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Sex and age related changes in the locomotor activity and phototactic behaviors of two closely related species of *Camponotus* ants

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ABSTRACT

A virgin ant queen has only one opportunity in her lifetime to realize her reproductive fitness when she leaves her nest for a mating flight. After successful mating she sheds her wings, excavates a nest and starts laying eggs to initiate her own colony. Here we report the results of our study on two related species of *Camponotus* ants – day active *Camponotus paria* and night active *Camponotus compressus* – aimed at investigating (i) if there exist inter-species differences in the activity and phototactic behaviors of males and queens, (ii) whether these behaviors in the queen change after mating, and (iii) whether the activity rhythm of queens changes with age. We find that while activity profiles differ between *C. paria* and *C. compressus* virgin males and queens, such differences in queens disappear after mating. Once mated, the activity rhythm of queens shows little change with age; the rhythm in virgin queens, on the other hand, changes considerably. As virgins, *C. paria* queens are positively phototactic, while *C. compressus* queens are negatively phototactic. After mating, *C. paria* queens become less phototactic, particularly during the subjective night, while *C. compressus* queens remain negatively phototactic. These results indicate that there are considerable differences in the activity and phototactic behaviors of virgin queens of the two related species of *Camponotus* ants. Most of these differences disappear after mating, which suggests that these behaviors may have evolved primarily for the proper execution of pre-mating events.

1. Introduction

The ability to keep time is of prime importance to social insects especially for the production of sexual castes, scheduling nuptial flight, and for establishing new colonies (Tauber et al., 1986). In ants, mating is once-in-a-lifetime event and therefore should be precisely timed and effectively executed to ensure maximum reproductive fitness (Hölldobler and Wilson, 1990). Time of mating in ants is species-specific and is largely influenced by environmental factors such as temperature, light intensity, and availability of food (Talbot, 1945; Hölldobler and Wilson, 1990). In the harvester ant *Veromessor andrei*, males leave the nest for mating flight just before dawn, while males of Argentine ant *Iridomyrmex humilis* leave during the last 2 h before night (Hölldobler and Wilson, 1990). The males of African *Dorylus* ant species *Dorylus moestus* fly out at sunset, *Dorylus burmeisteri* at sunrise, while of other species at different times during the night (Haddow et al., 1966). Similarly, virgin males and females of the fire ant species *Solenopsis saevissima* fly out for mating during the second half of the day, while those of *Camponotus clarithorax* do so during the first half of the day (McCluskey, 1965). These studies suggest that related species of ants temporally segregate their mating behavior (Haddow et al., 1966; Tauber et al., 2003), probably to secure better mates, and to avoid predators and competitors.

Long-term behavioral changes are critical in the life of most organisms, as it probably helps them to adapt to novel abiotic and biotic environments. Given that the societal roles of social animals change over age, young animals may use a different set of behavioral strategies compared to older ones (Julian and Gronenberg, 2002). Behavior of many animals changes during mating; elusive animals become apparent and submissive ones become aggressive (Alcock, 1989). After courtship is concluded they reduce the intensity of such behaviors or abandon them altogether. In many ant species, young winged queens are cared for by workers in their dark subterranean nests until they are ready for their mating flight. During such flights, winged males and queens from different colonies meet in-flight and copulate. While males die after this event, queens start a new and completely different phase of their life. In some species, young queens forage occasionally; while in other species they live a solitary life, laying eggs using her metabolic reserves (Wheeler and Buck, 1996). Mating in ants is followed by immediate, drastic long lasting changes in circadian

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rhythm (McCluskey, 1967). Since virgins do not return to the mother colony if the mating flight was not successful, and die within a few hours, it would be interesting to examine if such behavioral changes are due to the actual act of mating, or due to the flight associated with it, or due to aging.

Ants, like many other insects, are attracted towards light, a behavior which is believed to be of great adaptive value (Fraenkel and Gunn, 1961). Some ant species time their mating flight at different time of the day using ambient light intensity. In some species of ants light is perceived as an indicator of danger, and workers start moving pupae and larvae to the darker and safer portion of the nest. However, during mating flight young ant queens are likely to rely mostly on visual and chemical cues that they may not have experienced before. In some ant species, individuals collected from the upper part of the nest on the day of mating flight show greater preference for light, while those collected from the interiors of the nest show no preference for light or darkness (Julian and Gronenberg, 2002). Ant queens are less attracted by light prior to, and post mating than on the day of mating. Pre-flight virgins kept in the dark for several months continue to show positive phototactic behavior (Julian and Gronenberg, 2002), which suggests that change in light preference on the day of mating flight is because of mating, and not due to increase in age, and/or social structure of the colony. It would be interesting to study behavioral changes in ants at different times of the day, before and after mating flight, mating, and nest excavation. Further, it would be worthwhile to examine this in closely related but oppositely phased (day and night active) species of ants.

Many organisms undergo age related changes in their circadian rhythm (Brock, 1991; Valentinuzzi et al., 1997; McAuley et al., 2002). Such changes have also been reported in many insect species (Bloch and Robinson, 2001; Koh et al., 2006; Bloch, 2010). Among insects, ants are known to have extended lifespan; age of mated queens of some species has been reported to be as long as 20 years (Hölldobler and Wilson, 1990). Virgin ants do not live long in the wild and are often killed by predators, or die due to desiccation (Hölldobler and Wilson, 1990; Whitcomb et al., 1973; Levin et al., 2009). It would therefore be interesting to examine if ant queens undergo post-mating behavioral switching of circadian rhythm, and whether such changes are age-dependent, or a function of queen's mating status. To the best of our knowledge such a study has never been reported in any species of ant.

Our studies are based on two tropical species of carpenter ants, *Camponotus compressus* and *Camponotus paria*, which live in dark underground nests wherein light, temperature, and humidity are fairly constant (Hölldobler and Wilson, 1990). The workers of *C. paria* species are day active, while those of *C. compressus* are night active. Both species colonize fairly similar habitat; a colony of one species is frequently found adjacent to the other. In field conditions we have observed that virgin males and queens of *C. paria* leave their nest for mating flight at a specific time of the day, 30–45 min before *C. compressus*, which fly out exactly at dusk, and mate around dusk when the ambient light intensity falls a little below 10 lux (Shahnaz Rahman Lone and Vijay Kumar Sharma, personal observation). Although we know that circadian rhythms play a key role in the regulation of ant behaviors (McCluskey, 1967; North, 1987; Sharma et al., 2004a–c), our understanding of the consequence of mating on their circadian clocks is very preliminary. Since activity rhythm (McCluskey, 1967) and phototaxis behavior in ants (Julian and Gronenberg, 2002) have been shown to be closely associated with mating flights, it would be interesting to study these behaviors in the reproductives prior to and after mating.

Here we present the results of our study on two related species of *Camponotus* ants – the night active *C. compressus* and the day active *C. paria*, aimed at investigating (i) if there exist inter-species differences in activity and phototaxis behaviors of virgin males and queens, (ii) whether activity and phototaxis behaviors of queens change after mating, and (iii) whether the activity rhythm of queens change with age. The results provide interesting insights into sex and age related changes in circadian activity and phototactic behaviors of *Camponotus* queens.

2. Materials and methods

2.1. Background, collection, and maintenance of ants

Mating in *Camponotus* ants is a seasonal event; it occurs by the end of April or during May, soon after the first rains following a prolonged dry season (Lone et al., 2010). During this time, virgin males and queens are often seen shuttling in-and-out of their nests, probably to sample light. They eventually fly out of their nests in large numbers and are seen swarming near the mating sites. Males and females of *C. paria* leave their nests before dusk, when there is still some light, while those of *C. compressus* fly out in a short interval of time immediately after dusk.

On no occasion did we notice any inter-species mating between these two species, at least not under laboratory conditions. *C. paria* males do approach *C. compressus* queens but are chased, bitten, and eventually killed, while males of *C. compressus* were never seen to approach *C. paria* queens. Under laboratory conditions virgin queens of *C. compressus* survive up to a year, while those of *C. paria* only for a few months. Under a similar set of conditions, mated queens of both species survive for a minimum of 2 years. Males on the other hand survive for no more than 10 days. During the locomotor activity recordings, mated queens were found to lay eggs, which were cleared every 3–4 days (or as and when petri-plates were changed).

Virgin males and queens (winged) were collected from the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore campus (13°00′N, 77°32′E) while they were emerging from their nests for their mating flight. The mated queens were collected soon after mating, on the evening of their mating flight, while they were trying to dig into the soil. They were identified by the absence of wings, and by their ability to lay fertile eggs. To additionally confirm their reproductive status, plates in which they were kept were continuously monitored for fertile eggs. While we could easily identify the reproductive status of queens, their age remained unknown.

Freshly caught ants were immediately transferred individually into petri plates (diameter × height = 90 mm × 15 mm), and introduced under 12:12 h light/dark (LD) cycles, in an incubator maintained at constant temperature (25 ± 0.5 °C; mean ± SD), and humidity (~80%). The Petri-plates were cleaned on alternate days, at irregular hours with 5% ethanol. Care was taken to ensure minimal disturbance to the ants. Ants were provided with *ad libitum* food in the form of Bhatkar diet (Bhatkar and Whitcomb, 1970) and 10% dilute honey solution.

2.2. Assay and analyses of locomotor activity behavior

The locomotor activity behavior of ants was monitored individually using a recording device designed to track movements of small insects using two pairs of infrared emitters and receivers (Sharma, 2003). Data from the device was stored in 5 min bins for the analysis using CLOCKLAB software from Actimetrics, USA. The locomotor activity of virgin males was recorded for 4–5 days, while those of queens for 7–10 days, starting within 3–4 days of their collection from the field. The ants used in this study were – *C. paria* (CP) queens, CP males, *C. compressus* (CC) queens, and CC