

Mating triggers queen elimination by workers of Japanese harvester ant (*Messor aciculatus*)

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Abstract

During reproduction, ant colonies produce winged queens. These new queens usually leave the nest to mate and can then establish a new nest. If the new nest is close to an existing colony, it will be in competition with the existing colony. Therefore, workers will kill any mated queens they find outside the colony during the reproductive season. In this study, factors that might determine whether workers eliminate queens were investigated. Mating status (mated or unmated), colony origin (same or different to tested workers) and mating partners (inbred or outbred) of the queens of Japanese harvester ants (*Messor aciculatus*) were manipulated and the workers' behavior towards the queens was observed. Mated queens were always attacked by workers, though this was not affected by either colony origin or mating partners. These results suggest that mating status triggers elimination of queens by workers, and that the colony origin and mating partner are unlikely to be important roles in elimination of queens.

Keywords

harvester ant, intraspecific competition, mating partner

Introduction

Ants are important components of ecosystems across the globe (Folgarait 1998). Their population size is affected by intraspecific competition for limited resources (Connell 1961; Paine 1966; Dayton 1971; Ryti and Case 1984, 1988; Hölldobler and Wilson 1990). Intraspecific competition occurs directly (interference competition) and/or indirectly (exploitative competition) among colonies (Cerdá et al. 2013). Intensity of intraspecific competition is related to distance between their colonies (Wiernasz and Cole 1995; Gordon and Kulig 1996). Elimination of newly founded nests by established colonies affects intercolony distance (Fellers 1987; Savolainen and Vepsäläinen 1988; Andersen 1991), because many ants are sessile animals which cannot change the nest site once they established their nest (Hölldobler and Wilson 1990).

Seed harvester ants are ecologically important invertebrates in deserts and grasslands (Brown and Davidson 1977; Hölldobler and Wilson 1990). Foundress queens establish new colonies without assistance from workers. Since freshly mated queens have no workers to protect them, they are vulnerable to attack by workers from neighboring colonies (Levings and Franks 1982; Pfennig 1995; Gordon and Kulig 1996; Johnson 2001; Wiernasz and Cole 2003). As a result, a mortality of first year colonies is more than 99% in some species (Harrison and Gentry 1981; Ryti and Case 1988; Pfennig 1995; Wiernasz and Cole 1995; Gordon and Kulig 1996). On the other hand, the mortality of colonies survived to second year is as low as older colonies (Gordon 1991; Keeler 1993; Wiernasz and Cole 1995; Gordon and Kulig 1996). Therefore, it would be important for established colonies to eliminate the freshly mated queens arrived near their nest to decrease the intraspecific competitors.

Japanese harvester ants (*Messor aciculatus*) experience intraspecific competition for resources, including the seeds of Poaceae. Colonies of harvester ants can be found in close proximity to one another, sometimes only 1.5 m apart (Yamaguchi 1995). This results in overlapping foraging ranges and competitive interactions, including ritualized combat and food robbing, are performed toward individuals from the different colonies (Yamaguchi 1995). When a colony is removed, adjacent colonies decrease the frequencies of seed stealing and worker elimination, leading to an increased feeding rate (Yamaguchi 1995). This indicates that intraspecific interference occurs among colonies of *M. aciculatus*.

In spring, winged queens perform a mating flight and mate with males, then shed their wings. After mating, single or multiple queens may establish a nest, and these founding queens remain in the colony after the appearance of the first workers in *M. aciculatus*. The closer a founding nest is to a mature nest, the higher the chance that it will be in competition with that colony (Yamaguchi 1995). The workers in that colony therefore kill any newly mated queens. In this study, the factors that determine elimination of queens by workers were investigated. Colony origin (the same or different colonies to the workers), mating status (mated or unmated), and mating partners (inbred or outbred)

of the queens were experimentally manipulated, and the workers' behavior towards the queens was observed. If workers react less aggressively toward queens originated from their colony, it may cause spatial aggregation of related colonies.

Materials and Methods

Study Species and Collection

From April to May 2008, more than 30 unmated, winged queens and 50 males that appeared to their nest entrance before mating flight were collected from each of 8 colonies in a field area of 900 m² in Yachimata, Chiba, Japan. Males were also collected from an additional 7 colonies in the same area. The queens were randomly divided into 3 groups: those that remained unmated throughout the experiment (unmated), those mated with a male from a different colony (outbred), and those mated with a male from the same colony (inbred). The queens were caged (40 × 25 × 27 cm) with 100–150 males to mate and were observed for a maximum of 30 min. The queens that had mated were individually transferred from the cage to a plastic cup (height 4 cm, diameter 6 cm) and left to shed their wings. Mated queens that had shed their wings were used for the experiment (outbred and inbred). Unmated queens were also caged with males, but experimenters manually obstructed mating by sweeping males from queens with a brush when males attempted copulation. Workers were collected from 15 colonies after the winged individuals flew away. These workers were used to test aggression toward queens, and to make a group with workers (worker) in which the workers, instead of queens, were subjected to the attack from workers from the same or different colonies.

Aggression Test

Aggression tests were performed in a container (8 cm diameter, 4 cm height) at 20 ± 2 °C. One queen was placed in the container with 10 workers. The workers originated from the same or different colonies to the focal queens. Ants were observed using blind observations for 1 min or until the first attack on the queen by workers, whichever happened first. Attacking was defined as opening the mandibles and walking toward the focal ant to bite. The time until the first attack was recorded. If an attack did not occur, the trials were used only for comparisons of attack rate, but not time until attack. Observations were conducted within 30 min of mating and 60 min of the ants' collection from the colonies. The same observations were carried out with workers as the focal point instead of queens.

Statistical Analysis

Within each of the three treatment groups with workers from the same colonies, the proportion of trials where an attack occurred was compared to the proportion of trials where an attack did not occur using Fisher's exact test. Similar comparisons were conducted with the two treatment groups with workers from the different colonies and the worker group.

Mann-Whitney U tests were performed to test for significant differences in the time until the first attack among the three treatment groups with workers from the same colonies and among the two treatment groups with workers from the different colonies and the worker group. P values were corrected using the Bonferroni method.

Results and Discussion

Attacks by workers from the same colonies occurred in all trials in the group of individuals that were mated regardless of the queens' mating partners (Fig. 1). Attacks were only observed in 1 of 17 trials in the group of unmated individuals. The proportion of trials in which the attacks occurred was significantly higher with outbred or inbred queens than unmated queens (Fisher's exact test, outbred vs. unmated, $P < 0.001$; inbred vs. unmated, $P < 0.001$). No attacks by the workers from the same colonies were observed in the worker group (Fig. 2).

There was no significant difference in time until first attack toward outbred or inbred queens by workers from the same colonies (outbred, $n = 24$, $7.1 \text{ s} \pm 9.9$ (mean $\pm$ SD); inbred, $n = 21$, $6.5 \text{ s} \pm 8.3$, Mann-Whitney U test, $U = 238.5$, $P = 0.77$). One unmated queen was attacked by workers from the same colonies after 50 s.

Attacks occurred in all trials in the outbred and unmated groups with workers from different colonies, and 10 of 25 trials in the workers group. The proportion of trials in which the attacks occurred was significantly higher with queens than workers (Fisher's exact test, outbred vs. worker, $P < 0.001$, unmated vs. worker, $P < 0.001$).

There was no significant difference in time until first attack toward outbred and unmated queens by workers from the different colonies (outbred, $n = 27$, $9.5 \text{ s} \pm 9.6$, unmated, $n = 22$, $7.2 \text{ s} \pm 7.7$, Mann-Whitney U test, $U = 247.5$, $P = 0.98$). The first attack on workers from the different colonies happened after a significantly longer time than the first attack on queens (workers, $n = 10$, $35.7 \text{ s} \pm 10.6$; workers vs. outbred, Mann-Whitney U test, $U = 17.5$, $P < 0.001$; workers vs. unmated, $U = 8.5$, $P < 0.001$).

In this study, ant workers' reactions to new queens were investigated, depending on their mating experience, mating partner and colony origin. We found that both mated and unmated queens were attacked by the workers from the different colonies. The workers' aggression toward mated queens occurred even when the queens were originated from the same colonies to the workers. Unmated queens were not attacked by the workers from the same colonies. The origin of mating partners of queens did not alter these reactions by workers. These results suggest that whether the queens are future competitors affects workers aggression, and workers attack mated queens irrespective of relatedness to the queens. This pattern of queen elimination may contribute to decrease the number of neighboring competitors and would not cause spatial aggregation of related colonies.

In ant societies, established colonies with superior number of workers have an advantage in intraspecific competition and the outcome of the competition affect fitness components. *M. aciculatus* workers are usually observed outside of their nest during the foraging season in October and November (Terayama et al. 2014). However, workers can be seen wandering around their nest entrances before and after winged individuals take their mating flight. These workers may be patrolling and act aggressively toward mated queens. Since worker aggression is task dependent (Sturgis and Gordon 2012), this may explain why workers attack mated queens more often and faster than workers from different colonies. Elimination of mated queens prevents them from creating new colonies in close proximity, which can lead to avoidance of the future risk of competition. The colony may gain a fitness benefit by eliminating mated queens.

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Acknowledgements

We thank VAIC-CCI Yachimata centre for permitting us to collect ants. We are grateful to Michiko Fukumoto, Yuya Fukumoto and staff members of VAIC-CCI Yachimata centre for help collecting the queens. We thank two anonymous referees for their constructive comments.

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Figures



Figure 1 Proportion of attacks from workers. Numbers in parentheses indicate sample sizes.

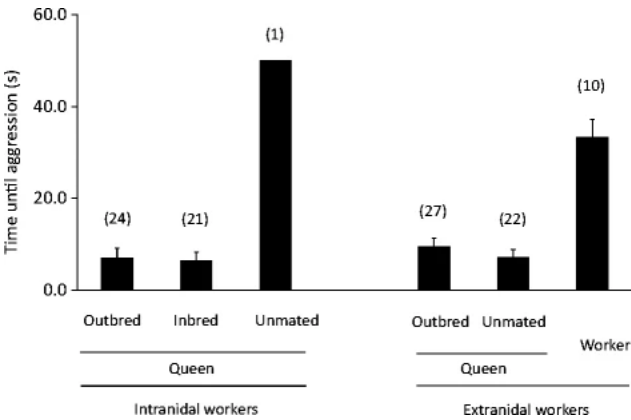


Figure 2 Time until aggression from workers. Numbers in parentheses indicate sample sizes. Error bars indicate SE.