A Comparative Study of the Ovaries in Some Brazilian Bees (Hymenoptera; Apoidea)

Gustavo Ferreira Martins[1]

José Eduardo Serrão[1,2]

ABSTRACT

The present paper concerns the morphological features of ovaries in 33 species of bees with different social behavior patterns. The ovaries of bees were examined under light microscope. They are polytrophic-meroistic ovaries formed for an anterior germarium and a basal vitellarium. The germarium houses the germ cells and in the vitellarium there are follicles arranged linearly. In general the follicle is constituted by a nutritive chamber (a cluster of nurse cells) and an oocyte chamber, both covered by a single epithelial layer of follicular cells. The number of ovarioles per ovary and the number of mature oocyte per ovary were analyzed. Measurements of ovariole length, oocyte size, oocyte width, follicular epithelial height and the intertegular distance were made to support the comparative study. Statistical analysis showed that representatives of Meliponini and Apini have the largest ovaries. On the other hand, in solitary bees were found the bigger oocytes. Furthermore, our results suggest that there is a tendency for increase in ovary size and ovariole number, with increasing level of sociality.

KEYWORDS: insect morphology, ovary, reproductive tract, social behavior.

INTRODUCTION

Insect ovaries are formed of several functional and elongated units called ovarioles (Bilinsk, 1998) and in Hymenoptera they are of meroistic polytrophic type (Chapman, 1998). A typical polytrophic ovariole consists of three regions: terminal filament, germarium and vitellarium (Chapman, 1998). The germarium contains the germ cells and their derivatives, the terminal filament continues from the anterior end of the germarium and constitute a suspensory apparatus of the ovarioles while the vitellarium occupies the proximal region of the ovariole where yolk uptake and oocyte growth occur (Chapman, 1998; Snodgrass, 1935).

Mitotic divisions of germ cells take place within the germarium, whereas the vitellarium contains developing egg chambers in a linear arrangement. Each chamber consists of an oocyte and the nurse cells that are formed by incomplete cytokineses from the same germ cell (Zacaro & Cruz-Landim, 1996; Bilinsk *et al.*, 1998; Patrício & Cruz-Landim, 2001). In the polytrophic ovariole, its own group of nurse cells accompanies each oocyte. In general, this structure is delimited by follicular epithelium constituting the follicles or egg plus nutritive chambers (Bilinsk, 1998).

[1] Departamento de Biologia Geral. Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brasil. E-mail: jeserrao@ufv.br. Phone: +553138991301. Fax: +553138992549.

[2] To whom all correspondence should be addressed.

The number of ovarioles per ovary is variable and shows interspecific differences (Jaglarz, 1998). The ovary morphology and its phylogenetic relationships have been studied by various authors (Simiczyjew *et al.*, 1998; Szklarzewicz, 1998; Jaglarz, 1998; Kubrakiewicz *et al.*, 1998; Bilinsk *et al.*, 1998).

Iwata (1955; 1965) studied the polymorphism of the ovaries in bees considering the change that may occur on the ovary structure, observing the number of mature and immature oocytes, the size of mature oocyte and the speed of oocyte maturation, showing that these characteristics have a distinct correlation with the different behavioral patterns found in this insect group. The following study reports on the variation of ovary morphology in bees to support a comparative study, in order to test the hypothesis that structure is indeed related to their sociality.

MATERIALS AND METHODS

Nineteen species of Apidae were analyzed with representatives from Apini, Bombini, Meliponini, Euglossini, Centridini, Eucerini, Ericrocidini, Emphorini and Xylocopinae, one species of Andrenidae, ten species of Halictidae and three species of Megachilidae (Table 1).

Bees were collected in the field in Viçosa, MG, Brazil, while for Meliponini and Apini were analyzed physogastric queens obtained from the Central Apiary, Universidade Federal de Viçosa, MG.

The specimens were dissected in insect saline solution and the pieces were removed from mated bees, what was determined by the presence of spermatozoa in their spermathecae. The ovaries were isolated from the dissected reproductive tract and transferred to 4% paraformaldehyde in phosphate buffer 0.1M, pH 7.2.

The samples were dehydrated in an ethanol series, embedded in historesin (Leica) and cut at 4 μm serial sections, which were stained with Dominici solution.

Some sections were submitted to the following histochemical tests: mercury-bromophenol blue for protein, Nile blue for lipids and methyl green-pyronin for cell death. These tests were performed as described by Pearse (1968) with few variations for historesin embedded tissues.

For each bee the following parameters were analyzed: number of ovarioles per ovary, ovariole length, number of mature oocytes per ovary, oocyte length, oocyte width, follicular epithelium thickness and the intertegular distance (as representative of the body size).

Measurements were performed with aid of the software Image Pro-Plus™, 4.0 version for Windows. For determination of the body size of bees, measurements of the intertegular distance were made with the same software. The number of mature oocyte per ovary was determined as proposed for Iwata (1955; 1965).

To determine the degree of dependency of body size and morphological parameters, we used a linear regression procedure following standard statistical tests described by Snedecor & Cochran (1980).

RESULTS

The general morphology of the ovaries is almost equal to that was described before for some species (Snodgrass, 1956; Cruz-Landim *et al.*, 1998), and therefore only a brief description will be given, emphasizing only the features that have not been detailed before.

All species studied have meroistic polytrophic ovarioles (Figs. 1-3).

A sheath, which is made up of two layers, encloses each ovariole: an outer peritoneal membrane and an inner non-cellular tunica propria. The former is constituted by a network of cells, including muscle cells, fat body cells, and tracheoles that do not penetrate the tunica propria (Figs. 1-3, 7, 9).

The height of the follicular epithelium of egg chamber changes according to the species, with measurements varying from 6.6 μm in *Plebeia* sp. to 45 μm in *Xylocopa frontalis* in mature basal follicles. In *Epicharis flava* and *E. affinis* extensive cell projections were found (64.08 ± 8.6 μm length) in follicular tissue and these structures penetrate the corion that is very thick (102.0 ± 7.8 μm length) (Figs. 7, 9).

The cytoplasm of nurse and follicular cells was strongly stained by methyl green-pyronin, mercury-bromophenol blue and nile blue, marking the following constituents in the cytoplasm: RNA, proteins and neutral-lipid (Figs. 3, 7). The follicular cells however show different staining tonalities for bromophenol blue in the mid-vitellogenic follicles (Fig. 7).

Among nurse cells there is the presence of groups of small cells, which are smaller than nurse cells, the somatic-like cells (Fig. 6).

Presence of accessory nuclei was observed in the oocytes during the previtellogenic and vitellogenic stages. In Meliponini accessory nuclei were found in the previtellogenic region (Fig. 8) while in Centridini and Halictidae they were found in the vitellogenic

FIGURE 1. Longitudinal section of the germarium position of *Melipona quadrifasciata*. The anterior region have undifferentiated cells and the posterior region have many oocyte-nurse cell complexes; o: young oocyte; on: oocyte nucleus; uc: undifferentiated cells; pm: peritoneal membrane; T: trachea; tp: tunica propria. Bar = 10 μm.
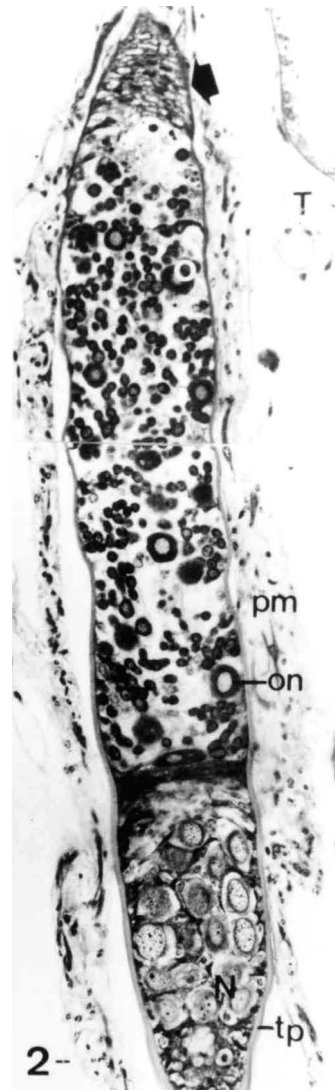

FIGURE 2. Longitudinal section of the anterior region of the ovariole of the *Xylocopa frontalis*, showing a short terminal filament (arrow) followed by a short germarium with few oocyte-nurse cell complexes followed by a nutritive chamber with the nurse cells (N); on: oocyte nucleus; pm: peritoneal membrane; tp: tunica propria; T: trachea. Bar = 10 μm.

region (Figs. 3-5). In Halictidae this structure was more developed in comparison with other species (Figs. 4, 5).

Xylocopa frontalis had the greatest oocyte (1.1 cm long), occupying almost the entire extension of the ovariole (1.3 cm length) (Table 1).

Apidae species have four ovarioles per ovary, while Andrenidae, Halictidae and Megachilidae have three ovarioles per ovary. These numbers were constant in both ovaries and bee group, except *A. mellifera*. However, in Apidae the number of mature oocytes per ovariole varied from 1 to many (Table 1).

Morphometrical data of the ovarioles showed some differences among the studied bees, with Meliponini and Bombini presenting the longest ovarioles (Table 1). In addition the germarium in solitary bees is reduced in comparison with the ones found in primitively and highly eusocial bees (Figs. 1, 2).

Ovarioles in solitary bees contain 1 to 3 follicles (nutritive plus egg chambers) and the sizes greatly differ among them, while in the ovaries of primitively and highly eusocial bees many follicles were found arranged in linear series with small differences in their size.
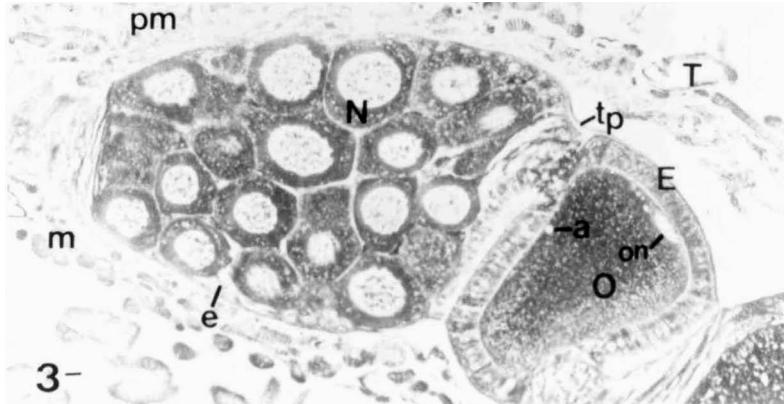
FIGURE 3. Longitudinal section of a follicle of *Epicharis affinis*, observed under phase contrast microscope, Methyl Green-Pyronin stained, showing one vitellogenic growing oocyte (o) that have the nucleus (on) and the accessory nuclei (a) placed in the peripheral region of the cytoplasm; E: follicular epithelium covering the oocyte chamber; e: follicular layer of the nutritive chamber; m: muscle; N: nurse cells; pm: peritoneal membrane; T: trachea; tp: tunica propria. Bar = 10 μm.
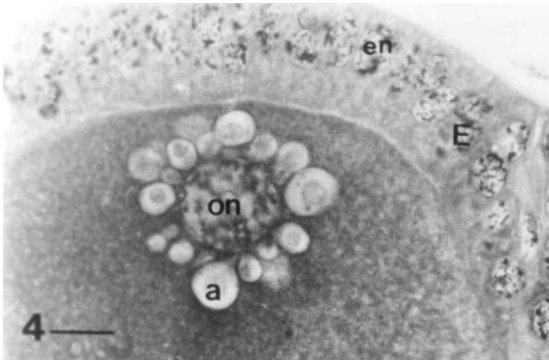

FIGURE 4. Longitudinal section of vitellogenic growing oocyte of *Pseudaugochlora graminea*, showing many accessory nuclei (a) surrounding the oocyte nucleus (on). The oocyte is covered by the columnar follicular epithelium (E). en: follicular epithelial cell nucleus. Bar = 10 μm.
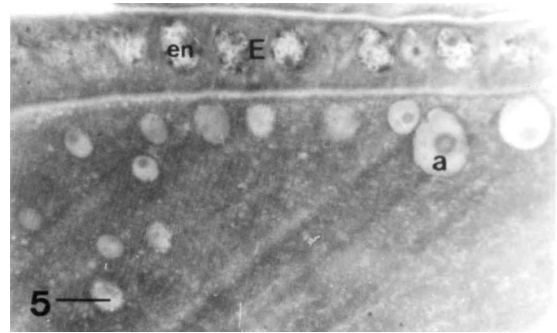

FIGURE 5. Longitudinal section of mature oocyte of *Pseudaugochlora graminea* with accessory nuclei placed in the peripheral oocyte region. The follicular cells are flattened; E: follicular epithelium; a: accessory nucleus; en: follicular epithelial cell nucleus. Bar = 10 μm.

Meliponini have the proximal region of the ovarioles with an accumulation of follicles with degenerative nutritive chamber.

The statistical analysis showed that the ovariole size [$R^2 = 0.010$, $F (1,31) = 0.030$, $p < 0.862$] have negative correlation with body size (Fig. 10), because Meliponini have the longest ovariole while the others have the largest body size (Table 1). On the other hand, when Meliponini is excluded from the analyses, there is a positive correlation of these features [$R^2 = 0.527$, $F (1,28) = 27.864$, $p < 0.00002$] (Fig. 11). Furthermore the statistical analysis showed that the oocyte size and oocyte width have a positive correlation with intertegular distance [oocyte size: $R^2 = 0.485$, $F (1,31) = 27.315$, $p < 0.0001$; oocyte width: $R^2 = 0.418$, $F (1,31) = 20.835$, $p < 0.00008$] (Figs. 12; 13).

DISCUSSION

The small cells scattered among nurse cells are similar to those found in the beetle *Badister bipustulatus*, which present characteristics of somatic cells derived from pre follicular cells (Jaglarz, 1998), in spite of their role is unknown.

In youngest follicles, the follicular cells acquire different bromophenol blue staining tonalities, suggesting that protein synthesis is an asynchronous process, similar to the one observed in the ant *Neoponera villosa* (Camargo-Mathias, 1993). It means that some cells start protein synthesis before the others. On the other hand, in post-vitellogenic follicles all follicular cells are equally stained, suggesting that all cells are synthesizing protein. Fleig (1995) observed that after

coriogenesis, follicular cell degenerates in *A. mellifera* that is in agreement with the results obtained in this study for others species of bees.

Accessories nuclei have been studied in *Eomenacanthus stramineus* (Mallophaga) (Bilinsk, 1989) and in the bee *M. quadrifasciata anthidioides* and in the ant *Atta sexdens rubropilosa* (Cruz-Landim, 1991) and their formation were very similar among them. It is evident that these

accessories nuclei arise from the protrusion of the nuclear envelope of the germinal vesicle as described in ants by Billen (1985) following migration to the anterior pole of the peripheral region of the oocyte cytoplasm. The accessories nuclei are involved in the transport and deposition of RNA and proteins into ooplasm (Büning, 1994; Cruz-Landim, 1991), but the function of accessory nuclei in bees needs further investigations.
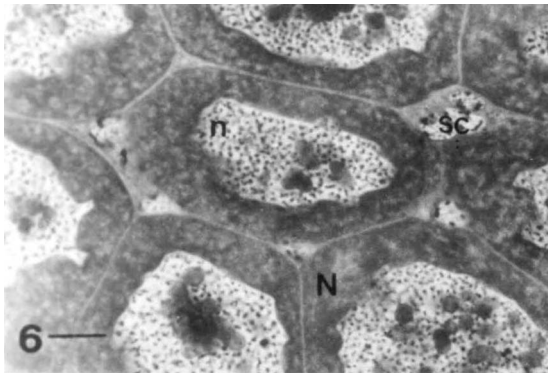

FIGURE 6. Longitudinal section of the nutritive chamber of *Pseudaugochlora graminea*, showing the nurse cells (N) with irregular nucleus (n) and multiple nucleoli. Between the nurse cells there are somatic-like cells (sc). Bar = 10 μm.
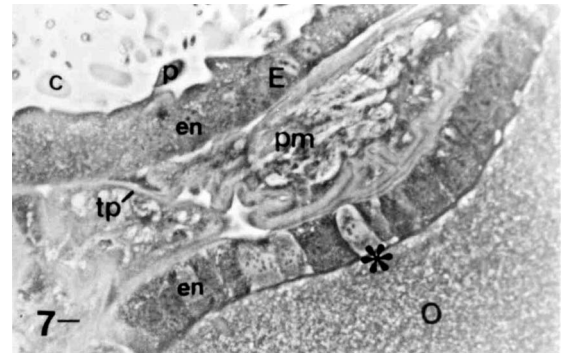

FIGURE 7. Longitudinal section of the ovariole of *Epicharis affinis* obeserved under phase contrast microscope, Bromophenol Blue stained, showing the follicular epithelium (E) of two successive follicles with different developmental stages. Covering the mature oocyte, the follicular projection (p) that penetrate the corion (c). Notice that the epithelium cover the vitellogenic growing oocyte (o) without projections and with cells presenting different staining tonalities (*); en: follicular epithelial cell nucleus; (tp) tunica propria; (pm) peritoneal membrane; en: follicular epithelial cell nucleus. Bar = 10 μm.
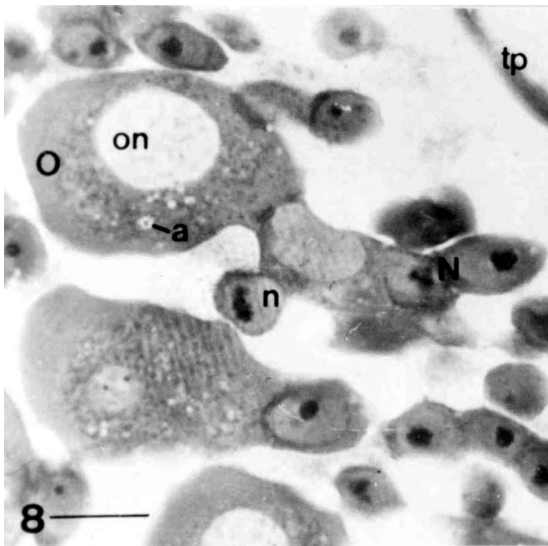

FIGURE 8: Oocyte-nurse cell complex in the posterior region of the germarium of *Melipona bicolor*. Notice the presence of accessory nuclei (a) into the oocyte cytoplasm. The nurse cells present a linear arrangement and they are connected with the oocyte; tp: tunica propria; (o): oocyte; (on): oocyte nucleus; (N): nurse cell; (n): nurse cell nucleus. Bar = 10 μm.
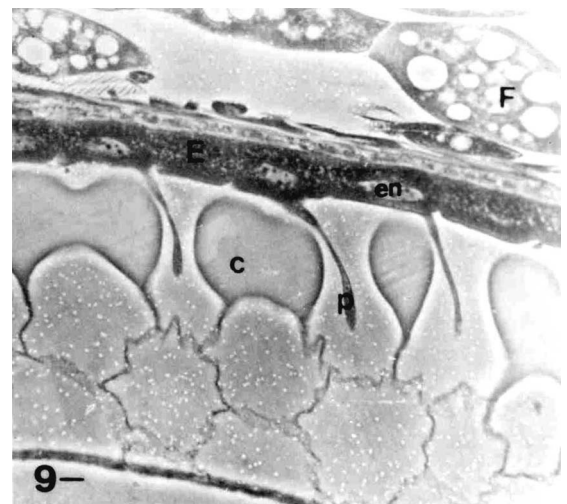

FIGURE 9: Longitudinal section of a mature follicle of *Epicharis affinis*, Methyl Green Pyronin, observed under phase contrast microscope. Notice the follicular cells (E) with extensive cellular projections (p) that penetrate the thick corion (c); en: follicular epithelial cell nucleus; F: fat body cells. Bar = 10 μm.
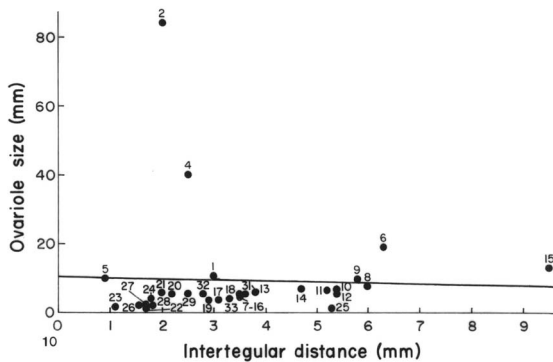
FIGURE 10. Relationship between the ovariole size and intertegular distance of bees (Linear regression). Numbers regards to number of the species listed in the Table 1.

FIGURE 12. Relationship between the oocyte size and intertegular distance of bees (Linear regression). Numbers regards to number of the species listed in the Table 1
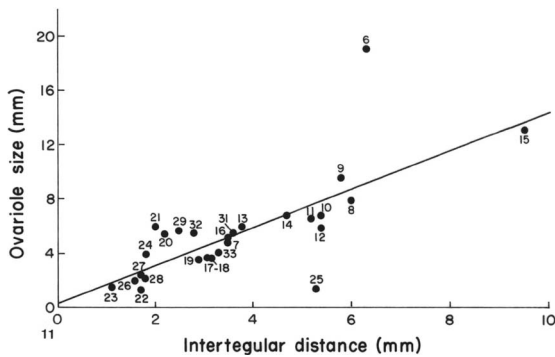

FIGURE 11. Relationship between the ovariole size and intertegular distance of bees (Linear regression). Data on Meliponini and Apini were excluded. Numbers regards to number of the species listed in the Table 1.

FIGURE 13. Relationship between the oocyte width and intertegular distance of bees (Linear regression). Numbers regards to number of the species listed in the Table 1.

In Apini, Meliponini and Bombini all ovarioles have mature oocyte suggesting a synchronism in the oocyte production in these ovaries what did not occur in the solitary bees that have only one mature oocyte per ovary. Social bees have an elongated germarium followed by many follicles in a linear arrangement while the solitary ones have short germarium followed by few mature follicles which can be correlated with egg production, because social bees lay many eggs, while solitary bees lay only few eggs.

Number of ovarioles per ovary was found to be a multi-state character. We, Iwatta (1955), Rozen (1986) and Alexander & Rozen (1987) found that Andrenidae, Megachilidae, Halictidae, Colletidae and Melittidae have three ovarioles/ovary, while Apidae presents four or more. Thus, three ovarioles per ovary is the plesiomorphic condition, the others being derived
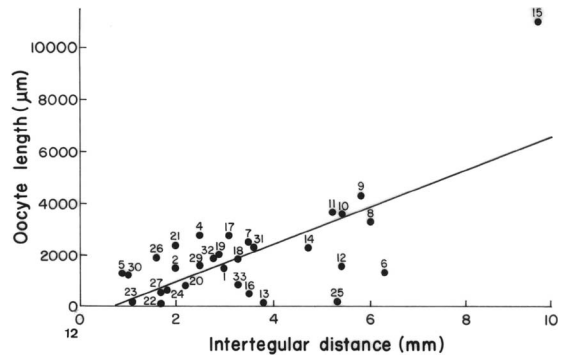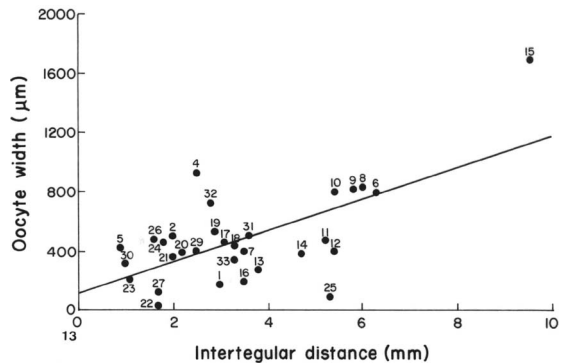
states. Whether these are ordered or unordered states is not known for sure, but it seems reasonable to believe that an increased feature derived from a smaller one, hardly would evolve to decrease, by the criterion of similarity. It seems more likely that four ovarioles/ovary, five ovarioles/ovary found in Nomadinae (Alexander & Rozen, 1987), six to 18 ovarioles/ovary in parasitic Bombini (Cumber, 1949) and hundreds ovarioles/ovary derived independently from the three ovarioles/ovary condition, because they are very discontinuous.

Among corbiculate Apinae, Euglossini is the most similar to the ancestor because this tribe presents few mature oocytes, such as found in others non-corbiculate Apinae and bee families, so that orchid bees can be considered as sister-group of other corbiculate Apinae, which corroborates the hypothesis of Roig-Alsina & Michener (1993), based on external morphology, and

TABLE 1: Morphometric parameters of ovaries in different species of bees.

Family	Subfamily/Tribe	Species*	Number of ovarioles per ovary	Ovariole lenght (mm)	Mature oocyte per ovary	Oocyte length (µm)	Oocyte width (µm)	Follicular epithelial heigh (µm)	Intertegular distance (mm)	Behavior***
Apidae	Apini	Apis mellifera (1)	100-180**	10.4	Many	1492.6	168.9	18.5	3	Highly eusocial
	Meliponini	Scaptotrigona sp. (2)	4	84	Many	1494.4	500.0	9.4	2	Highly eusocial
		Melipona bitolor (3)	4	56.2	Many	2143.4	987.8	7.8	-	Highly eusocial
		Melipona quadrifasciata (4)	4	40	Many	2779.0	926.6	12.9	2.5	Highly eusocial
		Plebeia sp. (5)	4	10	4	1275.8	420.0	6.6	0.9	Highly eusocial
	Bombini	Bombus morio (6)	4	19	4	1317.9	789.5	20.02	6.3	Primitively eusocial
	Euglossini	Euglossa sp. (7)	4	4.7	1	2514.4	396.3	41.93	3.5	Primitively eusocial
		Eulaema nigrita (8)	4	7.8	1	3303.8	832.2	26.60	6.0	Primitively eusocial
	Centridini	Epicharis flava (9)	4	9.5	1	4317.8	813.5	19.52	5.8	Solitary
		Epicharis affinis (10)	4	6.7	1	3610.0	800	17.74	5.4	Solitary
		Centris aenea (11)	4	6.5	1	3694.8	471.6	20.20	5.2	Solitary
		Centris fuscata (12)	4	5.8	1	1589.7	395.1	13.8	5.4	Solitary
		Centris tarsata (13)	4	5.8	1	127.3	269.5	21.91	3.8	Solitary
		Centris sp. (14)	4	6.8	1	2293.1	379.3	23.8	4.7	Solitary
	Xylocopini	Xylocopa frontalis (15)	4	13	1	11053.8	1687.2	45	9.5	Solitary
	Eucerini	Thygater analis (16)	4	5.1	1	507.9	188.2	16.85	3.5	Solitary
		Thygater palliventris (17)	4	3.6	1	2773.7	455.3	28.20	3.1	Solitary
	Ericrocidini	Mesocheira sp. (18)	4	4.0	1	1849.6	438.1	24.6	3.3	Solitary
	Emphorini	Melitoma segmentaria (19)	4	3.5	1	2039.9	530.1	25.8	2.9	Solitary
Andrenidae	Oxaeinae	Oxaea flavescens (20)	3	5.4	1	825.3	390.6	26.9	2.2	Solitary
Halictidae	Augochlorini	Augochlora sp. (21)	3	5.9	1	2364	360.5	13.9	2.0	Solitary
		Augochlora amphitrite (22)	3	1.3	1	76.9	26.1	11.3	1.7	Solitary
		Augochlora thalia (23)	3	1.5	1	191.4	206.4	13.9	1.1	Solitary
		Augochloropsis cockerelli (24)	3	3.9	1	624.9	460.6	15.8	1.8	Solitary
		Augochloropsis electra (25)	3	1.3	1	154.41	84.5	31.2	5.3	Solitary
		Augochloropsis patens (26)	3	2	1	1878.3	476.6	19.5	1.6	Solitary
		Augochloropsis callichroa (27)	3	2.4	1	527.7	114.4	15.1	1.7	Solitary
		Augochloropsis brachycephala (28)	3	2.1	1	-	-	-	1.8	Solitary
		Pseudaugochlora graminea (29)	3	5.6	1	1590.9	395.6	19.5	2.5	Solitary
	Halictini	Dialictus sp. (30)	3	-	1	1248.1	316.1	27.3	1.0	Solitary
Megachilidae	Megachilini	Megachile aureiventris (31)	3	5.4	1	2317.7	502.9	41.5	3.6	Solitary
		Megachile giraffa (32)	3	5.4	1	1888.2	722.6	30.1	2.8	Solitary
		Megachile paulistana (33)	3	4	1	861.1	338.4	22.7	3.3	Solitary

* Numbers in parenthesis indicates those plotted in the Figures 10-13. ** Data from Snodgrass (1956). *** Classification from Roubik (1992).

Serrão (2001) and Peixoto & Serrão (2001) based on digestive tract features, that placed Euglossini in a more basal position in their trees. In this sense, synchronism of egg production represented by accumulation of mature oocytes in the proximal region is a synapomorphy for Apini, Bombini and Meliponini.

Females of the solitary bee, *Andrena erythronii* lay approximately 8 diploid eggs in the reproductive lifetime (Michener & Rettenmeyer, 1956), while *Megachile rotundata* lay approximately 20 diploid eggs (Gerber & Klostermeyer, 1970). Primitively eusocial *Lasioglossum laevissimum* lay approximately 75 diploid eggs (Packer, 1992) and *L. marginatum* lay over 2000 eggs (Plateaux-Quénu, 1960). In the high social bees, *A. mellifera* queens lay hundreds of thousands of diploid eggs in the reproductive lifetime (Snodgrass, 1956). In Meliponini, *M. compressipes fasciulata* lay 25.6 to 30.43 eggs/day, *M. quadrifasciata anthidioides* lay 10-22 eggs/day (Kerr, 1949) and *P. remota* probably produce 60-180 eggs/day (Van Benthem *et al.*, 1995). According to Iwata (1955; 1964), Iwata & Sakagami (1966) and Alexander & Rozen (1987) an increased number of ovarioles functions to increase the reproductive potential of an individual. In this sense, Cruz-Landim *et al.* (1998) stated that the achievement of reproductive efficiency in bees is attained through the increase of ovariole numbers and length. In Meliponini it seems that the chosen mechanism is mostly that of the ovarioles length, because there is a negative correlation between body size and ovary length in this bee group (see Figs. 10, 11), while in *Apis* the achievement of reproductive efficiency is attained through the increase of ovariole number and length (see Table 1).

We suggest that the queen ovary of highly eusocial species are more efficient than that of the solitary and primitively eusocial ones, because in eusocial bees the germarial zone is more developed, housing a higher numbers of germinative cells, ovarioles and mature follicles.

The primitively eusocial *Bombus morio* have not the same reproductive efficiency in comparison to eusocial species, because its ovary has 4 ovarioles per ovary, one mature oocyte per ovariole, and are shorter than those found in the highly eusocial honey bee and stinglessbees.

We are in agreement with Cruz-Landim *et al.* (1998) with the possibility that the number of ovarioles and their length are related to the oviposition rate. Our results showed that the increasing of ovariole number, ovariole size, number of follicles per ovary and size of the germarium, follows the increasing of egg number laying and degree of sociability.

RESUMO

A morfologia do ovário em 33 espécies de abelhas apresentando diferentes graus de sociabilidade foi estudada. Todas as espécies apresentaram ovário do tipo meroístico politrófico formado por um germário anterior e um vitelário basal. No germário estão localizadas as células germinativas e o vitelário apresenta folículos arranjados linearmente. Cada folículo é constituído pela câmara nutridora e pela câmara ovocítica, ambas revestidas por uma camada única de células foliculares. O número de ovaríolos/ovário e de ovócitos maduros/ovário, o comprimento dos ovaríolos, o tamanho e a largura dos ovócitos, a altura do epitélio folicular e a distância intertegular foram analisadas, mostrando que os representantes das tribos altamente eussociais Meliponini e Apini têm os maiores ovários, enquanto as abelhas solitárias apresentam maiores ovócitos. Os resultados obtidos sugerem que há uma tendência para o aumento no tamanho do ovário e número de ovaríolos conforme há um aumento no nível de sociabilidade das abelhas.

PALAVRAS-CHAVE: comportamento social, insetos, morfologia, ovário, sistema reprodutor.

REFERENCES

Alexander, B. & Rozen, J.G. 1987. Ovaries, ovarioles and oocytes in parasitic bees (Hymenoptera: Apoidea). *Pan Pacific Entomologist,* 63(2):155-164.

Bilinski, S.M. 1989. Formation and function of accessory nuclei in the oocytes of the bird louse, *Eomenacanthus stamineus* (Insecta, Mallophaga) I. Ultrastructural and histochemical studies. *Chromosoma,* 97(4):321-326.

Bilinski, S.M. 1998. Introductory remarks. *Folia Histochemica Cytobiologica,* 36:143-145.

Bilinski, S.M.; Büning, J. & Simiczyjew, B. 1998. Neuropteroidea: different ovary structure in related groups. *Folia Histochemica et Cytobiologica,* 36:189-195.

Billen, J. 1985. Ultrastructure of the worker ovarioles in *Formica* ants. *International Journal of Insect Morpholoy and Embryology,* 14:21-32.

Büning, J. 1994. *The Insect Ovary. Ultrastructure, Previtellogenic Growth and Evolution.* Chapman & Hall, London.

Camargo-Mathias, M.I. 1993. *Histoquímica e ultra-estrutura dos ovários de operárias e rainhas de formigas Neoponera villosa (Hymenoptera: Ponerinae).* Tese (Doutorado), Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP.

Chapman, R.F. 1998. *The insects: structure and function.* 4.ed. Cambridge University, Cambridge.

Cruz-Landim, C. 1991. Accessory nuclei in Hymenoptera oocytes and the germ plasm, a study of *Melipona quadrifasciata anthidioides* Lep. (Hymenoptera, Apidae, Meliponinae). *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae, Attinae). *Naturalia,* 16:171-182.

Cruz-Landim, C.; Reginato, R.D. & Imperatriz-Fonseca, V.L. 1998. Variation on ovariole number in Meliponinae (Hymenoptera, Apidae) queen's ovaries, with comments on ovary development and caste differentiation. *Papéis Avulsos de Zoologia,* 40:289-296.

Cumber, R.A. 1949. Bumble-bees and commensals found within a thirty mile radius of London. *Proceedings of the Royal Entomological Society of London, Série* A, 24:119-127.

Fleig, R. 1995. Role of the Follicle Cells for Yolk Uptake in Ovarian Follicles of the Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae). *Internalional Journal of Insect Morphology and Embryology,* 24:427-433.

Gerber, H.S. & Klostermeyer, E.C. 1970. Sex control by bees: a voluntary act of egg fertilization during ovopositon. *Science,* 167:82-84.

Iwata, K. 1955. The comparative anatomy of the ovary in Hymenoptera. Part I. *Mushi,* 29:17-34.

Iwata, K. 1964. Egg giantism in subsocial Hymenoptera, with ethological discussion on tropical bamboo carpenter bees. *Nature Life Southeast Asia,* 3:399-435.

Iwata, K. 1965. The comparative anatomy of the ovary in Hymenoptera. *Mushi,* 38:101-110.

Iwata, K. & Sakagami, S.F. 1966. Giantism and dwarfism in bee eggs in relation to the modes of life, with notes on the number of ovarioles. *Japanese Journal of Ecology,* 16:4-16.

Jaglarz, M.K. 1998. The number that counts. Phylogenetic implications of the number of nurse cells in ovarian follicles. *Folia Histochemica et Cytobiologica,* 36:167-178.

Kerr, W.E. 1949. Algumas comparações entre a abelha européia (*Apis mellifera*) e as abelhas brasileiras (Meliponini). *O Solo,* 1:40-47.

Kubrakiewicz, J.; Jedrzejowska, I. & Bilinsk, S.M. 1998. Neuropteroidea: different ovary structure in related groups. *Folia Histochemica et Cytobiologica,* 36:179-187.

Michener, C.D. & Rettenmeyer, C.W. 1956. The ethology of *Andrena erythronii* with comparative data on other species. *University of Kansas Science Bulletin,* 37:645-684.

Packer, L. 1992. The social organization of *Lasioglossum laevissimum* (*Dialictus*) in southern Alberta. *Canadian Journal of Zoology,* 70:1767-1774.

Patrício, P. & Cruz-Landim, C. 2001. Tipos de ovários presentes nos insetos. Características morfológicas e ultra-estruturais: uma revisão. *Naturalia,* 26:53-68.

Pearse, A.G.E. 1968. *Histochemistry theoretical and applied.* Little Brown & Company, Boston.

Peixoto, E.B.M.I. & Serrão, J.E. 2001. A comparative study of the cardia and cardiac valves in corbiculate bees (Hymenoptera, Apinae). *Sociobiology,* 37:707-721.

Plateaux-Quénu, C. 1960. Nouvelle preuve d'un déterminisme imaginal des castes chez *Halictus marginatus* Brulle. *Compte Rendu de l'Academie des Sciences, Paris,* 250:4465-4466.

Roig-Alsina, A. & Michener, C.D. 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). *University of Kansas Science Bulletin,* 55:123-173.

Roubik, D.W. 1992. *Ecology and Natural History of Tropical Bees.* Cambridge University Press, New York.

Rozen, J.G. 1986. Survey of the number of ovarioles in various taxa of bees (Hymenoptera: Apoidea). *Proceedings of the Entomological Society of Washington,* 88(4):707-710.

Serrão, J.E. 2001. A comparative study of the proventricular structure in corbiculate apinae (Hymenoptera, Apidae). *Micron,* 32:397-385.

Simiczyjew, B., Ogorzalek, A. & Stys, P. 1998. Heteropteram ovaries: variations on the theme. *Folia Histochemica et Cytobiologica,* 36:147-156.

Snedecor, G.W. & Cochran, W.G. 1980. *Statistical Methods.* 7.ed. The Iowa State University Press, Iowa.

Snodgrass, R.F. 1935. *Principles of Insect Morphology.* McGraw-Hill Book Co., New York.

Snodgrass, R.F. 1956. *The Anatomy of Honey Bee.* Comstock Publ. Assoc., Ithaca.

Szklarzewicz, T. 1998. The ovaries of scale insects (Hemiptera, Coccenea). Morphology and phylogenetic conclusions. *Folia Histochemica et Cytobiologica,* 36:157-165.

Van Benthem, F.D.J.; Imperatriz-Fonseca, V.L. & Velthuis, H.H.W. 1995. Biology of the stingless bee *Plebeia remota* (Holmberg), observations on evolutionary implications. *Insectes Sociaux,* 42:71-87.

Zacaro, A.A. & Cruz-Landim, C. 1996. Ovogênese previtelogênica e diferenciação dos ovaríolos prepostura: considerações ultra-estruturais em *Apis mellifera*. In: Encontro sobre Abelhas, 2. *Anais.* Universidade de São Paulo, Ribeirão Preto, SP, p.94-104.