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Comparative study of mandibular glands of *Melipona bicolor* queens obtained from polygynic and monogynic colonies

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Key words: *Melipona bicolor*, mandibular gland, morphology, polygynic colonies.

ABSTRACT: The aim of the present study was analyze, by histological and morphometrical studies, mandibular glands of *Melipona bicolor* queens collected from monogynic and polygynic colonies and compare their level of development. The results showed that the glands of physogastric queens from monogynic colony present a higher level of activity in relation to the queens of polygynic colonies; this is explained by the fact that just a unique queen controls the monogynic colony. In the polygynic colonies, the queens may divide such control to each other.

Introduction

The most complete studies on the pheromone system of bees have been done with the queen mandibular glands of *Apis mellifera*, whose secretion constitutes the "queen substance" (Butler, 1957). This secretion is a complex of five compounds, with different effects, that include the worker court behavior around the queen (Slessor *et al*., 1988), inhibition of the worker ovary development (Pankiw *et al*., 1994), inhibition of the queen cell construction by the workers (Winston *et al*., 1989, 1990), suppression of the swarm, delay of the attack behavior of the foraging workers (Pankiw *et al*., 1996), and foraging activity and worker cell construction stimulation (Higo *et al*., 1992).

The queen meliponine dominance on the workers is not as evident as in *Apis*, and there are reports of occurrence of natural polygyny, as it is the case of *Melipona bicolor*, unlikely the colonies of *Apis* where the monogyny is a constant condition. The term polygyny was first used by Herbers (1993) and Imperatriz-Fonseca and Zucchi (1995) "to denote the mutual coexistence of two or more egg-laying queens in the same nest, (...) and the mixture of their offspring in a same social homogeneous unit." In this way, colonies of *Melipona bicolor* with a considerably high population may present until five physogastric queens involved simultaneously in oviposition, apparently without any agonistic behavior (Bego, 1989).

Amongst the pheromone producing glands, the mandibular glands are the most important ones whose role in *Apis* is the regulation of the worker reproductive activity. These glands, considered part of the salivary system, have digestive function (Cruz-Landim, 1967) being considered, essentially, pheromone-producing glands. They are very well studied in queens of *Apis*, under the different aspects, but in meliponines very few

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studies have been performed, were the interesting polygyny condition may be found naturally. For such reasons, in the present work the morphological and morphometrical differences of the mandibular glands among active and non-active physogastric queens from monogynic and polygynic colonies were evaluated, trying to establish possible differences of glandular development as regard to the characteristics of the colonies and the physiologic condition of the queens.

Materials and Methods

To perform the present study three physogastric queens of *Melipona bicolor* Lepeletier, 1836 were collected from colonies maintained in the apiary of the Bee Center of the University of São Paulo (USP - Brazil), in the following conditions:

- Queen 1: physogastric queen from monogynic colony.
- Queen 2: physogastric queen (without posture) from polygynic colony.
- Queen 3: physogastric queen from the same colony than queen 2.

For histological analyses, queens were dissected and mandibular glands removed in saline solution for insects (NaCl - 3.75 g, KH_2PO_4 - 1.76 g, Na_2HPO_4 - 1.98 g for 500 ml of distilled water) and fixed, for 24 h, in alcoholic Bouin. After fixation, glands were dehydrated in an increasing concentration of alcoholic series and then, included in resin JB - 4 (Polysciences). Sections were obtained in a BIO-RAD JB-4 microtome, and stained with hematoxylin and eosin to be examined and photographed in a photomicroscope (ZEISS) and to perform the morphometric analyses.

The secretory cell areas were obtained through a Axioskop microscope (Zeiss) coupled to a microcomputer, which is provided with the program Impact - Graphic Application, which allows the measurement and calculation of cell areas directly of the sections. A total of 50 areas of secretory cells were measured in each gland.

The averages and standard deviations of the cell areas were calculated by each gland measured. The parametric test of Tukey was applied, with significance level at 5% for comparison of the area average among the queens studied, according to Gomes (1981).

Results

The mandibular glands of *Melipona bicolor* are paired, located one on each side of the head and formed by group of secretory cells (Fig. 1A), which are linked to a sack-like reservoir.

The spherical secretory cells present a central nucleus also spherical. The glandular cell cytoplasm is rich in vacuoles (Figs. 1B, 1C). Each secretory cell shows a canaliculus presenting one of its extremities involved by the own secretory cell, and the other extremity connected to the reservoir. Around of the apparently intracellular portion of the canaliculus, secretion accumulation is observed (Figs. 1A, 1C), which is strongly stained by the hematoxylin in queen of monogynic colony (Fig. 1B).

Figure 1D shows the sack-like reservoir, with the wall covered by cuticle on the luminal surface and constituted by a low epithelium, whose cells are small and flat.

The results of the morphometric analyses, presented in the Table I, allow to verify the occurrence of significant differences among the areas of secretory cells of the physogastric queens, with the presence of larger secretory cells in the mandibular glands of the queens 1 and 2 (monogynic and polygynic without posture, respectively). The smallest cellular areas appear in the physogastric queen 3 (of a polygynic colony).

FIGURE 1. Light micrograph of secretory cells of the mandibular glands from physogastric queens of *Melipona bicolor*.
A. General aspect of secretory cells of a queen from a polygynic colony (without posture) (X 16).
B. Detail of secretory cells of a queen from a monogynic colony (X 100).
C. Detail of secretory cells of a queen from a polygynic colony (X 100).
D. General aspect of the cells that cover the reservoir of the mandibular glands (X40).
c= cytoplasm; n= nuclei; v= vacuoles; RL= reservoir lumen; cr= cytoplasm of reservoir epithelium; nr= nuclei of reservoir epithelium; *= canaliculus in secretory cells.

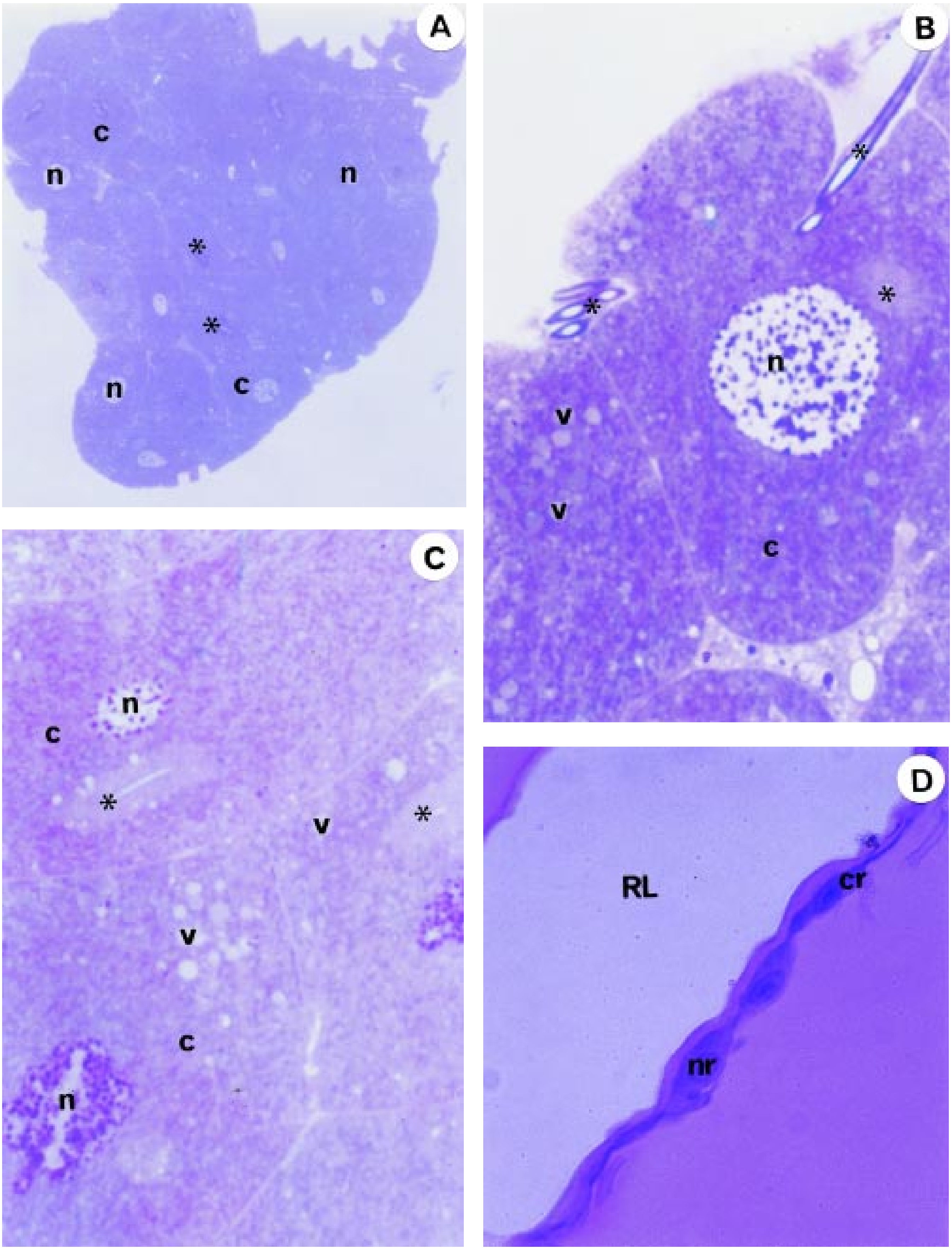
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TABLE I.

Results from the Tukey test for the verification of significant contrasts among values of the secretory cell areas (μm^2) of the mandibular glands among different physogastric queens of *Melipona bicolor*.

Individual	*N*	*X (SD)*	*Tukey **
Queen 1	50	3846 ± 905	A
Queen 2	50	2954 ± 648	B
Queen 3	50	2511 ± 589	C

N = number of individuals analyzed.
X = average
SD = Standard Deviation
Queen 1: physogastric queen from monogynic colony.
Queen 2: physogastric queen (without posture) from polygynic colony.
Queen 3: physogastric queen from the same colony than queen 2.
* Different letters mean significant differences at 5% of level of significance.

Discussion

The morphological results show that the mandibular glands of *Melipona bicolor* queens is constituted by a group of secretory cells united by a membrane in the mandible base, as already observed by Gracioli and Silva de Moraes (2002) in workers of the same species. The cells are associated to the wall of the proximal portion of a sack-like reservoir. The presence of a long and thin canaliculus going through the whole cytoplasm of the secretory cells, taking the secretion from the secretory cells until the reservoir lumen, confirms the observations of Cruz-Landim (1967) for other species of meliponines. Among the queens studied, the mandibular glands did not present morphologic differences, suggesting that this aspect is not affected neither for the posture activity nor for the colony condition (monogynic or polygynic).

The mandibular gland reservoir exhibits a sack-like morphology and is covered by a layer of small and flat cells, which forms a cuticular epithelium. Such morphology, also described for the mandibular glands of all inferior trigonines and other meliponines, and also for Bombini, Euglossini, Exomalopsini, Hemissini, Xylocopini, Centridini and *Trichocolletes venustus* (Cruz-Landim, 1967), is quite different from that found in *Apis*, where the own secretory cells form the epithelium that covers the reservoir. In *Apis*, flat and small cells, called cells of intima, are found between the lumen of the reservoir and the secretory cells (Gary, 1963; Cruz-Landim, 1967; Costa-Leonardo, 1980, 1982; Crewe and Velthuis, 1980; Lensky and Cassier, 1995; Gracioli, 1998).

Due to their structure, the secretory cells of the mandibular glands of bees is classified as class III, according to the classification of Noirot and Quennedey (1974, 1991) and Quennedey (1998). Although they are not in direct contact with the surface of the tegument, establish contact with the lumen of the glandular reservoir, through the canaliculi that conduct individually the secretion, what also happens in *Melipona* and in *Apis* (Cruz-Landim, 1967).

In all queens studied, the cytoplasm of the secretory cells of the mandibular glands showed portions quite stained by hematoxylin, having the secretion accumulated in the canaliculi stronger staining intensity in physogastric queen secretory cells of monogynic colony. The presence of numerous vacuoles in the cytoplasm may suggest the lipid accumulation, since this gland, in *Apis* and in this species, secretes lipids (Costa-Leonardo, 1981; Gracioli, 2003 np). These vacuoles seem to be concentrated around the canalicular area. According to Cruz-Landim and Puga (1967) there is, also, lipid presence in the cytoplasm of the secretory cells, as well as in the lumen of the mandibular gland reservoir of workers of *Trigona postica*, which intracellular material stayed constant and independent of the tasks exercised by the individuals.

The material secreted and accumulated around the portion of canaliculus that is involved by the secretory cell, may be in the cytoplasm of this cell and be contained in vesicles and/or in the space between the plasma membrane of secretory cell and canalicular wall, called pericanalicular area, likely occurs in the hypopharyngeal glands (Knecht and Kaatz, 1990). This pericanalicular space would function as a cellular reservoir for the secretion, before its elimination for the lumen of the gland reservoir.

The morphometric results showed that in physogastric queen of monogynic colony the mandibular glands are more developed than those present in queens of polygynic colony. Admitting that the mandibular glands are involved in the production of compounds corresponding to the "queen substance", likely the situation in *Apis*, and that the largest development of the secretory cells indicates larger glandular activity, this result was waited, since the queen in monogynic colony exercises alone the control of the colony. The opposite may happen in polygynic colonies, where all the physogastric queens may execute such task.

Caste-specific morphometric differences were already observed in mandibular glands of *Melipona bicolor* when these glands were compared among workers, virgin and physogastric queens, presenting the last ones larger cellular areas, followed by the virgin queens and, later by the workers, indicating larger activity in the physogastric queen glands (Gracioli and Silva de Moraes, 2002). The authors report these morphometrical differences to the glandular function in each individual.

According to Costa-Leonardo (1980), the mandibular glands of workers of *Apis mellifera* are characterized by secretion cycles, coincident with certain social functions carried out by the workers in the colony. These cycles seem to be influenced by the juvenile hormone that causes an increase of the cell area of the mandibular glands, when topically applied in newly-emerged workers of *Apis* (Gracioli, 1998; Salles and Gracioli, 2002), indicating that this hormone, when present in enough amounts, acts at the cellular level, modifying the normal metabolism and taking to a higher cell activity, approximating, under this aspect, the workers of the queens. Therefore, we may consider different cycles of development for the mandibular glands in queens under different conditions, where they execute different activities suggesting, also, different levels of juvenile hormone in their haemolymph.

In queens from the polygynic colony that do not present egg-laying activity, it was found intermediate value of the secretory cell areas as compared to physogastric queens from monogynic and polygynic colonies, suggesting a relationship between glandular development and colony condition.

References

Bego LR (1989). Behavioral interactions among queens of the polygynic stingless bee *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apidae). Brazilian J Med Biol Res. 22: 587-596.

Butler CG (1957). The process of queen supersedure in colonies of honeybees (*Apis mellifera*). Insects Sociaux. 4: 211-223.

Costa-Leonardo AM (1980). Estudos morfológicos do ciclo secretor das glândulas mandibulares de *Apis mellifera* L. (Hymenoptera, Apidae). Rev Bras Biol. 24(2): 143-151.

Costa-Leonardo AM (1981). Ultra-estrutura do ciclo secretor das glândulas mandibulares de operárias de *Apis mellifera* L. (Hymenoptera, Apidae). Rev Bras Zool. 41(2): 307-316.

Costa-Leonardo AM (1982). Ciclo de desenvolvimento das glândulas mandibulares de *Apis mellifera* L. (Hymenoptera: Apidae) e a regulação social na colônia. Doctor thesis, Universidade de São Paulo, USP, São Paulo, SP, Brazil.

Crewe RM, Velthuis HHW (1980). False queens: A consequence of mandibular gland signals in worker honeybees. Naturwissenschaften. 67(5): 467.

Cruz-Landim C (1967). Estudo comparativo de algumas glândulas das abelhas (Hymenoptera, Apoidea) e respectivas implicações evolutivas. Arq Zool. São Paulo. 15(3): 177-290.

Cruz-Landim C, Puga FR (1967). Presença de substâncias lipídicas nas glândulas do sistema salivar de *Trigona* (Hym., Apoidea). Papéis avulsos Zool. 20(7): 65-74.

Gary NE (1963). Observations of mating behavior in the honeybee. J Apic Res. 2: 3-13.

Gomes FP (1981). Estatística experimental. 9 ed. Piracicaba, SP: Livraria Nobel SA. 430pp.

Gracioli LF (1998). Efeito do hormônio juvenil sobre o desenvolvimento de algumas glândulas exócrinas de *Apis mellifera* (Hymenoptera; Apidae). Master thesis, Universidade Estadual Paulista, UNESP, Rio Claro, SP, Brazil.

Gracioli LF, Silva de Moraes RLM (2002). Histological and morphometric comparisons of worker and queen mandibular glands of *Melipona bicolor bicolor* (Hymenoptera, Meliponini). Sociobiology. 40(2): 449-456.

Herbers JM (1993). Ecological determinants in queen number in ants. In: Queen number and sociality in insects. L Keller. Oxford Sci. Publi., Oxford, UK, 262- 293 pp.

Higo HA, Colley SJ, Winston ML, Slessor KN (1992). Effects of honey-bee (*Apis mellifera* L.) queen mandibular gland pheromone on foraging and brood rearing. Can Entomol. 124: 409-418.

Imperatriz-Fonseca VL, Zucchi R (1995). Virgin queens in stingless bee (Apidae, Meliponinae) colonies: a review. Apidologie. 26: 231- 244.

Knecht D, Kaatz HH (1990). Patterns of larval food prodution by hypopharyngeal glands in adult woker honey bees. Apidologie. 27: 457- 468.

Lensky Y, Cassier P (1995). The alarm pheromones of queen and worker honey bees. Bee world. 76(3): 119-129.

Noirot C, Quennedey A (1974). Fine structure of insect epidermal glands. Annls Rev Entomol. 19: 61-80.

Noirot C, Quennedey A (1991). Glands, gland cells, glandular units: some comments on terminology and classification. Annls Soc Ent Fr (N.S.). 27(2): 123-128.

Pankiw T, Winston ML, Plettner E, Slessor KN, Pettis JS, Taylor OR (1996). Mandibular gland components of European and Africanized honeybee queens (*Apis mellifera*). J Chem Ecol. 22: 605-615.

Pankiw T, Winston ML, Slessor KN (1994). Variation in worker response to honey bee (*Apis mellifera*) queen mandibular pheromone (Hymenoptera: Apidae). J Insect Behav. 7: 1-15.

Quennedey A (1998). Insect epidermal gland cells: ultrastructure and morphogenesis. In: Microscopic anatomy of invertebrates. FW Harrison, M Locke. Wiley-Liss, Inc, 11A: 177-207pp.

Salles HC, Gracioli LF (2002). Glândulas mandibulares e Intramandibulares. In: Glândulas exócrinas das abelhas. C Cruz-Landim, FC Abdalla. FUNPEC, pp. 71-90.

Slessor KN, Kaminski LA, King GGS, Borden JH, Winston ML (1988). Semiochemical basis of the retinue response to queen honey bees. Nature. 332: 354-356.

Winston ML, Higo HA, Slessor KN (1990). Effect of various dosages of queen mandibular gland pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidae). Ann Entomol Soc Am. 83: 234-238.

Winston ML, Slessor KN, Willis LG, Naumann K, Higo HA, Wyborn MH, Kaminsky LA (1989). The influence of queen mandibular pheromone on worker attraction to swarm clusters and inhibition of queen rearing in the honey bee (*Apis mellifera* L.). Insect Sociaux. 36: 15-27.