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Resource allocation among worker castes of the leaf-cutting ants *Acromyrmex subterraneus subterraneus* through trophallaxis

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ABSTRACT

The division of labor between the different worker castes of leaf-cutting ants may reflect in their capacity to exchange liquids by trophallaxis. The crop capacity of and trophallactic exchanges between different size classes of worker leaf-cutting ants of the sub-species *Acromyrmex subterraneus subterraneus* were investigated. Size classes were defined from head capsule widths and crop capacity of each class was determined following *ad libitum* feeding on dye solution. Experiments were carried out to investigate trophallactic exchanges between donor ants and recipient ants of each class size combination on a one to one basis. An experiment was also performed to investigate dye distribution within mini-colonies following introduction of three classes of donor ants. Worker ants were categorized into four size classes from their head capsule widths (C1 = 0.8–1.0 mm; C2 = 1.2–1.5 mm; C3 = 1.6–2.0 mm; C4 = 2.1–2.4 mm). C1 ants crop capacity was 0.13 μL; C2: 0.21 μL; C3: 0.52 μL; C4: 1.03 μL. Ants of each class previously fed on the dye solution (donors) were placed individually with an unfed ant of each class (recipients) and the presence of dye solution, passed from the donor to the recipient by oral trophallaxis was observed after 1 h. Results showed that all classes of donor ants performed trophallactic exchanges with all recipient classes. However, statistically fewer exchanges were seen for C2 donor ants when placed with C3 recipient ants. Ten donor ants of each of three classes (C2, C3 and C4) were introduced into mini-colonies without queen ants. It was observed that C1 and C2 ants were poor recipients, whilst C3 and C4 received the highest percentages of dye. Within 10 h of introducing the donor ants, 14 to 20% of their nest-mates had received dye solution, with 58 to 77% of dye passed to recipients. These studies show the altruistic nature of "food-laden" leaf-cutters and indicate that ants involved in garden maintenance activity are less likely to receive liquids from foraging workers.

1. Introduction

One of the major factors reported to be responsible for the success of the ants is an efficient system of food distribution within the colony, allowing colony members that do not forage to receive vital nutrients. Nutrient exchange by trophallaxis is therefore considered a highly important behavioral repertoire. Hölldobler and Wilson (1990, 2008) describe this behavior as the regurgitation of liquids stored in the crop ("social stomach") that are shared with nest-mates, accompanied by a complex behavioral ritual of antenation, filmed by Moreira et al. (2006). This type of liquid exchange is called stomodeal or oral trophallaxis. Abdominal or anal trophallaxis has been observed during interactions between larvae and adults, where larvae secrete substances that are then ingested by the worker ants (Jaffé, 1993; Hölldobler and Wilson, 1990; Lopes et al., 2005).

In order to transport liquids from foraging sites to the nest, the workers normally have a well-developed crop, although it is possible for them to carry liquid droplets between their mandibles ("social bucket") as in the case of some Ponerinae species (Hölldobler and Wilson, 1990). Ants which feed on nectar, such as *Camponotus mus* have relatively large crop capacities, the largest worker ant in the colony were capable of ingesting up to 7 μL of a sucrose solution (Josens et al., 1998).

Paul and Roces (2003) investigated liquid uptake in a range of ant species and observed two types of behavior, licking and sucking. In order to suck liquid food sources, the glossa forms a tube. To supply their own needs ants were observed to suck liquids.

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However to transport liquid nutrients ants can use either sucking or licking techniques and then store the liquid in the crop. Returning to the nest, the ant regurgitates and delivers the liquid to nest-mates by trophallaxis. Paul and Roces (2003) observed that the leaf-cutting ant *Atta sexdens* used the sucking technique.

Foraging workers of the genus *Atta* and *Acromyrmex* collect fresh plant material for the cultivation of their mutualistic fungus and carry this back to the nest. Non-foraging smaller workers cut the leaves into small fragments, which are then extensively licked to remove wax and contaminating microorganisms and incorporated into the fungus garden (Andrade et al., 2002). During the act of cutting the leaves and their subsequent manipulation, the ants ingest plant sap, from which they are believed to obtain approximately 91% of their nutritional needs (Bass and Cherrett, 1995). Silva et al. (2003) questioned the nutritional contribution of plant sap in the leaf-cutting ants' diet, suggesting instead that the ants obtain at least 50% of their nutritional needs by licking the surface of the fungus garden, which is rich in glucose. Regardless of the actual source of nutrients, worker leaf-cutters are adapted to handle liquid nutrients.

Liquid ingestion by the leaf-cutting ant *A. sexdens* has been observed during leaf-cutting and leaf preparation (Forti and Andrade, 1999), as was also the case for *Atta cephalotes* (Littledyke and Cherrett, 1976). Solids and semi-solid components are ingested and stored in the infrabucal cavity and subsequently regurgitated in the form of pellets (Fowler et al., 1991).

The division of labor amongst the different ant worker castes improves efficiency and benefits the colony as a whole (Hölldobler and Wilson, 1990). Polymorphism and polyethism are evident in leaf-cutting ant colonies. In *A. sexdens*, tasks are divided between four physical castes of which three are further subdivided into temporal castes, resulting in work force comprising a total of seven castes overall (Wilson, 1980a). Age polyethism was observed in *Acromyrmex subterraneus brunneus* where young workers performed tasks inside the nest related to brood care and gardening activities, whereas older individuals performed activities outside the nest such as foraging and activities in the waste chamber (Camargo et al., 2007).

Worker caste polymorphism in *Acromyrmex echinatior* was studied by Hughes et al. (2003), who divided the worker castes into two distinct groups: small workers which performed tasks within the nest and large workers involved in foraging activities. Other studies divided the worker castes of *A. subterraneus brunneus* into four divisions based tasks performed by these ants in relation to head width and age (Camargo et al., 2007). Although Camargo and co-workers quantified a total of 29 different behavioral acts performed by the different worker castes of this species of leaf-cutting ant, surprisingly they did not observe trophallaxis among the ants repertoire.

Although it is believed that many derived ant species exchange food by trophallaxis (Wilson, 1971), only two studies to date have described and quantified this behavior in leaf-cutting ants (Moreira et al., 2006; Richard and Errard, 2009), despite extensive research on their complex social interactions (Wilson, 1980a,b, 1983a,b).

Here we investigate differences in crop volumes between different size classes and trophallactic exchanges between nest-mates of adult leaf-cutting ants of the sub-species *A. subterraneus subterraneus*, particularly looking at exchanges between size classes on a one to one basis and quantify the dispersion of liquids by trophallaxis within mini-colonies. Leaf-cutting ants are serious pests in the Neotropics and notoriously difficult to control (Fowler et al., 1989). Therefore a better understanding of their social behaviors may lead to the development of improved forms of pest management.

2. Material and methods

2.1. Insects

Colonies of *A. subterraneus subterraneus* were originally collected in the municipality of Bom Jardim, Rio de Janeiro State (22°09′07″S and 42°25′10″W). Worker ants were obtained for experiments from three colonies maintained in the laboratory at 25 °C and a photoperiod of 12L:12D (Della Lucia, 1993). In order to separate worker ants into class sizes, the head capsules were measured across their widest point with a stereoscopic dissecting microscope equipped with a calibrated eyepiece micrometer.

With practice it was possible to separate ants into size classes directly from the colony by visual observation without prior measurement of the head capsule. However, in order to check the accuracy of this empirical method, ants were randomly chosen and divided into four size classes and the head capsules measured. Statistical analysis confirmed the accuracy of the selection technique (results not shown). Thus, the ants were categorized into the following four class sizes in a manner similar to that used by Forti et al. (2004): C1 (head capsule width: 0.8–1.0 mm), collected from the fungus garden; C2 (head width: 1.2–1.5 mm), collected from the trail and foraging area; C3 (head width: 1.6–2.0 mm), collected from the trail and foraging area; C4 (head width: 2.0–2.4 mm), collected from the trail and foraging area.

2.2. Experiment 1: crop capacity determination

In order to compare the class size with crop volume capacity, ants (15 individuals for each class) were removed from colonies, placed in individual Petri dishes (diameter 9.5 cm) with 50 μL of a solution of Evans Blue (1%) and honey (10%) dissolved in sterile distilled water, and allowed to feed for a period of up to 1 h. Experiments showed that the dye had no adverse effects on ant survival using Kaplan–Meier survival analysis and Log-Rank test to compared survival of Evans Blue fed and control ants ($\chi^2 = 0.85$, 1; $p = 0.356$). No behavioral modifications were observed following feeding on this dye. Mini-colonies exposed to ants previously fed on Evans Blue developed normally. This dye is not absorbed during passage through the digestive tract (Erthal et al., 2004).

Immediately following feeding, the ants were dissected and dye volume was estimated. Each crop was individually placed in an Eppendorf tube containing 1 mL distilled water. The crops were then homogenized with a plastic pestle and centrifuged at $10{,}000 \times g$ for 10 min. The absorbance of the supernatant was read at 610 nm using a spectrophotometer (Pharmacia). A calibration curve was used to estimate volume of Evans Blue.

2.3. Experiment 2: occurrence of trophallaxis between different classes on a one to one basis

Ants from the different size classes were removed from colonies, marked with non-toxic acrylic paint on the dorsal thorax, placed in a individual Petri dishes (diameter 9.5 cm) with 50 μL of a solution of Evans Blue (1%) and honey (10%) and allowed to feed *ad libitum* for 1 h. These ants, denominated as "donor" ants, were then placed individually in a Petri dish containing an unfed "recipient" ant. All possible combinations of size classes were placed together on a one to one basis to verify the occurrence of trophallaxis. Following a 1-h period, the recipient ants were dissected to verify the presence or absence of dye in the crop. All 16 possible combinations of donor and recipient size classes were tested 20 times per combination, thus resulting in a total of 320 observations.