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SHORT NOTE

Mating Behavior of the Small Carpenter Ant, *Camponotus yamaokai*

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Abstract

Ants are known to show diverse mating behaviors across different species, but detailed reports on such behaviors are limited to only 0.7% of ant species. Therefore, we report on the mating behavior of the alate females and males in the small carpenter ant, *Camponotus yamaokai*. Its process was classified into two phases: mating attempt and copulation. Out of the 53 pairs we observed, 23 pairs engaged in a total of 36 mating attempts. The mating attempt durations in cases where copulation failed were significantly longer than those leading to copulation. Among these attempts, copulation was observed ten times in 9 pairs, including multiple mating in one pair. The copulation lasted for 24.0 ± 5.1 seconds (mean ± SD). At the end of copulation, males pulled out their genitalia quickly to avoid potential attacks from the females. This behavior might be a strategy of males to minimize the risk of mortality during a single mating and support them in engaging in multiple matings.

Formicidae has diverse mating systems, broadly categorized into three main types: male-aggregation syndrome (MA), female calling syndrome (FC), and intranidal mating (Hölldobler & Bartz, 1985). In species with MA, alate females and males leave their natal nest simultaneously. Males form large swarms in which females fly to mate (Hölldobler & Bartz, 1985). In species with FC, alate females attempt to attract males with pheromones, and alate males actively search for females during flight (Hölldobler & Bartz, 1985). Most Formicidae species have one of the two mating systems mentioned above. However, some engage in intranidal mating, which occurs within the nest without flight (Hölldobler & Wilson, 1990).

While mating systems are reasonably known, information on ant mating behavior remains limited (Baer, 2011). About 0.7% of the recognized >14,000 ant species have been reported on mating behavior (Baer, 2011; Shik et al., 2012). This low percentage of mating behavior description is because many ants mate on flight (Hölldobler & Wilson, 1990), limiting the possibility of observing copulations or conducting experimental works (Bear, 2011).

Formica aquilonia with FC is one of the few species for which mating behavior has been extensively reported. The mating behavior of this species is classified into a courting phase, a still phase, and a terminal phase, with the durations and general behaviors for each phase described (Fortelius, 2005). Similar behaviors have been observed in the genus *Formica*. Behaviors characteristic in the mating of genus *Formica* include the females' rejection behavior during the courting phase and the males' releasing legs and turning over on the back of the females once the genitalia is inserted into the females during the still phase. (Kannowski, 1963).

In *Cardiocondyla* species, which engage in intranidal mating, the mating behavior is classified into four phases:

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recognition, pre-copulatory behavior, copulation, and post-copulatory behavior (Mercier et al., 2007). Notable behaviors include the males' striking females' heads with mandibles and/or antennae during pre-copulatory behavior, the remarkably brief duration of mating (around 3 seconds in *C. elegans*), and the single distinctive 'kiss' in post-copulatory behavior. This kiss triggers the males to move forward on the females' backs and bring their mandibles in close contact with those of the females.

To better understand mating behaviors in ants, it is essential to accumulate descriptions of behaviors in various species. Thus, in this study, we provide data on the mating behavior of the small carpenter ant, *Camponotus yamaokai*. This species nests in the hollows of dead wood branches or bamboo stems and often dominates the arboreal ant communities in evergreen broad-leaved forests in Japan (Terayama & Satoh, 1990). The colony of this species contains multiple functional queens (Terayama et al., 2014). The alates are thought to mate inside the nest in spring without nuptial flight (Terayama et al., 2014). *Camponotus yamaokai* shows polydomy, where a colony consists of multiple nests (Satoh, 1991). Satoh et al. (1997) reported that colony members of *C. yamaokai* are genetically close, suggesting that this species reproduces via sib-mating, at least occasionally. However, mating behavior in *C. yamaokai* has not been observed. To clarify the mating pattern of *C. yamaokai*, we conducted laboratory observations of mating behavior using multiple pairs of alates, providing detailed descriptions of mating behaviors and their durations.

Nests of *Camponotus yamaokai* were collected in Hachioji, Tokyo, Japan, on October 10, 2011. The nests were fed on a semiweekly 10% sucrose solution, the commercial protein diet for honeybees (Bee Hatcher: protein 9.7%, carbohydrate 1.0%, fat 0.1%, fiber 0.1%; FEED ONE), and frozen honeybee drone adults (*Apis mellifera*). In May 2012, a single alate female and male (considered as one pair) from different nests were placed in a plastic container (1.5 cm × 5.5 cm × 8.5 cm) for observations (a petri dish with a diameter of 3.5 cm was used to create Fig 1). The ants were observed until copulation occurred, or both remained still for five minutes. The observations were conducted on 53 pairs at 22 ± 1 °C in light conditions.

We video-recorded and observed the mating behaviors of each pair of alates. The acting behaviors were classified into two phases: mating attempts and copulation. Mating attempts were defined as the period from "when a male recognizes a female and begins antennation until genitalia insertion". If genitalia insertion did not occur, it was defined as the time until a male dismounted from a female. Copulation was defined as the period from "genitalia insertion until the separation of a male and a female". Detailed descriptions of behaviors and time measurements were conducted for each phase of the mating attempt and copulation". The females that copulated with the males during the mating experiments were maintained with the three nestmate workers in captivity to verify the production of daughter workers via fertilized eggs.

The males antennated the females before mating attempts (Fig 1a). Once the mating attempt was started, the males jumped on and grasped the females. In contrast, the males' wings were raised and shaken (Fig 1b). Engaging in antennation with the females did not always lead to a mating attempt by the males. The females often escaped from the males after the encounter. Even when the females stayed after antennation by the males, the males did not always start the mating attempts. During the observations of the 53 pairs, 36 mating attempts were recorded in 23 pairs. When on the females, the males shook their bodies and kept antennating the females' bodies (Fig 1c). The males stroked the females' gasters slightly laterally with his gasters, attempting to insert genitalia into the females' genitals. The females sometimes raised their antennae to touch the males on the back. The duration of mating attempts was 68.5 ± 40.8 seconds (mean ± SD, range: 21–174 s, n = 26) for the attempts where genitalia insertion failed and 14.6 ± 17.1 seconds (range: 4–62 s, n = 10) for the attempts where genitalia insertion was successful.

Out of 36 mating attempts, genitalia insertion was observed ten times in 9 pairs, including a second mating in one pair, which occurred before the termination of observation. When pairs were in copula, the males opened their mid and hind legs and wings. The females walked in copulation, causing the males to turn over on their backs (Fig 1d,e). While the females dragged the males, the males slightly shook their antennae, with the tarsi of the forelegs touching each other and the mid and hind legs opened. The pairs remained in copula for 24.0 ± 5.1 seconds if the second mating was included and 22.9 ± 4.0 seconds if excluded (range: 17–34 s for both cases). At the end of copulation, the males started to move their legs. Then, the males assumed prone positions and pulled their genitalia from the females (Fig 1f). The females did not actively participate in the extraction of the males' genitalia, except for one case in which the females bit their mate males at the end of copulation.

The mated females, kept with the workers, laid eggs, and the eggs developed into the workers in a few months. These results indicate that genitalia insertion results in sperm transfer.

The intranidal mating in *C. yamaokai* is likely to influence the mating activity of alates. In species that engage in nuptial flights, mating opportunities are limited in the flight period, so alates of such species would be expected to attempt copulation immediately upon encounter. This behavior could be advantageous in reducing predation risk by natural enemies (e.g., O'Neill, 1994). However, as *C. yamaokai* engages in intranidal mating, mating opportunities are relatively unrestricted, and the associated predation risk during mating is expected to be low. Therefore, females and males of this species might not always exhibit high mating activity. Indeed, only 23 out of the 53 pairs attempted mating. What causes the rise in mating activity in *C. yamaokai* remains unclear.

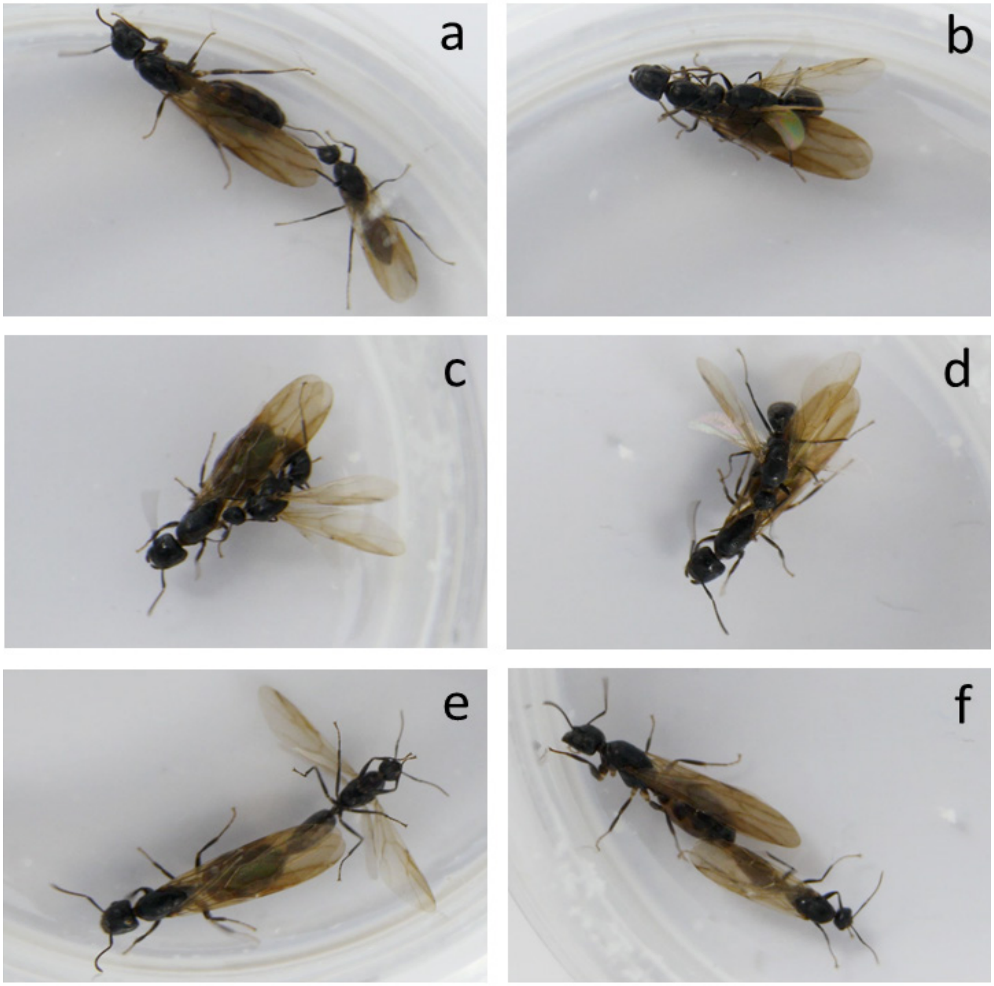

Fig 1. Mating behaviors of *Camponotus yamaokai*. a: the male antennating the female; b: the male jumping on and grasping the female; c: the male attempting to insert genitalia into the female's genitals; d, e: after successful genitalia insertion, causing the male to turn over on his back; f: the male assuming prone positions and withdrawing his genitalia from the female.

Studying the process leading to mating in the field may help to clarify the factors that increase mating activity in species that engage in intranidal mating.

Unlike mating activity, intranidal mating may not consistently impact copulation duration. The copulation duration varies depending on the ant species (Baer, 2011). While most species engage in copulation for approximately 30 seconds to 5 minutes, *Cardiocondyla* species show remarkably short copulation durations of 2–3 seconds. In contrast, *Diacamma* species show exceptionally long copulation durations, lasting from 1 hour to 2 days (Mercier et al., 2007; Fukumoto et al., 1989; Allard et al., 2007). In *C. yamaokai*, the copulation duration averages 24.0 seconds, somewhat shorter or approximately the same as other ant species (Baer, 2011). In species that also mate inside the nest, copulation durations have been reported for *Cardiocondyla* species and *Linepithema humile*: In *Cardiocondyla* species, it ranges from 255 seconds (Mercier et al., 2007), and in *Linepithema humile*, it is 6 minutes (Keller & Passera, 1992). Therefore, the intranidal mating system is not necessarily correlated with copulation duration.

The males' behaviors during mating attempts and copulations in *C. yamaokai*, such as grasping females' backs, spreading wings, and body posture, were similar to those reported in several *Formica* species (Fortelius, 2005; Kannowski, 1963). However, the behaviors at the end of copulation differed from those demonstrated in the previous studies. In *F. aquilonia*, the terminal phase is notably lengthy, accounting for 50% of the total mating duration. During this phase, the females display rapid movements and bite the males. Post-mating biting behavior by females has been observed in multiple ant species, such as *F. subintegra*, where the females begin biting the males' posterior gaster for 6 to 30 seconds after genitalia insertion, continuing for 4 seconds to over 2 minutes (Kannowski, 1963). In *F. rufa*, the females bite and sever the males' gaster during mating termination (Marikovsky, 1961). However, a clear terminal phase was not identified in *C. yamaokai*. Biting behavior by the female was observed in only one pair, with the males quickly separating from the females when he rose, suggesting that males may be avoiding physical damage by withdrawing their genitalia before being attacked by the females. Even if males engaging in MA or FC were capable of multiple mating, flying to search for other females after the initial mating poses risks of predation and the possibility of not finding other females.

Therefore, ensuring the success of the initial mating might be more adaptive for males even in the presence of the ability for multiple mating. In *Diacamma* species, which mate within the nest, males continue to be physically connected for several days until workers eventually dismember them, although it has been observed that sperm transfer is completed in 15 minutes in *D. pallidum* (Allard et al., 2007). This extreme form of mate guarding suggests that it is advantageous for males, facing limited mating opportunities, to invest fully in one mating (Allard et al., 2007). In contrast, it is expected to find multiple females within a single nest of *C. yamaokai* (Terayama et al., 2014). Additionally, because *C. yamaokai* conducts mating inside the nest, the risk of male death during female searching is low. Therefore, the quick withdrawal of the genitalia by the males before being attacked by the females might be a strategy that minimizes the investment required for a single mating event, supporting males to engage in multiple mating. In ants, multiple mating by males is uncommon. Species that are capable of multiple mating by males often show characteristics such as having the ability of sperm reproduction in males after mating, having a surplus of sperm in seminal vesicles beyond what females need for colony formation, and long male lifespan (Heinze & Holldobler, 1993; Shik et al., 2012). In future research on *C. yamaokai*, it will be necessary to verify the males' potential for multiple mating by investigating their lifespans, microscopic examinations of testicular tissues, and behavioral observations.

Multiple female mating has been observed in several ants (Hughes et al., 2007). It enhances genetic diversity within the colony, leading to increased colony performance (Keller & Reeve, 1995). However, females may not utilize the sperm from all mated males equally. For example, in *Leptothorax gredleri*, despite the single female mates with up to four males, the microsatellite analysis has revealed that the workers are descendants of only a single male (Oberstadt & Heinze, 2003). To reveal effective multiple mating in females of *C. yamaokai* it is crucial to verify whether the descendants of a single queen include multiple patrilines.

Author's Contribution

KC: Writing, Data curation;

K I: Review & Editing;

KN: Review & Editing;

KY: Review & Editing;

T S: Review & Editing;

SK: Investigation, Writing, Supervision.

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