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Short communication

A case of multiple mating in stingless bees (Meliponinae)

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Summary

In several stingless bee species many males aggregate in the vicinity of a nest when a virgin queen is present in the colony and is preparing for the nuptial flight. We report such male assemblage for *Tetragonisca angustula*. The departure of a virgin queen from the colony and the subsequent mating could be video-recorded, because the queen and the males that had mounted her fell to the ground. Since at least two males had lost their genitalia, multiple mating seems to have occurred. This is in contrast with the prevailing view found in literature concerning the mating biology of stingless bees.

In many species of stingless bees mating involves drone aggregations in front of the nests where queen supersedure or swarm settlement has taken place and where, consequently, a young queen will leave for her nuptial flight (Engels and Engels, 1988; Roubik, 1990). In the genus *Melipona*, such drone aggregation is reported for *M. favosa* (Sommeijer and de Bruijn, 1995), and *M. fasciata* (Veen et al., 1997) and here too the aggregation appeared to be linked to one or more colonies nearby.

Direct observations of natural matings do not exist, and therefore, our understanding of the process is only of an indirect nature. It is based on comparisons of the amount of sperm produced by a drone compared to the amount present in the spermatheca of a mated queen (Kerr and Krause, 1950; Kerr et al., 1962; Camargo, 1972; Almeida, 1981), and on studies concerning allozyme variation (Kerr, 1969; Contel and Kerr, 1976; Machado et al, 1984; Falcão and Contel, 1991). Except for the last study, in all cases the authors concluded that a single mating occurred. In their review, Page and Kerr (1990) take the same position.

During November, 1995, a large group of drones was hovering and resting around the entrances of some hives in the bee garden of the Instituto de Biociências of São Paulo University, each hive containing a colony of *Tetragonisca angustula angustula* (Latreille). Supposedly, queen supersedure was taking place. During

these days the concentration of the drones shifted from one colony to the other, an indication that queen supersedure in the colonies was somehow synchronized. We made video pictures of male behaviour and, by chance, recorded a virgin queen departing from the colony as well as subsequent queen-male interactions. As soon as the queen had taken flight, males were flying after her and pounced on her, with the consequence that she fell on the ground. An estimated 30–50 drones followed her. The soil being covered with low weeds, the queen frequently was out of sight when running on the underside of a leaf, and so were the drones running close after her. Other males were running on the upper side of the leaves, their location indicating her position, which could also be estimated from those males flying low above the vegetation. This made it possible to film the queen again as soon as she appeared to the upper side. All the time several bees were pursuing her at close range or had taken position on top of her. Several times it appeared as if copulation was taking place. Twice, in close vicinity to the queen, a male was seen having a white filament protruding from the abdomen. We take this as a sign that these males copulated with the queen, an act in which males lose their genitalia. We also consider it probable that more than two inseminations had taken place, given the many bees pursuing the queen and the proportion of time she was in sight of the camera.

The total duration of this mating flight was 5.5 min. This is slightly longer than in the observations of Ferreira (1995), who reports 4 mating flight durations between 1 min 45 sec and 2 min 23 sec for this species. In one case the queen dropped to the ground, like the case reported here, but that queen was able to resume flight, and mating was not observed. All males aggregated around the nest left with the queen, indicating the importance of queen pheromones in the attraction of the drones. After the return of the queen, the males gradually returned too, though further mating flights probably would not occur. In all these cases the drone aggregation contained several hundred males.

This observation, and the fact that quite large drone aggregations are common among the subgenera of *Trigona*, casts doubt on the widely accepted view that in stingless bees single insemination occurs. Such uniformity, however, appears to be in contrast with the large variation among species concerning worker reproduction (Sakagami, 1982), and kin selection theory predicts that worker reproduction would vary with the number of parents represented in the colony. Further inquiries are apparently needed.

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