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ULTRASTRUCTURE OF THE SPERMATHECAL GLAND OF *Melipona bicolor* LEP. (HYMENOPTERA, APINAE, MELIPONINI)

Carminda da Cruz-Landim[1] and José Eduardo Serrão[2]

[1]Department of Biology, Institute of Biosciences, Paulista State University (UNESP), 13506-900, Rio Claro, SP and [2] Department of General Biology, Federal University of Viçosa (UFV), Viçosa, MG, Brazil.

ABSTRACT

The ultrastructural features of the spermathecal gland of *Melipona bicolor,* a stingless bee, are described. The gland in this species is very small, but the organization of the cells and their ultrastructural features are very similar to those of *Apis mellifera.* The gland consists of two short tubular diverticuli connected directly to the spermatheca. The diverticuli are constituted by class III glandular cells. The only difference between the glands of virgin and mated queens is the presence of glycogen deposits in virgins, probably remnants of energetic reserves frequently present in young tissues. The physiological role of the secretion of this organ is not known since its lipid morphological aspect, does not seem support a nutritional function to the sperm cells.

Key words: Stingless bee, reproduction, queen, spermatheca, ultrastructure

INTRODUCTION

In most insects, the sperm transferred to females during mating are retained in one or more special storage organs known as spermatheca [3,7,13,15]. The anatomy of insect spermatheca varies from species to species, but generally consists of a pouch of ectodermal origin with walls formed by an inner epithelium, and an outer muscular layer [11,14]. In several species, the spermatheca has associated spermathecal glands that produce a secretion [5,18] which activates or nourishes the spermatozoa, or may simply serve to lubricate the spermatic ducts [21].

In honeybee queens, the spermatheca is a fairly large, globular sac lying over the vagina, to which it is connected by a short duct. "The upper end of the spermathecal duct is joined by the duct of a pair of tubular spermathecal glands, closely applied to the surface of the spermatheca" [19].

Happ and Happ [8,9] studied the differentiation and structural organization of spermathecal glands in several insects. According to these authors, three cell types form each secretory unit of the gland. The first cell type is secretory and produces the intracellular cuticular end apparatus. The second type secrets the cuticle of the efferent ductule and the third forms the epithelium that lines the gland lumen. This structural organization corresponds to the class III gland cells of insects, as proposed by Noirot and Quennedey [16].

Dallai [4] and Camargo and Mello [1] described the ultrastructure and secretion histochemistry of the honeybee spermathecal gland, respectively, and found the same structural pattern as that proposed by Happ and Happ [8] for other insects.

In this work, we examined the ultrastructural features of the spermathecal gland in *Melipona bicolor*, a stingless bee, in order to contribute to the comprehension of the spermathecal function in bees.

MATERIAL AND METHODS

The spermathecae of virgin and mated queens of *M. bicolor*, obtained from colonies maintained in Rio Claro, SP and Viçosa, MG, Brazil, were dissected and transferred to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for at least 2 h. After washing in cacodylate buffer, the spermathecae were post-fixed in 1% osmium tetroxide in the same buffer, then dehydrated in a graded acetone series and embedded in Epon Araldite. During dehydration, the spermathecae were stained with 2% uranyl acetate in 10% acetone and thin sections were stained with lead citrate. The sections were examined and photographed using a Philips transmission electron microscope (TEM). In some cases, 0.5% ruthenium red was included in the fixatives.

Sections 1 µm thick stained with 1% aqueous toluidine blue were used for light microscopy (LM).

Correspondence to : Dr. Carminda da Cruz-Landim
Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Avenida 24A, 1515, CP 199, CEP 13506-900, Rio Claro, SP, Brasil. Fax: (55) (19) 534-0009, E-mail: cclandim@rc.unesp.br

Some tissues were also fixed in Karnovsky solution, dehydrated in graded acetone, then dried in a CO_2 critical point dryer and coated with gold before examining with a Philips scanning electron microscope (SEM).

RESULTS

In virgin queens the spermatheca is a very small, whitish sphere, located at the end of the ovarian median oviduct. In mated queens the spermatheca is enlarged by the presence of sperm. The spermathecal glands in *M. bicolor* are very small and difficult to see during dissection under a stereomicroscope. Examination with SEM showed two short diverticula placed over the spermathecal surface (Fig. 1), beside the spermathecal duct. The diverticula open independently into the spermathecal reservoir (Fig. 2).

Each diverticulum consists of a row of class III glandular cells around a layer of intima formed by a flat epithelium coated on its luminal surface by a cuticle (Fig. 3). This epithelium is continuous with the spermathecal epithelium. Each of the glandular cells is provided with an intracellular ductule (Figs. 4 and 5), all of which are continuous with the efferent ductule that delivers the secretion to the gland lumen (Figs. 3-5). Although the glandular cells tend to be spherical, the compression of one against the other produces an "epithelium" around the gland lumen (Fig. 3,5).

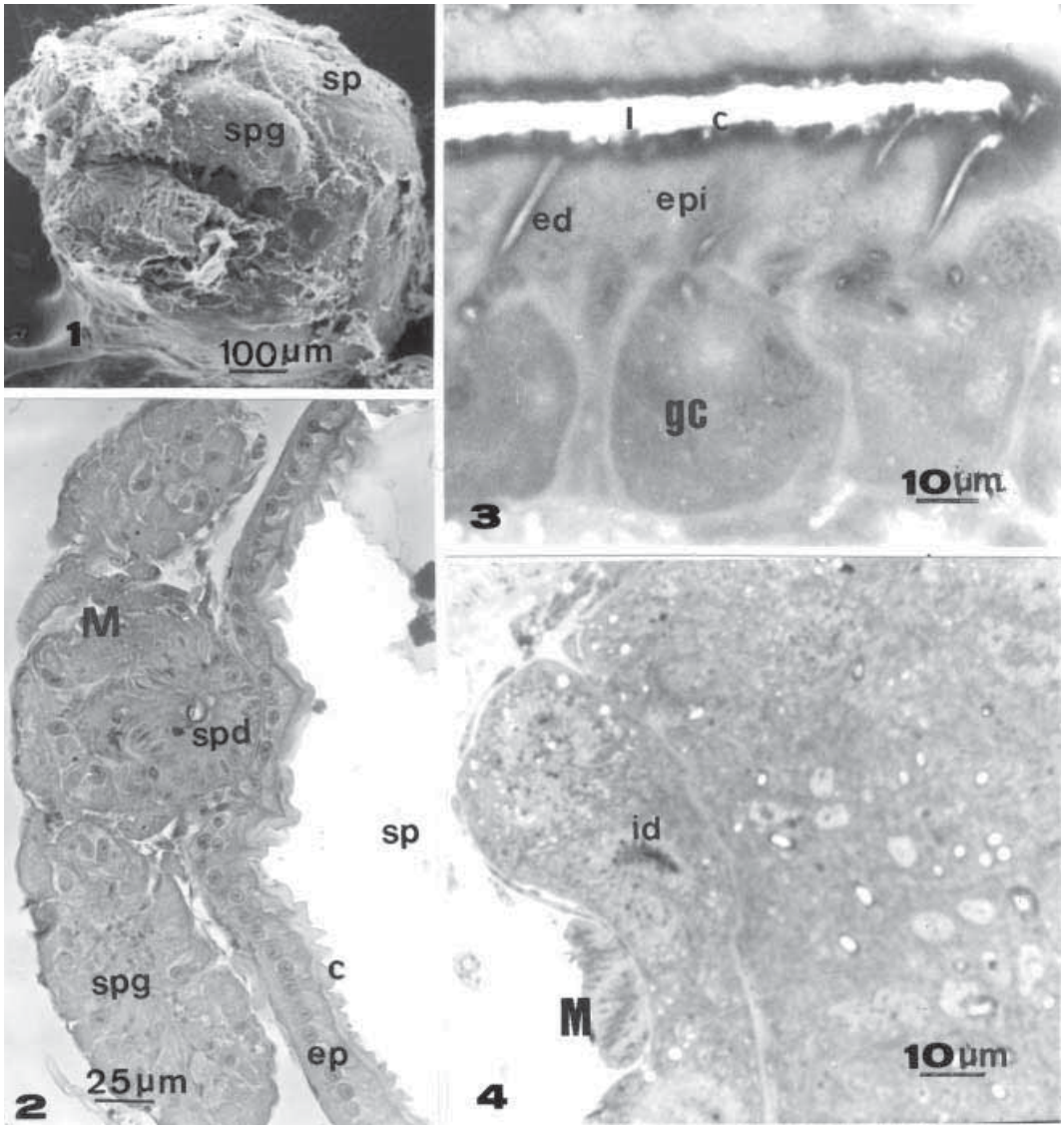


Figure 1. Scanning electron micrograph of the spermatheca of a mated of *M. bicolor* queen showing the location of the spermathecal glands (spg). sp = spermatheca. **Figures 2-4.** Light microscopy micrographs of spermathecae. **2.** Spermatheca (sp) and spermathecal gland (spg). **3.** Spermathecal gland showing the intima (epi), the gland cells (gc) a nd the efferent ductules (ed) of the gland cells. **4.** Section of a gland cell showing the intracytoplasmic ductule (id). c = cuticle; l = lumen; M = muscle; spd = spermathecal duct.

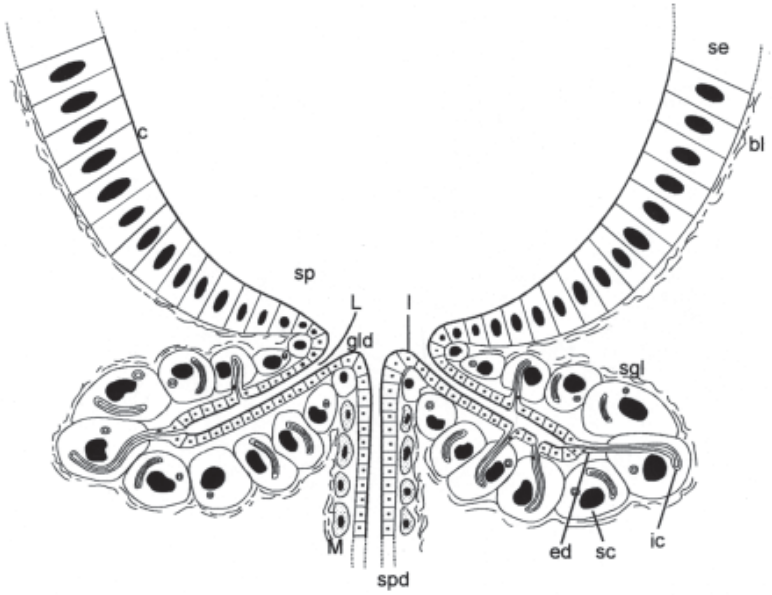


Figure 5. Schematic representation of the spermathecal gland of an *M. bicolor* queen. sp = spermatheca; sgl = spermathecal gland; spd = spermathecal duct; gld = glandular duct ; c = cuticle; se = spermathecal epithelium; M = muscle sheath; sc = secretory cell; ic = intracellular canal; ed = efferent duct.

Figure 5 is a schematic representation of the glandular morphology. An outer muscular layer surrounds the spermathecal duct and the very beginning of the glandular diverticula to form the sperm pump, but is absent in the glandular portion. The muscular sheath around the spermathecal duct consists of strong visceral muscle cells rich in glycogen (Figs. 6 and 7) and with tracheal and nerve branches (Fig. 7). A thick basal lamina separates the epithelium from the muscle cells and outlines each fiber and all of the gland (Figs. 6 and 7).

The gland has a complex structure. The epithelial cells that form the intima beneath the cuticle has many infoldings of the apical plasma membrane interspersed by mitochondria and electrondense membranous structures (Figs. 8 and 9). Beyond the intima lay the glandular secretory cells which are connected to the lumen by individual efferent ductules that pierce the intima cuticle to deliver the secretion (Figs. 8 and 9). The efferent ductule cuticle is continuous with the intima cuticle that lines the lumen of the gland. The basal region of this cuticle, in contact with the ductule cell, contains electron-transparent vesicles (Fig. 9) and infoldings of the plasma membrane (Fig. 10). In mated queens, the lumen of the efferent ductule gland contained fibrillar material (Figs. 8 and 9).

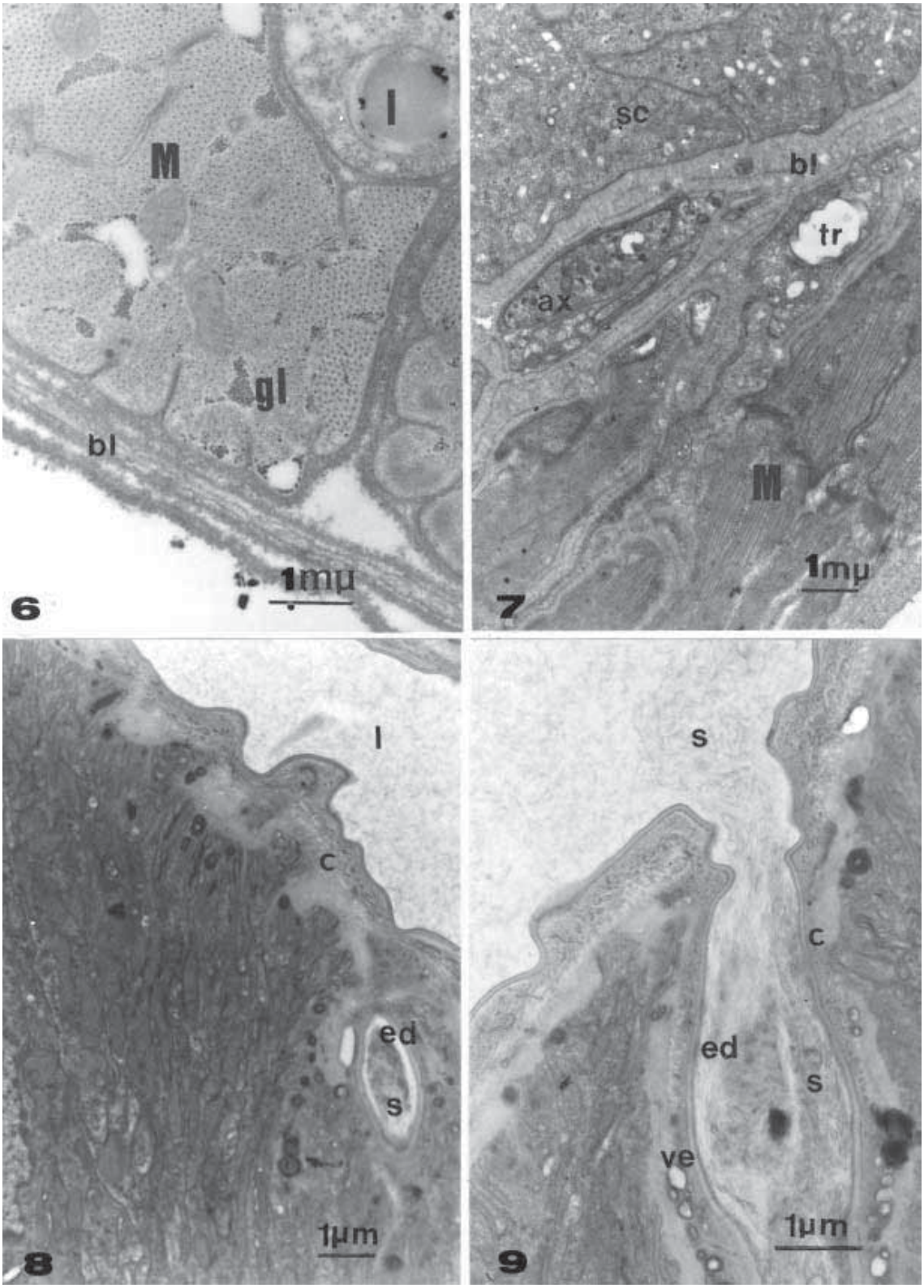
The secretory cells have an intracytoplasmic ductule (end apparatus) that initially collectes the secretion (Fig. 11). This ductule has a thin, discontinuous lining of cuticle and is surrounded by numerous, deep infoldings of the gland cell plasma membrane. The space of the infolding sometimes contained electron-dense material (Figs. 11 and 12) that occasionally showed a paracrystalline array (Fig. 13) or myelin-like figures (Figs. 12 and 13). The lumen of the intracellular canalicule is filled with filamentous, electron-dense material similar to that of the efferent ductule and gland lumen.

The gland cells are rich in mitochondria, have a well developed Golgi complex (Fig. 14), smooth endoplasmic reticulum, and free ribosomes, some of which are organized as polysomes (Figs. 14 and 17). The smooth endoplasmic reticulum has a localized distribution in the cytoplasm and, in virgin queens, displays a concentric array around glycogen deposits (Fig. 17). Glycogen deposits were not observed in mated queens.

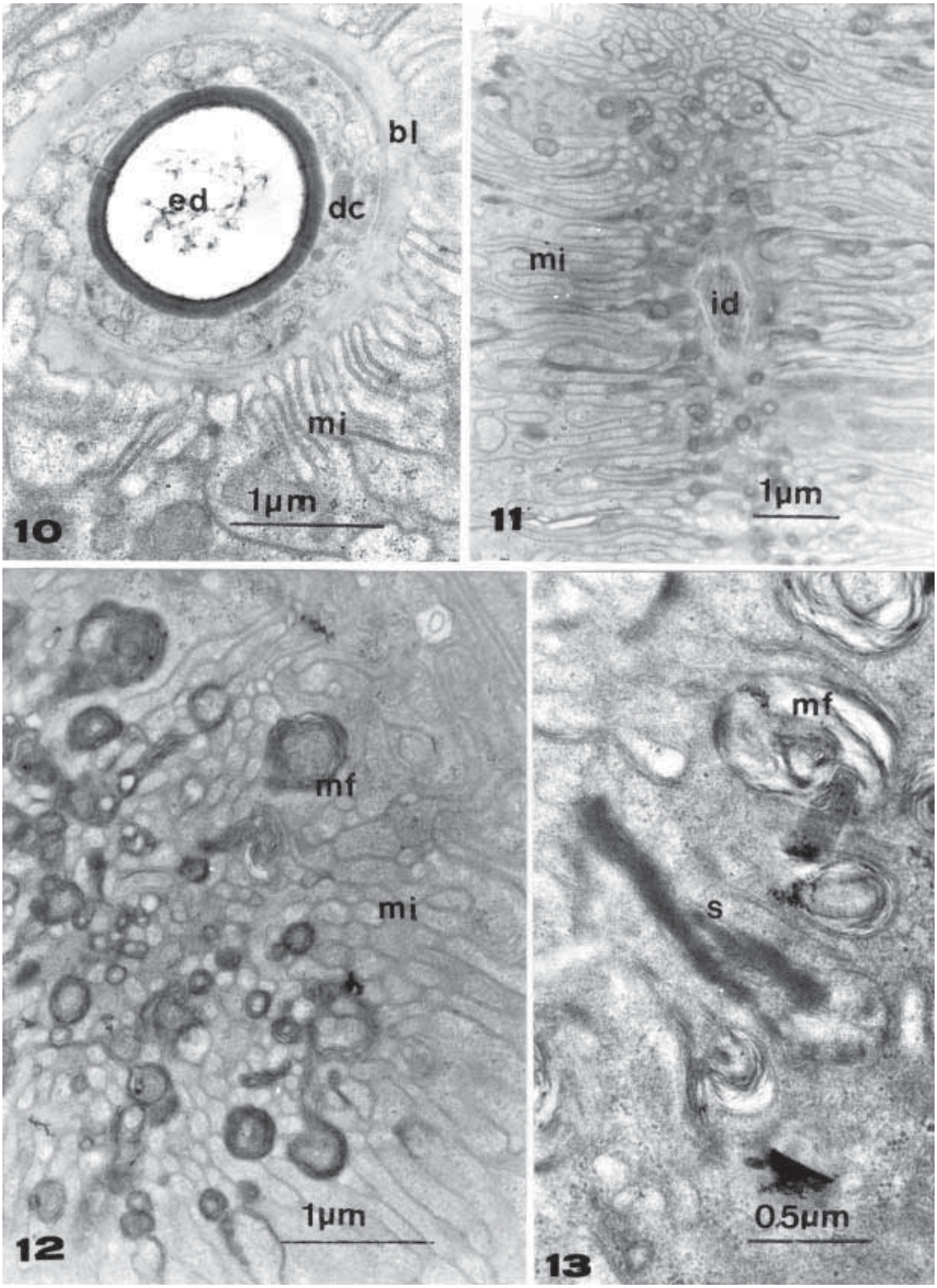
The basal side of the secretory cells shows many deep infoldings of the plasma membrane (Figs. 15 and 16), the inner surface of which appeared electron-dense in preparations treated with ruthenium red.

Only a basal lamina separates this epithelium from the hemolymph and other subjacent structures such as tracheoli and nerve branches (Fig. 7).

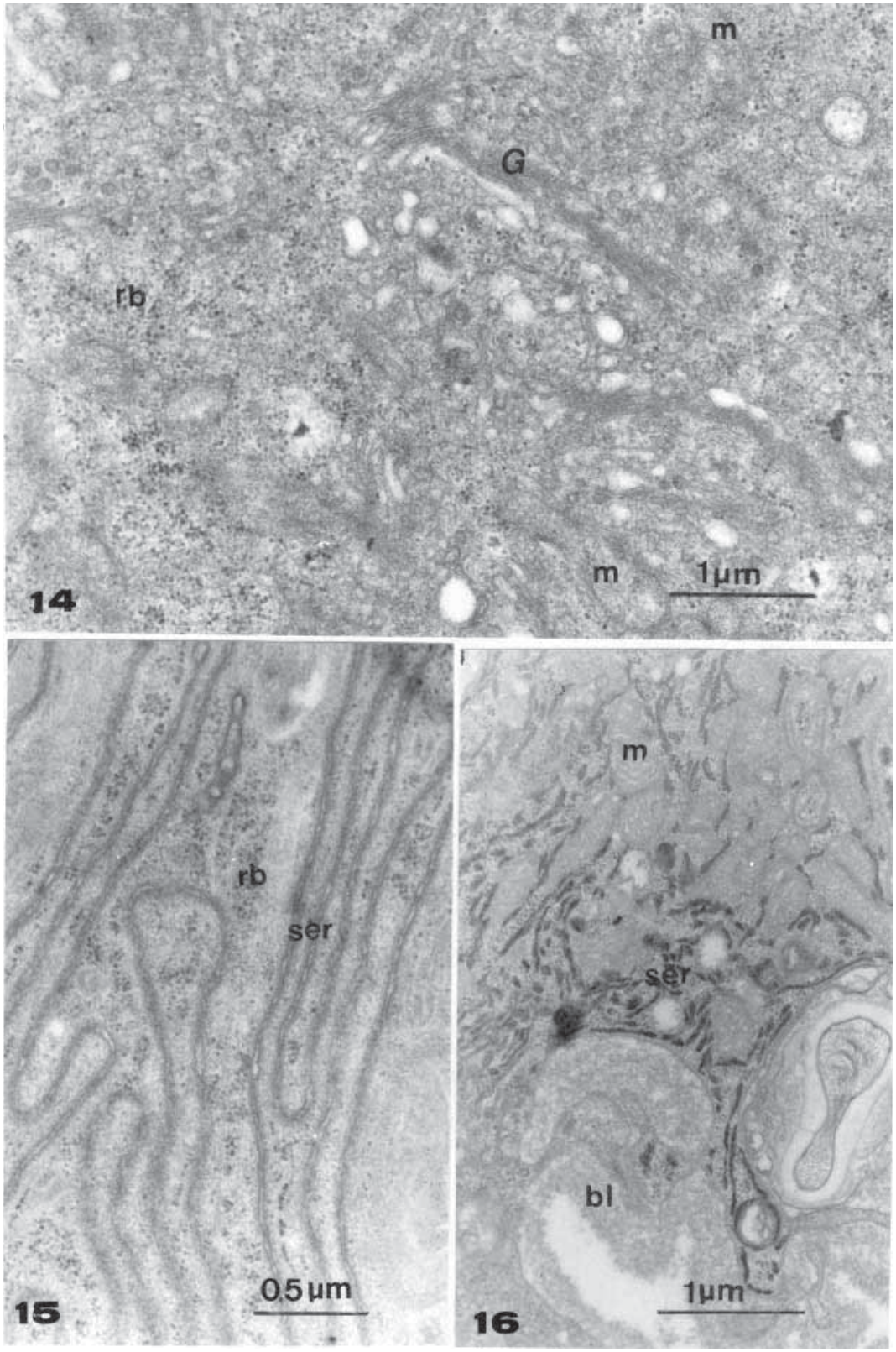
The glands of virgin and mated queens are very alike, except for clusters of glycogen on the basal side of the gland cells in virgin queens (Fig. 17), a large amount of material in the intracellular and efferent ductules (Figs. 8-11) and electron-dense material in the infoldings around the intracellular ductule in mated queens.



Figures 6-9. Transmission electron micrographs of spermathecae. **6.** Cross-section of a muscle fiber (M) showing glycogen (gl) and lipid (l) deposits. **7.** Muscle layer of the spermathecal duct showing tracheoli (tr) and nerves (ax). **8.** The gland duct intima cells showing infoldings of the epithelial plasma membrane. **9.** The end of an efferent duct (ed) in the gland lumen showing intracuticular vesicles (ve). s = secretion; l = lumen; bl = basal lamina; c = cuticle; se = spermathecal epithelium.



Figures 10-13. Transmission electron micrographs of spermathecae. **10.** Cross-section of an efferent ductule (ed) showing the infoldings of the plasma membrane (mi) and the ductule cells (dc). **11.** Intracytoplasmic ductule (id) with secretion and the surrounding infoldings (mi) of the gland cell plasma membrane **12.** Accumulation of dense material within the infoldings, sometimes with a concentric membranes arrangement (mf) **13.** Paracrystalline array (s) and myelin figures (mf) in the secretory space. bl = basal lamina.



Figures 14-16. Transmission electron micrographs of spermathecae. **14.** General aspect of a gland cell cytoplasm showing the Golgi complex (G), mitochondria (m) and ribosomes (rb). **15.** Basal smooth endoplasmic reticulum (ser) forming a labyrinth. **16.** The basal labyrinth filled with material stained by ruthenium red (ser). bl = basal lamina; m = mitochondria.

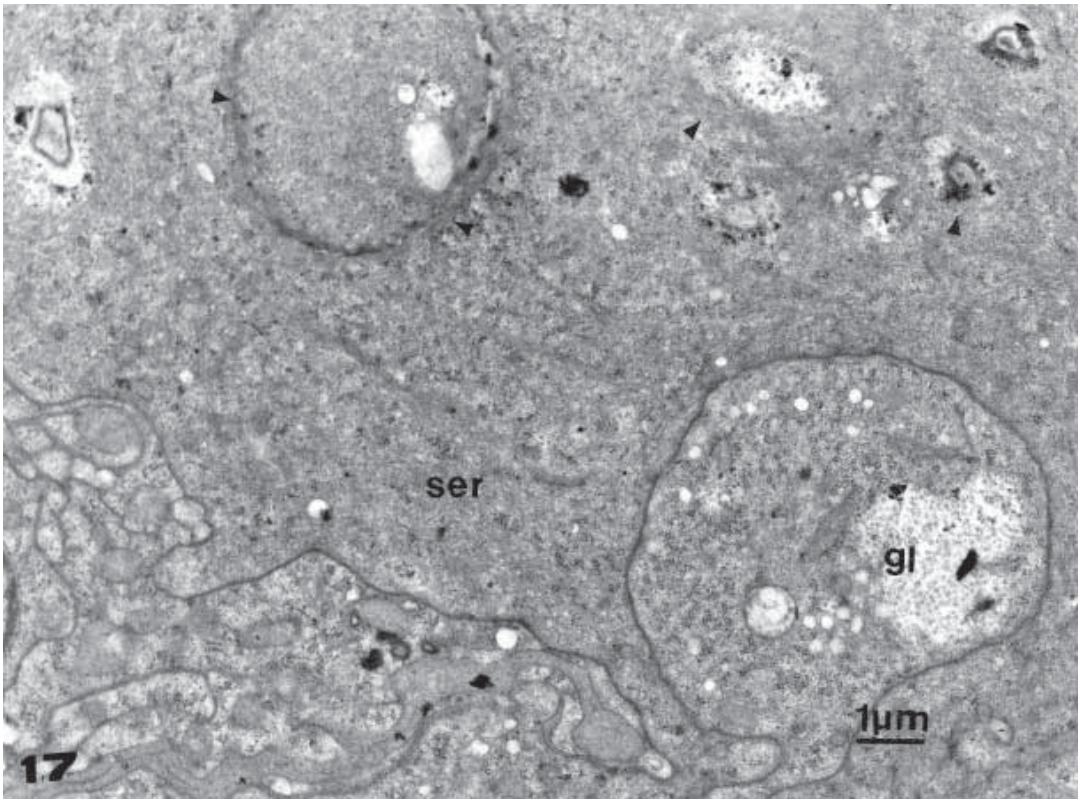


Figure 17. Transmission electron micrograph of the gland cell of a virgin queen showing glycogen deposits (gl) surrounded by smooth endoplasmic reticulum (arrowheads). ser = smooth endoplasmic reticulum.

DISCUSSION

Although the spermathecal gland in *M. bicolor* is very small compared to that of *A. mellifera,* it shows the same organization and cellular ultrastructure described by Dallai [4] for *A. mellifera*. Indeed, this structure appears to be common to most of the insect spermathecal glands [6,8,9,13]. The three types of cells described by Happ and Happ [9] are present in the gland of *M. bicolor*: glandular cells with an intracytoplasmic ductule or end apparatus, cells of the efferent ductule, and epithelial cells that form the intima lining the gland lumen and terminal duct of the gland. Noirot and Quennedey [16] classified the epidermal gland cells of insects with a similar morphology as class III glandular cells. In this case, the intima cells correspond to the tegument epidermis. The glandular cells do not have the ultrastructural characteristics necessary for producing secretion, despite their well developed Golgi apparatus. However, the presence of a well-developed smooth endoplasmic reticulum, and of material in the infoldings around the intracytoplasmic ductule and in the ductule itself are suggestive of secretory activity in these cells.

Dallai [4] reported that the gland cells in *A. mellifera* are involved in the secretion of polysaccharide-containing proteins. A role in polysaccharide synthesis or metabolism was suggested here by ruthenium red stained material inside the basal plasma membrane infoldings and by the paracrystalline structures sometimes seen in the infoldings around the intracytoplasmatic ductule.

The morphological features of the gland cell do not support the presence of protein secretory mechanisms. Indeed, the myelin figures observed in the ductule region could be a special type of lipid or hydrocarbonic secretion. Hefetz and Orion [10] and Quennedey [17] described a hydrocarbonic secretion that assumed this morphology in gland cells of ants and termites. The lipid nature of the secretion of *M. bicolor* spermathecal gland, would be compatible with the well-developed smooth endoplasmic reticulum. The fibrillar or lamellar morphology of the secretion in the ductules and gland lumen, also appears to be compatible with a lipid composition.

Various functions have been attributed to the spermatheca, including the nourishment of

spermatozoa. The presence of secretory cells in the spermathecal wall or its associated glands supports this hypothesis, whereas the type of secretion apparently does not. In addition to the small size of the spermathecal gland in this species, the spermathecal wall is not secretory, so if any nourishment is to be furnished to the sperm stored in the spermatheca, it must be provided by the spermathecal gland or by direct exchange between the spermatheca and the hemolymph through the spermatecal epithelium [14]. The impregnation of spermathecal gland cells basal infoldings by the ruthenium red, may be evidence of this intake route.

The presence of glycogen, as seen in the gland cells of virgin queens, is common in tissues of young bees and may provide a reserve of energy for the maturation and beginning of cell activity. The special arrangement of the smooth endoplasmic reticulum around these deposits indicates a role in glycogen mobilization since glycogenolytic enzymes are located in this type of reticulum.

The numerous infoldings of the plasma membrane below the intima cuticle and efferent duct of the gland, increase the cell surface area available for exchange and may indicate a role in the reabsorption of luminal contents.

A. mellifera queens practice multiple mating and can store large amounts of spermatozoa in the spermatheca; they can also lay approximately 1,500 eggs/day [19]. In contrast, multiple mating is rare in meliponines. In stingless bees, the average effective numbers of mates is 1.06 [20], and in *Melipona,* the clutch numbers range from 10 to 30 eggs/day [12]. Thus, the amount of spermatozoa stored in the spermatheca may be much lower in *Melipona,* which could explain the difference in size between the spermathecal glands of these two species.

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