Sociobiology 61(4): 483-489 (December, 2014) DOI: 10.13102/sociobiology.v61i4. 483-489

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Size variation in eggs laid by normal-sized and miniature queens of *Plebeia remota* (Holmberg) (Hymenoptera: Apidae: Meliponini)

MF Ribeiro[1,2], PS Santos Filho[1]

1 - Universidade de São Paulo, São Paulo, SP, Brazil

2 - Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Petrolina, PE, Brazil

Article History

Edited by
Vera L. Imperatriz-Fonseca, IBUSP, Brazil
Received 26 September 2014
Initial acceptance 27 October 2014
Final acceptance 25 November 2014

Keywords
Queen body size, egg morphometry, stingless bees.

Corresponding author
Márcia de Fátima Ribeiro
EMBRAPA SEMIÁRIDO, BR 428, Km 152
Zona Rural, C.P. 23, 56302-970
Petrolina - PE, Brazil
E-Mail: marcia.ribeiro@embrapa.br

Abstract

Miniature stingless bee queens have been studied concerning frequency distribution, production and egg laying performance. This study aimed to investigate size variation in eggs laid by *Plebeia remota* (Holmberg) queens and whether it is due to differences in queen size or colony conditions. A sample of 10 queens (8 of typical size and 2 miniature) was measured morphometrically (head width, interorbital distance, and intertegular distance) as well the eggs they laid (length, width and volume). Initially, eggs were analyzed when laid by queens in their own colonies. Significant differences were found for length, width and volume of eggs considering the total group of queens or both queen morphotypes. However, no significant correlations were found between queen size and egg size. Afterwards, two experiments were performed to evaluate the influence of colony conditions on egg size. Firstly, we shifted the queens from their original colonies (i.e., a typical queen was placed into a miniature queen colony, and vice-versa). Secondly, they were put into another colony (both types of queens, one each time, were placed on a third colony, a 'host colony'). In all situations, both queen morphotypes laid eggs of similar or different sizes than before, often with significant differences. The results indicate that variation in egg size is due to conditions imposed to queens in the colony (e.g. queen feeding status, number of cells available to be oviposited), and not due to variation in queen body size.

Introduction

Variation in queen body size has been registered for several species of stingless bees. Dwarf or miniature queens have been described as small sized queens that may be eventually present in the nests. However, details related to proportion of occurrence and behavior when heading colonies, have been investigated practically only for *Schwarziana quadripunctata* (Lepeletier) and *Plebeia remota* (Holmberg) (Ribeiro, 2002; Ribeiro & Alves, 2001; Ribeiro et al, 2003; Wenseleers et al., 2005; Ribeiro et al, 2006a, b). These studies reported that miniature mated queens occur naturally at a low frequency in the population and, at least for *P. remota*, they can be as efficient in laying eggs as typical-sized queens. Nevertheless, even when miniature queens lay the same amount of eggs as normal-sized queens, it is unknown whether the eggs of both queen morphotypes differ in size, and whether this variation is related to body size. In case the eggs produced by small queens are also small, this could result in smaller individuals, or when hatching out, the larvae would need larger amounts of food to develop into normal-sized adults. As suggested for honeybees (Henderson, 1992), the amount of food available to the larvae is, in fact, more important to determine adult size rather than egg size. However, to date, the question remains unclear and it is not possible to determine the implications of small and large eggs to the colony.

Actually, egg size in bees has been poorly studied. In honeybees, egg size varies due to several factors: between castes, i.e. queen and workers (Woyke & Wongsiri, 1992; Woyke, 1994; Gençer & Woyke, 2006); according to seasons (Henderson, 1992); and due to changes in the metabolic process (Woyke, 1998). In stingless bees, eggs differ in size according to the species (Velthuis & Sommeijer, 1991), but there is little information on intra-specific variation. Eggs can also differ in morphology due to their different functions: trophic eggs (laid by workers) or reproductive eggs (laid by queens) (Koedam et al., 1996; 2001). Variation in egg size produced by a single queen was studied only for *Scaptotrigona* aff. *depilis* Moure and *S. quadripunctata* (Lacerda, unpublished data; Lacerda & Simões, 2006a, b; Ribeiro et al., unpublished data).

In this context, this study aimed (i) to investigate the size of eggs laid by queens of *P. remota*; (ii) to check for the relationship between the variation in egg size and queen body size, and (iii) the verify influence of the environment (colony)

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

on egg size variation. The hypothesis is that small queens do lay smaller eggs than normal-sized queens, similarly to that found for *S. quadripunctata* (Ribeiro et al., unpublished data).

Material and Methods

Queen measurements

Ten *P. remota* queens of different sizes were collected with an insect aspirator, directly from colonies kept at the Bees Laboratory, University of São Paulo (USP), São Paulo. Their maximum width, average interorbital distance, and intertegular distance were measured under a stereomicroscope with a micrometer eyepiece (for details on the measuring method see Ribeiro & Alves, 2001). The queens were separated into two morphotypes: normal-sized (or typical) and miniature queens based on a previous study (Ribeiro et al., 2006a). A queen was classified as miniature using the equation HEAD < 2.76 – 0.378.IOD – 0.416 ITEG, where HEAD: head width; IOD: interorbital distance, and ITEG: intertegular distance. Although the group of queens used in this study does not represent the total range of sizes, they were the only ones available at the laboratory when the experiments were performed, and they were classified into both morphotypes using the formula described above without any restriction.

Egg measurement: non-experimental and experimental situation

Eggs were collected from the periphery of the upper combs, in which the cells have been oviposited recently. Once the hatching of the larvae is virtually identified by the horizontal position of the egg (Sommeijer et al., 1984), it was possible to collect eggs even without knowing exactly when the queen had laid them. In this way, we collected only eggs in the upright position, which therefore did not go through embryonic development. After opening the brood cell with a warmed entomological pin, the egg was collected using another pin, curved at the extremity. The egg was then immediately placed on aluminum foil, with a little amount of larval food, to prevent its dehydration. After that, the egg was measured under a stereomicroscope with a micrometer eyepiece, for length and width. Egg volume was then calculated considering the egg as a prolate spheroid, and using the formula: $V= 4/3.\pi.L/2.(L/2)^2$, where V= volume, L= length and W= width of the egg.

To analyze the possible effects of colony conditions, some experiments were performed considering other situations for eggs collection. Thus, in the first situation, non-experimental, queens were in their own colonies (Qown). In the second situation, we shifted the position of queens (Qexch), i.e., a miniature queen was placed into the colony of a typical sized queen, and vice-versa. In the third situation, queens (miniature and typical sized), one at time, were placed into a third colony, a 'host colony' (Qhost). The use of this third colony ensured that both queens (normal-sized and miniature) were subjected to the same colony conditions, which could be eventually different from their own colony, or the colony to which they were shifted to. The queen of the host colony was simultaneously placed in the colony from where the queen was removed to be tested. In order to provide time for adaptation of the queen time to a new colony, an interval of three days was allowed before a new egg collection. This method also assured that the sampled eggs were originated from the newly inserted queen and not from the former one; eggs were collected in the same way already described. In some cases, a new sample of eggs was obtained after the return of queens to their own colonies. In this way, in tables 3, 4 and 5, the numbers 1 and 2 after the code indicate the first and second times the queen was subjected to that situation. For example, Qown 2 means that the queen returned to its own colony, after a shift; Qexch 2 means the second time the queen was shifted, and so on. The table also mentions, in parentheses, in which colony the queen was at the moment of egg collection.

Statistical analysis

Statistical tests (Zar, 1999) were applied (1) to check for normality of the data (Kolmogorov-Smirnov); (2) to test the differences between the eggs laid by different queens (Kruskal-Wallis); (3) to compare the eggs laid by both morphotypes of queens (Mann-Whitney U test); and (4) to check for correlation between the queen size and the egg size (Spearman correlation). The software used to perform these tests was SPSS.

Results

Measurements of queens and eggs in own colony (Qown)

From the group of ten queens, eight were classified as normal-sized (numbered from one to eight) and two, as miniature (numbered from nine to ten). Their body measurements are presented in Table 1.

Considering all situations, a total of 642 eggs were collected, and for each individual queen, in each situation, up to 30 eggs were collected. Because data showed no normal distribution, non-parametric tests were applied. Table 1 lists the mean values (and SD) of egg measurements (length, width and volume) in Qown 1. Significant differences were found for all variables (Kruskal-Wallis, P= 0.000, N= 275 eggs, for length, width and volume). When comparing the two sets of queens, all variables analyzed for their eggs were also significant (Mann-Whitney, P= 0.000, N= 275 eggs, for length, width and volume).

Queen body size (head width, interorbital distance and intertegular distance) presented negative non-significant correlations with all variables of eggs (egg length, egg width and egg volume; Table 2).

Table 1. Morphometric measures of body and eggs (averages ± SD) fromnormal and miniature queens of *Plebeia remota* in their own colonies, in the first analyzed situation (Qown 1). (N = number of eggs).

Queens (morphotypes)	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)	Egg Length (mm) X ± SD	Egg Width (mm) X ± SD	Egg volume (mm^3) X ± SD
1 (Normal)	1.93	1.48	1.78	1.20 ± 0.05 (N=28)	0.45 ± 0.04 (N= 28)	1.13 ± 0.12 (N= 28)
2 (Normal)	1.93	1.48	1.78	1.24 ± 0.04 (N= 30)	0.46 ± 0.04 (N= 30)	1.20 ± 0.13 (N= 30)
3 (Normal)	1.85	1.41	1.70	1.21 ± 0.05 (N= 30)	0.47 ± 0.04 (N= 30)	1.18 ± 0.14 (N= 30)
4 (Normal)	1.85	1.41	1.70	1.20 ± 0.04 (N= 30)	0.46 ± 0.03 (N= 30)	1.16 ± 0.11 (N= 30)
5 (Normal)	1.78	1.33	1.63	1.24 ± 0.05 (N= 16)	0.52 ± 0.04 (N= 16)	1.35 ± 0.13 (N= 16)
6 (Normal)	1.78	1.41	1.63	1.25 ± 0.04 (N= 30)	0.47 ± 0.05 (N= 30)	1.24 ± 0.14 (N= 30)
7 (Normal)	1.78	1.33	1.56	1.20 ± 0.04 (N= 30)	0.44 ± 0.04 (N= 30)	1.10 ± 0.12 (N= 30)
8 (Normal)	1.70	1.33	1.48	1.22 ± 0.03 (N= 30)	0.45 ± 0.03 (N= 30)	1.14 ± 0.10 (N= 30)
9 (Miniature)	1.63	1.26	1.41	1.17 ± 0.04 (N= 21)	0.45 ± 0.03 (N= 21)	1.09 ± 0.08 (N= 21)
10 (Miniature)	1.48	1.11	1.18	1.16 ± 0.04 (N= 30)	0.43 ± 0.03 (N= 30)	1.04 ± 0.09 (N= 30)

On the other hand, when we included Qown2 in the analysis of all queens (i.e. Qown1 + Qown2), significant differences were also found for all variables (Kruskal-Wallis, P= 0.000, N= 275 eggs, for length, width and volume). This was not observed when the queens were compared between morphotypes, for egg length and volume (Mann-Whitney, P= 0.427, P= 0.517, respectively, N= 365 eggs). The differences for egg width were significant (Mann-Whitney, P= 0.050, N= 365 eggs).

Measurements of queens and eggs in another colony (Qexch)

The results found for egg measurements under Qexch situations (1 and 2), including Qhost and Qown (situations 1 and 2), as well as the P values for the statistical tests are shown in Tables 3, 4 and 5.

When queens were interchanged between colonies (Qexch 1 and Qexch 2), eggs presented significant differences (Kruskal-Wallis, P= 0.000, for all variables, N= 277 eggs). When both morphotypes were compared, differences were significant only for egg volume (Mann-Whitney, P= 0.879, P= 0.134, and P= 0.000, respectively for length, width and volume, N= 277 eggs).

Table 2. Spearman rank correlations (Rho) and P values for comparisons between queens' size (head width, interorbital distance and intertegular distance) and average values for eggs' measures (length, width and volume) of *Plebeia remota* queens in their own colonies (Qown1). (N = number of eggs).

Eggs measures	Head width (mm)	Interorbital distance (mm)	Intertegular distance (mm)
Average Length (mm)	Rho= - 0.127 P= 0.727 (N= 10)	Rho= - 0.211 P= 0.559 (N= 10)	Rho= - 0.166 P= 0.646 (N= 10)
Average Width (mm)	Rho= - 0.267 P= 0.456 (N= 10)	Rho= - 0.295 P= 0.408 (N= 10)	Rho= - 0.274 P= 0.444 (N= 10)
Average Volume (mm^3)	Rho= - 0.268 P= 0.454 (N= 10)	Rho= - 0.334 P= 0.346 (N= 10)	Rho= - 0.296 P= 0.406 (N= 10)

It is remarkable that the lowest and highest mean values of egg length were presented by a miniature queen (number 10), respectively, 1.16 mm and 1.29 mm. In relation to egg width, the lowest mean value (0.43 mm) was once again presented by the queen 10, but the highest (0.52 mm) was exhibited by a normal-sized queen (number 5). With respect to egg volume, the lowest mean value was found for the queen 10 (1.04 mm^3) and the highest by the queen 5 (1.35 mm^3; Tables 2, 3 and 4).

Fig. 1 illustrates the changes observed in egg volume for the queens subjected to more than two different experimental situations. Two normal-sized queens (numbers 2 and 5) laid smaller eggs after the shift, while the others (one normal-sized: 1, and both miniatures: 9 and 10) laid larger eggs after the shift. When they returned to their own colonies,

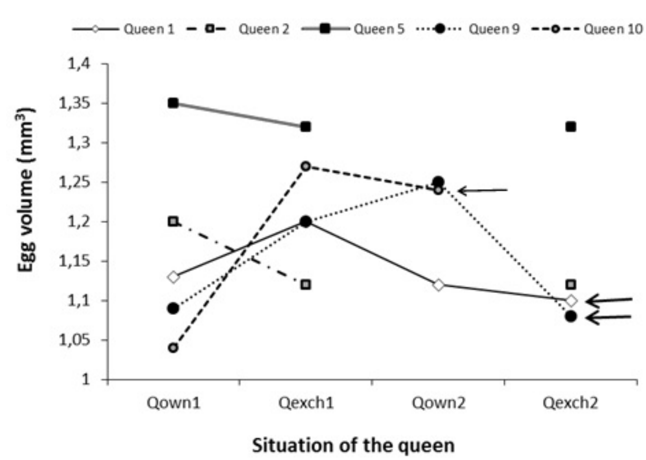

Fig 1. Average volume of eggs (mm3) laid by *Plebeia remota* queens (number 1, 2 and 5: normal-sized; 9 and 10: miniature) in the different situations (own colony or another colony). Legend: Qown 1: queen in her own colony, and analyzed for the first time; Qown 2: queen returned to her own colony, after being in another colony; Qexch 1: queen in another colony, for the first time; Qexch 2: queen in another colony, for the second time. Arrows show the host colony.

Table 3. Comparisons of morphometric measures (averages ± SD) obtained for eggs laid by the different normal-sizedqueens of *Plebeia remota* in their colonies and other colonies. The number after Qown or Qexch refers to the situation, i.e., the first or second time the queen was in that situation. (Legend: Qown = Queen in own colony; Qexch = Queen exchanged, in another colony; N = number of eggs; col. = colony).

Queens	Comparison Experimental condition	Egg Length (mm) x ± SD	Mann-Whitney test *P*	Egg Width (mm) x ± SD	Mann-Whitney test *P*	Egg Volume (mm³) x ± SD	Mann-Whitney test *P*
1	Qown 1 (col. 1) vs. Qexch 1 (col. 9)	1.20 ± 0.05 (N=28) 1.25 ± 0.04 (N=24)	*P*= 0.001**	0.45 ± 0.04 (N= 28) 0.46 ± 0.05 (N= 24)	*P*= 0.181	1.13 ± 0.12 (N= 28) 1.20 ± 0.14 (N= 24)	*P*= 0.009**
1	Qown 1 (col. 1) vs. Qown 2 (col. 1)	1.20 ± 0.05 (N=28) 1.22 ± 0.03 (N= 30)	*P*= 0.014*	0.45 ± 0.04 (N= 28) 0.44 ± 0.01 (N= 30)	*P*= 0.771	1.13 ± 0.12 (N= 28) 1.12 ± 0.04 (N= 30)	*P*= 0.105
1	Qexch 1 (col. 9) vs. Qown 2 (col.1)	1.25 ± 0.04 (N=24) 1.22 ± 0.03 (N= 30)	*P*= 0.024*	0.46 ± 0.05 (N= 24) 0.44 ± 0.01 (N= 30)	*P*= 0.034*	1.20 ± 0.14 (N= 24) 1.12 ± 0.04 (N= 30)	*P*= 0.040*
2	Qown 1 (col. 2) vs. Qexch 1 (col. 1)	1.24 ± 0.04 (N=30) 1.23 ± 0.04 (N=30)	*P*= 0.196	0.46 ± 0.04 (N= 30) 0.44 ± 0.03 (N= 30)	*P*= 0.002**	1.20 ± 0.13 (N= 30) 1.12 ± 0.10 (N= 30)	*P*= 0.030*
2	Qown 1 (col. 2) vs. Qexch 2 (col. 9)	1.24 ± 0.04 (N=30) 1.24 ± 0.03 (N=30)	*P*= 0.938	0.46 ± 0.04 (N= 30) 0.43 ± 0.02 (N= 30)	*P*= 0.002**	1.20 ± 0.13 (N= 30) 1.12 ± 0.08 (N= 30)	*P*= 0.049*
2	Qexch 1 (col. 1)vs. Qexch 2 (col. 9)	1.23 ± 0.04 (N=30) 1.24 ± 0.03 (N=30)	*P*= 0.169	0.44 ± 0.03 (N= 30) 0.43 ± 0.02 (N= 30)	*P*= 0.603	1.12 ± 0.10 (N= 30) 1.12 ± 0.08 (N= 30)	*P*= 0.146
5	Qown 1 (col. 5) vs. Qexch 1 (col. 3)	1.24 ± 0.05 (N=16) 1.24 ± 0.05 (N=30)	*P*= 0.626	0.52 ± 0.04 (N= 16) 0.51 ± 0.04 (N= 30)	*P*= 0.399	1.35 ± 0.13 (N= 16) 1.32 ± 0.13 (N= 30)	*P*= 0.388
5	Qown 1 (col. 5) vs. Qexch 2 (col. 10)	1.24 ± 0.05 (N=16) 1.24 ± 0.05 (N=30)	*P*= 0.921	0.52 ± 0.04 (N= 16) 0.51 ± 0.04 (N= 30)	*P*= 0.258	1.35 ± 0.13 (N= 16) 1.32 ± 0.12 (N= 30)	*P*= 0.466
5	Qexch 1 (col. 3) Qexch 2 (col. 10)	1.24 ± 0.05 (N=30) 1.24 ± 0.05 (N=30)	*P*= 0.597	0.51 ± 0.04 (N= 30) 0.51 ± 0.04 (N= 30)	*P*= 0.811	1.32 ± 0.13 (N= 30) 1.32 ± 0.12 (N= 30)	*P*= 0.852

Table 4. Comparisons of morphometric measures (averages ± SD) obtained for eggs laid by the different miniature queens of *Plebeia remota* in their colonies and other colonies. The number after Qown or Qexch refers to the situation, i.e., the first or second time the queen was in that situation. (Legend: Qown = Queen in own colony; Qexch = Queen exchanged, in another colony; N = number of eggs; col. = colony).

Queens	Comparison Experimental condition	Egg Length (mm) x ± SD	Mann-Whitney test *P*	Egg Width (mm) x ± SD	Mann-Whitney test *P*	Egg Volume (mm³) x ± SD	Mann-Whitney test *P*
9	Qown 1 (col. 9) vs. Qexch 1 (col. 1)	1.18 ± 0.04 (N=21) 1.22 ± 0.05 (N=20)	*P*= 0.001**	0.44 ± 0.03 (N= 21) 0.47 ± 0.04 (N= 20)	*P*= 0.027*	1.09 ± 0.08 (N= 21) 1.20 ± 0.12 (N= 20)	*P*= 0.001**
9	Qown 1 (col. 9) vs. Qown 2 (col. 9)	1.18 ± 0.04 (N=21) 1.28 ± 0.04 (N= 30)	*P*= 0.000**	0.44 ± 0.03 (N= 21) 0.47 ± 0.04 (N= 30)	*P*= 0.004*	1.09 ± 0.08 (N= 21) 1.25 ± 0.10 (N= 30)	*P*= 0.000**
9	Qexch 1 (col. 1) vs. Qown 2 (col.9)	1.22 ± 0.05 (N=20) 1.28 ± 0.04 (N= 30)	*P*= 0.000**	0.47 ± 0.04 (N= 20) 0.47 ± 0.04 (N= 30)	*P*= 0.799	1.20 ± 0.12 (N= 20) 1.25 ± 0.10 (N= 30)	*P*= 0.028*
10	Qown 1 (col. 10) vs. Qown 2 (col. 10)	1.16 ± 0.04 (N=30) 1.29 ± 0.07 (N=30)	*P*= 0.000**	0.43 ± 0.03 (N= 30) 0.46 ± 0.05 (N= 30)	*P*= 0.011*	1.04 ± 0.09 (N= 30) 1.24 ± 0.17 (N= 30)	*P*= 0.000**

queens 1 (normal-sized) and 10 (miniature) laid smaller eggs again, but the queen 10 (the smallest queen) laid even larger eggs. After Qexch2, all queens laid similar or smaller eggs than in the previous situation.

Regarding the third situation (Qhost: colonies 2 and 5), queens of both morphotypes showed similar performance (Table 5). Thus, no significant differences were detected between the eggs from normal-sized (1 and 3) and miniature queens (9 and 10), when considered both morphotypes for egg length and width, but not for egg volume (Mann-Whitney, *P*= 0.147, *P*= 0.306, and *P*= 0,004, respectively, N= 113 eggs). When queens were considered in pairs, i.e., queens 1 and 9 in host colony 2, the results were similar (Mann-Whitney, *P*= 0.658, *P*= 0.366, P= 0.000, respectively for length and width, and volume, N= 60 eggs). However, when queens 3 and 10 in the host colony 5 were compared, no differences were significant for all analyzed variables (Mann-Whitney, *P*= 0.056 and *P*= 0.160, and P= 0.601, respectively for length, width, and volume N= 53 eggs).

Discussion

The results for the situation in which the queens remained in their own colonies (Qown1) could suggest that normal-sized queens lay larger eggs than miniature queens (Table 1). However, analyzing in detail the results of queens in other colonies (especially Qhost, Table 5; Fig 1), it was verified that sometimes miniature queens are able to lay larger eggs than normal-sized queens. In fact, queens of different sizes laid, in all situations analyzed (i.e., in Qown 1 and 2, Qexch 1 and 2, or Qhost), eggs of similar sizes, smaller or larger than before, and often these differences were significant (Tables 3, 4 and 5; Fig. 1). Moreover, non-significant correlations between body size and egg size evidenced that the variation in egg size is not due to differences in the queen size. Likewise, in different honeybee species, Woyke et al. (2003) found no relationship between eggs' size among queens of different species and sizes.

Table 5. Comparisons of morphometric measures (averages ± SD) obtained for eggs laid by the different queens of *Plebeia remota* in their colonies and other colonies. The number after Qown or Qexch refers to the situation, i.e., the first or second time the queen was in that situation. (Legend: Qown = Queen in own colony; Qexch = Queen exchanged, in another colony; Qhost= Queen in host colony; N = number of eggs; col. = colony).

Queens (morphotypes)	Comparison Experimental condition	Egg Length (mm) x ± SD	Mann-Whitney test *P*	Egg Width (mm) x ± SD	Mann-Whitney test *P*	Egg Volume (mm^3) x ± SD	Mann-Whitney test *P*
1 (Normal)	Qown 1 (col. 1) vs. Qexch 2 (col. 2) = Q host	1.20 ± 0.05 (N=28) 1.20 ± 0.04 (N= 30)	*P*= 0.974	0.45 ± 0.04 (N= 28) 0.44 ± 0.03 (N= 30)	*P*= 0.355	1.13 ± 0.12 (N= 28) 1.10 ± 0.10 (N= 30)	*P*= 0.627
1 (Normal)	Qexch 1 (col. 9) vs. Qexch 2 (col. 2) = Q host	1.25 ± 0.04 (N=24) 1.20 ± 0.04 (N= 30)	*P*= 0.000**	0.46 ± 0.05 (N= 24) 0.44 ± 0.03 (N= 30)	*P*= 0.026*	1.20 ± 0.14 (N= 24) 1.10 ± 0.10 (N= 30)	*P*= 0.001**
1 (Normal)	Qown 2 (col.1) vs. Qexch 2 (col. 2) = Q host	1.22 ± 0.03 (N= 30) 1.20 ± 0.04 (N= 30)	*P*= 0.007*	0.44 ± 0.01 (N= 30) 0.44 ± 0.03 (N= 30)	*P*= 0.307	1.12 ± 0.04 (N= 30) 1.10 ± 0.10 (N= 30)	*P*= 0.015*
9 (Miniature)	Qown 1 (col. 9) vs. Qexch 2 (col. 2) = Q host	1.18 ± 0.04 (N=21) 1.20 ± 0.05 (N= 30)	*P*= 0.112	0.44 ± 0.03 (N= 21) 0.43 ± 0.02 (N= 30)	*P*= 0.102	1.09 ± 0.08 (N= 21) 1.08 ± 0.09 (N= 30)	*P*= 0.862
9 (Miniature)	Qexch 1 (col. 1) vs. Qexch 2 (col. 2) = Q host	1.22 ± 0.05 (N=20) 1.20 ± 0.05 (N= 30)	*P*= 0.029*	0.47 ± 0.04 (N= 20) 0.43 ± 0.02 (N= 30)	*P*= 0.001**	1.20 ± 0.12 (N= 20) 1.08 ± 0.09 (N= 30)	*P*= 0.001**
9 (Miniature)	Qown 2 (col.9) vs. Qexch 2 (col. 2) = Q host	1.28 ± 0.04 (N= 30) 1.20 ± 0.05 (N= 30)	*P*= 0.000**	0.47 ± 0.04 (N= 30) 0.43 ± 0.02 (N= 30)	*P*= 0.000**	1.25 ± 0.10 (N= 30) 1.08 ± 0.09 (N= 30)	*P*= 0.000**
3 (Normal)	Qown 1 (col. 3) vs. Qexch 1 (col. 5) = Q host	1.21 ± 0.05 (N=30) 1.25 ± 0.06 (N=23)	*P*= 0.012*	0.46 ± 0.05 (N= 30) 0.49 ± 0.04 (N= 23)	*P*= 0.008**	1.18 ± 0.14 (N= 30) 1.29 ± 0.15 (N= 23)	*P*= 0.001**
10 (Miniature)	Qown 1 (col. 10) vs. Qexch 1 (col. 5) = Q host	1.16 ± 0.04 (N=30) 1.28 ± 0.05 (N=30)	*P*= 0.000**	0.43 ± 0.03 (N= 30) 0.48 ± 0.05 (N= 30)	*P*= 0.000**	1.04 ± 0.09 (N= 30) 1.27 ± 0.15 (N= 30)	*P*= 0.000**
10 (Miniature)	Qown 2 (col. 10) vs Qexch 1 (col. 5) = Q host	1.29 ± 0.07 (N=30) 1.28 ± 0.05 (N=30)	*P*= 0.477	0.46 ± 0.05 (N= 30) 0.48 ± 0.05 (N= 30)	*P*= 0.058	1.24 ± 0.17 (N= 30) 1.27 ± 0.15 (N= 30)	*P*= 0.329

Thus, our results indicate that it is not the size of the queens that influences the size of the eggs, but rather the conditions imposed to the queens. Probably the condition of each colony was different and influenced the egg size, whether positively (larger eggs) or negatively (smaller eggs). On the other hand, when queens laid similar-sized eggs in both situations, all the colonies probably provided similar conditions.

A possible explanation for the differences in egg size is the development of the embryos. Thus, changes in egg size would imply in embryonic development. The exact moment of egg laying by the queen was not observed in this work. Nevertheless, eggs were always collected in very new combs, i.e., with cells recently operculated and, therefore, the possibility of collecting eggs at an advanced stage of development is excluded. Moreover, as already mentioned, when eggs hatch they did not stay in a vertical position, but lay down on the larval food and only eggs in vertical position were collected.

Egg size variation may be caused by nutritional conditions to which queens were exposed. Therefore, in colonies with abundant food reserve, queens probably received more food, and could produce larger eggs. Simultaneously, queens placed in colonies with fewer reserves, and supposedly lower food intake, would produce smaller eggs. Opposite results, however, were found for *S.* aff. *depilis* (Lacerda, unpublished data; Lacerda & Simões, 2006b). Queens of this species in 'weak' colonies produced larger eggs than in 'strong' ones. However, the authors suggested that these eggs were haploid eggs, which are larger than diploid eggs. In *P. remota*, most haploid eggs are produced by the queen (Tóth et al., 2002). Nevertheless, the variation in egg size found herein was not due to male production, since adult males were not registered in the studied colonies after the experimental period.

So, if the variation in egg size is associated to queen feeding status, this would be imposed by colony condition, and in some way, by the workers. Queens get food through three different ways: by eating larval food from the open cell just before laying the egg, by eating trophic eggs laid by the workers or by trophallaxis with workers. This food ingestion could be important for the production and development of eggs inside the queen ovarioles. In this way, the workers, besides controlling the number of cells available for egg laying (Ribeiro, 2002), indirectly are also responsible for the size of the eggs laid by queens.

On the other hand, as suggested by J. Woyke (personal communication, March 8, 2008) egg size may still be related to the cell production rate of the colony. Analyzing the relationship between the number of cells available for oviposition in each colony with egg size, negative correlations were recorded for egg length and width (Spearman, rho= -0.040, rho= -0.144, respectively, *P*= 0.01, N= 642 eggs). The longer the queen had to wait to lay eggs, the larger these eggs could become, probably by accumulation of vitellogenin. Certainly, before the end of egg development, during vitellogenesis, a deposit of vitello can be established thus increasing egg volume up to the moment of ovulation (i.e. the expulsion of the egg through the oviducts) (Cruz-Landim, 2009). In this work, when the queen was transferred to a smaller colony, this would result in a delay in the oviposition and consequently could result in an increased size of the eggs ready to be laid. In an opposite situation, one could expect a contrary result.

Therefore, the results of the present study indicate that egg size is influenced by colony conditions (and consequently, food supply for the queens and/or rate of cell construction) imposed to the queen, rather than by body size.

Acknowledgments

We are grateful to Dr. J. Woyke, Dr. Vera L. Imperatriz-Fonseca, Dr. Dirk Koedam, Dr. Beatriz A. J. Paranhos and two anonymous referees for the critical reading of the manuscript; Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, proc. n. 98/01679-5), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP, proc. n. 35.0781/2004-4) for financial support; and Erica M. T. de Alencar, for language advice.

References

Cruz-Landim, C. (2009). Aparelho reprodutor e gametogênese. In C. Cruz-Landim (Ed.), Abelhas - Morfologia e função de sistemas (pp. 15-57). São Paulo: Editora UNESP.

Gençer, H.V. & Woyke, J. (2006). Eggs from *Apis mellifera caucasica* laying workers are larger than from queens. J. Apicult. Res. 45(4): 173-179. doi: 10.3896/IBRA.1.45.4.02

Henderson, C.E. (1992). Variability in the size of emerging drones and of drone and worker eggs in honey bee (*Apis mellifera* L.) colonies. J. Apicult. Res., 31(3/4): 114-118. doi:

Katzav-Gozansky, T., Soroker, V., Kamer, J., Schulz, C. M., Francke, W. & Hefetz, A. (2003). Ultrastructural and chemical characterization of egg surface of honeybee worker and queen-laid eggs. Chemoecology 13: 129-134. doi: 10.1007/s00049-003-0238-0

Koedam, D., Velthausz, P.H., Krift, T.V.D., Dohmen, M.R. & Sommeijer, M.J. (1996). Morphology of reproductive and trophic eggs and their controlled release by workers in *Trigona* (*Tetragonisca*) *angustula* Illiger (Apidae, Meliponinae). Physiol. Entomol. 21: 289-296. doi: 10.1111/j.1365-3032.1996.tb00867.x

Koedam, D., Velthuis, H.H.W., Dohmen, M.R. & Imperatriz-Fonseca, V.L. (2001). The behaviour of laying workers and the morphology and viability of their eggs in *Melipona bicolor bicolor*. Physiol. Entomol. 26: 254-259. doi: 10.1046/j.0307-6962.2001.00241.x

Lacerda, L.M. & Simões, Z.L.P. (2006a). Ovos produzidos por rainhas e operárias de *Scaptotrigona depilis* (Hymenoptera, Apidae, Meliponina): morfometria e aspectos relacionados. Iheringia, Sér. Zool. 96: 89-93. doi: 10.1590/S0073-47212006000100016

Lacerda, L.M. & Simões, Z.L.P. (2006b). Effects of internal conditions on the size of eggs laid by queens of *Scaptotrigona depilis* (Moure, 1942) (Apidae, Meliponinae). Sociobiology 47: 85-97. doi: 10.13102.

Pereira, R.A., Morais, MM., Gioli, L.D., Nascimento, F.S., Rossi, M.A. & Bego, L.R. (2006). Comparative morphology of reproductive and trophic eggs in some *Melipona* bees (Apidae, Meliponini). Braz. J. Morphological Sci. 23: 349-354.

Ribeiro, M.F. (2002). Does the queen of *Plebeia remota* (Hymenoptera, Apidae, Meliponini) stimulate her workers to start cell construction after winter? Insect. Soc. 49: 38-40. doi: 10.1007/s00040-002-8276-0

Ribeiro, M.F. & Alves, D.A. (2001). Size variation in *Schwarziana quadripunctata* queens (Hymenoptera, Apidae, Meliponinae). Rev. Etologia 3(1): 59-65.

Ribeiro, M.F., Santos-Filho, P.S. de & Imperatriz-Fonseca, V.L. (2003). Exceptional high queen production in the Brazilian stingless bee *Plebeia remota*. Stud. Neotrop. Fauna Envir. 38: 111-114. doi: 10.1076/snfe.38.2.111.15925.

Ribeiro, M.F., Santos-Filho, P.S. de & Imperatriz-Fonseca, V.L. (2006a). Size variation and egg laying performance in *Plebeia remota* queens (Hymenoptera, Apidae, Meliponinae). Apidologie 37: 653-664. doi: 10.1051/apido:2006046

Ribeiro, M.F., Wenseleers, T., Santos-Filho, P.S. de & Alves, D.A. (2006b). Miniature queens in stingless bees: basic facts and evolutionary hypotheses. Apidologie 37: 191-206. doi: 10.1051/apido:2006023

Sommeijer, M.J., van Zeijl, M. & Dohmen, M.R. (1984). Morphological differences between worker-laid eggs from a queenright colony and a queenless colony of *Melipona rufiventris paraensis* (Hymenoptera: Apidae). Entomol. Berichten, 44: 91-95.

Velthuis, H.H.W. & Sommeijer, M.J. (1991). Morphogenetic hormones in caste polymorphism in stingless bees. In A.P. Gupta (Ed.), Mophogenetic Hormones of Arthropods (pp. 346-382). News Brunswick: Rutgers Univ. Press.

Wenseleers T., Ratnieks, F.L.W., Ribeiro, M.F., Alves, D.A. & Imperatriz-Fonseca, V.L. (2005). Working-class royalty: bees beat the caste system. Biol. Lett. 1: 125-128. doi: 10.1098/rsbl.2004.0281

Wheeler, D. (1996). The role of nourishment in oogenesis. Annu. Rev. Entomol., 41: 407-431. doi: 10.1146/annurev.en.41.010196.002203

Woyke, J. (1994). Comparison of the size of the eggs from *Apis mellifera* L. queens and laying workers. Apidologie, 25: 179-187. doi: 10.1051/apido:19940206.

Woyke, J. (1998). Size change of *Apis mellifera* eggs during the incubation period. J. Apicult. Res. 37: 239-246.

Woyke, J. & Wongsiri, S. (1992). Occurrence and size of laying worker eggs in *Apis florea* colonies. J. Apicult. Res. 31: 124-127.

Woyke, J., Chanchao, C., Wongsiri, S., Wilde, J. & Wilde, M. (2003). Size of eggs from queens of three Asian species and laying workers of *Apis cerana*. J. Apicult. Science 47: 57-71.

Zar, J.H. (1999). Biostatistical Analysis. 4th ed. New Jersey: Prentice Hall, 663 p.