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Relationship between lateral oviduct morphology and reproductive strategy in earwigs

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ABSTRACT

The earwig (Dermaptera) order contains around 2200 insect species with extremely diverse reproductive strategies. The vast majority of free-living earwigs are oviparous, i.e. the females lay eggs and the embryonic development takes place in an external environment. In contrast, the two known epizoic groups (Arixeniina and Hemimerina) are viviparous, i.e. embryogenesis takes place inside the female, which "gives birth" to the young offspring. There are also examples of another reproductive strategy, i.e. ovoviviparity, when the embryos develop inside eggs retained in the mother's body. The ovoviviparous earwigs are free-living and belong to the family Spongiphoridae. Here I present the results of ultrastructural analysis of lateral oviducts in three earwig species belonging to three different reproductive strategy groups: (1) oviparous *Forficula auricularia* (Forficulina, Forficulidae), (2) ovoviviparous *Chaetospania borneensis* (Forficulina, Spongiphoridae) and (3) viviparous *Arixenia esau* (Arixeniina, Arixeniidae), and show that the lateral oviducts of viviparous species undergo extensive structural and functional modifications, which facilitate gas exchange, nourishment and protection of developing embryos.

1. Introduction

The vast majority of insect species are oviparous. Females lay eggs and the embryogenesis takes place in an external environment. In these insects the embryo development relies on reserve materials (yolk proteins and lipids) accumulated in the oocyte cytoplasm during oogenesis (see Kunkel and Nordin, 1985; Wheeler, 2003 for a review). Two other reproductive strategies observed among insects are viviparity and ovoviviparity (Wheeler, 2003). The viviparity is relatively rare. In this type of reproduction, the embryonic development and the nourishment of embryos take place within the female body (Hagan, 1951; Andrews and Rose, 1994; Retnakaran and Percy, 1985). In the case of ovoviviparity, the embryos develop inside the eggs that are retained in the mother's body until they are ready to hatch (Wheeler, 2003). The larvae hatch inside the mother's reproductive system or the hatching occurs immediately after oviposition. Although the ovoviviparity is deceptively similar to viviparity it does not require any nourishment of the embryos by the mother because the unborn offspring use the reserve materials accumulated in the egg cytoplasm.

Insect female reproductive system is composed of paired ovaries and an out-leading tract that opens through the genital external opening. The ovaries are composed of elongated units termed the ovarioles (see Buning, 1994; Bilinski, 1998). As a rule, the fully developed ovariole consists of four easily recognizable parts: the terminal filament, germarium, vitellarium and the ovariolar stalk. The terminal filament is a stack of flattened somatic cells oriented perpendicular to the long axis of ovariole. The germarium contains dividing and differentiating germ line cells, and the vitellarium consists of a linear arrangement of developing ovarian follicles (Bilinski, 1998). The ovariolar stalks are composed of several layers of closely apposed somatic cells and connect ovarioles to the lateral oviducts. The latter are the tube-shaped structures composed of one-cell thick epithelium. The epithelial cells of the lateral oviducts lie on the basement lamina and are surrounded by numerous muscle fibers and tracheal branches (Lehane and Laurence, 1978). The lateral oviducts lead to a common, muscular, oviduct. The muscle fibers are arranged along and circumferentially around the oviduct (Chapman, 2013). In lower orders of insects, the common oviduct is short and opens directly to the outside. In advanced, higher groups of insects, rear part of the common oviduct is transformed into the genital chamber, which narrows into the vagina (Chiang and O'Donnel, 2009; Chiang, 2010). The female reproductive tract of insects is usually accompanied by spermathecae and accessory glands (Gschwentner and Tadler, 2000; Marchini et al., 2010). The

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spermathecae are used for the sperm storage after copulation. They are lined with epithelia and are variable in shape: some are thin and heavily coiled tubes, and some are short outpocketings extending from the main reproductive tract (Marchini et al., 2010). Very often the female reproductive system also contains highly specialized structures called the accessory glands. These include, for example, the adhesive glands, which produce a substance allowing the adhesion of eggs to the substrate. In some insects (e.g. cockroaches or mantises) accessory glands produce materials for the cocoons (oothecae) or, like in the hymenopterans, they are transformed into the venom glands (Chapman, 2013).

The earwigs (Dermaptera) are a relatively small insect order with about 2200 species classified in three suborders: the most abundant free-living Forficulina and two epizoic groups, the Hemimerina and Arixeniina (Popham, 1985; Haas and Kukalová-Peck, 2001). The majority of earwig species are, like most insects, oviparous. The females lay eggs in the nests, and, in some species, they look after the offspring, which is an example of a very simple maternal care. The representatives of epizoic groups (the Arixeniina and Hemimerina) are viviparous and females "give birth" to the larvae (Hagan, 1951; Nakata and Maa, 1974). It is believed that in these groups the viviparity evolved to provide nymphs an immediate contact with the preferred host and to accelerate the life cycle (Hagan, 1951; Nakata and Maa, 1974). Another reproductive strategy, the ovoviviparity, occurs in free-living earwigs from the Spongiphoridae (Forficulina) family (Herter, 1943; Kocarek, 2009). Our previous studies showed that in bat-associated epizoic, viviparous *Arixenia esau* the female reproductive system undergoes several important modifications that allow internal embryonic development (Tworzydlo et al., 2013a,b). One of these modifications is a distention of the lateral oviducts and their transformation into the uteri (see Tworzydlo et al., 2013a,b and Section 4 for details).

While there is an extensive literature on the structure of the ovaries (and ovarioles) and the course of oogenesis in different systematic groups of insects, the studies of the morphology and function of the remaining parts of the female reproductive system are relatively scarce. In this context, and to address the question whether the morphology of the lateral oviduct is related to insect reproductive strategy, I analyzed and compared the morphology of lateral oviduct in three earwig species: (1) an oviparous *Forficula auricularia* (Forficulina, Forficulidae), (2) an ovoviviparous *Chaetospania borneensis* (Forficulina, Spongiphoridae) and (3) the viviparous species *A. esau* (Arixeniina, Arixeniidae).

2. Materials and methods

2.1. Animals

Three species of dermapterans from three different families were studied: *F. auricularia* (Forficulidae), *Chaetospania borneensis* (Spongiphoridae) and *A. esau* (Arixeniidae). The specimens of *Forficula* were collected in neighborhood of Krakow (southern Poland), *Chaetospania* were collected in Gunung Raya in Langkawi Island (western Malaysia) and *Arixenia* in Bintulu District area (Sarawak, Malaysia) where numerous bat colonies are present.

2.2. Light and electron microscopy

The female reproductive systems were dissected and fixed in 100% ethanol (*Chaetospania*), a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0. 1 M phosphate buffer (*Forficula*) or in 4% formaldehyde (*Arixenia*). Different fixation methods are related to the difficulty of obtaining some of the specimens. The ethanol and formaldehyde fixed samples were rinsed in PBS, dehydrated in series of ethanol and embedded in a Histocryl acrylic resin (Agar Scientific Ltd., Stansted, Essex, UK). Material fixed in a mixture of formaldehyde and glutarldehyde was postfixed in 2% osmium tetroxide and 0.8% potassium ferrocyanide for 30 min at 4 °C. After dehydration in the series of ethanol and acetone the material was embedded in an epoxy resin glycid ether 100 (formerly known as Epon 812; Serva, Heidelberg, Germany). Histocryl and epon semithin (0.7 μm thick) sections were stained with 1% methylene blue and examined under a Leica DMR (Heidelberg, Germany) or Nikon Eclipse Ni (Nikon, Japan) microscopes (LM). Ultrathin (80 nm thick) epon sections were contrasted with uranyl acetate and lead citrate according to standard protocols and analyzed with a Jeol JEM 2100 transmission electron microscope at 80 kV.

2.3. Ag-NOR technique

The Ag-NOR technique is a very good procedure showing synthetic activity of the cells. The staining of semithin Histocryl sections was performed according to Howell and Black (1980) modified by Bilinski and Bilinska (1996). The sections were stained for 17 min at 37 °C with a 1:2 mixture of 2% gelatin in 1% formic acid and 50% $AgNO_3$ (Sigma, St. Louis, MO, USA). After rinsing with distilled water the slides were analyzed with a Leica DMR or Nikon Eclipse Ni microscopes.

2.4. Scanning electron microscopy (SEM)

For the SEM, the material was fixed and postfixed as described above. After dehydration in graded series of ethanol, the material was critical-point dried, coated with gold and examined with a JSM 5410 scanning electron microscope at 25 kV.

3. Results

3.1. Forficula

The female reproductive system of *F. auricularia* is composed of paired ovaries, lateral oviducts, and a single common oviduct. The ovaries are elongated and contain numerous (up to 40) very short ovarioles that "surround" the lateral oviducts (for detailed description of the ovaries in this species see Tworzydlo and Bilinski, 2008; Tworzydlo and Kisiel, 2010). The ovarioles are attached to the oviducts by ovariolar stalks (Fig. 1A). The stalks are composed of several layers of closely apposed flattened cells. The oviducts are elongated, tube-shaped structures. In very young females the diameter of the oviduct is about 90 μm. During female maturation the diameter of the oviduct slightly increases to about 120 μm. Analysis of the serial semithin sections showed that the oviducts are slightly folded (Fig. 1A). The structure of the lateral oviduct remains the same along its whole length. The wall of the lateral oviduct is relatively thin and simple. It is composed of a single layer of a columnar epithelium lying on a basement lamina (Fig. 2A and B). The epithelial cells are equipped with relatively large nuclei, which contain patches of heterochromatin irregularly located underneath the nuclear envelope (Fig. 2A and B). The epithelial cell cytoplasm contains numerous free ribosomes and the polysomes, mitochondria, elements of endoplasmic reticulum, different size vacuoles and microtubules (Fig. 2). The apical surface of the epithelial cells is furnished with short, irregular microvilli. At the base of the microvilli sparse endocytotic vesicles are observed (Fig. 2C). Neighboring epithelial cells are closely opposed and remain connected by apically located adherens junctions (Fig. 2C–E, arrows) and more distant (in relation to the cell surface) septate junctions (Fig. 2D and E, arrowheads). The septa of the latter junctions are especially well visible in sections tangential to the membranes of the neighboring cells (Fig. 2E).