

Feeding behavior and social interactions of the Argentine ant *Linepithema humile* change with sucrose concentration

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Abstract

Liquid sugar baits are well accepted by the Argentine ant *Linepithema humile* and are suitable for the chemical control of this invasive species. We evaluated how sugar concentrations affect the foraging behavior of *L. humile* individuals. We quantified feeding variables for individual foragers (ingested load, feeding time and solution intake rate) when feeding on sucrose solutions of different concentrations, as well as post-feeding interactions with nestmates. Solutions of intermediate sucrose concentrations (10–30%) were the most consumed and had the highest intake rates, whereas solutions of high sucrose concentrations (60 and 70%) resulted in extended feeding times, low intake rates and ants having smaller crop loads. In terms of post-feeding interactions, individuals fed solutions of intermediate sucrose concentrations (20%) had the highest probability of conducting trophallaxis and the smallest latency to drop exposure (i.e. lowest time delay). Trophallaxis duration increased with increasing sucrose concentrations. Behavioral motor displays, including contacts with head jerking and walking with a gaster waggle, were lowest for individuals that ingested the more dilute sucrose solution (5%). These behaviors have been previously suggested to act as a communication channel for the activation and/or recruitment of nestmates. We show here that sucrose concentration affects feeding dynamics and modulates decision making related to individual behavior and social interactions of foragers. Our results indicate that intermediate sucrose concentrations (ca. 20%), appear to be most appropriate for toxic baits because they promote rapid foraging cycles, a high crop load per individual, and a high degree of stimulation for recruitment.

Keywords: argentine ant, Trophallaxis, intake rate, feeding behavior, foraging, Nectivorous ants

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Introduction

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), is a global invasive species and is one of the most studied species in the world (Sanders & Suarez, 2011). Carbohydrates from hemipteran honeydew and extrafloral nectar are an important part of its diet and are a great driver

of its invasive abilities (Lach, 2003, 2005, 2007; Tillberg *et al.*, 2007). For many decades considerable attention has been devoted to research on nectar feeding behavior, the distribution of sugar solutions within the colony and recruitment triggered by sugar baits (Heller *et al.*, 2008; Silverman & Brightwell, 2008; Nyamukondiwa & Addison, 2014). Consequently liquid sugar baits have proven to be highly effective for control of *L. humile* (Boser *et al.*, 2014; Buczkowski *et al.*, 2014; Rust *et al.*, 2015).

In most eusocial insects, foraging tasks are performed by subsets of workers. Even in species with massive or group

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65 foraging, the coordination of such behavior is based on deci-
 66 sions made by each individual and on the communication be-
 67 tween nestmates. These decisions are affected by the
 68 nutritional requirements of the colony and the quality of the
 69 food source (Cassill & Tschinkel, 1999; Josens & Roces, 2000;
 70 Cassill, 2003; Detrain & Deneubourg, 2008; Dussutour &
 71 Simpson, 2008; Falibene & Josens, 2008). For nectivorous
 72 ants, sugar concentration affects feeding behavior; e.g. nectar
 73 acceptance, feeding time and volume ingested (Josens *et al.*,
 74 1998; Schilman & Roces, 2006; Dussutour & Simpson, 2008).
 75 Furthermore, ingestion dynamics (i.e. intake rate) is affected
 76 by the physical properties of the nectar, particularly nectar vis-
 77 cosity (Josens *et al.*, 1998; Paul & Roces, 2003), as well as by in-
 78 sect morphometry (Kingsolver & Daniel, 1979; Heyneman,
 79 1983; Roubik & Buchmann, 1984; Harder, 1986; Josens &
 80 Q3 Farina, 2001). When leaving the nectar source, foragers also
 81 decide whether to recruit other nestmates or not (De Biseau
 82 & Pasteels, 1994; Detrain & Deneubourg, 2008). One of the
 83 main recruitment mechanisms is by trophallaxis, whereby a
 84 donor ant regurgitates the ingested solution which is offered
 85 as a drop between her mandibles from where one or more
 86 nestmates (the receiver ants) can drink.

87 Trophallaxis and recruitment are considered to be the link
 88 between individual behavior and group organization (Wilson,
 89 1971; Breed *et al.*, 1996; Farina, 1996; Mailleux *et al.*, 2000;
 90 Gordon, 2007; Hölldobler & Wilson, 2008). This is because
 91 trophallaxis does not just serve as a means of food distribution;
 92 it also allows worker ants to acquire information, particularly
 93 for recruitment (McCabe *et al.*, 2006; Provecho & Josens, 2009).
 94 In addition, other communicational channels could be in-
 95 volved in recruitment depending on the species. For example,
 96 different motor displays were described for specific species as
 97 fast walking or head waggle, which are modulated by the
 98 scout based on food quality for *Solenopsis invicta* (Cassill,
 99 2003).

100 Although feeding behavior has already been studied in
 101 small colonies or ant groups in *L. humile* for different sucrose
 102 concentrations (Baker *et al.*, 1985; Silverman & Roulston, 2001;
 103 Silverman & Brightwell, 2008), there is no information avail-
 104 able on decision-making in individual workers for this species.
 105 Here we provide the first quantitative analysis of the how su-
 106 crose concentrations affect individual feeding dynamics, behav-
 107 ior, and post-feeding interactions among nestmates of
 108 *L. humile*.

111 Materials and methods

112 Laboratory conditions

114 Experiments were performed using four *L. humile* colonies
 115 that had been collected from their native range in Argentina at
 116 the Campus of the University of Buenos Aires (34°32'48.3"S;
 117 58°26'21.0'W). Each colony was estimated to contain about
 118 4000–5000 workers. The colonies were housed in our labora-
 119 tory for at least 2 months prior to conducting the experiments.

120 Colonies were kept in artificial nests that consisted of large
 121 plastic boxes (30 × 50 × 30 cm³) with the sides painted with
 122 fluon to prevent the ants from escaping. The colonies were
 123 maintained in a temperature-controlled environment (25 ± 3°C)
 124 under a natural light-dark cycle. Ants were fed daily with
 125 honey-water and three times a week with fresh cockroaches
 126 (*Blattica dubia*) or tinned meat. Water was provided *ad libitum*.
 127 For all experiments, the trials were performed over many days
 128 to achieve the required number of replication. Prior to

conducting trials the colonies were subjected to a carbohydrate
 starvation period of 72–96 h. This period of starvation main-
 tained a constantly high motivation for feeding during the
 trials. Colonies used in assays were given normal diets for at
 least 5 days before they were used again.

Individual feeding behavior

For each trial, one ant at a time was gently placed on a
 bridge (2 × 50 mm²) that ended in a feeding arena containing
 a drop (10 µl) of sucrose solution. This volume constituted an
ad libitum source for this species as their crop load is between
 0.1 and 0.5 µl (preliminary estimations). Ants were offered one
 of seven different sucrose concentrations: 5, 10, 20, 30, 40, 60
 and 70% w/w. To avoid bias, we haphazardly chose the
 order of sucrose solutions used, and individual ants were
 only used once. We measured individual crop load volume
 by filming the ants from a lateral view whilst they were drink-
 ing using a camera-fitted stereomicroscope (Leica MZ8 – 25×
 magnification – with a Leica ICA camera). The amount of so-
 lution ingested was estimated as the difference in gaster vol-
 ume before and after feeding (Mailleux *et al.*, 2000) which
 was calculated from the maximal length and height of the gas-
 ter before and after feeding. The width of the abdomen could
 not be seen on these lateral images. In order to estimate the re-
 lationship between this axis and the height in the lateral view,
 we performed preliminary measurements on 40 ants fed in
 similar conditions but filmed laterally and from above. We
 found that the relationship between width:height was 1.0:1.1
 for both empty and filled gasters. We therefore approximated
 the abdomen to be an ellipsoid in order to calculate the volume
 of the gaster of each forager before (V_i , initial volume) and
 after (V_f , final volume) drinking. The volume of solution in-
 gested (µl) was calculated as the difference between initial
 and final volumes ($V = V_f - V_i$). Feeding time (s) was also ob-
 tained from the videos and was defined as the time during
 which the ant's mandibles were in contact with the solution.
 A total of 271 ants were recorded feeding on the sucrose solu-
 tions ($N_5 = 35$; $N_{10} = 48$; $N_{20} = 35$; $N_{30} = 55$; $N_{40} = 35$; $N_{60} = 35$,
 $N_{70} = 28$).

Of the 271 ants that ingested the sucrose solution, the feed-
 ing time was measured only on 164 individuals, which were
 video-recorded uninterruptedly during feeding. From these
 recordings we measured the total feeding time. We compared
 these values among sucrose concentrations ($N_5 = 20$, $N_{10} = 20$,
 $N_{20} = 20$, $N_{30} = 36$, $N_{40} = 20$, $N_{60} = 20$, $N_{70} = 28$). From the vo-
 lumes ingested and feeding durations we calculated intake
 rates (nl s⁻¹) for individual ants and compared values
 among concentrations.

Trophallaxis and other social interactions

This experiment aimed to compare the post-feeding inter-
 actions between a donor and a group of receivers for four dif-
 ferent sucrose concentrations: 5, 20, 40 and 60% w/w. For each
 trial, a group of ten ants was separated from one of the colonies
 and placed in a flask (diameter, 4.5 cm; height, 2 cm) that had
 plaster of paris on the floor and the inner sides coated with
 fluon. After an acclimation time of no <15 min, we removed
 one ant, the 'donor ant'. This ant was allowed to climb onto
 a feeding arena (similar to the one describe in the previous ex-
 periment) to feed on one of the four sucrose solutions. When
 the ant stopped feeding for 10 s, we put a toothpick close to the
 ant for it to climb onto and we returned this ant to the flask.

From this moment, the flask was filmed from above with a digital camera (Sony Handycam HDR-SR11) for 300 s. We chose this timeframe based on our observations in preliminary trials.

Once returned to the flask, donors immediately walked actively. Generally, after a short time, the donor ant would stop walking and expose a drop of regurgitated solution which other ants would ingest by trophallaxis.

The proportion of trophallaxis was calculated as the number of donor ants that offered their crop load within 300 s after being placed in the flask out of the total number of ants that accepted the solution offered ($N_5 = 40$; $N_{20} = 40$; $N_{40} = 31$; $N_{60} = 40$).

Drop exposure latency was the time that each donor ant took to offer a small drop of the ingested solution to the nestmates. This behavior invariably led to trophallaxis between the donor ant and the receiver ants. The proportion of receivers was the number of ants that performed trophallaxis with the donor out of the nine ants present in the flask during the recording time. Delivery duration was measured as the uninterrupted time that the donor spent with at least a receiver in a delivery event of trophallaxis regardless of how many receivers were involved. During the 300 s of recording only one delivery event took place per donor ant. This event could involve one or several receivers.

We characterized and quantified the donor behavior from the moment the ant was returned to the flask until 30 s or drop exposure, whichever occurred first. We calculated Walking Activity as the number of times the ant crossed a line in a square grid dividing the arena, either until drop exposure latency or until 30 s within the arena. The 4×4 grid used was drawn on transparent acetate and placed on the monitor while playing the video (the side of each square of the grid represented 1.1 cm on the arena). We distinguished three behaviors performed by the donor ant; two of them necessarily involved a partner: Head Contacts and Head Jerking, whereas the other was performed by the donor alone: Gaster Waggle. These behaviors could occur in the recording period even if food offering did not.

Head Contacts were counted when the donor ant touched another ant's head; these recordings included any kind of contact (i.e. mandibular, antennal, etc.). Head Jerking occurred when the donor ant performed vigorous longitudinal jerking movements during head contacts. Therefore, Head Jerking is a subset of head contacts. Gaster waggle was a side-to-side movement of the donor ant's gaster that was sometimes performed before trophallaxis while the ant walked.

We calculated the proportion of the donor ants that performed each of these three behaviors from the total donor ants. Head contact and Head jerking instances were counted as discrete events and then divided by the time of the active period (i.e. time the ant remained in motion) to present them as rates (number of events per time unit). Gaster waggle was presented as an index (gaster waggle index) which was the time during which the ant moved her gaster relative to the time of the active period.

Statistical analyses

Feeding variables were analyzed using one-way ANOVA or Kruskal–Wallis (K–W) tests when normality assumptions were not met. In cases of significant differences, *post hoc* Tukey's for pairwise multiple comparisons (ANOVA), or two-tailed *post hoc* comparisons of mean ranks (K–W), were

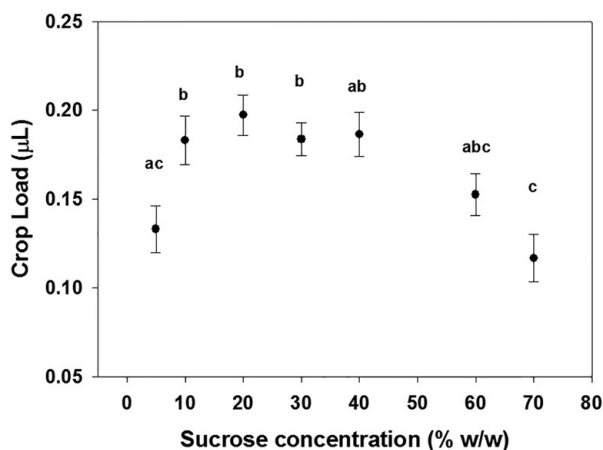


Fig. 1. Crop load (μL , mean \pm SE) as a function of sucrose concentration for foraging ants collecting at an *ad libitum* source. Points with different letters differ significantly (Tukey, $P < 0.05$). $N_{\text{total}} = 271$.

applied. Feeding time was fitted to a linear regression. Proportions for different solutions were compared using G-tests with corrected alpha when performing pairwise comparisons. ANOVA values were calculated for regression curves.

Results

Individual feeding behavior

Feeding behavior varied among the solution concentrations. Mean crop load had a unimodal relationship with sucrose concentration, being lowest (0.11 and 0.13 μL) at the highest and lowest sucrose concentrations respectively, and highest (0.20 μL) at 20% sucrose concentration, with this variation differing significantly (ANOVA: $F_{6,264} = 5.51$, $P < 0.0001$, fig. 1). The highest volume values recorded were about 0.39 μL , for 10 and 20% solutions, close to our maximum crop load observed for this species (data not shown).

Feeding time increased linearly and significantly with increasing sucrose concentration (regression ANOVA; $F_{1,162} = 94.31$, $P < 0.0001$, fig. 2a). Mean feeding time on the highest concentrate solution was 308 s (mean), with a maximum value of 934 s.

Feeding dynamics was also affected by the concentration of the solution ingested. Intake rate decreased with increasing sugar concentration with rates for concentrations of 40% or higher being significantly different than rates for concentrations of 30% or less (ANOVA: $F_{6,157} = 16.77$, $P < 0.0001$). The highest intake rate was 0.09 $\mu\text{L min}^{-1}$, being for 10% w/w, and the lowest rate was 0.029 $\mu\text{L min}^{-1}$, being for 70% w/w.

Trophallaxis and other social interactions

Of the 151 donor ants that ingested the solution only 85 offered a regurgitated drop. Of these 85, in 84 cases a receiver ant approached and drank from this drop (i.e. trophallaxis was established) within 300 s. Ants that had ingested intermediate sucrose concentrations of 20 and 40% were significantly more likely to perform trophallaxis (G test, $G = 12.84$; $P = 0.005$, fig. 3).

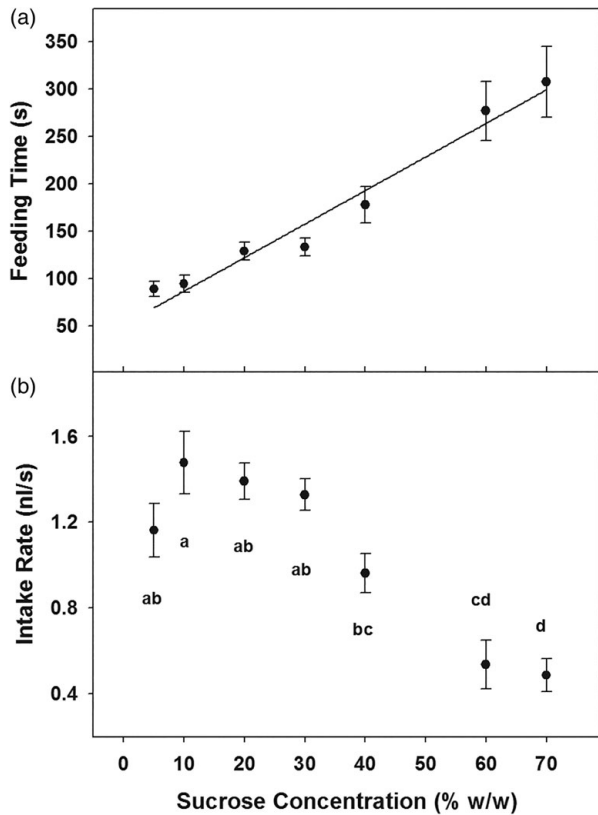


Fig. 2. (a) Feeding time (s, mean \pm SE) increased linearly with sucrose concentration ($y = 51.5 + 3.5x$) and (b) Intake rate (nl s^{-1} , mean \pm SE) decrease with sucrose concentration. Points with different letters differ significantly (Tukey, $P < 0.05$). $N_{\text{total}} = 164$.

Sucrose concentration also affected the drop exposure latency. Ants that had ingested 20% sucrose solution were the quickest to offer a drop and were significantly faster (mean 25.9 ± 3.2 s) than the slowest which were those that ingested 5% sucrose (mean 77.7 ± 12.0 s) (K–W: $H_{3,85} = 9.52$, $P = 0.023$, fig. 4a).

The proportion of receiver ants did not differ significantly with sucrose concentration (K–W: $H_{3,85} = 3.24$, $P = 0.355$, fig. 4b). Delivery duration increased with sucrose concentration, with that of the lowest concentration (5% sucrose, $81.5 \text{ s} \pm 5.9$) being significantly shorter than for the highest concentrations of 40 and 60% (125.7 ± 9.5 s and 153.5 ± 10.9 s, respectively) (K–W: $H_{3,85} = 15.58$, $P = 0.0014$, fig. 4c).

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Donor behavior prior to food offering

Walking activity did not differ with the sucrose concentration being for 10%: 1.04 ± 0.5 ; for 20%: 1.00 ± 0.4 ; for 40%: 1.03 ± 0.6 and for 60%: 1.22 ± 0.6 line crossings (ANOVA: $F_{3,120} = 0.98$, $P = 0.41$; for each concentration $N = 31$).

Head contacts, Head jerking (interactions between individuals) and gaster waggle (individual behavior) differed among the sucrose concentrations, predominantly with ants consuming the most dilute solution (5%) conducting the smallest proportion of behaviors (fig. 5) and also conducting significantly fewer movements than ants consuming all other sucrose concentrations (fig. 5) (proportion of head contacts: $G = 12.377$, $P = 0.0062$; head contact rate: $H_{3,118} = 7.997$,

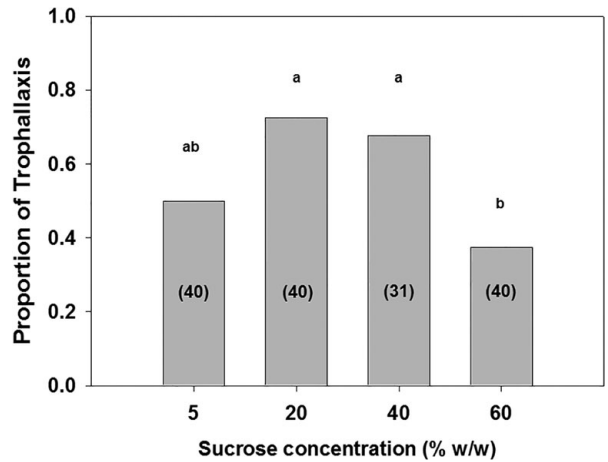


Fig. 3. Probability of trophallaxis after donor ants ingested sucrose solutions and returned to the recording arena. Different letters above bars indicate statistical differences (G -test pairwise comparisons with corrected alpha; $P = 0.005$). Brackets indicate the number of ants for each treatment.

$P = 0.0461$), (proportion of jerking: $G = 25.996$, $P < 0.0001$; jerking rate: $H_{3,90} = 11.394$, $P = 0.0098$), (proportion of gaster waggle: $G = 8.606$; $P = 0.0350$; gaster waggle index $F_{3,120} = 7.952$, $P < 0.0001$).

Discussion

Modulation of individual ingestion

Our study showed that sugar concentration can affect individual feeding behaviors as well as subsequent interactions between nestmates in the Argentine ant. Crop loads were largest for intermediate sucrose concentrations. Similar results have been observed in other ants (*Camponotus mus*: Josens *et al.*, 1998; and *Rhytidoponera metallica*: Dussutour & Simpson, 2008), in which dilute solutions also produced partial crop loads.

It has commonly been observed that nectivorous insects increment feeding time with increasing sucrose concentration (Josens & Farina, 1997; Josens *et al.*, 1998; Detrain & Prieur, 2014). This was also the case of *L. humile* in which feeding time increased linearly with increasing sucrose concentration. This linear pattern of increment differs from that observed in other ant species in which ingestion time increased exponentially (*C. mus*: Josens *et al.*, 1998; Falibene *et al.*, 2009; *Lasius niger*: Bonser *et al.*, 1998; Detrain & Prieur, 2014), coinciding with the increment of viscosity with concentration (Wolf *et al.*, 1984). In the case of the ant *Odontomachus chelifer*, longer ingestion times were reported for medium and low concentrations (5–30%) compared with those observed for high concentrations (50–60%) (Ávila Núñez *et al.*, 2011); this could have been a consequence of their feeding habits, because they are predominantly predators even though they can have an opportunistic diet (Raimundo *et al.*, 2009).

The intake rates also varied with sugar concentration. Theoretical models on feeding dynamics predict a decrement in intake rates with increasing sugar concentration due to the exponential increment in viscosity (Kingsolver & Daniel, 1979, 1995; Harder, 1986; Kim *et al.*, 2011). Our results are in agreement with this concept and also aligned with studies on

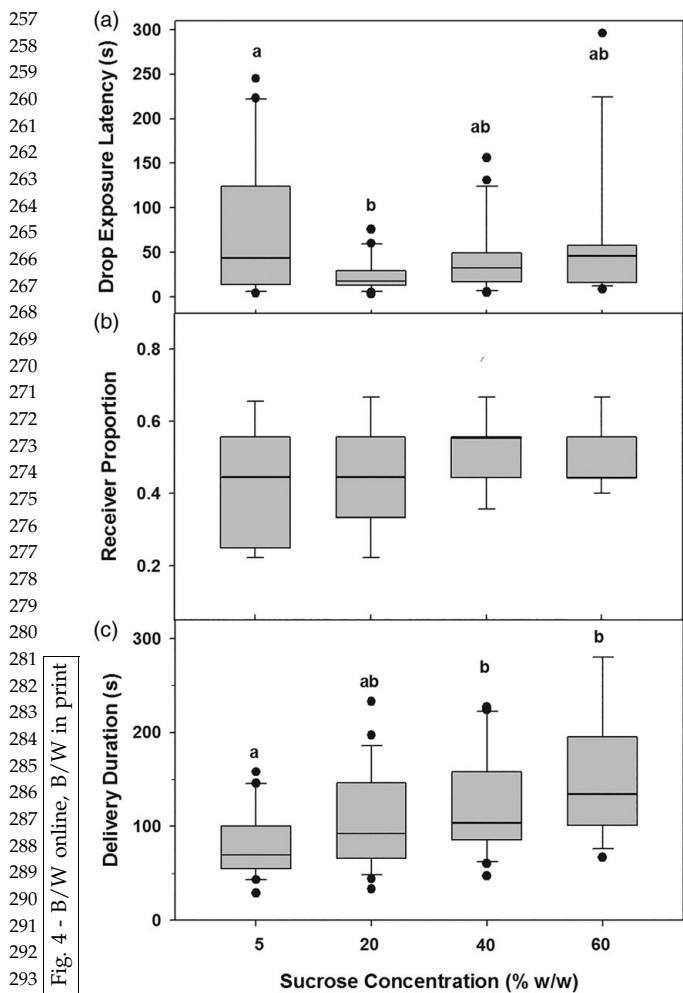


Fig. 4. (a) Drop exposure latency (s), (b) Receiver proportion and (c) Trophallaxis duration (s) as functions of sucrose concentration. Drop exposure latency showed a minimum for 20% sucrose, significantly less than for 5%. Receiver proportion did not vary with sucrose concentration. Trophallaxis duration increased with sucrose concentration, with significant differences between 5 and 40% and between 5 and 60%. N : 5% = 20, 20% = 29, 40% = 21, 60% = 15. Boxes show quartiles, horizontal lines within each box represent medians, whiskers provide the extreme values and dots indicate outliers. In all graphs, different letters indicate statistical differences (K-W pairwise comp., $P < 0.05$).

individual food intake in which other ant species ingested solutions at different rates according to sucrose concentration (Josens *et al.*, 1998; Paul & Roces, 2003; Ávila Núñez *et al.*, 2011).

Body size affects feeding performances; particularly, intake rate of sucrose solution increments with increasing ant size within a polymorphic species (*C. mus*: Josens, 2002; *C. rufipes* and *Atta sexdens*: Paul & Roces, 2003) as well as among species (Davidson *et al.*, 2004). Because of the small size of *L. humile* and its mechanism of ingestion by suction, it is expected that viscosity becomes a critical factor limiting intake rate for the more concentrated solutions (Kingsolver & Daniel, 1995; Kim *et al.*, 2011). Here, concentrations >30% w/w had significantly lower intake rates than 10%, with the highest values of intake rates being for 10 and 20%.

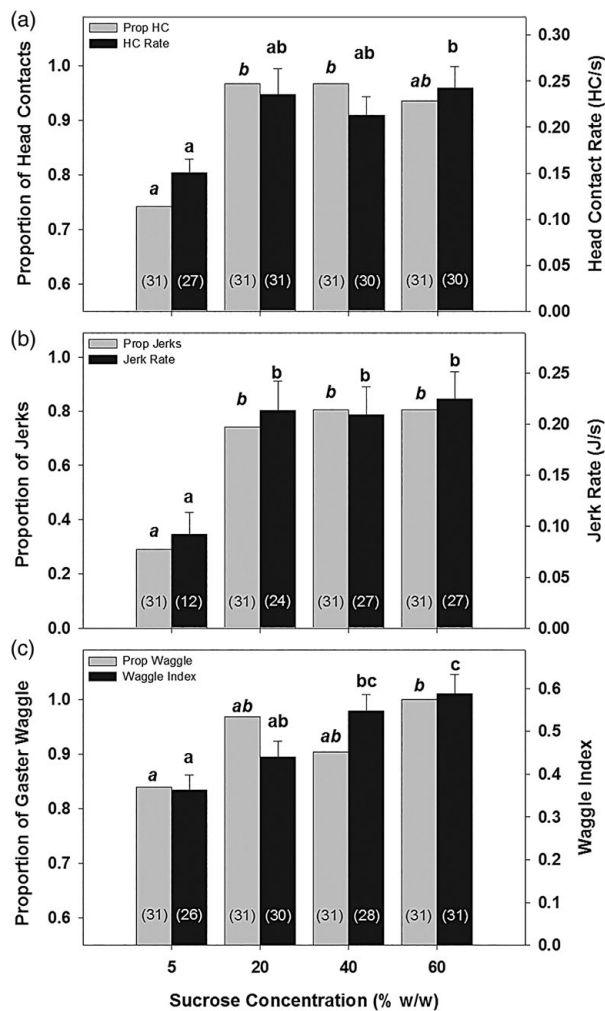


Fig. 5. Donor ant behavior. Gray columns represent the proportion of total donor ants which performed the behavior, black columns represent the rate at which the behavior occurred (i.e. number of events during latency time). The gaster waggle index takes into account the time the ant wagged her gaster over the total walking time. All behaviors were affected by sucrose concentration; in particular, low concentration showed lower rates and proportions. (a) Head contact proportion and rate, (b) jerking proportion and rate and (c) gaster waggle proportion and index. Different letters above bars indicate statistical differences (G-test pairwise comparisons with corrected alpha; $P < 0.05$). Brackets indicate the number of ants in each treatment.

Modulation of trophallaxis and other social interactions

Contact between individuals within a colony enables information transfer (Wilson, 1971; Farina, 1996; Hölldobler, 1999). Trophallactic contact in particular, allows foragers to empty their crop, distribute food, and exchange information about a particular food source (Farina & Grüter, 2009). Even brief contacts with the drop offered are enough for the receiver ant to associate an odor with the sucrose present in the nectar, as has been shown in bees (De Marco & Farina, 2001) and the ant *C. mus* (Provecho & Josens, 2009). Additionally, brief contacts with a donor ant can stimulate foraging; in harvester ants,

inactive foragers are activated to forage depending on the rate of interactions with successful foragers when they return to the nest (Gordon *et al.*, 2008, 2011). In a similar way, honeybee foragers are stimulated to leave the hive by contact with returning foragers (Farina, 1996).

Our experiments showed quantitatively for *L. humile* how the highest sucrose concentrations promote donor ants to perform a repertoire of motor displays and tactile interactions with nestmates. The proportion of donors that performed head contacts, head jerks and gaster waggles was lower for 5% w/w and those ants which did so, showed a lower rate. In the nest context, this would imply that a lower number of potential foragers would be stimulated when a scout returns from a low concentrate source rather than when she returns from a high concentrate source. In other species, high sucrose concentrations lead to an increase in the number of recruited ants (Detrain *et al.*, 1999; Detrain & Deneubourg, 2008). Therefore, our results might explain possible mechanisms that might be involved in triggering the recruitment of *L. humile* workers to a sucrose source.

Head jerking and contacts plateaued at 20%, both in their rate and proportion. That could reflect that these variables reach a saturation value at a sucrose concentration of 20% w/w. It is possible that these behaviors may depend on some threshold of resource assessment, above which are triggered by the donor ant. However, other conditions, such as a shorter starvation, a different season of the year, etc. may result in a shift of the curves and reach saturation values at a different concentration.

The modulation of behaviors related to recruitment and also to nestmates' response to these stimuli plays a key role not only in the selection of suitable food resources to forage, but also in the regulation of recruitment optimization according to colony's needs. Waggle motor displays and vibrations alert nestmates, which subsequently follow the recruiting leader ant to the food source in *Camponotus socius* and jerking movements are involved during recruitment to new nest sites (Hölldobler, 1971). Head jerking has been suggested to increase recruitment efficiency in other ant species (Sudd, 1957; Szlep & Jacobi, 1967; Szlep-Fessel, 1970; Möglich & Hölldobler, 1975; Van Vorhis Key & Baker, 1986; Hölldobler, 1999). In *L. niger*, the starvation level was found not to affect the trail-marking intensity (Mailleux *et al.*, 2006), but the recruits' response to the recruiter's signal was: if the starvation level increased, this induced more recruits and thus more workers foraging in starved colonies (Mailleux *et al.*, 2010).

Some studies on ant recruitment refer to an 'excited walk' exhibited by the recruiter, especially after ingestion of a rich source (Sudd, 1957; Szlep & Jacobi, 1967; Szlep-Fessel, 1970). In our recordings, we were able to observe that a greater proportion of donors that drank on a higher concentration also depicted a different, more 'agitated' walking style. Initially, we assumed that the difference was due to speed, but, at least in the conditions of the arena, we could not find any differences when comparing the walking activity among the groups. Nonetheless, after more detailed observation, we were able to characterize a new walking behavior, an intermittent waggle of the gaster from side to side. The gaster waggle occurred while the ants were walking, and its oscillation frequency proved to be very difficult to quantify as they were not filmed in high speed in order to be analyzed later in slow motion. In *C. socius*, the recruiting ant vibrates with the head and thorax between 6 and 12 strokes s^{-1} (Hölldobler, 1971). However, most studies referring to 'excited walks' were performed

through naked-eye observations (Sudd, 1957; Szlep & Jacobi, 1967; Szlep-Fessel, 1970); therefore, if any gaster waggle occurred, it could have not been detected as such. The donors that had ingested richer solutions exhibited this behavior more frequently; in the case of 60% w/w sucrose solutions, 100% of donors 'waggled'. The function of this behavior remains unclear, though it is possible that in the nest, where density of individuals may be very high, the probability of encounters between the donor and inactive foragers would increase with this waggle. In addition, it could also facilitate the propagation of pheromones being released by the donor, which are essential for recruitment in this ant (Van Vorhis Key & Baker, 1986).

Finally, trophallaxis was also influenced by sugar concentration. The probability of trophallaxis occurrence was minimal and the latency maximal for both 5 and 60% sucrose solutions, but the underlying mechanisms were probably different. Although low motivation for establishing contacts may explain the delay for donors that consumed 5% sucrose donors, the high motivation that triggered jerking and waggles before trophallaxis may explain the delay for 60% donors. In the nest context, donors that return from a rich nectar source would delay trophallaxis by performing contacts and motor displays to recruit inactivate foragers.

Liquid sugary baits laced with an active compound are generally preferred to solid or gel baits for controlling *L. humile* (Baker *et al.*, 1985; Klotz *et al.*, 1998, 2002; Rust *et al.*, 2000; Silverman & Roulston, 2001). Our results indicate that sugar solutions being used for baits should not be too dilute or too concentrated. On the one hand, dilute baits can generate low motivation, low rates of trophallaxes and reduce recruitment. On the other hand, too highly concentrated baits would lead to high time of feeding and trophallaxis, which would result in very long foraging cycles and partial crop loads. Thus, baits of intermediate sugar concentrations, ca. 20%, appear to be appropriate to add an active compound because they promote rapid foraging cycles, a high crop load per individual, and a high degree of stimulation for recruitment.

Because dilute baits require periodic cleaning to prevent sugar from fermenting and therefore need to be monitored and refilled frequently (Boser *et al.*, 2014; Buczkowski *et al.*, 2014), most commercially manufactured baits contain additives that increase viscosity or gelling improving bait stability, durability and ease of application. Our results put in evidence that one of the big challenges for Argentine ant control is the development of a device able to deliver dilute and stable baits.

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