Foraging in the ant *Camponotus mus*: nectar-intake rate and crop filling depend on colony starvation

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Abstract

The effects of colony starvation on the dynamics of nectar collection were studied in individual workers of the ant *Camponotus mus*. A laboratory colony was first deprived of carbohydrates for 15 days, and thereafter fed daily ad libitum with diluted honey until satiation. During these two successive experimental phases, the probability of feeding, crop filling and fluid-intake rates were recorded daily for individual foragers collecting a 10% (w/w) sucrose solution. The feeding responses of individuals varied with the nutritional state of the colony. When the colony was deprived of sugar, acceptance of the sucrose solution was higher than under satiation. Feeding time increased with increasing starvation. During deprivation workers fed nearly continuously on the solution, whereas a number of feeding interruptions occurred under satiation. Crop filling also increased with increasing starvation, and showed a marked decrease when the colony was satiated. Fluid-intake rate during the deprivation phase was roughly twice that during the satiation phase. This matched well with the difference in sucking frequency recorded during ingestion in satiated and starved workers, which was also higher during starvation. Results indicate that the responsiveness of foragers, determined by the nutritional state of the colony, influenced both foraging decisions and the dynamics of fluid intake. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Ants of the genus *Camponotus* feed on insects and carbohydrate solutions obtained from Homopteran honeydew and extrafloral nectaries (e.g., Levieux and Louis, 1975). Even though these sources are composed of sugars (principally sucrose, glucose and fructose), amino acids, lipids and secondary compounds (Baker and Baker, 1982; Bentley and Elias, 1983), sugar concentration is one of the main variables affecting the individual foraging behavior of ants. In particular, the fluid-intake rate — i.e., the gross rate of energy gain — is largely determined by the sugar concentration of the fluid being ingested, and by its effects on feeding dynamics (Josens et al., 1998).

Several theoretical models based on the Poiseuille equation have been developed to account for the dynamics of ingestion in fluid-feeding animals (Kingsolver and Daniel, 1979, 1983; Heyneman, 1983; Harder, 1983, 1986). Such models have taken into account the effects of the mechanical properties of the fluid (e.g., its density, viscosity, surface tension, etc.), the morphometry of the buccal apparatus, and the pressure differences exerted by the feeding system during intake. Regarding the feeding mechanics, Kingsolver and Daniel (1979, 1983) have distinguished two principal modes of fluid intake in insects: by suction as in butterflies, and by licking as in bees (and hummingbirds). While butterflies are assumed to feed continuously, bees extend and retract the glossa into the fluid, so that it adheres to the surface and subsequently reaches the functional mouth. In both cases, insects need to generate a negative pressure in the alimentary canal in order to transport the liquid into the cibarium. Such a difference is generated by the rhythmic contraction of transverse muscles that are inserted between the inner (buccal) and the outer (cranial) walls.
of the clypeus of the head (Snodgrass, 1984). The cibarium, therefore, can be compressed and dilated, constituting the so-called cibarial pump responsible for the ingestion of liquids.

Because of the movement of fluid occurring during feeding, fluid viscosity, which increases exponentially with increasing concentration of dissolved sugars, dramatically affects the intake rates achieved by nectarivorous insects. A high viscosity leads to a marked decrease in fluid-intake rate. As a consequence, the intake rate, expressed in volume per unit time, remains high and more or less invariant for low sugar concentrations but only up to a given critical value. Beyond this concentration, which depends on both the feeding mechanics and the species-specific morphometry of the buccal apparatus, fluid-intake rate decreases with increasing sugar concentration (honeybees: Núñez, 1966; Roubik and Buchmann, 1984; bumblebees: Harder, 1986; butterflies: May, 1985; nectar-feeding bats: Roces et al., 1993).

In the ant Camponotus mus, however, a different dependence between fluid-intake rate and sucrose concentration has recently been found (Josens et al., 1998). The difference with the pattern described above occurred in the range of lower sucrose concentrations [from 5% to 30% weight/weight (w/w)]. After two days of sugar deprivation, fluid-intake rate was observed to be low for very dilute concentrations (5% w/w), and increased with increasing sucrose concentration up to a maximum at 31% w/w. Beyond this value, fluid-intake rate began to decrease for higher concentrations, as should be expected due to the negative effects of viscosity. To account for the low intake rates observed for dilute solutions, which cannot be explained by viscosity effects, Josens et al. (1998) postulated a new hypothesis suggesting that the low chemosensory stimulation provided by a dilute sucrose solution would negatively influence the worker’s responsiveness. This would affect not only the fluid-intake rate of the foraging ant, but also the decision when to give up the source and return to the nest; i.e., the final crop load. In fact, workers feeding on dilute solutions were observed not only to collect them at lower rates, but also return to the nest with partial crop-filling (Josens et al., 1998). Returning to the nest with partial crop loads has been recorded widely in social insects (Núñez 1966, 1982; Varjú and Núñez, 1991; Farina, 1996).

In that sense the feeding process in Camponotus ants appears to be influenced by a motivational system as defined by McFarland (1971) — i.e., “a system that controls a group of functionally related activities”, the variables of such a “feeding system” relate to behavior as a function of time. A typical example of a motivational drive is “hunger”, in which the behavioral responses depend on both the internal state of the animal and on the external stimulus that, in turn, affects the motivational state. Since the term “motivation” is controversial and it is used in different contexts, we shall use it here to refer to quantitative changes in the behavioral responses. Thus, as McFarland (1971) defined, “the appropriate experimental conditions for studying motivational systems correspond to a familiar and unchanging external environment in which the animal is presented with an unchanging stimulus”.

The aim of this work was to address the effects of food deprivation on the dynamics of fluid intake by the ant Camponotus mus. Instead of changing the stimuli presented to the ants as in the study mentioned above (Josens et al., 1998), the feeding motivation was changed by modifying the nutritional conditions of the colony. To that aim, a laboratory colony was deprived of sugar solution for 15 days, and then fed ad libitum. During both deprivation of sugar and satiation, the feeding responses of workers presented with a 10% (w/w) sucrose solution as standardized food source were quantified. If the individual feeding responses to the same stimulus depend on colony starvation, it can be concluded that changes in motivational thresholds are involved (McFarland, 1971).

2. Materials and methods

A colony of C. mus Roger composed of one queen, approximately 500 workers and brood was used during this study. The nest was collected near Buenos Aires, Argentina, where this species is very common (Kusnezov, 1963). The colony was kept in the laboratory inside glass boxes at a temperature of 27°C by means of electric heaters, and under the natural light/dark cycle (12 h light/12 h dark, approximately). During the assays, ants foraging on the experimental arena experienced a mean room temperature of approximately 20°C. The nest boxes were located on a tray where workers could move freely, having access to an ad libitum water source.

Individual foraging behavior was analyzed throughout two different experimental phases. In the first phase, the colony was deprived of carbohydrates for 15 days (sugar-deprivation phase). Afterwards, it was provided daily with honey water ad libitum until satiation (satiation phase). During these two phases, the feeding behavior of workers collecting 10% sucrose solution was compared. The colony was given insect parts as protein food throughout these two phases.

Each day single ants were allowed access to a small foraging arena (5 cm x 4 cm) via a wooden bridge connected to the nest. A large droplet of 10% w/w sucrose solution, which represented an ad libitum source for a single foraging worker, was offered on the plastic floor of the arena. Single foraging workers running towards the arena were captured gently with a small cap on the bridge and weighed to the nearest 0.01 mg (initial body weight=W_i). After being weighed, the ant was placed
again on the bridge, where it resumed its trip to the feeding place. The weighing procedure took approximately 1 min. Once at the source, ants that discovered the drop could initiate drinking or not. The acceptance index was defined as the ratio between the number of ants that fed on the drop of sucrose solution and the total number of ants that tasted the drop; i.e., the ants that fed and those that discovered the droplet but did not feed.

Visit time (s) was recorded as the interval from the first contact of a forager with the droplet to the time at which its mouthparts definitely left the fluid, it finished feeding and then left the arena, with the crop not necessarily replete. During the assays, workers usually performed a number of brief feeding interruptions, sometimes moving back and forth around the solution droplet. This allowed us to define the feeding time (s), which represents the time actually spent in contact with the droplet of sucrose solution. When no feeding interruptions occurred, visit and feeding times were identical. A feeding index was calculated by dividing the feeding time by the visit time; this index varied between 0 and 1.

When the forager stopped feeding, it ran back to the bridge in order to reach the nest. At this point the ant was caged again and weighed (final body weight = Wf). The weight of the load (mg) was calculated as the difference between Wf and W0. The crop load carried (µl) was obtained by dividing the weight load by the density of the 10% w/w sucrose solution, which was obtained from tables (Wolf et al., 1984). Independent data from different workers were obtained daily throughout both experimental phases (between nine and 15 workers per day). Ants captured and weighed on their way back to the nest were not allowed to return to the nest.

In order to quantify fluid-intake rates, expressed in µl/min, the absolute crop loads (µl) and the corresponding feeding times (expressed in min for this calculation) measured for different workers during a day were plotted. Fluid-intake rate was calculated as the slope of the linear regression between these measurements for each day.

The movements of the feeding apparatus in workers collecting the dilute sucrose solution were recorded by means of a video camera, under both deprivation and satiation. This analysis was done in order to provide information about the mechanics of fluid intake and their possible modulation through the nutritional state of the colony. Recordings focused on the rhythmical movements of the labial prementum, which result from the alternate compression and extension of the preoral cavity occurring during fluid sucking. This was considered a reliable measure of the activity of the cibarial pump, and similar approaches have been used to measure this parameter in different insect species (e.g., Bennet-Clark, 1963). Video recordings were analyzed afterwards in slow motion, and the frequency of the rhythmical movements was quantified. Two examples were digitized frame by frame (25 frames/s).

3. Results

The feeding behavior of individual ants depended on the nutritional state of the colony, which affected feeding motivation. Fig. 1 shows that the acceptance index, which represents the probability of ingesting the 10% (w/w) sucrose solution, increased over the initial days of the sugar-deprivation phase (white area). From the ninth day onwards, the acceptance of that solution reached its maximum (1), and remained constant until the end of the deprivation phase. The beginning of the satiation phase led first to a slight decrease, and after two days to a marked decrease in the acceptance of the dilute solution, which reached a level of ca. 0.6.

The feeding time (FT) increased during the last days (D) of the sugar-deprivation phase (Fig. 2, white area; analysis of variance (ANOVA) of regression: \( FT = 35.5 + 0.8D; F_{1:164}(1) = 12.33, P < 0.001 \)). In the satiation phase (gray area) there was a high variability in the measurements, so that the mean feeding time was similar in both experimental phases [test between phases: \( t_{(201)} = 0.8 \), not significant (NS)].

The feeding index (FI) represents the time actually spent in contact with the droplet of sugar solution, relative to the total time at the food source. A value of 1
[FT=visit time (VT)], as a consequence, indicates that the ant was extremely motivated to collect the fluid. The index varied significantly among phases (t-test between phases: \( t_{201} = 8.31, P < 0.001 \)). Fig. 3 shows that in the sugar-deprivation phase (white area) it reached values around 1, indicating that workers spent practically all the visit time drinking, and that no or only very brief feeding interruptions occurred when the colony was deprived. The slight variations around this value were not significant for the days of this phase (ANOVA of regression:
\[ F_{(1;164)} = 0.54, \text{NS} \]. In the satiation phase, the feeding index decreased significantly to values around 0.4 (gray area: ANOVA of regression, \( FT = 2.96 - 0.145D, F_{(1;35)} = 13.52, P = 0.001 \)). This was not caused by differences in the actual feeding time, but by differences in both the frequency and duration of feeding interruptions (t-test between phases: \( t_{124} = 5.8; P < 0.001 \), which led to extended visit times during satiation.

Between the seventh and 10th days of the sugar-deprivation phase, a small decrease in the feeding index and a concomitant increase in the variability (SE) were observed (Fig. 3). This resulted from feeding interruptions longer than 20 s observed in some individuals, between 10 and 25% of the recorded ants. From this time onwards, roughly no ant performed feeding interruptions during the deprivation phase. In the satiation phase, the daily percentage of individuals performing interruptions equal or longer than 20 s increased from 20% at day 17 to ca. 70% at the subsequent days.

Not only the duration and frequency of feeding interruptions varied with the nutritional state of the colony, but also the behaviors displayed during these interruptions. As indicated, only a few individuals interrupted their feeding activity during the deprivation phase. In this case, workers were observed to perform conspicuous scent-marking behaviors around the food source. This could be easily recognized as the ants constantly walked around the droplet of sugar solution pressing the tip of their abdomen against the substrate, indicating that the source was profitable enough and was worth communicating to other nest mates. During the satiation phase, although the proportion of individuals performing interruptions was higher, no ant was engaged in scent marking. They mostly remained motionless near the food source during the feeding interruptions, indicating that when the colony received honey water supply, the food source did not constitute a particularly attractive resource.

There was a considerable variation in the size of Camponotus mus ants. Although foragers collecting 10% sucrose solution were of similar size, in order to allow comparisons, crop load was normalized to the body weight of the foragers involved. The relative crop load was calculated by dividing the collected volume by the initial body weight of the ant. This index assumes that the collected volume varies uniformly with ant body size, which was shown to be valid by Josens et al. (1998).

Fig. 4 shows the variation of the relative crop load (RCL, \( \mu l/mg \) body weight) for the experimental phases. During the sugar-deprivation phase (white area), the RCL increased with the deprivation time \( (D) \) (ANOVA of regression: \( RCL = 0.275 + 0.009D; F_{(1;164)} = 14.74, P < 0.001 \)). In the satiation phase (gray area), in contrast, the relative crop load decreased with time (ANOVA of regression: \( RCL = 1.214 - 0.057D; F_{(1;35)} = 4.29, P < 0.05 \)).
The intake rate (IR) — i.e., the volume of solution ingested per unit time (μl/min) — also differed between the deprivation and the satiation phases (Fig. 5, t-test between phases: \( t_{124} = 4.67, P < 0.001 \)). While IR did not change with the days in the deprivation phase (ANOVA of regression: \( F_{(1,164)} = 0.03 \), NS), it showed a marked decrease after the second day in the satiation phase, reaching a minimum of ca. 1 μl/min on day 18.

Video recordings of ants ingesting 10% sucrose solution showed two different feeding mechanisms: lapping and suction, which depended on the amount of fluid available. Ants drinking from a very small volume of solution, or from a liquid film extended on a solid surface, lap it with the glossa conspicuously extended, with movements of the head and of retraction of the glossa, similar to the licking behavior described for bumble bees, honey bees and flies. When drinking from large volumes of solution, workers contact the solution with their mandibles and simply suck the liquid by means of their cibarial pump, without moving their glossa. The same two feeding behaviors have been observed in honey bees under similar situations (Núñez, personal communication). Since ant workers were presented with a large droplet as in our previous study (Josens et al., 1998), licking was not observed. Thus, the rhythmical movements of the labial prementum were recorded and analyzed, which may indeed reflect the activity of the cibarial pump. Our video sequences were chosen such that conspicuous movements of the palps during sucking were explicitly avoided, as they may affect spuriously estimation of the pumping activity.

Workers were observed to modify the pumping frequency of their cibarium depending on the nutritional state of the colony; i.e., on their own feeding motivation. The mean frequency of sucking movements (peaks/s), obtained by counting 20 consecutive movements in three deprived workers, averaged 8 Hz (\( n = 3; \text{SE} = 0.4 \)) and was approximately twice that of satiated workers, which averaged 4 Hz (\( n = 3; \text{SE} = 0.8 \)). These movements induced rhythmical deformations of the illuminated surface of the drop that were sometimes easier to digitize. Two representative examples of such records in deprived and satiated workers are presented in Fig. 6.

4. Discussion

It has been demonstrated that the foraging responses of individual Camponotus mus ants confronted with a standardized stimulus (10% sugar solution) depended on the nutritional state of the colony and, therefore, on their own feeding motivation. When changing the nutritional conditions of the colony from starvation to satiation, the change in the individual responses was not immediate. A delay of one day was observed before the animals reacted to the new experimental situation. On day 16 the colony was provided with honey water for the first time. Recruitment to this food source was massive during the first two hours after food supply but was reduced drastically during the third hour. Although a high incorporation of sugar was then attained, changes in responsiveness with respect to the deprivation phase were recorded only from day 18 onwards.

Sugar deprivation resulted not only in an increase of responsiveness, but also in the collection of larger loads,
Fig. 6. Activity of the cibarial pump during ingestion, recorded as rhythmical movements of light reflected on the drop, is shown as a function of time, over 5 s. Examples obtained in a satiated (A) and a deprived (B) worker. Within each 1 s period, slight displacements along the ordinates correspond to changes in the position of the ant head relative to the fluid drop.

a result previously shown in Formica aquilonia ants (Cosens and Toussaint, 1986). Since the set point of crop filling varied with the nutritional conditions, it can be argued that crop filling is also influenced by the feeding motivation, as suggested by Núñez (1973, 1987) and analyzed extensively in foraging honeybees (Núñez, 1982; Varjú and Núñez, 1991).

Sugar deprivation also led to high fluid-intake rates, which were independent of deprivation time. Since deprivation was maintained over 15 days, it may a priori be argued that a progressive increase in the intake rate should be expected. This was not the case, probably reflecting the fact that the first measurements were actually performed at the third day of deprivation, and the previous feeding conditions of the colony were not standardized.

The most striking result of our study was the observation of differences in the fluid-intake rates as a function of colony conditions, irrespective of the physical properties of the ingested solution. Until now, fluid-intake rate had always been studied as depending on the physical properties of the fluid being ingested (e.g., viscosity or sugar concentration), or on the morphometry of the feeding apparatus involved. The present study shows that, in addition to these factors (see also Josens et al., 1998), the feeding motivation of the individual ant needs to be considered. What are the mechanisms responsible for the observed modulation in the fluid-intake rates? Three potential explanations, not mutually exclusive, can a priori be considered: (1) the frequency of cibarial pumping may be altered; (2) the volume ingested per pumping cycle may be changed; and (3) drinking pauses in which the mouth parts of the animal remain in contact with the fluid may be introduced, thus leading to an erroneous calculation of the actual feeding times. This was not observed in our recordings.

Our records of feeding mechanics indicate that the first explanation is the most likely. The frequency of rhythmical movements of the labium did vary with foraging motivation and, during the deprivation phase, it was twice that observed in the satiation phase. A similar change in the fluid-intake rates was recorded as a function of colony nutrition (during deprivation roughly twice as much as during satiation, Fig. 5), indicating that the activity of the sucking pump was the variable under motivational control which led to different intake rates. Potential changes in the volume ingested per sucking cycle cannot be completely excluded; yet, if such changes occur, any contribution to the modulation of the rate of fluid intake appears to be minimal as judged by our measurements of pumping frequency. In fact, using the recorded frequencies of sucking movements for deprived and satiated workers (8 and 4 Hz, respectively) and the measured fluid-intake rates (2.4 and 1.2 ml/min, see Fig. 5), the fluid volume ingested per sucking movement averaged 5 nl under both conditions.

The frequencies of rhythmical movements of the labial prementum measured in our study further support the idea that this parameter was a proper indicator of the actual pumping activity of the cibarium. They varied between a minimum of 3.5 Hz and a maximum of 9 Hz. This range is coincident with those of pumping activity known for different insect species. In the bug Rhodnius prolixus, the pumping frequency (pulsation of the pharyngeal pump), measured through quantification of the movements of the top of the head, reached 3 Hz (Bennet-Clark, 1963). Similarly, Smith (1979) studied the action of the pharyngeal pump in the same insect using an electrical resistance technique developed by Smith and Fri-end (1970). By decreasing the viscosity of the blood being ingested, he recorded values between 3.8 and 7.3 strokes/s. In bumble bees, the lapping rate was observed to remain constant at ca. 4.8 laps/s for a broad range of concentrations of sucrose solution (from 20 to 70% w/w) (Harder, 1986). Although the lapping rate may not reflect the activity of the sucking pump, a
hypothesis worth testing is that both frequencies could be coupled.

Different hypotheses have been proposed to explain how the activity of the pharyngeal pump can be controlled: a control through peripheral feedback (Rice, 1970), e.g., through stretch receptors located adjacent to the mouth parts; a direct control through a central oscillator (as in many rhythmic patterns of behavior in insects, Wilson, 1966); or a combination of both possibilities (Smith, 1979). In the latter two models, the feeding response was assumed to be an all-or-none process, and no modulation of the pumping frequency could occur. An alternative hypothesis was advanced by Núñez (1973, 1987) who stated that the feeding process is controlled by a “motivational feeding system”, so that modulation during feeding would be possible. Our results are consistent with this idea, since the frequency of movements of the labial prementum varied with the feeding motivation.

Social insect foraging behavior has been discussed as resulting from a trade-off between maximizing individual foraging efficiency and maximizing social information exchange with nest mates (for honey bees: Núñez, 1982; Núñez and Giurfa, 1996; for leaf-cutting ants: Roces and Núñez, 1993). Food searching by scout workers constitutes an individual activity, whilst exploitation of the discovered food sources is a collective behavior mediated through communication signals (in Camponotus ants: Hölldobler, 1971; Traniello, 1977; reviewed by Hölldobler and Wilson, 1990). In this sense, individual foraging behavior is affected by colony needs which regulate, via negative feedback, food-source exploitation. For C. mus workers, it was demonstrated that not only the dynamics of fluid intake but also their foraging decisions about the load size are directly modulated by the nutritional state of the colony. However, what kind of feedback mechanisms are actually acting to regulate foraging patterns at the colony level, remain to be investigated.

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