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# VARIATION ON OVARIOLE NUMBER IN MELIPONINAE (HYMENOPTERA, APIDAE) QUEEN'S OVARIES, WITH COMMENTS ON OVARY DEVELOPMENT AND CASTE DIFFERENTIATION

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#### **ABSTRACT**

The present paper concerns the variation of the number of ovariole per ovary in the females of some Meliponinae species. In workers, the number of ovarioles was always of 4 per ovary, but in some species the queens had a variable number of ovarioles, from 4 to 15 per ovary (as in Plebeia remota, Nannotrigona testaceicornis, Trigona spinipes, Schwarziana quadripunctata). The variation of the number of ovarioles was also found in the same species from individual to individual and among the ovaries of a single individual.

Keywords: ovariole number, stingless bees, queen, worker.

#### Introduction

During the evolution the bees sociality, fundamental differences in the social role of queens and workers developed. These are also reflected in a marked dimorphism in the development of the ovaries of queens and workers. In Apis mellifera, queens have ovaries with more than one hundred ovarioles in each

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ovary. Snodgrass (1956) reports 160 to 180 ovarioles in each ovary of A. mellifera and Morini and Bueno (1995) found an average of  $349,865 \pm 11.60$  ovarioles in A. m. caucasica. The worker's number of ovarioles rarely reaches 20 (Oertel, 1930; Snodgrass, 1956; Chaud-Netto and Bueno, 1979). Not only the number of ovarioles is greater in queens, but the ovarioles of queens are also much longer than in workers, besides being functional, while in workers, they are inactive in the queen's presence.

In Meliponinae there are also developmental differences concerning the ovaries of queens and worker, but the numeric differences are not so great. The differences seem to be more related with the ovariole length, than to its number. Sakagami et al. (1963) believed that all meliponine species have four ovarioles per ovary, but Camargo (1974) showed that queens of different sizes in Schwarziana quadripunctata have 6 to 9 ovarioles per ovary. Intermediate queens are not known in A. mellifera but in Meliponinae some species Nannotrigona testaceicornis, Plebeia remota, P. juliani have normal sized and small queens.

This paper reports on the variation of ovariole number in queens of some Meliponinae species.

#### MATERIAL AND METHODS

Queens and workers were studied in: Melipona quadrifasciata anthidioides, Melipona bicolor bicolor, Plebeia sp, Plebeia remota, Tetragonisca angustula, Trigona spinipes, Nannotrigona testaceicornis, Scaptotrigona postica, Paratrigona subnuda and Schwarziana quadripunctata.

The number of queens was small and variable, depending upon the availability of these individuals. The number of workers was always 10. Whenever small queens were available they were also studied. For the queens all counts were made in virgin individuals because the presence of developed oocytes renders the couting difficult.

The queens and workers gasters were fixed in the Dietrich or Bouin fixative mixtures and processed routinely for histological examination on slides stained with hematoxylin and eosine. All counts were made under optic microscope.

## RESULTS AND DISCUSSION

Table I shows the number of ovarioles found in the studied species. In workers there were always four ovarioles each ovary, but in the queens this number presented some variation.

The non functional ovaries of newly emerged workers and virgin queens are more suitable for ovarioles counting, since the presence of grown ovocytes makes their visualization difficult mostly in workers, where they are short.

The number of queens examined for each species was low and the results presented here may eventually change with the addition of new countings. However, based upon the available data, M.q. anthidioides, M. bicolor, Plebeia sp, T. angustula, S. postica and P. subnuda have 4 ovarjoles in each ovary (Figs. 1A, B; 2B, D), while in the other studied species the number is variable (Table I, Figs. 1D and 2A). The specie that showed the greatest number of ovarioles was T. spinipes, in which, up to 15 ovarioles (Fig. 2A) were counted in one ovary. The number of ovarioles is rarely the same in both ovaries, unless in the species that have only four ovarioles in each ovary.

The queens have very long ovarioles (Fig. 3A) generally rolled up in the distal extremities. Of those some that could be measured had lengths varying from 3.5 to 5.5 mm, while in workers they are much shorter. Newly emerged workers have an ovariole length between 0.4 and 0.7mm, while in nurse workers they may reach 1mm, when the ovocytes are developed (Figs. 1C and 3B).

The miniature queens have intermediate ovaries in the sense that the ovariole number in each one is lower than in the corresponding normal sized queen (Table I). However the histological aspect of these ovarioles is the same (Fig. 2C). As these queens seem to have all features of a normal queen, only being smaller, they cannot be considered as intercastes.

In Apis mellifera the control of ovary development, and other characteristics of the female castes, are determined by the type of food. The workers suffer an alimentary castration (Michener, 1974). Similar mechanism may be found in those stingless bees species with trophic caste determination. While in Apis the achievement of reprodutive efficiency is attained through the increase of ovariole number and lenght (Morini and Bueno, 1995, found \$\inc\$.34mm in laying queens), in meliponines it seems that the chosen mechanism is mostly that of the ovarioles' lenght.

In virgin queens, developing oocytes were absent from the ovarioles, and from the proximal to the distal end, they presented an organization which in laying queen is found only in the germary (Fig. 2C, D), showing mitosis and several phases of cysts formation along all the length. This indicates that probably the vitelogenesis starts only after queen mating, an expected condition since the weight of a young queen that can still fly is an important factor. A great number of vitelogenic eggs will cause an unwanted increase in weight.

It is possible that the number of ovarioles and their length are related to the oviposition rate of the species, as well as to the colony population. *Apis* mellifera, according to Snodgrass (1956), may, in optimal conditions lay until

Table 1. Ovariole number in Meliponinae

Workers	Number of specimens counted	10	10	10	10	10	10	10	10	10	10
	Ovariole numbers	4	4	4	4	4	4	4	4	• 4	4
Miniature Queen	Number of specimens counted	No.		•	-		•	2	•		
	Ovariole numbers				4		•	8-4		•	
Queen	Number of specimens counted	3	1	2	3	1	3	3	3	2	4
ð	Ovariole numbers	4	4	4	8-4	4	10-15	10-12	4	4	8-4
Species		Melipona quadrifasciata anthidioides	Melipona bicolor	Plebeia sp	Plebeia remota	Tetragonisca angustula	Trigona spinipes	Nannotrigona testaceicornis	Scaptotrigona postica	Paratrigona subnuda	Schwarziana quadripunctata

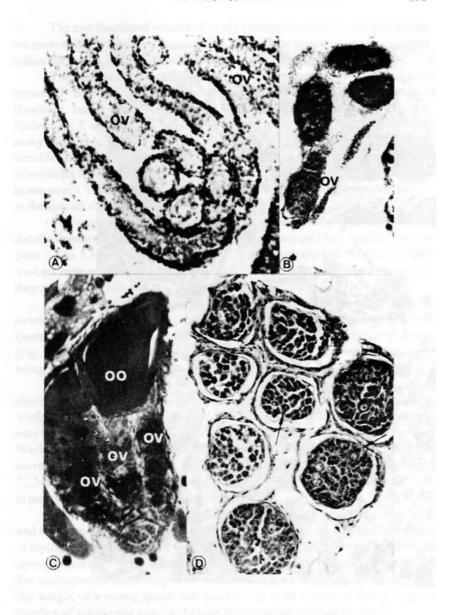


Fig. 1. Light micrographics of ovaries of some Meliponinae. A, B. longitudinal and cross section, respectively of *S. postica* ovaries showing 4 ovarioles (ov); 200x. C. nurse worker of *S. quadripunctata* showing developing ovocytes (oo) and 3 of the 4 ovarioles (ov).200x. D. Ovary of *S. quadripunctata* queen showing ovocytes differenciation (white arrows). 450x.

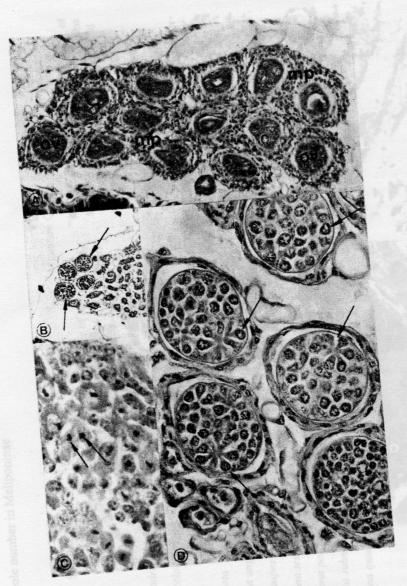


Fig. 2. Queens' ovaries. A. Cross section of an ovary of a *T. spinipes* showing 13 ovarioles (ov); 200x. B. Cross section of ovary of *P. remota* showing 4 ovarioles (arrows); 80x. C. Longitudinal section through an ovariole of a miniature queen of *S. quadripunctata* showing germinative cells in mitosis (arrows). 650x. D. Cysts differ entiation in ovarioles of *P. remota* (arrows). 500x.

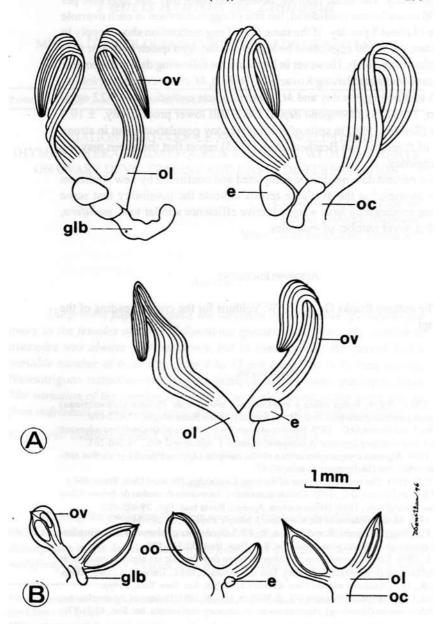


Fig. 3. Schematic representation of the ovaries of 3 virgin queens (A) and 3 nurse workers (B) of *S. quadripunctata*. ov=ovaries; e=spermatheca; ol=lateral oviduct; glb=Dufour's gland; oc=common oviduct; oo=oocyte.

3.000 eggs a day. The usual number, however is close to 1.500 eggs laid per day. If 300 ovarioles are considered, the rate of egg maturation in each ovariole should be of about 5 per day. If the same rate of egg maturation should apply to meliponines, 40 or 150 eggs could be laid in one day by a queen with 8 or with 30 ovarioles respectively. However in literature the following data are reported: for species of Melipona (having 8 ovarioles, Table I), M. compressipes fasciculata lays 25.6 to 30.43 eggs a day and M. quadrifasciata anthidioides 10-22 eggs/day (Kerr, 1949). Scaptotrigona depilis has a still lower productivity, ± 10.4 eggs/day (Santos, 1996) in spite of the high colony populations. But in strong colonies of P. remota van Benthem et al. (1995) report that the queen may lay 60-180 eggs/day.

The present data need to be completed and confirmed by new studies in these bee ovaries, but the available results indicate the possibility that some meliponine species may have a reproductive efficience similar to A. mellifera, even with a lower number of ovarioles.

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