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Multiply Mating Males in *Gnamptogenys striatula* Mayr (Hymenoptera, Formicidae)

D. Allard · M. Van Hulle · J. Billen · B. Gobin

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Abstract Multiply mating ant males are rare, because in most ant species, multiple mating opportunities for males are scarce. *Gnamptogenys striatula* is an exception to this rule, as intranidal mating offers protection and a local female-biased sex ratio. In a behavioral experiment, we confirm the males' ability to inseminate several receptive workers, with four females being the observed maximum.

Keywords Formicidae · males · multiple mating · insemination

Introduction

The role of males in social Hymenoptera biology has long been neglected, as these males do not take part in the daily household tasks of a colony. However, in recent years, new fields of study have emerged in which males do play an important role, like sexual selection and sexual conflict (see the recent review by Boomsma et al. 2005). Detailed studies of male life history and mating systems are now needed to test these sexual selection hypotheses.

This study shows that males of the polygynous ant *Gnamptogenys striatula* Mayr can inseminate several females. While multiple mating by females has been well documented (Strassmann 2001), multiple male mating is exceptional in ants (Bourke and Franks 1995), with the best documented example being *Cardiocondyla*

D. Allard is a Research Assistant for the Fund of Scientific Research—Flanders.

D. Allard · M. Van Hulle · J. Billen (✉) · B. Gobin
Zoological Institute, University of Leuven, Naamsestraat 59,
3000 Leuven, Belgium
e-mail: johan.billen@bio.kuleuven.be

B. Gobin
Zoology Department, pcfruit Research, Fruittuinweg 1,
3000 Leuven, Belgium

(Kinomura and Yamauchi 1987; Heinze and Hölldobler 1993). The male-biased local sex ratios and consequent harsh competition, together with the multiple dangers encountered during a mating flight, are invoked as the selecting forces behind this single-mating phenomenon (Bourke and Franks 1995; Thornhill and Alcock 2001). The constrained sperm supply of ant males (ant testes degenerate before sexual maturity of the male and sperm is stored in the seminal vesicles (Hölldobler and Bartz 1985)) might have evolved as a consequence of this almost obligatory single mating (Bourke and Franks 1995). However, given the opportunity to mate with several females, males are expected to do so, because of the following advantages: an increased amount of offspring, and an increased probability of success of at least one mate. By success we mean survival and production of offspring.

This theory is supported by the discovery of multiply mating males in a few species with either female-biased operational sex ratios (*Formica aquilonia* (Pamilo et al. 1978; Pamilo and Rosengren 1983), *Formica polyctena* (Yamauchi et al. 1994)), or with female-biased sex ratios and with mating taking place in the safe environment of the nest (*Technomyrmex albipes* (Yamauchi et al. 1991), *Epimyrma kraussei* (Winter and Buschinger 1983), *Monomorium pharaonis* (Allard et al. 2006), *Cardiocondyla* species with continuous spermatogenesis (Heinze and Hölldobler 1993)).

G. striatula is a South American ponerine ant with polygynous colonies, headed either by several queens or several inseminated workers (gamergates; Blatrix and Jaisson 2000, 2001). Gamergates appear after a colony has lost its queens (orphaning), increasing colony lifespan and resource inheritance (Peeters and Ito 2001). New nests, both queen-right and queenless, seem to be founded by budding (Giraud et al. 2000). This type of social organization implies that males mate both with queens and workers. Blatrix and Jaisson (2000) studied mating with workers: within 9–15 days after removal of the queens from a colony, several workers started performing sexual calling inside the nest. Foreign males introduced into the foraging arena of such a nest were seized by foragers and transported inside the nest chamber, where other workers groomed the males. The males then explored the nest and mated with the workers previously performing sexual calling. Conditions are thus favorable for male multiple mating with workers: after orphaning, several receptive workers are present in the same nest simultaneously, and copulations take place inside the safety of the nest. Blatrix and Jaisson (2000), based on dissection data, indeed reported a total of 14 inseminated workers after the introduction of five *G. striatula* males into a recently orphaned colony; however they could not rule out the possibility that some workers were already inseminated at the start of the experiment.

We repeat this mating experiment with workers eclosed in the absence of males, precluding the possibility of females being inseminated before the start of the experiment, and replicate it with several colonies.

There are no field observations of males mating with the larger, winged queens. Several virgin alate queens can be present simultaneously in the same nest (field data from Blatrix and Jaisson (2001) and Giraud et al. (2001)). Giraud et al. (2000) suggest the following mating scenario, based on genetic data and lab observations: virgin queens leave the nest to perform sexual calling in the vicinity of the nest

entrance, whereas males leave their natal nest and fly some distance to a calling female and mate with her on the substrate; after mating, the queen returns to her maternal colony.

Therefore, conditions for multiple mating with queens seem less favorable. Nevertheless, we test multiple mating with young virgin queens eclosed in our stock colonies.

Material and Methods

Twenty-nine colonies of *G. striatula* were collected in Costa Rica during October 1999 and October 2003. They were kept in translucent plastic boxes (10×20×5 cm) with a plaster floor. A small nest chamber (4×4×0.5 cm) was carved out in the plaster and covered with a glass slide and red plastic foil. Nests were kept at a constant temperature (25°C) and constant relative humidity (75%) and exposed to a 12 h light/12 h dark photo-cycle. The ants were fed three times a week with small mealworms, crickets, crumbled cake and crushed almonds and provided with honey water ad lib.

For our mating experiments, we reared young virgin males by isolating groups of workers from queen right colonies. After approximately 2 months, males started eclosing in these groups. To test for maturity, males were allowed to fly briefly before introduction by dropping them from a forceps. Only males that readily flew were used in further experiments.

To test multiple mating with workers, virgin workers were reared by isolating 13 groups of 15 adult workers and 20 worker pupae from 13 different stock colonies. The adult workers were marked with paint. As the young workers eclosed, we gradually removed the marked workers from the groups. Two weeks after the last callow eclosed, and upon removal of the last marked worker, we introduced a single male in each nest. These males were collected from multiple stock colonies other than those from which the female groups originated.

To test multiple mating with queens, young virgin alate queens were collected from the stock colonies in groups of six, together with 12 workers. The groups were allowed to settle for 2 days, after which we introduced a single foreign male in each nest. We performed this on 18 queen groups, originating from four different stock colonies.

After a male died, all workers and queens were dissected and their spermathecae examined for the presence of sperm. We also recorded their degree of ovarian development.

Results

We found inseminated workers in six of the 13 worker groups. Three groups had one inseminated worker, two groups had two, and one group had four inseminated workers (see Table 1). All inseminated workers had developing yolky oocytes in their ovaries, as well as conspicuous yellow bodies. Of the non-inseminated workers,

Table 1 Numbers of Inseminated Females (Workers or Queens) from Experimental Colonies with Virgin Females in Which a Single Male Was Released

Type of colony (worker or queen)	Nr of inseminated females	Nr of colonies
W	0	7
	1	3
	2	2
	4	1
Q	0	18

only a few had active ovaries (one in a colony with one inseminated worker, one in a colony with two inseminated workers, one in a colony with four inseminated workers, and two in a colony with four inseminated workers).

Of the 18 queen groups, none contained inseminated queens.

Discussion

Six males successfully mated with workers. The inseminated workers always had active ovaries, and the presence of yellow bodies showed their egg-layer status in the colony. This suggests that males are attracted to dominant egg-laying workers in *G. striatula*. It has been demonstrated in this species that fertility status is indicated by differences in the cuticular hydrocarbon profile (Lommelen et al. 2006), which acts as a signal of a workers' position in the reproductive division of labour (Lommelen et al. 2008). Two of the males were able to inseminate two workers; one of them even inseminated four workers. Even when laboratory colonies readily reared males, multiple insemination and the observation that foragers indiscriminately retrieve males to the nest (Blatrix and Jaisson 2002) suggests male scarceness for mating in nature. *G. striatula* is a polygynous species that has multiple queens or gamergates. Fathering offspring from multiple workers is then a straightforward way to ensure sufficient colony growth.

We did not manage to create the appropriate conditions for mating between males and queens, as none of the young alate queens were inseminated. This dysfunction is likely due to the queen's behavior, as the males readily mated when presented with workers. Inducing natural mating in captive ants is exceedingly difficult, only successful in a few species that mate on the ground (e.g. *Cardiocondyla* (Kinomura and Yamauchi 1987); *Diacamma* (Allard et al. 2007); *Gnamptogenys* (Gobin et al. 2001); *Monomorium* (Allard et al. 2006) and only further trials and experiments can reveal what conditions and factors are required to induce mating in artificial conditions.

Nonetheless, multiple mating with queens seems unlikely, for mating opportunities are scarcer, and predation risks are high. Moreover, queens of *G. striatula* require more sperm than gamergates for their lifetime production of offspring as they likely have a higher lifetime fecundity. Queens have double the number of ovarioles than gamergates (4.41 vs 2.16; Blatrix and Jaisson 2001) and

most ant queens have a longer lifespan than workers (Hölldobler and Wilson 1990). Furthermore, they generally mate only once (Giraud et al. 2000), which means their entire sperm supply comes from one male. Possibly, males transfer their entire sperm supply to a single queen, and divide it among several mates when presented to workers. Testing these different insemination strategies would require precise sperm counts.

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