MINIATURE QUEENS IN STINGLESS BEE SPECIES – A REVIEW

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ABSTRACT

Miniature virgin queens have been described for several species by many authors previously. However, only in the last 7 years miniature mated queens were also investigated. These populational studies determined size variation, described the frequency distribution of miniature virgin and mated queens, investigated their viability after mating, and their egg laying capability as heading normal colonies. The production of two morphotypes of queens and caste determination is discussed here, as well the possibilities to explain the mechanisms involved on it.

INTRODUCTION

Caste determination in stingless bees is still not completely understood. Basically there are two groups of stingless bees, and caste determination mechanisms differ in these two groups. In *Melipona* there is a genetic influence, although trophic aspects are also important (Kerr, 1950, 1997; Velthuis & Sommeijer, 1991). In this manner, female larvae, since quite early in development, may follow diverse pathways. Queens and workers are reared in identical cells.

In the other group of stingless bees, however, there are three possibilities for queen production. The caste determination is, in principle, trophic. Nevertheless, more complexity is involved in the process of production of new queens, according to the genus. In *Frieseomellita* and *Leurotrigona* a female larva is able to perforate the wall of a contiguous cell and eat extra larval food, becoming a queen (Terada, 1974; Faustino et al., 2002). In all other genera, royal cells are specially built by the workers. These cells are larger than cells from where workers (and males) emerge, and therefore, contain more food (Engels & Imperatriz-Fonseca, 1990). Besides, in several species new queens can emerge from normal sized cells as well. These queens, ingesting less food as larvae, are of smaller size: they are the so-called "miniature" or "dwarf" queens. This suggests that the amount of food is not enough for explaining the determination of a female larva to a queen. In this case the quality of food could be also have some influence. Previous works on food quality (Hartfelder, 1987; Hartfelder & Engels, 1989), however, discarded this possibility, keeping the problem unsolved. These authors found no differences in the amount of protein in the larval food of different cells. Nevertheless, studying the larval food of individual cells in *Schwarziana quadripunctata,* we recently found that the amount of protein varied among them. In fact, this variation was so large as a factor of about 70 times (Ribeiro et al., in preparation). In this way, although this finding do not prove that the quality of food is important for caste determination, it reinforces this idea and keeps the discussion open.

In the last few years an alternative theory has been formulated. During development, the female larvae would have the decision of becoming a worker or a queen, and therefore, would have the power of self-determination. Microgyny would be a selfish strategy, since these larvae would gain a higher pay-off becoming a small queen than a worker (Bourke & Ratnieks, 1999; and see also: Ratnieks, 2001; Wenseleers & Ratnieks, 2003; Wenseleers et al., accepted).

Miniature virgin queens of several species (*Cephalotrigona capitata*, *C. femorata*, *S. quadripunctata*, *Plebeia julianii, P. remota*, *P. emerina, Nannotrigona testaceicornis*) have been reported by several authors since long ago (Ihering, 1903; Nogueira-Neto, 1951; Juliani, 1962; Camargo, 1974; Imperatriz-Fonseca & Darakjian, 1993; Nogueira-Ferreira et al., 2000; Kleinert, pers. com.; Imperatriz-Fonseca et al., 1975; Imperatriz-Fonseca et al., 1997, respectively). Nevertheless, the first studies on miniature queens, considering mated queens, and not only the virgins, were done in our laboratory (Laboratory of Bees – Depto. de Ecologia, USP), only recently, from 1998 up to now (Ribeiro & Alves, 2001; Ribeiro et al., 2003; Ribeiro et al., submitted; Wenseleers et al., submitted).

The goal of this work is to make a review on the subject, and discuss the possible mechanisms of production and determination of miniature queens in stingless bees.

MATERIAL & METHODS

Many colonies of *P. remota* (n= 61), and *S. quadripunctata* (n= 54 colonies), have been studied in the past 7 years. A new method was developed in order to obtain morphometric measurements of alive adult queens without harming them. Using a very simple apparatus (fig. 1a), a queen was placed in it (fig. 1b) and taken under a stereomicroscope with an ocular micrometer. Three measurements were obtained: maximum head width, medium interorbital distance and intertegular distance (see details in Ribeiro & Alves, 2001).

In the last studies, pupae ready to emerge was also checked and measured, relating caste and cell origin, i.e., royal or normal-sized cells (Alves et al., 2003; Wenseleers et al., submitted).

(a) (b)

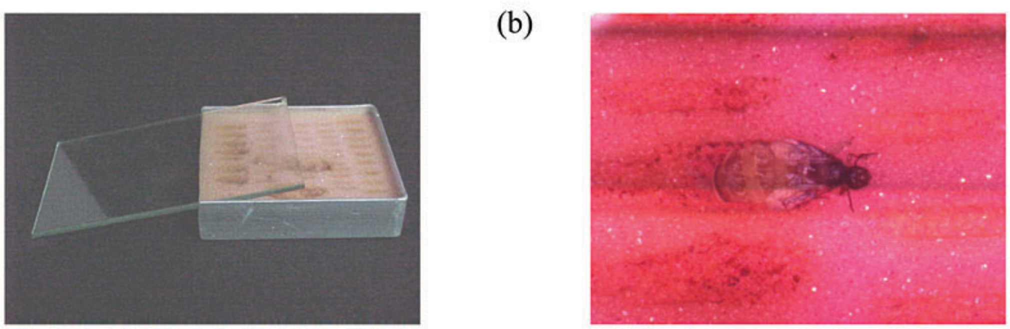

Fig.1- (a) Apparatus used to measure the queens under the stereomicroscope; (b) *P. remota* mated queen placed into it, ready to be measured. (Photos by M. de F. Ribeiro).

Queens (virgin and mated) were classified into miniature or normal, for *P*. *remota* and *S. quadripunctata,* through 2-means cluster procedure, and discriminant analysis, respectively (Ribeiro et al., submitted; Wenseleers et al., submitted). Other statistical methods were also applied to test differences and establish correlations (Zar, 1999).

RESULTS AND DISCUSSION

Table 1 shows the values for two morphometric measurements (head width and intertergular distance) used to distinguish between the two queen morphotypes for *P. remota* (n= 183 queens) and *Schwarziana quadripunctata* (n= 51 queens).

Bee	**Head width (mm)**	**Intertegular distance (mm)**
P. remota	≤ 1.70	≤ 1.48
S. quadripunctata	< 2.28	< 1.82

Table 1- Values found for head width and intertegular distance (mm) in order to discriminate miniature queens in *P. remota* and *S. quadripunctata*. (Ribeiro et al., submitted and Wenseleers et al., submitted, respectively).

Studies on frequency distributions of queens showed there is a bimodality for both species, i.e., it is possible to distinguish two morphotypes of queens: miniature and normal with almost absolute confidence: ~99% (Ribeiro et al., submitted; Wenseleers et al., submitted). An exceptional production of miniature queens may occur by a few colonies (Ribeiro et al., 2003), although most of colonies produce just a few queens in a certain period (Alves et al., 2003).

In both species, all colonies produced more miniature queens than normal sized ones. In this manner, virgin queens are mostly miniature, while mated queens are mostly normal-sized (fig. 2). Therefore, although a large amount of miniature queens are produced only a small percentage of them are able to mate and head colonies (fig. 2; Ribeiro & Alves, 2001, Ribeiro et al., submitted, and Wenseleers et al., submitted, respectively). However, we should consider that this is based on data that did not include experimental work, testing the capability of mating of both kinds of queens, but on percentages of mated queens heading colonies (i.e., indirect observations on mating success).

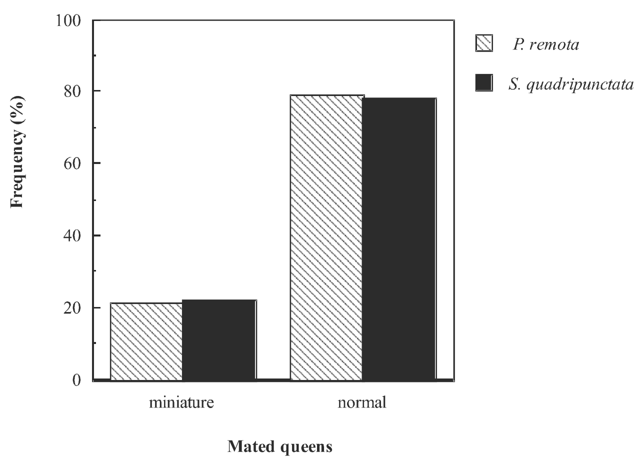

Fig. 2- Frequency distribution (%) of miniature and normal queens among mated queens in *P. remota* and *S. quadripunctata*. (Data were extracted from Ribeiro et al., submitted and Wenseleers et al., submitted, respectively).

All this led us to suppose miniature virgin queens are less successful than normal ones. There is a possibility that they are "selectively" eliminated by the workers, as in *S. quadripunctata* (Imperatriz-Fonseca & Kleinert-Giovannini, 1989; Imperatriz-Fonseca, 1990), or have lower probability of mating, induced by discriminant males, or still they have lower capability to fly and do the nuptial flight. Several attempts of mating in artificial conditions using miniature and normal-sized virgins queens of *P. remota* (n= 24 queens), did not succeed and we still do not have this answer (unpublished data).

After mating, however, normal and miniature *P. remota* queens seem to have equal chances of replacing mother-queens (being these miniature or normal). Through direct observation of supersedure (n= 14 cases), in 5 cases (~ 36%), the queen that replaced the mother-queen was smaller than she was. In 5 other times (~36%), she was larger than the mother-queen, and finally, in the rest (4 cases; 28%) both were similar in size (Ribeiro et al., submitted). Moreover, is remarkable that in one colony a miniature mother-queen survived, i.e., she was not replaced by 10 virgin normal-sized daughters, over a period of almost two years (unpublished data). This indicates that size, in this species, is not related to queen replacement.

Aspects of egg laying capability and fecundity were also studied, and here both species differ. *S. quadripunctata* miniature queens seem to have a lower capability to lay eggs, probably because they have a smaller amount of ovarioles than the normal-sized queens (Camargo 1974; Ribeiro & Alves, 2001; Wenseleers et al., submitted). But in *P. remota*, miniature and normal queens lay similar amounts of eggs. No significant correlation was found between oviposition rate and morphometric variables (table 2), demonstrating that the size of the queen is not related to her egg laying capability (Ribeiro et al., submitted). This is reinforced by the fact that the number of ovarioles is the same in queens of different sizes (Ribeiro et al., 1998; Ribeiro & Alves, 2001).

Morphometric variables (mm)	**Correlation coefficients**	**p values**
Head width	rs= - 0.049	p= 0.907
Interorbital distance	rs= - 0.098	p= 0.817
Intertegular distance	rs= - 0.048	p= 0.910

Table 2- Pearson correlation coefficients between oviposition rate of queens of *P. remota* (n=8 queens) and their morphometric variables. (Significant values for $p < 0.05$).

Concerning the size of eggs laid by queens of different sizes, we have data only on *S. quadripunctata* for while. The results showed there is a strong correlation between the size of the queens and the size of their eggs (Ribeiro & Castilho-Hyodo, in preparation). This could have as a consequence the production of smaller individuals, unless the amount of food available for their development would be large. Further investigation may clarify this.

CONCLUSIONS

Even for *Melipona*, where ideas on caste determination (i.e., genetic influence plus environment) are currently accepted, it has been suggested that the theory should be refined, considering the clustered distribution of queens in the comb (Koedam, 1999).

In relation to other species, the situation seems to be more complex. Data on *P. remota* and *S. quadripunctata* showed that miniature queens are produced in high percentages, and only a relatively small part of them may become effective queens. However, the existence of dwarf queens in the population proves their viability, and could indicate that is an evolutionary stable strategy (ESS).

On the other hand, concerning egg laying capability, the payoff of miniature queens is different for the two species. In *P. remota* both normal and miniature queens lay similar amounts of eggs (Ribeiro, 1998; Ribeiro et al., submitted), but in *S. quadripunctata* miniature queens are less fecund (Camargo, 1974; Ribeiro & Alves, 2001; Wenseleers et al., submitted). The reason is probably related to the number of ovarioles, which do not differ for both morphotypes in the first species, but do vary in the second one.

We finally reach the crucial point. How are the miniature queens then produced? The theory of self-determination seems to solve at least part of this dilemma. For a larva would be much more interesting to become a queen, even a small queen, rather than a worker (Bourke & Ratnieks, 1999). And data on *S. quadripunctata* at least, corroborates this idea (Wenseleers et al., submitted).

However, there is no explanation why occasionally we find giant workers emerging from royal cells (Imperatriz-Fonseca, 1976; Imperatriz-Fonseca et al., 1975). These larvae, even with a large amount of food available, may choose to become workers. In case the quality of food is also important (Ribeiro et al., in preparation), a larger quantity of food (found in the royal cell) could be not enough to ensure a larva will become a queen. Another possibility is that "the decision to become a worker or a queen depends upon an interaction of individual benefits and costs to the whole colony" (Wenseleers et al., 2003).

It is possible that depending on colony condition, or environmental constrains, or even on the species, different strategies can be adopted, demonstrating the flexibility and complexity of caste determination in stingless bees.

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