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To drink or grasp? How bullet ants (*Paraponera clavata*) differentiate between sugars and proteins in liquids

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Abstract Flexibility in behavior can increase the likelihood that a forager may respond optimally in a fluctuating environment. Nevertheless, physiological or neuronal constraints may result in suboptimal responses to stimuli. We observed foraging workers of the giant tropical ant (also referred to as the "bullet ant"), *Paraponera clavata*, as they reacted to liquid solutions with varying concentrations of sugar and protein. We show that when protein/sucrose concentration is high, many bullet ants will often try to grasp at the droplet, rather than gather it by drinking. Because *P. clavata* actively hunt for prey, fixed action patterns and rapid responses to protein may be adaptively important, regardless of the medium in which it is presented. We conclude that, in *P. clavata*, food-handling decisions are made in response to the nutrient content of the food rather than the texture of the food. Further, we suggest that colonies that maintain a mixture of individuals with consistent fixed or flexible behavioral responses to food-handling decisions may be better adapted to fluctuating environmental conditions, and we propose future studies that could address this.

Keywords Bullet ants · Central place foraging · Protein concentration · Sugar concentration

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Introduction

Plasticity in behavior can lead to a greater likelihood of responding optimally in a given situation. For example, optimal foraging theories have been developed to predict how an individual will adjust its foraging decisions, given the handling or search time to find the food source (Stephens and Krebs 1986). Still, physiological or neuronal mechanisms may constrain an individual's response, resulting in fixed behaviors that are functional in typical conditions but may not be functional in uncommon or novel circumstances. Further, the ability of an individual forager to collect food may be constrained by the physical properties of the food or ambient conditions. These constraints may overshadow the positive effects of compensatory behaviors demonstrated by individuals (Jandt et al. 2010).

Most social insect foragers carry both liquid and solid food sources back to the nest and share them with the colony. In most lineages, liquids are transported internally within the crop, and solids are transported externally, either on the legs or held in the mandibles. Liquid food sources for social insects tend to be richer in carbohydrates, whereas solids are generally higher in protein. By ingesting carbohydrates before returning to the nest, the quality (i.e., sugar concentration) might be easier to determine while still at the food source (Seeley 1995). Protein quality, however, may not be as easily determined at the food source (Pernal and Currie 2002). Instead, in honeybees for example, only after the pollen has been stored and assessed in the nest will foragers adjust their protein foraging behavior (Pernal and Currie 2001).

The giant tropical ant, *Paraponera clavata*, is known for a sting worthy of its alternative common name, the bullet ant. *P. clavata* forages for carbohydrates in the form of nectar/honeydew within the canopies of trees in tropical rainforests, and predates upon arthropods for protein (Carroll and Janzen 1973; Breed and Bennett 1985; Dyer 2002). Depending on the

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nutritional and developmental status of the colony (i.e., proportion of developing larvae relative to adults) or season, foragers may adjust the ratio of carbohydrates to proteins that they bring back to the nest (Kaspari and Yanoviak 2001; Dussutour and Simpson 2009; Cook and Behmer 2010; Cook et al. 2011). Even though the carbohydrates are most often in liquid form and proteins are most often in solid form, *P. clavata* carries both externally in their mandibles (and therefore not in their crop, Fig. 1; see also Hölldobler 1985; Dyer 2002). It is likely, then, that foragers are assessing quality or palatability of a food item by examining it with their antennae or labial palps (Fritz et al. 1981; Dyer and Floyd 1993). This explains how foragers can assess, preferentially collect, and be more likely to recruit nestmates to high-concentration sugar solutions (Breed et al. 1987; Fewell et al. 1996) as well as how they can discriminate among foods with varying protein/carbohydrate nutrient ratio when presented in a solid form (Dussutour and Simpson 2009).

P. clavata workers typically encounter protein as a solid prey item, which requires a different handling technique than a liquid. Even though ants can discriminate between different nutritional content of food (Dussutour and Simpson 2009), it is unknown whether they will adjust their handling technique if the protein is presented in a different form, as it might be if they were scavenging upon a liquefied corpse. By manipulating the nutrient content of liquid foods, we set out to determine whether *P. clavata* foragers' handling decisions are flexible if they appropriately respond to a liquid protein source by drinking or if they are fixed or canalized if they inappropriately grasp at the solution.

Methods

Ten colonies of *P. clavata* were located throughout La Selva Biological Station, Costa Rica, in July 2011 (see Larson 2012 for details on how colonies were located and determined to be independent of one another). *P. clavata* primarily nest at the base of trees and forage in the canopy, using trees and lianas to access nectar and prey in the arboreal environment. Nests are identified by excavated dirt at the base of trees with at least one foraging entrance where the dirt appears to pull away from the tree. The foraging entrances handle heavy traffic; ants can be readily observed carrying foraged booty directly into their nests. Although foragers rarely exhibit inter-colony aggression while foraging (Breed et al. 1991), we made sure that behaviors were collected on one colony of ants at a time by working directly above a foraging entrance. Therefore, colonies were only used if a steady trail of ants leading out of the foraging entrance could be established. Once the foraging path was determined, 2 M of sucrose was dripped along the path on the tree trunk and a dropper filled with 2 M of sucrose solution was mounted on the tree, with the opening facing down.

Based on data from Fewell et al. (1996) and Breed et al. (1987), we used concentrations of sucrose within the range to which *P. clavata* workers are responsive (0 M, 0.05 M, 0.5 M), and casein concentrations reflecting the concentrations of protein found within the tissue of prey (0 M, 0.1 mM, 0.5 mM, 1 mM, 1.5 mM). Once foragers were coming regularly to the 2-M sucrose dropper, we replaced the dropper with a dropper filled with one of the 15 combinations of sucrose/casein. The order in which the solutions were presented was randomized, and the observer was blind to the concentration of sucrose/casein in the solution.

We recorded the first ten behaviors (drinking, grasping, inspecting, aggression, or ignoring; Table 1, see also Online Appendix A: Video of *P. clavata* feeding behaviors) of individuals that approached the dropper. Emphasis was placed on the data that was related to how an individual tried to collect the solution (drinking or grasping), but we also collected data on brief inspecting of the solution

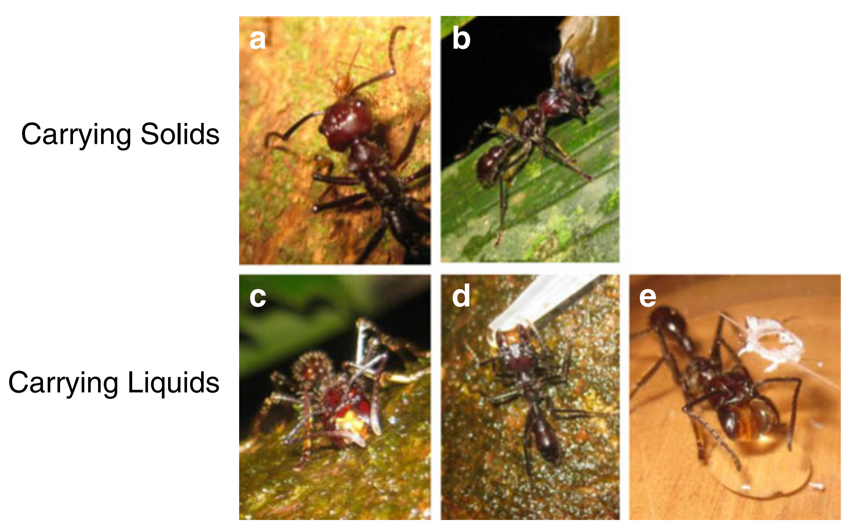


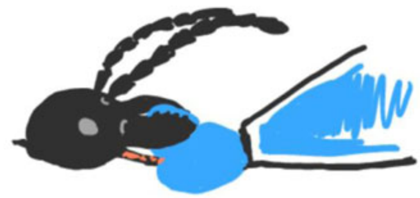
Fig. 1 Foragers carry solids (**a**, **b**) and liquids (**c**–**e**) externally in their mandibles back to the nest. **a** A forager carrying a leaf-cutter ant after capturing it from an ant trail on a tree; **b** a forager carrying a bee after capturing it in flight; **c** a forager collecting nectar/honeydew along the tree above the nest; **d** a forager collecting sucrose solution at our experimental feeder; and **e** a forager with a full load of sucrose solution held externally in her mandibles

Table 1 Ethogram of feeding behaviors in *P. clavata* (see Online Appendix A: Video of *P. clavata* feeding behaviors). Illustrations by Amy Geffre

Collecting Behaviors

Drinking

Individuals used palps (shown in orange) to collect a droplet of solution that they would hold and carry externally in their mandibles



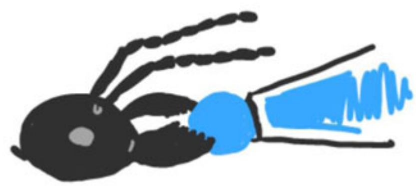
Grasping

Individuals would use mandibles to grab and pull at a liquid solution, occasionally succeeding in collecting an entire droplet of solution.

Inspecting / Ignoring

Tasting / Mandibulating

Mandibles contact the solution, but the ant does not collect any of the solution



Antennating

Individuals antennate the solution, but leave without tasting or collecting the solution

Ignoring

Ant approaches the feeder but turns away before antennating or tasting.

Aggressive Behaviors

Biting

The ant clasps the dropper at the base or near the center with its mandibles and holds tightly

(tasting–placing mandibles on solution without drinking or antennating) or biting the dropper. If a worker came within 1 cm of the dropper without interacting with the solution or the dropper, she was recorded as "ignoring" the dropper. After ten behaviors were recorded from individuals interacting with or ignoring the solution, the test dropper was replaced with the 2 M sucrose solution to re-recruit ants to the dropper. Once ants regularly started visiting and feeding from the dropper again (~2–20 foragers consistently, depending on colony activity level), we replaced the dropper with a dropper containing a different sucrose/casein mixture. This pattern continued until all 15 solutions were presented to the colony, and 10 behaviors were recorded for each. To control for pseudoreplication, we analyzed the behavior of the individuals as a collective response of the colony. Therefore, each colony was assigned a binary response as to whether it drank or grasped at least once, or if it ignored the solution (=ignored, tasted, or antennated, then left dropper without drinking or grasping).

Drinking behavior was scored if at least one individual drank the solution from the droplet at the end of the dropper (Fig. 1d) or the solution along the side of dropper. Grasping behavior was only scored if an individual grasped at the droplet. "Ignore" was recorded for those colonies where no individual attempted to collect the solution (e.g., they may have antennated or tasted the solution, but they did not drink or grasp at the solution). Individuals either drank or grasped at the solution during a visit; we did not observe them switching strategies, in mid-collection attempt, or sharing their collected resource via trophallaxis with others along the foraging trail. See Table 1 for an ethogram and diagrams of behaviors.

For each of the measured variables (drinking, grasping, or ignoring), we used a generalized linear model, with a binary response (drinking and grasping: 1=at least one individual from the colony engaged in behavior, 0=no individual engaged in that behavior; ignore: 1=no individual drank or grasped throughout trial, 0=at least one individual drank or grasped at the liquid) and logit function to evaluate the differential effects of sucrose or casein on worker behavior. In each model, we included the nest, order that the solutions were presented, sucrose concentration, casein concentration, and the interaction of sucrose and casein concentration. All analyses were run using JMP Pro v. 10 (SAS Institute).

Results

We anecdotally observed little evidence of recruitment behavior toward any of our 15 prepared samples. Instead, foraging rates often waned throughout each sampling period. Whether or not ants were recruiting additional foragers to the 2 M sucrose solution that we placed on the tree in between our prepared samples is unknown. However, passing individuals exposed to 2 M sucrose stopped to investigate the experimental droppers—the intended purpose of its usage.

Drinking behavior

There was a significant effect of sucrose (χ^2_1=29.57, P<0.001) and casein concentration (χ^2_1=4.52, P=0.03), but no interaction of the two (χ^2_1=0.85, P=0.36) on whether or not an individual would drink the solution. Ants were more likely to engage in drinking behavior at solutions with high sucrose and low casein concentration (Fig. 2a). There were no differences between colonies (χ^2_9=10.88, P=0.28) or the order in which the solutions were presented (χ^2_{14}=10.07, P=0.76).

Grasping

There was no effect of sucrose concentration (χ^2_1=0.81, P=0.37) or casein concentration (χ^2_1=2.37, P=0.12) on grasping behavior; however, the interaction of the two was significant (sucrose × casein: χ^2_1=4.08, P=0.04). Ants were more likely to engage in grasping behavior at a droplet with higher casein/sucrose concentration ratios (Fig. 2b). There were significant differences among colonies in grasping behavior (χ^2_9=27.31, P=0.001), but no evidence that the presentation order affected likelihood of grasping behavior (χ^2_{14}=13.11, P=0.52).

No interest in collecting the fluid

Ants were more likely to ignore (approach but not attempt to collect) the solution when sugar concentration was low (χ^2_1=17.53, P<0.0001; Fig. 2d). Neither casein concentration (χ^2_1=0.30, P=0.58) nor the interaction between sucrose and casein concentration (χ^2_1=0.53, P=0.47) could be used to predict whether individuals from the colony would ignore the solution. Again, there were differences between colonies in whether the solution was ignored (χ^2_9=19.85, P=0.02), but no effect of presentation order (χ^2_{14}=10.14, P=0.75).

Discussion

We found that when protein/sucrose concentrations were high, bullet ants often attempted to grasp at a droplet, rather than gather it by drinking. On occasion, we observed an individual grasp at a protein droplet and successfully hold the droplet in its mandibles (see Online Appendix A: Video of *P. clavata* feeding behaviors), but in most occasions, grasping behavior did not result in the collection of liquid. Bullet ants carry their booty externally in their mandibles, regardless if it is a solid or a liquid (Fig. 1). Nevertheless, they were able to assess the concentration of the protein without needing to ingest it or bring it back to the colony first, a phenomenon also described in *Rhytidoponera* ants (Dussutour and Simpson 2009). Why though, if all foragers can collect a fluid resource by drinking, only some do flexibly respond to and properly drink from a droplet with a high protein/sucrose concentration?

Ants from many colonies grasped at the solution when protein was present, but the probability that they would drink that solution increased as the concentration of sucrose increased. Bees, and other insects that ingest forage materials,

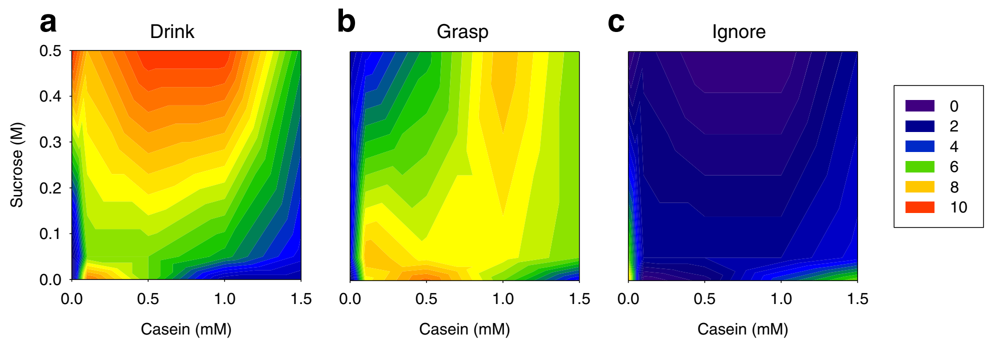


Fig. 2 Graphical representation of how ten different colonies reacted to liquids of varying sucrose/casein concentration ratios. **a** A colony was recorded as "drinking" the solution if at least one individual drank from the droplet at the end of the dropper or from the solution along the dropper. **b** A colony was recorded as "grasping" at the solution if at least one individual grasped at the droplet with her mandibles. **c** A colony was recorded as "ignoring" the solution if no individual attempted to collect the solution, either by drinking or grasping at it. *Each color* represents the number of colonies (0–10) observed performing these behaviors when confronted with each of the 15 different solutions

can assess the direct energy benefits of the nectar before leaving the foraging patch (Seeley 1995). Protein content of pollen, however, remains unknown until the external load is distributed and processed inside the hive (Pernal and Currie 2001). Since bullet ants collect and carry both liquids and solids externally in their mandibles (Hölldobler 1985; see also Fig. 1 "Carrying Liquid"), how then do they assess the quality of the material and subsequently adjust their behavior based on content? Even though they are carrying the material externally, bullet ants likely assess quality of forage material through their antennae or labial palps (see Online Appendix A), a mechanism that also allows them to reject unpalatable or poisonous prey items before carrying them back to the nest (Fritz et al. 1981; Dyer and Floyd 1993).

There were most likely a mixture of responses within a colony, as many colonies both grasped and drank at various solutions; however, our methods were not designed to assess within group variation. A mixture of behavioral types within a colony may affect colony-level performance and may also be a mechanism to allow the colony to respond flexibly to changing environmental conditions, whereas the individuals in the nest are constrained to a more inflexible behavioral type (Scheiner et al. 2004; Jandt et al. 2013; Jandt and Dornhaus 2013). We also observed differences between colonies in their probability to grasp at or ignore the solution altogether. This may have been due to limitations in nitrogen processing capabilities of the different colonies (Tillberg and Breed 2004) or variation in colony nutrient status or nutrient need (Dussutour and Simpson 2009). Honeybee foragers and fire ants have been shown to adjust their preferences for sugars or proteins based on protein content present in the colony or time of the year (Seeley 1995; Pernal and Currie 2001; Cook et al. 2011). Therefore, variation in colonies in terms of the degree to which foragers properly and improperly reacted to solutions with higher protein/sucrose ratios may reflect different internal needs of those colonies. This was not explored in here.

Why do most *P. clavata* properly drink liquid carbohydrates, but some improperly grasp at liquid proteins? Although a few foragers succeeded at gathering liquid by grasping at it (see Online Appendix A: Video of *P. clavata* feeding behaviors), this was rarely a successfully collecting strategy. Bullet ants may occasionally exploit carcasses for protein, but they are most commonly observed carrying recently killed insects back to the nest (Dyer 2002). The ability to quickly respond, i.e., maintain a fixed action sequence, to a moving prey item may be why these predators are so successful at capturing prey. Still, individuals from some colonies did properly drink a liquid solution with high protein concentration. This mixture of behavioral response types (fixed and flexible) within a colony may be an adaptation to allow the colony to respond flexibly to changing environmental conditions (Scheiner et al. 2004; Jandt et al. 2013). For example, in a predictable environment, fixed behavioral responses allow individuals to quickly exploit a resource. However, when environmental conditions fluctuate, fixed behaviors may lead to mistakes, and flexible individuals may make more accurate, albeit slower, decisions. The mixture of speed and accuracy within a colony has been described in bumble bees to increase foraging efficiency under some ecological conditions (Burns and Dyer 2008). It is unknown from our study whether individuals within a *P. clavata* colony consistently exhibit fixed or flexible behavioral types. If this is indeed the case, future studies can assess the adaptive function of maintaining multiple behavioral response types in a colony by examining colony performance under different ecological conditions and across colonies with different ratios of fixed/flexible behavioral types.

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