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MORPHOLOGY AND ULTRASTRUCTURE OF THE MALE ACCESSORY GLANDS OF *Achroia grisella* (FABRICIUS) (LEPIDOPTERA, PYRALIDAE)

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ABSTRACT

The adult male reproductive system of *Achroia grisella* consists of paired testes in a common oval sheath, paired deferent ducts, accessory glands, seminal vesicles and a single ejaculatory duct. In this work, we used light and transmission electron microscopy to study the morphology of the male reproductive accessory glands of *A. grisellla*. The accessory gland consisted of glandular cells with a well-developed rough endoplasmic reticulum and Golgi apparatus, although no secretion was seen in the cells. Histochemical analysis showed that the accessory gland secretion consisted of glycoproteins. The variable morphological appearance of the secretion (globular, amorphous and fibrillar) present in the gland lumen and its staining properties, which changed along the gland tract, suggested that the secretion underwent some form of processing that was possibly associated with its maturation before release from the gland. We suggest that the accessory gland secretion may have a role in the maintenance of spermatozoa and/or in plug formation, as already reported for other lepidopteran species.

Key words: Histochemistry, histology, reproduction, secretion, ultrastructure

INTRODUCTION

The moth *Achroia grisella* (Pyralidae) is a pest of *Apis mellifera* bee wax, but also occurs in meliponine colonies [5]. Since this species is a very common pest of several *A. mellifera* subspecies, its biological cycle is very well-known. In general, the egg stage lasts from 2 to 4 days, and the larval phase consists of 10 stages that last 34 to 48 days.

The male reproductive system of *A. grisella* consists of an oval testicular mass (formed by fusion of the two testicles), paired deferent ducts, seminal vesicles, accessory glands and an ejaculatory duct. Male accessory glands are common in insects and vary considerably in form [9,12,15]. Several functions have been attributed to the secretion produced by these glands, including spermatophore formation, the supply of nutrients to spermatozoa while stored in seminal vesicles or in the female genital tract, and the activation of spermatozoa [6,13-15]. The accessory gland secretion also contains substances that can change the female reproductive behavior and female physiology after copulation [7,17,18,24,25].

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The primary function of the secretion produced by the male accessory gland is in spermatophore formation in those species that produce spermatophores [10,12]. The functions of the accessory glands can be classified as structural, biochemical, behavioral and physiological. Physiologically, the secretion can facilitate the transfer of spermatozoa to the female spermatheca or, in cases of multiple mating, can inactivate the spermatozoa of other males that subsequently mate with the female [16]. In *Drosophila,* the secretory product of the accessory glands is transferred to the female tract during mating where it forms a plug and modifies the behavior and physiology of the female [7,17,18,25], including the rejection of other males [6]. In some cases, the secretion is transferred during the early moments of mating, prior to transfer of the spermatozoa, although in most cases the order of these events is reversed [23].

In *A. mellifera,* the accessory glands are known as mucus glands because of the consistency of their secretion. The composition of the secretion, which changes during male sexual maturation, is probably controlled by ecdysteroidal hormones [8], and attains a mucous consistency at the time of copulation. The mucus produced is released during copulation after

transfer of the spermatozoa and hardens as soon as it comes into contact with air [24]. The main role of this mucus is to prevent the reflux of sperm [24], although *Bombus* mucus contains a non-specific fatty acid that prevents females that have already mated from remating [2].

In most insects, the accessory gland can vary in form from a simple tube, identical to other conductive channels of the reproductive tract, to histologically complex tubes with regional differentiation, as occurs in most lepidopterans [20,22], or several pairs of morphologically distinct ducts, as occurs in Orthoptera [9]. Ultrastructurally, the accessory gland consists of epithelial cells with a morphology typical of glandular cells that secrete proteins. There is abundant rough endoplasmic reticulum, a well-developed Golgi apparatus, a large nucleus with several nucleoli and a luminal surface covered with microvilli [9]. In addition to proteins, the secretion may contain lipids, glycogen, aminoacids, peptides, and prostaglandins [6,10].

In this study, the morphology of the accessory glands of the lesser wax moth *Achroia grisella* was examined using light microscopy and transmission electron microscopy (TEM) as part of an investigation into the characteristics of the reproductive tract in this species.

MATERIAL AND METHODS

Adult males of *A. grisella* reared on an artificial diet in the Animal House of the Instituto de Biociências (UNESP, Rio Claro, SP) were used for the histological and ultrastructural analyses. Five mature, non-mated males were used for light microscopy and five for TEM. Mated adult females were placed in plastic containers along with a small piece of honeybee comb wax on which they laid their eggs. After hatching, the larvae were transferred to another container with an artificial diet consisting of soya flour, beer yeast, honey, glycerin, powdered milk and water, where they developed until emergence as adults.

Light microscopy

Male moths were anesthetized by cooling and, with the aid of a stereomicroscope, the abdomen was opened by a mid-ventral incision and a small volume of insect saline solution (0.75% NaCl, in phosphate buffer, pH 6.5) was pipetted into the abdominal cavity to facilitate dissection of the reproductive apparatus and separation of the accessory glands.

The accessory glands were fixed in 4% paraformaldehyde in sodium phosphate buffer, pH 7.4, for about 2 h and then washed in the same buffer for 20 min prior to dehydration in increasing concentrations of ethanol. After dehydration, the glands were infiltrated and subsequently embedded in historesin. Sections 6 µm thick were cut with a glass knife and transferred to histological slides, dried and stained with hematoxylin and eosin (HE). Histochemical tests were done by staining the glands with bromophenol blue for proteins, alcian blue for acid polysaccharides, and periodic acid-Schiff together with Alcian blue (PAS-AB) for carbohydrates such as glycogen and glyco-conjugates (glycoproteins and glycolipids). To facilitate comparison, alternate slides of serial sections from all specimens were stained with one of each of the procedures described above.

Transmission electron microscopy

The glands were fixed in Karnovsky solution (2% glutaraldehyde plus 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) for 2 h and post-fixed in 0.5% osmium tetroxide containing 0.8% potassium ferrocyanide in the same buffer. Dehydration was done using an increasing acetone series after which the samples were embedded in Epon-Araldite resin that was polymerized at 60°C for 24 h. The sections were stained with uranyl acetate for 45 min and lead citrate for 10 min prior to examination in a Philips transmission electron microscope operated at 80 kV.

RESULTS

The accessory glands of *A. grisella* consisted of long, narrow tubules that terminated distally in a cul-de-sac and were connected proximally to the seminal vesicles.

Histology and histochemistry

The glandular tubules consist of a simple, columnar epithelium and an underlying layer of visceral muscle. The cells have a weakly basophilic cytoplasm in which the secretory deposits were poorly distinguishable and small pycnotic nuclei were seen at the cell base (Fig. 1A). The gland lumen contains a large number of secretory globules of various sizes immersed in amorphous material. The aspect of this secretion varies along the gland length and from the lumen periphery to the central region. At the periphery, the globules are small and well-structured, but tend to form amorphous masses in the central region (Figs. 1A-D).

The secretion in the lumen of the distal extremity of the gland consists of small globules that stained with hematoxylin. Near the junction of the gland with the seminal vesicle, the granules become acidophilic and increase in size (Fig. 1A).

Proximally, the globules fuse to each other and form amorphous masses (Fig. 1C). Alcian blue did not stain the cytoplasm of these cells, but the secretion in the lumen was strongly stained (Fig. 1B). Generally, only small globules stained homogenously in blue. The amorphous masses resulting from globule fusion stained strong blue at the periphery but were almost colorless in the center. Alcian blue did not stain the cell cytoplasm, although some very finely granulated material in the apical portion of the cell was stained

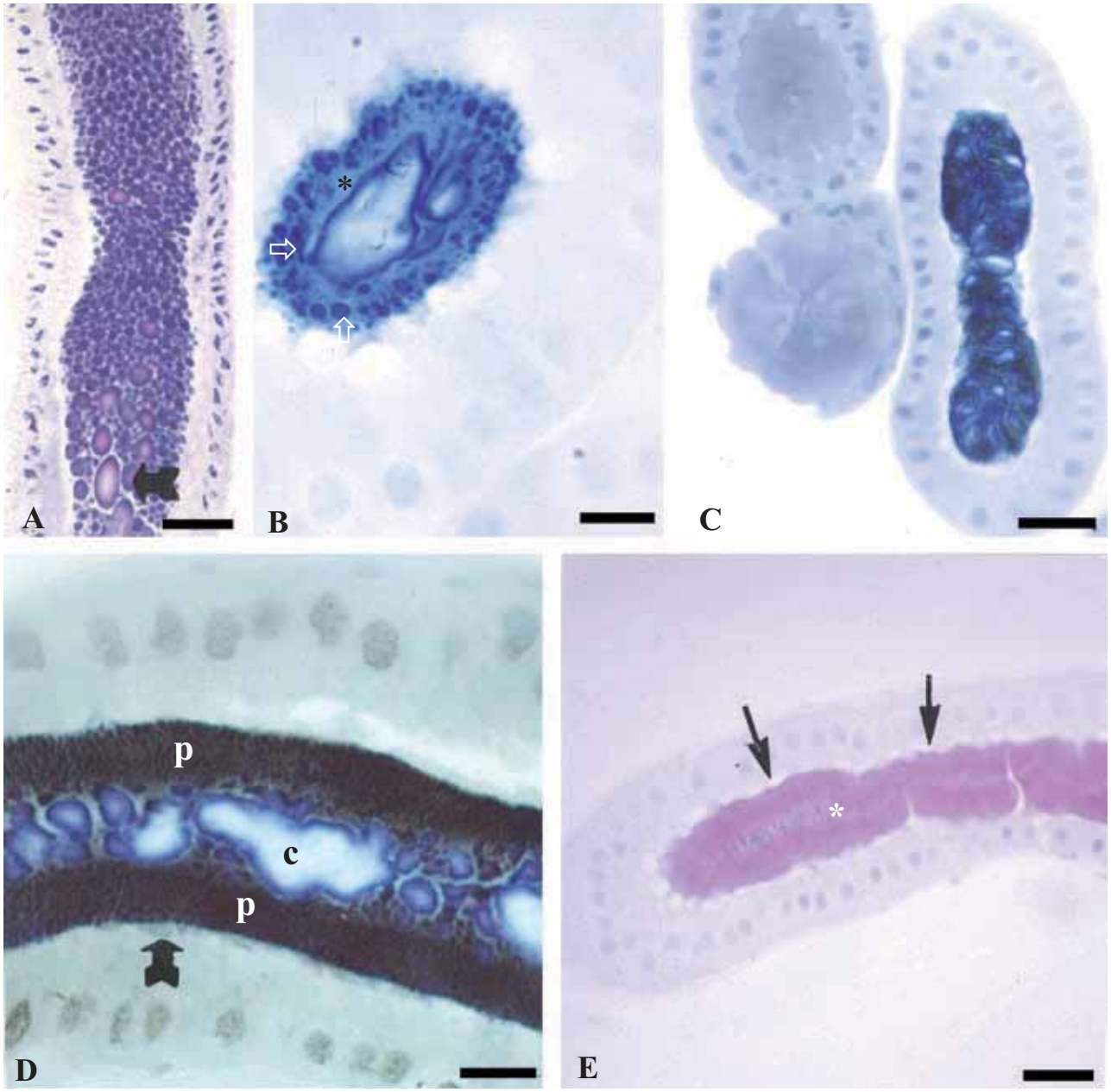

Figure 1. A. Light micrograph of a longitudinal section of *A. grisella* accessory gland showing the secretion in the lumen. Note the poor cellular basophilia and the large, acidophilic secretory globules in the lumen (**arrow**). HE. Bar = 60 µm. **B.** Alcian blue staining showing the background material in light blue (*****) and the secretory globules in deep blue (**arrows**). Note that the globules coalesce and become discolored in the central region. Bar = 15 µm. **C.** Oblique section of the glandular tubules showing the secretion stained with bromophenol blue. The left section is from the proximal region and the right one from the gland distal part. Bar = 40 µm. **D.** Staining with bromophenol blue showing secretion discharged at the cell luminal surface (**arrow**) and the different staining intensity between the coalescent periphery (**p**) and central region (**c**) of the secretory globules. Bar = 15 µm. **E.** PAS-AB staining. Note that only the background material (*****) is stained. The arrows indicate the cell apices delineated by Alcian blue. Alcian blue-positive material is present in central region of the lumen. Bar = 40 µm.

(Fig. 1D). The secretion in the lumen stained strongly, with the staining being much stronger in the peripheral region in contact with the epithelium (Fig. 1D). In the central region, where the globules coalesced, the outer limits of the masses stained deep blue, with the intensity of staining decreasing towards the almost colorless center. PAS produced uniform staining of the secretion in the lumen, with no difference among the globules. The combination of PAS with Alcian blue stained only the apical limits between cells and the lumen secretion. Some blue staining was also observed in the central region of the lumen (Fig. 1E).

Bromophenol blue stained the cell cytoplasm but did not highlight any intracellular structures, except the nuclei. The lumen secretion was stained, indicating its proteinaceous nature, although the reactions varied along the gland (Fig. 1C). In the proximal part, where the globules were coalesced, the staining was weak (Fig. 1C), while in the distal region, where the secretory globules were more structured, the staining was stronger.

Ultrastructure

The accessory gland epithelial cells of *A. grisella* has an ultrastructure characteristic of protein-secreting cells. Rough endoplasmic reticulum is abundant and occupies almost all of the cytoplasm; this organelle consists of cisternae in the basal portion of the cells and is vesicular in the apical region (Fig. 2A). The nucleus has an irregular outline, is located centrally in the cell and contains irregularly distributed heterochromatin. A thin muscular layer covers the epithelium (Fig. 2A).

The cisternae of the endoplasmic reticulum are dilated and contain fine granular material of medium electron density (Figs. 2B and 3C). The Golgi complexes are randomly distributed throughout the cytoplasm, with no polarization. These complexes consist of irregular, electron-dense lamellae, with numerous associated vesicles and small clear vacuoles (Figs. 2B and 3A). The numerous mitochondria tend to be spherical and are evenly distributed throughout the cells.

The cells show a few, irregular microvilli on the luminal surface that were generally compressed by the secretion that had accumulated in the lumen (Figs. 2A and 3A-B). In some cases, the apical cytoplasm form expansions towards the lumen (Fig. 3B).

The secretion do not form granules in the cytoplasm, as already shown by light microscopy. This lack of accumulation suggests a continuous discharge. The presence of clear vesicles (Fig. 3A) among the apical microvilli, together with the apical cell projections, suggests apocryne elimination.

The secretion in the lumen do not have a uniform appearance (Figs. 3A and 4). Proximally, there are small electron-dense clots and large globules of varying electron density surrounded by fibrillar or finely granular material (Fig. 3A). Distally, the secretion contains electron-dense globules of various sizes (small at the periphery and large or coalescent in the central region) intercalated with fibrils immersed in amorphous material of average electron-density (Fig. 4). These findings generally agree with the morphology seen by light microscopy.

DISCUSSION

The reproductive system of several Lepidoptera has been described, including that of economically important species, such as *Ephestia kuhniella* [13], *Heliothis zea* [4] and *Diatraea grandiosella* [11]. The male reproductive system of *A. grisella* is similar in morphology to that of other Lepidoptera [14,20,22], with the accessory gland of this species consisting of two long, narrow tubules.

Although there were no anatomical and cellular variations along these tubules, there were suggestions of regional functional differentiation, such as also seen in *Aedes* mosquitos [21] and most lepidopterans [20]. These differences in appearance may simply reflect changes in the chemical composition of the secretion as it accumulates in the lumen, especially when stained for proteins and glycosaminoglycans using bromophenol blue and alcian blue, respectively.

The secretion in the lumen contained mainly glycoproteins since it stained with bromophenol blue, PAS and Alcian blue. The secretion organized in globules was essentially proteinaceous, whereas the fibrillar and amorphous material that occupied the spaces between the granules consisted of carbohydrates. These results agreed with the composition found for most insects, including lepidopterans, i.e., predominantly proteinaceous, but also containing glycogen and lipids [3,13,14,19,23]. Although glycogen has been reported as a frequent component of this gland secretion [6], and staining indicated the presence of carbohydrates, no glycogen was observed in the ultrastructural analysis. Hence,

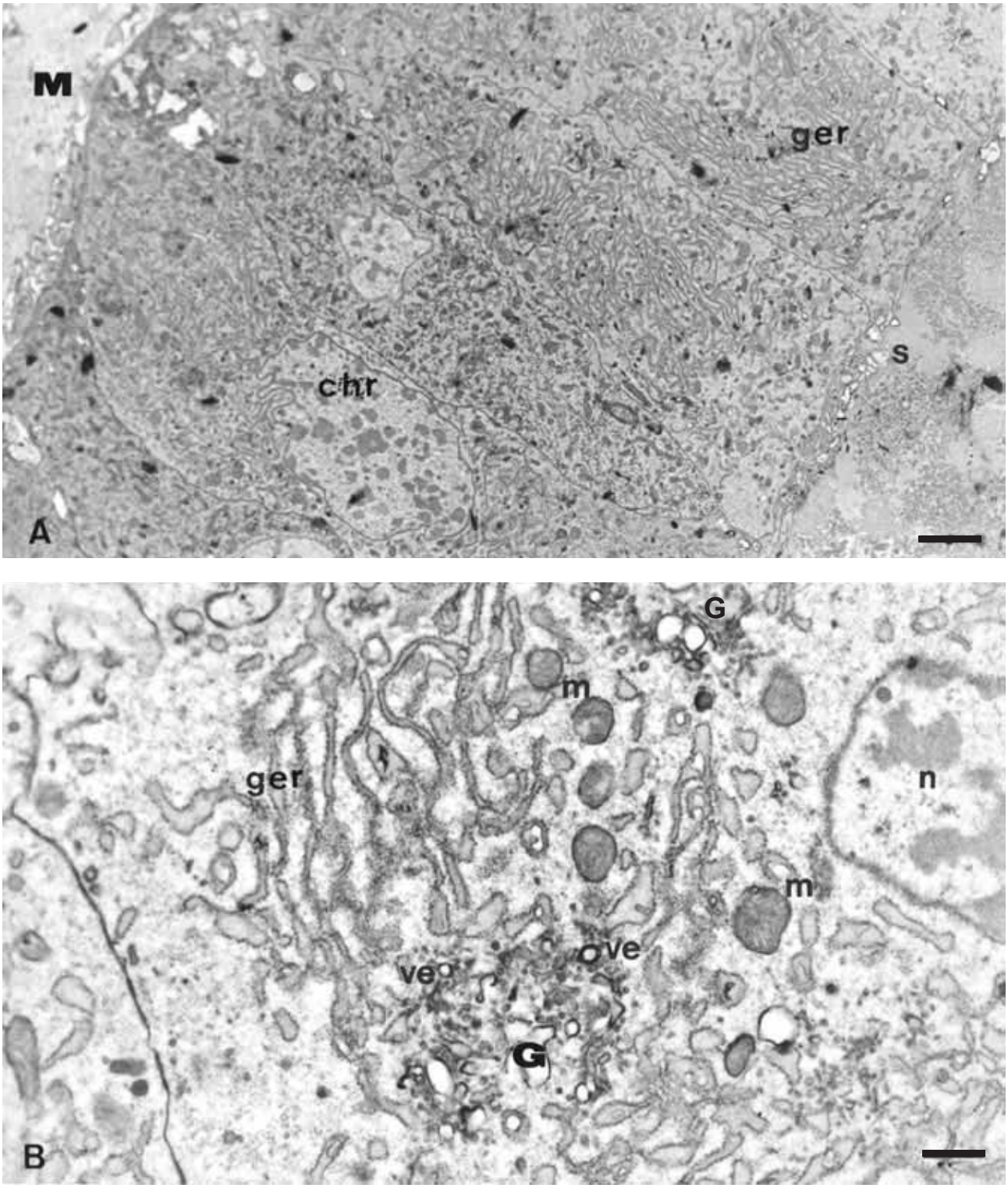

Figure 2. A. Transmission electron micrograph showing a general view of the gland wall, including the prismatic epithelial cells and the outer muscular layer (**M**). Note the nuclei containing clots of heterochromatin (**chr**) and a well-developed, granular endoplasmic reticulum (**ger**). **s** = secretion. Bar = 1 μm. **B.** Transmission electron micrograph showing details of the granular endoplasmic reticulum (**ger**) with its dilated lumen and the Golgi (**G**) complexes surrounded by vesicles (**ve**). **n** = nucleus; **m** = mitochondria. Bar = 0.5 μm.

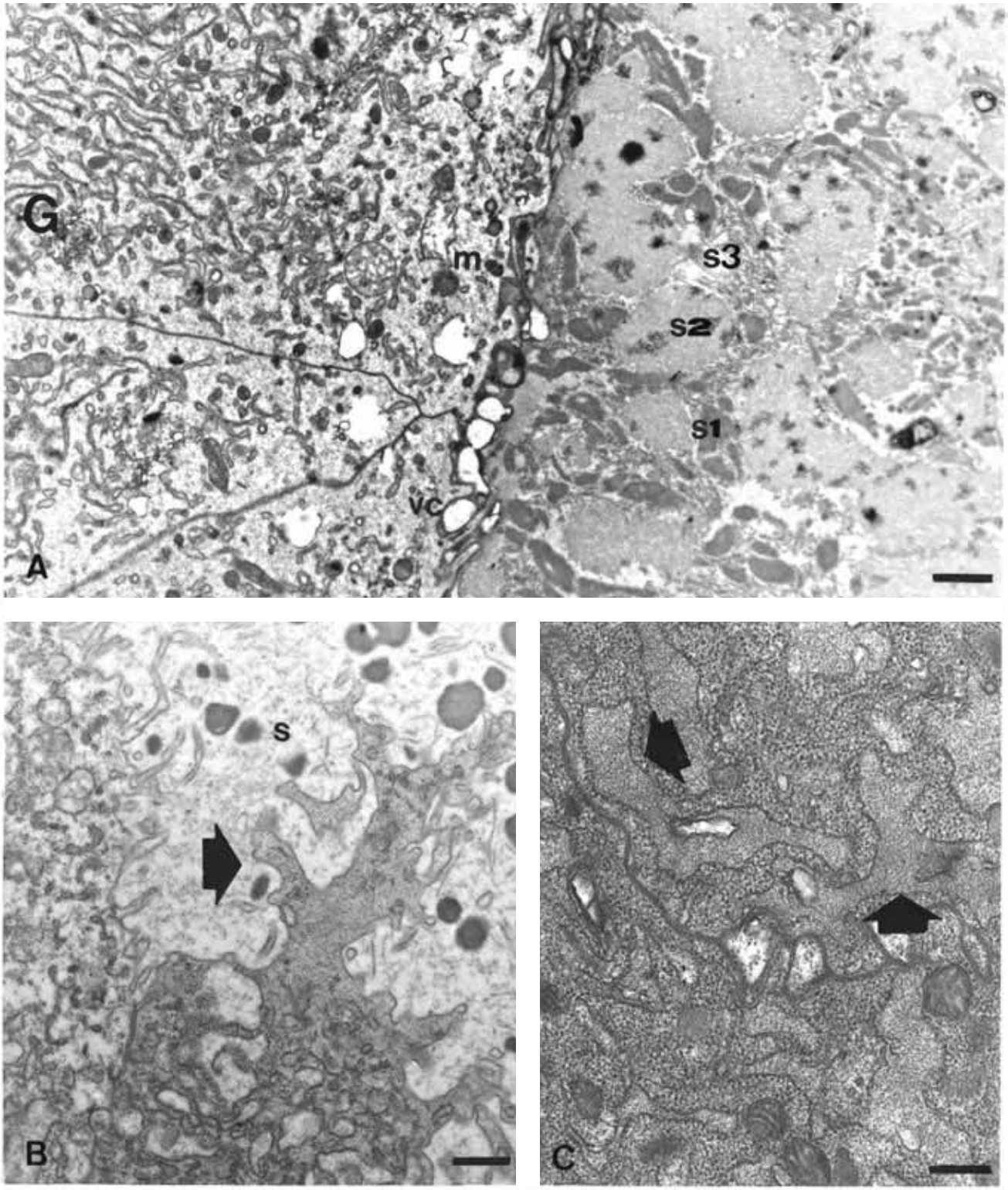

Figure 3. A. Transmission electron micrograph showing the heterogeneous morphology of the gland secretion present in the lumen. **s1** = small granules; **s2** = globules; **s3** = fibrils. Note the clear apical vacuoles (**vc**) at the cell luminal surface. **G** = Golgi; **m** = mitochondria. Bar = 1.5 μm**. B.** Transmission electron micrograph of the cell luminal surface showing the accumulation of secretion (**s**) in the lumen, as well as microvilli and cytoplasmic projections into the lumen (**arrow**). Bar = 1 μm. **C.** Transmission electron micrograph of the rough endoplasmic reticulum with a dilated lumen containing a huge amount of granular material (**arrows**). Bar = 0.5 μm.

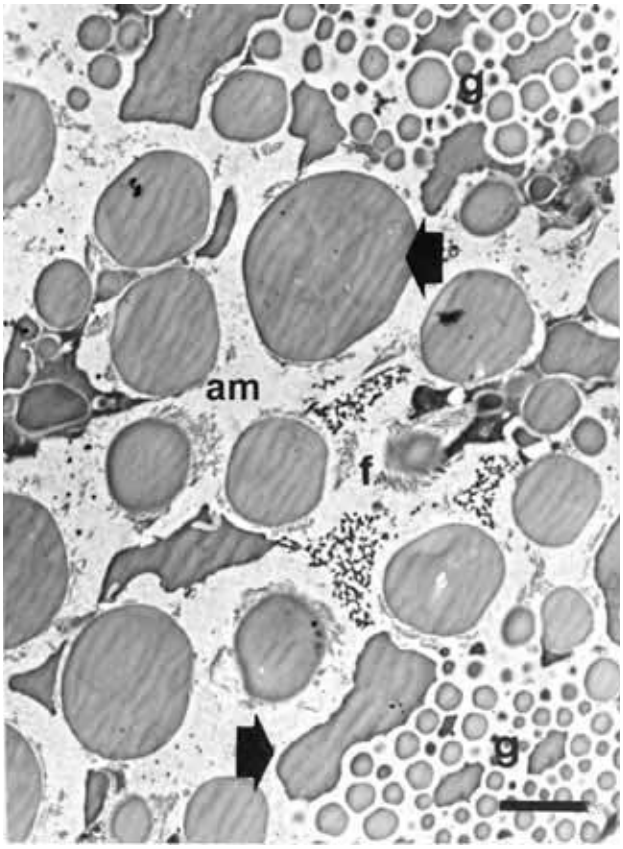

Figure 4. Transmission electron micrograph of secretion in the gland lumen showing the different structural components. **g** = globules; **f** = fibrils; **am** = amorphous material. The arrows indicate the masses formed by the coalescence of globules. Bar = 1 μm.

the material stained by PAS probably corresponded to glycoproteins, while the Alcian blue-stained material between the globules was probably glycosaminoglycans. The occurrence of staining only at the granule periphery reflected the differences between the granule and the nature of the material in which the globules were immersed. Although no tests were done for lipids, we inferred that they may have been represented by the membranous structures present in the secretion stored in the lumen, as seen in Bombus [2].

The secretory cells of *A. grisella* had poorly developed luminal microvilli that may be involved in the partial resorption of substances eliminated in the secretion. The different appearance of the secretion close to the cells compared to that in the central part of the gland lumen suggested that the secretion in this region underwent some processing that modified its composition and structure, perhaps through the resorption of some components by the epithelial cell microvilli.

In some insects, the accessory gland secretion exerts physiological and biochemical control of the female reproductive physiology [1,6], in addition to other functions. Although the function of this gland in *A. grisella* was not investigated here, the glycoprotein nature of the secretion suggests a nutritional role in the spermatozoa, and/or in plug formation in the female genital tract after mating. A role in female reproduction may also be possible, as already reported in other lepidopteran species [13,14].

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