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Avoid mistakes when choosing a new home: Nest choice and adoption of *Leptothorax* ant queens

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ABSTRACT

In ants, mating and colony founding are critical steps in the life of ant queens. Outside of their nests, young queens are exposed to intense predation. Therefore, they are expected to have evolved behavior to accurately and quickly locate a nesting place. However, data on the early life history of female reproductives are still lacking. *Leptothorax gredleri* is a suitable model organism to study the behavior of young queens. Reproductives can be reared under artificial conditions and readily mate in the laboratory. After mating, *L. gredleri* queens have the options to found solitarily, seek adoption into another colony, or return into their natal nest. In this study, we investigated the decision-making processes of female sexuals before and after mating. In particular, we tested whether female sexuals use chemical cues to find their way back to the nest, studied if they prefer their own nest over other nesting sites and followed the adoption dynamics of mated queens over 8 weeks (plus hibernation and spring). We showed that female sexuals and freshly mated queens spent more time on substrate previously used by workers from their own colony and from another colony than on a blank substrate. This discriminatory capability of queens appears to be lost in old, reproductive queens. Nest choice experiments showed that female sexuals and freshly mated queens can distinguish their own nest while old mated queens' do not. When reintroduced in their maternal colony, young queens were readily adopted, but a few weeks later aggression against young queens led to their emigration from the maternal nest and eventually also death.

1. Introduction

Mating and colony founding are usually critical steps in the life of ant queens (e.g., Whitcomb et al., 1973; Forti et al., 2012; Staab and Kleineidam, 2014). Outside of their nests, young queens are exposed to various predators, including workers from nearby ant colonies (Pontin, 1960; Whitcomb et al., 1973; Nichols and Sites, 1991; Forti et al., 2012). In order to avoid external threats, queens therefore attempt to shorten the time needed to find a mating place, a mating partner, and a suitable nest site. While queens of some species found new societies independently after mating, others shorten the risky phase of solitary founding by seeking adoption into their natal nests or by taking over another conspecific colony. They thus are protected and supported by nestmate workers during the initiation of their own colony (Foitzik and Heinze, 2001; Peeters and Molet, 2010; Cronin et al., 2013). Dependent colony founding is a common strategy particularly in species from temperate or boreal habitats (e.g., Buschinger, 1974; Heinze, 1993). Though it decreases the risks of solitary founding, it is also not without obstacles. If mating occurs away from the immediate vicinity of the natal nest, finding the way back to it might be a difficult task. Furthermore, adoption is also a sensitive stage. Whether young queens are allowed to enter a nest or not may be influenced by factors such as the type of sexuals produced by the colony (i.e., male-producing colonies execute most of the young queens that are introduced in the colony, Brown et al., 2003), their reproductive status (mated or unmated: Sundström, 1997; Holzer et al., 2008) and their origin (nestmate or foreign queens: Stuart et al., 1993; Meunier et al., 2011; Chernenko et al., 2012).

Young queens are expected to have evolved ways to accurately locate a nesting place and, in species with dependent founding, to increase their chances of being adopted (Wilson and Hunt, 1966; Kaspari and Vargo, 1994; Schooley and Bestelmeyer, 2000). Despite the obvious importance of post-mating decisions for social evolution, behavior of young queens has only rarely been studied (e.g., Julian and Gronenberg, 2002; Lone et al., 2012; Bernadou and Heinze, 2013). This is because data are not easily accessible: mating is often limited to a few days per year, reproductives are

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difficult to follow in the field, and sexuals of most ant species do not mate under laboratory conditions (Wilson and Hunt, 1966; Dunn et al., 2007).

The small myrmicine ant *Leptothorax gredleri* Mayr, 1855 is a suitable model organism to study the behavior of young queens. Reproductives can be reared under artificial conditions and readily mate in the laboratory (Oberstadt and Heinze, 2003; Oppelt and Heinze, 2009). Female sexuals of *L. gredleri* leave the natal nest, climb on nearby plants and release sexual pheromones from their extruded sting to attract males (“female calling”, Buschinger, 1968, 1971; Heinze et al., 1992). After mating, they have the options to found solitarily, seek adoption into another colony, or return into their natal nest (Heinze et al., 1992). In the latter cases, colonies may end up with several related queens that form a hierarchy in which only the top-ranking queen reproduces (“functional monogyny”, Buschinger, 1968; Heinze et al., 1992).

The aim of this study was to investigate the decision-making processes of *L. gredleri* female sexuals before and after mating. First, we checked if *L. gredleri* workers release chemical cues, e.g., cuticular hydrocarbons (CHCs), into their environment and tested whether female sexuals use them to find their way back to the nest and/or to avoid potentially dangerous areas (Devigne and de Biseau, 2012). Second, we studied if female sexuals prefer their natal nest site over other nesting sites. Because their preference might change with reproductive status we compared used and unused substrates and nest choice across different life stages (female sexuals with dark pigmentation, freshly mated queens, and old, reproductively active queens, Bernadou and Heinze, 2013; Devigne and de Biseau, 2012). Finally, we studied the adoption dynamics of mated queens over a long time period (8 weeks plus hibernation and subsequent spring). Our aim was not to quantify the formation of hierarchies or the fighting behavior among queens, which has been studied previously but to document the decision-making processes and the adoption success (Heinze and Lipski, 1990; Heinze et al., 1992; Heinze and Oberstadt, 2003).

2. Methods

2.1. Study organisms and life stages

Colonies of *L. gredleri* were collected from their nests in rotting twigs in small groups of pine trees in Erlangen, Germany (49° 35′ N, 11° 01′ E, elevation 290 m; see Bernadou and Heinze, 2013). Colonies were transferred to the lab in plastic bags and subsequently housed in incubators in three-chamber plastic nest boxes (9.5 cm × 9.5 cm × 3 cm) with a plaster floor under standard conditions (Heinze and Ortius, 1991; Oppelt and Heinze, 2009). A cavity provided by a plastic frame with a cardboard floor, sandwiched between two microscope slides served as nest (Heinze and Ortius, 1991). The colonies were fed with honey and cockroaches and the plaster moistened every 4–6 days. Incubator temperatures were kept at 10 °C night/20 °C day (spring) and 15 °C night/25 °C day (summer) (Oppelt and Heinze, 2009). Under these conditions, sexuals develop 2–3 months after the end of hibernation (Oppelt and Heinze, 2009). In total, 28 colonies were used for the experiments.

To study the behavior of female sexuals and queens, four female “stages” were used: dark female sexuals (with a fully pigmented body and a reduce activity), mature female sexuals (who were ready to mate and had actively left their nests), freshly mated queens (3–24 h after mating – see below), and old mated queens (egg-laying queens collected in the field or that had spent hibernation in the laboratory, for more details see Bernadou and Heinze, 2013).

2.2. Mating

Female and male sexuals from different colonies that had left their nest on their own accord during the peak period of sexual activity (on the morning between 10:00 and 12:00) were transferred into Petri dishes and exposed to bright sunlight. Sexuals readily mated under these conditions. A mating attempt was considered to be successful if males after the insertion of their aedeagus tilted backwards and remained immobile in this position for few seconds (Oberstadt and Heinze, 2003). Furthermore, successful mating immediately changes the behavior of the queen (e.g., Bernadou and Heinze, 2013). After mating, young queens were isolated in glass vials with a humid cotton plug for experiments 1–3.

2.3. Behavioral experiments

Behavioral experiments were done at a temperature of 24.5 ± 2 °C. We investigated if dark female sexuals, freshly mated queens, and old mated queens differ in their behavior (experiments 1–2). Freshly mated queens were tested in experiments 1 and 2 either on the same day or 24 h after mating. Dark female sexuals were never used twice on the same day in the same experiment. As we did not mark dark female sexuals we cannot exclude that several individuals were used in the same experiment on different days. As colonies contained dozens of female sexuals and not all colonies were used in experiments 1 and 2, the number of female sexuals that had been used twice is likely negligible. Old mated queens were tested in March–April and October while dark female sexuals and freshly mated queens were tested in May–June and July–August according to their availability in nests (experiments 1–2).

2.3.1. Experiment 1: locomotor behavior on substrate previously used or unused by ants

In this experiment, we tested whether queens and female sexuals discriminate between clean, unused substrates and substrates that had previously been walked on by workers from their own colony, another conspecific colony, or a different species, *Temnothorax nylanderi* (Förster, 1850). We recorded the spontaneous locomotor behavior of ants by tracking their movement in a circular arena (diameter: 15 cm) with Fluon® coated walls. The floor of the arena was covered by filter paper: half of it was unused and the other half previously walked on by 20–25 workers for 24 h prior to the experiment. These workers were provided with water *ad libitum* (a piece of cotton soaked with water and placed on a clean watch glass, diameter: 3 cm). Access to the other half of the filter paper was prevented by a Plexiglas® barrier coated with Fluon®, which was removed after the workers had been returned to their nest of origin just before the discrimination experiment started. A filter paper was used in two consecutive trials within a time period of 30–40 min. This was necessary because the number of marked filter papers available at the same time was limited. It is unlikely that the short period during which female sexuals or queens walked on the filter paper during the first trial would have significantly changed the markings made by exposure to workers for 24 h. An individual female sexual was placed in a small plastic cylinder (diameter: 2 cm) into the center of the observation arena. The cylinder was removed after a few seconds and thereafter the female sexual could move freely in the arena. Its spontaneous trajectory was recorded for 300 s with a DNT® DigiMicro 2.0 Scale camera (resolution: 800 × 600 pixels) centered 70 cm above the arena floor. The position of used and unused substrates was pseudo-randomized between trials. To homogenize the light and to mask any visual cues that might have influenced the ants’ trajectory, we surrounded the arena by a white cardboard and lighted it from above by white neon lights.