SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Tegumentary Epithelial Glands in the Abdomen of Virgin and Physogastric Queens of the Stingless Bee *Scaptotrigona postica* Latreille (Meliponini: Trigonina)

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Glândulas Tegumentares Epiteliais no Abdome de Rainhas Virgens e Fisogástricas de *Scaptogrigona postica* Latreille (Meliponini: Trigonina)

RESUMO - Nas operárias das abelhas eussociais, regiões do epitélio tegumentar do abdome podem hipertrofiar-se e tornarem-se glandulares, sendo responsáveis pela produção de cera usada na contrução dos alvéolos de cria. Nos meliponíneos, epitélio com essas características também foi observado nas rainhas, porém sua função ainda continua desconhecida. Teria esse epitélio função homóloga nas operárias? As rainhas, além da função reprodutiva, desempenhariam funções na colônia até então consideradas exclusivas das operárias? Para tentar colaborar no esclarecimento dessas questões realizou-se um estudo histológico e ultra-estrutural das glândulas epiteliais tegumentares do terceiro tergito em rainhas virgens e fisogástricas de *Scaptotrigona postica* Latreille. Os resultados morfológicos mostraram que o epitélio glandular existe e é facilmente discernível no terceiro tergito das rainhas, sendo muito mais desenvolvido nas fisogástricas do que nas virgens. A ultra-estrutura mostrou que há diferenças no tipo de organização do retículo endoplasmático liso entre as rainhas, o que, juntamente com os resultados da histologia e morfometria, indicam poder haver diferenças funcionais desse epitélio entre elas. Como já observado na literatura, nas rainhas virgens tal epitélio pode estar envolvido na produção de cera e confecção de alvéolos de cria, porém sua função nas rainhas fisogástricas é totalmente desconhecida. A hipótese lançada no presente estudo é de que esse epitélio pode estar envolvido na produção de feromônios que irão atuar nas interações sociais da rainha poedeira.

PALAVRAS-CHAVE: Cera, abelha, meliponíneo, histologia, microscopia

ABSTRACT - In the eusocial worker bees, portions of the epithelium of the abdominal epidermis may become hypertrophied and glandular, and produce the wax used to build the brood alveoli. In meliponine bees, the presence of similar epithelium was already observed in the queens, but its function is still unknown. Would this epithelium have homologous function in the workers? May the queens display other functions, besides reproduction, related to those considered exclusive of workers? In order to contribute to solve these questions, a histological and ultrastructural study of the tegumentary epithelial glands of the third tergite was carried out in virgins and physogastric queens of *Scaptotrigona postica* Latreille. The glandular tegumentary epithelium was easily discernible in the third tergite of the queens, being much more developed in physogastric than in virgin queens. The ultrastructural differences in the smooth endoplasmic reticulum between the queens, additionally to the histological and morphometrical data, indicate that this epithelium may have functional differences between the queen types. As already known, the virgin queens may produce wax and build brood alveoli, but the role of this epithelium in physogastric queens is totally unknown. A hypothesis considered in the present study is that the epithelium may be involved in production of pheromones, which may play roles in the social interaction of the laying queen.

KEY WORDS: Wax, meliponine, histology, microscopy

Bees present tegumentary glands distributed along the whole body (Bordas 1908, Cruz-Landim 1996). In the abdomen, the glands are placed dorsally and/or ventrally, depending on the species (Cruz-Landim 1967; Cruz-Landim *et al.* 1980a,b; Cruz-Landim & Mota 1990; Guerino & Cruz-Landim 2003). These glands are of epidermal origin and are grouped into two basic types: 1) glands composed of class I cells, which are regions of hypertrophied epidermis that became secretory and 2) unicellular glands composed of class III cells, which are modified epidermal cells and can be considered a glandular unit or Class III gland cells. These latter are cells detached from the epithelial layer that form free globular secretory units connected to the exoskeleton through individual conducting canals, which are formed by a canal cell (Cruz-Landim 1967, 2002; Noirot & Quennedey 1991).

The function of the class III abdominal exocrine glands is not well known, but in eusocial bees they may be involved in pheromone production (Cruz-Landim 1987, Wilson 1963, Blum & Brand 1972, Velthuis 1970), being in this case important for communication and for the establishment of sociality, reinforcing the colony hierarchy and the caste differentiation (Guerino & Cruz-Landim 2003). In eusocial species the wax used to build the nest is secreted in the glands composed of class I cells. In other species, the function of these glands is unknown and the possibility that they produce pheromones may not be discharged.

In workers of *Apis mellifera* L., the epithelial glands are located on sternites III to VI of the abdomen (Dreyeing 1903). Glands of the same type occur dorsally in stingless bee workers, in tergites III to VI (Cruz-Landim 1967, Cruz-Landim & Mota 1993, Guerino & Paes de Oliveira 2002). In meliponine workers, these glands also produce wax, but in queens their function is unknown.

In some meliponine species the queens may present abdominal epithelial glands more developed than in workers (Cruz-Landim *et al.* 1980a,b; Cruz-Landim & Mota 1993). The production of wax by virgin queens has been reported by some authors (Ihering 1903, Koedam *et al.* 2002) and Imperatriz-Fonseca (1973) observed young virgin queens of *Schwarziana quadripunctata* Lepeletier**,** building wax alveoli, supporting a wax production function for these glands in the virgin queens, although wax production and manipulation for building the nest is a typical worker task.

Epithelial tegumentary glands are also present in social and solitary bees that do not build wax nest (Ramos *et al.* 2002, Guerino & Cruz-Landim 2003), suggesting that the glands may have other functions.

To contribute to the knowledge of the queen tegumentary epithelial glands in stingless bees, a comparative morphological study between virgin and physogastric queens of *Scaptotrigona postica* Latreille was performed.

Material and Methods

The virgin and physogastric queens of *S. postica* L. were collected from colonies maintained at the Apiary of the Departmento de Biologia, Instituto de Biociências, Unesp (Rio Claro – Brazil). For all experiments four glands of each queen type were used.

Light Microscopy (LM). Third tergites of virgin and physogastric queens were fixed in 4% paraformaldehyde for at least 2h and then embedded in historesin, following the usual procedures. The histological sections were stained with haematoxylin and eosin (HE) and observed and photographed in a photomicroscope.

Transmission Electron Microscopy (TEM). Third tergites of virgin and physogastric queens were fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.2, for at least 2h. After fixation, the tergites were washed twice in 0.1 M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer and stained with 2% uranyl acetate in 10% ethanol during 2h, before being dehydrated in a series of acetone (30-100%). The tergites were embedded in Epon Araldite resin. Thin sections were cut with a diamond knife, stained with lead citrate, and examined by electron microscopy.

Morphometry and Statistical Analysis. The height of the glandular epithelium of four virgin and four physogastric queens was measured with aid of a Leica™ microscope that allows measurement and calculation of the epithelium heights directly from histological sections. For each glandular epithelium 11 measurements were done. The data were submitted to an ANOVA test followed by Tukey-test at 5% level of significance to detect differences among the queen groups studied. The averages and standard deviations obtained for each queen groups studied were also calculated.

Results and Discussion

In *S. postica*, both virgin and physogastric queens present epithelial tergal glands in the third tergite of the abdomen, in accordance with Cruz-Landim & Mota (1993), who also verified the presence of this epithelium in the tergites III to VII of workers (wax gland). In males these glands are absent.

This glandular tegumentary epithelium is hypertrophied and easily discernible from common epidermis (Fig. 1A). In virgin queens, the hypertrophied epithelium occurs in the lateral sides of the tergite (Fig. 1A), and is composed of tall cells with wide intercellular spaces (Fig. 1B). In physogastric queens, the glandular epithelium occurs along the whole extension of tergite III. The height of the lateral tergite portions is very similar to that of the virgin queens (Fig. 1C), but in the tergite median portion the cells are taller than in virgin queens (Figs. 1B,D). The average height of the tegumentar cells in physogastric queens is higher than in virgins (Tukey Test $P > 0.05$, 300 mm and 200 mm, respectively). Apparently the number of cells is also larger in physogastric queens, resulting in folds of the epithelium to accommodate all of them in the tergite (Fig. 1D). In physogastric queens, the cells appear closer one to another, with closed intercellular spaces (Figs. 1C,D).

Below the whole epithelium of both queens types, class III unicellular glands, enocytes and trophocytes are frequently observed (Fig. 1E), being the two later cell types more abundant in physogastric queens. Cruz-Landim & Mota (1993) have already registred the occurrence of these cells in

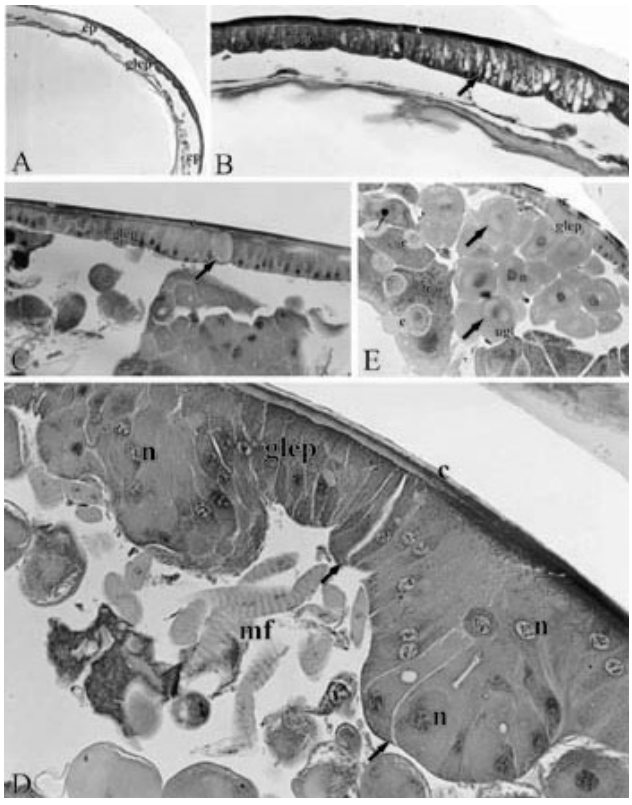

Figure 1. Micrographies of the queen epithelial glands of the tergite III. A. Virgin queen tergite III, showing the simple epidermis (ep) and the glandular epithelium (glep). 25x. B. Detail of the virgin queen glandular epithelium (glep), showing many wide opened intercellular spaces (arrows). 50x. C. Physogastric queen glandular epithelium (glep), where very wide open intercellular spaces (arrow) may bee seen, this structure is very specialized as seen physogastric tergite III of a physogastric queen. The glandular epithelium (glep) is more hypertrophied and folded on the cuticle (c). mf = muscle fibers. 26x. E. Detail of trophocytes (tr) associated with oenocytes (e) and units of class III glands (ugl). arrows = intracellular canals of the class III glands. 70x.

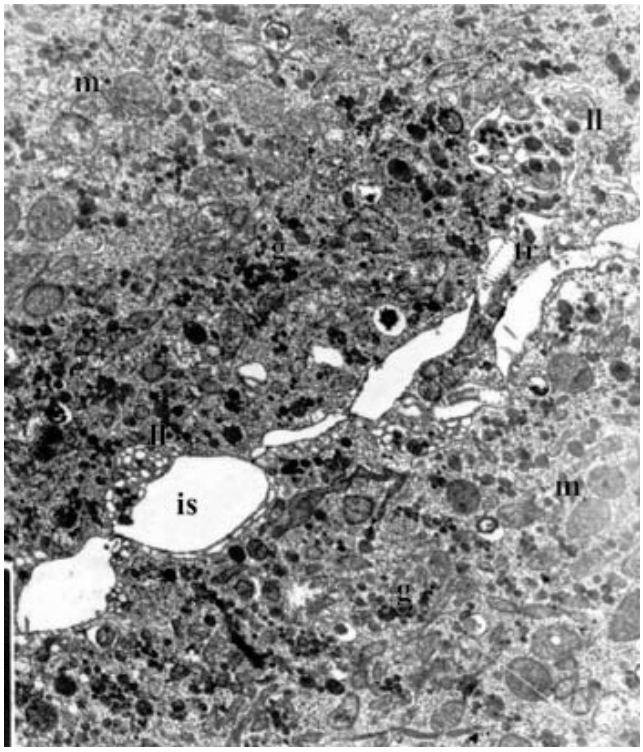

Figure 2. TEM of the epithelial gland of the tergite III of a virgin queen, showing the wide-open intercellular space (is) and various types of electrondense granules (g) and mitochondria (m). Note tracheoles (tr) inside the intercellular space and the lateral labyrinth (ll) formed by lateral invaginations of the plasmic membrane. 8,000x.

queens and workers of *S. postica* and observed that the Class III glands are much more developed in queens.

In virgin queens, the cytoplasm of the epithelial cells is full of small electrondense granules and polymorphic mitochondria (Fig. 2A). In some cells, vesicles containing filamentous or flocculent material can also be seen (Fig. 3B). Smooth endoplasmic reticulum is present.

The intercellular spaces are wide open (Fig. 2A), as observed by the histological sections. Near from intercellular spaces appear small electronlucid vesicles and canals suggesting that occasionally the lateral plasma membrane folds, forming an intercellular lateral labyrinth (Figs. 2A,3A). The presence of tracheolar ramifications into some of the labyrinth canals (Figs. 2A) supports this interpretation and signalizes high oxygen consumption by these cells, in other words, high energy requirement. The apical portion of the cells presents many invaginations, through which secretion is discharged into the subcuticular space and across the cuticle through canal pores (Fig. 3B). The intercellular space in this region is closed (Fig. 3B).

In physogastric queens, intercellular spaces are often closed. In some portions of the epithelium intercellular spaces are however rather wide and full of fuzzy filamentous material. To this wide intercellular space, the plasma membranes fold and produce short and irregular microvilli like (Figs. 1C, 4A,B). This structure seems to be highly specialized and is not observed in virgin queens. The cell cytoplasm contains groups of electrondense granules and vesicles with flocculated material, similar to the ones seen in virgin queens, in addition to small mitochondria (Figs. 4A,B,C). In physogastric queens, the smooth endoplasmic reticulum seems to be more developed, being present in stacks with parallel organization (Fig. 4C).

The epithelium morphology, cell ultrastructure and morphometric results indicate that the epithelial gland of the third tergite may have different functions in virgin and physogastric queens.

In both phases of the queen's life there are strong indications of extensive interchange of the gland cells with the haemolymph, through the basal invaginations and open intercellular spaces, maybe allowing substances absorption. Nevertheless, as the intercellular space is closed

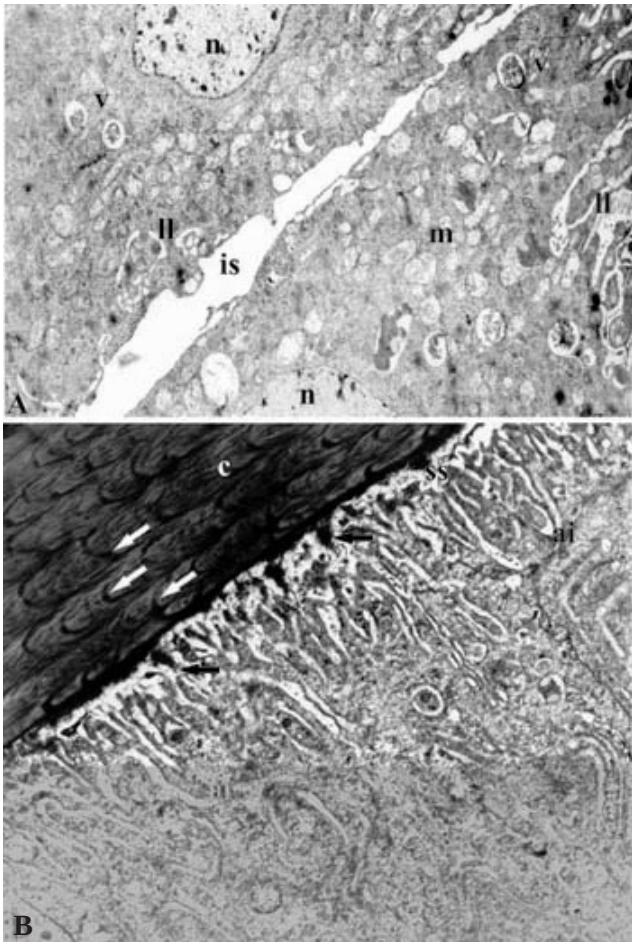

Figure 3. TEM of virgin queen showing in A. Wide, open intercellular space (is) and vesicle with filamentous material (v), granules (g) and mitochondria (m). ll = lateral labyrinth. 8,000x. B. The elimination of secretion (s) from the subcuticular space (ss) and through the pore canals (pc) in the cuticle (c). 14,100x.

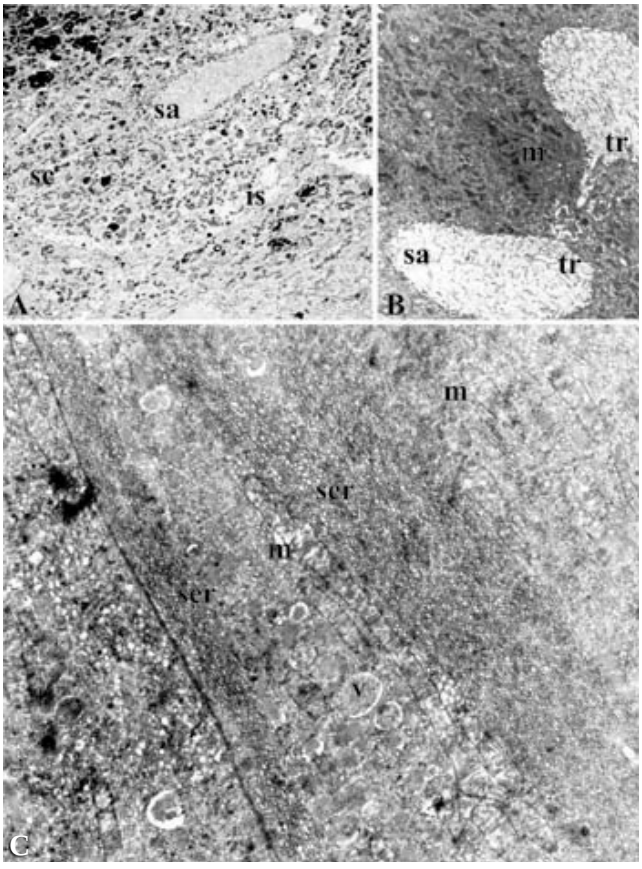

Figure 4. TEM of the cells of the epithelial gland of a physogastric queen. A. General view of a transversal section of the epithelium, showing a cell with an intercellular secretory apparatus (sa) like. is = intercellular space, sc = secretory cell. 4,600x. B. Detail of the intercellular secretory apparatus (sa), tracheoles (tr) inside this structure. m = mitochondria. 10,200. C. High magnification of the cytoplasm of a cell showing smooth endoplasmic reticulum (ser) associated with long mitochondria (m) placed in parallel and with secretion vesicles (v). 21,000x.

apically, the substances absorbed from the haemolymph do not reach the cuticle without passing through the cells. They may be barred in the apical portion. As evidences of pinocytosis were not observed in the basal or lateral labyrinth, the substances absorbed migth have a molecular size compatible with free transit through the membrane or migth be crossed by membrane transporters. It is unknown if the material absorbed by the cell is modified or not in the intercellular medium. Nevertheless, the presence of electrondense granules, vesicles containing several morphological types of material, and the developed smooth endoplasmic reticulum demonstrates that, in queens, mainly in the physogastric ones, the cells are secretory. These cells not only discharge exogenous material, as do class II gland cell of Noirot & Quennedey (1991), but also produce lipids, and this function seem to be more intense in physogastric queens, which have this organelle better organized.

The production of wax by meliponine virgin queens was observed in *Melipona bicolor* (Koedam *et al.* 2002), but not in the species examined in this study. Nevertheless, the production of wax, and even its manipulation (Imperatriz-Fonseca 1973) in meliponines may be more frequent than it was though.

In physogastric queens this tergal epithelium may be involved in pheromone production, since the cells present a developed smooth endoplasmic reticulum, which is compatible with volatile producing glands. The gland secretion may constitute in surface pheromones, which help in caste and reproduction status identification, therefore playing roles in the establishment of queen identity, contributing to the queen fitness and social dominance (Abdalla *et al.* 2003).

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