CAN THE GHOST ANT (HYMENOPTERA: FORMICIDAE) SELECT AND SEPARATE FOOD INSIDE THE BODY?

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Abstract This investigation aimed at analyzing the passage of food inside the digestive system of *Tapinoma melanocephalum*. A water-soluble dye and a lipid-soluble dye were added to 3 different diets as tracers, to be later offered to different laboratory colonies of the ghost-ant. We collected 80 workers which had ingested the diet, and past set periods of time they were dissected under a stereomicroscope. The ingested quantity of tracers was measured in the infrabuccal pocket, postpharyngeal glands, crop and ventriculus of each ant. Using a video camera, we also recorded 120 min. of the trophallaxis process between the members of one colony. Solid food was retained in the infrabuccal pockets; only liquid diets were actually ingested. Water-soluble tracers were found in infrabuccal pockets, crops and ventriculi, while lipid-soluble tracers were found in infrabuccal pockets, postpharyngeal glands and crops. Carbohydrates mixed up with lipids do not reach the midgut. After 30 min., about half of the colony received food, except the larvae. In 50 min., all workers and queens got food inside their crops. Results suggest that workers can control the passage of food through their digestive system. We also think lipids ingested might be stored and metabolized in the postpharyngeal glands.
Key Words Carbohydrates, digestive system, infrabuccal pocket, lipids, postpharyngeal glands; trophallaxis.

INTRODUCTION

The insects were among the first organisms to colonize terrestrial ecosystems, however, social insects (including ants) blossomed as "dominant taxa" over other insect species about 50-60 million years ago (Hölldobler and Wilson, 1994). Among the strategies used by ants during its evolution, the feeding behavior occupies a prominent place. Feeding involves many chemical and physiological processes that turn the food into energy and animal tissue (House, 1974). Then, the capacity of ants in exploring a variety of food resources has direct impacts on the growth and reproduction of the colony (Fowler et al., 1991). In this context, the morphology of the digestive system and glands associated to it got an important role to play upon the feeding habits in ants.

The infrabuccal pocket is a rounded structure located right before the opening of the mouth, its function is retain any solid material that reach the oral cavity during the feeding or grooming (Eisner and Happ, 1962; Quinlan and Cherrett ,1978). These solid materials are later eliminated as a small pellet (Janet, 1895). The crop of ants is located at the end of the esophagus and it is responsible to store all liquid food ingested. In the species that perform trophallaxis, the crop is considered the colony's "social stomach", because its contents can be shared among the nestmates (Hölldobler and Wilson, 1990). In ants, the main functions of the midgut are related to the production and secretion of digestive enzymes, absorption of nutrients and production of the peritrophic membrane (Chapman, 1998).

Like other organisms, ants have many types of glands, although the postpharyngeal gland (PPG) is unique to them. The PPG is the largest exocrine gland in their head and they are located dorsally at the end of pharynx, near the transition to the esophagus (Caetano, 1998; Eelen et al., 2006). The postembryonic morphological development and anatomy of the PPG in ants was already described by Emmert (1968) and Peregrine et al. (1973), whereas the functions of this gland have been the target of numerous investigations they are still disputed and a spectrum of suggested functions has been put forward (Eelen et al., 2006).

Over evolution ants developed distinct strategies to find, consume and use food resources. Studies about feeding in ants are important as they allow us to identify where storage, digestion and absorption of nutrients occur in their body. Then, the present investigation aimed at analyzing the passage of food inside the digestive system of the ghost ant *Tapinoma melanocephalum*.

MATERIALS AND METHODS

Ant Colonies
Colonies of *T. melanocephalum* were collected in urban areas and transferred to artificial nests made out of red cellophane covered plastic Petri dishes. These nests were kept inside plastic trays (37 x 33 x 7 cm) coated with Teflon® (in order to prevent ants from escaping) at laboratory conditions of 27ºC and 75% relative humidity. The adults were fed upon water and honey *ad libitum*, and also fresh *Tenebrio mollitor* and *Apis mellifera* larvae fragments.

Flow of Diets
Water-soluble dye Rodamin B and the lipid-soluble dye Sudan Black were added to 3 different diets as tracers. A solid food was prepared by mixing equal parts of dehydrated bovine liver, Pullman® sponge-cake, peanut oil with Sudan Black (0.1%) and aqueous honey solution (1:1) with Rodamin B (0.2%). Two liquid diets were also used: aqueous honey solution (1:1) with Rodamin B (0.2%), and peanut oil with Sudan Black (0.1%). Each diet was offered to laboratory colonies of *T. melanocephalum* over a small piece of foil (4cm x 4cm) after a starving period of 24 hr (aqueous honey solution diet), 72 hr (solid diet) or 9 days (peanut oil diet). We collected 80 workers which had ingested the diet, then, workers were divided into eight groups (10 ants per group) and placed into plastic Petri dishes. After past set periods of time (10 min, 30 min, 1 hr, 4 hrs, 12 hrs, 24hrs, 48hrs and 72hrs) one of Petri dishes were placed into the freezer (-4ºC) to anesthetize the ants. They were dissected under a stereomicroscope and the ingested quantity of tracers was measured in the infrabuccal pocket (IBP), postpharyngeal glands (PPG), crop and ventriculus (VENT) of each ant.

We made a visual evaluation from the quantity (intensity of color) of the tracers as follows: 0 = without dye; 1 = small quantity of dye; 2 = medium quantity of dye; 3 = totally colored. We also estimated the turgidity from the crop and PPG, as follows: 0 = empty lumen; 1 = flaccid (25% full); 2 = half filled (25-50% full); 3 = fairly expanded (50-75% full); 4 = complete full (75-100% expanded). Based on the data obtained the mean standard deviation and an ingestion index were calculated. The index was calculated as follows:

$$I = \frac{C x T}{P} \ x100$$

Where: I – Ingestion index; C – Quantity of dye; T – Turgidity of the organ; P – Maximum value obtained from CxT (PPG and Crop = 12; IBP and VENT = 3).

Trophallaxis Process
One colony containing 15 queens, 600 workers and a varied number of immatures (eggs, larvae and pupae) remained 72 hours without food and then received a droplet of aqueous honey solution (1:1) with Rodamin B (0.2%) over a foil (4 cm x 4 cm). All colonies were monitored using a video camera for 120 minutes to evaluate the distribution of food between nestmates.

RESULTS AND DISCUSSION

Liquid Diet (Aqueous honey solution + Rodamin B)
The tracer was found in all structures analyzed except in the PPG. The IBP and crop presented the dye immediately after the ingestion of the food, whereas the VENT showed signs of the dye after 1 hour.

The Figure 1 shows the variation observed on the ingestion index calculated to *T. melanocephalum* workers. A number of 70 workers had solid particles inside the IBP, all the others eliminated pellets during the assays or already had an empty IBP. According to Febvay and Kermarrec (1981) this behavior prevent the block of proventriculus, what could stop the flow of food from the crop to the ventriculus.

The absence of Rodamin B in the PPG's suggests that the aqueous honey solution did not enter these glands. This corroborates other investigations aimed at the feeding habits of many ant species, which have never found water-soluble dyes inside the PPG (Peregrine and Mudd, 1974; Phillips and Vinson, 1980; Bueno, 2005).

The region with highest amount of Rodamin B was the crop. Until 30 minutes, the crop from workers is totally colored and expanded, however after 30 minutes we observed a marked reduction in the quantity of dye and turgidity. This reduction was caused by the regurgitation of food on the surface of the Petri dishes and the
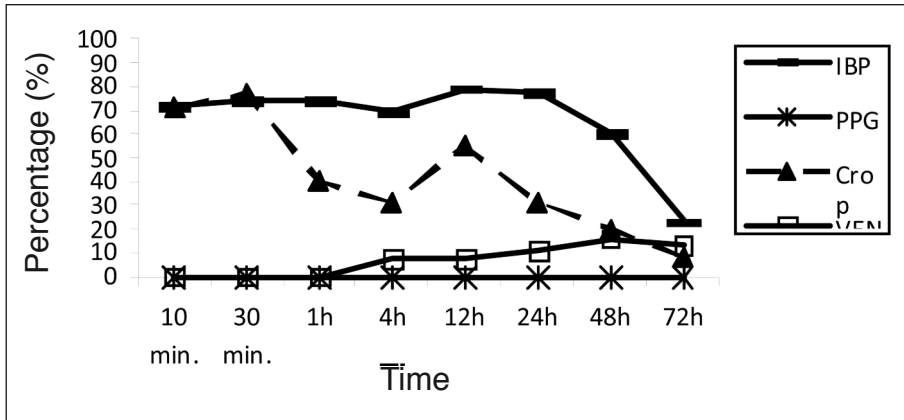
Figure 1. Ingestion index calculated for the flow of the peanut oil containing Sudan Black in the digestive system of workers of *Tapinoma melanocephalum* (n=80). **IBP** – Infrabuccal pocket; **PPG** – Postpharyngeal gland; **VENT** – Ventriculus.
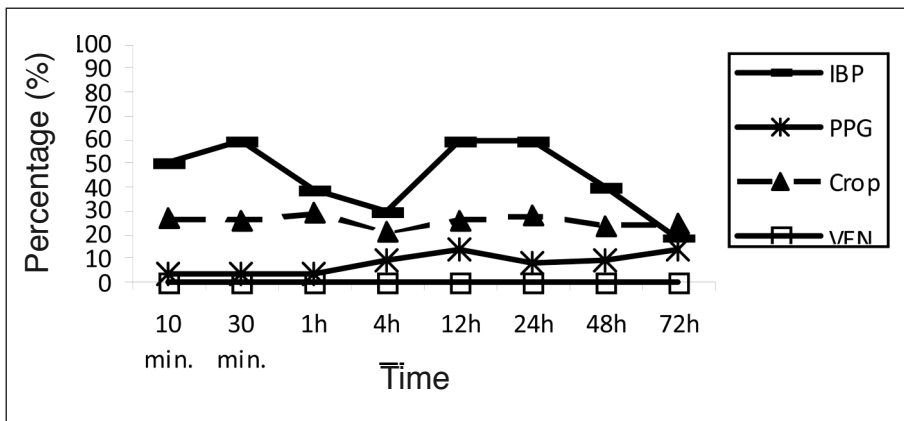

Figure 2. Ingestion index calculated for the flow of the aqueous honey solution containing Rodamin B in the digestive system of workers of *Tapinoma melanocephalum* (n=80). **IBP** – Infrabuccal pocket; **PPG** – Postpharyngeal gland; **VENT** – Ventriculus.

ingestion of water by workers, affecting both the color and crop's turgidity. This can be observed on the Figure 2, where the ingestion index from the crop decreases several times after 30 minutes. The same behavior was already observed for *Monomorium pharaonis* and *Linepithema humile* workers (Jesus, 2006).

After 1 hour, small quantities of dye were observed in the ventriculus and the greatest values reached after 24 hours from the food ingestion. This suggests that food is quickly used by workers, but in small quantities, and that the most of it is stored in the crop or shared with nestmates. There are only few studies regarding the necessary period of time to start the food absorption inside the ventriculus. In *Solenopsis invicta* it is necessary only few seconds to start it, although the highest levels occur between 6 and 24 hours. For workers of *Camponotus pennsylvanicus* it was demonstrate that the digestion begins between 4 to 16 hours, reaching its maximum activity after 20 hours (Cannon, 1998).

Liquid Diet (Peanut oil + Sudan Black)
The tracer was found in all structures analyzed except in the VENT, IBP, PPG and crop presented the dye immediately after the food ingestion.

The Figure 3 shows the variation observed on the ingestion index calculated to *T. melanocephalum* workers. Only 36 workers had solid particles inside the IBP, all the others eliminated pellets during the assays or already had an empty IBP. Workers did not regurgitate the peanut oil during the time analyzed in this investigation

At the end of the pharynx part of the Sudan Black ingested entered the PPG and part went to the crop. The ingestion index calculated for the PPG varied between 3 and 15%, demonstrating the little amount of peanut oil that entered in this gland. Probably this occurred due to the presence of other substances inside it, since other species like *M. pharaonis*, *L. humile* and *Paratrechina fulva* presented the same ingestion index ranging from 70% to 85% (Jesus, 2006). It is import to emphasize that a slight decrease in the ingestion index for the crop is followed by an increase in the PPG, suggesting a flow of food from the crop to the head of workers. This corroborates the findings of Phillips and Vinson (1980) in a study using queens and workers of *S. invicta*.

It was expected that after a long period of fasting (72 hours) workers would consume a larger quantity of food, however, the maximum quantity of peanut oil found in the crop filled up only 25% of its total volume (Figure 3). Considering the small quantity of lipids found in the crop of ants collected in nature (Tennant and Porter, 1991; Cannon and Fell, 2002), it is possible that lipids are used on a smaller scale, since they are not easily found in nature.
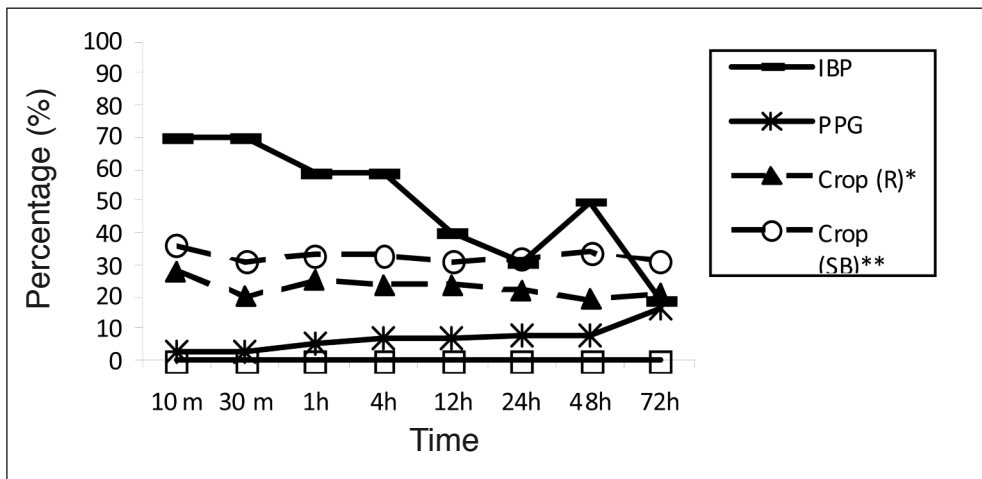

Figure 3. Ingestion index calculated for the flow of the solid diet containing Rodamin B and Sudan Black in the digestive system of workers of *Tapinoma melanocephalum* (n=80). **IBP** – Infrabuccal pocket; **PPG** – Postpharyngeal gland; **VENT** – Ventriculus; * Rodamin B; ** Sudan Black.

Moreover, the peanut oil might have only a small participation on feeding processes of *T. Melanocephalum*. If this is true, workers avoid the excess of lipids and consume preferably carbohydrates. It was not observed Sudan Black in the ventriculus, therefore the peanut oil does not reach the worker's midgut, it is stored in the crop and in the PPG.

Solid Diet (mixture containing Rodamin B and Sudan Black)
The dyes were found in all structures analyzed except in the vent. The IBP and crop presented both Rodamin B and Sudan Black, while the PPG was marked only by the Sudan Black. The solid particles were retained in the IBP and only the liquid part of diet was actually ingested.

The Figure 3 shows the variation observed on the ingestion index calculated to *T. melanocephalum* workers. Only 44 workers had solid particles inside the IBP, all the others eliminated pellets during the assays or already had an empty IBP.

All PPG's were colored by the Sudan Black immediately after the ingestion of food and the quantity of dye and the turgidity of this gland increased along the time (Figure 3). Like the observed in the other assays, we didn't find any trace of Rodamin B inside the PPG.

Compared to all other structures from the digestive system, the crop of workers showed the highest quantity of both Rodamin B and Sudan Black. On the other hand, dyes did not reach the ventriculus and workers did not regurgitated the food. Hence it follows that the presence of lipids mixed with carbohydrates interferes on the flow of food to the midgut and/or trophallaxis process.

Trophallaxis

Immediately after the ingestion of food workers came back quickly to the nest. Inside the nest all workers shared the food stored on its crop with queens and other workers, but not with the larvae (120 minutes of observation). This result can be compared with other investigations which demonstrate that food is not shared homogeneously between the castes. Generally, carbohydrates and lipids are shared between the adults, whereas the proteins are given to the larvae (Vinson, 1968; Weeks et al., 2004)

It was observed that a single worker can shared food with 1to 4 nestmates at the same. After 30 minutes 9 queens and at least 50% of workers received the aqueous honey solution, and after 50 minutes almost all nestmates, except the larvae, showed traces of Rodamin B inside their bodies. We also observed that after 35 minutes many workers regurgitate food on the surface of the nest.

CONCLUSIONS

Results suggest workers can control the passage of foods through their digestive system. Solid particles are retained in the infrabuccal pocket and are later eliminated as small pellets; only liquid substances are actually ingested. After the ingestion of food, workers kept away from the colony regurgitate the carbohydrates but not the lipids. The carbohydrates flow through the infrabuccal pocket, crop and ventriculus, while lipids flow through the infrabuccal pocket, postpharyngeal glands and crop. The lipids stored in the crop are sent back to postpharyngeal gland. Carbohydrates mixed up with lipids do not reach the midgut. Lipids ingested might be stored and metabolized in the postpharyngeal gland before its consumed or shared with nestmates.

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