

Ultrastructure of the ovariole sheath in *Diatraea saccharalis* (Lepidoptera: Pyralidae)

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ABSTRACT: The ultrastructure of the ovariole sheath along the *Diatraea saccharalis* ovariole was studied by scanning and transmission electron microscopy. Each ovariole is surrounded by an epithelial sheath, a tunica propria and scattered lumen cells. These three components of the ovariole sheath show different ultrastructural features along the ovariole, in the germarium or in the vitellarium; these differences are more evident in the epithelial sheath cells. The epithelial sheath is composed by two layers of cells, the external one running longitudinally and the internal one running circularly in the ovariole. These cells, in vitellarium, present cytoplasmic bundles of myofilaments that are arranged parallel to the long axis of the cells; these myofilaments are apparently related to the contraction movements of the follicles within the ovariole. The acellular tunica propria, composed of finely filamentous material, is attached to the adjacent follicle cells by adhesive dense plates. Between the epithelial sheath and the tunica propria there is a population of lumen cells, with morphological features of secretory activity.

Introduction

The great majority of insects has each of their two ovaries encompassed by an ovarian capsule, in the beginning of the female reproductive system development (Machida, 1926; Miya *et al.*, 1970b; Büning, 1994).

During the growth of the ovaries, this ovarian capsule tears at its attachment points to the oviduct and the ovarioles emerge gradually by elongation into the abdominal cavity of the insect (Machida, 1926; Snodgrass,

1935; Koch *et al.*, 1967; King and Aggarwal, 1965; Miya *et al.*, 1970a, b; Büning, 1994).

Each ovariole is enclosed separately by an ovariole sheath, which is composed by: the cellular epithelial sheath on the outside, the amorphous tunica propria on the inside and a layer of isolated lumen cells between the epithelial sheath and tunica propria. (Bonhag and Arnold, 1961; King and Koch, 1963; King and Aggarwal, 1965; Koch and King, 1966; Miya *et al.*, 1970a, b; Cruickshank, 1973; Gregório *et al.*, 1990; Büning, 1994). In this paper, we describe the ultrastructural organization of the ovariole sheath along the germarium and vitellarium of *Diatraea saccharalis* ovarioles. There are no ultrastructural studies on ovarian sheath organization in *Diatraea saccharalis*, although this insect has long been considered the most destructive pest attacking sugarcane in several Latin American countries.

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Material and Methods

The larvae of *Diatraea saccharalis* were reared on an artificial diet (Hensley and Hammond, 1968), and the pupae were maintained in recipient, without the artificial diet, until the emergence as adults. Both larvae and pupae were maintained under controlled temperature (25–27°C) and humidity (70%).

Ovaries removed from insects of the last larval instar and from pupae (5–7 days) were fixed for 24h in 2% glutaraldehyde - 4% paraformaldehyde solution buffered in 0.1M buffer phosphate (pH 7.3).

For the transmission electron microscopy (TEM) observations, the ovaries were post-fixed in 1% osmium tetroxide for 2h, dehydrated through a graded series of acetone and embedded in Araldite. Ultra-thin sections were double stained with uranyl acetate and lead citrate.

For the scanning electron microscopy (SEM) observations, the pre-fixed ovarioles were immersed in 10% NaOH solution for 17h, post-fixed in 1% osmium tetroxide for 2h, dehydrated through a graded series of acetone, critical point dried with liquid CO₂ and sputtered gold-coated (10nm thick).

Results

Each ovariole of *Diatraea saccharalis* is surrounded by a continuous sheath composed of the epithelial sheath, the lumen cells and the tunica propria. These three components of the ovariole sheath show different ultrastructural features along the ovariole.

In the germarium, the epithelial sheath cells are flat and could be visualized as juxtaposed “disks”, not easily distinguishable from the cells of the adjacent connective tissue (Figs. 1, 3, 4). Their cytoplasm present elongated mitochondria, few rough endoplasmic reticu-

lum, glycogen deposits and some dense vesicles (Fig. 3). It is not possible to distinguish a basal membrane separating the epithelial sheath cells from the adjacent connective tissue (Fig. 4). The tunica propria is very thin and is composed of a homogeneous layer of finely filamentous material (Figs. 1–5). There is a discreet but progressive increase in the thickness of the tunica propria along the germarium. The fluid-filled lumen which extends between the tunica propria and epithelial sheath contains small populations of ameoboid cells, the lumen cells. These cells possess dense mitochondria, few cisterns of rough endoplasmic reticulum, heterogeneous vacuoles and vesicles, Golgi complexes, in addition to free ribosomes (Figs. 1–3). Their nuclei are ovoid in shape, exhibiting uncondensed chromatin and evident nucleoli. There are no adhesive junctions between lumen cells, except interdigitations (Figs. 1 and 5); finely flocculated material is observed in the intercellular spaces, when the interdigitations are enlarged (Figs. 1 and 5).

In the vitellarium, the cells of the epithelial sheath are disposed in two layers (external and internal cellular layer) (Figs. 6, 7, 8). SEM of the NaOH digestion preparation shows that in the external layer the cells are arranged parallel to the long axis of the ovariole and in the internal layer they run circularly around the ovariole (Figs. 11–13). TEM shows that these cells in both layers present large amount of cytoplasmic myofilaments parallel to the longest axis of the cells; there is an increase in the amount of myofilaments along the vitellarium (Figs. 6 and 7). The epithelial sheath is surrounded by both inner and outer continuous basal membranes (Figs. 6 and 7). There are interdigitations and desmosomes attaching the epithelial sheath cells of the same layer as well as between the cells of the two adjacent layers (Figs. 6, 7, 9). Besides myofilaments, the cytoplasm shows mitochondria, rough endoplasmic reticulum, small vesicles with flocculent material and free ribosomes (Fig. 7). The tunica propria is thicker

Figs. 1–5 Transmission electron micrograph of the ovariole sheath adjacent to the germarium.

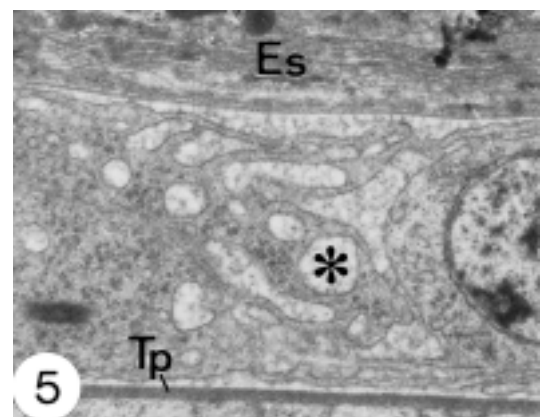
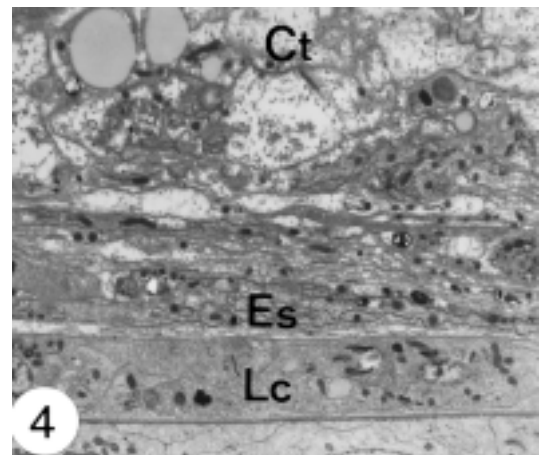
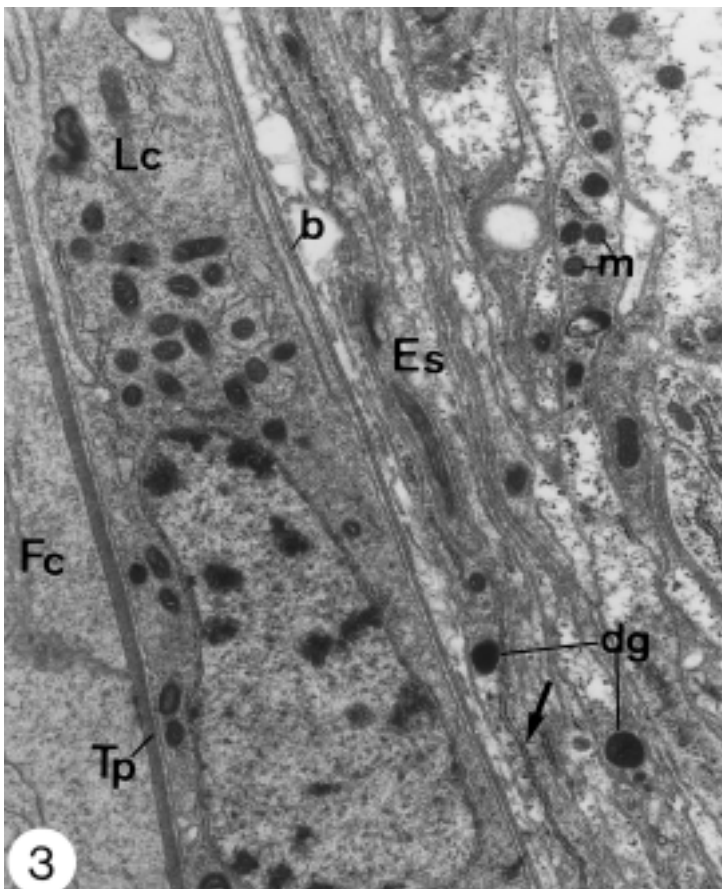
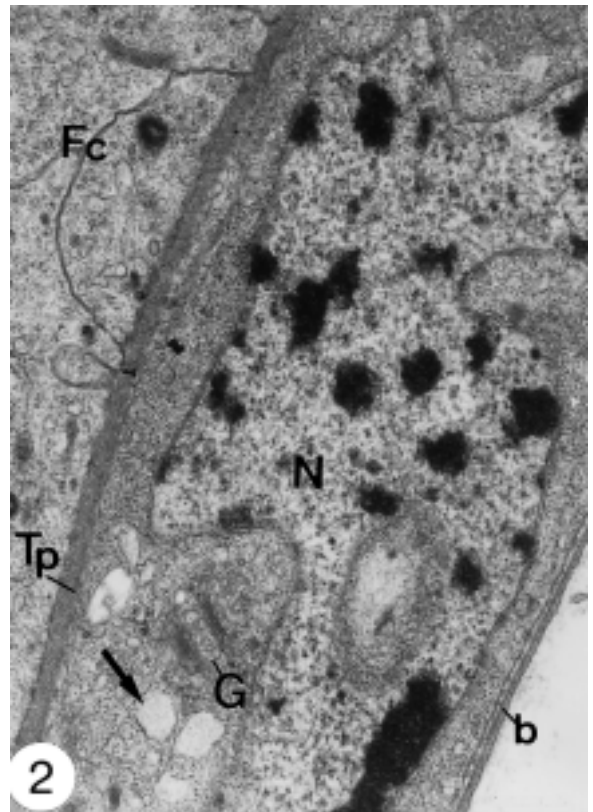
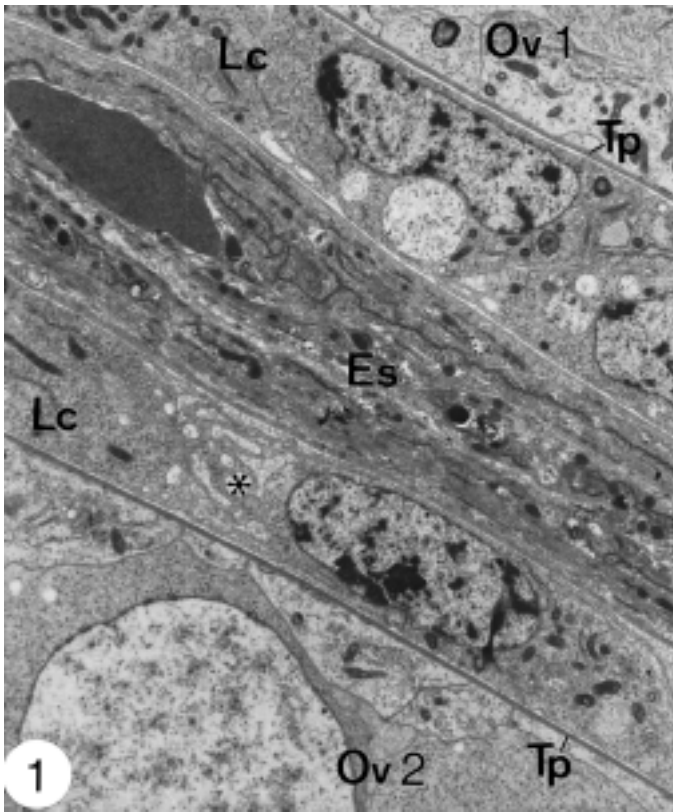
FIGURE 1. Epithelial sheath (Es) between adjacent ovarioles (Ov1 and Ov2); lumen cells (Lc); thin tunica propria (Tp). Finely flocculated material (*) among the lumen cells. (X 8.000).

FIGURE 2. Lumen cell with irregular nucleus (N), cytoplasm with Golgi complex (G) and some vesicles (arrow). Basal membrane (b); tunica propria (Tp) and follicle cell (Fc). (X 21.000).

FIGURE 3. Epithelial sheath (Es) with few rough endoplasmic reticulum cisternae (arrow) and some dense granules (dg). Lumen cells (Lc); basal membrane (b); tunica propria (Tp); dense mitochondria (m); follicle cell (Fc). (X 16.500).

FIGURE 4. Connective tissue (Ct) not easily distinguishable from the epithelial sheath (Es); lumen cells (Lc). (X 6.000).

FIGURE 5. Lumen cells with finely flocculated material (*) in the intercellular spaces. Epithelial sheath (Es); tunica propria (Tp). (X 17.600).



than that in the germarium and exhibits bundles of tubular structures in addition to the filamentous material (Fig. 10). There are electron-dense adhesive plates attaching the tunica propria to the adjacent follicle cells, with adjacent microtubules (Fig. 10). The lumen cells show the same ultrastructural morphology as those described in the germarium (Fig. 8).

Discussion

The ovarioles in *Diatraea saccharalis* are individually covered by an epithelial sheath, a population of lumen cells and a tunica propria. These three elements of the ovariole coating were also observed in many other insects (Bonhag, 1958; King and Aggarwal, 1965; Koch and King, 1966; Miya *et al.*, 1970b; Cruickshank, 1973), although the morphologic variations along the ovariole coating were not described in a systematized way.

In the germarium of the *Diatraea saccharalis* ovariole, the epithelial sheath cells are not distinguishable from those of the adjacent connective tissue; we did not detect basal membrane separating the two tissues, as observed by Miya *et al.* (1970b) in *Bombyx mori*. The epithelial sheath cells present a flat shape, with oval nuclei and reduced cytoplasm, with small elongated mitochondria, few cisterns of rough endoplasmic reticulum, small glycogen deposits and some dense vesicles; these ultrastructural features composing the cytoplasm are very similar to those found in *Bombyx mori* (Miya *et al.*, 1970b).

In the vitellarium of the *Diatraea saccharalis* ovariole, the long epithelial sheath cells are disposed in two layers: an external cellular layer and an internal cellular layer, both attached by desmosomes. Adjacent to previtellogenic follicles, these epithelial sheath cells show characteristics of muscle cells, exhibiting bunches of

myofilaments, distributed in the following way: longitudinally (external cellular layer) and transversally (internal cellular layer) to the long axis of the ovariole. There is an increase in the amount of myofilaments along the vitellarium. These characteristics of the epithelial sheath cells observed along the vitellarium of *Diatraea saccharalis* seem to be a common feature in the great majority of the insects (Machida, 1926; Bonhag, 1958; King and Koch, 1963; King and Aggarwal, 1965; Koch and King, 1966; Koch *et al.*, 1967; Miya *et al.*, 1970a; Mahowald, 1972; Cruickshank, 1973; Mandelbaum, 1980; Cook and Peterson, 1989; Carcupino *et al.*, 1992; Büning, 1994). The authors relate the presence of these myofilaments to the capacity of the contraction of the ovarioles for the deposition of eggs. Pulsation and synchronized contractions of muscle fibers of the epithelial sheath were recorded in complete cycles of oscillations ranging from 200ms to 4s (Büning, 1994).

The lumen cells observed between epithelial sheath and tunica propria do not show morphologic variations along the germarium or the vitellarium of the *Diatraea saccharalis* ovarioles. Lumen cells are cuboid in shape, with various interdigitations; the nucleus is oval and its cytoplasm exhibits mitochondria, rough endoplasmic reticulum, some vacuoles and small vesicles, small glycogen deposits and Golgi complexes. In the extracellular spaces between adjacent lumen cells, we observed a considerable amount of finely flocculated material. The lumen cells described above are similar to those found in other insects. The same holds true for the flocculent material found here and there between lumen cells of *Hyalophora cecropia* (King and Aggarwal, 1965), *Drosophila melanogaster* (Koch and King, 1966), *Bombyx mori* (Miya *et al.*, 1970a) and *Anagasta kuhniella* (Cruickshank, 1973). Some of these authors described these cells as blood cells (macrophages) (Koch

Figs. 6–10. Transmission electron micrograph of the ovariole sheath adjacent to the vitellarium.

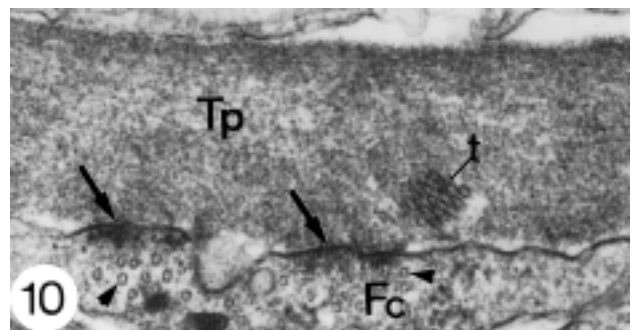
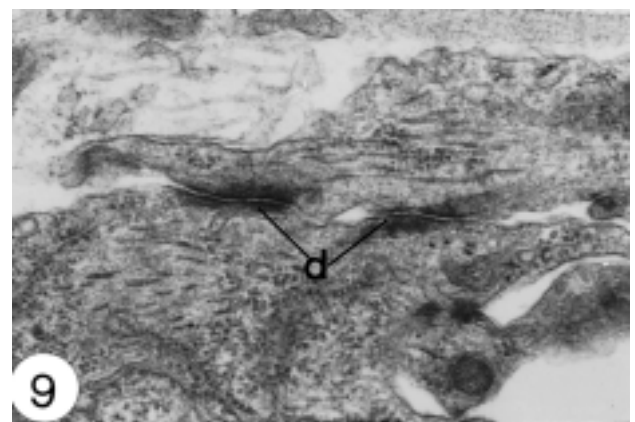
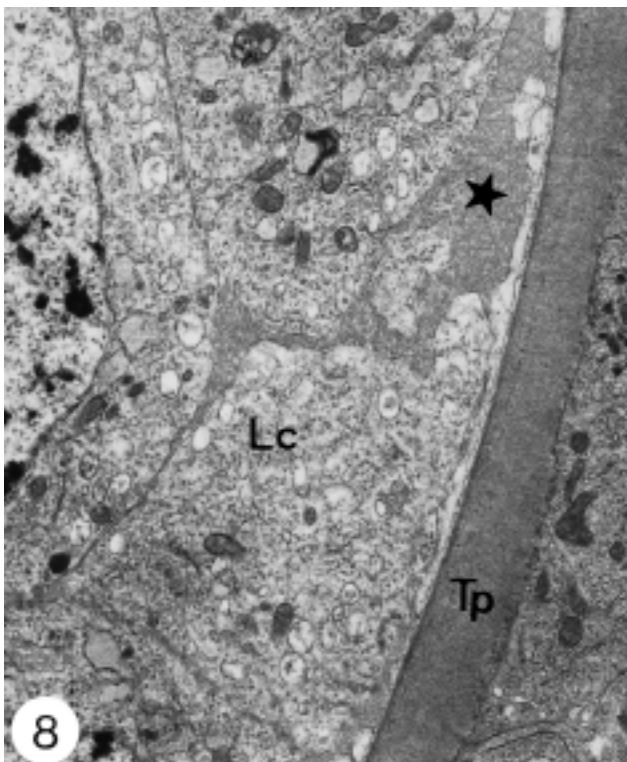
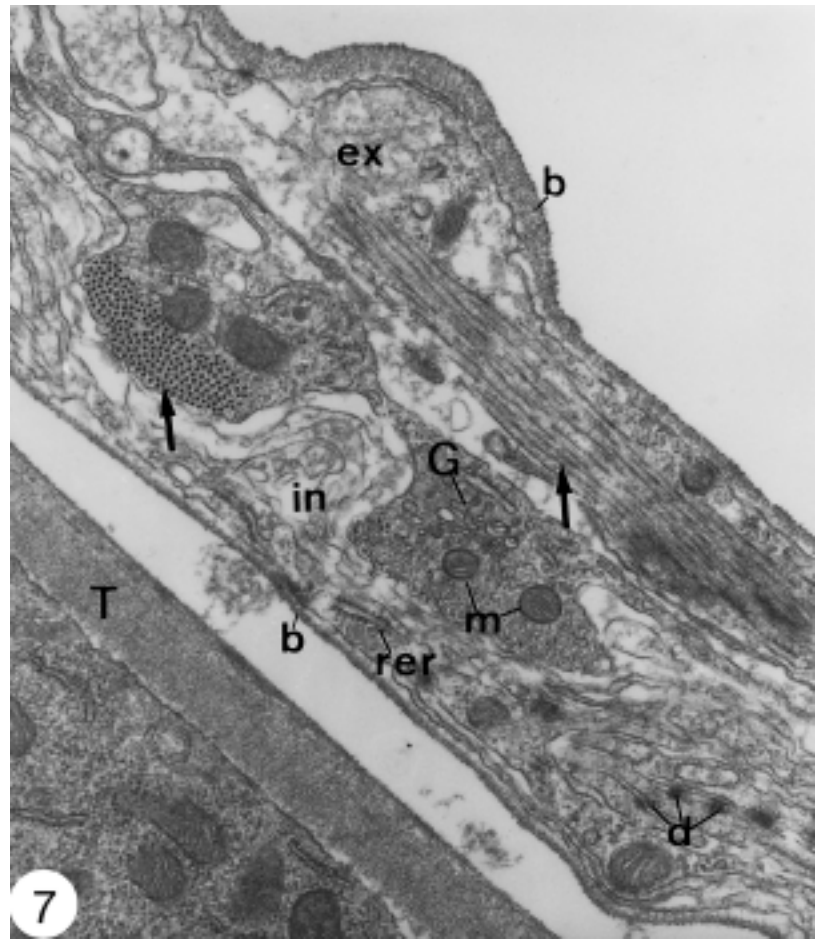
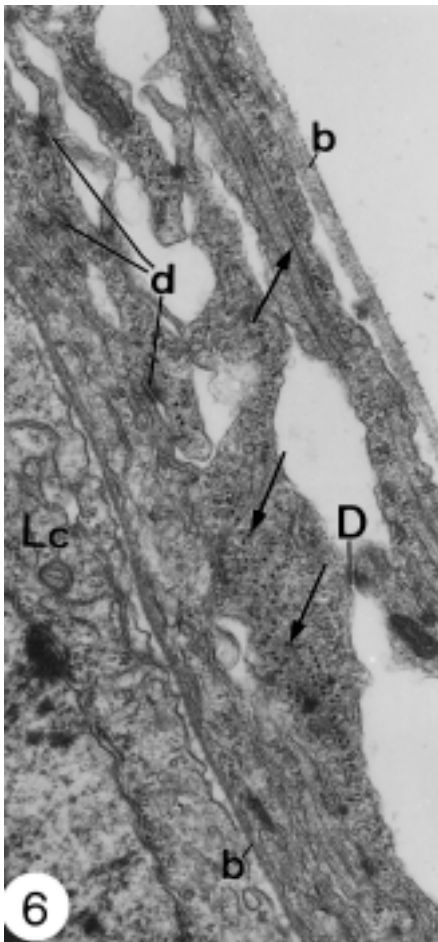
FIGURE 6. Ovariole sheath near previtellogenic follicle: epithelial sheath cells with desmosomes between adjacent cell (d) and the two layers (D); few cytoplasmic myofilaments (arrows); lumen cell (Lc); basal membranes (b). (X 27.500).

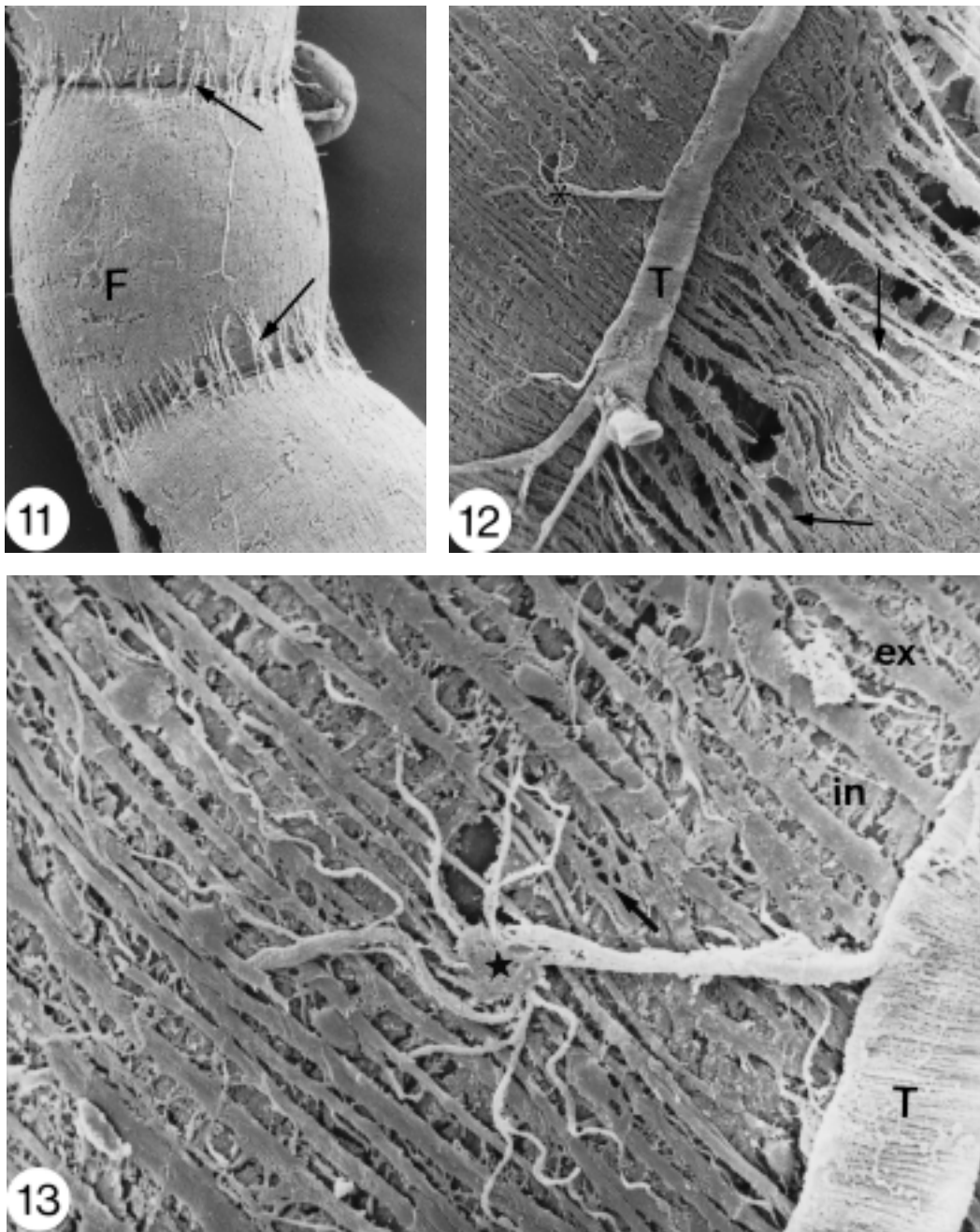
FIGURE 7. Ovariole sheath near vitellogenic follicle: epithelial sheath cells with large amount of cytoplasmic myofilaments (arrows) in the external (ex) and internal (in) layers. Mitochondria (m); rough endoplasmic reticulum (rer); Golgi complex (G); desmosomes (d); basal membranes (b); tunica propria (Tp). (X 13.000).

FIGURE 8. Flocculated material (★) among lumen cells (Lc). Tunica propria (Tp). (X 31.500).

FIGURE 9. Detail of desmosomes (d) between epithelial sheath cells. (X 36.000).

FIGURE 10. Detail of the filamentous tunica propria with bundle of tubular structures (t). Adhesive plates (arrows) attaching the tunica propria (Tp) to the adjacent follicle cells (Fc); microtubules (arrow heads). (X 57.500).





Figs. 11–13. Scanning electron micrograph of NaOH digested ovariole sheath in the vitellarium.

FIGURE 11. General view of three ovarian follicles (F). Cells of the epithelial sheath external layer (arrows) connecting adjacent follicles. (X 90).

FIGURE 12. Cleft between adjacent follicles with the epithelial sheath cells (arrows) of the external layer; tracheole (T) with branches (*). (X 330).

FIGURE 13. Detail of the epithelial sheath surface. The cells of the circular internal layer (in) are partially hidden by the ones of the longitudinal external layer (ex). The cells of external layer are intensively anastomosed (arrows). Traqueole (T) with branch (★) going through the epithelial sheath cells. (X 1.200).

and King, 1966; Cruickshank, 1973), capable of secreting material to repair the tunica propria at its torn points due to ovariole growth and to the moment of eggs deposition; Cruickshank (1973) confirms this function for the lumen cells, suggesting that there seems to be a correlation between its secretory activity and the increase in thickness of the tunica propria during development. Although we did not observe significant modifications in the ultrastructural organization of the lumen cells, their morphological features indicate its contribution to the tunica propria thickness, that occurs along the *Diatraea saccharalis* ovariole.

In the germarium, the tunica propria is extremely thin, composed of finely filamentous material. There is an increase in the tunica propria thickness from the germarium to the vitellarium, where some dense structures were visualized among its filamentous material. The ultrastructural aspect of the tunica propria is very similar in many insects (Machida, 1926; Bonhag, 1958; King and Koch, 1963; King and Aggarwal, 1965; Koch and King, 1966; Koch *et al.*, 1967; Miya *et al.*, 1970a; Mahowald, 1972; Cruickshank, 1973; Gregório *et al.*, 1990; Büning, 1994). According to these authors, the nature of the tunica propria material is still uncertain. It is known that this acellular layer presents proteins and carbohydrate complexes (Bonhag and Arnold, 1961) and that these proteins are rich in S-S groups (Cruickshank,

1973). Büning (1994) suggests that collagen type I, laminine and proteoglycans compose the tunica propria of insects. This author describes that, adjacent to the vitellogenic follicles, the tunica propria exhibits structures in stick shape, oriented parallel to the long axis of the ovariole, as we saw in *Diatraea saccharalis* at the end of previtellogenesis. However, Büning (1994) does not suggest a function for these structures. The basal membranes (tunica propria) of the insect ovarioles present three functions, as proposed by Büning (1994): (1) they serve as a mechanical structure to keep the elongated structure of the ovarioles stable; (2) they serve as physical sieves against the haemolymph; (3) they have some chemical selectivity for macromolecules which play a role in hormone access during previtellogenesis. The presence of adhesive plates attaching the tunica propria to the follicle cells, and their relation to the cytoplasmic microtubules, suggest that the tunica propria becomes stretched during the oogenesis.

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