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Ovary structure and transovarial transmission of endosymbiotic microorganisms in *Clethrobius comes*, *Myzocallis walshii* and *Sipha maydis* /Hemiptera, Aphididae: Drepanosiphinae/

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Abstract

We examined ovary structure and transovarial transmission of endosymbiotic microorganisms in aphid subfamily Drepanosiphinae: *Clethrobius comes* (Walker, 1848)*, Myzocallis walshii* (Monell in Riley& Monell, 1979) and *Sipha maydis* (Passerini, 1860). Ovaries of examined aphids consist of 4 or 5 telotrophic ovarioles. In each ovariole one can distinguish: terminal filament, tropharium, vitellarium and pedicel. The tropharium contains nurse cells (trophocytes) and oocytes early previtellogenic also called arrested oocytes. The central part of the tropharium is taken by trophic core which is connected with trophocytes by means of processes. Oocytes develope in the vitellarium, and they are surrounded by one-layered follicular epithelium. Oocytes communicate with troparium via nutrtive cords, which run in the depression of oocyte cytoplasm in subfamily Drepanosiphinae. The number of germ cells per ovariole is constant and amounts to 32 cells in the viviparous generations of examined aphids and oviparous generations of *Myzocallis walshii*, whereas in the oviparous generations of *Clethrobius comes* and *Sipha maydis* this number is variable and much larger (i.e. – 42-60 and – 31-49, respectively). In the body cavity of examined spacies endosymbiotic microorganisms are present. Endosymbionts are transovarially transmitted from mother to the offspring. In the oviparous generations bacteria infect choriogenic oocytes, whereas in the viviparous generations endosymbionts infest young embryos.

Introduction

The paired ovaries of insects are composed of functional units termed ovarioles. The ovariole number is usually species specific (MATSUDA, 1976). Traditionally, two types of ovarioles are recognized: panoistic and meroistic. In panoistic ovarioles only oocytes arise, whereas in meroistic ones germ cells differentiate into oocytes and trophocytes (=nurse cells). The trophocytes do not transform into functional gametes. The basic function of these cells is to synthesize macromolecules (rRNA, mRNA). Within meroistic ovarioles two types have been distinguished: polytrophic and telotrophic. In the polytrophic ovariole several units termed egg chambers occur. Each egg chamber consists of a single oocyte and several trophocytes. In telotrophic ovarioles all trophocytes occupy its apical region forming the tropharium (=trophic chamber) (see e.g. BÜNING, 1994; BILIŃSKI, 1998 for further details). In aphids, like in other hemipterons, ovaries consist of telotrophic ovarioles (BÜNING, 1985; SZKLARZEWICZ *et al.*, 2000; PYKA-FOŚCIAK & SZKLARZEWICZ, 2008; SZKLARZEWICZ *et al.*, 2009). The individual ovariole is differentiated into a terminal filament, tropharium, vitellarium and ovariolar stalk (pedicel). Tropharium contains individual trophocytes and previtellogenic oocytes (=arrested oocytes). The vitellarium is composed of a small number of oocytes. Studies on aphid ovaries have revealed that in advanced aphid subfamily Aphidinae ovarioles may contain 32 germ cells (e.g. *Metopolophium dirhodum* 24 trophocytes and 8 oocytes) (BÜNING, 1985), whereas in the primitive aphid families this number is much larger, not constant, and ranges from 50 to 92 in family Adelgidae (SZKLARZEWICZ *et al.*, 2000) and from 49 to 64 in family Phylloxeridae (SZKLARZEWICZ *et al.*, 2009). According to SZKLARZEWICZ *et al.* (2000), during aphid phylogeny, like in scale insects, the gradual reduction of the number of germ cells per ovariole took place.

In the body cavity of most aphids and other hemipterans endosymbiotic microorganisms are present. The endosymbionts can be prokaryotic or eukaryotic (BUCHNER, 1965; SZKLARZEWICZ & MOSKAL, 2001; ISHIKAWA, 2003). There are two categories of endosymbionts: primary endosymbionts (P-endosymbionts) and secondary endosymbionts (S-endosymbionts) (BUCHNER, 1965; ISHIKAWA, 2003). The primary endosymbionts are necessary for the survival and reproduction of the host insect because they provide them with essential amino acids. The role of the secondary endosymbionts remains still unknown. However, recent studies have shown that aphids which in the body cavity possess secondary endosymbionts are more resistant to heat stress (MONTLOR *et al.*, 2002) as well to an attack of parasitic hymenopterans (SCARBOROUGH *et al.*, 2005).

Material and methods

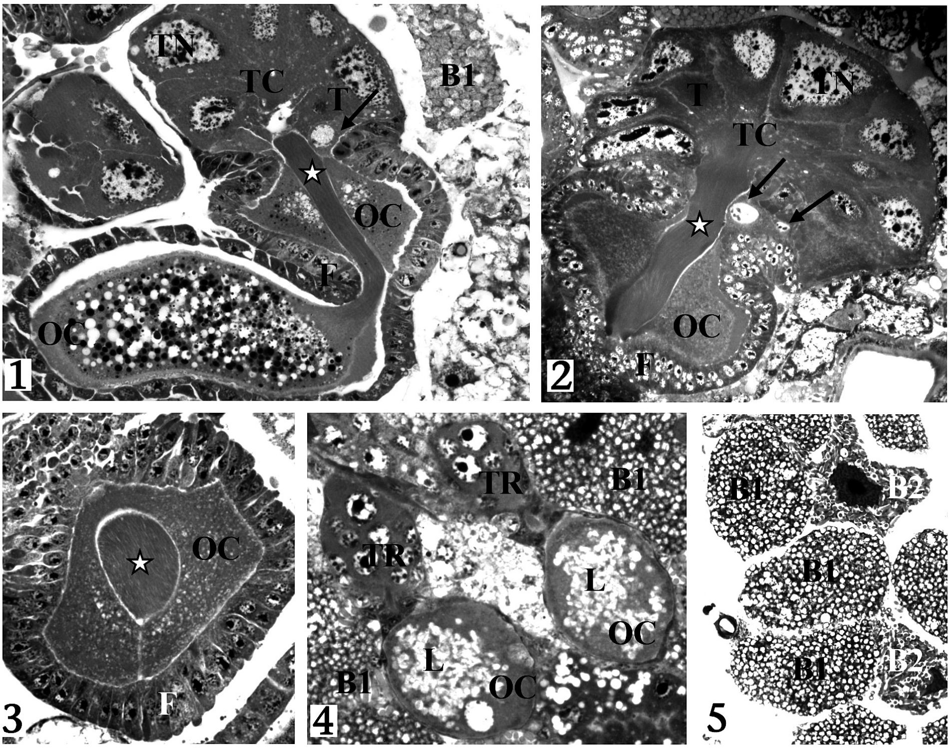
Adult females of *Clethrobius comes* were collected in Katowice. Viviparous generations were collected in April and August 2009, oviparous generations in September 2005 and October 2008. Females of *Sipha maydis* were collected in October 2009 in Zawada (near Dąbrowa Górnicza) (viviparous generation) and September 2009 in Dąbrowa Górnicza (oviparous generation). Oviparous females of *Myzocallis walshii* were collected in October 2005 in Katowice. The collected insects were fixed in 2.5% glutaraldehyde in 0.1 M phosphorate buffer (pH 7.4) at room temperature, rinsed in the phosphate buffer with addition of sucrose and postfixed in 1% osmium tetroxide. Next the material was dehydrated in a series of ethanols and acetone and embedded in epoxy resin Epox 812 (Fullam Inc., Latham, N.Y., USA). Semithin sections were stained with 1% methylene blue in 1% borax and photographed in a Jenalumar (Zeiss Jena) microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in JEM 100 SX electron microscope at 80 kV.

Results

In oviparous and viviparous generation of *Clethrobius comes* and oviparous generation of *Myzocallis walshii* ovaries are composed of 5 ovarioles of telotrophic type, whereas in both generations of *Sipha maydis* they consist of 4 ovarioles. In each ovariole a terminal filament, tropharium, vitellarium and pedicel can be distinguished (Figs. 1, 2, 4). The tropharium houses individual trophocytes and early previtellogenic oocytes termed arrested oocytes. The latter are located at the basal part of the tropharium (Figs. 1, 2). The analysis of semithin section of 10 ovarioles has shown in viviparous generations of *Clethrobius comes* and *Sipha maydis* as well as in the oviparous females of *Myzocallis walshii* a stable number of 32 germ cells per ovariole (in *Cletrhrobius comes* 24 trophocytes and 8 oocytes, in *Sipha maydis* 22 trophocytes and 10 oocytes and in *Myzocallis walshii* 16 trophocytes and 16 oocytes). In ovarioles of oviparous generations of *Clethrobius comes* and *Sipha maydis* the number of germ cells is not constant and ranges from 31 to 49 in *Sipha maydis* (18-35 trophocytes and 6-15 oocytes) and from 42 to 60 in *Clethrobius comes* (30-46 trophocytes and 12-14 oocytes).

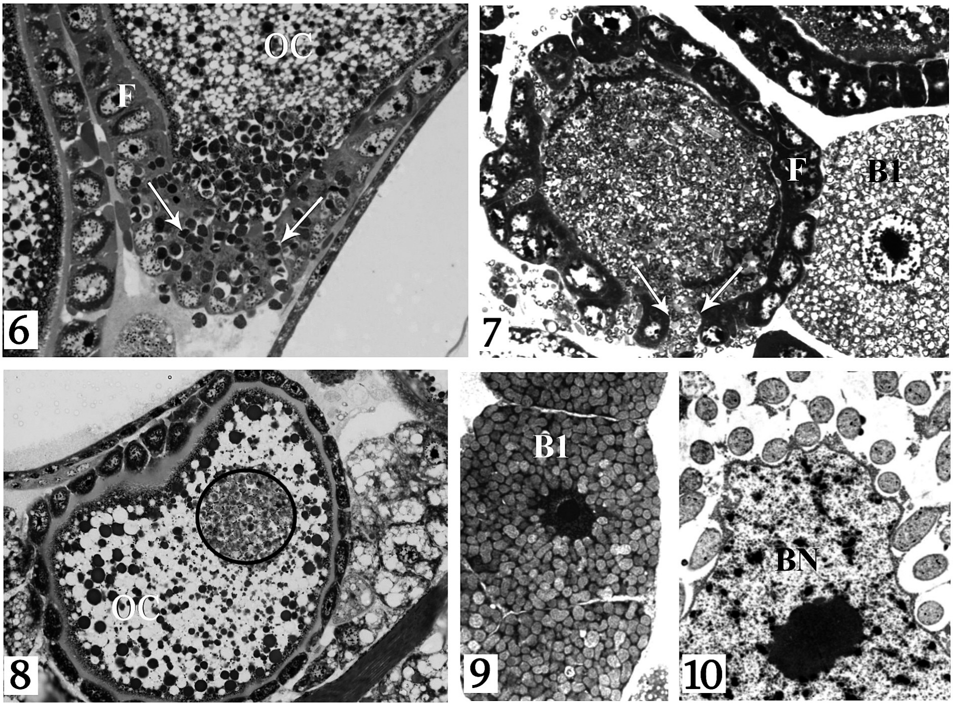
The central region of the tropharium is occupied by a trophic core (Figs. 1, 2) which is connected both with trophocytes and with oocytes (arrested oocytes and oocytes in the vitellarium). The vitellarial oocytes communicate with the trophic core by means of broad nutritive cords (Figs. 1, 2). The nutritive cord runs in the deep depression of the oocyte cytoplasm (Figs. 1, 2, 3). Both trophic core and nutritive cords are tightly packed with microtubules (not shown).

Trophocytes contain large, lobated nuclei (Figs. 1, 2). The vitellaria of adult females may contain 1-3 linearly arranged oocytes that are surrounded by a one-layered follicular epithelium. Follicular cells do not undergo diverification into subpopulations (Figs. 1, 2, 3, 6). In oviparous generations the vitellarial oocytes develop through three stages: previtellogenesis, vitellogenesis and choriogenesis. During vitellogenesis oocytes accumulate reserve substances: yolk granules and lipid droplets (Figs. 1, 8). In viviparous generations soon after previtellogenesis, the oocyte nucleus starts to divide to form the embryo. In the oocytes of viviparous females only lipid droplets are accumulated (Fig. 4).



Key to figures: B1 – bacteriocyte containing bacterium *Buchnera aphidicola*; **F** – follicular epithelium; **L** – lipid droplets; **OC** – oocyte; **T** – trophocyte; **TC** – trophic core; **TN** – trophocyte nucleus; **TR** – tropharium; **arrow** – arrested oocytes; **asterisk** – nutritive cord

Figure 1. *Myzocallis walshii.* Oviparous generation. Longitudinal section through the ovariole. Methylene blue, X 230. **Figure 2.** *Clethrobius comes*. Oviparous generation. Longitudinal section through the ovariole. Methylene blue, X 350. **Figure 3**. *Clethrobius comes*. Oviparous generation. Cross section through the oocyte. Methylene blue, X 620. **Figure 4**. *Sipha maydis*. Viviparous generation. Longitudinal section through the ovariole. Methylene blue, X 730. **Figure 5**. *Sipha maydis*. Oviparous generation. Bacteriocytes containing primary (B1) and secondary endosymbionts (B2). Methylene blue, X 200.



Key to figures: B1 – bacteriocyte containing bacterium *Buchnera aphidicola*; **BN** – bacteriocyte nucleus; **F** – follicular epithelium; **OC** – oocyte; **arrow** – arrested oocytes

Figure 6. *Clethrobius comes*. Oviparous generation. Fragment of the ovariole. Bacteria (arrows) invade cytoplasm of the posterior pole of the oocyte. Methylene blue, X 630. **Figure 7.** *Sipha maydis*. Oviparous generation. Cross section through the posterior pole of the oocyte. Primary and secondary endosymbionts migrate to the oocyte between neighbouring follicular cells (arrows). Methylene blue, X 700. **Figure 8**. *Myzocallis walshii*. Oviparous generation. Longitudinal section through the oocyte. Endosymbiotic microorganisms encircled. Methylene blue, X 300. **Figure 9**. *Clethrobius comes*. Oviparous generation. Fragment of the bacteriocyte containing bacterium *Buchnera aphidicola* (B1). Methylene blue, X 380. **Figure 10**. *Sipha maydis*. Oviparous generation. Fragment of the bacteriocyte filled with secondary endosymbionts (B2). TEM, X 1900.

The gonads are surrounded by large cells termed bacteriocytes (Figs. 4, 7). The cytoplasm of these cells is filled with endosymbiotic bacteria (Figs. 5, 7, 9). In the body of *Clethrobius comes* and *Myzocallis walshii* only primary endosymbionts *Buchnera aphidicola* occur (Fig. 9), whereas in *Sipha maydis* apart from P-endosymbionts the secondary endosymbionts are present (Figs. 5, 10). The endosymbiotic microorganisms are transovarially transmitted from one generation to the next. In oviparous generations bacteria invade choriogenic oocytes (Figs. 6, 7). The endosymbionts migrate into the perivitelline space (i.e. space between the oocyte and follicular epithelium) beetwen neighbouring follicular cells

(Fig. 7) or transverse their cytoplasm (Fig. 6). Then, they enter the perivitelline space and gather in the cytoplasm of the posterior pole of the oocyte (Fig. 8). In viviparous generations endosymbionts infest embryos (not shown).

Discussion

The obtained results have shown that the number of germ cells constituting the ovarioles in members of the subfamily Drepanosiphinae is not stable. BÜNING (1985) reported that in *Drepanosiphum platanoidis* both in viviparous and oviparous females ovarioles contain 32 germ cells. Thus, in *Drepanosiphum platanoidis* the total number of germ cells per ovariole is not fixed and follows the $N=2^n$ rule (where N = total number of germ cells, n = number of divisions of germ cells). Our results indicated that only in viviparous generations of *Clethrobius comes* and *Sipha maydis* and oviparous generation of *Myzocallis walshii* the ovarioles consist of 32 germ cells, while in oviparous generations of *Sipha maydis* and *Clethrobius comes* ovarioles contain larger number of germ cells. JAGLARZ (1992) suggested that the exceptions to the $N=2^n$ rule in insect ovaries may be a consequence of a lack of synchrony of mitotic divisions or degeneration of some germ cells. It should be noted that similar situation as in ovarioles of *Sipha maydis* and *Clethrobius comes* has also been observed in *Stomaphis quercus* belonging to the subfamily Lachninae (PYKA-FOŚCIAK & SZKLARZEWICZ, 2008). The ovarioles of viviparous females of *Stomaphis quercus* contain 32 germ cells, whereas ovarioles of oviparous females consist of 45-60 germ cells. Since ovarioles of primitive families of aphids as Phylloxeridae and Adelgidae (see Introduction) contain larger and not fixed number of germ cells, it may be speculated that the large number of germ cells represents ancestral condition within aphids.

Like in other so far examined aphids, the trophocytes constituting the trophic chamber are characterized by the presence of large nuclei. Such ultrastructure is connected with the synthetic activity of these cells. The most characteristic feature of the ovarioles of examined species is the unusual location of the nutritive cords. In oviparous generations of *Myzocallis walshii*, *Cletrhrobius comes* and *Sipha maydis* nutritive cords are situated in the depression of oocyte cytoplasm, while in other so far examined aphids (as well as in other insects possessing telotrophic ovarioles) the nutritive cords run in the perivitelline space (SZKLARZEWICZ *et al.*, 2000; PYKA-FOŚCIAK & SZKLARZEWICZ, 2008; SZKLARZEWICZ *et al.*, 2009). Since such location of nutritive cord has not been observed in other aphids, it may be suggested that this character may represent synapomorphy of the Drepanosiphinae subfamily.

Endosymbiotic microorganisms have been described in many insect species (BUCHNER, 1965; ISHIKAWA, 2003). The presence of endosymbionts is connected with the restricted diet of host insect, e.g. the diet of plant sap-sucking hemipterans is poor in essential amino acids. It is known that endosymbiotic microorganisms provide their hosts with essential amino acids which are absent in phloem sap of plants (DOUGLAS, 1989). Numerous ultrastructural as well as molecular investigations have shown that advanced aphids harbor the primary endosymbionts belonging to the species *Buchnera aphidicola* (DOUGLAS, 1989; 1998). Some aphids, e.g. *Sipha maydis* besides *Buchnera aphidicola* possess secondary endosymbionts. In contrast to advanced aphids, in members of the primitive family Phylloxeridae that feed on parenchyma (which contains all the essential nutrients) endosymbionts do not occur (SZKLARZEWICZ *et al.*, 2009).

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Struktura jajnika oraz transowarialny przekaz endosymbiotycznych mikroorganizmów u *Clethrobius comes*, *Myzocallis walshii* i *Sipha maydis* /Hemiptera, Aphididae: Drepanosiphinae/

Streszczenie

Przedmiotem badań była organizacja jajnika oraz transport endosymbiotycznych mikroorganizmów u mszyc z podrodziny Drepanosiphinae (*Clethrobius comes*, *Myzocallis walshii* i *Sipha maydis*). Jajniki badanych mszyc zbudowane są z 4 lub 5 owariol typu meroistycznego-telotroficznego. W każdej owarioli można wyróżnić: filament terminalny, trofarium, witelarium oraz pedicel. W trofarium znajdują się komórki odżywcze (trofocyty) oraz oocyty wczesnoprewitelogeniczne zwane oocytami zatrzymanymi w rozwoju. Centralną część trofarium zajmuje rdzeń odżywczy, z którym trofocyty łączą się za pomocą wypustek cytoplazmatycznych.

W witelarium rozwijają się oocyty otoczone jednowarstwowym nabłonkiem folikularnym. Kontakt oocytów z trofarium zapewniają sznury odżywcze, które u mszyc z podrodziny Drepanosiphinae biegną w zagłębieniu cytoplazmy oocytu. Liczba komórek płciowych w owariolach mszyc z pokoleń żyworodnych oraz pokolenia jajorodnego *Myzocallis walshii* jest stała i wynosi 32 komórki, natomiast owariole mszyc z pokoleń jajorodnych cechują się znacznie wyższą liczbą komórek w gronie (*Clethrobius comes* – od 42 do 60, *Sipha maydis* – od 31 do 49). W jamie ciała badanych gatunków mszyc obecne są endosymbiotyczne mikroorganizmy. Endosymbionty są przekazywane z organizmu matki do potomstwa na drodze transowarialnej. W pokoleniu jajorodnym bakterie infekują oocyty późnowitelogeniczne, natomiast w pokoleniu żyworodnym infekcji ulegają zarodki we wczesnym stadium rozwoju.