

Size Variation in *Schwarziana quadripunctata* Queens (Hymenoptera, Apidae, Meliponini)

MÁRCIA DE F. RIBEIRO AND DENISE DE A. ALVES

Universidade de São Paulo

Queen size variation has been mentioned for some species of stingless bees. However, up to now it was considered a phenomenon that occurs only for virgin queens. There is no information on the frequency of the different sizes. Moreover, it is unknown whether small individuals can mate and are able to head colonies as normal queens. In order to verify queens' size, we weighed and measured morphometrically (head width, interorbital distance, and intertegular distance) 35 mated and 59 virgin queens of *Schwarziana quadripunctata*. The results showed that there is size variation in both groups of queens. The finding of small mated queens confirms their viability. Small virgin queens can thus mate, lay eggs and produce normal colonies. We also followed the oviposition rate of three mated queens of different sizes during 25 days. The two queens with similar size laid an equivalent number of eggs ($p=0.3765$, Mann-Whitney). The smallest queen, however, laid fewer eggs than the other two. The differences were statistically significant (respectively, $p=0.0017$, and $p=0.0001$, Mann-Whitney). The possibility that oviposition performance, among other factors, is related to the queens' number of ovarioles, is discussed.

Index terms: Size variation. Queens. Stingless bees. *Schwarziana quadripunctata*.

Varição de tamanho em rainhas de *Schwarziana quadripunctata* (Hymenoptera, Apidae, Meliponini). A variação de tamanho entre rainhas tem sido descrita para algumas espécies de abelhas sem ferrão. Entretanto, acredita-se que este fenômeno ocorra apenas para as rainhas virgens. Não existe informação sobre a frequência dos diferentes tamanhos. Além disso, não se sabe se os indivíduos pequenos podem se acasalar e se são capazes de atuar como rainhas normais. Para verificar o tamanho das rainhas, pesamos e medimos morfometricamente (largura da cabeça, distância entre os olhos e distância entre as tégulas), 35 rainhas fisogástricas e 59 rainhas virgens de *Schwarziana quadripunctata*. Os resultados mostraram que há variação de tamanho em ambos os grupos de rainhas. O fato de existirem rainhas fecundadas pequenas, confirma que elas são viáveis. Portanto, rainhas virgens pequenas podem se acasalar, ovipositar e produzir colônias normais. Também observamos a taxa de postura de três rainhas fecundadas de diferentes tamanhos, durante 25 dias. As duas rainhas que possuíam tamanhos semelhantes, colocaram um número equivalente de ovos ($p=0.3765$, Mann-Whitney). A rainha menor, porém, colocou menos ovos que as outras duas rainhas. As diferenças foram significativas (respectivamente, $p=0.0017$, e $p=0.0001$, Mann-Whitney). A possibilidade de que a capacidade de oviposição, além de outros fatores, esteja relacionada ao número de ovaríolos das rainhas, é discutida.

Palavras-chave: Variação de tamanho. Rainhas. Abelhas sem ferrão. *Schwarziana quadripunctata*.

Caste determination and differentiation in stingless bees are not yet completely understood. In *Melipona*, caste determination seems to be genetic, although the trophic aspect is also important. In *Trigona* and other genera, the amount, rather than the quality of food is

the determining factor (see Velthuis & Sommeijer, 1991 for a review).

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Márcia de F. Ribeiro. Depto. de Ecologia, IB-USP, R. do Matão, trav. 14, n. 321, 05508-900, São Paulo, Brazil, e-mail: marib@ib.usp.br

In *Melipona*, virgin queens are frequently produced and emerge from cells similar to those of workers and males. In *Trigona* and other genera, virgin queens are occasionally produced and emerge from larger cells, the royal cells. However, they may also emerge from smaller cells. In this case, because they ingest less food as larvae, they are smaller than queens from royal cells. This variation in the virgin queens' size was registered for some species as *Cephalotrigona femorata* (Nogueira-Neto, 1951), *Plebeia julianii* (Juliani, 1962), *P. remota* (Imperatriz-Fonseca, Oliveira & Iwama, 1975), *P. emerina* (A. de M. P. Kleinert, personal communication), *Nannotrigona testaceicornis* (Imperatriz-Fonseca, Cruz-Landim, & Moraes, 1997), and *Schwarziana quadripunctata* (Camargo, 1974; Costa, 1977).

Nevertheless, the knowledge on the subject is still quite poor and comprises just a few species. External and internal morphology, and behavioural aspects were studied in virgin queens of *N. testaceicornis* of different sizes (Imperatriz-Fonseca et al., 1997). Concerning *S. quadripunctata*, the development of female larvae was analysed under the effects of juvenile hormone and amount of food (Campos, 1979; Campos & Costa, 1989). However, the virgin queens produced were not measured. In other studies, the morphology (Camargo, 1974; Cruz-Landim, Reginato & Imperatriz-Fonseca, 1998) and the behaviour (Imperatriz-Fonseca & Darakjian, 1993) of virgin queens of different sizes were described. Larval growth of virgin queens of different sizes was also investigated (Nogueira-Ferreira, Baio, Noll, Tidon-Sklorz, & Zucchi, 2000). Although Imperatriz-Fonseca and Kleinert-Giovannini (1989) observed one small queen of *S. quadripunctata*, no detailed work has yet been done on the frequency of small queens or their egg laying capacity.

It has been suggested that the queen oviposition rate is related, among other factors, to the number of ovarioles. Moreover, this number seems to be variable in some species (Cruz-Landim et al., 1998). In *P. remota*, the same authors found that the number of ovarioles varied from four to eight (n = 4 virgin

queens). We found, however, that queens of different sizes had a constant number: four ovarioles (n = 16 virgin queens and one mated queen; Ribeiro, Patrício & Imperatriz-Fonseca, 1998). We also found that *P. remota* queens of different sizes had an equivalent egg laying capacity (Ribeiro & Imperatriz-Fonseca, submitted). In *S. quadripunctata* there is variation on the number of ovarioles among virgin queens. Camargo (1974) found queens with six to nine ovarioles (n = 29 virgin queens), and Cruz-Landim et al. (1998) mentioned four to eight ovarioles (n = 4 virgin queens). However, no study on the oviposition rates from queens of different sizes has been done.

This work aimed at investigating size variation in mated and virgin queens of *S. quadripunctata*, and the oviposition performance of mated queens of different sizes.

Methods

We used 35 colonies of *S. quadripunctata* from Southeast and South Brazil (Cunha: 23°05'S 44°55'W, Cotia: 23°39'S 46°47'W, and Prudentópolis: 25°13'S 50°59'W). The nests were maintained in observation boxes (Sakagami, 1966), under controlled temperature (28° C), with free access to outdoors, at the Bee Laboratory (University of São Paulo, in São Paulo: 23°33'S 46°43'W). We collected the mated queens (n = 35) and several virgin queens (n = 59) from these colonies

In order to determine their size, queens were weighed¹ in an analytical balance (Ohaus, 0,1 mg precision) and measured morphometrically. Each queen was immobilised by low temperature (one minute at -8.0° C) and then put into a concavity made on a spongy material supported by a metal structure. Once in the cavity, a piece of glass was placed over the queen to keep her head and thorax in an adequate position for the measurements. Their

¹ The sample size related to the queens' mass (Tables 1 and 2) is different because two individuals (one mated queen and one virgin queen) could not be measured.

maximum head width, medium interorbital distance, and intertegular distance were measured under a stereomicroscope equipped with an ocular micrometer (see Michener, 1965 for details on bee morphometry). Manipulation caused no harm to the queens and they were returned to their colonies soon after the measurements, except for some virgin queens that were used in other experiments.

Averages and standard deviations were calculated for mass and morphometric measurements of virgin queens, mated queens, and both groups of queens together. In order to know whether the mass would be a good indicator of size, we calculated the Spearman's rank correlation coefficients (Zar, 1999) between this variable and the morphometric measurements for mated and virgin queens. We did not calculate these coefficients for both groups of queens together because mated queens are obviously much heavier than virgin queens due to physogastry. We used Spearman's instead of Pearson's rank correlation coefficients because the data distributions proved not be normal. The frequency distributions of the morphometric measurements were tested for normality using the Kolmogorov-Smirnov test (Zar, 1999).

Morphometric measurements of virgin and mated queens were compared using a Mann-Whitney U test (Zar, 1999).

The oviposition rates of three queens (from colonies A, B and C) were observed. These colonies were selected because of their similar general conditions (food pots and population), the differences in the queens' sizes (see in Results), and the horizontal layers of the comb (the brood cell construction is easier to follow in this situation than when the comb layers are in spiral). The oviposition process in *S. quadripunctata* was studied in detail by Nogueira-Ferreira, Zucchi, Silva-Matos & Sakagami (1998). In this work we simply counted how many cells were oviposited by the queens. We did this daily from March 12 to April 05 1998 (n= 25 days). A sheet of paper with hexagonal figures was used to represent the comb. The cells that had been sealed every 24h were filled using coloured pencils. The average number of cells oviposited per day by each queen was compared with Kruskal-Wallis and afterwards, in pairs with Mann-Whitney (Zar, 1999). During the entire period of observation these colonies were provided with extra food (sugar water solution 60%, sunflower pollen and *Apis mellifera* honey) twice a week.

Results

Size variation of virgin and mated queens

We found variation in the size of the queens (Table 1). The mass showed much

Table 1. Average and standard deviation ($x \pm sd$) obtained for mass (mg) and morphometric measurements (head width, interorbital distance, and intertegular distance, in mm) for *S. quadripunctata* virgin queens, mated queens, and virgin and mated queens together (n= number of individuals). * Two individuals (one virgin and one mated queen) could not be weighed. ** The large standard deviation is due to the obvious differences in mass of virgin and mated queens (physogastry).

Queens	Mass (mg)	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)
Virgin	30.37 \pm 8.82 (n=58)*	2.17 \pm 0.14 (n=59)	1.54 \pm 0.11 (n=59)	1.73 \pm 0.17 (n=59)
Mated	129.63 \pm 32.00 (n=34)*	2.41 \pm 0.19 (n=35)	1.75 \pm 0.14 (n=35)	2.02 \pm 0.21 (n=35)
Virgin and Mated	67.05 \pm 52.35** (n=92)*	2.26 \pm 0.20 (n=94)	1.62 \pm 0.16 (n=94)	1.84 \pm 0.23 (n=94)

variation, being the largest mated queen (172.3mg) more than three times larger than the smallest queen (55.3mg). Mass varies due to factors such as amount of fat, crop and glandular contents, and mainly, degree of ovarian development. However, it presented high Spearman correlation coefficients with the morphometric measurements indicating that mass may be a relatively good indicator of size. The values found for r_s in all comparisons were significant. Mass x head width: virgin queens ($r_s = 0.461$, $p < 0.001$), mated queens ($r_s = 0.612$, $p < 0.001$); mass x interorbital distance: virgin queens ($r_s = 0.401$, $p < 0.01$), mated queens ($r_s = 0.663$, $p < 0.001$); mass x intertegular distance: virgin queens ($r_s = 0.368$, $p < 0.01$), mated queens ($r_s = 0.715$, $p < 0.001$).

In relation to the morphometric measurements, virgin queens were generally smaller than mated queens (Table 1, Figures 1, 2, and 3). These differences were significant ($p = 0.0001$, Mann-Whitney, in all comparisons).

The frequency distributions of the morphometric measurements were significantly different from the normal curves for virgin queens ($p = 0.000$), and for mated queens ($p = 0.01$). Nevertheless, there was a tendency to bimodality, especially concerning the intertegular distance (Figure 3).

Size variation and oviposition rate of mated queens

Table 2 shows the mass, morphometric measurements and the average number of cells

oviposited by the queens during observation. The queen from colony A was the smallest, and the queens from colonies B and C were similar in size (Figure 4).

Comparing the average number of cells oviposited by the three queens (Table 2), we found a significant difference ($p = 0.0001$, Kruskal-Wallis). When we compared the queens in pairs, we found that the smallest queen (A) laid a significant lower amount of eggs than the largest queen (C) ($p = 0.0001$, Mann-Whitney), and than the other large queen (B) ($p = 0.0017$, Mann-Whitney). The two large queens, which were more similar in size (B and C), however, showed no significant difference ($p = 0.3765$, Mann-Whitney) concerning the amount of oviposited eggs.

Discussion

Our results for virgin queens ($n = 59$, Table 1), for head width, interorbital distance, and intertegular distance, differed from those obtained by Camargo (1974): $2.12 \pm 0.05\text{mm}$, $1.58 \pm 0.03\text{mm}$, and $1.50 \pm 0.05\text{mm}$ ($n = 29$), respectively. The mated queen of that work was larger in head width and interorbital distance, but smaller in the intertegular distance (2.48mm, 1.80mm, and 1.88mm, respectively) than the queens of our study ($n = 35$, Table 1). This divergence could be due to the different geographic origin of the colonies (in the case of Camargo, Caldas: $21^{\circ}92'S$ $46^{\circ}39'W$) or still, to the wide size variation present in the species.

Table 2. Mass (mg), morphometric measurements (head width, interorbital distance, and intertegular distance, in mm), and average number of cells oviposited by the three queens of *S. quadripunctata* during the observation period ($n =$ number of days).

Queens	Mass (mg)	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)	Average number of oviposited cells
A	89.3	2.07	1.48	1.85	23.64 ± 8.01 ($n = 25$)
B	139.3	2.37	1.70	2.07	36.64 ± 15.33 ($n = 25$)
C	143.1	2.44	1.78	2.07	41.68 ± 8.21 ($n = 25$)

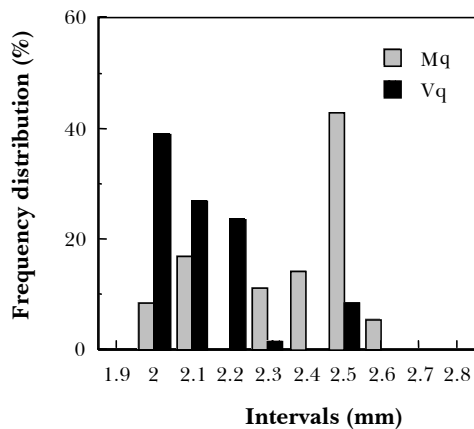


Figure 1. Frequency distribution of intervals of head width (mm) of mated queens (Mq) and virgin queens (Vq).

We did not find a clear bimodality in the distributions of the queens' different sizes as in ants (see Ruppel & Heinze, 1999 for a review on size polymorphism in ants). Maybe this would be found in a much larger sample. Even though there is a tendency for the existence of two queens' sizes (small and large), especially regarding the intertegular distance (Figure 3). Visually splitting the distributions for the three morphometric measurements into two groups (Figures 1, 2 and 3), and calculating an average, we found that around 31% of the mated queens were small.

Nogueira-Ferreira et al., (2000), analysing three colonies of *S. quadripunctata*, whose mated queens were large, suggested that size is important to promote queen dominance. However, we found several small mated queens (31%) in our 35 colonies. Therefore, small virgin queens of *S. quadripunctata* are viable. They certainly are able to do a nuptial flight, mate,

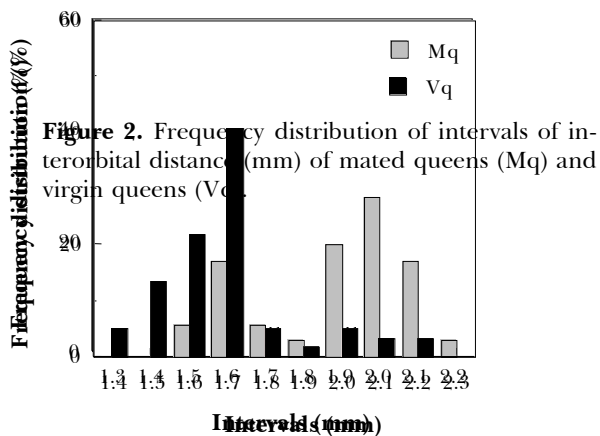


Figure 2. Frequency distribution of intervals of interorbital distance (mm) of mated queens (Mq) and virgin queens (Vq).

Figure 3. Frequency distribution of intervals of intertegular distance (mm) of mated queens (Mq) and virgin queens (Vq).



Figure 4. Mated queens of *S. quadripunctata* of different sizes used in the oviposition rates observation. Above: the smallest (from colony A). Below: the largest (from colony C). Note the thorax width of the two queens. Photos by Dirk Koedam.

and head normal colonies later on. Moreover, as in *P. remota* (Ribeiro & Imperatriz-Fonseca, submitted), we observed that *S. quadripunctata* queens, being small or large, may produce virgin queens of different sizes, and larger daughters do not necessarily replace their smaller mothers.

In this work we found that the smallest queen laid significantly less eggs than the two larger queens. Considering that Camargo (1974) and Cruz-Landim et al. (1998) found a variation in the number of ovarioles of queens, one may suppose that small queens have less ovarioles, and consequently, lower oviposition capability than large ones. Cruz-Landim (2000) suggested that the number of ovarioles per ovary is related to the amount of food a larva consumes in the differentiation phase. In this way a small queen, born from a normal-sized cell, would have less ovarioles than a large queen, that emerged from a royal cell. Unfortunately, we were not able to check the number of ovarioles of each mated queen of our observation because they died after the colonies were used for other studies.

However, other factors have to be considered as well. For instance, the number of available cells to be oviposited, which is directly related to colony condition, and can also affect the queen's oviposition performance. Further investigations are necessary to test this hypothesis.

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