to  
57 be expandable to best store liquid food, either temporarily for transport or over the long term in the  
58 case of repletes. The extreme example of morphological specialisation are honeypot ants  
59 *Myrmecocystus* (Formicinae), where replete workers have a massive ball-like distended gaster full of  
60 food to the point where they can barely move [19]. Such species rely on trophallaxis to redistribute  
61 food from the repletes to the rest of the colony. Although trophallaxis is considered a safe and reliable  
62 liquid transportation method for ants, the crop load (i.e., liquid food intake) strongly depends on these  
63 morphological constraints.

64         Some ants do not have these morphological specialisations but nonetheless consume liquid  
65 food: Ectatomminae (*Ectatomma*), Ponerinae (*Diacamma*, *Neoponera Odontomachus*, *Paraponera*,  
66 *Pachycondyla*, *Rhytidoponera*). These ants typically use mandibular pseudotrophallaxis (hereafter  
67 called pseudotrophallaxis) as their method of liquid transport [15,20]. Instead of storing liquid inside  
68 the crop, foragers hold liquid food between their mandibles where it forms a droplet because of surface  
69 tension. After foragers return to the nest, they pass the liquid food to nestmates without regurgitation.

70 Previous studies in ponerine ants have reported how this behaviour allows liquids to be distributed in  
71 the nest [21,22]. This liquid transport method is sometimes referred to as the ‘social bucket’ method  
72 and has been suggested to be an evolutionary precursor to ‘true’ trophallaxis [7].

73 Handling time is crucial for efficient foraging. For liquid food, the handling time includes  
74 both the speed of food collection (i.e., drinking time or grabbing time) and the transport time to the  
75 nest. Drinking time in ants has been shown to depend on food quality such as sugar concentration and  
76 viscosity itself [23]. Previous studies found that drinking time increased linearly with increasing  
77 sucrose concentration [23–27]. Individuals need to decide when to stop drinking, considering the  
78 balance between energy gain and predation risk. The handling time of pseudotrophallaxis have not  
79 been investigated. In the case of the transport time, once foragers store food in the crop, they can  
80 transport the liquid food safely. When using pseudotrophallaxis, there is the possibility to lose the  
81 liquid food along the return path. Also, keeping mandibles open adequately may reduce walking  
82 speed and increase likelihood of predation.

83 In summary, to provide liquid resources for nestmates, ants must collect, transport, and share  
84 liquid food through a series of behaviours. Generally, ants either drink, and internally store liquid and  
85 share it through regurgitation and trophallaxis, or they grab liquid into a mandibular droplet and share  
86 through pseudotrophallaxis (Figure 1). Some ants use both behaviours. The ponerine ant *Diacamma*  
87 *cf. indicum* from Japan performs both trophallaxis and pseudotrophallaxis[28], and has a simple  
88 proventricular morphology and has a rigid, non-extensible gaster. Thus, *Diacamma cf. indicum* is an  
89 ideal model species to investigate efficient foraging strategies regarding liquid food because it allows  
90 us to investigate foraging strategies without morphological specialisation.

91 The aim of the present study is to reveal what leads ants use the collection mode of  
92 mandibular grabbing instead of drinking and whether ants’ liquid collection modes mechanisms  
93 maximise calorie intake rates for their colony. We hypothesize that viscosity triggers a switch in  
94 collection behaviour between drinking and mandibular grabbing, where mandibular grabbing being  
95 more efficient to collect high viscosity solutions. To test this hypothesis, we conducted behavioural  
96 experiments to investigate 1) the volume and speed of liquid food collection depending on sugar  
97 concentration, 2) whether ants change their transportation method depending on sugar concentration  
98 or viscosity, and 3) the foraging efficiency for each approach by estimating the total amount of sugar  
99 carried per trip.

## 100 **Method**

### 101 *Colony collection and colony keeping*

102 12 Colonies of *Diacamma* cf. *indicum* from Japan were collected from Kenmin-no-mori (Onna) and  
103 Sueyoshi park (Naha), Okinawa, Japan. The colonies were kept in plastic artificial nests filled with  
104 moistened plaster (9 cm diameter  $\times$  1.5 cm height). Each colony contained a mated gemma-possessing  
105 female (i.e., functional queen or gamergate), 50–150 workers, and brood. The artificial nests (90 mm  
106 in diameter) were placed in a plastic arena (diameter: cm, height: cm). Nests were maintained at  
107 25 °C under a 12h/12h light-dark regime (light phase: 0800–2000 hours). Reared colonies were fed  
108 with chopped frozen crickets three times per week. Water and 10% sugar water were provided ad  
109 libitum.

110

### 111 *Behaviour Definitions*

112 The ethogram of the social bucket method (encompassing mandibular grabbing, transport and  
113 pseudotrophallaxis) of *Diacamma* ant is shown in Figure 1. Based on a previous study [29] three  
114 behaviours for liquid feeding and collection were defined 1) tasting: placing mandibles on a solution  
115 without drinking or antennating, 2) drinking: individuals drink (i.e., mouthpart, labrum, attached to a  
116 liquid solution), 3) grabbing: individuals use (open) mandibles to grab and pull at a liquid solution,  
117 occasionally succeeding in collecting a droplet. We confirmed that the focal ant species showed the  
118 same actions. However, tasting was difficult to see by video observation, and thus we only considered  
119 drinking and grabbing.

120 All behavioural experiments were conducted between 12:00-19:00 under the light condition at  
121 25°C. Colonies were starved for 3-4 hours before experiments (starvation time based on preliminary  
122 behavioural observations). We placed an artificial nest on one side and a plate-shaped feeder (40  $\times$   
123 40 mm) on the other side of the foraging arena (460  $\times$  260  $\times$  100 mm) (Supp. figure 1). In the  
124 preliminary observation, all foraging trips and pseudotrophallaxis bouts were counted by watching the  
125 recorded video for 30 minutes (Supp. Figure 2). Ants clearly preferred sugar water over water.

126

### 127 *Measurement of volume of water internally and externally carried*

128 The volume of sugar water drunk was estimated as the difference in body mass before and after  
129 drinking. We chose ants outside of the nest and measured their body mass (before drinking). Then,  
130 each individual was separately placed into a plastic box (4.5  $\times$  4.5  $\times$  2 cm), containing a drop  
131 (approximately 100  $\mu$ l) of sugar water. Ants were offered one of six different sucrose concentrations:  
132 10, 20, 30, 40, 50, 60 % w/w. The concentrations we used were within the range reported for natural  
133 nectar sources for ants (extrafloral nectars: 4.7–76% w/w [30]). After 10 minutes, or when ants  
134 stopped drinking, we dried the ant mouths and measured the ant's body mass to determine the volume

135 of liquid food inside its crop. We estimated the volume ( $\mu\text{L}$ ) of sugar water using the average weight  
136 of sugar water per 1  $\mu\text{l}$  (Supp. table 1). Individuals were never tested more than once a day.  
137 The volume of sugar water carried by mandibular grabbing was measured with a microcapillary tube.  
138 We used the same setup of the experimental arena above (Supp. figure 1). Ants were offered one of  
139 six different sucrose concentrations: 10, 20, 30, 40, 50, 60 % w/w. The ants freely accessed the  
140 offered food. After an ant succeeded in grabbing a droplet of the solution, we collected the droplet  
141 with a microcapillary tube during a return trip to the nest. The weight of the dispensed volume was  
142 measured to calculate the volume of sugar water the ant carried. We estimated the volume ( $\mu\text{L}$ ) of  
143 sugar water using the average weight of sugar water per 1  $\mu\text{l}$  (Supp. table 1).

144

#### 145 *Measurement of grabbing and drinking time*

146 We placed an artificial nest on one side and a plate-shaped feeder ( $40 \times 40$  mm) on the other side of  
147 the foraging arena ( $460 \times 260 \times 100$  mm) (Supp. figure 1). Ants were offered one of the six sucrose  
148 concentrations: 10, 20, 30, 40, 50, 60 % w/w. We video-recorded the area around the sucrose droplet  
149 for 1 hour. We manually recorded one type of the interaction which foragers had with the droplet only  
150 drinking, grabbing after drinking (both), or only grabbing and accumulated grabbing/drinking time for  
151 each foraging trip by an observer analysing the videos.

152

#### 153 *Reaction to viscosity*

154 To test the effects of sweetness and viscosity on foraging methods used, we modified the viscosity of  
155 the solution using carboxymethylcellulose sodium salt (Medium viscosity) (Sigma-Aldrich). The  
156 carboxymethylcellulose sodium salt (CMC) is a non-toxic inert viscosity modifier [31]. By adding  
157 this product, we increase the viscosity of the solution without changing its sugar concentration. We  
158 used: 10% w/w sugar solution with CMC 0.25% w/w (10CMC) as a viscosity-altered solution. We  
159 confirmed that the sugar concentration of CMC additive solution was not changed using a  
160 saccharimeter (Refractometer RBR32-ATC).

161

#### 162 *Dynamic viscosity measurement*

163 We prepared six sugar solutions, 10, 20, 30, 40, 50, 60 % w/w, and in addition, two viscosity-altered  
164 solutions, 10% sugar solution with CMC 0.25% (10CMC) and 30% sugar solution with CMC 0.25%  
165 w/w (30T). We measured their dynamic viscosity at  $25^\circ\text{C}$ . The dynamic viscosity is determined in a  
166 commercial stress-controlled rheometer (ANTON PAAR MCR 300) with a plate-plate geometry of 5  
167 cm diameter and 0.5 mm gap. The bottom plate was roughened by sandblasting to prevent slip  
168 artefacts and the temperature was fixed at  $25^\circ\text{C}$  by a Peltier hood. We applied constant shear rates,  
169 and stress was computed when the steady-state regime had been reached for each shear rate. The  
170 resulting stress versus shear rate experiments exhibited linear behaviour, as expected for Newtonian

171 liquids, and the dynamic viscosity was directly read from the slope evaluated by least-square  
172 minimisation for each sample, for more detail see Rhee and Lee (1994).

173

#### 174 *Intake rate and estimation of sugar intake*

175 Following a previous study [33], intake rate was calculated as the slope ( $\mu\text{L}/\text{sec}$ ) of the linear  
176 regression of crop load and corresponding feeding time. Thus, we measured these two for each sugar  
177 concentration. For crop loads ( $\mu\text{L}$ ), we used the same procedure as ‘measurement of the volume of  
178 liquid drunk’. For the corresponding feeding time, we filmed the behaviour of ants when drinking and  
179 measured the time spent in contact with the droplet.

180 We calculated the total liquid load per trip based on the foraging action used. The total crop  
181 load per trip was estimated by multiplying the intake rate by the accumulated time drinking. The  
182 mandible load (collected by grabbing) was defined as the average volume of liquid carried for each  
183 sugar concentration in the experiment ‘Measurement of volume of water drunk and water carried’.  
184 The average volume can change depending on sugar concentration. When ants performed both  
185 drinking and grabbing, we summed the average volume of water carried and the estimated total crop  
186 load. Using the average weight of sugar water per 1  $\mu\text{l}$  (Supp. table 1), we converted the total liquid  
187 load per trip ( $\mu\text{L}$ ) to weight of the liquid load (mg). From the liquid load weight (mg), we calculated  
188 the total sugar intake per trip (mg) for each sugar concentration.

189

#### 190 *Statistical analysis*

191 Generalized linear regression models were used to investigate the relationship between the liquid  
192 volume or loading speed with the food quality variables and foraging actions. Pairwise chi-square  
193 tests with a Bonferroni correction were used for comparing foraging action on different sugar  
194 concentrations. Generalized linear regression models were used to investigate the relationship of the  
195 load with the food quality variables and foraging actions. A significance general level of 5% was used  
196 in all comparisons. All analyses were run in R studio 2022.02.3 (package: ggplot2).

## 197 **Results**

198 To understand whether ants altered their liquid collection and transport behaviour according to the  
199 concentration of sugar they encounter, we measured multiple variables to find what the ants are  
200 optimising: volume carried, time spent per trip, frequencies of different foraging actions, and sugar  
201 load per trip.

202 First, we analysed the liquid food collection method and volume of liquid food collected by  
203 637 ants from eight colonies feeding on six different sugar concentrations (Figure 2). We observed an  
204 interaction between sugar concentration and foraging action (Figure 2a, Table 1a, GLM, sugar  $\times$   
205 foraging action:  $p < 0.001$ ), and therefore analysed the foraging methods separately. When ants drank  
206 liquid, the amount of sugar water imbibed decreased as sugar concentration increased (Figure 2a,  
207 Table 1b, LM:  $p < 0.001$ ), suggesting that with increasing sugar concentration, drinking becomes  
208 more difficult. For the amount of liquid grabbed within the mandibles, there was no significant trend  
209 in the across the different sugar concentrations (Figure 2a. Table 1b, LM:  $p = 0.41$ ). Thus, the most  
210 effective method to bring liquid food home to the colony, in terms of volume, depended on sugar  
211 concentration.

212 Regarding the collection time of drinking or mandible grabbing, we again found an  
213 interaction between sugar concentration and foraging action (Figure 2b, Table 1a, GLM, sugar  $\times$   
214 foraging action:  $p < 0.001$ ). When ants drank, the drinking time also decreased with increasing sugar  
215 concentration (Figure 2b, Table 1b, LM:  $p < 0.001$ ). Grabbing time slightly increased with sugar  
216 concentration (Figure 2b. Table 1b, LM:  $p < 0.01$ ), though mandible grabbing generally took less time  
217 when compared to drinking.

218 Ants often collected sugar water in their mandibles after drinking sugar water and rarely  
219 performed only mandible grabbing without drinking (Figure 3). The proportion of these foraging  
220 actions was significantly different across different sugar concentrations (Figure 3a, chi-sq test with  
221 Bonferroni correction). The proportion of the mandible grabbing after drinking (both) and mandible  
222 grabbing alone (both of which results in pseudotrophallaxis) increased with increasing sugar  
223 concentration (Figure 3a). This indicates that ants switch to grabbing and pseudotrophallaxis when  
224 they feed on liquid food with higher concentrations of sugar. This could come about because this  
225 high-sugar food is more valuable or because high viscosity liquids are difficult for them to drink.

226 To test whether ants react to changes in sugar concentration or viscosity, we altered the  
227 viscosity of a low-sugar solution using a viscosity-modifying additive CMC (carboxymethylcellulose  
228 sodium salt). The viscosity level of 10% sugar water with CMC (CMC10) was comparable to the one  
229 between 40% and 50% sugar water (Supp. Table 2, Figure 4). When we offered ants CMC10, the  
230 proportion of drinking was significantly decreased and equivalent to the high viscosity 50% sugar  
231 solution (Figure 3b, chi-sq test,  $p < 0.001$ ). This result suggests that ants switch collection method in  
232 response to viscosity, and not to sweetness.

233 To investigate whether this transition toward grabbing over drinking with increasing viscosity  
234 was optimal for the colony, we estimated the total sugar load per trip by combining intake rate and  
235 load per trip across the different sugar concentrations. The intake rate was the highest with 20% sugar  
236 water (Supp. Table 3, Figure S3, 20%:  $0.21 \mu\text{L}/\text{min}$ ), and decreased dramatically with increasing  
237 sugar concentration to just 8% of maximum at the highest tested viscosity (Supp. Table 3, Figure S3).  
238 The total crop load per trip was estimated using the intake rate and time spent drinking. The total  
239 liquid load had the largest volume when ants used both drinking and grabbing in the same trip (Figure  
240 5a). There was a significant interaction between sugar concentration and foraging action on the liquid  
241 load (Table 2a, GLM, sugar  $\times$  foraging action:  $p < 0.01$ ). The crop load decreased with increasing  
242 sugar concentration (Table 2b, LM:  $p < 0.001$ ). On most trips, the total liquid load of grabbing was  
243 7.5 times larger than the crop load (Table 2b, GLM, foraging action:  $p < 0.01$ ). To examine how much  
244 energy ants can bring back to the nest through these methods, we transformed the liquid load to sugar  
245 load. We found that the difference in efficiency between drinking and grabbing increased with sugar  
246 concentration (Figure 5b). There was a significant interaction between sugar concentration and  
247 foraging action (Table 2a, GLM, sugar  $\times$  foraging action:  $p < 0.01$ ). While there were no significant  
248 linear associations between sugar concentration and drinking (Table 2b, LM:  $p = 0.39$ ), the total sugar  
249 load acquired by grabbing significantly increased with sugar concentration (Table 2b, LM:  $p < 0.001$ ).  
250 These results suggest that grabbing, and consequently pseudotrophallaxis, are more efficient methods  
251 to collect high-viscosity liquid than drinking and regurgitation.



## 252 **Discussion**

253 Behaviour in a given species is highly adapted to that organism's context, and these behavioural  
254 adaptations often involve precise forms of behavioural plasticity. In this study, we analysed the  
255 flexibility of foraging behaviours in response to biophysical constraints in *Diacamma cf. indicum*. The  
256 ant used two liquid collection actions – drinking and grabbing – when collecting liquid food. In this  
257 study, we aimed to quantify dynamic switching between of two types of liquid food collection used by  
258 a single ant species. *Diacamma cf. indicum* is a part of a clade of ants that rarely specialise on liquid  
259 food. Given this species' phylogenetic context, these behaviours are likely to be relatively recent  
260 specialisations [15]. However, it remains unclear when ants use pseudotrophallaxis as opposed to true  
261 trophallaxis to collect, transport, and share liquids, whether the use of these behaviours varies  
262 according to food quality, and whether one is an evolutionary step toward another.

### 264 ***Viscosity dictates behaviour***

265 Here, we clearly observed mandibular grabbing and pseudotrophallaxis in the lab in *Diacamma cf.*  
266 *indicum* and we see that their use of this collection behaviour changes with viscosity. Our results are  
267 consistent with previous study in other ponerine ants, where ants stopped drinking [34] and tended to  
268 use mandibular grabbing at higher sugar concentrations (> 40%) [29]. Our work revealed that ants  
269 made this switch in collection mode according to viscosity (Figure 3b). Viscosity has been seen to  
270 reduce the liquid intake rates in many insects, including ants [23,34–37], consistent with our results  
271 (Figure S3). The viscosity of the solution makes drinking more time-consuming and this causes the  
272 ant to switch toward grabbing behaviour. When *Diacamma* ants used mandibular grabbing, total sugar  
273 load clearly increased at higher sugar concentrations. We also found that ants used mandibular  
274 grabbing after drinking liquid (Figure 3a). This maximises liquid load per trip because ants can  
275 transport internally and externally. Multiple trips can be costly as they involve loss of energy and  
276 increased predation risk.

### 278 ***Why do so few ants perform mandibular grabbing at high sugar concentration?***

279 Mandibular grabbing and pseudotrophallaxis are mostly performed by ponerine ants (Ponerinae), with  
280 only a few noted exceptions in other major ant subfamilies [38] One possibility is that mandibular  
281 grabbing is a risky, but high pay-off collection method. For example, if ants encounter predators, they  
282 might not react quickly enough, ending up lose their mandibular droplet and/or being preyed upon  
283 because they are less agile. Ants might not use mandibular grabbing in dangerous sites where they  
284 encounter predators. On the contrary, if there are competitors around the food site, ants need to  
285 compete against other ant species. *Pheidole megacephala* soldiers reacted to the presence of  
286 competitors. The soldiers performed more mandibular grabbing on the territory of other ant species in  
287 order to rapidly gathering and transporting large loads of liquid food [38] In our study, we did not  
288 measure transportation time from feeding site to the nest or impact of predation or competition.

289 Future studies with different distances and ecological contexts while analysing transport time could  
290 help elucidate the cost of transportation of pseudotrophallaxis through surface tension.

291

### 292 ***Morphological adaptations and biophysical constraints***

293 Another possible reason why some ants use pseudotrophallaxis and others use true trophallaxis is that  
294 many ants have internal morphological adaptations for liquid intake, storage and regurgitation, such as  
295 an expandable gaster, an elastic crop and a highly developed proventriculus [7,15]. These are likely to  
296 speed intake rate, allow for larger internal capacity and possibly allowing for greater flexibility  
297 regarding the intake of high viscosity solutions [16,39]. Thus, species with these morphological  
298 adaptations may not need pseudotrophallaxis.

299 A third possibility is that there might be biophysical restrictions on whether an ant can collect  
300 a liquid drop between her mandibles. It is likely that small body size makes interactions with liquid  
301 droplets more dangerous due to the strong forces of surface tension [40]. We observed that ants make  
302 a ‘hasty’ motion at the end of the extraction of the droplet. The strong force is likely needed to  
303 break the droplet away. If the ants pull with a constant force or weak force, they struggle to grab a  
304 droplet. For small ants, it may be difficult to exert force to extract the droplet. Whether any  
305 relationship exists between the ability to perform pseudotrophallaxis and biophysical restrictions,  
306 related to body size, has not yet been studied.

307 Not only body size, but also head and mandible shape could be relevant to the performance of  
308 liquid collection. Like *Platythyrea conradi*, soldiers of *Pheidole megacephala* use corporal  
309 pseudotrophallaxis wherein liquid is held under the head and thorax through surface tension [38],  
310 while minor workers do not perform pseudotrophallaxis. Soldier of *Pheidole* are much larger and have  
311 disproportionately large heads compared with minors. These morphological traits of soldiers might be  
312 related to their performance of corporal pseudotrophallaxis. Some ant species have unique and/or  
313 exaggerated mandible shapes, for example, some army ants [41], desert ants [42], and trap-jaw ants  
314 [43]. Given that the trap-jaw ant *Odontomachus* can collect droplets using uniquely shaped mandibles  
315 [22], this indicates that several types of mandible shapes allow ants to hold liquid between mandibles.  
316 However, the interaction between ant head and mouth-part morphology, body size and liquid  
317 collection modes is unclear, making this area well poised for study from a biomechanics perspective.

318

### 319 ***Share with nestmates: diffusion in the colony***

320 After foragers go back to the nest, they share the liquid food through trophallaxis including  
321 (regurgitation), or through pseudotrophallaxis. Trophallaxis should more rapidly distribute food in the  
322 colony because a receiver can become a donor and continue to distribute liquid food by regurgitation,  
323 also allowing the formation of a more complex social network[44–46]. In the carpenter ant  
324 *Camponotus*, foragers give food to a receiver, proportional to the available capacity in the receiver’s

325 crop. This trophallactic interaction helps the forager to sense colony satiation level and decide when  
326 to leave the nest and bring in more food [47]. In case of pseudotrophallaxis, a donor can provide  
327 liquid food to several receivers at same time. However, the distribution dynamics of liquid by  
328 pseudotrophallaxis has not been studied. We also do not know whether *Diacamma* ants share both  
329 internal and external liquid foods to nestmates. The observation of liquid distribution in the focal  
330 species that use both trophallaxis and pseudotrophallaxis is needed to understand these dynamics of  
331 liquid distribution and the regulation of foraging effort.

332

### 333 ***Share with nestmates: social circulatory system***

334 Trophallaxis allows for medium- to long-term food storage before redistribution while  
335 pseudotrophallaxis does not. Thus, ecological contexts and environmental harshness may also tilt an  
336 ant to engage in one behaviour or another. Another valuable feature of trophallaxis is that donors can  
337 alter the contents of what they pass to nestmates, either through partial digestion or through more  
338 complex signaling [48,49], which may bias a species or even a single ant to use one or another  
339 behaviour. Recent studies reveal that ants' regurgitated fluid contained more than food [48,49]. For  
340 example, trophallactic fluid in carpenter ants contains hormones, nestmate recognition cues, small  
341 RNAs, and various proteins. In *Diacamma*, it is unclear whether foragers regurgitate the contents of  
342 their crop during pseudotrophallaxis, or if they only regurgitate when they do trophallaxis. Future  
343 studies could examine whether they add any endogenous materials during these behaviours.

344 **Table 1. Influence of sugar concentration and foraging action on volume and handling time.**

345 Results of generalized and general linear regression (a) and linear regression model (b) between two  
 346 explanatory variables and volume of carried or drunk water and handling time. Sugar concentration  
 347 water is 10, 20, 30, 40, 50, and 60% (w/w) and foraging actions are drinking (DR) and mandibular  
 348 grabbing (GR).

349 (a) generalized linear regression

	Explanatory variables	Estimate	Std. Error	p-value
<b>Volume</b>				
	Sugar concentration	-0.028	0.003	< 0.001
	Foraging actions (DR or GR)	-1.153	0.231	< 0.001
	Sugar * Type of actions	0.030	0.005	< 0.001
<b>Handling time</b>				
	Sugar concentration	-0.999	0.172	< 0.001
	Foraging actions (DR or GR)	-85.573	13.375	< 0.001
	Sugar * Foraging actions	1.118	0.320	< 0.001

350

351 (b) linear regression

		Explanatory variables	Estimate	Std. Error	t-value	p-value
<b>Volume</b>	Drinking	(Intercept)	1.965	0.139	13.656	< 0.001
		Sugar concentration	-0.028	0.003	-7.647	< 0.001
	Grabbing	(Intercept)	0.812	0.118	6.832	< 0.001
		Sugar concentration	-0.999	0.172	0.839	0.41
<b>Handling time</b>	Drinking	(Intercept)	91.41	7.964	11.477	< 0.001
		Sugar concentration	-9.999	2.112	-4.733	< 0.001
	Grabbing	(Intercept)	5.836	1.884	3.097	< 0.01
		Sugar concentration	1.1822	0.4349	2.718	< 0.01

352

353 **Table 2. Influence of sugar concentration and foraging action on liquid and sugar load.**

354 Results of generalized and general linear regression (a) and linear regression model (b) between two  
 355 explanatory variables and liquid load or sugar load per trip. Sugar concentration water is 10, 20, 30,  
 356 40, 50, and 60% (w/w) and foraging actions are drinking (DR) and both drinking and mandibular  
 357 grabbing (Both).

358 (a) generalized linear regression

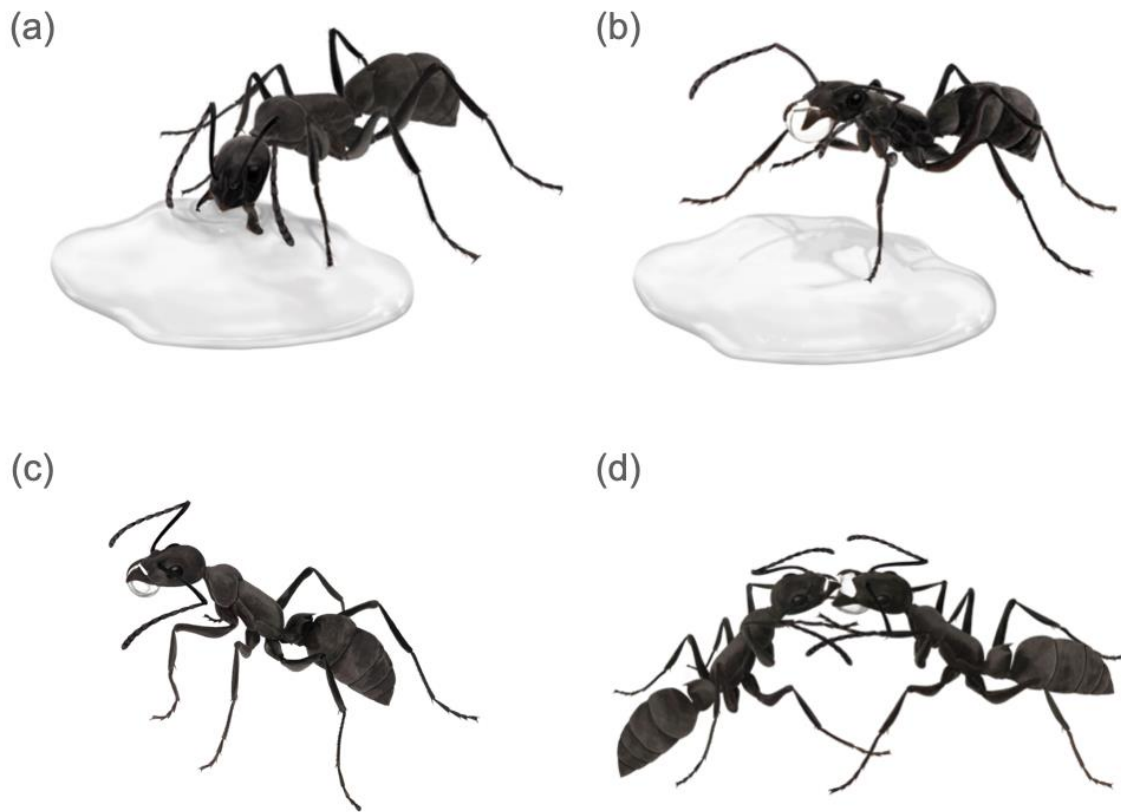
	Explanatory variables	Estimate	Std. Error	p-value
Liquid load	(Intercept)	1.035	0.032	< 0.001
	Sugar concentration	-0.0005	0.0007	0.451
	Foraging actions (DR or Both)	-0.784	0.038	< 0.001
	Sugar * Type of actions	-0.003	0.0009	< 0.001
Sugar load	(Intercept)	-0.035	0.01	< 0.001
	Sugar concentration	0.011	0.0002	< 0.001
	Foraging actions (DR or Both)	0.066	0.011	< 0.001
	Sugar * Foraging actions	-0.011	0.0003	< 0.001

359

360 (b) linear regression model

		Explanatory variables	Estimate	Std. Error	t-value	p-value
Liquid load	Drinking	(Intercept)	0.25	0.017	14.207	< 0.001
		Sugar concentration	-0.004	0.0005	-7.558	< 0.001
	Both	(Intercept)	1.035	0.038	27.062	< 0.001
		Sugar concentration	-0.0005	0.0008	-0.648	0.518
Sugar load	Drinking	(Intercept)	0.031	0.004	7.388	< 0.001
		Sugar concentration	-0.0001	0.0001	-0.860	0.391
	Both	(Intercept)	-0.035	0.0135	-2.599	0.01
		Sugar concentration	0.01	0.0003	37.494	< 0.01

361

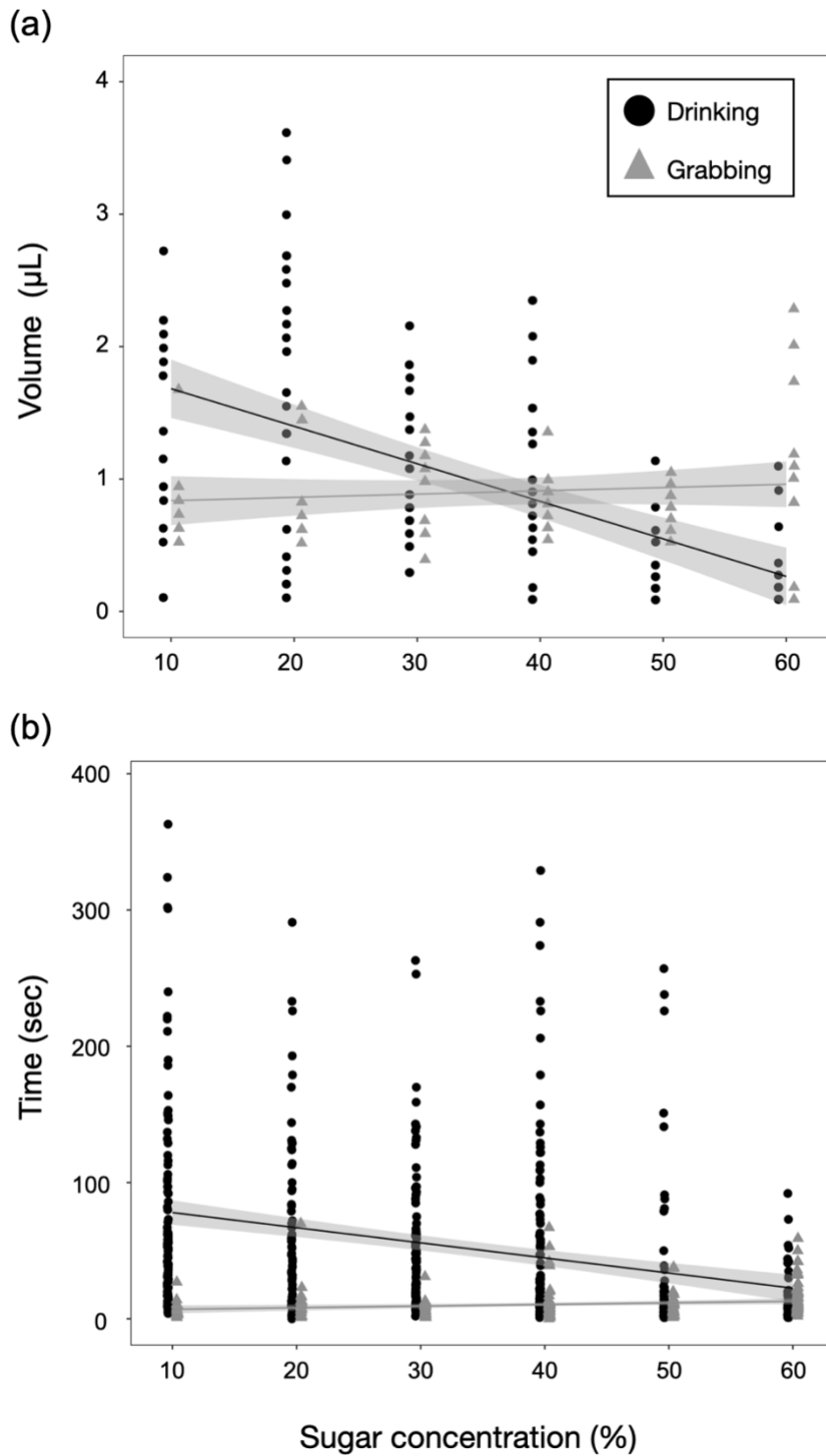


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362

363 **Figure 1. Ethogram of mandibular pseudotrophallaxis (social bucket).**

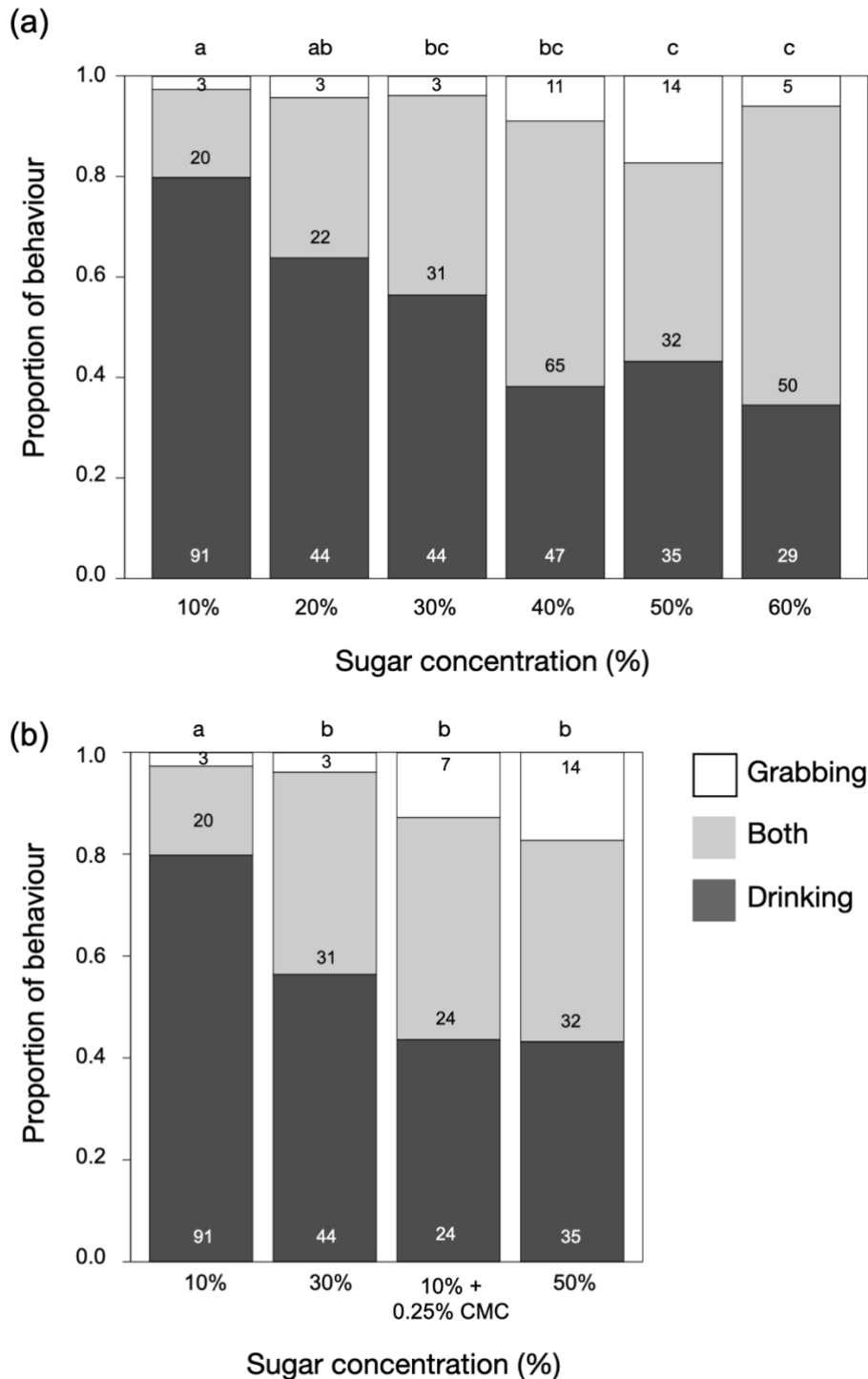
364 An ant collects (a, b), transports (c) and shares (d) liquid food through pseudotrophallaxis. The ant  
365 touches a solution with her antennae and mouthparts (tasting). After tasting, the individual opens her  
366 mandibles to grab and pull at the liquid solution (a). The ant occasionally succeeds in collecting a  
367 droplet of liquid between her mandibles (b). The ant returns to her nest (c). Inside the nest, the doner  
368 ant (right) shares the droplet with other nestmates (d). When the receiver (left) begs, their antennae  
369 move rapidly. Several nestmates can drink from the donor's droplet at the same time. Illustrations by  
370 Ken Naganawa.



371

372 **Figure 2. Effect of sugar concentration on foraging action.**

373 (a) The volume of liquid drunk or grabbed, and (b) the time spent drinking and grabbing, by an ant for  
374 each concentration of sugar water (% w/w). Black circles and grey triangles indicate drinking and  
375 grabbing, respectively. The shading is 95% confidence limits for the regression line.



376

377 **Figure 3. The proportion of pseudotrophallaxis depends on sugar concentration (a) and**

378 **viscosity (b).**

379 Grabbing (white), both (grey) and drinking (black) indicate the behaviour of only mandibular

380 grabbing, mandibular grabbing after drinking, and only drinking. The x-axis is sorted by viscosity (b).

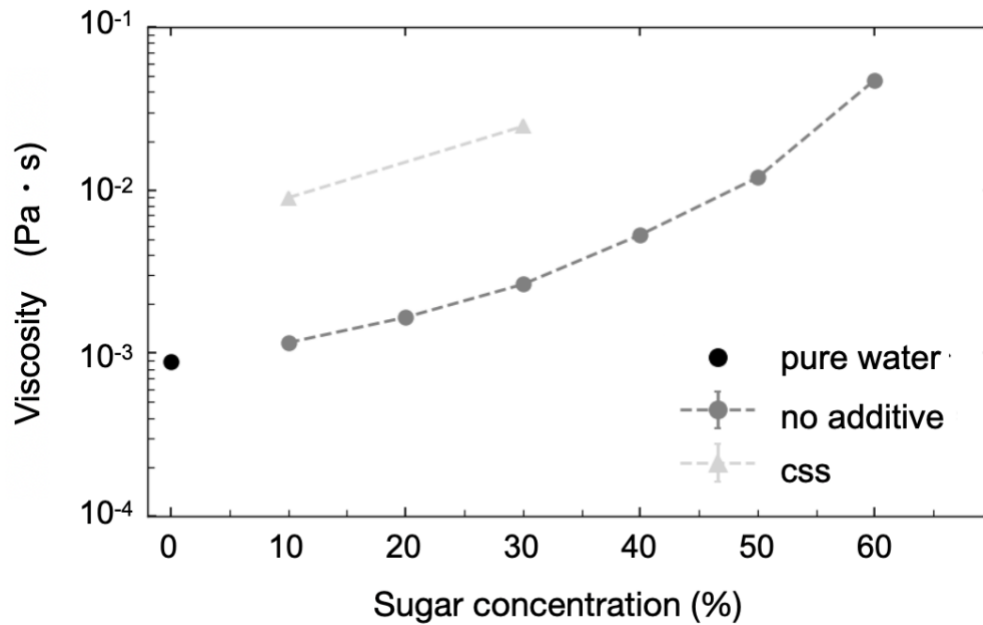
381 Different letters on the top of the bar mean they were significantly different at  $p < 0.05$  (chi-square

382 test with Bonferroni correction).

383



384



385

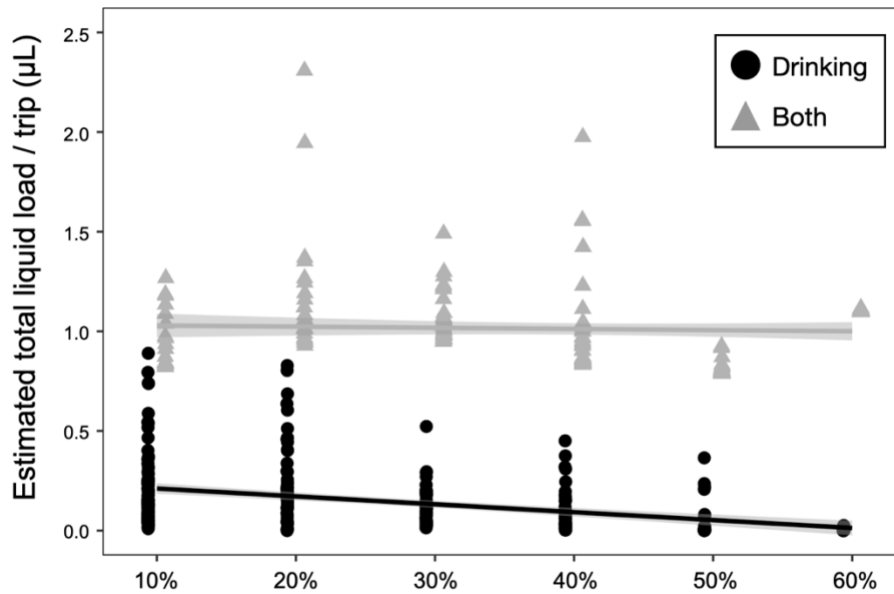
386 **Figure 4. Dynamic viscosities at 25°C for different sugar water solutions as a function of the**  
387 **sugar concentration (w/w) and viscosity-altering materials.**

388 The different colors correspond to pure water and viscosity-altered solutions. The error bars denote  
389 the quality of the linear fit applied to stress versus shear rate experiments for each solution (see  
390 Methods). Noted that the error margin was very small in our measurement.

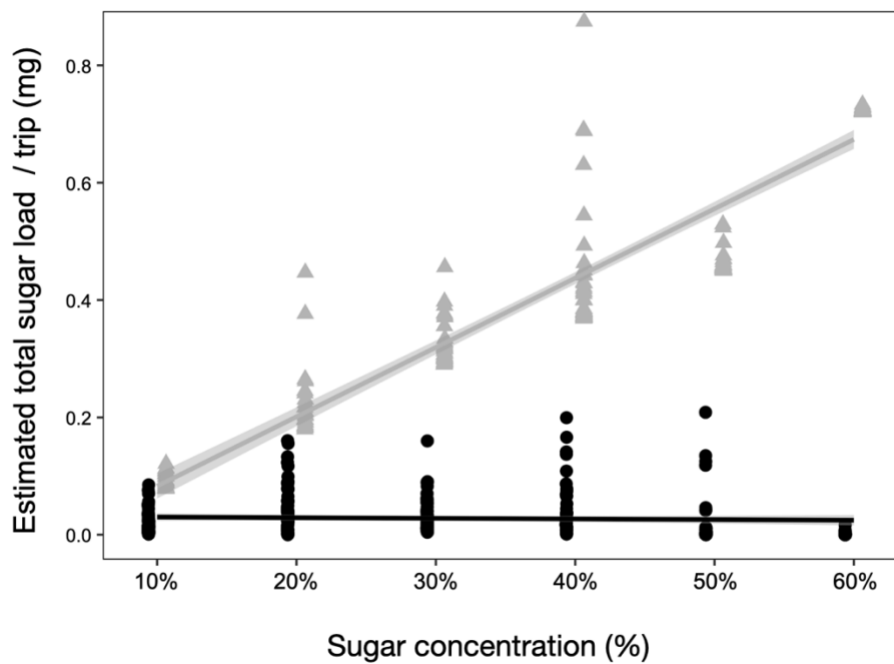
391

392

(a)



(b)



393

394 **Figure 5. Foraging efficiency of different modes of liquid collection and transport.**

395 Estimated total liquid(a) and sugar load per trip (b) are estimated based on drinking time and foraging

396 action. Dark grey, grey, and light grey indicate the action of drinking, both and grabbing. The shading

397 is 95% confidence limits for the regression line. The volume of liquid by grabbing is the mean of

398 carried volume by an ant, calculated in Figure 2a.

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- 531
- 532

547 **Supp. Table 1. Weight of each concentration of sugar water per 1  $\mu$  L.**

548 Weight is the average weight of corresponding 1  $\mu$  L sugar water (n=10).

Sugar concentration % (w/w)	Weight (mg/ $\mu$ L)
10	0.955
20	0.968
30	1.020
40	1.107
50	1.143
60	1.094

549

550 **Supp. Table 2. Viscosity.**

551 CMC is a non-toxic inert viscosity modifier. 10 CMC and 30 CMC are 10% sugar solution with CMC

552 0.25% and 30% sugar solution with CMC 0.25% w/w.

Sugar concentration % (w/w)	Viscosity (mPa•sec)
10	1.158 $\pm$ 0.002
20	1.656 $\pm$ 0.002
30	2.641 $\pm$ 0.003
40	5.308 $\pm$ 0.005
50	11.919 $\pm$ 0.009
60	47.34 $\pm$ 0.004
10 CMC	8.97 $\pm$ 0.002
30 CMC	24.6 $\pm$ 0.2

553

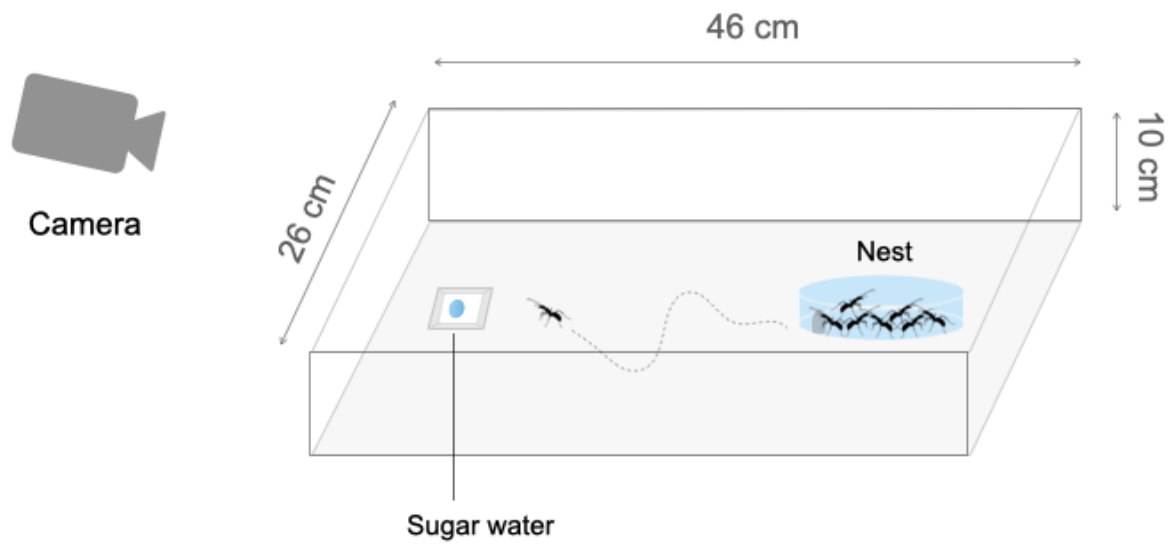


554 **Supp. Table 3. Intake rate of each concentration of sugar water.**

555 Intake rate is the slope ( $\mu\text{L}/\text{sec}$ ) of the linear regression of crop load and corresponding feeding time.

Sugar concentration % (w/w)	Intake rate ( $\mu\text{L}/\text{sec}$ )
10	0.00245
20	0.00355
30	0.00206
40	0.00136
50	0.00091
60	0.00027

556



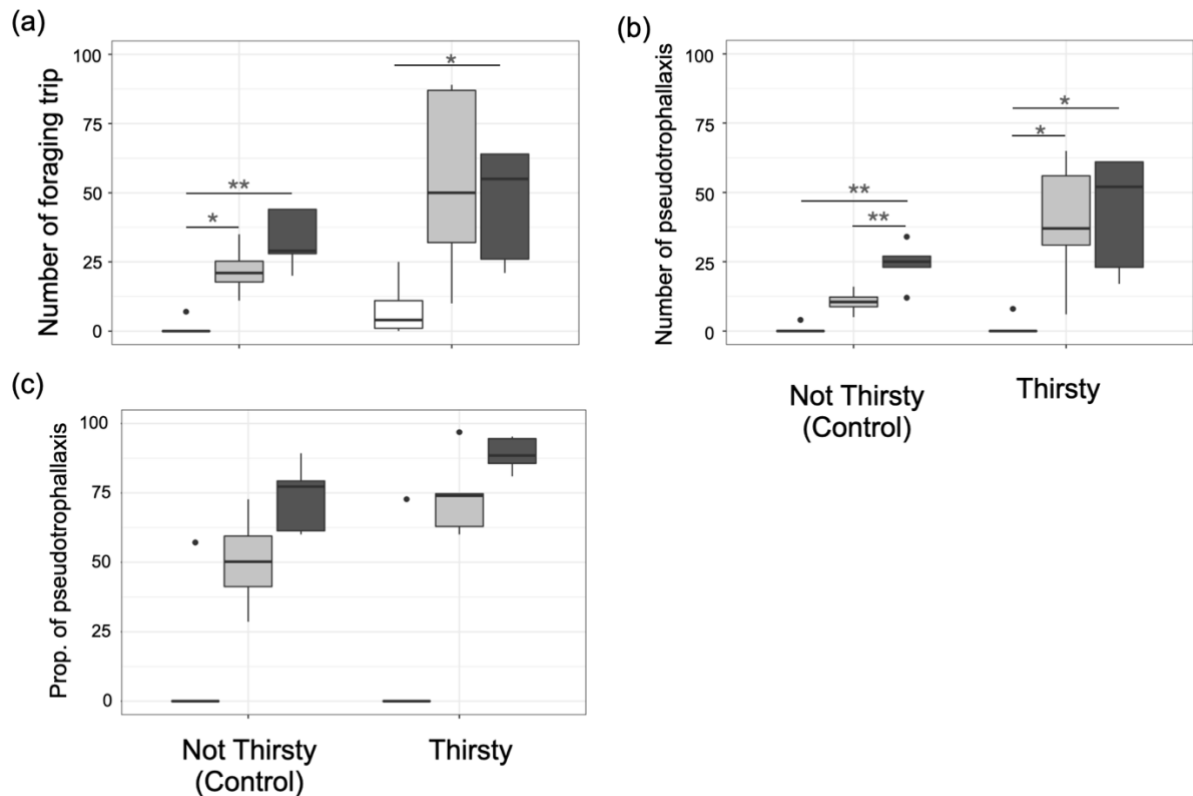
557

558 **Supp. Figure 1. Experimental arena design.**

559 An artificial nest (diameter = 9 cm) is placed on a container (48\* 26\* 10 cm). The nest was covered

560 with a red film to darken inside the nest. The distance between the nest and food is about 30 cm.

561 Fluon was applied to the wall of the foraging arena to prevent ants from escaping.



562

563 **Supp. Figure 2. Preference and foraging actions of different sugar concentrations on non-thirsty**  
564 **and thirsty colonies.**

565 Colonies were starved for 3-4 hours before the experiment (thirsty condition). Ants were offered three

566 different sugar concentrations: 0, 30, and 60 % w/w. (a) Total foraging bouts and (b) the number of

567 pseudotrophallaxis that an ant grabbed a drop by mandibles and transported it were counted during

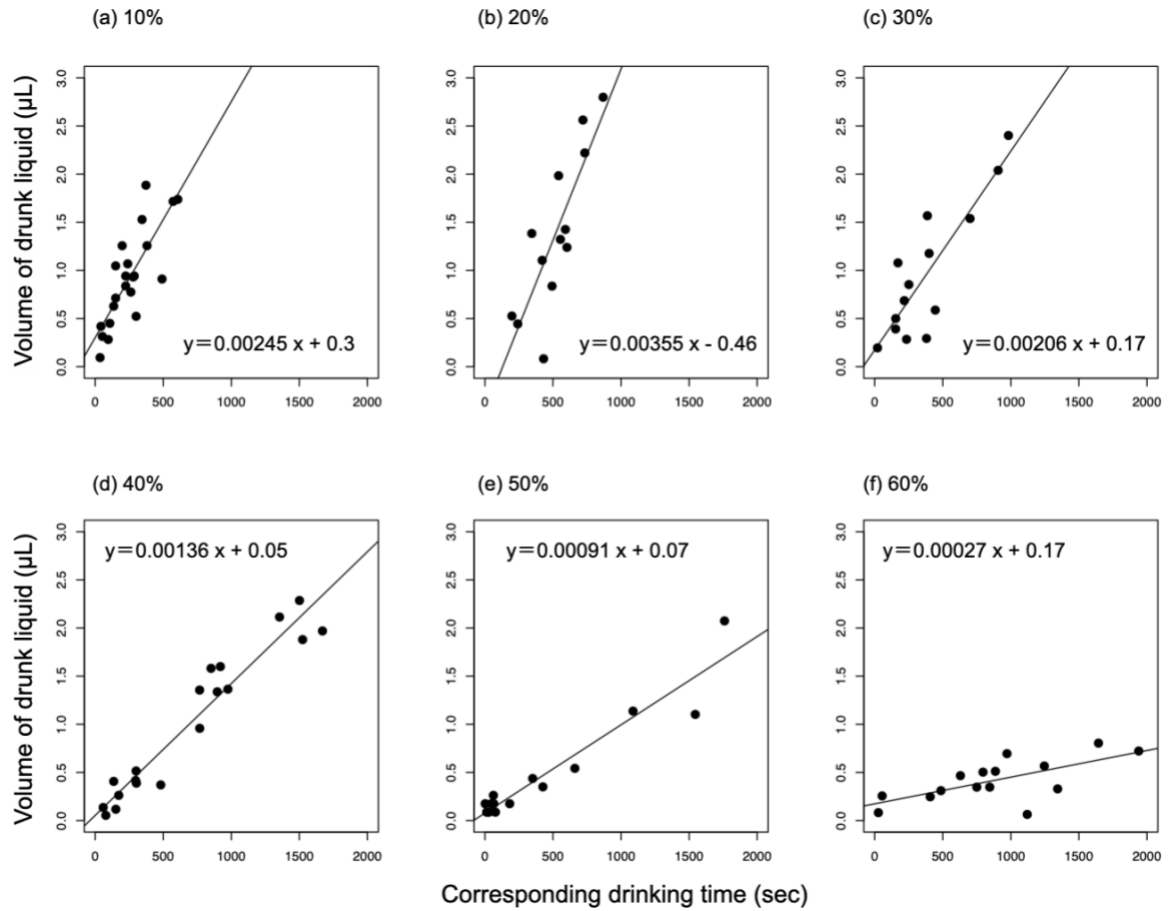
568 30-min observation. (c) The proportion of pseudotrophallaxis ants used was calculated for each

569 colony. The color of white, grey, and dark grey represents 0, 30, and 60 % w/w sucrose

570 concentrations. Asterisk indicates significant difference (Tukey–Kramer test, \*  $p < 0.05$ , \*\*  $p < 0.01$ ).

571 Ants clearly preferred sugar water compared to water. The number of foraging trips was high in the

572 thirsty condition.



573

574 **Supp. Figure 3. Intake rate, plotting crop load ( $\mu\text{L}$ ), and corresponding drinking time (sec).**

575 (a-f) Each column indicates different sugar concentrations from 10% to 60%. Linear regression

576 models are shown inside the column.