Speciation, development, and the conservative egg of the stingless bee genus *Melipona*

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Species of the stingless bee genus *Melipona* have different-sized adults, while their eggs have a rather uniform volume. Since the larvae of these species are probably the same size at the onset of their development, they differ considerably in the amount of food they need to ingest in order to complete the four larval stages. This implies that moulting from one larval stage to the next is not a response to the amount of food ingested relative to gut capacity, but may instead be triggered by a time-related mechanism.

Keywords: *Melipona,* egg size, larval development, food composition, speciation

The topic of this paper is the unexpected result of a comparative study on the biophysical properties of the stingless bee eggs. This outcome has lead to as yet unanswered questions with regard to the relationship between developmental differences and speciation.

The elongated eggs of stingless bees are deposited vertically on liquid food. In *Melipona bicolor,* for example, only five percent of the egg's volume is below the surface of the fluid. This is caused by surface tension forces that keep it upright (Velthuis & Velthuis, 1998). The bottom part of the egg is hydrophilic and smooth, while the part above the food surface is hydrophobic with a reticulated chorion. This reticulation has already been reported in other species (Koedam *et al.*, 1996). The reticulate part has a coating consisting of fatty acids and long-chain hydrocarbons (Jungnickel *et al.*, 2001). Air is trapped in this reticulate structure, and promotes the egg's return to a vertical position if it should become tilted. The abrupt transition zone from the smooth to the reticulate part forms a ring near the egg's base and is the site where surface tension exerts its lifting force.

If surface tension is eliminated experimentally, using alcohol or a droplet of detergent, the egg immediately sinks under the surface. It does not, however, reach the bottom, indicating that the egg's density must equal that of the food (Velthuis & Velthuis, 1998).

At the start of the present study it was hypothesized that the strength of the force exerted by surface tension on the egg depends on the circumference of the egg at the transition zone and that the weight to be lifted depends on the volume of the egg. Circumference is a linear character, while weight is a three-dimensional property. It can be expected, therefore, that the bigger the egg, the bigger its instability on the food and, consequently, there must be a maximum egg size.

Because some of the species of *Melipona* comprise the largest stingless bees known, and others are distinctly smaller, a comparative study was undertaken on egg size, shape and stability on the food in the cell. Seven species, encompassing the whole array of sizes in the genus, were included in the study. The outcome was a surprise: the eggs of these seven species did not differ considerably in size. This paper presents the results and discusses the consequences for the developmental process from egg to adult in light of the speciation of the genus.

MATERIAL AND METHODS

The seven species studied included four from Acre, West-Amazonia (*M. fuliginosa, M. crinita, M. eburnea fuscopilosa*, and *M. rufiventris flavolineata*) and three from the Atlantic rain forest of São Paulo and adjacent Paraná (*M. quadrifasciata, M. bicolor,* and *M. marginata*). The colonies in Acre were located in the meliponary of Dr. Paulo Nogueira-Neto, while those from São Paulo belonged to the Laboratório de Abelhas of the University of São Paulo.

In order to characterize the size of the bees, the head dimensions of a few workers of each species were noted. These measurements included the distance between the eyes at the level of the antennae and the width of the head. A view of the dorsal side of the head was taken for the latter measurement; the site where the margin of the eye bent downward along the side of the head was then marked and the distance between these points was measured. A frontal view of the head of one specimen of each species was also drawn.

Eggs taken from young cells were measured under a binocular microscope using an ocular micrometer and their average shape was drawn.

The volume of food inside the cells of four of the species (*M. crinita, M. quadrifasciata, M. bicolor* and *M. marginata*) was measured using a calibrated capillary. The composition of this food, separated into free proteins, carbohydrates, water content, and the number of pollen grains was also determined. This was done at the Depto. de Genética, Faculdade de Medicina, USP, Ribeirão Preto. For the methods employed, see Pereboom (2000).

RESULTS

Figure 1 shows the frontal views, drawn to the same scale, of the heads of a worker of each species. Head sizes are given in Table 1 based on the two dimensions discussed above. The figure and the table show that, over the size range of these seven species, linear dimensions differ by a factor 1.8.

Figure 2 shows the average shapes of the eggs. Note that a number of the eggs were slightly curved: this is not depicted in the figure. Only a few cells of *M. fuliginosa* could be used as the single colony at our disposal had no physogastric queen and only a small brood nest. We suspect that the eggs in the combs of this species were laid by the workers. With regard to the other species, there were several good colonies per species at our disposal. The data presented here, however, are from eggs from just one colony per species, all collected from the same comb.

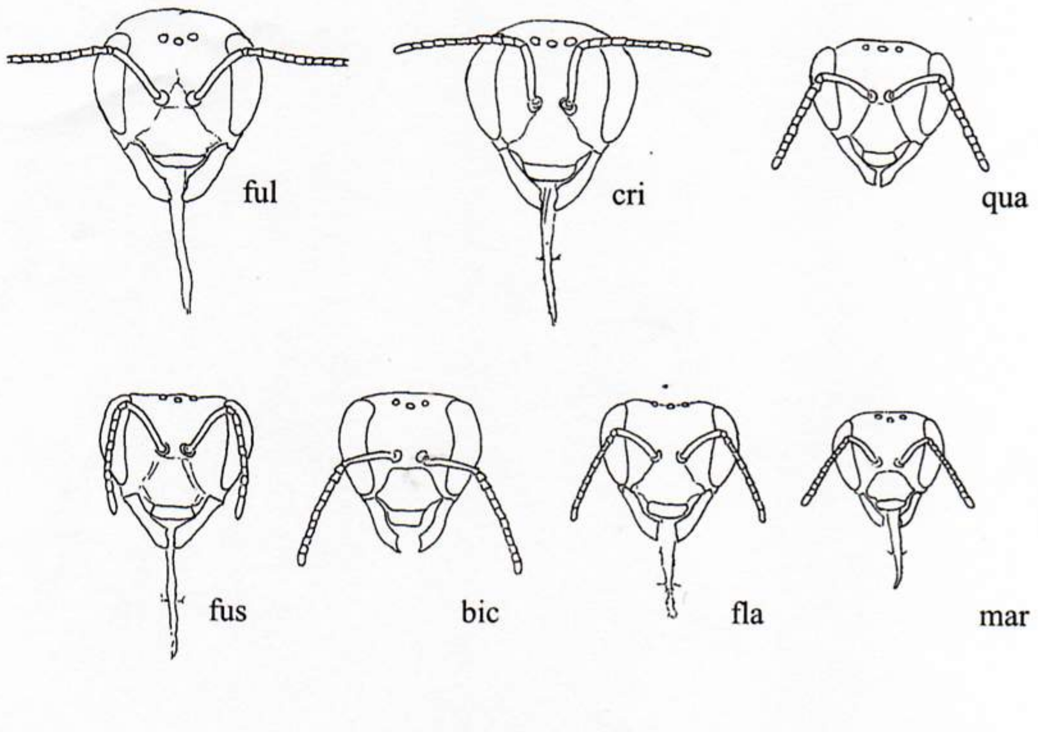

Figure 1. Frontal view of the heads of *Melipona fuliginosa* (ful), *M. crinita* (cri), *M. quadrifasciata* (qua), *M. eburnea fuscopilosa* (fus), *M. bicolor bicolor* (bic), *M. rufiventris flavolineata* (fla) and *M. marginata* (mar). All are drawn to the same scale.

Because of their specific shape, the dimensions of the eggs had to be measured at several places. Table 2 gives the actual average lengths and maximum diameters of these eggs. The volume was calculated from these measurements by distinguishing sections of the eggs that were either parts of a round ball, cone-shaped, or approximately cylindrical. These calculations showed that, except for *M. fuliginosa*, the volumes of all the eggs were remarkably similar.

Table 3 gives the average volumes of food encountered in the brood cells of the seven species. In accordance with the differences in adult size, the cells contained quite different amounts of food, the extremes differing by a factor 4.3.

Table 1. Average dimensions of the heads of the bees depicted in Fig. 1. *M. fuliginosa*: ful; *M. crinita*: cri; *M. quadrifasciata anthidioides*: qua; *M. eburnea fuscopilosa*: fus; *M. bicolor bicolor*: bic; *M. rufiventris flavolineata*: fla; *M. marginata marginata*: mar.

	Species						
	ful	cri	qua	fus	bic	fla	mar
Between eyes (mm)	3.03	2.75	2.55	2.45	2.16	2.21	1.63
Head width (mm)	4.81	4.25	3.85	3.90	3.67	3.44	2.68
Number	10	9	10	10	10	10	10

Table 2. Average length and maximum width of the eggs depicted in Fig. 2.

	Species						
	ful	cri	qua	fus	bic	fla	mar
Length (mm)	2.67	2.69	2.97	2.81	3.01	2.77	2.67
Width (mm)	1.47	1.21	1.20	1.28	1.15	1.25	1.29
Volume (mm^3)	3.08	2.15	2.15	2.31	2.37	2.22	2.29
Number	5	17	12	17	7	18	9

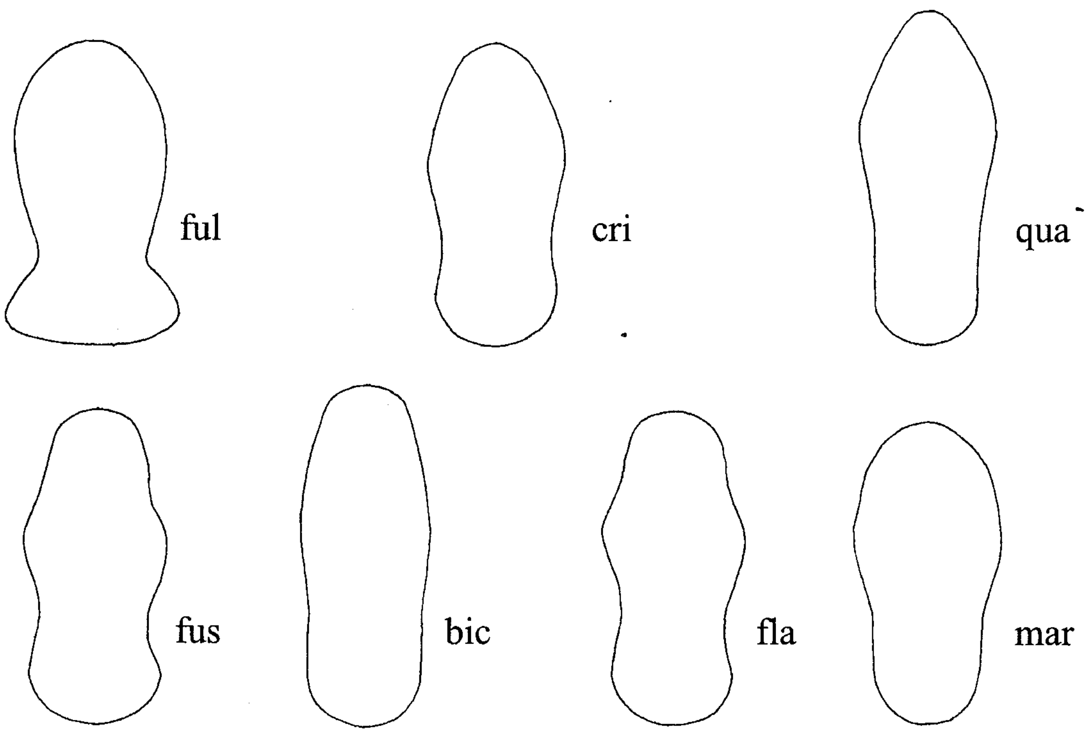

Figure 2. The shapes of the eggs. For abbreviations, see Fig. 1.

Table 3. Average amounts of food recovered from the brood cells containing eggs. For abbreviations, see Fig. 1.

	Species ful	cri	qua	fus	bic	fla	mar
Amount (µl)	196	175	126	131	106	83	46
Number	5	15	13	17	24	18	10

Table 4. Composition of the food in the brood cells of four species of *Melipona*: water content concentrations of carbohydrates and free proteins, and number of pollen grains per unit volume (average ± standard error). 'SE (%)': standard errors given as a percentage of the averages.

	M. crinita	*M.quadrifasc*	*M. bicolor*	*M. marginata*
Water (%)	58.63 ± 1.76	61.54 ± 0.91	47.74 ± 1.37	47.16 ± 0.96
n	12	21	13	15
SE (%)	3.0	1.5	2.9	2.0
Carbohydrates (µg/µl)	108.09 ± 33.60	77.51 ± 32.17	118.30 ± 52.93	199.90 ± 62.36
n	20	22	22	27
SE (%)	31.0	41.5	44.7	31.2
Free proteins (µg/µl)	2.38 ± 0.30	2.86 ± 0.77	2.97 ± 0.91	3.20 ± 0.84
n	22	24	26	29
SE (%)	12.6	26.9	30.6	26.2
Pollen grains (n/µl)	101364 ± 10542	90478 ± 10767	110721 ± 10186	93492 ± 9630
n	6	10	12	16
SE (%)	10.4	11.9	9.2	10.3

Data on the food composition of four species is presented in Table 4. *M. crinita* was considered representative of the large species, *M. quadrifascita* and *M. bicolor* the middle-sized species, and *M. marginata* the small species. These size differences, however, do not seem to be correlated with differences in food composition. Free proteins, for example, were always present in low concentrations. The bulk of proteins in all four species was in the pollen grains. The water content of the larval food did vary between the species, but appeared to be rather constant within each species. In contrast, the amount of carbohydrates differed greatly not only between the species, but it varied considerably also within the species.

DISCUSSION

Contrary to our expectation, egg size in the genus *Melipona* is not a function of adult worker size. One possible explanation for the absence of this correlation could be that, given the biophysical limitations resulting from the eggs' circumference/volume ratio, the eggs of the smaller species already reached the maximum size, and that other adaptations occurred during the evolution towards still larger sizes of members of the genus that compensate for the egg being unable to grow along with adult body size. Given the shapes of the eggs, however, there does not appear to be a maximization of the upward lifting force. In fact, the bottom part of the egg in most species is relatively narrow and the egg is at its broadest at 2/3rd or 3/4th of its height. Therefore, the hypothesis that *Melipona* eggs, given their remarkable orientation on food, are at the maximum size attainable loses its initial attractiveness.

Only in *M. fuliginosa* did we find the egg to have a broad foot, resulting in a large circumference at the transition zone from the smooth to the reticulate surface. This shape could, however, be atypical for the species, since these eggs were probably laid by workers and worker reproductive eggs usually have a less well-developed chorion, leading to a less-fixed shape (see Koedam *et al.*, 2001). Presumably, these eggs were somewhat compressed compared to the eggs of a queen, which would cause them to become somewhat shorter and broader than those of the other species. It is known that worker eggs, especially trophic eggs, are often bulky and this could be why our *M. fuliginosa* eggs had a larger volume. We maintain our conclusion, therefore, that because the eggs of *Melipona* species are all about the same size, the egg is a conservative factor in the differentiation of the genus.

The consequence of the eggs being rather uniform while the adults are of quite different sizes is that development during the larval stage must be regulated differently. We expect that the larvae of these species are about the same size at eclosion from the egg. The development of bees into pupae involves four larval stages (according to Lucas de Olivera, 1960, there are five larval stages in the stingless bees), during which food is consumed. Like in other insects, moulting from one stage to the next is supposedly triggered by the amount of food consumed, so that temporary starvation during a larval stage leads to a prolonged duration of that particular stage. In our *Melipona* species, however, the larvae encounter quite different amounts of food in the cell. Moulting therefore must be programmed in such a way that there is food left for each of the following larval stages. These species appear to be distinct in the amounts of food consumed during the same larval stages. Are there large differences in developmental time, or are there great differences in the speed of eating? Since we did not find an essential difference in the composition of the food, we do not suppose that the amount consumed by each of the larval stages is regulated by quality aspects. Perhaps if the larvae of the larger species have big mouths, while the larvae of the smaller species trifle with their food, moulting would occur on a similar time-based schedule rather than on the basis of having a full gut?

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