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Ovariole structure in viviparous and oviparous generations of *Glyphina betulae* (Linnaeus 1758) (Insecta, Hemiptera, Aphidinea: Thelaxidae)

Marta Kot

Department of Developmental Biology and Morphology of Invertebrates,
Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland
marta.kot@uj.edu.pl

ABSTRACT

Glyphina betulae (Linnaeus 1758) is a representative of the advanced aphid family Thelaxidae. The ovaries of the viviparous generations are composed of five telotrophic - meroistic ovarioles, whereas ovaries of the oviparous generation contain four ovarioles. Each ovariole consists of a terminal filament, tropharium (trophic chamber), vitellarium and ovariolar stalk. The tropharium houses individual trophocytes and arrested oocytes. The vitellaria of oviparous and viviparous females contain single oocytes. The ovarioles of viviparous generations contain 32 germ cells (trophocytes, arrested oocytes and developing oocyte), whereas in oviparous generations the number of germ cells is not stable and ranges from 55 to 90. Numerous embryos develop within the body of viviparous females. The ovaries are accompanied by large cells termed bacteriocytes. The bacteriocyte cytoplasm is packed with endosymbiotic microorganisms. The endosymbionts are transmitted from one generation to the next transovarially.

KEY WORDS: aphids, *Glyphina betulae*, ovarioles, oocytes, trophocytes, endosymbiotic microorganisms

INTRODUCTION

Ovaries of insects are paired organs located in the abdomen. The ovaries are composed of tubular units termed ovarioles. There are two categories of ovaries:

panoistic and meroistic (see e.g. Büning, 1985; Biliński, 1998 for further details). Like in other hemipterans, ovaries of aphids are composed of meroistic ovarioles of a telotrophic type (Büning, 1985). In individual ovariolae of aphids, four elements are distinguished: a terminal filament, tropharium (trophic chamber), vitellarium and ovariolar stalk (Büning, 1985; Szklarzewicz *et al.,* 2000; Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2011). The tropharium is located in the anterior part of the ovariole and contains numerous individual trophocytes (nurse cells) and early previtellogenic oocytes (arrested oocytes). The main function of trophocytes is to synthesize different kinds of RNAs that are next transferred to the developing oocyte (Büning, 1985). The central part of the trophic chamber occupies a trophic core. The vitellarium contains a small number of oocytes. Each oocyte is surrounded by a single layer of follicular cells that are responsible for the synthesis of precursors of egg envelopes. The ovariolar stalk joins the ovarioles with lateral oviduct. Extensive studies on ovaries of primitive (Szklarzewicz *et al.,* 2000; 2009) and advanced aphids (Büning, 1985; Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2010; 2011) have shown that the number of all germ cells (oocytes + trophocytes) constituting ovarioles is variable. Moreover, in advanced aphids, the course of oogenesis in viviparous generations significantly differs from the course of this process in oviparous generation (Büning, 1985; Pyka-Fościak & Szklarzewicz, 2008; Michalik*,* 2011).

In contrast to other aphid families, the organization of ovaries in representatives of the family Thelaxidae is poorly known, because there is only one observation concerning the ovaries in viviparous generations of *Thelaxes dryophila* (Schrank 1801) (Michalik, 2011). Since the ovary structure in oviparous females of representatives of the family Thelaxidae is not known, the present study was undertaken to provide further information about the anagenesis of ovaries of aphids.

MATERIAL AND METHODS

G. betulae is a widely distributed pest of the birch. It feeds on young shoots of *Betula pendula* Roth *(= B. verrucosa* Ehrh.) and *Betula pubescens* Ehrh. It is a holocyclic and monoecious species. Adult females of viviparous and oviparous generations of *G. betulae* were collected in June in the Nowotarska Valley. The dissected abdomens of five viviparous females and one oviparous female were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature, rinsed in the 0.1 M phosphate buffer with the addition of sucrose and postfixed in 1% osmium tetroxide. Subsequently, the material was rinsed in water and dehydrated in a graded series of alcohol and acetone. Finally, the material was embedded in epoxy resin Epox 812 (Fullam Inc., Latham, N.Y., USA). Semithin sections (0.7 µm thick) were stained with 1% methylene blue and examined and photographed through a Nicon Eclipse 80i microscope. Ultrathin sections (90 nm thick) were contrasted with uranyl acetate and lead citrate and analysed under a JEM 100 SX transmission electron microscope at 80 kV.

RESULTS

The paired ovaries in oviparous females of *G. betulae* consist of 4 telotrophic ovarioles (Fig. 1). In females of viviparous generations, ovaries are composed of 5 ovarioles (Fig. 6). Each ovariole consists of four elements: a terminal filament (not shown), tropharium (Figs. 1, 6), vitellarium (Figs. 1, 6) and ovariolar stalk (pedicel) (not shown). The tropharia are of spherical shape and contain individual trophocytes and arrested oocytes (Fig. 1). The latter are localized in the posterior region of the trophic chamber (Fig. 1). The trophocytes contain huge, spherical nuclei with giant nucleoli (Figs. 1, 6). Analysis of serial sections of 10 ovarioles has shown that ovarioles of viviparous females contain 32 germ cells: 24 trophocytes and 8 oocytes. In ovarioles of oviparous females, this number is not fixed and varies from 55 to 90 cells (47-81 trophocytes and 7-9 oocytes). In the vitellaria of viviparous females as well as oviparous females, single oocytes develop (Figs. 1, 6). In both generations, the developing oocyte is connected with the trophic chamber by a nutritive cord which in ovarioles of oviparous females is very broad (Fig. 1). The developing oocyte is surrounded by a monolayered follicular epithelium (Figs. 1, 2). The follicular cells do not undergo differentiation into distinct subpopulations (Figs. 1, 2). In oviparous generation, yolk granules and lipid droplets are accumulated in the oocyte cytoplasm (Fig. 2), whereas in the oocytes of viviparous females, only lipid droplets are present (Fig. 7). In viviparous generations, shortly after termination of the previtellogenesis, the oocyte nucleus undergoes a series of mitotic divisions that lead to the formation of embryos (Fig. 7). As a consequence, in the abdomen of adult viviparous females, numerous embryos occur (Figs. 7-10). In the body of these embryos, the next generations of embryos develop (Figs. 7, 10). In the very young embryos, cells located in the posterior pole differentiate into bacteriocytes (Fig. 9). Next, the bacteriocyte cytoplasm is invaded by symbiotic microorganisms. In the older embryos, the bacteriocyte cytoplasm is tightly packed with symbiotic bacteria (Fig. 8). The bacteria reproduce by binary fission (Fig. 5). In the body of *G. betulae,* two types of bacteriocytes occur: numerous bacteriocytes containing primary symbiont - bacterium *Buchnera aphidicola* (Figs. 2-5) and less numerous bacteriocytes filled with small coccoid bacteria - secondary symbionts (Figs. 2, 3, 5). Both kinds of bacteriocytes form large structures called bacteriomes that are localized in the close vicinity of the ovaries (Figs. 2, 3).

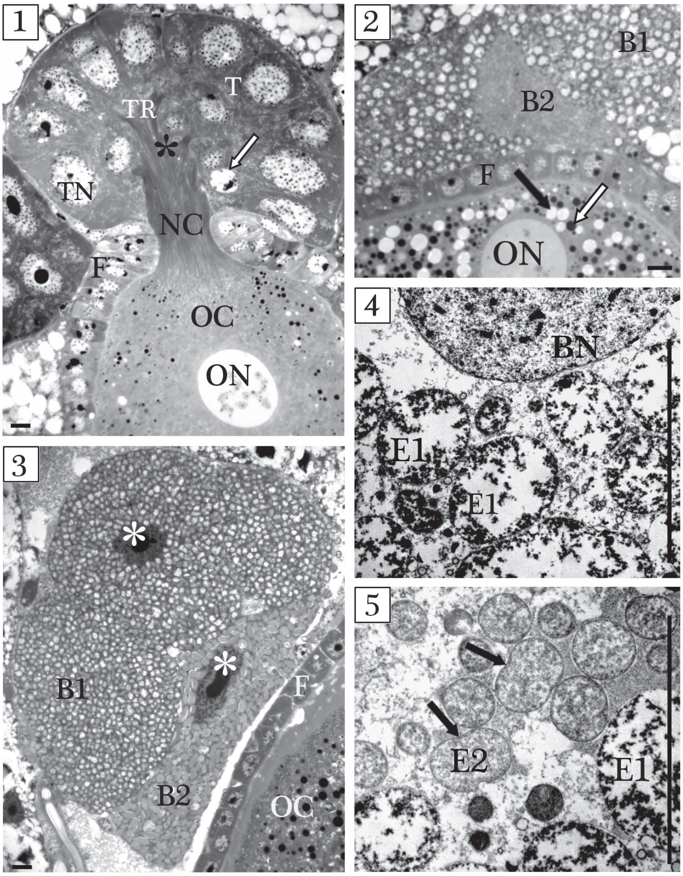

Figure 1. Longitudinal section thorough the ovariole of an oviparous female; arrow - arrested oocyte; asterisk - trophic core. Methylene blue. Bar = 10 µm. **Figure 2.** Fragment of the oocyte in an oviparous female. In the oocyte cytoplasm, lipid droplets (black arrow) and yolk granules (white arrow) are accumulated. In the close vicinity of the ovary, a bacteriome is localized. Methylene blue. Bar = 10 µm. **Figure 3**. Bacteriome containing two kinds of bacteriocytes in the neighborhood of the ovary of an oviparous female. Methylene blue. Bar = 10 µm. **Figure 4.** Fragment of the bacteriocyte containing bacterium *Buchnera aphidicola*. TEM. Bar = 1 µm. **Figure 5.** Fragment of the bacteriocyte filled with small, coccoid bacteria (secondary symbionts) and bacteriocyte containing bacterium *Buchnera aphidicola*; arrows - dividing bacteria. TEM. Bar = 10 µm.

Key to figures. B1 - bacteriocyte containing bacterium *Buchnera aphidicola*; **B2** - bacteriocyte with coccoid bacteria; **BN, white asterisks** - bacteriocyte nucleus; **E1** - bacterium *Buchnera aphidicola*; **E2** - small, coccoid bacteria (secondary endosymbiont); **F** - follicular epithelium; **NC** - nutritive cord; **OC** - oocyte; **ON** - oocyte nucleus; **T** - trophocyte; **TN** - trophocyte nucleus; **TR** - tropharium.

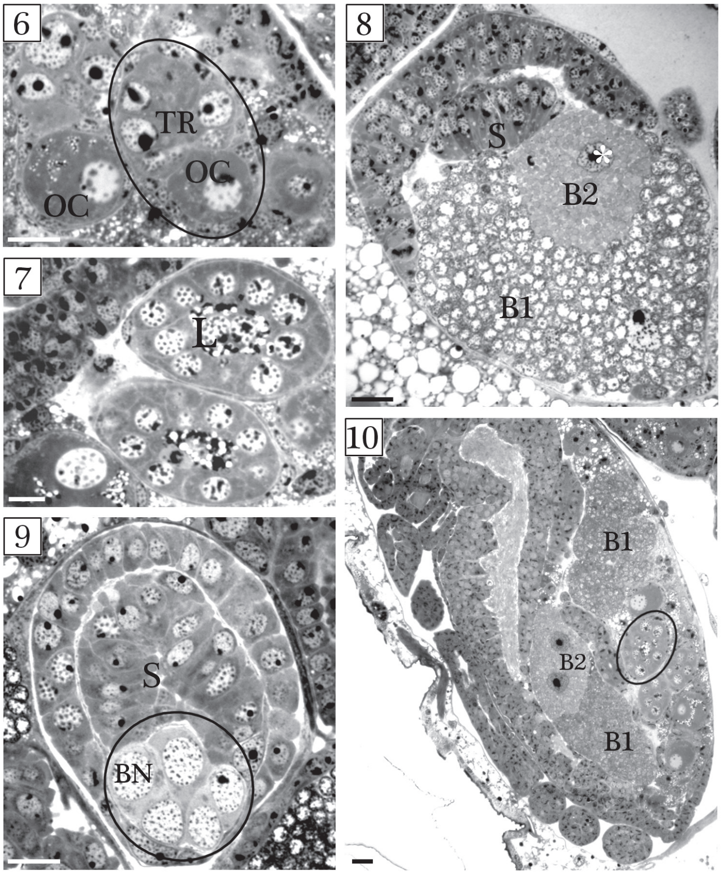

Figure 6. Fragment of the ovary of a viviparous female; **encircled** - ovariole. Methylene blue. Bar = 10 μm. **Figures 7-10.** Embryos within the body of a viviparous female in different stages of development. Methylene blue. Bar = 10 μm. **Figure 8.** The embryo containing two kinds of bacteriocytes. Methylene blue. Bar = 10 μm. **Figure 9**. Bacteriocyte (encircled) localized in the posterior pole of the embryo where the bacteriocytes are localized. Methylene blue. Bar = 10 μm. **Figure 10.** The older embryo containing the next generations of embryos (encircled). Methylene blue. Bar = 10 μm.

Key to figures: B1 - bacteriocyte containing bacterium *Buchnera aphidicola*; **B2** - bacteriocyte with coccoid bacteria (secondary endosymbiont); **BN, white asterisks** - bacteriocyte nucleus; **OC** - oocyte; **S** - somatic cells; **TR**- tropharium;

DISCUSSION

Previous studies on aphid ovaries concerned the advanced families Aphididae: *Metopolophium dirhodum* (Walker 1848), *Longicaudus trirhodus* (Walker 1848), *Macrosiphum rosae* (Linnaeus 1758), *Macrosiphum euphorbiae* (Thomas 1878) and Drepanosiphidae: *Drepanosiphum platanoides* (Schrank 1801) (Büning, 1985) and primitive, oviparous families Adelgidae and Phylloxeridae (Szklarzewicz *et al.,* 2000; 2009). Present studies have shown that the general structure of ovaries of *G. betulae* is similar to the ovaries of other advanced aphids (Büning, 1985; Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2010; 2011). It has also been confirmed that the main difference between ovaries of advanced and primitive aphids concerns the number of germ cells per ovariole (Szklarzewicz *et al.,* 2000; 2009). Szklarzewicz *et al.* (2000) have shown that for ovarioles of all generations of primitive aphid families, a relatively large number of germ cells is characteristic, e.g. in ovarioles of *Adelges laricis* (Vallot 1836) (Adelgidae) up to 90 germ cells may occur. Büning (1985) examined ovaries of aphids belonging to the most advanced families Aphididae and Drepanosiphidae and showed that all their generations have the same and stable number of 32 germ cells per ovariole. The similar situation has been observed in viviparous females in *Stomaphis quercus* (Linnaeus 1758) (Lachnidae) (Pyka-Fościak & Szklarzewicz, 2008), *Anoecia corni* (Fabricius 1775) (Anoeciidae) (Michalik, 2011), *Prociphillus fraxini* (Fabricius 1777) (Eriosomatidae) (Michalik, 2011) and in representatives of the family Thelaxidae: *G. betulae* (this study) and *T. dryophila* (Michalik, 2011).

In contrast to viviparous generations, the oviparous generations of advanced aphids are characterized by a variable and larger number of germ cells per ovariole (Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2011), e.g. in *S. quercus* (Lachnidae), ovarioles of oviparous females consist of 45-60 germ cells (Pyka-Fościak & Szklarzewicz, 2008), in *A. corni* (Anoeciidae) from 115 to 192 (Michalik, 2011), in *P. fraxini* (Eriosomatidae) from 70 to 139 (Michalik, 2011). Thus, obtained results confirm the hypothesis of Szklarzewicz *et al*. (2000; 2009) and Michalik (2011) that in aphids, a tendency to reduce the number of germ cells per ovarioles (Szklarzewicz *et al.,* 2000) took place. According to Michalik (2011), the large number of germ cells in individual ovariole represents an ancestral condition within aphids.

The telescopic development of embryos is characteristic for viviparous generations of *G. betulae*. This peculiar phenomenon has also been observed in other viviparous aphids (Büning, 1985; Dixon, 1987; Moran, 1992; Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2011). According to Moran (1992), this strategy of reproduction allows aphids to increase their population in a short period of time.

Aphids, like other plant sup-sucking hemipterans, harbor obligate intracellular bacterial symbionts (see e.g. Buchner, 1965; Douglas, 1989; Baumann, 2005 for further details). These endosymbionts are housed in large cells termed bacteriocytes (Buchner, 1965; Douglas, 1989; Baumann, 2005). In advanced aphids, two types of endosymbionts may occur (Buchner, 1965). Primary endosymbionts (P-symbionts) belong to the genus *Buchnera* (Baumann, 2005). Primary symbionts are always present in the bodies of host insects and are necessary for their growth and development (Buchner, 1965; Baumann, 2005). The bacterium *Buchnera* is responsible for synthesis of the nutrients missing in the diet of aphids (Douglas, 1989; Baumann, 2005). Apart from bacterium *Buchnera,* aphids may have secondary symbionts (S-symbionts). The role of S-symbionts is still unclear (Bauman, 2005). The endosymbiotic microorganisms are transmitted to the next generation transovarially (Buchner, 1965).

Present studies have shown that in the bodies of *G. betulae* females, two kinds of endosymbiotic microorganisms occur. In the bodies of examined species, apart from the bacterium *Buchnera aphidicola,* other small, coccoid bacteria are present. The function of these bacteria (S-symbionts) remains unknown, however, their presence in all examined specimens and their transovarial transmission suggest that they play an important role for the host insect. It should be noted that within aphids only in representatives of the family Phylloxeridae, endosymbionts do not occur (Szklarzewicz *et al.*, 2009), because phylloxerids feed on parenchyma that contain all essential nutrients. Similar to other hemipterans, the endosymbionts in aphids are transmitted transovarially (vertically) to the host progeny, through the female germ cells (Buchner, 1965; Szklarzewicz *et al.,* 2000; Michalik, 2011). In viviparous generations, embryos are infected, whereas in oviparous generations, endosymbionts enter into the oocyte (Pyka-Fościak & Szklarzewicz, 2008; Michalik, 2011).

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Budowa owariol żyworodnych i jajorodnych pokoleń *Glyphina betulae* (Linnaeus 1758) (Insecta, Hemiptera, Aphidinea: Thelaxidae)

STRESZCZENIE

Przedmiotem badań były jajniki żyworodnych i jajorodnych pokoleń mszycy *Glyphina betulae* (Linnaeus 1758) należącej do rodziny Thelaxidae. Pojedynczy jajnik żyworodnych samic zbudowany jest z 5, a jajorodnych samic z 4 owariol typu meroistycznego - telotroficznego. W każdej owarioli można wyróżnić filament terminalny, trofarium, witelarium oraz nóżkę owarialną (pedicel). W trofarium znajdują się trofocyty, które zachowują swoją indywidualność oraz oocyty wczesnoprewitelogeniczne, zwane oocytami zatrzymanymi w rozwoju. Centralną część komory odżywczej zajmuje rdzeń odżywczy. Trofocyty kontaktują się z rdzeniem za pomocą wypustek cytoplazmatycznych, a oocyty za pośrednictwem sznura odżywczego. Liczba komórek płciowych w owariolach samic żyworodnych wynosi 32 (24 trofocyty i 8 oocytów). Owariole samic jajorodnych zawierają od 55 do 90 komórek płciowych. W witelarium rozwija się pojedynczy oocyt otoczony jednowarstwowym nabłonkiem folikularnym. W ciele żyworodnych samic rozwijają się liczne zarodki, wewnątrz których wzrasta kolejne pokolenie zarodków („zarodek w zarodku"). W jamie ciała *G. betulae* znajdują się także liczne bakteriocyty zawierające dwa rodzaje endosymbiotycznych mikroorganizmów: duże i bardziej liczne bakterie z rodzaju *Buchnera* oraz mniejsze bakterie kuliste. Bakterie endosymbiotyczne przekazywane są do następnego pokolenia na drodze transowarialnej.