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COMPARATIVE MORPHOLOGY OF REPRODUCTIVE AND TROPHIC EGGS IN *Melipona* BEES (APIDAE, MELIPONINI)

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ABSTRACT

The process of cell provisioning and oviposition (POP) in stingless bees involves highly complex interactions between the queen and workers. During this process, workers can usually lay two types of eggs, referred to as reproductive and trophic. Reproductive worker-laid eggs are unfertilized but develop into males whereas trophic worker-laid eggs are eaten by the queen immediately after oviposition. Although the egg-laying activity of reproductive workers varies considerably among species, the laying of trophic eggs appears to be the rule in *Melipona* bees. In this work, we examined whether the morphology of eggs laid by workers and queens of *M. scutellaris*, *M. compressipes fasciculata* and *M. asilvai* was similar to that of other *Melipona* species. Egg morphology was examined by scanning electron microscopy whereas egg size was measured by light microscopy. In all of the species studied, the chorion of queen and worker reproductive eggs showed a characteristic reticulate pattern. The surface of trophic eggs was not reticulate and had an irregular appearance following fixation. Trophic eggs were also invariably smaller than queen-laid eggs and were sometimes smaller than worker-laid reproductive eggs. These findings indicate that trophic eggs can be smaller than the eggs of functional workers, which suggests that the development of this type of egg is probably associated with different physiological adaptations.

Key words: Eggs, *Melipona*, Meliponini, morphology, oviposition

INTRODUCTION

In stingless bees (Apidae, Meliponini), the process of cell provisioning and oviposition (POP) is an important phenomenon for understanding the sociobiology of these insects because of the highly complex, species-specific, stereotypical queen-worker interactions involved [7,17,18]. During POP, each cell is filled with liquid larval food regurgitated by a number of workers (reviewed in Zucchi *et al*. [27]) and the queen then lays an egg on top of this liquid, after which one or more workers seal the cell [17]. In many species, workers can lay reproductive eggs (thereby contributing to the colony's production of males) and trophic eggs, which are eaten by the queen [7,13,21,23,25,26]. The workers usually lay reproductive and trophic eggs during POP [2-4,19].

Trophic eggs are thought to be a derived character in eusocial insects since their sole function is to provide nourishment for the queen [8]. Previous studies based on morphology [1,24], cytochemistry and ultrastructure [10] have confirmed that trophic eggs in stingless bees are unlikely to develop normally. However, only a few studies have documented morphological differences between reproductive and trophic eggs in *Melipona* bees [9,11,12,24]. In this work, we compared the morphology and size of worker-laid trophic and reproductive eggs, and of eggs laid by queens in three *Melipona* species.

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MATERIAL AND METHODS

Egg collection

Eggs were collected from 11, nine and seven colonies of *Melipona scutellaris*, *M. compressipes fasciculata*

and *Melipona asilvai*, respectively, maintained in the Laboratório de Ecologia, Departamento de Biologia, at USP, Ribeirão Preto. The workers of all colonies were able to forage freely. In queenright *Melipona* colonies, the workers always lay trophic eggs immediately before oviposition by the queen, whereas reproductive eggs are normally laid after oviposition. Consequently, the process of provisioning and oviposition (POP) was monitored closely so that eggs could be collected immediately after oviposition. Because oviposition by reproductive workers can be infrequent, POPs were monitored for 12 consecutive months (June 2001 to July 2002). Throughout this period, no reproductive eggs were laid by *M. c. fasciculata* workers.

Morphological analysis:
scanning electron microscopy (SEM)

Reproductive and trophic eggs were fixed in 2% glutaraldehyde buffered in 0.05 M sodium cacodylate plus 0.15 M sucrose (pH 7.2) for 2 h [15], rinsed in distilled water and post-fixed in 2% picric acid for 1 h. The eggs were subsequently dehydrated in an ethanol series, critical point dried with CO_2 and sputtered with gold. The eggs were examined by SEM using a Jeol JSM-5.200 scanning electron microscope (Departamento de Morfologia da Faculdade de Medicina, USP, Ribeirão Preto).

Egg measurements

Immediately after collection, the egg size was measured using a binocular stereomicroscope with a coupled eyepiece. Four measurements were obtained for each egg: length (L), anterior width (W_1), medial width (W_2), and posterior width (W_3). The three widths were taken at predetermined spots along the long axis of the egg. For this, each egg was partitioned into three sections of equal length and the widths were measured in the middle of each section (Fig. 1). For the size measurements, 20 reproductive eggs of *M. asilvai* queens (QEs) and workers and five trophic eggs were used. For *M. scutellaris*, 10 reproductive worker-laid and queen-laid eggs and 10 trophic eggs were used, whereas for *M. c. fasciculata*, 10 trophic eggs and 10 queen-laid eggs were measured. The Mann-Whitney *U* test was used to determine whether the egg types differed in size [22].

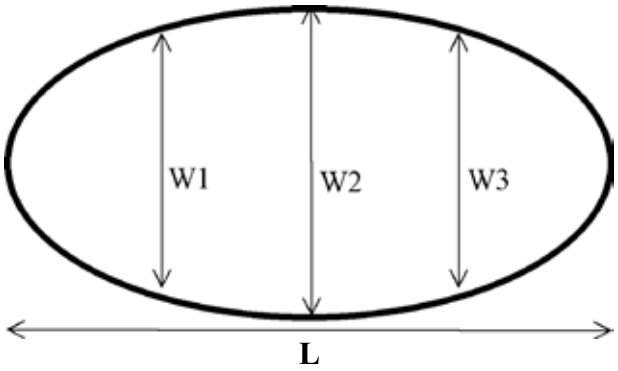

Figure 1. Schematic representation of the egg measurements used in this work: length (**L**), anterior width ($\mathbf{W_1}$), medial width ($\mathbf{W_2}$) and posterior width ($\mathbf{W_3}$).

RESULTS

Egg morphology

Queen-laid eggs and worker-laid reproductive eggs of all of the species were pale white and very similar in shape (ellipsoidal, elongated, and asymmetrical from the anterior to the posterior region) (Figs. 2-4). Both types of eggs had a network pattern or reticulated chorion (Fig. 2C,D, Fig. 3C,D). The anterior region was wide and reticulate while the posterior region was slender and non-reticulate at the apex (Figs. 2C and 3C). The chorion of queen and worker reproductive eggs had a polygonal format (Fig. 2C,D, Fig. 3C,D, Fig. 4D).

In all of the species, worker-laid trophic eggs were oval-shaped and yellow (Figs. 2A, 3A and 4B). The chorion was always non-reticulate and the distribution of yolk mass was irregular. Trophic eggs proved extremely fragile when prepared for SEM, with those of *M. c. fasciculata* being the most resistant to manipulation. As in other species, these worker-laid eggs lacked the typical patterned chorion, although fixation probably resulted in a more corrugated appearance (Fig. 4D).

Egg size

Significant differences in size were observed between queen-laid and worker-laid eggs in *M. asilvai* (Table 1). Queen-laid eggs and reproductive worker-laid eggs were longer than trophic worker-laid eggs (QE *vs.* TWE: $Z = 5.41$, $p < 0.01$; RWE *vs.* TWE: $Z = 3.39$, $p < 0.01$). The anterior region of queen-laid eggs was significantly wider than that of trophic eggs ($Z = 2.61$, $p < 0.01$), whereas the central portion of trophic eggs was significantly thinner than that of worker-laid reproductive eggs ($Z = 3.39$, $p < 0.01$). The posterior region of worker-laid reproductive eggs was wider than that of trophic eggs ($Z = 2.24$, $p < 0.01$) and queen-laid eggs ($Z = 2.25$, $p < 0.01$).

In *M. scutellaris*, queen-laid eggs and worker-laid trophic eggs differed significantly in most measurements (Table 1). Queen-laid eggs were more elongated (L: $Z = 2.22$, $p < 0.05$) and wider (W_1: $Z = 1.96$, $p < 0.05$; W_2: $Z = 3.77$, $p < 0.001$) than trophic eggs. The width of the posterior region did not differ

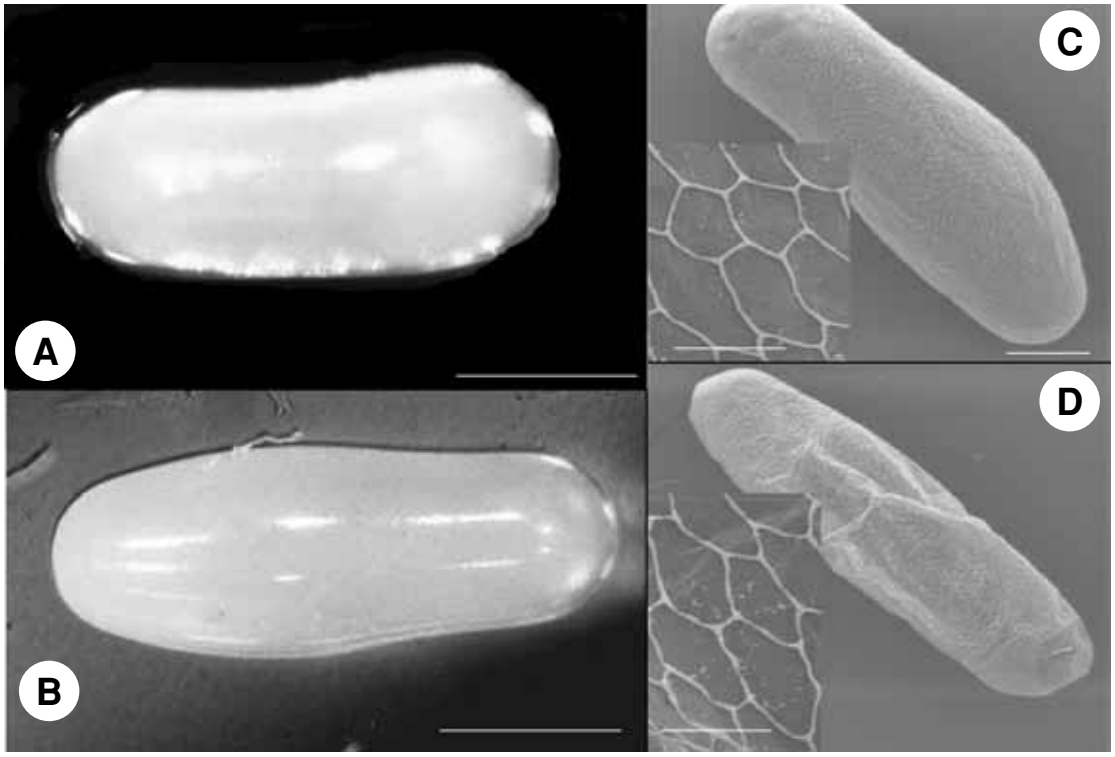

Figure 2. *Melipona asilvai* eggs. Light micrographs of a worker-laid trophic egg (**A**) and a queen-laid egg (**B**). Bars = 1 mm. SEM images showing reproductive eggs laid by a queen (**C**) and workers (**D**). Bars = 300 μm. A detail of the reticulate chorion is also shown. Bar = 90 μm.

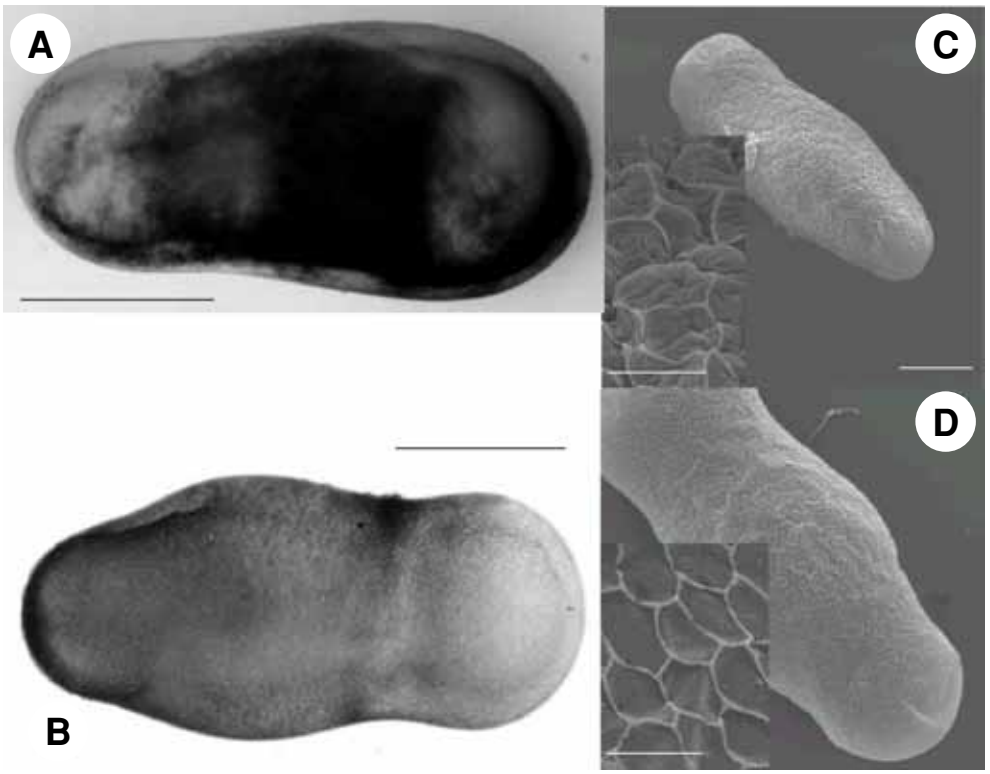

Figure 3. *Melipona scutellaris* eggs. Light micrographs of a worker-laid trophic egg (**A**) and a queen egg (**B**). Bars = 1 mm. SEM images showing reproductive eggs laid by workers (**C**) and a queen (**D**). Bars = 300 μm. A detail of the reticulate chorion is also shown. Bar = 90 μm.

Table 1. Morphometric data for eggs of three *Melipona* species.

Type of egg	Length (L)	Anterior width (W_1)	Medial width (W_2)	Posterior width (W_3)
Melipona asilvai				
Queen (N = 20)	2.87 ± 0.10	0.82 ± 0.04	1.04 ± 0.06	0.70 ± 0.03
Worker-laid reproductive egg (N = 5)	2.93 ± 0.13	0.79 ± 0.04	1.04 ± 0.05	0.69 ± 0.01
Worker-laid trophic egg (N = 20)	2.22 ± 0.16	0.78 ± 0.03	0.85 ± 0.04	0.73 ± 0.04
Melipona scutellaris				
Queen (N = 10)	3.29 ± 0.18	1.01 ± 0.10	1.48 ± 0.09	1.24 ± 0.08
Worker-laid trophic egg (N = 10)	3.03 ± 0.21	1.09 ± 0.08	1.10 ± 0.07	1.17 ± 0.10
Melipona compressipes fasciculata				
Queen (N = 10)	3.46 ± 0.14	0.97 ± 0.07	1.38 ± 0.10	1.12 ± 0.09
Worker-laid trophic egg (N = 10)	2.81 ± 0.22	0.93 ± 0.05	1.08 ± 0.05	1.05 ± 0.07

The data (in mm) are the mean ± SD of the number of eggs indicated.

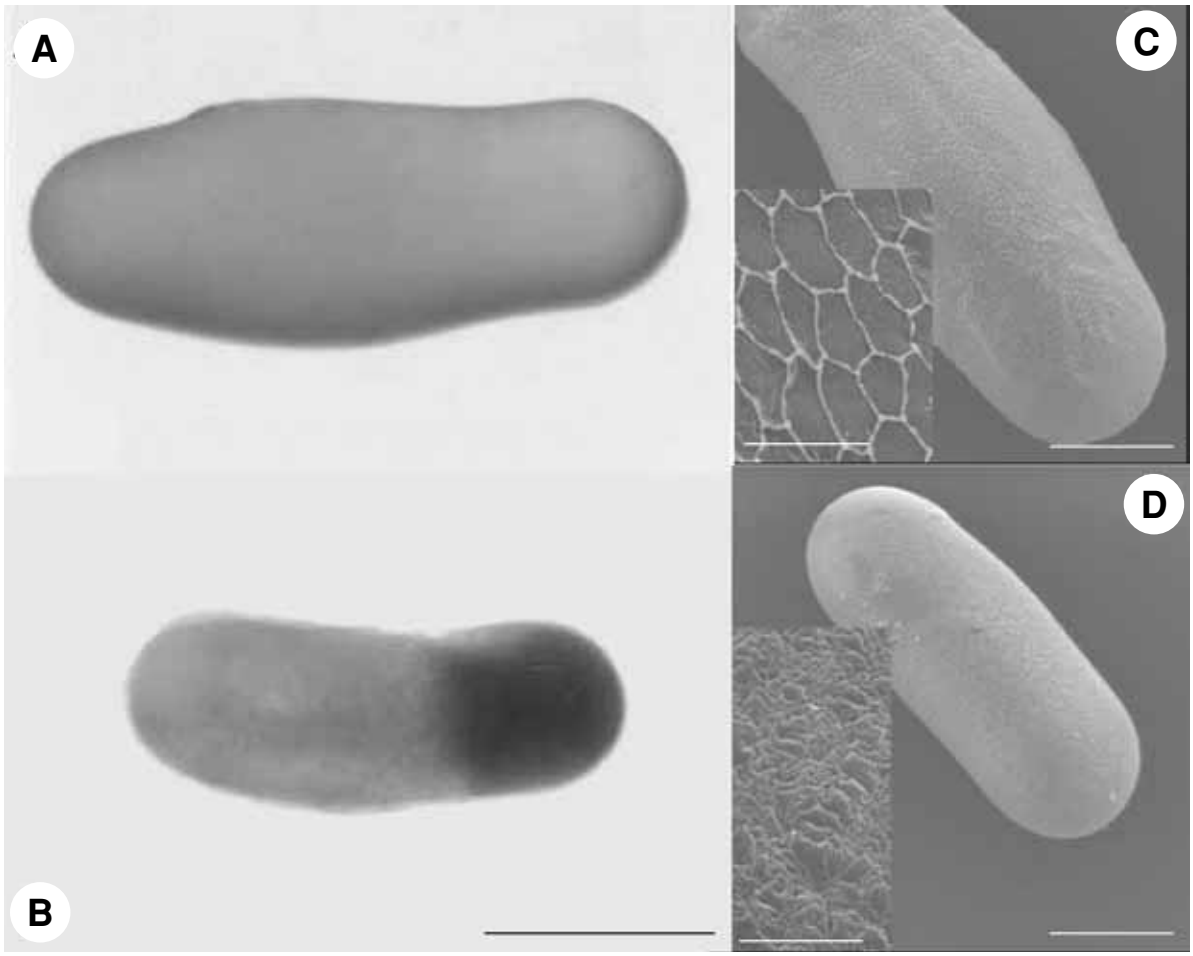

Figure 4. *Melipona c. fasciculata* eggs. Light micrographs of a queen-laid egg (**A**) and worker-laid trophic egg (**B**). Bars = 1 mm. SEM images showing a queen egg (**C**) and a worker-laid trophic egg (**D**). Bars = 300 μm. Details of the reticulate and corrugated chorions are also shown. Bars = 90 μm.

significantly among the eggs (W_3: Z = 1.28, p = 0.19). It was not possible to obtain precise measurements of worker reproductive eggs, although they appeared to be longer than trophic eggs.

The queen-laid eggs and worker-laid trophic eggs of *M. compressipes fasciculata* also differed in size (Table 1), with the former being longer (L: Z = 3.57, $p < 0.001$) and wider (W_2: Z = 3.57, $p < 0.001$; W_3: Z = 2.07, $p < 0.05$) than the latter. However, the width of the anterior region of these eggs did not differ significantly (W_1: Z = 1.54, p = 0.12).

DISCUSSION

Our results confirm previous observations that the reproductive eggs of queens and workers of stingless bees have a chorion with a reticulate pattern, whereas trophic eggs have a smoother surface. This characteristic difference in the pattern covering the egg surface has also been reported for *Tetragonisca angustula* [12,15] *Scaptotrigona aff. depilis* [16], *Scaptotrigona postica* and *Melipona quadrifasciata anthidioides* [9,11], *Melipona rufiventris paraensis* [24] and *Melipona bicolor bicolor* [14]. SEM also showed that the posterior region of reproductive eggs, which is normally in contact with the larval food after oviposition, had a smooth, non-reticulate surface.

The trophic eggs were smaller than queen-laid reproductive eggs, and this appears to be a general pattern for *Melipona* bees [6,19,20], although none of the latter studies provided quantitative data to support this conclusion. Our measurements also corroborated with previous findings that worker-laid reproductive eggs of *M. q. anthidioides* were smaller than the corresponding queen-laid eggs [5], although additional studies are needed to determine whether this is characteristic of stingless bees. It is also unclear whether the larger size of queen-laid eggs applies equally to eggs destined to produce males and females. This is a particularly important consideration since if male-destined eggs laid by workers are smaller than those laid by the queen this could adversely affect the ontogeny of the former and compromise their fitness compared to males derived from the queen. The existence of fitter queen-derived males would be another demonstration of queen dominance over workers.

Research on queen-worker interactions during POP has shown that the evolution of trophic eggs is characterized by marked variation in egg-laying behavior [27]. The novel finding reported here is that, in contrast to previous findings, trophic eggs can be smaller than worker-laid reproductive eggs, which suggests that the development of this type of egg is probably also associated with different physiological adaptations.

In agreement with previous studies, trophic eggs showed small morphological alterations during preparation for SEM, probably because of their relatively fragile structure. This fact could explain the corrugated surface seen in SEM. Although the reproductive eggs of workers and queens differed in size, eggs destined for males and females were similar in appearance. Future studies should assess whether there are morphological differences between queen-laid eggs destined to produce males or females.

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