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Development of ovary structures in the last larval and adult stages of psyllids (Insecta, Hemiptera, Sternorrhyncha: Psylloidea)

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ABSTRACT

The development and organization of the ovaries of ten species from four Psylloidea families (Psyllidae, Triozidae, Aphalaridae and Liviidae) have been investigated. The ovaries of the last larval stage (i.e. fifth instar) of all examined species are filled with numerous clusters of cystocytes which undergo synchronous incomplete mitotic division. Cystocytes of the given cluster are arranged into a rosette with polyfusome in the centre. These clusters are associated with single somatic cells. At the end of the fifth instar, the clusters begin to separate from each other, forming spherical ovarioles which are surrounded by a single layer of somatic cells. In the ovarioles of very young females all cystocytes enter the prophase of meiosis and differentiate shortly thereafter into oocytes and trophocytes (nurse cells). Meanwhile, somatic cells differentiate into cells of the inner epithelial sheath surrounding the trophocytes and into the prefollicular cells that encompass the oocytes. During this final differentiation, the trophocytes lose their cell membranes and become syncytial. Oocytes remain cellular and most of them (termed arrested oocytes) do not grow. In the ovarioles of older females, one oocyte encompassed by its follicle cells starts growing, still connected to the syncytial tropharium by a nutritive cord. After the short phase of previtellogenesis alone, the oocyte enters its vitellogenic the growth phase in the vitellarium. At that time, the second oocyte may enter the vitellarium and start its previtellogenic growth. In the light of the obtained results, the phylogeny of psyllids, as well as phylogenetic relationships between taxa of Hemiptera: Sternorrhyncha are discussed.

1. Introduction

Jumping plant lice (psyllids) are small phloem feeding insects which belong to the suborder Sternorrhyncha within the Hemiptera order. According to Li (2011), the world fauna of psyllids comprises of about 3850 species distributed worldwide from Arctic areas to tropical regions. According to Burckhardt and Ouvrard (2012), the superfamily of Psylloidea consists of 8 families: Aphalaridae, Carsidaridae, Calophyidae, Homotomidae, Liviidae, Phacopteronidae, Psyllidae, and Triozidae. The life cycle of psyllids includes five larval stages and sexually reproducing adults of both sexes in almost equal numbers. Parthenogenetic reproduction is rare and occurs in only some populations. Depending on the climatic zone, psyllids may have only one generation or multiple generations per year (Gullan and Martin, 2003). The great majority of psyllids are associated with dicotyledonous plants and they have a very narrow host–plant specialization (Hodkinson, 2009). Most of them are free living, except for about 15% of species which live inside galls. Jumping plant lice play a considerable role in agriculture as pests of cultivated plants and vectors of serious plant diseases (Gullan and Martin, 2003; Hodkinson, 2009).

The ovaries of insects are composed of units termed ovarioles, in which full growth of the eggs takes place. Traditionally, two basic categories of ovarioles are distinguished: panoistic and meroistic (Brandt, 1874). The divisions of germ cells in the panoistic ovarioles are terminated with complete cytokinesis, thereby all oogonia transform into functional gametes (=oocytes) (Büning, 1994;

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Biliński, 1998). In meroistic ovarioles mitotic divisions of germline cells are followed by incomplete cytokineses which lead to the formation of clusters of germ cells (=cystocytes) interconnected by intercellular bridges. The divisions of cystocytes are generally synchronous, therefore the number of cells belonging to one clone is usually determined by the $N = 2^n$ rule (i.e. Giardina's rule, where "N" indicates the cystocyte number and "n" – the number of series of cystocyte divisions). The values of "N" and "n" are generally species-, family- or even order specific (Telfer, 1975; Štys and Biliński, 1990; Büning, 1994; Biliński, 1998). When the divisions are complete, the cystocytes in each cluster (=cyst) differentiate into trophocytes and oocytes. The trophocytes do not transform into functional gametes and, instead, provide the oocytes with different macromolecules (mainly rRNA) and organelles. In meroistic ovarioles of the polytrophic type, only one cystocyte differentiates into the oocyte, while in the ovarioles of the telotrophic type more germ cells become oocytes.

An individual ovariole usually consists of a terminal filament, germarium (in panoistic and polytrophic ovarioles) or tropharium (=trophic chamber) (in telotrophic ovarioles), vitellarium and pedicel (ovariolar stalk) (for further details concerning ovariole organization see Štys and Biliński, 1990; Büning, 1994; Biliński, 1998). As a rule, the terminal filaments of all the ovarioles combine, forming a single ligament that anchors the ovary in the fat body, whereas a pedicel joins the ovariole with the lateral oviduct. The vitellarium contains linearly arranged, sequentially growing oocytes (through three stages: previtellogenesis, vitellogenesis and choriogenesis) which are surrounded by a single layer of follicular cells.

Psyllids, like other hemipterans (see e.g. Huebner, 1981; Książkiewicz, 1980; Büning, 1985; Książkiewicz-Kapralska, 1985, 1991; Biliński et al., 1990; Szklarzewicz, 1998a; Szklarzewicz et al., 2000, 2007; Simiczyjew et al., 1998; Štys et al., 1998; Michalik et al., 2013a,b), are characterized by the occurrence of telotrophic ovarioles. The tropharium in the telotrophic ovariole encloses trophocytes and less numerous early previtellogenic oocytes, termed arrested oocytes (except for some scale insects in which only trophocytes are present). The oocytes, which develop in the vitellarium, are connected with the trophic chamber by nutritive cords (i.e. elongated cytoplasmic extensions), which are filled with bundles of microtubules. Comparative studies on hemipteran ovaries have revealed that they exhibit several synapomorphies: (1) the centre of the tropharium is occupied by a branched area, free from germ cell nuclei, termed trophic core that is connected with both trophocytes and oocytes; (2) the trophic core and nutritive cords contain numerous microtubules; (3) in each cluster, more than one oocyte differentiates (Książkiewicz, 1980; Büning, 1985; Książkiewicz-Kapralska, 1985, 1991; Biliński et al., 1990; Büning, 1994; Szklarzewicz and Biliński, 1995; Simiczyjew et al., 1998; Szklarzewicz, 1998a,b; Štys et al., 1998; Niżnik and Szklarzewicz, 2007; Szklarzewicz et al., 2000, 2007, 2009, 2013, 2014; Pyka-Fosciak and Szklarzewicz, 2008; Michalik et al., 2013a,b). The results of these studies have also shown that the ovarioles of hemipterans significantly differ in the organization of their trophic chambers: (1) the tropharium may be composed of individual trophocytes (in scale insects, aphids, whiteflies, cicadomorphans, basal heteropterans) or may be syncytial (in psyllids, fulgoromorphans and advanced heteropterans); (2) the trophocytes may undergo mitotic divisions (in cicadomorphans and most heteropterans) or not divide (in scale insects, aphids, whiteflies, psyllids and some heteropterans). Additionally, there are considerable differences in the number of trophocytes constituting the tropharia: from three in advanced scale insects to several hundred in heteropterans.

In contrast to other groups of hemipterans, the organization and development of the ovaries of psyllids is poorly known. Preliminary studies on ovaries of adult females of three psyllid species: *Rhinocola speciosa* (Aphalaridae), *Psylla alni* (Psyllidae) and *Diaphorina citri* (Liviidae) revealed clear differences from ovaries of other hemipterans: (1) the trophocytes are not polyploid and remain in the 4C-status; (2) nutritive cords are devoid of microtubules; (3) the tropharium is syncytial; (4) the trophic core is strongly reduced (Büning, 1994; Dossi and Consoli, 2014). Except for a short summary on the development of the *Psylla alni* ovary (Büning, 1994), there are no comparative data concerning the development of ovaries of other psyllids. The goal of the current study was to provide a comprehensive description of the ontogeny of ovaries in Psylloidea. We present a detailed description of ovary development in ten species representing four psyllid families: Psyllidae, Aphalaridae, Triozidae and Liviidae.

2. Materials and methods

2.1. Insects

The ovaries of the fifth instar larvae and adult females of ten species were investigated. The selected species represent four families which occur in Poland: Psyllidae, Aphalaridae, Triozidae, Liviidae. The specimens were collected in southern Poland. All of the species are oviparous. *Trioza urticae* (Triozidae) and *Psyllopsis fraxinicola* (Liviidae) have two generations per year, whereas the remaining species have only one generation yearly. The examined species, their host plants and the collection details are listed in Table 1.

2.2. Light and transmission electron microscopy (TEM)

The dissected ovaries (or entire abdomens) of all investigated species were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 months at room temperature. Next, the material was rinsed several times in the phosphate buffer with the addition of sucrose (5.8 g/100 ml) and postfixed in 1% osmium tetroxide in the same buffer (for 1.5 h). After rinsing in water, the ovaries or entire abdomens were dehydrated in a graded series of ethanol (30%, 50%, 70%, 90%, 100%) and acetone. Then, the material was embedded in epoxy resin Epon 812 (Serva, Heidelberg, Germany), and cut into semithin (1 μm thick) or ultrathin (90 nm thick) sections. The semithin sections were stained with 1% methylene blue in 1% borax and analyzed and photographed using light microscopes: Nikon Eclipse 80i and Leica DMR. The ultrathin sections were doubly contrasted with uranyl acetate and lead citrate and then examined and photographed in electron microscopes: JEOL JEM 100 SX (at 80 kV) and JEOL JEM 2100 (at 75 kV).

The number of ovarioles per ovary and the number of arrested oocytes per ovariole were counted using the analysis of serial semithin sections of 10 females of each species.

2.3. Nomarski interference contrast

The isolated ovaries were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h, washed several times in PBS and photographed in Leica DMR light microscope equipped with Nomarski (differential interference contrast) optics.

2.4. Scanning electron microscopy (SEM)

The isolated ovaries were fixed in glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for about 1 month. Next, the material was rinsed twice in PBS, dehydrated in graded series of ethanol and acetone (in the same manner as it was described above), dried in critical-point CO_2, and coated with gold. The ovaries were