

PHYSOGASTRIC-QUEEN MATING IN *Melipona quadrifasciata* LEP. (HYMENOPTERA, APIDAE)

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ABSTRACT

The influence of the number of matings on the survival of physogastric queens after they have copulated was investigated. Five physogastric queens of *Melipona quadrifasciata* were put to copulate with one drone each and two queens with four drones each. It took one to three days for single-mated queens to get rid of the male genitalia and restart oviposition. The multiple mated queens were found dead within their nests on the third day after matings. Some of the male genitalia remained attached to these queens. The death of multiple mated queens appears to be caused by the perforation of the genital chamber wall by male genitalia valves. Two of the one-male matings mentioned above were carried out using genetically marked queens and drones. Subsequent analysis of progenies have shown that spermatozoa had entered the physogastric-queen spermatheca and mixed with those already present there. The attractiveness of physogastric queens to males in the absence of light was also demonstrated.

Reproductive pheromones appear to be important to the colony life of stingless bees, because the queens continue producing at least some of them even after mating. Tergal glands are suggested as a possible source of the pheromones involved in physogastric-queen attractiveness to the males.

INTRODUCTION

Female mating patterns of social Hymenoptera have received increasing attention since the proposal of the haplodiploid hypothesis (Hamilton, 1964) for the evolution of reproductive altruism in this insect order. This hypothesis is based on the sex determination system of the Hymenoptera which allows a 3/4 relatedness among female siblings when the mother is single mated. Multiple mating lowers the average relatedness among siblings and therefore would weaken the tendency to evolve

worker behavior (Hamilton, 1964). However, polyandry has been reported for several species of solitary and social Hymenoptera (Garófalo, 1980; Page and Metcalf, 1982; Cole, 1983; Page, 1986).

Queens of highly eusocial bees and other social Hymenoptera mate only during a short period at the beginning of their reproductive lives. Generally, *Apis mellifera* queens copulate with an average of 17 drones during one or more mating flights and then start oviposition (Adams *et al.*, 1977). *Apis cerana* mates with an average of 30 males (Ruttner *et al.*, 1973).

Stingless bee queens, in contrast, are thought to mate only once (Kerr, 1969; Silva *et al.*, 1972). This assumption is based on sperm counts (Kerr *et al.*, 1962), permanency of male genitalia in the queen genital chamber after mating (Kerr and Krause, 1950; Kerr *et al.*, 1962; Silva *et al.*, 1972) and segregation of genetic markers (Kerr, 1969; Contel and Kerr, 1976). Nevertheless, only a reduced number of species have been investigated for their mating habit.

Egg-laying in stingless bees begins 5 to 40 days after the mating flight, depending on the species and on the strength of the colony (Kerr *et al.*, 1962; Silva, 1972; Inoue *et al.*, 1984a; Kerr, 1987a). The queen's ovaries develop rapidly from mating to the onset of oviposition and the queen becomes physogastric. Due to their greatly swollen metasoma, laying meliponine queens cannot fly any and they stay in the nest until supersedure. Inoue *et al.* (1984b) report the only known possible exception to this condition.

Physogastric-queen mating in stingless bees was first reported by Sakagami and Laroca (1963) in *Lestrimelitta ehrhardti*. The mating pair was found inside the nest during the manipulation of a colony. Sakagami (in Sakagami and Laroca, 1963) also mentions that a laying queen of *Scaptotrigona postica* exposed experimentally to a drone aggregation, elicited mating attempts. A few observations on mating attempts with physogastric queens have been reported since.

Sakagami (1982) observed that several males attempted to copulate with a physogastric queen when a colony of *Schwarziana quadripunctata*, kept in a hive, was accidentally disturbed. Engels and Engels (1988) working on *Scaptotrigona postica*, have noticed that queens 15 or more days old, including laying queens up to 50 days old, are unattractive or weakly attractive to the males. On the other hand, these authors found two physogastric, laying queens, 17 and 24 days old respectively, that were highly attractive, comparable to 10-14 day old virgin queens.

In our laboratory, we have made observations on *S. quadripunctata*, similar to those of Sakagami (1982). Moreover, we also have found physogastric queens of *Melipona quadrifasciata* having up to 6 male genitalia attached to their genital chamber, when feral colonies with a high number of drones were transferred to wood boxes. The queens died following the removal of male genitalia.

It is worth noting that all observations mentioned above were made under anomalous circumstances. Some factor may preclude the occurrence of matings with

physogastric queens under normal circumstances. V.L. Imperatriz-Fonseca (personal communication) has suggested that stingless bee males would not copulate in the absence of light. Thus, darkness could possibly prevent matings from occurring within the nests.

In this paper, the results of studies on the effect of single and multiple matings on physogastric queens of *M. quadrifasciata*, insemination and sperm utilization after such matings, and the influence of light on the attractiveness of these queens, are reported.

MATERIALS AND METHODS

The *M. quadrifasciata* bees used in our experiments were obtained from colonies maintained in hives at the Federal University of Viçosa, Minas Gerais State, Brazil. Only mature males, judged from the dark pigmentation of their bodies, were used. The queens were fully physogastric, with ages ranging from six months to two years.

In order to test the influence of the number of matings on queen survival following copulations, five queens were put to copulate with one drone each (one-male mating), and two others with four drones each (multiple mating). The matings took place in a small wooden box (11 x 8 x 3 cm). The queens were removed from their colonies just before the matings. For multiple matings, each drone was introduced into the box after the preceding had finished copulation. After the matings, the queens were examined to observe the position of the male genitalia and were marked with a paint spot on their mesoscutum before being returned to their respective colonies. The combs under construction were sketched to record oviposition and the queens were observed daily.

Two of the one-male matings described above were carried out with genetically marked queens and males. This species is subdivided into at least two subspecific morphs (Moure and Kerr, 1950): one of them, *M. q. quadrifasciata*, has four continuous yellow tergal bands, while in the other, *M. q. anthidioides*, the tergal bands are interrupted by a large black gap in their central portion. These yellow bands occur only in males and workers (queens have brown metassoma). The hybrids are phenotypically intermediate and the genetic differences between the two morphs show quantitative inheritance (Melo and Campos, unpublished results). Two laying queens that were producing only interrupted banded workers in their progeny were crossed with continuous banded males. If the spermatozoa of the second male would enter the spermatheca, then hybrid workers should be produced in subsequent progeny. This was verified by examining emerging workers and by collecting samples of foragers at the nest entrance. The foragers were returned to the colony after phenotypic frequencies were recorded. These samples were used to determine sperm utilization, under the assumption that the two morphs have the same viability.

The attractiveness of physogastric queens to the males in the absence of light was also tested. Four males were placed in one of the chambers of a dimidiated box (11 x 8 x 3 cm) and one queen in the other chamber. The box was placed in a dark room and after one minute, the separation wall was removed and the bees were allowed to interact for about 15 min. Still in the dark room, the bees were anesthetized with CO₂ and were examined to verify the occurrence of copulations. Two replications of this test were performed.

RESULTS

Effects of single and multiple matings on physogastric queens

The one-male and multiple mating experiments showed that physogastric queens are very attractive to the drones. The males promptly mounted the queens and copulated. The first male to copulate leaves his genitalia attached to the queen genital chamber in the same position observed for matings with virgin queens. In multiple matings, the genitalia of the subsequent males were inserted above the first without, however, a regular spatial arrangement.

The attached male genitalia preclude egg-laying in queens, probably acting both as blocking and stress agents. All single mated queens succeeded in getting rid of the male genitalia one to three days after copulation and restarted oviposition. This did not occur in the experiments with multiple mated queens. These queens were found dead within the nest the third day after matings. One of the queens got rid of 3 male genitalia and the other got rid of only 2. The remaining genitalia were attached to the queen genital chamber wall by means of the valves. *M. quadrifasciata* male genitalia are almost entirely sclerotized, with long and pointed valves. It is not known whether the queen removes the male genitalia by herself or is assisted by workers.

Insemination of physogastric queens and subsequent sperm utilization

Phenotypically hybrid workers were produced in the two genetically marked crosses. This indicates that laying queens remain physiologically capable of receiving more spermatozoa.

Sperm utilization was followed in one of the colonies during one year (Figure 1). Sampling was initiated only at the 16th week after the mating. Samples of forager workers were not collected from the second colony because it became very weak.

Figure 1 shows that the frequency of the hybrid phenotype in the progeny of the physogastric queen oscillated around 7% until the 27th week after mating. Subsequently, the frequency of hybrids increased gradually, approaching 30% at the end

of the sampling period. Despite the fluctuations between weeks, our data suggest that the proportion of fertilizations achieved by the spermatozoa from the second male increases with time, the variation of this proportion being higher among the initial samples.

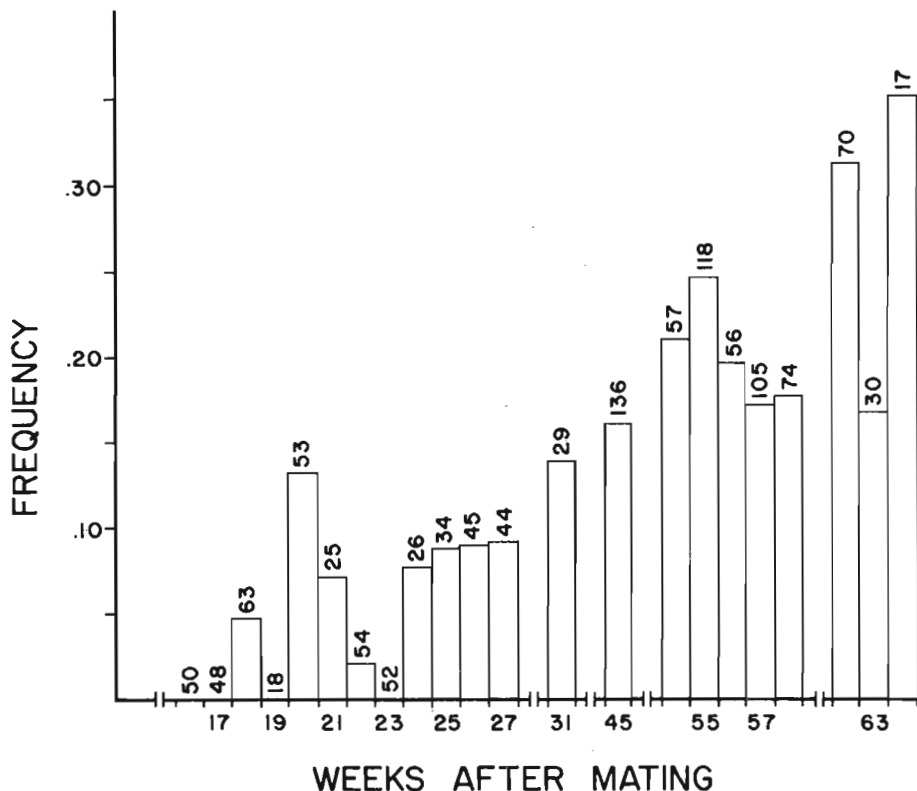


Figure 1 - Frequency of the hybrid phenotype obtained in the progeny of a cross between a continuous tergal banded male and a physogastric-queen that was previously producing only interrupted tergal banded workers. The sample sizes are indicated above the columns.

Influence of light on the attractiveness of physogastric queens

The bees mate even in the absence of light. Two and three males copulated, respectively, in the replicated experiment. Provided that the bees were not affected by the exposure to light during manipulation, we conclude that nest darkness does not prevent physogastric-queen mating in undisturbed colonies of *M. quadrifasciata*.

DISCUSSION

The importance of sex pheromones in mediating queen-worker interactions has been verified in honey bees (Velthuis, 1985). Reproductive pheromones are apparently important for stingless bee colony life, since the queens of several species continue producing at least some of them even after mating. This question should be further investigated because a shift in the kind of pheromone produced by the queens along their life cycle may be involved.

Analysis of cephalic volatiles of *S. postica* has revealed the following pattern: secondary alcohols predominate in young virgin queens, while several alkenes increase in amount in newly mated queens and become predominant in physogastric queens (Engels *et al.*, 1987). This pattern has led these authors to suggest that sex pheromones are probably not involved in queen-worker interactions within stingless bee colonies. Nevertheless, since the laying queens of *S. postica* and of other meliponines are attractive to males, a possible role of reproductive pheromones on intranidal interactions should not be dismissed.

The tergal glands of stingless bees seem to be involved in pheromone production (Cruz-Landim, 1987). In *Paratrigona subnuda*, these glands are most developed in physogastric queens, decreasing their development in queens near supersedure (Cruz-Landim *et al.*, 1980). Regarding the results of Engels *et al.* (1987), the attractiveness of stingless bee physogastric queens to the males could perhaps be attributed to the products of the tergal glands. The mandibular gland pheromone of honey bee queens attracts males at long distances, while the pheromones of epidermal tergal glands are effective only at a short distance (Renner and Vierling, 1977). Tergal and mandibular gland pheromones could also have distinct roles during mating in stingless bees.

The death of multiple mated queens appears to be due to the perforation of the queen's genital chamber wall by male genitalia valves. Perforations may cause a continuous hemolymph loss as well as microbial contaminations. Successive matings increase the chance that the queen's genital chamber wall will be damaged because subsequent male genitalia may push forward the preceding ones. Consequently, the probability of queen death after multiple matings would increase.

Our data suggest that the spermatozoa of the second mating mixes with those already present in the spermatheca. The gradual increase in the proportion of phenotypically hybrid workers could be explained by slow mixing of the spermatozoa within the spermatheca, during the entire sampling period. Frequency fluctuation may indicate heterogeneity of the distribution of the two kinds of sperm (first and second mating) within the spermatheca. Alternatively, the spermatozoa from the second male may rapidly achieve a proportion of fertilizations determined by the amount of sperm that entered the spermatheca at the second mating, and this proportion gradually increases because the competitive ability of the spermatozoa from the

first mating may decay with age. The natural supersedure of the queen has occurred one month after our last sampling.

It remains unsettled if physogastric-queen matings or other forms of multiple mating actually occur in stingless bees under natural conditions. At first sight, the actual possibility of insemination when drones copulate with physogastric laying queens could be interpreted as a selective pressure favoring drones that adopt this type of behavior: it provides them an additional choice of female partners for successful mating. On the other hand, an increase in the proportion of drones presenting this behavior could result in a higher number of deaths of physogastric queens due to multiple mating. Also, the occurrence of successful physogastric-queen mating is rather complex. It requires from the male the ability to invade a colony and to discriminate among physogastric-queens of different ages. A young laying queen has little available space in the spermatheca while an old one is coming close to supersedure. In addition the male should distinguish among colonies and avoid inbreeding. Otherwise, such a mating would increase the chance that diploid males are produced in the progeny (Camargo, 1979). Emergence of diploid drones induces the workers to kill the queen in *M. quadrifasciata* (Camargo, 1979) and *M. compressipes* (Kerr, 1987b).

Studies on stingless bee biology in which observation hives were used, do not refer to mating events involving physogastric queens (e.g. Sakagami and Zucchi, 1963; Sakagami *et al.*, 1965; Camilo-Atique, 1977; Zucchi, 1977; Lacerda, 1986). This may be regarded as evidence that the observed matings involving meliponine laying queens are ultimately induced by disturbance.

Additional studies are necessary to elucidate the mating pattern of stingless bee queens, principally in view of the great diversity among species, both in terms of morphology and behavior. Reliable data must be obtained through the use of genetic markers (Page and Metcalf, 1982; Page, 1986). Falcão (1984) has provided evidence of multiple mating in stingless bees, using allozyme markers at the malate dehydrogenase and phosphoglucosmutase loci in *Plebeia droryana*. Her data on three out of 22 colonies show that queens had been inseminated at least twice. Again, the multiple mating of these queens may have occurred as a consequence of the manipulation of the colonies.

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RESUMO

Foi investigada a sobrevivência de rainhas fisogástricas de *Melipona quadrifasciata* com 6 a 24 meses de idade após terem sido submetidas à cópula. Cinco rainhas fisogástricas foram colocadas para copular com um macho cada e duas outras com quatro machos cada. As rainhas que copularam com um

único macho levaram de 1 a 3 dias para se livrarem da genitália masculina e iniciarem novamente a oviposição. As rainhas que copularam com quatro machos foram encontradas mortas dentro de suas colônias no terceiro dia após as cópulas. A morte destas rainhas parece estar relacionada às perfurações na parede de sua câmara genital causadas pelas valvas das genitálias dos machos. Dois dos cruzamentos mencionados acima envolvendo um único macho foram feitos com machos e rainhas geneticamente marcados. Análise posterior da progênie revelou que espermatozóides destes cruzamentos penetram na espermateca das rainhas fisogástricas e se misturam com os espermatozóides já presentes. Foi investigada também a atratividade de rainhas fisogástricas em ausência de luz. Nas duas repetições conduzidas, as abelhas foram capazes de copular. Feromônios reprodutivos aparentemente são importantes nas interações sociais em colônias de meliponíneos, uma vez que as rainhas continuam a produzir pelo menos alguns destes feromônios.

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