

Size variation and egg laying performance in *Plebeia remota* queens (Hymenoptera, Apidae, Meliponini)**

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Abstract – Stingless bees may produce both large queens and "miniature" queens, but the adaptive significance of this is uncertain. Using biometry and statistical analysis we determined the proportion of miniature queens in *Plebeia remota* that were either mated (14%) or virgin (45%). Mated queens were mostly normal-sized which indicated that they were more successful. Nevertheless, the presence of miniature mated queens heading colonies confirmed that they also *are* viable. The egg-laying capacity of mated queens of different sizes was determined using colony exchange experiments. The results showed that egg laying is not influenced by size differences. Therefore, miniature mated queens may be as successful in laying eggs as normal-sized queens. Finally we discuss some ultimate hypotheses for why miniature queens are produced.

queen size variation / miniature queen / egg laying / stingless bee / *Plebeia remota*

1. INTRODUCTION

Stingless bees show a variety of ways in which queens are reared. Trophic aspects are extremely important for caste determination, although genetic factors also seem to play a role (at least for *Melipona* species; for a review see Velthuis and Sommeijer, 1991).

There are two main methods of rearing queens. In the genus *Melipona* virgin queens emerge from cells that are of the same size as those used to rear workers and males. In contrast, in *Trigona* and related genera, virgin queens are generally produced from larger, specially constructed royal cells. Nevertheless,

in some trigonine species, virgin queens may occasionally also be reared from worker-sized cells, as in *Melipona* (Imperatriz-Fonseca et al., 1997). Moreover, in *Leurotrigona muelleri* and *Frieseomelitta varia* queens may be produced in a special way. The cells of the comb are arranged in clusters and when two cells are contiguous a larva also may eat the contents of the neighbouring cell. After that the workers transform the cell into a royal cell (Terada, 1974; Faustino et al., 2002). The queens produced from normal-sized cells are smaller than the ones reared from royal cells, and are referred to as miniature or dwarf queens (Engels and Imperatriz-Fonseca, 1990; Imperatriz-Fonseca and Zucchi, 1995). Consequently, these species produce queens of two sizes: miniature and normal-sized queens.

So far, miniature queens have been observed in six species: *Cephalotrigona femorata* (Nogueira Neto, 1951), *Plebeia juliani* (Juliani, 1962), *Schwarziana quadripunctata* (Camargo, 1974; Imperatriz-

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Fonseca and Darakjian, 1993, Nogueira-Ferreira et al., 2000), *Plebeia remota* (Imperatriz-Fonseca et al., 1975), *P. emerina* (A.M.P. Kleinert personal comm.), and *Nannotrigona testaceicornis* (Imperatriz-Fonseca et al., 1997). In most of these species, the production of miniature queens is considered a rare phenomenon. In *S. quadripunctata*, however, miniature queen production can reach high levels (Camargo, 1974; Imperatriz-Fonseca and Zucchi, 1995), with as many as 86% of all queens reared being miniature (Wenseleers et al., 2005). A large number of miniature queens were also found to be reared in *P. remota* (Ribeiro et al., 2003a). For the other species, no information on the production of miniature virgin queens is available.

Most virgin queens are killed by workers soon after emergence. Those that survive may swarm with some workers to start a new colony, or replace the old queen. Therefore, only a small percentage of virgin queens become effective queens (Engels and Imperatriz-Fonseca, 1990; Imperatriz-Fonseca and Zucchi, 1995). Except for *S. quadripunctata* (Ribeiro and Alves, 2001; Wenseleers et al., 2005) it has not been reported whether a miniature gyne may supersede a normal-sized mother-queen, mate and lay eggs normally.

The aim of this study was to compare the performance of miniature and normal-sized queens in *P. remota*. To do this, we first compared the proportion of virgin and mated queens that were miniature. We classified queens by using detailed morphometry and discriminant analysis. Second, we compared egg laying performance of miniature and normal-sized queens using colony exchange experiments. Finally, we also discussed some evolutionary hypotheses as to why miniature queens are produced.

2. MATERIALS AND METHODS

2.1. Size variation

During 5 years (from 1998 to 2002), a total of 61 colonies of *P. remota* were maintained at the Bee Laboratory (University of São Paulo) and inspected regularly. To study queen size variation we

collected 112 virgin queens and 71 mated queens which were heading colonies. Virgin queens were mostly collected from chambers where they may be kept for a variable period of time (Imperatriz-Fonseca and Zucchi, 1995). The following morphometric measurements were taken from each individual using a stereomicroscope fitted with an ocular micrometer (12 xs): head width (HEAD), medium interorbital distance (IOD), and intertegular distance (ITEG). All measurements were taken non-invasively from live bees using an apparatus described in Ribeiro and Alves (2001).

The size of virgin and mated queens was compared using nonparametric Mann-Whitney U test, since HEAD, IOD, and ITEG were not normally distributed (Kolmogorov-Smirnov test, mod. Lilliefors, see Results).

To distinguish miniature and normal-sized queens based on our biometrical measurements we developed the following analytical protocol. First, we established that there was bimodality by showing that the size distributions were not normally distributed, characterised by a test for normality through usual statistical tests; absence of normality indirectly indicates non-unimodality. Second, we tested for kurtosis of the distributions against that expected under normal distribution; a significant negative kurtosis is an indicator of bimodality (Wyszomirski, 1992). Once these two steps were applied and sufficient evidence for bimodality was found, we performed a third step, a k-means clustering using Euclidean distances based on nearest centroid sorting (Anderberg, 1973), with two a priori groups (i.e. $K = 2$, normal sized and miniature queens) including all three variables previously found to be bimodal, and using iterative estimation of cluster centroids. As a final check on the efficacy of the discrimination of morphotypes, a discriminant analysis was applied as a fourth step using the cluster membership as the classification variable. The degree of correct allocation of individuals to morphotypes was assessed through jackknife cross validation, and by examining the between-groups to within-groups variability with Wilk's lambda. Equality of group covariance matrices and multinormality were tested by the omnibus Box's M test.

In addition, a Principal Components Analysis (PCA) using the three bimodal variables (HEAD, IOD, ITEG) was made, and the first principal component used as an overall index of size. The PCA scores were classified using the cluster membership, and the linear discriminant analysis was applied to the PCA scores as described above. Both

k-means procedure, the linear discriminant analysis, and the principal component analysis are relatively robust to the type of departures from normality displayed by the studied variables (Tabachnick and Fidell, 1989).

2.2. EGG LAYING PERFORMANCE

2.2.1. *Observation on daily oviposition*

Two colonies, Pr1 and Pr2, in similar conditions with respect to food storage and number of workers, but with a miniature and a normal-sized queen, respectively, were used for observations on oviposition rate. The number of cells into which queens oviposited was counted daily over a period of 19 days (March, 13th to 31st, 1998) just before the typical interruption of cell construction (Bentham et al., 1995; Ribeiro et al., 2003b). A sheet of paper with hexagonal figures was used to represent the comb. The cells which had been operculated every 24 h were completed using coloured pencils. The total number of cells that were oviposited in per day by both queens was compared using a Chi-square test (Zar, 1999). The variation in daily oviposition rate between the two queens could not be directly compared statistically due to time and intra-individual data dependencies, but Spearman rank correlation and the Sign test were used descriptively (Zar, 1999).

2.2.2. *Queen exchange experiment*

During a period of 4 days (in November 1998), we made a continuous video recording of the comb area of two other colonies, Pr3 and Pr4, which contained a miniature and normal-sized queen, respectively. The provisioning and oviposition process (POP) (Zucchi et al., 1999) of *P. remota* has previously been studied by van Bentham et al. (1995) and Drumond et al. (1996). Here we simply counted the number of cells oviposited in by the queens. The queens were then exchanged between the colonies. After 2 days another continuous recording was made for a period of 4 days. In this way we could test whether each queen would have the same oviposition rate in another colony. The switching allowed us to determine whether queens' size influenced egg laying, independently of colony conditions. A Chi-square test was used to compare the performance of the two queens before and after their exchange. Due to the presence of a virgin

queen, which emerged on the second day of recording after the queen exchange in col. Pr3, we compared the first 24 h only. At this time the situation in both colonies was the same (i.e. no virgin queens were present).

2.2.3. *Host colony experiment*

We performed another experiment with 5 other colonies. One of them, Pr5, was chosen as a host colony to receive queens of different sizes from 4 other colonies, Pr6 through Pr9, one at a time. In this way we guaranteed the environment was the same for all queens. The host colony was in very good condition and was fed ad libitum with fresh pollen of *Tetragonisca angustula* and *Apis mellifera* honey during the days of the experiment. This was done to stimulate a high cell construction rate. When the cells were almost ready, just before the POP, the queen of the host colony was replaced by an alien queen. This queen was allowed to oviposit and the number of cells oviposited in was counted. Immediately after the POP the queens were exchanged again. This procedure was repeated 3 times on subsequent days, with the 3 other queens. Since no aggressive behaviour by the workers was observed, the introduced queens were put directly on the comb. The number of cells oviposited in by the two miniature and normal-sized queens was compared with Mann-Whitney U test, using exact p values obtained by permutation. We also counted the number of cells under construction in the original colony of each queen at the moment of exchange.

2.2.4. *Pooled data*

Finally, to obtain a larger sample size for the comparison between normal-sized and miniature queens we pooled the three previously described experiments. We used the average daily oviposition rates for queens from colonies Pr1, Pr2, Pr3 and Pr4, and used the observed oviposition for one day for queens from colonies Pr6, Pr7, Pr8 and Pr9. Daily oviposition rate was compared with the Mann-Whitney U test with exact p values obtained by permutation.

All analyses reported here were carried out using Systat v. 8.0.

Table I. Morphometric measurements of *P. remota* queens and statistical tests (with *P* values). (A) Mean and standard deviation (sd) and statistical tests for pooled sample of both virgin and mated queens ($n = 183$, for each morphometric variable). (B) Mean and standard deviation (sd) and statistical test for normal-sized ($n = 123$, being 62 virgins and 61 mated) and miniature queens ($n = 60$, being 50 virgins and 10 mated), considering all queens (i.e., mated and virgin). (C) Mean and standard deviation (sd) and statistical test for mated ($n = 71$, being 10 miniature and 61 normal-sized) and virgin queens ($n = 112$, being 50 miniature and 62 normal-sized). For (B) and (C) considering the contrast between virgin and fecundated queens, the Bonferroni alpha was $0.05/5 = 0.01$. ($n =$ number of individuals).

Statistics	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)
(A)			
Mean \pm sd for all queens	1.72 \pm 0.16	1.32 \pm 0.11	1.50 \pm 0.17
Range	1.41–2.00	1.11–1.56	1.11–1.85
Skewness	–0.510	–0.416	–0.354
T test (<i>P</i>)	$t = 2.83$ ($P < 0.01$)	$t = 2.31$ ($P < 0.05$)	$t = 1.97$ ($P < 0.05$)
Kurtosis	–0.827	–0.571	–0.976
T test (<i>P</i>)	$t = 2.32$ ($P < 0.05$)	$t = 1.60$ ($P > 0.05$)	$t = 2.73$ ($P < 0.01$)
Kolmogov-Smirnov test (<i>P</i>)	0.233 ($P = 0.0005$)	0.183 ($P = 0.0005$)	0.182 ($P = 0.0005$)
(B)			
Mean \pm sd for normal-sized queens	1.82 \pm 0.07	1.39 \pm 0.06	1.60 \pm 0.09
Mean \pm sd for miniature queens	1.52 \pm 0.08	1.20 \pm 0.06	1.29 \pm 0.08
Mann-Whitney's U (<i>P</i>)	12.500 ($P < 0.0005$)	95.000 ($P < 0.0005$)	65.500 ($P < 0.0005$)
(C)			
Mean \pm sd for mated queens	1.78 \pm 0.12	1.35 \pm 0.09	1.57 \pm 0.14
Mean \pm sd for virgin queens	1.68 \pm 0.17	1.31 \pm 0.12	1.45 \pm 0.18
Mann-Whitney's U test (<i>P</i>)	2659.5 ($P = 0.0005$)	3203.5 ($P = 0.023$)	2431.0 ($P = 0.0005$)

3. RESULTS

3.1. Production and viability of miniature queens

3.1.1. Classification of queens based on biometrical measurements

Biometrical results for the pooled sample of virgin and mated queens are shown in Table IA. The hypothesis of a normal distribution was rejected for all variables, and all distributions were significantly skewed to the left. In addition, both HEAD and ITEG were found to be significantly platykurtic, corroborating the visual detection of bimodality (Fig. 1), and IOD only tended to be platykurtic. The ranked variability of all morphological variables as

judged by the coefficient of variability (cv) was as follows: ITEG (cv = 11.8%), HEAD (cv = 9.2%) and IOD (cv = 8.2%).

Of the 183 total queens, the 2-means cluster procedure classified 123 as normal-sized queens and 60 as miniature queens (Tab. IB). A considerable distance was found between clusters (0.47138). The between-cluster to within-cluster ratio was very high for HEAD, IOD and ITEG, as shown in Table II. The between-cluster to within-cluster ratio was very high for HEAD, IOD and ITEG ($F_{1,181} > 380.0$ for the three variables, $P < 0.0005$, Tab. II). Group centroids of the two clusters and minimum and maximum values were: normal-sized queens – HEAD: 1.82 (1.63–2.00), IOD: 1.39 (1.26–1.56), ITEG: 1.60

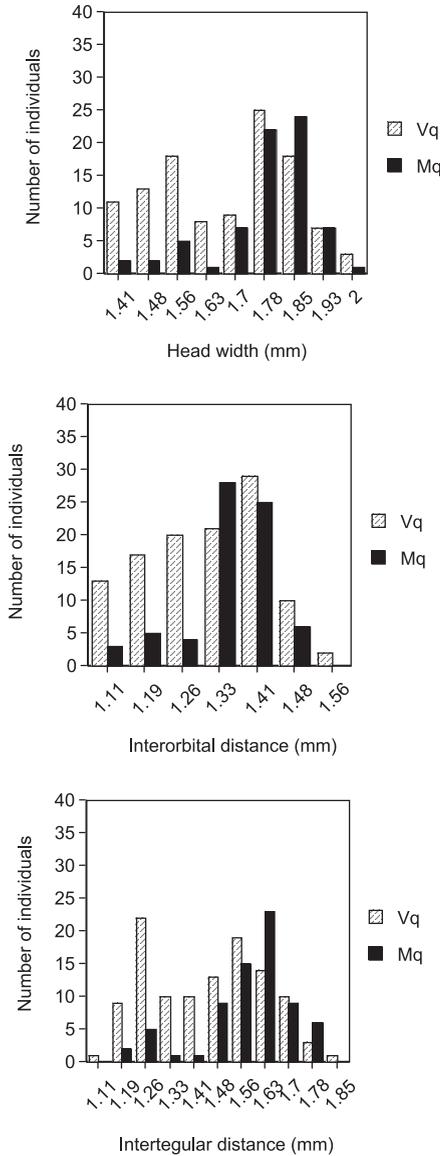


Figure 1. Frequency distributions (%) found for virgin (Vq) and mated (Mq) *P. remota* queens for the three morphometric measurements (head width, interorbital distance, and intertegular distance, in mm).

(1.33–1.85); miniature queens – HEAD: 1.52 (1.41–1.70), IOD: 1.20 (1.11–1.33), ITEG: 1.29 (1.11–1.48). Note that group centroids are the average values for each variable. Using this procedure we classified the queens into normal-sized and miniature queens despite the

fact that we did not know from which cell (i.e. royal cell or normal cell) they emerged.

The results of the discriminant analysis are shown in Table II. The Box’s test (10.305, $P = 0.121$) indicated equal covariance matrices for the two groups, and the low and significant values of Wilk’s lambdas for individual variables indicated that most of the total variability could be attributed to differences between groups. The discriminant classification functions showed that any particular queen was a miniature queen when $HEAD < 2.76 - 0.378 \cdot IOD - 0.416 \cdot ITEG$ (with all sizes measured in mm) (Tab. II). The discriminant analysis gave a good correspondence to the cluster analysis, with 182 out of the 183 queens being classified the same way. The first component (an overall index of size) obtained through principal component analysis, which accounted for 92.7% of the total variance in the three linear measurements, was able to perfectly separate the miniature from the typical queens (100% correct classification through jackknife cross-validation).

3.1.2. Comparison of the proportion of virgin and mated queens that were miniature

The frequency distributions of HEAD, IOD, and ITEG, for virgin and mated queens, are shown at Figure 1. None was normally distributed. In addition, the bimodality of the distributions was visually evident for all variables within each group of queens. The morphometric measurements (mean \pm sd) are shown in Table IC.

When we used the discriminant function to classify queens as either miniature or normal-sized, we found that 50 out of 112 (44.6%) virgin queens and 10 out of 71 (14.1%) mated queens were miniature. The proportion of miniature queens among the mated queens was significantly lower compared to the virgin queens ($\chi^2 = 18.41, P < 0.0001$). This indicates that miniature queens would be less successful than normal-sized ones in heading colonies. On the other hand, when the virgin queens from colonies with excessive queen production were excluded from the analysis

Table II. Results of the discriminant analysis and classification functions to classify queen morphotypes of *P. remota* using biometry. Lambda = Wilk's lambda; $F_{df1,df2}$ = F values of the tests of equality of group means; r = the pooled within-groups correlation between each variable and the standardized canonical discriminant function; discriminant coefficient = unstandardized canonical discriminant function coefficient. Note: (*): all $P < 0.0005$.

Variables and constant	Linear discriminant function			Linear classification function coefficients #		
	Lambda	$F_{1,181}^{(*)}$	r	Discriminant coefficient	Normal-sized queens	Miniature queens
Head width	0.220	641.510	0.961	8.282	219.336	184.973
Interorbital distance	0.320	384.705	0.839	3.127	192.275	179.299
Intertegular distance	0.270	488.580	0.744	3.447	4.993	-9.310
constant				-23.550	-337.235	-242.484

Wilk's lambda = 0.207, $\chi^2 = 282.904$, 3df, $P < 0.0005$.

Levene statistics = 0.018, df = 1.181, $P = 0.894$.

These functions can be solved to give the linear classification discriminant function: HW < 2.76 - 0.378 IOD - 0.416 ITEG.

(Ribeiro et al., 2003a, i.e., n = 40 miniature and 4 normal-sized queens), we found that 10 out of 68 (14.7%) virgin queens were miniature, a proportion which was practically the same of that of mated queens (14.1%). Indeed these proportions were not significantly different ($\chi^2 = 0.069$, $P > 0.90$).

3.2. Egg laying capacity of miniature queens

3.2.1. Observations on daily oviposition

The total number of cells oviposited in by two queens, one miniature and one normal-sized, and their size are given in Table IIIA. The total number of cells oviposited in by the miniature queen was significantly lower ($\chi^2 = 6.406$, $P < 0.02$). This suggests that the miniature queen had a lower egg-laying capacity. Nevertheless, the difference could also have arisen from differences in cell building rates, which are determined by the workers and by colony conditions (see also Ribeiro, 2002). Indeed, there were more cells available for egg laying in colony Pr2, which was headed by a normal-sized queen. To exclude the effects of colony conditions, we performed queen exchange experiments.

3.2.2. Queen exchange experiment

The results of the queen exchange experiment are presented in Table IIIB and Figure 2. The performance of miniature and normal-sized queens before their exchange was equivalent: the total number of cells oviposited in during 4 days was not significantly different ($\chi^2 = 0.0637$, $P > 0.80$), 381 and 388 cells in 4 days, respectively (Tab. IIIB). However, after exchange the normal-sized queen oviposited in significantly fewer cells compared to the miniature one (282 in 4 days vs. 416 for the miniature one, $\chi^2 = 25.7249$, $P < 0.001$, Tab. IIIB). The low number of cells oviposited in by the normal-sized queen was due to the presence of a virgin queen, as mentioned before (marked by an asterisk in Fig. 2b). When considering only the first day, the total number of cells oviposited in by both queens was not significantly different ($\chi^2 = 0.0215$, $P > 0.80$) (94 and 92 cells, respectively, Fig. 2b).

The performance of the miniature queen in her own colony was similar and not significantly different from that in the other colony (oviposition in 381 and 416 cells, respectively, $\chi^2 = 1.5370$, $P > 0.20$). The performance of the normal-sized queen, however, significantly reduced when it was placed in the other colony because of the presence of the virgin queen (from 388 to 282, respectively, $\chi^2 = 16.7701$, $P < 0.001$). When only the first 24 h were considered, the number of cells oviposited in

Table III. Morphometric measurements (mm) and number of cells oviposited in by the *P. remota* queens. (A) observation on daily oviposition, (B) queen exchange experiment, (C) host colony experiment. (N = number).

Colony	Queen morphotype	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)	N. of cells in own colony	N. of cells in other colony
(A)	miniature	1.41	1.11	1.19	808	–
Pr1	normal-sized	1.70	1.33	1.56	913	–
Pr2						
(B)	miniature	1.41	1.11	1.19	381	416
Pr3	normal-sized	1.70	1.26	1.56	388	282
Pr4						
(C)	normal-sized	1.85	1.33	1.56	–	–
Pr5	miniature	1.48	1.11	1.26	17	34
Pr6						
Pr7	normal-sized	1.70	1.33	1.48	32	33
Pr8	miniature	1.48	1.19	1.33	20	30
Pr9	normal-sized	1.93	1.48	1.70	18	40

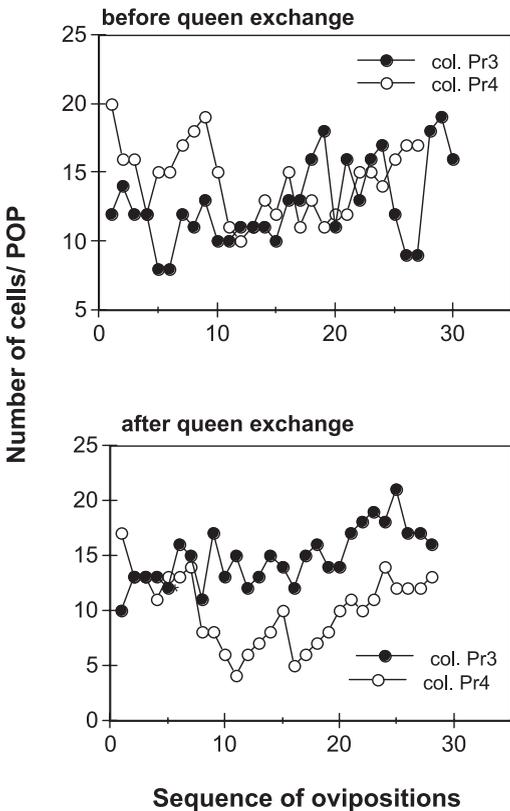


Figure 2. Number of cells oviposited into that were observed in each POP during 4 days by two *P. remota* queens (Pr3 and Pr4) before and after their colony exchange. The sign * shows when a virgin queen was born (for details see text).

(94 and 92, respectively) was not significantly different ($P > 0.80$). Once again, in a colony in a better condition, a queen (miniature, in this case) was able to lay more eggs than in her own colony (416 and 381 eggs, respectively).

3.2.3. Host colony experiment

Table IIIC shows the number of cells oviposited in, the number of cells available for oviposition in their own colonies, and the sizes of eight queens used for the host colony experiments. Independent of their size, queens oviposited in all cells that were available at that moment in the host colony. Comparing the number of cells oviposited in by queens of different sizes, no significant difference was found ($U = 1.0$, $P = 0.667$). Moreover, in all cases the number of cells in which they oviposited in within the host colony was higher than the number of cells available in their own colony (in case of col. Pr6 and Pr9, this number was about the double).

3.2.4. Pooled data

No difference in daily oviposition rate was found between normal-sized and miniature queens (Mann-Whitney $U = 6.00$, $P = 0.686$, $n_1 = 4$, $n_2 = 4$, $N = 8$). There also was no correlation between oviposition rate and any of the three morphological variables (HEAD $r_s = -0.049$, $P = 0.907$, IOD

$r_s = -0.098$, $P = 0.817$, ITEG $r_s = 0.048$, $P = 0.910$). Overall, there was no difference in the oviposition rate of normal-sized and miniature queens.

4. DISCUSSION

4.1. Size variation

Our results showed that all variables analysed (HEAD, IOD, and ITEG) were bimodal. *P. remota* queens are indeed two well-defined groups with similar variability in linear measurements. The procedure for classifying queen morphotypes through morphometric measurements was very successful, as practically all individuals were correctly assigned to the morphotype into which they belonged. Miniature queens were found to be smaller with respect to all morphometric measurements. Hence, this technique should prove useful in non-invasive studies of stingless bees which have queen morphs.

Virgin queens were mostly miniature while mated queens were mostly normal-sized. This indicates that normal-sized queens are the most successful. However, we should stress that these data included two exceptional colonies which produced large numbers of gynes (Ribeiro et al., 2003a). The majority of those gynes were miniature and might be produced as a strategy used by a few colonies (large numbers, small individuals). But since this behaviour was occasionally observed (in several years, and considering many colonies), we analyzed also the data considering only colonies with normal gyne production. In this case we found an almost identical proportion of miniature mated and virgin queens (~14%). This finding suggests that miniature virgin queens are indeed viable and have similar chances of mating and heading colonies as compared to normal-sized virgin queens.

Importantly, we found that miniature virgin queens were much more common than previously assumed. On the other hand, using another method for collecting virgin queens (i.e. monthly samples of pupae extracted from combs of 4 colonies over one year, queens were found to be produced at a very low rate

($n = 9$ queens, in 11,184 individuals analyzed), and among them only one (~11%) was miniature (Alves et al., 2003; unpublished data). This suggests that gyne production is variable, and may be influenced by as yet unknown factors.

4.2. Egg-laying capacity of miniature queens

Miniature queens had the same egg laying performance as normal-sized queens. One important feature that may determine queen oviposition rate is the number of ovarioles (Cruz-Landim et al., 1998). In two species (*S. quadripunctata* and *N. testaceicornis*) this number varies, as well the size of the virgin queens (Camargo, 1974; Imperatriz-Fonseca et al., 1997). Preliminary studies indicated that a small queen of *S. quadripunctata* indeed may lay fewer eggs than a larger one (Ribeiro, 1998; Ribeiro and Alves, 2001). In *P. remota* Cruz-Landim et al. (1998) found that the number of ovarioles varied from 4 to 8 ($n = 4$ virgin queens). However, analysing individuals of different sizes, we found a constant number: 4 ovarioles ($n = 16$ virgin queens and 1 mated queen; Ribeiro et al., 1998; Ribeiro and Alves, 2001).

Besides the number of ovarioles, the number of available cells ready to be oviposited in is important to the queen's egg laying performance (Tab. III, Ribeiro, 2002). The cell construction rate may be influenced by several factors including the amount of workers performing this task, the amount of stored food and the presence of the queen (mated and/or virgin, Fig. 2). Nevertheless, our results showed that queens of different sizes under the same circumstances can be equally active in laying eggs. An overall comparison of the performance of all available queens ($n = 8$) allowed us to conclude that the oviposition rate of miniature queens did not differ from that of normal-sized queens. Therefore, miniature queens can successfully mate and are able to head normal colonies.

4.3. Why are miniature queens produced?

From all this one crucial question arises: why are miniature queens produced in the

first place? One possibility is that under certain conditions, such as low food storage, it could be useful to produce small-sized virgin queens, which could represent an economy for the colony. In another stingless bee species where there is no queen size variation (i.e. *Melipona beecheii*), it was found that colonies with experimentally reduced food stores indeed produced fewer queens when compared to untreated colonies and colonies which received extra food. However, the latter ones did not produced significantly more queens compared to the control colonies (Moo-Valle et al., 2001). However, miniature queens were found to be produced at a high rate in two *P. remota* colonies which had ample supplies of food (Ribeiro et al., 2003a), which is evidence against the low food storage hypothesis.

Another hypothesis is related to queen/worker conflict. Assuming single mating of the queen (Peters et al., 1999; Tóth et al., 2002), the relatedness of workers to their full-sisters is of 0.75 (Hamilton, 1964; Trivers and Hare, 1976; Queller and Strassmann, 1998). Workers would prefer to rear virgin queens (who mate and pass their genes) instead of other workers (who never mate and only occasionally may have sons, which are less related to them anyway). Therefore, the workers' indirect fitness would be greater when they rear new queens. On the other hand, the mother-queen does not have interest in producing new queens since she can be replaced by one of them. Therefore, conflicts occur between the queen and workers. Since workers build and provision the cells, they could increase queen production, rearing small individuals in normal-sized cells and "cheating" the mother queen. Thus, tiny differences in larval food could result in virgin queens of different sizes. Of course this hypothesis implies food quantity/quality recognition by the workers but not by the queen. Differences in larval food quality were indeed found in *S. quadripunctata* (Castilho-Hyodo, 2001; Ribeiro, 2004), and should be better investigated.

Another ultimate hypothesis proposes that miniature queens arise as a result of a conflict over the developmental fate of female larvae (Bourke and Ratnieks, 1999; Wenseleers

et al., 2003, 2004; Ratnieks and Wenseleers, 2005; for a review see Ratnieks et al., 2006). Bourke and Ratnieks (1999) suggested that a female larva might have the power of self-determination. They also suggested that microgyny may occur as a selfish strategy of female larvae when they gain a higher pay-off by becoming a small queen than a worker. This seems to be the case at least in *S. quadripunctata* (Wenseleers et al., 2005).

These hypotheses have yet to be carefully and extensively examined. Possibly there are different strategies involved in queen size variation. For a colony of *P. remota* it might be favourable to invest in the production of small-sized queens, depending on colony situation, and/or particular ecological pressures. In any case, it is extremely relevant that queens of different sizes may be equally successful in laying eggs.

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Résumé – Variation de la taille et performance de ponte chez les reines de *Plebeia remota* (Hymenoptera, Apidae, Meliponini). La variation de la taille a déjà été mentionnée chez les reines vierges de certaines espèces d'abeilles sans aiguillon (Apidae, Meliponini). Il n'existe pourtant presque aucune information sur la variabilité des ouvrières miniatures ni sur leur capacité à pondre. Le but de ce travail était de combler ces lacunes pour des reines vierges et des reines fécondées de *Plebeia remota*. Grâce à un appareil simple on a pu mesurer sous un stéréomicroscope des reines vivantes sans les blesser. Les mesures ont porté sur la largeur de la tête et du thorax. Des analyses statistiques ont permis de séparer 2 groupes de reines : celles de taille normale et les reines miniatures. Dans 2 colonies, contenant chacune soit une reine miniature, soit une reine de taille normale, on a dénombré durant 19 j les cellules dans lesquelles un œuf avait

été pondue. Une différence significative du nombre total d'œufs produits a été trouvée. Mais ce résultat peut avoir été influencé par l'état de la colonie plutôt que par la capacité de ponte des reines. Dans 2 autres colonies, on a échangé la reine miniature et la reine de taille normale. Les 2 reines ont montré une même capacité de ponte. Enfin 4 reines, deux de chaque catégorie, ont été placées dans une colonie hôte et leur processus de ponte a donc été observé dans les mêmes conditions. Aucune différence significative n'a été trouvée. Donc le ponte n'a pas été influencée par la taille de la reine. La variation du nombre d'ovarioles pourrait avoir une influence sur la capacité de ponte. Pourtant une étude antérieure avait montré que des reines de différente taille possédaient le même nombre d'ovarioles. Nous discutons les raisons à produire des reines miniatures. La première raison pourrait être de faibles réserves de nourriture dans les colonies, mais seules des études à long terme sur la fréquence de la production de gynés en fonction de la condition de la colonie pourraient tester cette hypothèse. La seconde raison est en rapport avec le conflit reine/ouvrière. Contrairement à la reine, les ouvrières pourraient avoir un intérêt plus grand à élever de nouvelles reines (avec lesquelles elles obtiendraient une valeur adaptative globale plus grande). Puisque les ouvrières construisent et approvisionnent les cellules, elles pourraient accroître la quantité (ou la qualité) de la nourriture larvaire pour tromper la reine mère et élever des reines miniatures dans des cellules de taille normale. Nous signalons la stratégie d'auto-détermination des larves femelles qui retirent un plus grand bénéfice en devenant des petites reines plutôt que des ouvrières. De toutes façons le fait d'avoir confirmé que des reines fécondées ayant des tailles différentes existent de façon naturelle dans la population, qu'elles sont viables et peuvent être capables de pondre comme des reines de taille normale, est très utile.

***Plebeia remota* / abeille sans aiguillon / reine miniature / ponte / variation de la taille**

Zusammenfassung – Größenvariationen und Eilegeverhalten von *Plebeia remota* Königinnen (Hymenoptera, Apidae, Meliponini). Die Größenvariation von jungfräulichen Königinnen wurde bereits bei einigen Arten der Stachellosen Bienen beschrieben. Es gibt aber fast keine Informationen über die Lebensfähigkeit dieser Miniaturköniginnen oder über ihre Fähigkeit, Eier zu legen. Ziel dieser Arbeit war, die Größenvariationen von jungfräulichen und begatteten Königinnen zu bestimmen und Daten über die Eilegetätigkeit von begatteten Miniaturköniginnen zu erhalten. Die Größe der Königinnen wurde durch Biometrie bestimmt. Mit einem einfachen Apparat konnten lebende Königinnen unter einem Stereomikroskop ohne Verletzung vermessen werden. Die

Kopf- und Thoraxbreite wurde bestimmt. Mit statistischen Analysen unterschieden wir zwei Gruppen: Miniaturköniginnen und normal große Königinnen. In zwei Völkern mit je einer Miniatur- bzw. normal großen Königin wurde die Anzahl von Zellen mit Eiern über einen Zeitraum von 19 Tagen täglich bestimmt. Es ergab sich ein signifikanter Unterschied in der Gesamtzahl der gelegten Eier. Dieses Ergebnis könnte jedoch mehr vom Zustand des Volkes abhängen als von der Fähigkeit der Königinnen, Eier zu produzieren. Deshalb wurde in zwei anderen Völkern die Miniatur- und die normal große Königin experimentell ausgetauscht. Beide Königinnen zeigten gleichwertiges Eilegeverhalten. Schließlich wurden vier Königinnen, zwei Miniatur- und zwei normal große Königinnen, in ein Gastvolk eingeführt, also unter gleichen Bedingungen gehalten. Der Prozess der Eilage wurde beobachtet. Es wurden keine signifikanten Unterschiede gefunden. Die Variationen in der Anzahl der Ovariole könnte einen Einfluss auf die Eilegefähigkeit haben. Eine frühere Studie zeigte jedoch, dass verschieden große Königinnen die gleiche Anzahl Ovariole haben. Wir diskutieren die Gründe für die Erzeugung von Miniaturköniginnen. Die erste Möglichkeit wären geringe Futterreserven im Volk. Eine Langzeitstudie über eine Abhängigkeit der Frequenz der Produktion von Geschlechtstieren vom Volkszustand könnte diese Hypothese testen. Die zweite Möglichkeit könnte mit dem Königin- /Arbeiterinnen-Konflikt zusammenhängen. Im Gegensatz zu Königinnen müssten Arbeiterinnen Interesse haben, neue Königinnen aufzuziehen, durch die ihre inklusive Fitness gesteigert würde. Da Arbeiterinnen die Zellen bauen und verproviantieren, könnten sie die Menge (oder Qualität) des Larvenfutters erhöhen, um die Mutterkönigin zu betrügen und Miniaturköniginnen in normal großen Zellen aufzuziehen. Wir gehen auf die mögliche Strategie der Selbstbestimmung von weiblichen Larven ein, bei der diese als kleine Königinnen einen höheren Gewinn erlangen als Arbeiterinnen. Wie auch immer, unser Nachweis, dass begattete Königinnen unterschiedlicher Größe natürlicherweise in der Population vorkommen, dass sie lebensfähig sind und wahrscheinlich auch gleiche Fähigkeiten beim Eierlegen besitzen wie normal große Königinnen, ist von großer Relevanz.

Größenvariation bei Königinnen / Miniaturköniginnen / Eilage / Stachellose Bienen / *Plebeia remota*

REFERENCES

- Alves D.A., Ribeiro M.F., Imperatriz-Fonseca V.L. (2003) A conservação das abelhas sociais nativas (Apidae, Meliponinae) e a produção de rainhas em *Plebeia remota* Holmberg 1903, VI Congr. de Ecologia do Brasil, pp. 217–219.

- Anderberg M.R. (1973) Cluster analysis for applications, Academic Press, New York.
- Bentham F.D.J. van, Imperatriz-Fonseca V.L., Velthuis H.H.W. (1995) Biology of the stingless bee *Plebeia remota* (Holmberg): observations and evolutionary implications, *Insectes Soc.* 42, 71–87.
- Bourke A.F.G., Ratnieks F.L.W. (1999) Kin conflict over caste determination in social Hymenoptera, *Behav. Ecol. Sociobiol.* 46, 287–297.
- Camargo J.M.F. de (1974) Notas sobre a morfologia e a biologia de *Plebeia* (*Schwarziana*) *quadripunctata* (Hymenoptera, Apidae: Meliponinae), *Studia Entomol.* 17, 433–470.
- Castilho-Hyodo V.C.C. (2001) Rainha ou operária? Um ensaio sobre a determinação de castas em *Schwarziana quadripunctata* (Lepeletier, 1836) (Hymenoptera, Apidae, Meliponini), Ph.D. Thesis, University of São Paulo, São Paulo, 134 p.
- Cruz-Landim C. da, Reginato R.D., Imperatriz-Fonseca V.L. (1998) Variation on ovariole number in Meliponinae (Hymenoptera, Apidae) queen's ovaries, with comments on ovary development and caste differentiation, *Pap. Av. Zool. S. Paulo* 40, 289–296.
- Drumond P.M., Zucchi R., Yamane S., Sakagami S.F. (1996) Oviposition behaviour of the stingless bees. XVIII. *Plebeia* (*Plebeia*) *emerina* and *P. (P.) remota*, with a Preliminary Ethological Comparison of Some *Plebeia* Taxa (Apidae, Meliponinae), *Bull. Fac. Educ., Ibaraki Univ. (Nat. Sci.)* 45, 31–55.
- Engels W., Imperatriz-Fonseca V.L. (1990) Caste Development, Reproductive Strategies, and Control of Fertility in Honey Bees and Stingless Bees, in: Engels W. (Ed.), *Social Insects*, Springer Verlag, Berlin, pp. 167–230.
- Faustino C.D., Matos E.V.S., Mateus S., Zucchi R. (2002) First record of emergency queen rearing in stingless bees, *Insectes Soc.* 49, 111–113.
- Hamilton W.D. (1964) The genetic evolution of social behaviour, I, II, *J. Theor. Biol.* 7, 1–52.
- Imperatriz-Fonseca V.L., Darakjian P. (1993) Notas sobre o comportamento das rainhas de *Schwarziana quadripunctata* (Apidae, Meliponinae), *Ciênc. Cult.* 45, 912.
- Imperatriz-Fonseca V.L., Zucchi R. (1995) Virgin queens in stingless bee colonies: a review, *Apidologie* 26, 231–244.
- Imperatriz-Fonseca V.L., Oliveira M.A.C. de, Iwama S. (1975) Notas sobre o comportamento de rainhas virgens de *Plebeia* (*Plebeia*) *remota* Holmberg (Apidae, Meliponinae), *Ciênc. Cult.* 27, 665–669.
- Imperatriz-Fonseca V.L., Cruz-Landim C. da, Moraes R.L.M.S. de (1997) Dwarf gynes in *Nannotrigona testaceicornis* (Apidae, Meliponinae, Trigonini), Behaviour, exocrine gland morphology and reproductive status, *Apidologie* 28, 113–122.
- Juliani L. (1962) O aprisionamento de rainhas virgens em Trigonini, *Bol. Univ. Paraná, Zool.* 20, 1–11.
- Johnson R.A., Wichern D.A. (1998) *Applied Multivariate Statistical Analysis*, 4th ed., Prentice Hall, Upper Saddle River, New Jersey.
- Moo-Valle H., Quezada-Euán J.J.G., Wenseleers T. (2001) The effect of food reserves on the production of sexual offspring in the stingless bee *Melipona beecheii* (Apidae, Meliponini), *Insectes Soc.* 48, 398–403.
- Nogueira-Ferreira F.H., Baio M.V., Noll F.B., Tidon-Sklorz R., Zucchi R. (2000) Morphometric study in *Schwarziana quadripunctata* with emphasis on virgin queens (Hymenoptera, Apidae, Meliponinae), *Sociobiology* 35, 99–108.
- Nogueira-Neto P. (1951) Stingless bees and their study, *Bee World* 32, 73–76.
- Peters J.M., Queller D.C., Imperatriz-Fonseca V.L., Roubik D.W., Strassmann J.E. (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees, *Proc. R. Soc. London B* 266, 379–384.
- Queller D.C., Strassmann J.E. (1998) Kin selection and Social Insects, *Bioscience* 48, 165–175.
- Ratnieks F.L.W., Wenseleers T. (2005) Policing insect societies, *Science* 307, 54–56.
- Ratnieks F.L.W., Foster K.R., Wenseleers T. (2006) Conflict resolution in insect societies, *Annu. Rev. Entomol.* 51, 581–608.
- Ribeiro M.F. (1998) Size polymorphism among queens of stingless bees, XIII Int. Congr. IUSSI, Adelaide, Australia, p. 394.
- Ribeiro M.F. (2002) Does the queen of *Plebeia remota* (Hymenoptera, Apidae, Meliponini) stimulate her workers to start brood cell construction after winter? *Insectes Soc.* 49, 38–40.
- Ribeiro M.F. (2004) Miniature queens in stingless bee species – a review, in: Proc. 8th IBRA Int. Conf. on Tropical Bees and VI Encontro sobre Abelhas, pp. 280–286.
- Ribeiro M.F., Alves D. de A. (2001) Size variation in *Schwarziana quadripunctata* queens (Hymenoptera, Apidae, Meliponinae), *Rev. Etol.* 3, 59–65.
- Ribeiro M.F., Patricio E.F.L.R.A., Imperatriz-Fonseca V.L. (1998) Exceptional production of gynes in a *Plebeia remota* (Hymenoptera, Apidae, Meliponinae) colony, Proc. XIII Int. Congr. IUSSI, Adelaide, Australia, p. 395.
- Ribeiro M.F., Santos Filho P. de S., Imperatriz-Fonseca V.L. (2003a) Exceptional high queen production in the Brazilian stingless bee *Plebeia remota*, *Studies Neotrop. Fauna Environm.* 38, 111–114.

- Ribeiro M.F., Imperatriz-Fonseca V.L. Santos Filho P. de S. (2003b) A interrupção da construção de células de cria e postura em *Plebeia remota* (Holmberg) (Hymenoptera, Apidae, Meliponini), in: Melo G.A.R., Alves I. dos. (Eds.), *Apoidea Neotropica: Homenagem aos 90 anos de Jesus Santiago Moure.*, Unesc., Criciúma, pp. 177–188.
- Tabachnick B.G., Fidell L.S. (1989) *Using multivariate statistics*, Harper Collins Publishers, Inc., New York.
- Terada Y. (1974) Contribuição ao estudo da regulação social em *Leurotrigona muelleri* e *Frieseomelitta varia* (Hymenoptera, Apidae), Msc. Thesis, University of São Paulo, Ribeirão Preto.
- Tóth E., Strassman J.E., Nogueira-Neto P., Imperatriz-Fonseca V.L., Queller D. (2002) Male production in stingless bees: variable outcomes of queen-worker conflict, *Mol. Ecol.* 11, 2661–2667.
- Trivers R.L., Hare H. (1976) Haplodiploidy and the evolution of the social insects, *Science* 191, 249–263.
- Velthuis H.H.W., Sommeijer M.J. (1991) Roles of Morphogenetic Hormones in Caste Polymorphism in Stingless Bees, in: Gupta A.P. (Ed.), *Morphogenetic Hormones of Arthropods*, New Brunswick Rutgers Univ. Press, New Jersey, pp. 346–383.
- Wenseleers T., Ratnieks F.L.W., Billen J. (2003) Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis, *J. Evol. Biol.* 16, 647–658.
- Wenseleers T., Hart A.G., Ratnieks F.L.W., Quezada-Euán J.J.G. (2004) Queen execution and caste conflict in the stingless bee *Melipona beecheii*, *Ethology* 110, 725–736.
- Wenseleers T., Ratnieks F.L.W., Ribeiro M.F., Alves D.A., Imperatriz-Fonseca V.L. (2005) Working-class royalty: bees cheat the caste system, *Biol. Lett.* 1, 125–128.
- Wyszomirski T. (1992) Detecting and displaying size bimodality: kurtosis, skewness and bimodalizable distributions, *J. Theor. Biol.* 158, 109–128.
- Zar J.H. (1999) *Biostatistical analysis*, 4th ed., Prentice Hall, Upper Saddle River, New Jersey.
- Zucchi R., Silva-Matos E.V. da, Nogueira-Ferreira F.H., Azevedo G.G. (1999) On cell provisioning and oviposition process (POP) of the stingless bees, nomenclature reappraisal and evolutionary considerations (Hymenoptera, Apidae, Meliponinae), *Sociobiology* 34, 65–86.