

# Influence of Brood on the Nutritional Preferences of the Tropical Ant Species, *Pheidole megacephala* (F.) and *Ochetellus glaber* (Mayr)<sup>1</sup>

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**ABSTRACT** The influence of brood on the nutritional preferences of two ant pests, *Pheidole megacephala* (F.) and *Ochetellus glaber* (Mayr), was examined. Both ant species preferred fruit fly pupae and diamondback moth larvae to tuna and peanut butter. The foraging rates of *O. glaber* for proteins and carbohydrates depended upon both the food type and the amount of brood present. When *O. glaber* foragers were presented with a choice of tuna (a less preferred source of protein) and sucrose, the foraging rate was always greater on sucrose, regardless of the presence of brood. However, when ants were presented with fly pupae (a more preferred source of protein) and sucrose, the foraging rate on pupae was greater than on sucrose for colonies containing brood and the foraging rate on protein and sucrose was similar for colonies without brood. The foraging rate of *P. megacephala* for proteins and carbohydrates depended upon the type of protein, but was not affected by the presence of brood.

**Key Words** Ants, brood, foraging, baits, *Pheidole megacephala*, *Ochetellus glaber*

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In Hawaii, ants are alien species that have flourished due to the favorable climate, abundant food sources, and lack of natural enemies (Reimer 1994). Ants in Hawaii are pests in urban, agricultural, and natural environments. Toxic baits are currently a popular method for control of ant infestations in Hawaii because baits are more likely to kill queens and brood than insecticidal sprays, especially when ant colonies are difficult to locate (Williams et al. 1990, Knight and Rust 1991, Williams and Vail 1993). Toxic baits are also considered to be less likely to cause environmental contamination than liquid insecticide applications. However, baits need to be highly acceptable in order to attract foraging workers in the presence of other food sources. Thus, research on food preferences of the major pest ants of Hawaii is necessary to improve toxic bait efficacy.

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Studies of food flow in ant colonies have revealed that proteins are generally utilized by larvae and queens, and sugars are consumed primarily by workers (Markin 1970, Howard and Tschinkel 1981, Sorensen and Vinson 1981). Therefore, it is expected that there would be changes in food preferences depending upon the composition of the colony. Developing a better understanding of how brood production influences feeding preferences will improve our ability to control ant infestations by using baits. In the current study, we examined the preferences of two ant pests, *Pheidole megacephala* (F.) and *Ochetellus glaber* (Mayr), for different sources of protein and how differences in brood levels affected ant foraging behavior for proteins and carbohydrates.

These two species share common attributes of tramp species such as polygyne, colony reproduction by budding, and the ability to colonize disturbed habitats and relocate nesting sites rapidly (Passera 1994). All of these attributes contribute to difficulties in controlling ant infestations. *Pheidole megacephala* is a well-known invasive pest with an almost worldwide distribution in tropical and semitropical habitats. It originated in Africa and has been transported by humans to many parts of the world. It has been established in Hawaii for over 100 years (Smith 1879), and it is almost always the dominant species in areas where it is found (Reimer 1994). Although it is a nuisance pest in homes and gardens, *P. megacephala* is also a serious agricultural pest. It causes problems by tending homopterous pests which spread plant diseases in pineapple, coffee, and sugar cane (Reimer et al. 1990) and by damaging drip irrigation tubes in sugar cane fields (Chang and Ota 1990). In addition, *P. megacephala* has been implicated as a major cause of the disappearance of almost all of the native invertebrates in lowland ecosystems (Perkins 1913).

In contrast, *O. glaber* is a much more recent immigrant to Hawaii. It was first collected in Hawaii in 1977 (Beardsley 1980), and it has been spreading rapidly throughout the island of Oahu since (Reimer, pers. comm.). It originated in Australia and is also currently found in New Zealand and parts of Asia. This ant species is primarily an urban pest in Hawaii, and its biology is largely unknown.

### Materials and Methods

Ant colonies were collected at various locations on the island of Oahu and maintained in the laboratory at ambient conditions (23 to 25°C) in uncovered 30 by 16 cm plastic boxes. The sides of the boxes were coated with liquid teflon (Fluon®, Northern Products, Woonsocket, RI) to prevent ants from escaping. Each box contained a 4.5-cm diam plastic Petri dish with a layer of plaster-of-Paris in the bottom and a red cellophane-covered lid to provide a suitable nesting site for ants. Ants were able to freely enter and leave the dish through a hole in the lid. They were provided with a constant supply of water from a water-filled 15-dram plastic vial which contained small holes in the sides of the container and was positioned upside down in the ant box so that ants could collect water droplets when needed. Ants were provided with honey-water by soaking a piece of absorbent paper towel and placing it in a 4.5-cm diam plastic Petri dish. They also were provided with adult tephritid fruit flies (*Bactrocera dorsalis* Hendel and *B. cucurbitae* Coquillett) as a source of protein. Food preference tests were performed in a second Fluon-coated 30 by 16 cm plastic box which was connected to the nest box with

1-cm diam tygon tubing, 10 cm in length. Standard food items were removed 48 h before tests were performed. The numbers of ants feeding on each food type were compared using Least-Squares means (LSM) in the General Linear Models (GLM) procedure of SAS (SAS Institute 1987).

**Feeding preferences for different sources of protein.** Feeding preferences of ants were examined for four different sources of protein: three *Bactrocera* fruit fly pupae, two fourth to fifth instars of the diamondback moth, *Plutella xylostella* (L.), 0.5 g tuna (68% protein, 32% fat, and 0% carbohydrates) (Coral Hawaiian®, Bumble Bee Seafoods, San Diego, CA), and 0.5 g peanut butter (23% protein, 23% carbohydrates and 54% fat) (Skipppy®, Best Foods, Englewood Cliffs, NJ). Ants were deprived of protein for 48 h before each trial. The four food items were each presented in the cap of a Falcon® test tube (12 × 75 mm). The caps were arranged 2 cm apart in a square formation. The location of food items was altered in each replicate to avoid any positional bias. Numbers of ants foraging on each food were counted every 15 min for 6 h. There were 8 colonies of *O. glaber* consisting of 300 workers, 1 queen and 100 larvae. There were 2 trials for this experiment. There were 20 colonies of *P. megacephala* consisting of 500 minor workers, 25 major workers, 1 queen and 100 larvae. Statistical analyses were conducted as described above.

**Effect of brood on ant preference for proteins versus carbohydrates.** A two-way choice test was performed to compare foraging on proteins versus carbohydrates of colonies with different amounts of brood of *O. glaber*. In the first experiment, there were 15 colonies comprised of 300 workers and 1 queen that were divided into 3 treatments with 5 colonies for each treatment. The 3 treatments consisted of brood (ant larvae of various instars) of 200, 50 and 0 brood per colony. The two food types were each presented on a 2.3-cm diam Whatman No. 3 filter paper disk. The protein consisted of 0.5 g of tuna packed in soybean oil (Coral Hawaiian®, Bumble Bee Seafoods, San Diego, CA). The carbohydrate consisted of 100 µl of 25% sucrose water. The two disks were placed 2 cm apart in the center of the second box and left for 15 min to attract foraging ants. After 15 min, the number of ants on each disk was recorded on videotape (Sony®, CCD-TR101 Video Camera Recorder) at 30-s intervals for 15 min. There were 6 trials for this experiment. Before each trial, ants were starved for 48 h.

A second experiment was performed to determine the feeding preferences of ants over a longer time interval. There were 10 colonies comprised of 300 workers and 1 queen. There were 5 colonies with brood (100 larvae of various instars) and 5 colonies without brood. Tuna (0.5 g) was placed in the cap of a Falcon test tube (12 × 75 mm) and 25% sucrose water was placed in a 5-dram plastic vial with a cotton plug. The two food types were placed 2 cm apart in the second box. Numbers of ants foraging on each food were counted every 30 min for 6 h. There were four trials performed on consecutive days.

In a third experiment, feeding preferences of ants for protein versus carbohydrate was determined using a different source of protein, *Bactrocera* fruit fly pupae. There were 8 colonies with 300 workers and 1 queen. There were 4 colonies with brood (100 larvae of various instars) and 4 colonies without brood. Numbers of ants foraging on each food were counted every 30 min for 6 h. Three previously frozen pupae were cut in half and placed in the cap of a Falcon test tube (12 × 75 mm) and 25% sucrose water was placed in a 5-dram plastic vial with a

cotton plug. The two foods were placed 2 cm apart in the second box. Statistical analyses of data resulting from all three experiments were performed as previously described.

In the first experiment of the foraging activity of *P. megacephala*, there were 16 colonies comprised of 500 minor and 25 major workers and 1 queen and 8 colonies with 100 larvae of various instars and 8 colonies without brood. Tuna (0.5 g) was placed in the cap of a Falcon test tube (12 × 75 mm) and 25% sucrose water was placed in a 5-dram plastic vial with a cotton plug. The two foods were placed 2 cm apart in the second box. Numbers of ants foraging on each food were counted every 30 min for 6 h. There were two trials performed on consecutive days. A second experiment was performed as described above, except that the protein consisted of three previously frozen *Bactrocera* pupae, cut in half and placed in the cap of a Falcon test tube (12 × 75 mm). Statistical analyses of data from both experiments were performed as previously described.

## Results

**Feeding preferences for different sources of protein.** There was a greater number of *O. glaber* foraging on the fruit fly pupae and the *Plutella* larvae than on tuna or peanut butter (Table 1). Foragers of *P. megacephala* were able to drag *Plutella* larvae back to the nest very quickly. Larvae were dragged away by only 1 or 2 ants. In 15 of 20 replicates, ants dragged both larvae away within 1 h and, in 19 of 20 replicates, ants dragged both larvae away within 3 h. In contrast, ants did not attempt to drag away the other three sources of protein, but rather fed on them in the caps. Because *P. megacephala* dragged larvae back to the nest before feeding on them, *Plutella* larvae were not included in the statistical analysis comparing foraging upon the four food items. Thus, although larvae appeared to be a highly acceptable food, we cannot compare their acceptability to that of the other sources of protein from the results of this bioassay. In a comparison of the remaining three sources of protein, there were greater numbers of ants foraging on the fruit fly pupae than on tuna or peanut butter (Table 1).

**Effect of brood on ant preference for proteins versus carbohydrates.** Greater numbers of *O. glaber* fed on sucrose than on tuna, regardless of differences in the amount of brood in the first and second experiments. In the first experiment, there were greater numbers of ants feeding on sucrose in colonies with 200 brood than in colonies with 50 or 0 brood; and in the second experiment, there were greater numbers of ants feeding on sucrose in colonies with brood than in colonies without brood (Table 2). In the second experiment, the number of ants foraging was greatest on the first day compared with the following 3 d; however, there were no changes in ant preferences for proteins versus carbohydrates on the different days ( $P < 0.0001$ ). In the third experiment, there were greater numbers of ants foraging on protein (fruit fly pupae) than sucrose for colonies with brood and equal numbers of ants foraging on protein and sucrose for colonies without brood (Table 2).

There were greater numbers of *P. megacephala* foraging on sucrose than tuna for both colonies with brood and colonies without brood in the first experiment. In the second experiment, there were greater numbers of ants foraging on the fruit fly pupae than the sucrose for both colonies with brood and colonies without brood

**Table 1. Acceptability of four different sources of protein to *Ochetellus glaber* and *Pheidole megacephala*.**

	Numbers of Ants Feeding on Each Food Type ( $\pm$ SD)*	
	<i>O. glaber</i>	<i>P. megacephala</i>
Fruit fly pupae	45.7 $\pm$ 28.3 a	95.5 $\pm$ 53.2 a
Diamondback moth larvae	43.8 $\pm$ 34.7 a	—**
Tuna in oil	6.3 $\pm$ 5.8 b	18.5 $\pm$ 29.4 b
Peanut butter	0.2 $\pm$ 0.5 b	1.85 $\pm$ 2.2 b

\*Means within a column followed by the same letter are not significantly different (GLM, LSM [SAS Institute 1987]).

\*\*In 15 of 20 replicates, ants dragged both larvae away within 1 h and in 19 of 20 replicates, ants dragged both larvae away within 3 h. Because *P. megacephala* dragged larvae back to the nest before feeding on them, larvae were not included in the statistical analysis comparing the four sources of protein.

(Table 2). There were no differences in the foraging behavior of ants on different days.

## Discussion

Both ant species preferred insects as a source of protein to tuna or peanut butter, which is not surprising because arthropods are a primary part of their diets in nature. Also, the fruit fly pupae and diamondback moth larvae may have been more attractive to ants because insect hemolymph is a liquid food source and can be consumed directly by foraging workers. Solid food is not consumed by workers, but fed directly to larvae and queens. Liquid protein was distributed equally among workers and larvae in colonies of the imported fire ant; whereas, the distribution of solid protein was 20% in the workers and 80% in the larvae (Sorensen and Vinson 1981). Sanders et al. (1992) reported that workers of *P. megacephala* ingested only the liquid portion of radio-labeled protein. Foragers of the native fire ant, *Solenopsis geminata* (F.), and the imported fire ant, *S. invicta* Buren, collected liquid food much more frequently than solid food (70 to 80% of the diet). Although most of the liquid food was probably derived from plant saps and honeydew, insect hemolymph accounts for some portion of the liquid component of the diet (Tennant and Porter 1991). In feeding tests, workers of the Argentine ant, *Linepithema humile* (Mayr), preferred honey water or sucrose water to tuna meal and other solid foods (Baker et al. 1985).

Although there was no nutritional analysis performed on the fruit fly pupae or diamondback moth larvae, these two sources of protein presumably contained less fat than tuna packed in oil (32% fat) and peanut butter (54% fat). The amount of

**Table 2. Acceptability of protein and carbohydrate foods to *Ochetellus glaber* and *Pheidole megacephala* foragers from colonies containing different amounts of brood.**

Numbers of Ants Feeding on Each Food Type ( $\pm 50$ )*			
	Protein	Carbohydrate	Protein vs Carbohydrate**
<i>Ochetellus glaber</i>			
<b>Experiment 1</b>	<b>Tuna</b>	<b>Sucrose</b>	<b>P</b>
Brood: 200	51.2 $\pm$ 52.3 a	252.1 $\pm$ 391.8a	0.0001
Brood: 50	71.4 $\pm$ 69.0 a	164.5 $\pm$ 183.9 b	0.0186
Brood: 0	73.6 $\pm$ 80.9 a	102.7 $\pm$ 94.5 b	0.4584
<b>Experiment 2</b>	<b>Tuna</b>	<b>Sucrose</b>	
Brood: 100	8.15 $\pm$ 13.8 a	21.4 $\pm$ 12.9 a	0.0001
Brood: 0	4.0 $\pm$ 9.6 a	14.5 $\pm$ 9.3 b	0.0002
<b>Experiment 3</b>	<b>Fly Pupae</b>	<b>Sucrose</b>	
Brood: 100	92.8 $\pm$ 44.9 a	20.3 $\pm$ 12.3 a	0.0085
Brood: 0	42.0 $\pm$ 18.6 b	45.3 $\pm$ 41.8 a	0.8904
<i>Pheidole megacephala</i>			
<b>Experiment 1</b>	<b>Tuna</b>	<b>Sucrose</b>	
Brood: 100	1.0 $\pm$ 1.7a	32.7 $\pm$ 24.7 a	0.0001
Brood: 0	1.0 $\pm$ 1.8 a	27.3 $\pm$ 29.1 a	0.0001
<b>Experiment 2</b>	<b>Fly Pupae</b>	<b>Sucrose</b>	
Brood: 100	94.3 $\pm$ 47.6 a	50.8 $\pm$ 29.5 a	0.0002
Brood: 0	84.7 $\pm$ 63.4 a	32.6 $\pm$ 26.9 a	0.0001

\*Means within a column, within an experiment, followed by the same letter are not significantly different ( $P \leq 0.05$ ; GLM, LSM [SAS Institute 1987]).

\*\*Paired comparisons of numbers of ants feeding on protein vs carbohydrates for each treatment ( $P \leq 0.05$ ; GLM, LSM (SAS Institute 1987)).

oil and lipids required by a colony is relatively small. For example, the feeding rate of the imported fire ant, which is characterized as a "grease-loving ant," on oil was much lower than on two aqueous solutions, sugar and rat serum (Glunn et al. 1981). In another study with the imported fire ant, soybean oil was taken in smaller quantities than sugar water and casein hydrolysate and the amount of oil consumed was least affected by colony starvation (Howard and Tschinkel 1981). Workers of the pharaoh ant, *Monomorium pharaonis* (L.), did not recruit to peanut oil or beef fat until after 7 d starvation, but they actively recruited to sucrose and egg yolk powder after only 2 d starvation (Haack et al. 1995). Therefore, workers in the present study may have fed more on peanut butter and oil-packed tuna, if colonies had been starved for longer. Our previous results indicate that *O. glaber* is not attracted to oils (Cornelius et al. 1996). However, oils are attractive to *P. megacephala* (Sanders et al. 1992, Cornelius et al. 1996), and it is not clear why workers did not feed more on oil-packed tuna in this study. Food preferences among ant colonies are highly variable (Glunn et al. 1981). Because of the variability in ant feeding behavior, bait matrices that include a mixture of proteins, oils, and sugars would be more likely to be effective than those containing only one or two food types.

The flow of proteins in ant colonies is generally directed towards larvae and queens, the flow of oils to workers and larvae, and the flow of sugar solutions to workers (Markin 1970, Howard and Tschinkel 1981, Sorensen and Vinson 1981). Insect prey are essential for brood production in fire ant colonies (Porter 1989). Therefore, it is expected that foraging rates for proteins would be affected by the amount of brood present in a colony. The foraging rates of *O. glaber* for proteins and carbohydrates depended on both the food type and the amount of brood present. When ants were presented with a choice of tuna (a less preferred source of protein) and sucrose, the foraging rate was always greater on sucrose, regardless of the presence of brood. However, when ants were presented with fly pupae (a more preferred source of protein) and sucrose, the foraging rate on pupae was greater than on sucrose for colonies containing brood and the foraging rate on protein and sucrose was similar for colonies without brood. The foraging rate of *P. megacephala* for proteins and carbohydrates depended upon the type of protein, but was not affected by the presence of brood. The foraging rate was greater on sucrose than on the less preferred source of protein (tuna), but greater on the more preferred source of protein (fly pupae), compared to sucrose.

Although workers generally feed solid protein directly to larvae and queens, workers also receive protein from larvae through brood cannibalism and by feeding on larval secretions which contain amino acids (Sorensen et al. 1983b). Therefore, ant larvae enable workers to utilize solid proteins that they cannot ingest themselves and serve the colony as a source of stored protein through cannibalism in times of stress. Brood cannibalism in starved colonies of the imported fire ant can be severe (Howard and Tschinkel 1981). Brood cannibalism in laboratory colonies of the imported fire ant was higher for colonies fed only oil and egg yolk than colonies fed insects and honey (Sorensen et al. 1983a). In the current study, the colonies containing brood had an additional source of protein available. Therefore, the number of workers from colonies without brood feeding on fruit fly pupae, a partially liquid protein, was relatively high. Because brood can also serve as a source of protein for workers, increases in brood biomass do not directly correspond

to higher foraging rates on protein. Other studies have found that brood biomass does not consistently increase in response to increasing protein availability. Queens of *Camponotus floridanus* (Buckley) produced more eggs as more protein became available; however, brood biomass and pupae production were greater in colonies provided with protein every fourth day compared to colonies provided with protein every day (Nonacs 1991). In a study examining food requirements of the imported fire ant, colony biomass increased as protein consumption increased, but brood biomass was not correlated with protein intake (Macom and Porter 1996). The foraging rates of workers on different food types are not only influenced by the differences in colony composition, but they are further complicated by brood cannibalism and by the ability of ant colonies to store proteins for later use.

The use of toxic baits is an important strategy to control ant infestations. One of the major stumbling blocks in using ant baits effectively to eliminate colonies is bait acceptance, especially when other food sources are readily available. It is important to examine how colony composition affects the acceptability of different food types to ants in order to maximize bait acceptance by foraging workers under different conditions. Our results indicate that bait matrices containing a combination of sucrose and insect proteins would be attractive to *P. megacephala* and *O. glaber* colonies, regardless of their levels of brood production.

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