Ants dynamically adjust liquid foraging strategies in response to biophysical constraints

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Abstract

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

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

Introduction

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

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

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

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

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

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

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

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

Method

Colony collection and colony keeping

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

Behaviour Definitions

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

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

Measurement of volume of water internally and externally carried

The volume of sugar water drunk was estimated as the difference in body mass before and after drinking. We chose ants outside of the nest and measured their body mass (before drinking). Then, each individual was separately placed into a plastic box (4.5 × 4.5 × 2 cm), containing a drop (approximately 100 µl) of sugar water. Ants were offered one of six different sucrose concentrations: 10, 20, 30, 40, 50, 60 % w/w. The concentrations we used were within the range reported for natural nectar sources for ants (extrafloral nectars: 4.7–76% w/w [30]). After 10 minutes, or when ants 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 (μL) of sugar water using the average weight
136 of sugar water per 1 μl (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 (μL) of
143 sugar water using the average weight of sugar water per 1 μl (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 × 40 mm) on the other side of
147 the foraging arena (460 × 260 × 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℃. 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°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

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

Intake rate and estimation of sugar intake

Following a previous study [33], intake rate was calculated as the slope (μL/sec) of the linear regression of crop load and corresponding feeding time. Thus, we measured these two for each sugar concentration. For crop loads (μL), we used the same procedure as 'measurement of the volume of liquid drunk'. For the corresponding feeding time, we filmed the behaviour of ants when drinking and measured the time spent in contact with the droplet.

We calculated the total liquid load per trip based on the foraging action used. The total crop load per trip was estimated by multiplying the intake rate by the accumulated time drinking. The mandible load (collected by grabbing) was defined as the average volume of liquid carried for each sugar concentration in the experiment 'Measurement of volume of water drunk and water carried'. The average volume can change depending on sugar concentration. When ants performed both drinking and grabbing, we summed the average volume of water carried and the estimated total crop load. Using the average weight of sugar water per 1 μl (Supp. table 1), we converted the total liquid load per trip (μL) to weight of the liquid load (mg). From the liquid load weight (mg), we calculated the total sugar intake per trip (mg) for each sugar concentration.

Statistical analysis

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

Results

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

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

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

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

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

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

Discussion

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

Viscosity dictates behaviour

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

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

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

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

Morphological adaptations and biophysical constraints

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

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

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

Share with nestmates: diffusion in the colony

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

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

Share with nestmates: social circulatory system

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

Table 1. Influence of sugar concentration and foraging action on volume and handling time. Results of generalized and general linear regression (a) and linear regression model (b) between two explanatory variables and volume of carried or drunk water and handling time. Sugar concentration water is 10, 20, 30, 40, 50, and 60% (w/w) and foraging actions are drinking (DR) and mandibular grabbing (GR).

(a) generalized linear regression

	Explanatory variables	Estimate	Std. Error	p-value
Volume				
	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
Handling time				
	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

(b) linear regression

		Explanatory variables	Estimate	Std. Error	t-value	p-value
Volume	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
Handling time	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

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

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

(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

(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

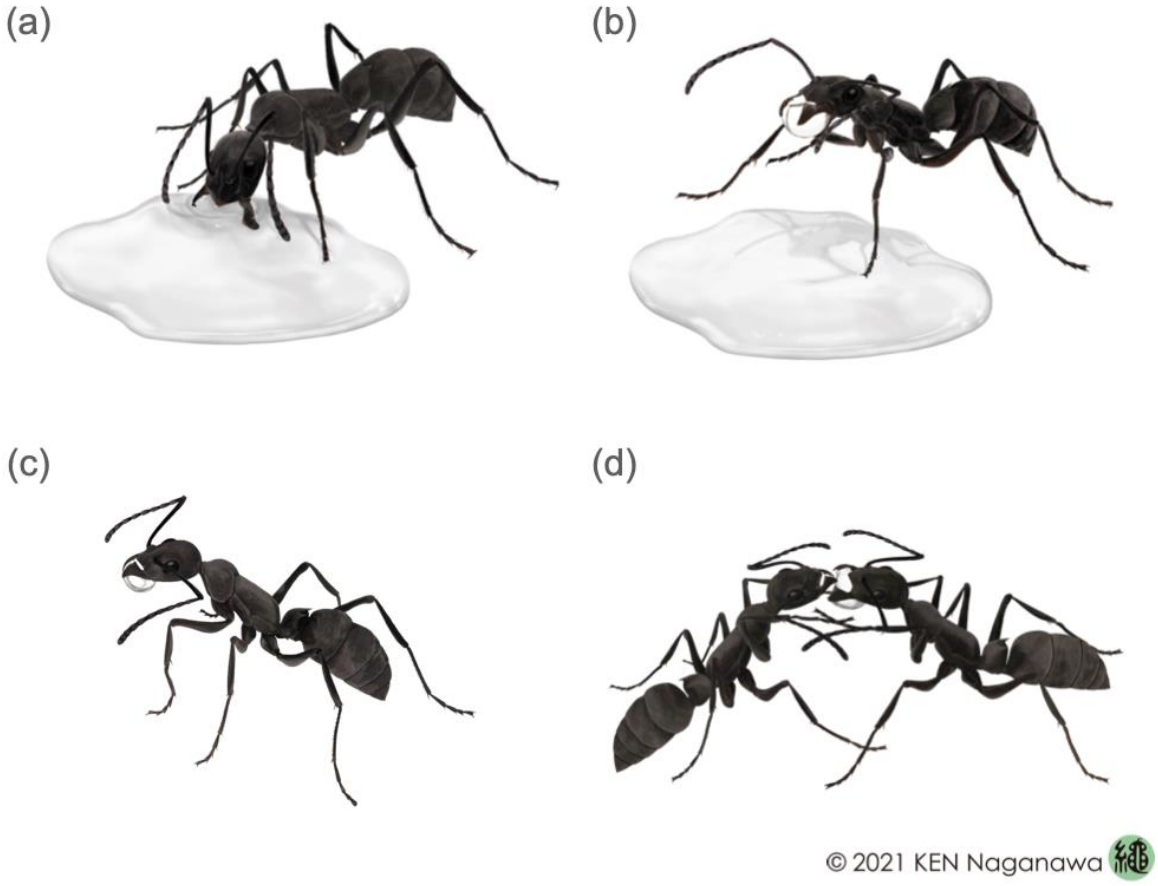

Figure 1. Ethogram of mandibular pseudotrophallaxis (social bucket).

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

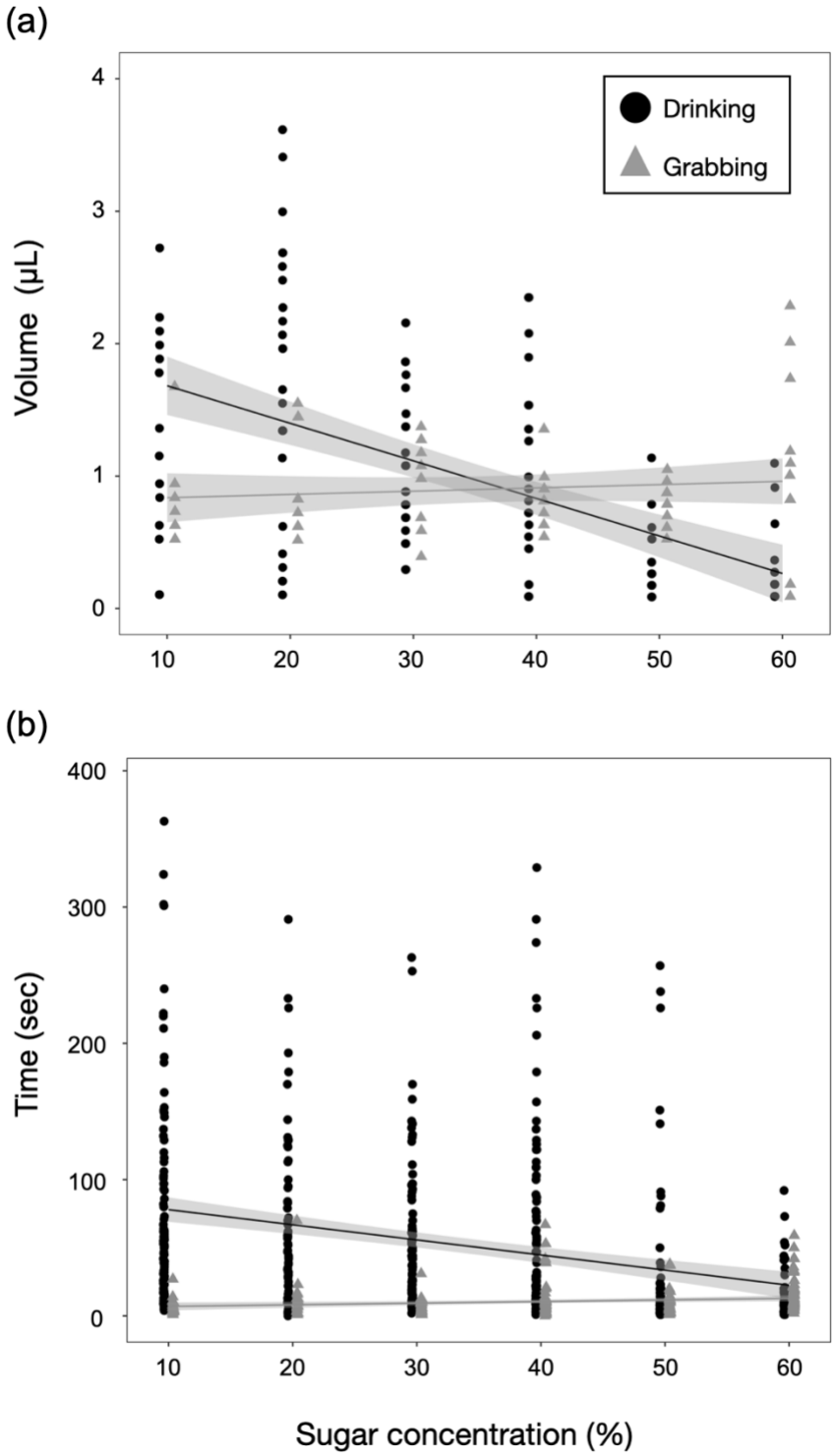

Figure 2. Effect of sugar concentration on foraging action.

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

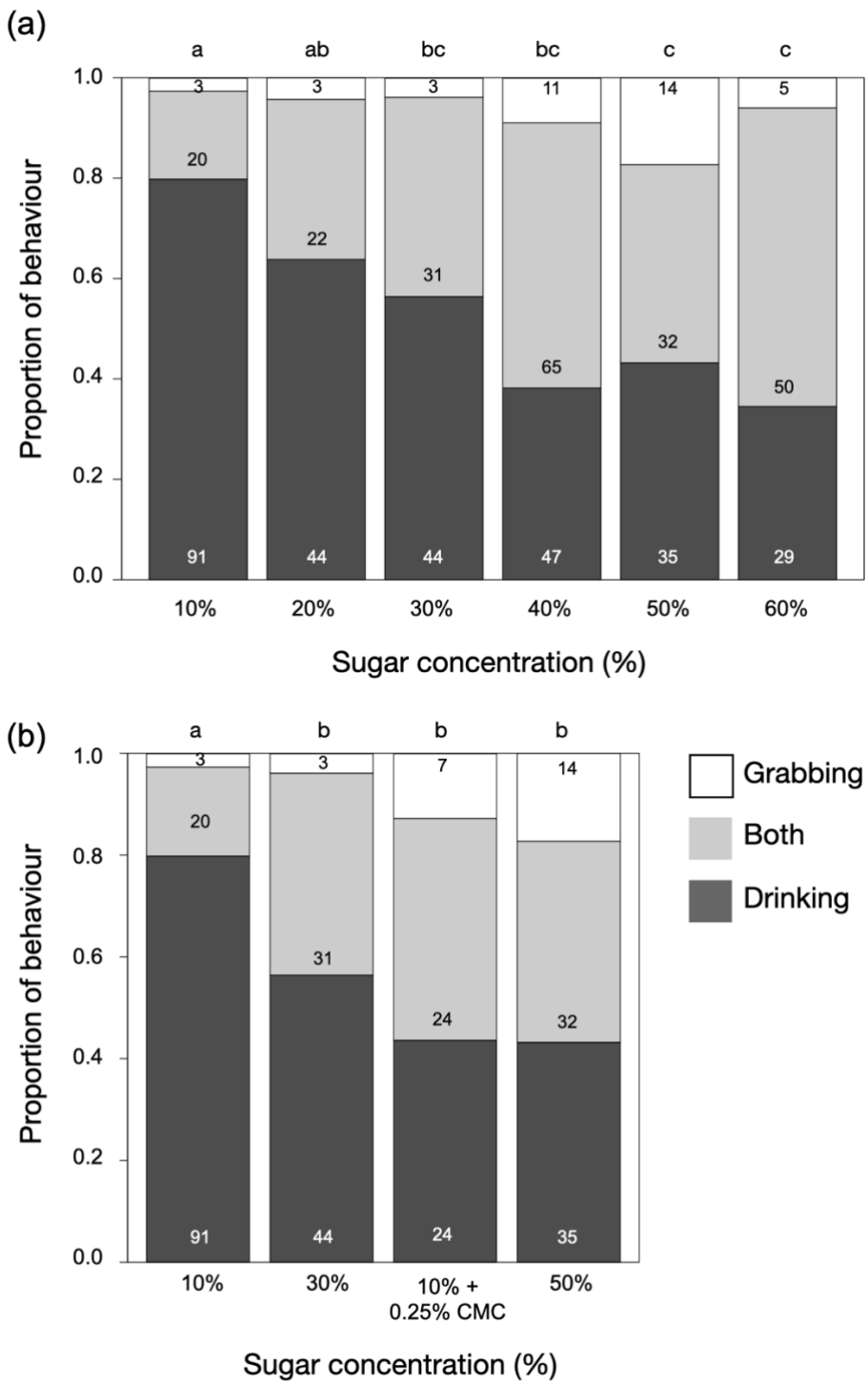

Figure 3. The proportion of pseudotrophallaxis depends on sugar concentration (a) and viscosity (b).

Grabbing (white), both (grey) and drinking (black) indicate the behaviour of only mandibular grabbing, mandibular grabbing after drinking, and only drinking. The x-axis is sorted by viscosity (b). Different letters on the top of the bar mean they were significantly different at $p < 0.05$ (chi-square test with Bonferroni correction).

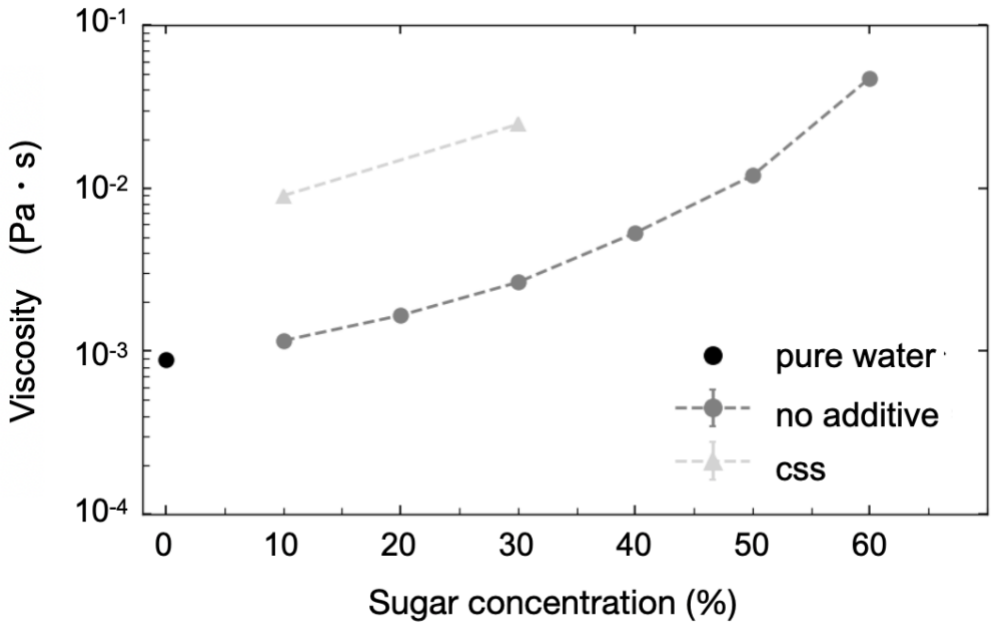

Figure 4. Dynamic viscosities at 25°C for different sugar water solutions as a function of the sugar concentration (w/w) and viscosity-altering materials.

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

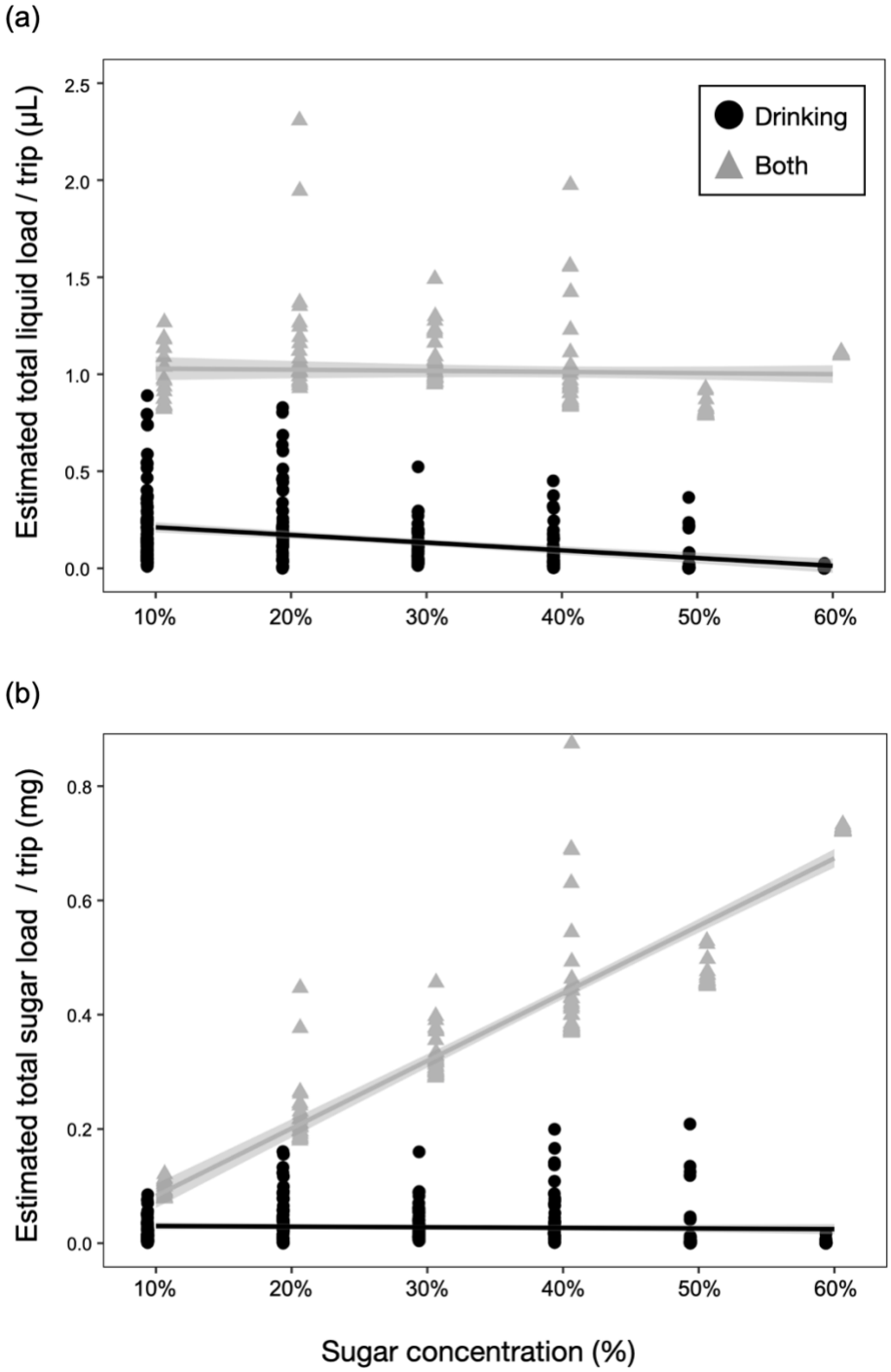

Figure 5. Foraging efficiency of different modes of liquid collection and transport. Estimated total liquid(a) and sugar load per trip (b) are estimated based on drinking time and foraging action. Dark grey, grey, and light grey indicate the action of drinking, both and grabbing. The shading is 95% confidence limits for the regression line. The volume of liquid by grabbing is the mean of carried volume by an ant, calculated in Figure 2a.

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547 **Supp. Table 1. Weight of each concentration of sugar water per 1 μ L.**

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

Sugar concentration % (w/w)	Weight (mg/μL)
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 ± 0.002
20	1.656 ± 0.002
30	2.641 ± 0.003
40	5.308 ± 0.005
50	11.919 ± 0.009
60	47.34 ± 0.004
10 CMC	8.97 ± 0.002
30 CMC	24.6 ± 0.2

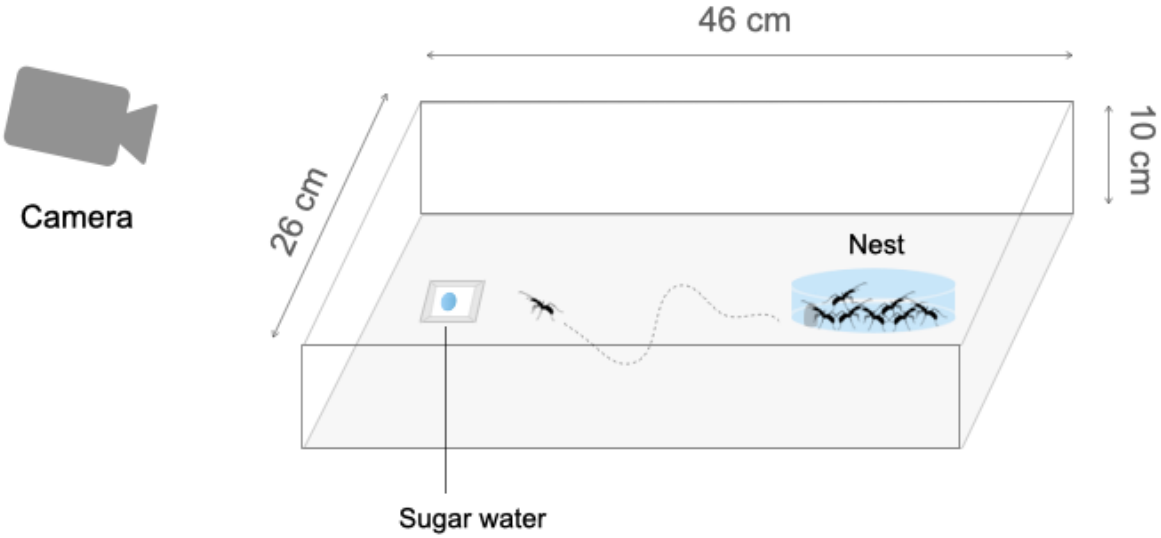
553

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

555 Intake rate is the slope (μL/sec) of the linear regression of crop load and corresponding feeding time.

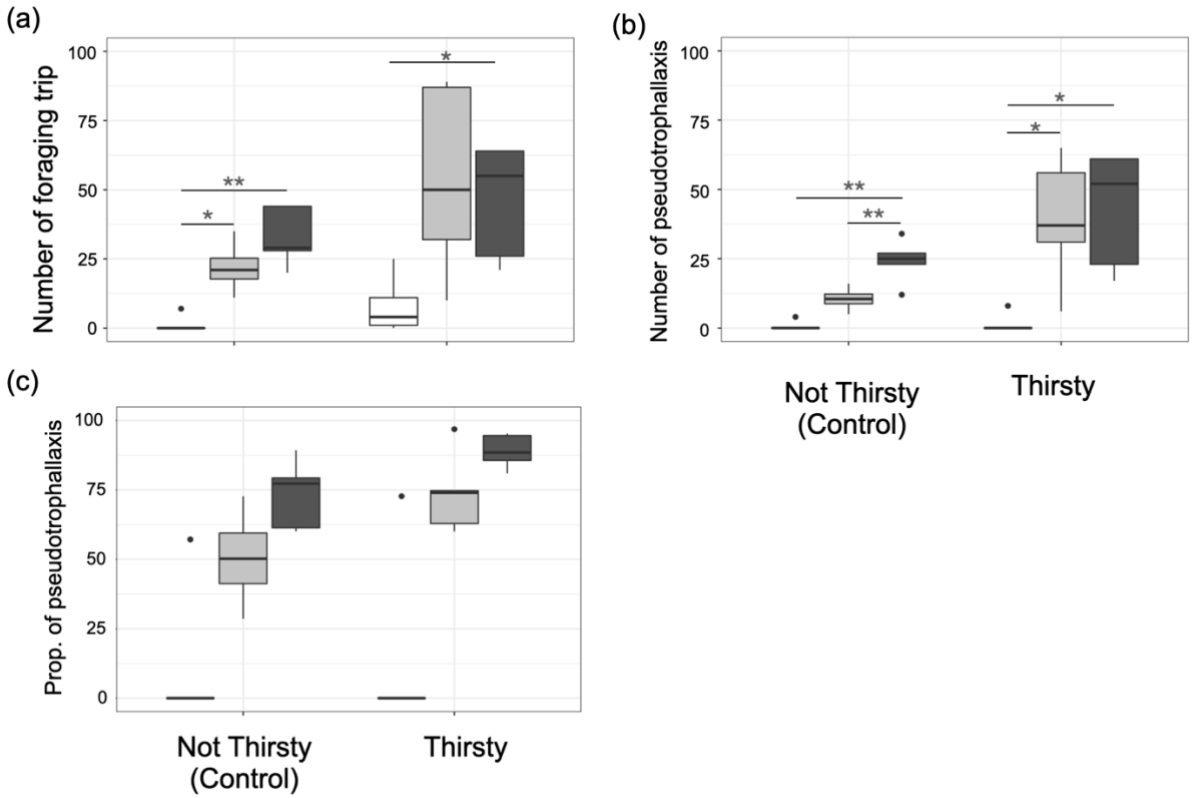
Sugar concentration % (w/w)	Intake rate (μL/sec)
10	0.00245
20	0.00355
30	0.00206
40	0.00136
50	0.00091
60	0.00027

556



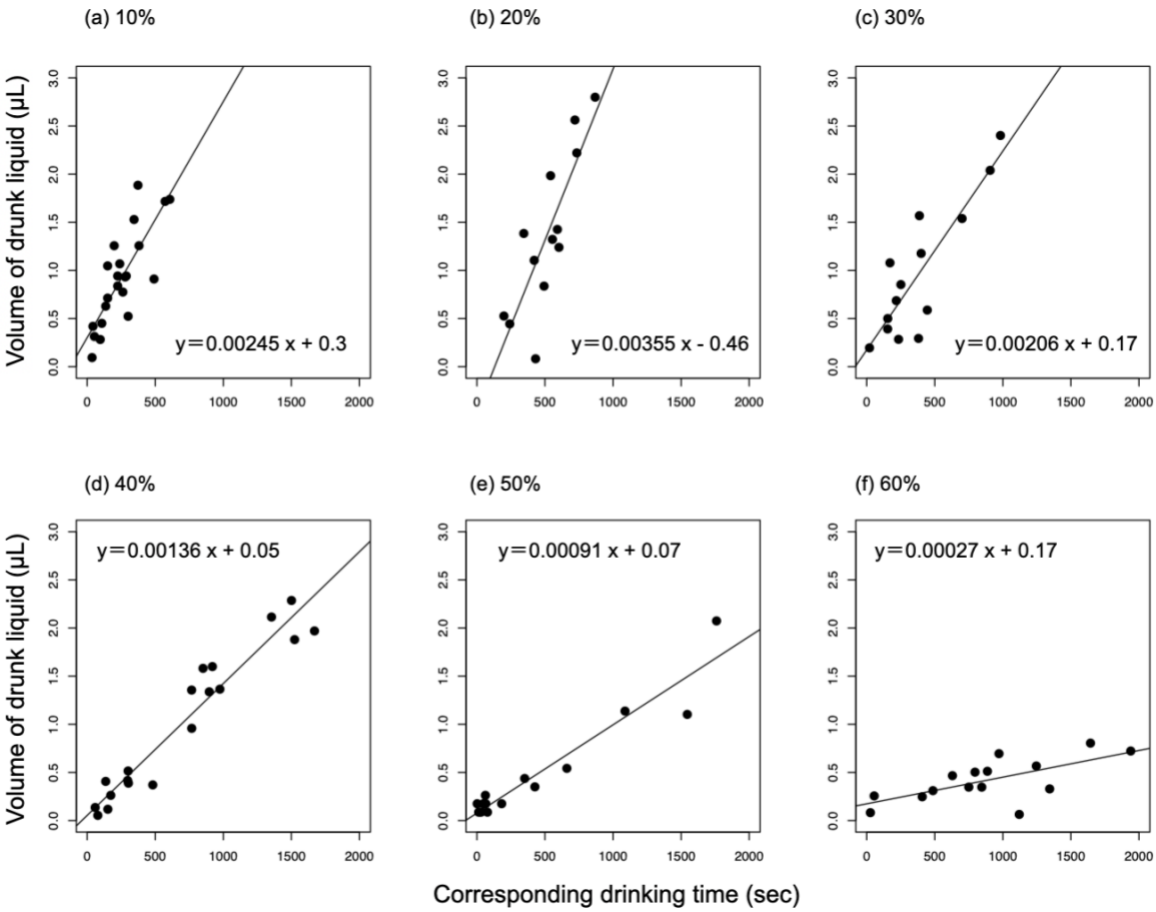
Supp. Figure 1. Experimental arena design.

An artificial nest (diameter = 9 cm) is placed on a container (48* 26* 10 cm). The nest was covered with a red film to darken inside the nest. The distance between the nest and food is about 30 cm. Fluon was applied to the wall of the foraging arena to prevent ants from escaping.



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

Colonies were starved for 3-4 hours before the experiment (thirsty condition). Ants were offered three different sugar concentrations: 0, 30, and 60 % w/w. (a) Total foraging bouts and (b) the number of pseudotrophallaxis that an ant grabbed a drop by mandibles and transported it were counted during 30-min observation. (c) The proportion of pseudotrophallaxis ants used was calculated for each colony. The color of white, grey, and dark grey represents 0, 30, and 60 % w/w sucrose concentrations. Asterisk indicates significant difference (Tukey–Kramer test, * $p < 0.05$, ** $p < 0.01$). Ants clearly preferred sugar water compared to water. The number of foraging trips was high in the thirsty condition.



Supp. Figure 3. Intake rate, plotting crop load (µL), and corresponding drinking time (sec). (a-f) Each column indicates different sugar concentrations from 10% to 60%. Linear regression models are shown inside the column.