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Exceptional High Queen Production in the Brazilian Stingless Bee *Plebeia remota*

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Abstract

In *Plebeia remota* only a few gynes (young virgin queens) are usually reared per year. However, in two laboratory colonies an extraordinarily high number of gynes emerged within a short period of time. These gynes were significantly smaller than those produced in low numbers by other colonies. Morphometric characters and behaviour of the gynes were recorded. Possible reasons for the phenomenon of exceptional high gyne production are discussed.

Keywords: *Plebeia remota*, stingless bees, queen production, queen size variation, Brazil.

Introduction

In honey bees, the production of gynes is regulated by queen pheromones which inhibit the construction of royal cells (for a review see Visscher, 1998). It is unknown whether in stingless bees the queen has a similar influence (Imperatriz-Fonseca & Zucchi, 1995). In contrast to honey bees, stingless bees rear queens even under queenright conditions. After emergence, however, most gynes are killed by the workers sooner or later. The few individuals that survive eventually supersede the mother queen or leave with a swarm. In some trigonine species, the workers build large royal chambers in which the virgin queens are isolated (Imperatriz-Fonseca & Kleinert-Giovannini, 1987; Engels & Imperatriz-Fonseca, 1990; Imperatriz-Fonseca & Zucchi, 1995).

Queen brood cells differ among the stingless bees. In the genus *Melipona,* queens are produced in comb cells of the same size and shape as those that give rise to workers and males. In *Trigona* and related genera, queen cells are larger, in length and width, than those of workers and males (Engels & Imperatriz-Fonseca, 1990). In addition, queens may emerge from normal-sized cells that are smaller than those from queen cells. Such a size polymorphism has been observed in some species including *Plebeia remota* (Imperatriz-Fonseca et al., 1975; unpublished data).

The production of queens in stingless bees occurs in small numbers in *Trigona* and related genera, or in large numbers in the genus *Melipona* (Imperatriz-Fonseca & Zucchi, 1995). Specifically in *P. remota*, a colony usually produces just a few gynes per year. However, we observed that an exceptionally high number of gynes emerged within a short period of time in two colonies. Details of this extraordinary occurrence are reported here together with notes on the behaviour of these gynes and data on their body size, in comparison to gynes regularly produced in other colonies. Possible reasons for this high queen production are discussed.

Materials and methods

Origin of the bee nests and their maintenance in the laboratory

Colonies of *P. remota* from southern Brazil were maintained at the Bee Laboratory of the University of São Paulo in thick wooden boxes at ambient temperature. The bees were allowed to forage outside and rarely received extra food, but when they were fed, they were given a sugar water solution (1 : 1 v/v) or honey bee honey, and fresh pollen. During the study period of almost two years (January 1998–August 1999), the total number of colonies fluctuated as some of them perished and new ones were brought to the laboratory.

Observations on the production of gynes

The period of observation for each colony varied between 6 and 18 months. They were inspected at intervals of one day

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to nearly one month. Most of the colonies produced a few gynes. Only two of them (colonies A and B) reared many gynes within a short period of time. In order to compare the rates of queen production, we calculated the average number of gynes produced by each colony per month during the period of observation for colonies (n = 13) which regularly reared a few gynes. We also recorded the number of queen cells, minimising any possible underestimation in gyne rearing. Colonies in which no gynes or royal cells could be recorded were not taken into account.

Behaviour and size of the gynes

The behaviour of the gynes was studied by direct observation (colony A) and through video recording (colony B). Morphometric data (maximum head width, medium interorbital distance, and intertegular distance) were obtained after the gyne had been immobilised by placing her in the freezer for 1 min. She was then transferred to a chamber under a piece of glass in such a way that head and thorax were in a suitable position to be measured under a stereomicroscope with a graduate ocular (Michener, 1965; Nogueira-Ferreira et al., 2000). This procedure allowed quick measurements without harming the queen. All gynes were individually paint-marked on the thorax and returned to their colonies. In addition, a few recently dead gynes, the mother queens of colonies A and B, and gynes produced by other colonies were also measured. The morphometric data were compared in pairs using the Mann–Whitney *U* test (Zar, 1999).

Results

Normal gyne production

In each of the 13 colonies that reared gynes at normal rates, we never recorded more than a total of five gynes during its respective period of observation. No more than one gyne emerged on the same day, and normally this occurred at intervals of some days. For the total of queens produced by all these colonies, we calculated a mean rearing rate of 0.13 (SD 0.09) gynes/month. Considering also the royal cells, the mean increased to 0.17 (SD 0.14) gynes/month. However, gynes may also have emerged from normal-sized brood cells not recognised by us.

Exceptional gyne production

Within two months (March 30 to May 28, 1998), we recorded 46 gynes emerging in colony A. This colony had been observed since February and perished in August of the same year. Therefore, the average was 6.57 gynes/month of observation. The mother queen was found dead on April 25 together with two gynes inside a honey feeder. The reason for her death is unknown. The previous days she had been very excited and had chased gynes throughout the colony.

In October 1998, during an inspection of colony B, we removed part of the comb (about 1/4 of the total amount of the brood cells) and placed it into a Petri dish. This piece of comb had six royal cells and 34 normal-sized cells. From November 10 to 14, from these 40 cells 21 gynes emerged. Colony B had been under observation for 10 months, became queenless before this extraordinary gyne production, and perished 1.5 months later. Over a total of 11.5 months of observation, on average 1.83 gynes/month were reared.

Behaviour and fate of the gynes

In all colonies, most of the gynes were attractive to workers, provoking great excitation and disturbance in the colony, whereas some gynes were not attractive at all.

From the 46 gynes reared by colony A, 10 died soon after emergence, 24 were returned to their colony, and 12 were used for other studies. In the colony, most of the gynes survived for one or two days, but some lived for up to a week. They were eventually killed by the workers or other gynes, or maybe left the colony on a nuptial flight and never returned. The last gyne was observed for 3.5 months. Apparently she did not mate and the colony finally perished. No royal chambers were built for the virgins.

From the 21 gynes reared by colony B, three died, 12 were used for other studies and six were put back into their colony. One of these gynes mated, but for an unknown reason the workers did not start to build brood cells and the colony also perished. Analysis of the video recordings revealed that the attractive gynes attacked one another. They also inflated the abdomen and solicited trophallaxis to workers. In addition, they were also seen feeding by themselves.

The size of mother queens and their gyne progeny

In both colonies A and B, most of the gynes produced at high rates were smaller (Fig. 1) than the mother queens (Table 1), but a few were larger. In colony A, the last surviving gyne was very small. In colony B, the gyne that survived and mated was the largest. There was considerable variation in the size of gynes within and between all colonies Of the three characters measured, the intertegular distance was found to differ in particular between gynes of the colonies A and B in comparison to the others (Fig. 1c). Colony A produced the smallest gynes according to all evaluated characters (Fig. 1). Colony B also produced gynes mostly smaller than those reared in the other colonies. These differences were statistically significant (Mann–Whitney *U* test).

Table 1. Size of the mother queens of the two colonies of *P. remota* which produced gynes at high rates.

Colony	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)
A	1.70	1.26	1.48
B	1.70	1.33	1.56

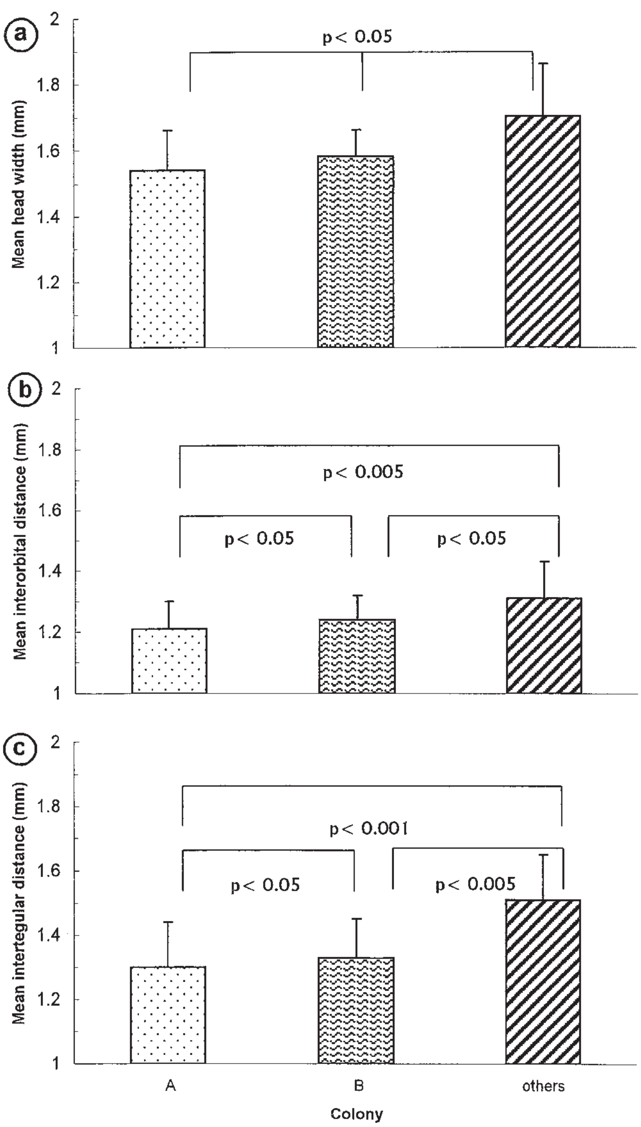

Fig. 1. Comparison of the size of gynes produced by colonies of *P. remota* at high (A and B) or at regular rates (13 others). Means of measurements taken from 27, 21 and 26 individuals, respectively. Bars indicate standard deviation. Significance of differences calculated with the Mann–Whitney *U* test.

Discussion

Normal versus exceptional gyne production in other species of stingless bees

The production of large numbers of queens by trigonine stingless bees has been recognised by several researchers. Already Ihering (1903) counted 20 gynes in a colony of *Cephalotrigona capitata*. A similar high rate of gyne production ($n = 29$) by a colony of *Schwarziana quadripunctata*, a species related to *Plebeia,* was reported by Camargo (1974). We also observed in our laboratory that two colonies of this species reared around 25–30 gynes in about 10 days, whereas the usual number is 5–6 gynes at a time (unpublished data). Moreover, Imperatriz-Fonseca et al. (1997) found nine gynes in a small comb of *Nannotrigona testaceicornis.* Therefore, the phenomenon of extraordinarily high rates of gyne production might be more common than documented to date.

Gyne size and caste differentiation

In *Trigona* and other trigonine genera, caste differentiation is dependent on the quantity of larval provisions (Hartfelder, 1987; Hartfelder & Engels, 1989). However, the mechanism by which small gynes are produced in normal-sized cells in *P. remota*, as clearly seen in our colony B, with a smaller amount of food than larvae have in royal cells, remains to be elucidated. Maybe a slightly larger amount of food is already enough to induce queen differentiation. Indeed, a small increase of larval provisions was enough to produce queens in *Nannotrigona testaceicornis*, although intercastes and workers also developed (Campos et al., 1986).

As a measure of size of gynes reared at different rates, we used the intertegular distance. The average values, calculated for samples of gynes produced by colonies with a particular rate of queen rearing (A with 46 gynes emerging, B with 21 and 13 others with 1–5 gynes), indicate that if queens were produced at high rates, they became smaller in size (Fig. 2). The generality of a relationship between the rate of production and size of the resulting gynes has to be confirmed by future studies.

Size difference, behaviour, and fate of gynes

Gynes of colonies A and B behaved in a similar way as those of other colonies and as is commonly reported (Imperatriz-Fonseca et al., 1975; Imperatriz-Fonseca & Zucchi, 1995), except that they attacked each other. The lack of royal chambers (Benthem et al., 1995) was probably due to the fact that the colonies were queenless, and there was no need to separate the gynes.

In colony A, the last gyne that survived for some months was small and was chosen by the workers instead of larger gynes. Therefore, small gynes can survive and apparently compete with large ones. Observations of queen supersedure in several colonies showed that gynes which replaced dominating queens could in fact be smaller or larger than their mother (unpublished data). We can conclude that size is not decisive for the acceptance of a gyne or for successful mating. Indeed, we observed small *P. remota* queens heading colonies of normal size, and even laying eggs in numbers similar to larger queens (unpublished data).

Possible reasons for high gyne production

The extreme investment in gyne production as observed by us should be related to colony conditions. It has been suggested that food storage/input and physiological status of the dominant queen are important factors for the rate of queen rearing (Engels & Imperatriz-Fonseca, 1990). But other

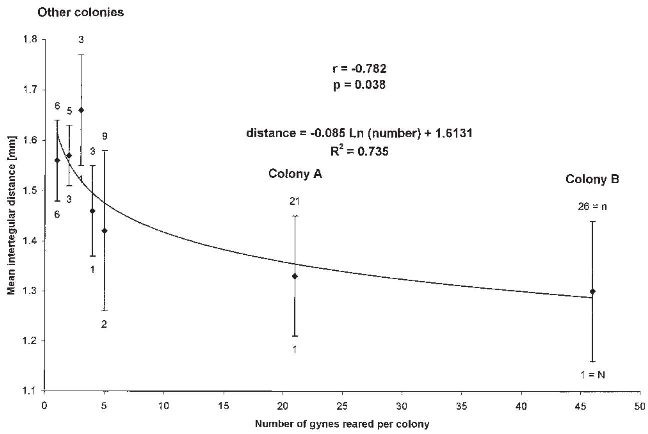

Fig. 2. Relationship between colony rate of queen rearing and a morphometric character in gynes of *P. remota*. Means and standard deviation. N = number of colonies per category of queen production, n = number of measured individuals per category. The regression curve indicates a negative correlation between rearing rate and body size of the gynes.

colonies maintained by us in the same environment and with similar internal conditions did not react like colonies A and B. Other colonies in even better conditions never produced so many gynes as did these two.

The queens of *P. remota* colonies A and B were in different phases of their egg-laying activity. The queen of colony A was passing through the typical interruption of oviposition, which occurs in fall and winter (Benthem et al., 1995), and was not laying eggs when the numerous gynes emerged. In colony B the queen had already passed this quiescent phase and was ovipositing again but died before the gynes emerged. In both cases, colony homeostasis was evidently affected through the loss of the queen. The workers might have responded to this situation by investing massively in queen production. However, in several other supersedure processes observed by us (n = 15), no increased gyne production occurred. The mechanism of queen control of the production of new queens, a well documented fact in honey bees (Engels et al., 1993), remains an open question in stingless bees and requires experimental analysis of the dominance signals and the reproductive processes involved.

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