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Ultrastructure of the female reproductive apparatus of the egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae)

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ABSTRACT

The growing interest in *Leptoglossus occidentalis*, the conifer seed bug pest accidentally introduced into Europe in the 1990s, led us to investigate the female reproductive structures of the hymenopteran platygastrid *Gryon pennsylvanicum*, which is its candidate antagonist for biological control programmes. Our study revealed a genital apparatus with some characteristic features, such as an unusual length of the oviduct (divided into a long proximal and a short distal tract), the absence of accessory glands and the presence of a spermatheca provided with a small spermathecal gland. The ultrastructural investigation revealed that the shorter part of the common oviduct is involved in ion uptake whereas the longer part has two cell types with secretory function: the former with dense bodies and the latter with granular particles. The secretory contents of both are released into the oviduct lumen. The granular particles are formed in a complex of modified endoplasmic reticulum and appear as virus-like particles.

1. Introduction

Hymenoptera is a very important insect order for several reasons, including medical and economic relevance, especially biological control of several phytophagous insects by means of hymenopteran parasitoids. The entire host–parasitoid system is worthy of study, both to evaluate the damage caused by the pest and to evaluate possible actions for biological control programmes. *Gryon pennsylvanicum* (Ashmead), an egg parasitoid wasp, is important for the biological control of the western conifer seed bug *Leptoglossus occidentalis* Heidemann, an invasive alien species that feeds mainly on conifers causing cone abortion and seed loss (Bates et al., 2000; Roversi et al., 2011; Strong et al., 2001). To better understand the host–parasitoid interaction, it is essential and useful for the management and handling of mass rearing to know the biology of *G. pennsylvanicum*, such as the life cycle (Sabbatini-Peverieri et al., 2012) and the internal anatomy of the male reproductive system and the sperm ultrastructure (Paoli et al., 2013). To complete this research, we have conducted a morphological analysis of the female reproductive system to ascertain the ultrastructure of the different genital organs and to observe the fate of the sperm after mating. Moreover, many molecular studies dealing with host immune system suppression have revealed that a large amount of venom and peculiar symbiotic virus-like particles (VLPs) are injected by the female of larval and egg-larval parasitoid wasps into the host during oviposition (Beckage and Drezen, 2012; Falabella et al., 2003; Pennacchio et al., 2001; Soller and Lanzrein, 1996; Strand, 2012; Turnbull and Webb, 2002; Webb, 1998). In light of our observation, we also debate the possible presence of VLPs in the oviduct of this egg parasitoid wasp.

2. Materials and methods

2.1. Origin and rearing of the insects

G. pennsylvanicum (Ashmead) (strain GP-BC-1, GenBank code JX968492) was collected in Canada and after introduction into Italy in 2010 a colony was established in the laboratory of the CRA-ABP (Florence), reared in a climatic chamber (Binder KBWF 720,

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Tuttlingen, Germany) under standard conditions of 26 ± 1 °C, 75 ± 5% RH and 18:8 L:D (Roversi et al., 2011).

2.2. Light and electron transmission microscopy

To remove the female reproductive system, we dissected specimens of *G. pennsylvanicum* under light microscopy in phosphate buffer (PB) 0.1 M, pH 7.2. The whole reproductive system was photographed with Leica DMRB and Nikon Eclipse TE 2000-U light microscopes both equipped with an interference-contrast system.

The material was then fixed in 2.5% glutaraldehyde in PB to which 3% of sucrose was added. After washing with PB, the material was post-fixed with OsO_4 for 1 h, rinsed in PB, dehydrated in a graded alcohol series and embedded in a mixture of Epon-Araldite resin (see Paoli et al., 2013). Semi-thin and thin sections obtained with Reichert and RMC PowerTome ultramicrotomes were stained with 1% toluidine blue for light microscopy observations and with uranyl acetate and lead citrate in succession for transmission electron microscopy. Thin sections were observed with a Philips CM 10 electron microscope operating at 80 kV and with a Jeol Jem 1011 operating at the same voltage.

3. Results

3.1. General organization of the female reproductive system

The female reproductive system of *G. pennsylvanicum* is composed of a pair of ovaries, two short lateral oviducts and a long common oviduct that opens into the ovipositor (Fig. 1A and B). Accessory glands are not present. Each ovary, 500 μm long, is provided with 5–6 ovarioles. The lateral oviducts, 120 μm long and 60 μm in diameter, are transparent in the region close to the ovary and whitish in their distal region where secretory activity is performed. The common oviduct, a tubular structure 450 μm long and 70 μm in diameter, is also whitish for most of its length and presents a bottleneck before entering the ovipositor (Fig. 1A and B). The main part of the long common oviduct runs towards the anterior abdominal region; then, its distal end bends towards the posterior abdominal region where the valves of the ovipositor are inserted (Fig. 1A, B and G). The diameter of this tract is almost uniform for the whole oviduct length. Only its terminal portion (about 85–90 μm long) is narrower (about 45–50 μm) and exhibits a transparent appearance. In that place, a small spermatheca opens (Fig. 1A–D and J).

The spermatheca is a flattened tubular structure that bends distally, giving origin to an open ring (Fig. 1J and see also supplementary material). It has a diameter of 50 μm (Fig. 1B, C, G and I) and is connected to the common oviduct by a long spermathecal duct about 100 μm long (Fig. 1C, F, H, I and J and see also supplementary material). A single spermathecal gland is present in the apical part of the spermathecal duct close to the spermathecal receptacle (Fig. 1C and J). This exocrine gland is composed of some efferent ducts that deliver secretions into a bagpipe-like cistern, from which another cuticular canal opens into the spermathecal duct close to the connection with the spermathecal receptacle (Fig. 1C, J and K).

3.2. Ultrastructure of the oviducts

3.2.1. The lateral oviducts and the proximal part of the common oviduct

The lateral oviducts, 120 μm long, are short tracts connecting the ovarioles to the proximal part of the common oviduct (Fig. 1A). They are narrower than the common oviduct, with which they share a similar appearance in their distal part. Their diameter varies from 40 μm in the proximal region to about 60 μm in the distal whitish region (Fig. 1A). The epithelial cells of distal whitish region are elongated (about 9 μm length) and conical in cross section (Fig. 2A). The cells are very close to each other, separated by straight limiting plasma membrane. They are lined apically by a thin cuticle only 17–20 nm thickness, likely the outer epicuticle layer (Fig. 2A). Beneath this cuticle, a layer of microvilli (0.5–0.8 μm high) delimits a region under which dense spheroid bodies are stored (Fig. 2A). Beneath this cell region, mitochondria, some cisterns of rough endoplasmic reticulum and free ribosomes are visible (Fig. 2B). The nucleus is centrally located in these cells (Fig. 2B); it is elongated and measures about 5.3 μm × 3.8 μm. The basal region of the cells is rich in cisterns of rough endoplasmic reticulum and Golgi apparatus. Scattered cells are characterized by clusters of electron-transparent vesicles (0.15–0.23 μm long and 70–80 nm wide), provided with dense wall, which can be seen close to the nucleus, embedded in a dense matrix (Fig. 2B). This cluster extends for about 2.5–3 μm in the cytoplasm. Some vesicles contain dense flocculent material. Intermingled with these vesicles tubular structure with a membrane surrounding an inner tubule 60–70 nm wide are present (Fig. 2B). The basal cell region lies on a very thin basal lamina. Numerous small dense granular particles are often visible in the small spaces created by infoldings of the plasma membrane (Fig. 2B).

The epithelium of the main part of the common oviduct is characterized by long cells 30–35 μm high (Fig. 2C) separated by straight junctional complexes consisting of apical zonulae adhaerentes, beneath which long pleated septate junctions interrupted by gap-junctions are visible (Fig. 2D and E). In a cross section the two opposite epithelia delimit a very reduced lumen, only 0.7 μm wide, appearing as a narrow slit (Fig. 2C).

3.2.2. Atypical endoplasmic reticulum and vesicle budding process

Two types of cells are identified along the common oviduct epithelium; the first type of cells, more numerous, corresponds to typical secretory cells rich in rough endoplasmic reticulum and appears to be filled with dense spheroid vesicular bodies 0.6 μm in diameter (Figs. 2C, D and 3A, B, H). Intermingled with these cells are scattered cells characterized by vesicles filled with granular secretion particles (Figs. 2C, D, E and 3A–J). This second type of cells is characterized by cisterns of endoplasmic reticulum often arranged longitudinally in a tightly parallel fashion, Golgi apparatus and microtubules (Fig. 3A, B and I). Large clusters of small elliptical vesicles embedded in a dense matrix are visible in the basal region of these cells (Fig. 3A and B). In the neighbouring region, numerous larger vesicles (0.5–0.7 μm in diameter) filled with dense granular particles are present (Fig. 3A–I). In some areas the whole basal region is involved in the production of these structures. These areas, up to 4–5 μm large, consist of intermingled cisterns of endoplasmic reticulum giving rise to a dense matrix, sometimes with a paracrystalline appearance; bundles of long cisterns of endoplasmic reticulum provided with a few ribosomes on their membranes are visible at the periphery of the areas (Fig. 3B). These cisterns are in continuity with other cisterns, about 80–90 nm in diameter, containing tubular dense structures about 60–70 nm wide. In cross section, these tubular structures are provided with a dense bilayered wall (Fig. 3B–G). At the extremity of the endoplasmic reticulum cisterns, it is not rare to observe budding processes originating vesicles provided with a dense wall (Fig. 3B, C and E–G); they undergo maturation giving rise to elliptic electron-transparent vesicles (Fig. 3C, E and G) lately containing flocculent material (Fig. 3G). By fusion, the vesicles produce large units within which more structured particles appear evident. At the end of the process the vesicles, reaching up to 0.6 μm, are filled with distinctly structured granular particles (Fig. 3A–C and G–J). At higher magnification, these granular particles, about 50 nm long and 30–40 nm