Induced Disturbances Cause *Monomorium pharaonis* (Hymenoptera: Formicidae) Nest Relocation

JIA-WEI TAY AND CHOW-YANG LEE

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Abstract: Budding and relocation of nests are important characteristics of the Pharaoh ant, *Monomorium pharaonis* (L.), an important pest of artificial structures. Pharaoh ant colony movements induced by several types of disturbances were evaluated in the laboratory. The percentages of workers and brood in the source and new nest sites were determined at Days 0, 1, 3, and 5 following physical disturbance (temporal removal of nestmates), chemical disturbance (application of pyrethroid insecticide), invasion by heterospecific ants, food depletion, and moisture depletion in the laboratory. All disturbances were performed in the source nest, which was connected to an empty new nest site. Almost all workers moved and carried the entire brood to the new nest site when subjected to physical disturbance, chemical disturbance, and ant invasion on Day 1, whereas only <5% of workers were present in the new nest site in the undisturbed control. After these disturbances, the brood was never relocated back to the original nest site in this 5-d study. When subjected to food depletion, ~60% of the brood were found in the new nest site and ~40% of the brood remained in the original nest on Day 5, resulting in a polydomous population. In contrast, moisture depletion did not show any significant effect on colony movement. These results provide useful information about the causes of Pharaoh ant colony budding and guidance about how to develop effective control and prevention strategies.

Key Words: *Monomorium pharaonis*, induced disturbance, physical disturbance, chemical disturbance, nest relocation.

Frequent nest relocation is well documented in social insect colonies, especially in ants (Tsuji 1988, McGlynn et al. 2004, McGlynn 2012). Budding, also known as sociotomy, is defined as the gradual process of colony division of a group of queens and worker ants from the original colony to establish a new colony at a new location (Vail and Williams 1994), and it functions as a reproduction strategy (Hölldobler and Wilson 1990). Numerous biotic and abiotic factors can initiate budding, including changes in weather and humidity (Gordon 1992, Gordon et al. 2001, Heller et al. 2008), exposure to pyrethroid insecticides (Lee et al. 1999, Buczkowski et al. 2005), physical nest disturbance (Möglicht 1978), increases in foraging distance (Holway and Case 2000), intra- and interspecific competition (Smallwood 1982), escape from parasites (Gordon 1992), and predation (McGlynn et al. 2004). Under such circumstances, budding is vital to ensure the survival of the colony (Buczkowski and Bennett 2009).

Pharaoh ant exhibits extensive budding behavior but does not perform nuptial flights; hence, it could be initiated at any time (Wilson 1971, Passera 1994). Reproduction by budding have advantage because in this reproductive strategy, each nest must produce fertile queens independently (Buczkowski et al. 2005, Tay et al. 2014), which, in turn, increases the rate of colony growth, which may worsen an infestation. Pharaoh ant colonies are also prone to budding as a result of various induced disturbances (Buczkowski and Bennett 2009). During budding, the original nest site is frequently abandoned, resulting in nest relocation. Nest relocation is considered to be an important component of the life history of an ant colony (McGlynn 2012), involving highly coordinated process and complex decision making considering the costs and benefits of the relocation (Tsuji 1988). Despite the energy to locate new nest site, it is necessary for the survival of the colony (Buczkowski and Bennett 2009). In some cases, budding may lead to polydomy, in which ants live within a network of multiple related nests near the main colony, usually within a confined area (Passera 1994, Tsutsui and Suarez 2003).

The Pharaoh ant, *Monomorium pharaonis* (L.), is an important structure-inesting pest ant species. Flexibility in colony size and caste number gives Pharaoh ants the ability to establish a new colony rapidly with a small numbers of individuals (Eötvös et al. 2004, Buczkowski and Bennett 2009, Schmidt et al. 2011, Tay et al. 2014). In addition, they can nest in various temporary nesting sites prior to budding (Wheeler 1910), and have the tendency to abandon the old nest and migrate to a new nest site (Passera 1994). The management of Pharaoh ants is challenging because their ability to bud to establish new colonies limits the effectiveness of residual
insecticide sprays (Buczowski et al. 2005). The frequent budding behavior in Pharaoh ant colonies makes them difficult to eradicate, resulting in their success in human living environments (Buczkowski et al. 2005)

Little is known about the causes and consequences of budding of Pharaoh ant colonies. Information about the human-induced disturbances that cause budding will provide us with a better understanding of the process of sociotomy that could ultimately be used to develop ant management and prevention strategies. Buczkowski and Bennett (2009) described nest movement of Pharaoh ants after different intensities of physical nest disturbances. However, no experimental and comparative study has been conducted to evaluate the effects of various types of disturbances that trigger colony budding of *M. pharaonis*. Therefore, in this study, we evaluated the effects of different types of induced disturbances on nest relocation behavior in Pharaoh ant laboratory colonies.

**Materials and Methods**

**Ant Colonies.** Experimental colonies were obtained from established populations of *M. pharaonis* cultured since 2000 at the Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia. Late instars of the lobster cockroach, *Nauphoeta cinerea* (Olivier), canned tuna fish (TC Boy Marketing Sdn. Bhd., Shah Alam, Malaysia), hard-boiled egg yolk, a moisture source (an inverted cup filled with water), and 10% sucrose solution (5.5 cm in diameter and 1.7 cm in height) provisioned for all treatments except for moisture depletion (an inverted cup filled with water), and 10% sucrose solution were provided ad libitum in all experimental colonies. The colonies were maintained under environmental conditions of 26 ± 1°C and 60 ± 5% relative humidity.

**Experimental Design.** The experimental arenas consisted of two round polyethylene containers (12.5 cm in diameter and 10.0 cm in height) connected to each other (a source nest and a potential bud nest site) by a Tygon tube (5.0 cm; Fig. 1); the inner sides of the containers were coated with luhn (BioQuip, Rancho Dominguez, CA) to deter the ants from escaping. A Petri dish (9.5 cm in diameter and 1.7 cm in height) provisioned with folded corrugated paper (8 by 3 cm²) served as the artificial nest site in each container. In each experimental arena, 800 workers, 2 queens, and 0.3 g of brood were introduced into the source nest; the other container served as an empty potential nest site for budding. The ants were left to acclimate for 3 d.

The following five treatments were evaluated: physical disturbance, chemical disturbance, invasion by ghost ants, *Tapinoma melanocephalum* (F.), food depletion, and moisture depletion. A sixth undisturbed treatment served as the control. All disturbances were performed in the source nest. Physical disturbance consisted of removing all colony members from the Petri dish and placing them randomly within the round polyethylene container. For the chemical disturbance treatment, 100 Pharaoh ant workers were separated from established colonies cultured at the laboratory and sprayed with pyrethroid insecticide (0.001% of deltamethrin w/v; WellTech HealthCare Co., Ltd., Bangkok, Thailand). The nest covering of the source nest was removed, and the pyrethroid-treated ants were transferred into the nest. Subsequently, the nest covering was put back in its original position. For the invasion treatment, 100 live ghost ant workers that were obtained from established colonies cultured at the laboratory were introduced into the source nest. For the food or moisture depletion treatments, the resources were removed from the source nest. Four replicates were performed for each treatment and control. From photographs of the artificial nest sites taken from various angles to expose the colonies, the numbers of workers and the relative volumes of brood in the source and new nest sites were determined at 0 (before the treatment), 1, 3, and 5 d after the treatment.

**Statistical Analysis.** The percentages of workers (number of workers in the new nest site divide by total number of workers in both nest sites × 100%) and the estimated percentages of brood volumes in the new nest sites were arcsine square root transformed to meet the assumption of normality and homogeneity of variance. The differences in percentages of worker numbers and estimated percentages of brood volumes among the treatments at Day 5 were analyzed using one-way ANOVA. The differences in percentages of worker numbers and estimated percentages of brood volumes among Days 0, 1, 3, and 5 in each treatment were analyzed using one-way ANOVA. Means were then compared with Tukey's honestly significant difference (HSD) test. All analyses were performed using SPSS version 11.5 at *z* = 0.05 (SPSS Inc 2002).

**Results**

In control colonies, relatively low number of workers (<5%), which probably consisted of foragers, were present at the new nest site during the observation period. At Day 5 posttreatment, significantly higher percentages of workers and percentages of brood were present in the new nest site compared with the control for all treatments except for moisture depletion (worker, *F* = 131.81, df = 5, 18, *P* < 0.05; brood, *F* = 607.36, df = 5, 18, *P* < 0.05; Fig. 2). At Day 5, a significantly higher percentage of workers was recorded in the new nest site in colonies subjected to chemical disturbance compared with colonies subjected to other forms of disturbance and the control. The next highest percentages were found in colonies subjected to physical disturbance and invasion by *T. melanocephalum*, which did not differ significantly from each other. At Day 5, a significantly higher percentage of brood was recorded in the new nest site in colonies subjected to chemical disturbance, physical disturbance, and invasion by *T. melanocephalum* compared with colonies subjected to other forms of disturbance and the control, and there was no significant difference among those three treatments. The next highest percentage was found in colonies subjected to food depletion. In colonies subjected to moisture depletion, only 6.16 ± 1.68% of workers and no brood were found in the new nest site, and these values did not differ significantly from those of the control (Fig. 2).
Compared with Day 0, significantly higher percentages of workers and percentages of brood were recorded in the new nest site at Days 1, 3, and 5 in colonies subjected to physical disturbance (worker, $F = 218.68$, df = 3, 12, $P < 0.05$; brood, $F = 640.66$, df = 3, 12, $P < 0.05$), chemical disturbance (worker, $F = 408.43$, df = 3, 12, $P < 0.05$; brood, $F = 1816.10$, df = 3, 12, $P < 0.05$), and invasion by ghost ants (worker, $F = 136.05$, df = 3, 12, $P < 0.05$; brood, $F = 1948.59$, df = 3, 12, $P < 0.05$; Fig. 3). In these treatments, the entire brood was relocated to the new nest site at Day 1 and was never relocated to the original nest. The workers were also observed mainly in the new nest site from Day 1 onwards.

Fig. 1. The experimental arenas.

Fig. 2. Percentage of workers and brood (mean ± SE) in the new nest site at Day 5 after being subjected to various induced disturbances: (A) control, (B) physical disturbance, (C) chemical disturbance, (D) invasion by T. melanocephalum, (E) food depletion, and (F) moisture depletion. For each caste, means with the same letter are not significantly different at $P = 0.05$ (Tukey’s HSD).
In the food depletion treatment, no significant difference in percentages of workers and percentages of brood were observed in the new nest site between Day 0 and Day 3. However, \( \approx 55\% \) of workers and \( \approx 64\% \) of brood had moved to the new nest site by Day 5, resulting in a polydomous state (worker, \( F = 60.40, \text{df} = 3, 12, P < 0.05 \); brood, \( F = 241.79, \text{df} = 3, 12, P < 0.05 \); Fig. 3).

Discussion

Physical and Chemical Disturbances. The physical and chemical treatments tested in this study, which simulated anthropogenic disturbances, and invasion by *T. melanocephalum* workers, triggered immediate movement of the Pharaoh ant colonies from the source nest to the new nest site. The colony movements following physical disturbance can explain the frequent nest moving behavior of opportunistic Pharaoh ant colonies in the field, where they often nest in human living environment that are prone to frequent physical disturbance due to human activities. Pharaoh ant colonies bud to seek a safer nest site immediately after being subjected to physical and environmental disturbances (Buczkowski and Bennett 2009, Schmidt et al. 2011). Our results also suggest that bait stations should not be placed at locations that are subject to frequent
physical disturbance in order to maximize the number of ants that feed on the bait (Buczkowski and Bennett 2009).

The chemical treatment caused immediate one-way movement of the entire brood, which showed that *M. pharaonis* is strongly repelled by deltamethrin. Budding following a chemical treatment helps Pharaoh ant colonies survive insecticide applications (Buczkowski et al. 2005). Chemical insecticidal spraying is a major factor promoting budding and should be avoided in Pharaoh ant management because it can worsen the state of infestation. Bait stations contaminated with insecticides or other chemicals may also repel the ants.

**Invasion by Heterospecific Ants.** Competition and interspecific aggression often occur between sympatric ants, including tramp species (Smallwood 1982, and interspecific aggression often occur between sympatric species (Smallwood 1982)). Chemical insecticidal spraying is a major factor promoting budding and should be avoided in Pharaoh ant management because it can worsen the state of infestation. Bait stations contaminated with insecticides or other chemicals may also repel the ants.

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**Food and Moisture Depletions.** When the food source was removed from the source nest in our study, about half of Pharaoh ant workers (~55%) carrying brood (~64%) moved to the new nest site, although it took longer (5 d) to initiate budding compared with when subjected to physical, chemical, and heterospecific ant disturbances. Further investigation is needed to determine whether division of the colony into two equally sized nests worsens infestation compared with complete relocation of a whole nest. The observed delayed response to food depletion is comparable with results of a previous study showing that *Linepithema humile* (Mayr) colonies moved to a location close to food 10 d after food depletion (Holway and Case 2000). In our study, the *M. pharaonis* colonies probably still had a sufficient amount of food in the source nest at Day 3, but it likely became insufficient by Day 5. Budding may be initiated when colonies are deprived of food, as budding reportedly can enhance foraging efficiency and rapid food distribution in *M. pharaonis* (Buczkowski and Bennett 2009) and *L. humile* (Holway and Case 2000). Because Pharaoh ant colonies are well adapted to the human living environment, colonies probably bud to facilitate access to human food in the field. Hence, cultural practice, such as removal of all competing food debris prior to bait placement, is an important strategy in ant management programs.

In contrast to food depletion, Pharaoh ants were not affected by moisture depletion, as the majority of the workers remained in the source nest with the brood and queens after treatment. The relative humidity in the laboratory environment might be sufficient for the colonies and the sucrose solution probably has provided enough moisture for the colonies. Only a few workers (<5%) were found in the new nest site close to food and water, and these ants likely were foraging workers (Fig. 3). This result is in striking contrast to the extremely sensitive response of another tramp species, *T. melanocephalum*, to desiccation (Appel et al. 2004). Moisture level or rainfall was also found to affect nest moving of the outdoor species *Tapinoma sessile* (Say) (Buczkowski and Bennett 2008) and *Pogonomyrmex barbatus* (F. Smith) (Gordon 1992). However, Pharaoh ant colonies usually nest in the human environment (Lee 2002) and have a high tolerance to a wide range of relative humidity values (Leow 2013), which may contribute to this species’ worldwide distribution and high pest status (Wetterer 2010).

This study revealed that for *M. pharaonis*, physical and chemical disturbances and invasion by heterospecific ants can be major factors that trigger abandonment of the source nest and movement to the new nest site within a short period of time, whereas the colonies split to become polydomous upon food depletion. Because Pharaoh ant colonies nest in artificial nest, the laboratory environment does not differ very much from the actual situation of field colonies; hence, the results obtained in the laboratory are useful for providing insights about how the colonies respond to various artificially induced disturbances. Budding is a major cause of the invasiveness of this important urban pest ant, and our results may aid in the development of methods to control and prevent reinfestation of this polydomous species in the future. We found that application of pyrethroid insecticides may promote budding and may result in more severe infestations. Alternatively, use of the slow acting juvenile hormone analogue pyriproxifen can be useful for controlling Pharaoh ants in locations where numerous colonies exist following a budding event (Vail et al. 1996, Oi et al. 2000, Tay and Lee 2014). This study provides simple ideas about the immediate responses of the colonies to various induced disturbances. However, it is possible that the colonies would have re-occupied the abandoned source nest given a longer time (Harris 1991, Vail 1996). Future research should be performed in a longer duration of study. Future work for chemical treatment should also be performed by using insecticides that exhibit delay in mortality to simulate a scenario where the foraging ants return to the nest upon exposure to residual treatment.

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