EFFECTS of STARVATION on NUTRIENT DISTRIBUTION IN THE PHARAOH ANT, *MONOMORIUM PHARAONIS* (HYMENOPTERA: FORMICIDAE) WORKERS and VARIOUS LARVAL STAGES

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Abstract The Pharaoh ant, *Monomorium pharaonis* (L.) is an important pest ant species in many parts of the world. Knowledge and understanding of feeding behavior of this species are essential to the success of baiting. Transfer of a toxicant in a bait to kill a targeted colony depends on the ability of the nutrient to move within various castes and stages of a colony. Here we report the effects of nutrient distribution in workers and larvae of laboratory colonies of the Pharaoh ant. Three types of baits: 10% sucrose solution (representing carbohydrate), corn oil (representing lipid), and boiled egg yolk (representing protein) were stained with an artificial food-coloring agent and offered to colonies undergoing 1, 3, and 6 days of starvation. Larvae were divided into three stages: stage I (1 – 5 days after hatching), stage II (5 – 10 days after hatching), and stage III (10 – 15 days after hatching). The number of larvae fed by their workers at 1, 8, and 24 hours after introduction of baits were counted. Results indicated that workers actively donate carbohydrate and protein-based food to all larval stages, irrespective of their starvation period; however, oil was transferred only upon a lengthened period of starvation. Larval stage III also showed the highest percentage of individuals oil-fed by the workers. We also observed that starved colonies showed a higher number of larvae receiving food from their workers. Food was more actively transferred to the latter two stages when compared to the youngest one. The function of each nutrient for colony development, role of larval stages as storage and digestion component of an ant colony, and the implication of our findings towards baiting strategies for Pharaoh ant control are discussed.

Key Words *Monomorium pharaonis* trophallaxis starvation feeding behavior nutrient distribution

INTRODUCTION

The Pharaoh ant, *Monomorium pharaonis* (L.) has been recognized as a major pest ant species world-wide. Infestation by this species usually occurred in large office buildings, houses, apartments, factories, food premises, and hospitals (Edwards, 1986a; Edwards and Baker, 1981; Yap and Lee, 1994). Pharaoh ant workers are also capable of mechanically transmitting various pathogens of human diseases, causing contamination of sterile surgical instruments and infection of patient wounds (Beatson, 1972), and foraging on bedridden patients' body and feeding on their wounds (Eichler, 1990).

Control of the Pharaoh ants relies heavily on the use of insecticides. The common spraying and dusting method are often ineffective against this species, as only a small percentage of foraging workers are affected. The presence of residual insecticides, especially pyrethroids, may also cause insecticide repellency (Lee et al., 1999). Pharaoh ant colonies with a portion of its workers killed by residual treatment, will often respond with budding, where a number of workers will transport a small quantity of brood to another area to nest, thus amplifying the infestation prevalence (Vail and Williams, 1994).

Baiting foraging workers with food mixed with slow-acting insecticides has been proven as a better control option against Pharaoh ants (Edwards and Clarke, 1978; Newton, 1980; Lee, 2000). This method provides the added advantage of enabling the toxicant to be transported back to the nest, which will eventually result in colony elimination (Edwards and Abraham, 1990). Field and laboratory studies of food baits containing slow-acting actives such as fenoxycarb and pyriproxyfen, against this species had been reported with encouraging results (Williams and Vail, 1993; Vail and Williams, 1995).

The success of baiting as a control method against household ants depends on the knowledge and understanding of their feeding and foraging behavior. As social insects, household ants are consistently involved in food exchange with other members in their colony. Thus, the transfer of an insecticide used inside a bait to poison the targeted colony will be determined by the nutrient movement within various castes and life stages found within the nest itself. This paper reports our work on the effects of colony starvation on nutrient distribution in adult workers and the larvae of the Pharaoh ant.

MATERIALS and METHODS

The *M. pharaonis* colonies used in this study were obtained from established colonies cultured at the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia since 1994. These colonies were reared in metal food pans (24 x 38 x 8 cm) with their upper surfaces smeared with a thin layer of hair cream to prevent the ants from escaping. A wooden box (10 x 5 x 2 cm) with lid was placed as harborage for the ants. Moisture was supplied through an inverted beaker on a petri dish. The Pharaoh ant colonies were provided with formulated agar food (Bhatkar and Whitcomb, 1970) daily at *ad libitum* and were maintained under 12-hour photoperiod, 26 $\pm$ 2°C and 70 $\pm$ 5% relative humidity. Each colony consisted of 30-50 queens and 3000-5000 workers.

A total of 10 queens, 1 g of brood (mixed stages of eggs, larvae, and pupae) and an estimated number of 1000 workers were separated and placed in a new pan to form a single experimental colony. A plastic petri dish with holes drilled along its side was also placed in an inverted position inside the pan for ants to build their nest. The usage of petri dish enabled the observation needed for our investigation on the feeding activities that occurred within the nest.

We observed the feeding of Pharaoh ant larvae by their workers when different types of food were offered to the experimental colonies. Changes in feeding activities with colonies undergoing different starvation levels were also studied. Three different types of food:10% sucrose solution, corn oil, and boiled egg yolk were used as bait. These baits were stained with a food dye (Artificial Blue Star®, Bush Boake Allen) at the rate of 0.1% v/v for sucrose solution and corn oil, and 1% v/w for egg yolk. Preliminary study on Pharaoh ant colonies fed with food dyed with this agent indicated that it was harmless and does not change their feeding behavior and activities.

A total of 3.0 ml was offered to an experimental colony. At 1, 8, and 24 hours after baiting, an approximate total of 0.5 g larvae was carefully removed from the colony using a small spatula under a dissecting scope. Larvae fed with bait by their workers could be easily identified by the blue coloration seen on their bodies. The percentage of fed-larvae was scored for each of the three larval stages: stage I (1-5 days after hatching), stage II (5-10 days after hatching), and stage III (10-15 days after hatching). These stages were differentiated according to the individual larval size and by the amount of setae covering its external body surface (Petralia and Vinson, 1978; 1979). This experiment was replicated three times with other similar experimental conditions. The study was also conducted with colonies baited with 3.0 ml corn oil and 3.0 g egg yolk. The experimental procedures mentioned above were then repeated with different colonies undergoing a starvation period of 1, 3, and 6 days.

Differences observed in feeding rate for different larval stages and starvation level for each type of food were analyzed separately. All data in percentages were subjected to arc-sine transformation before analysis of variance, and means were separated using Tukey's HSD using Statistix® software.

RESULTS

In our study, results showed that a lengthened starvation period caused a significant increase in the amount of larvae fed with sucrose solution by their workers (Table 1). Colonies starved for six days had the highest number of fed-larvae as compared to other starvation levels. Results indicated that colonies deprived of food for three days did not show any significant difference in sucrose feeding of larvae than those unstarved. In addition, sucrose feeding for all three larval stages occurred without preferences of larval age, with no significant difference in amount of stained larvae detected at 24 hours post-baiting (Table 1). Thus, feeding of Pharaoh ant larvae with carbohydrate increased after a prolonged starvation period, irrespective of larval stages.

For corn oil, results showed that distribution was at a much lower rate than that of the other two types of baits. It was also found that a prolonged starvation period will also increase the allotment of corn oil to the larvae (Table 2). This study, however, demonstrated that, unlike sucrose, there was a variance among stages of larvae receiving lipid-based bait, with the oldest larval stage having the highest percentage of individuals fed by the workers (Table 2).

The egg yolk was most actively distributed to Pharaoh ant larvae. Feeding rates were higher in starved colonies. The workers also actively fed protein to larvae in unstarved colonies, despite the fact that agar food was provided daily during the experiments (Table 3). Higher activities were also recorded in the later stage of larvae.

Table 1. Sucrose solution bait – mean percentage of different larval stages of *M. pharaonis* stained at different hours after subjected to increasing period of starvation

Hours after introduction	Larval stage**	% larvae stained days of starvation* 0	3	6
1				
	I	1.9 a (a)	2.2 a (a)	12.7 b (a)
	II	1.7 a (a)	4.6 a (ab)	28.4 b (ab)
	III	1.0 a (a)	12.0 a (b)	49.4 b (b)
8				
	I	3.0 a (a)	7.2 a (a)	30.3 b (a)
	II	2.8 a (a)	12.4 b (a)	40.5 c (b)
	III	5.6 a (a)	22.5 b (a)	55.9 c (b)
24				
	I	3.5 a (a)	9.8 a (a)	36.5 b (a)
	II	8.8 a (a)	9.9 a (a)	53.4 b (a)
	III	7.8 a (a)	19.6 a (a)	60.1 b (a)

*Mean values followed by the same letter within the same row are not significantly different (P > 0.05; Tukey HSD).
**Mean values in parentheses followed by the same letter within the same column are not significantly different (P > 0.05; Tukey HSD).

Table 2. Corn oil bait – mean percentage of different larval stages of *M. pharaonis* stained at different hours after subjected to increasing period of starvation

Hours after introduction	Larval stage**	% larvae stained days of starvation* 0	3	6
1				
	I	0 a (a)	0 a (a)	0 a (a)
	II	0 a (a)	0 a (a)	3.1 a (a)
	III	1.2 a (a)	1.8 a (a)	14.3 b (b)
8				
	I	0 a (a)	0 a (a)	1.5 a (a)
	II	0 a (a)	0 a (a)	5.6 a (a)
	III	0.9 a (a)	13.2 a (b)	40.5 b (b)
24				
	I	0 a (a)	0 a (a)	3.8 a (a)
	II	1.0 a (a)	1.1 a (a)	15.8 b (a)
	III	3.4 a (a)	27.6 b (b)	54.6 c (b)

*Mean values followed by the same letter within the same row are not significantly different (P > 0.05; Tukey HSD).
**Mean values in parentheses followed by the same letter within the same column are not significantly different (P > 0.05; Tukey HSD).

Table 3. Egg yolk bait - mean percentage of different larval stages of *M. pharaonis* stained at different hours after subjected to increasing period of starvation

Hours after introduction	Larval stage**	% larvae stained days of starvation* 0	3	6
1				
	I	0 a (a)	7.1 a (a)	7.7 a (a)
	II	32.9 a (b)	34.7 a (b)	46.8 a (b)
	III	31.8 a (b)	64.3 b (c)	73.0 b (c)
8				
	I	3.9 a (a)	32.3 a (a)	40.2 a (a)
	II	48.9 a (b)	58.6 a (b)	74.5 a (b)
	III	68.1 a (b)	71.3 b (b)	93.8 b (b)
24				
	I	32.5 a (a)	40.8 b (a)	58.6 b (a)
	II	70.9 a (b)	75.6 a (b)	83.3 a (b)
	III	80.8 a (b)	94.1 b (b)	96.9 b (b)

*Mean values followed by the same letter within the same row are not significantly different (P > 0.05; Tukey HSD).
**Mean values in parentheses followed by the same letter within the same column are not significantly different (P > 0.05; Tukey HSD).

DISCUSSION

McMahan (1963) stressed that food exchange activity in social insects is one of the main mechanism for integration between members of a large and complex colony. To effectively utilize different types of nutrient for different metabolic needs such as development and reproduction for the whole colony, ants must possess an efficient method of transferring and distributing food. An important part of food exchange behaviour or trophallaxis in ants is the feeding of brood by the adult workers. Wheeler (1994) noted that larval nutrition in Hymenoptera will influence the body size of various adult forms, development of reproductive systems and also the existence of various caste forms. Although the immature stages of social insects are generally immobile and do not participate directly in feeding activities, they play an important role in feeding as a nutrient storage and enrichment locality for a colony. Nutrients isolated from larvae of wasps and ants for instance have been reported to have an increased concentration after storing and were believed to be of greater nutrition benefits to other colony members (Hunt et al., 1982; Sorenson et al., 1983a).

Results obtained from our study demonstrated that both protein and carbohydrate were important components of *M. pharaonis* larval nutrition. It was also confirmed that unlike other pest species such as the fire ant (*Solenopsis* sp.) (Lofgren et al., 1964), Pharaoh ant showed a preference for oily type baits. Lipids are generally able to provide energy to insects, but they require more effort to be broken down into a more readily used energy form. This may explain the low intake of corn oil baits by workers that were provided with daily supplement of carbohydrates in this experiment.

A bait selection study conducted with the same species in the field showed that foraging workers generally preferred protein and sucrose-rich baits (Edwards and Abraham, 1990). Carbohydrate is taken by social insects to provide energy to workers, while protein is utilized for growth and development of brood stages (Sorensen et al., 1983a). Haack and Vinson (1990) reported from laboratory observations that liquid carbohydrate is utilized mainly for adult *M. pharaonis* workers, with trophallaxis occurring actively among workers, while solid proteinous bait is usually gathered back into the nest for storing purposes. Findings from field studies during control operations also showed that infesting Pharaoh ants can be effectively controlled by using a mixture of protein and carbohydrate baits (Granovsky and Howell, 1983).

Though larval stages of insects are generally inactive and therefore do not require a high amount of energy, social insects, nevertheless, have been known to be able to store various forms of carbohydrates in physical structures in their nest and also inside living tissues of their brood. Cmelik and Douglas (1970) found various types of carbohydrates in the nest of the fungus-growing termites. This behavior enables adult workers to have a readily available supply of carbohydrates. Hunt et al. (1982) reported that larva to adult trophallaxis constantly occur in wasps where saliva containing a concentrated amount of carbohydrates and amino acids were transferred from larvae to soliciting adults. Our data revealed that Pharaoh ant workers stored carbohydrate within their larvae without partiality to the larval stages. This is probably due to sucrose solution being used as a ready-to-use energy source and could be easily stored in all larval stages.

White (1978) hypothesized that the most important single factor limiting both vertebrates and invertebrates in their attempt for niche establishment, is the shortage of nitrogenous food for their young. Studies conducted with other ant species have shown that protein is most actively donated to their young for developmental purposes. Markin (1970) reported that mirex, a slow acting insecticide mixed with sugar was distributed actively among adult workers of *Iridomyrmex humilis* while distribution to the larval stages occurred when egg yolk was used. Fire ant (*Solenopsis invicta*) workers were reported to distribute protein more rapidly to their brood when compared to lipid or carbohydrate (Sorensen et al., 1981). Howard and Tschinkel (1981a) also reported that carbohydrate was usually retained and stored in the *S. invicta* workers' guts, while they actively regurgitate protein to their larvae.

High protein requirements for larvae could also be due to the fact that at certain times, workers may have to raise sexual castes from these larvae. Edwards (1986b; 1991) stressed that in Pharaoh ant colonies, caste-regulation by workers occurs constantly. Workers cannibalize sexual larvae when there is abundance of queen and male individuals to utilize extra nutrients for producing workers. Alternately, in situations where sexual castes are required, workers will react by feeding higher quality and quantity of food to selected larvae to develop it to reproductive individuals. In the former case, workers will have to feed larger amounts of protein to their queens while, in the latter situation, the nutrient will have to be channeled to the selected queen-to-be larvae. This probably explains why protein is actively foraged and gathered by Pharaoh ants. Abott (1978) has reported that ant larvae with protein under nourishment usually fail to develop to a sexual adult.

Besides the purpose of fulfilling the need of growth for their larvae, ant workers are also known to distribute protein to their immature stages for digestive purposes. Protein gathered by adult workers is usually from dead animals, seeds, cannibalized eggs and larvae, and other forms of large and solid food. The ant larvae will therefore act as a 'stomach' for the colony by breaking down these materials into digestible amino acids that can then be distributed to other members of the colony. Ant larvae have been reported to be able to digest solid proteins, forming soluble amino acids that will be passed to workers through oral trophallaxis, glandular and hemolymph fluid secretions for distribution to younger larval stages (Wheeler, 1994). In this study, we observed that the older two stages of Pharaoh ant larvae have more individuals fed with egg yolk by their adults when compared to the younger larvae stage. In another study, it was observed that the youngest larval stage depended on a more digestible form of nutrients obtained from older larvae (A. Chong, unpublished).

Trophallaxis within a social insect colony is a dynamic mechanism, which can be modified under different situations in order to respond to physiological changes that may occur within the colony. Sudden depletion in food reserves for instance, could led to lower rate of fecundity within an ant colony. Ants often react to a period of food scarcity by increasing the activities of food foraging, intake, feeding, and storing within the colony as a counter measure (Wheeler, 1994). Starvation is demonstrated in this study, as an important factor in influencing the distribution of food by Pharaoh ant workers to their brood. Foraging ants that find a fresh source of carbohydrate often rapidly feed on it as these active workers are constantly in an energy deficient state (Sorensen et al., 1981). The increase in larval feeding with carbohydrate observed here after starvation could be due to the need to find additional storage space besides the usual storage place inside the crop of workers. Larvae filled with liquid carbohydrate can therefore be another option for a future energy resource for their active workers. Our findings also showed that feeding corn oil to larvae increased with starvation, as Pharaoh ant workers probably use their larvae for digestion and storage purposes as a response to a lengthened-stress period of food deficiency. Haack and Vinson (1990) also reported laboratory Pharaoh ant colonies have to undergo a period of starvation before they could be stimulated to accept oily type of baits. Fire ant workers have also been reported to increase protein donation to their brood after starvation had occurred in the colony (Howard and Tschinkel, 1981b). Sorensen et al. (1983b) also reported that in a situation when food sources are scarce, *S. invicta* workers baited with egg yolk will share the food with their brood first, followed by their queens, whereas in a situation where food is abundant, proteinous food were retained by workers.

The success of baiting for ant control will depend on factors such as choice of insecticide; type, quality, and quantity of food attractant used as bait; nutritional requirements of the targeted colony; and the strategy of bait placement (Granovsky and Howell, 1983; Edwards and Abraham, 1990). Reinfestation occurs as the active ingredient used earlier only poisoned the adult stages, sparing the immature stages which with enough nutrient storage will grow gradually into another new batch of workers. Here, we speculate that insecticides placed in protein- and carbohydrate-enriched bait will have a higher probability to be transferred to various larval stages of the Pharaoh ants. Results from this study also indicated that in a situation where colonies were deprived of food, the intake of bait by the larval stages also increased, indicating a potential for better control programs. More studies, particularly under field conditions, should be conducted in future to further substantiate current findings.

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