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Ovarian Structure and Oogenesis of the Webspinner *Oligotoma japonica* (Embioptera, Oligotomidae)

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Abstract Ovarian structure and oogenesis of the webspinner *Oligotoma japonica* OKAJIMA are described by using light microscopy. The ovary consists of 5 pairs of panoistic ovarioles connecting with each lateral oviduct regularly arranged. The ovarial ligament of each ovarioles is attached to the dorsal blood vessel. An ovariole contains a linear array of 8 developing oocytes in different stages. Anterior 5 oocytes (1–5) show previtellogenic slow growth (stage 1). The fatty yolk globules appear in the ooplasm of the 6th oocyte (stage 2). In the 7th oocyte, the deposition of proteid yolk begins within the cortical and subcortical ooplasm (stage 3). Fully grown or the 8th oocyte is at the posterior end of the ovariole, and the vitelline membrane and chorion are produced on the oocyte surface by surrounding follicular cells (stages 4–5).

Key words: Embioptera; Oligotomidae; ovarian structure; panoistic type; oogenesis.

Introduction

The Embioptera belonging to the orthopteroid group of insects form a small order, with about 300 species recorded in the world (RICHARDS & DAVIES, 1977). The Japanese webspinner *Oligotoma japonica* OKAJIMA is found in the Kyushu district.

It is well known that orthopteroid orders except for the Dermaptera have the panoistic ovarioles (BONHAG, 1958; KING & BÜNING, 1985), and the Embioptera have been considered to have panoistic ovarioles (RICHARDS & DAVIES, 1977; KING & BÜNING, 1985). There has been only one study by MUKERJI (1927) on the ovarian structure of the Indian webspinner *Embia minor*, but descriptions of its oogenesis were omitted.

Our paper describes anatomical and histological features of the ovary and the oogenesis of *Oligotoma japonica*.

Materials and Methods

Gravid females of *Oligotoma japonica* OKAJIMA reared (NAGASHIMA *et al.*, 1991) in the laboratory were used. The insects were dissected in a fixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde, buffered at pH 7.5 with sodium cacodylate. Whole ovaries removed from the insects were observed with a stereoscopic microscope.

For histological observation, the ovaries were prefixed in the same fixative for 4 hrs at room temperature. Then the ovaries were washed thoroughly in0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide solution, buffered at pH 7.5 with sodium cacodylate for 2 hrs at room temperature. After usual dehydration by ethanol, the ovaries were embedded in Epon 812. Semi-thin sections (0.75 μm), cut with glass knives on the Reichert OMU-2 ultramicrotome, and stained with Azur B, were observed with a bright field light microscope.

Results and Discussion

1. *Gross morphology of the ovary*

The ovaries of *Oligotoma japonica* lie on both sides of the alimentary canal in the abdomen. An ovary consists of 5 ovarioles (Fig. 1), each enclosed in an epithelial sheath (Figs. 2–3). All ovarioles of *O. japonica* are arranged segmentally and each of them opens separately into the inner side of the lateral oviduct (Fig. 1), so that the ovary is "comb-shaped" type, which has been known in the Orthoptera, Phasmida, Isoptera, Dermaptera (RICHARDS & DAVIES, 1977; MATSUDA, 1976) and Grylloblattodea (MATSUZAKI *et al.*, 1979) in orthopteroid orders. In the case of the Plecoptera, according to BRINCK (1956) and our unpublished data, the numerous ovarioles are also arranged along the lateral oviducts, and their bases remain separately. However, the oviducts arch above the alimentary canal and are united anteriorly. Hence, the ovary of the Plecoptera differs from the typical "comb-shaped" ovary of the Embioptera in the present observation.

The ovariole of *O. japonica* is composed of an anterior terminal filament, germarium and vitellarium. The fully developed ovariole is about 2.5 mm long from the tip of the germarium to the base of the vitellarium. The ovariole is of the panoistic type having only oocytes with follicular cells and no nurse cells (Fig. 2). The terminal filaments of the ovarioles are all connected to the horseshoe-shaped ovarial ligament (Fig. 1). Sometimes, fusions of neighboring terminal filaments are observed, and usually many fat body cells are attached to this area. The ovarial

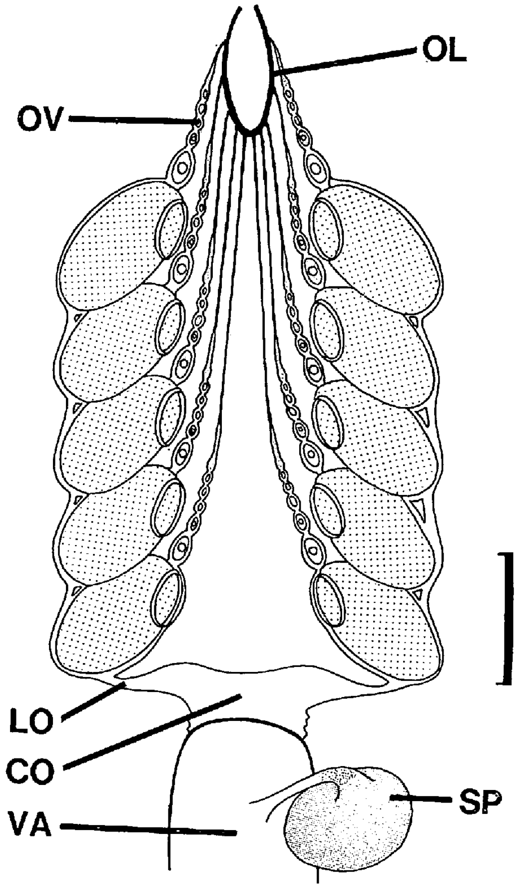


Fig. 1. Diagrammatic representation of gravid female ovary of *Oligotoma japonica*, showing a metameric arrangement of ovarioles (OV). CO, Common oviduct; LO, lateral oviduct; OL, ovarial ligament; SP, spermatheca. Scale: 1 mm.

ligament attaches to the ventral wall of the dorsal blood vessel. Similar structure has been found in the ovary of the Phasmida (De Sinety, 1901; Daiber, 1905; Marshall & Severin, 1906; Maki, 1935). Hence, the ovary of the Embioptera resembles that of the Phasmida. The globular spermatheca (approx. 1 mm in diameter) is reddish brown in color and opens into the dorsal side of the vagina (Fig. 1). In *O. japonica*, the accessory gland is absent as in the Indian embiopteran species (Mukerji, 1927). In the case of the fully grown ovary, each ovariole has one chorionated egg (Fig. 1). This fact suggests that a female produces about 10 eggs at the same time. Since the swollen egg calyx at the anterior part of the lateral oviduct is not observed, it is considered that each matured terminal egg is deposited singly. Observations made by Nagashima *et al.* (1991) on the oviposition of *O. japonica* agree with this. The eggs are oval, creamy white, about 1.15×0.6 mm in size, and have a conspicuous elliptical operculum (about 0.4×0.3 mm) at one side of their anterior end. The periphery of the operculum, as seen in Fig. 1, is enclosed with an embankment-like structure.

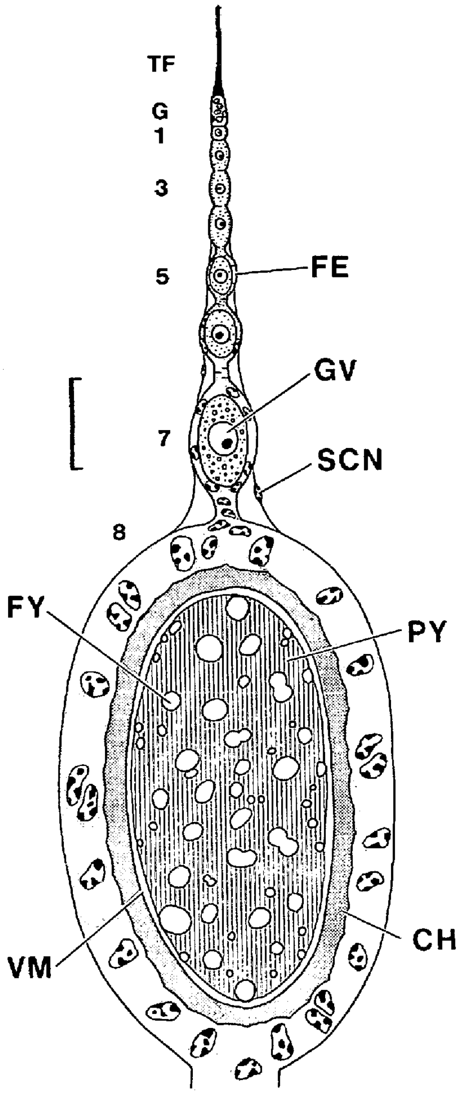
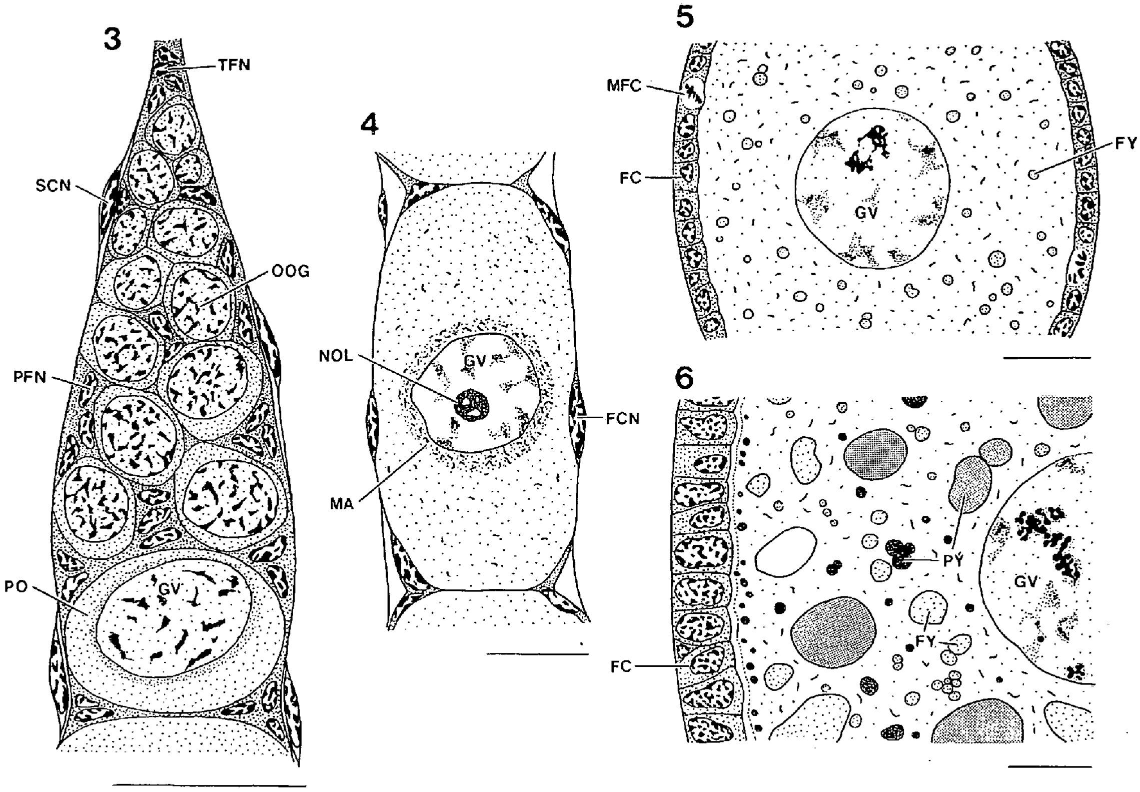


Fig. 2. Diagrammatic representation of a single ovariole of *Oligotoma japonica*. An ovariole contains terminal filament (TF), germarium (G), and 8 developing oocytes. —— 1–6, Previtellogenic growing oocytes; 7, penultimate oocyte in early stage of vitellogenesis; 8, matured terminal oocyte. CH, Chorion; FE, follicular epithelium; FY, fatty yolk; GV, germinal vesicle; PY, proteid yolk; SCN, nucleus of epitherial sheath cell; VM, vitelline membrane. Scale: 0.2 mm.

2. *Structure of developing oocytes in germarium and vitellarium*

In the gravid females, the germaria which contain the oogonia, primary oocytes and prefollicular cell nuclei are short (ca. 65 μm long and 25 μm in maximum width). The oogonia occupy the anterior to middle region of the germarium, and their nuclei are spherical in shape and measure 8–10 μm in diameter. The primary oocyte in the posterior region of germarium increases in volume, and its nucleus enlarges to about 20 μm in diameter. On the other hand, ellipsoidal prefollicular cell nuclei (ca. 3.5×2.0 μm in size) are scattered in the spaces between the oogonia and primary oocytes, and in the peripheral zones of the germarium (Fig. 3).

In the vitellarium, there are 8 developing oocytes arranged in a single row, and each oocyte is surrounded by a follicular epithelium (Fig. 2). Dimensions of these cells are summarized in Table 1. For convenience of description, the oogenesis of *O. japonica* is divided into 5 developmental stages on the basis of results of light



Figs. 3–6. Diagrammatic representation of germarium and developing oocytes in ovariole of *Oligotoma japonica*. —— 3, Germarium containing oogonia (OOG), primary oocytes (PO), prefollicular cell nuclei (PFN); 4, middle previtellogenic 3rd oocyte (stage 1) in vitellarium; 5, central part of 6th oocyte at late previtellogenic stage 2; 6, half of central part of vitellogenic 7th oocyte (stage 3). FC, follicular cells; FCN, follicular cell nuclei; FY, fatty yolk; GV, germinal vesicle; MA, mitochondria accumulation; MFC, mitosis of follicular cell; NOL, nucleolus; PY, proteid yolk; TFN, nuclei of terminal filament. All scales: 20 μm.

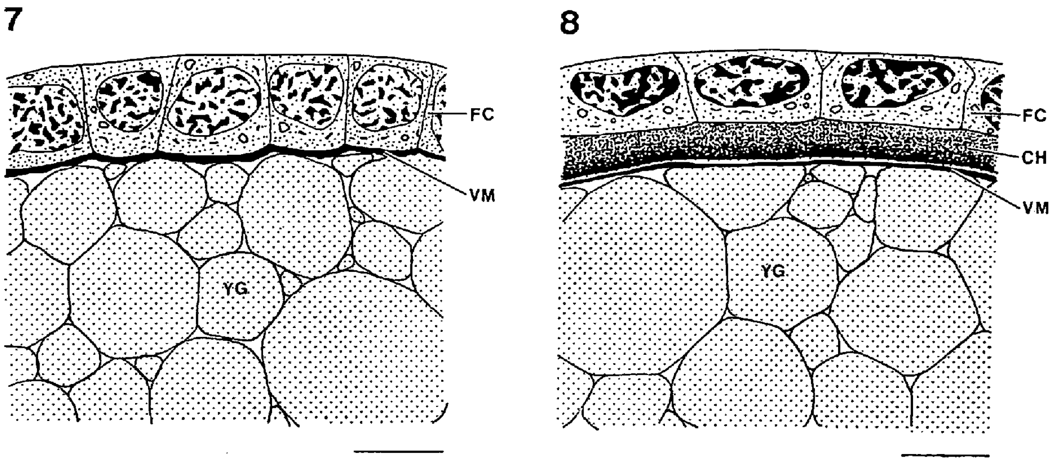
microscopy. The structural features of the oocytes and follicular cells in each stage are as follows:

Stage 1 (1st–5th oocytes): Early to middle previtellogenic oocytes in the vitellarium. During this stage, as seen in Table 1 and Fig. 2, the oocytes gradually increase in size, and their shapes also change from spherical to ovoidal. The nucleus or germinal vesicle of each oocyte possesses a large nucleolus (ca. 6 μm in diameter). In the ooplasm the mitochondria are distributed especially abundantly around the germinal vesicle (Fig. 4). A few follicular cells surrounding the 1st to 4th oocytes are irregular in thickness (Table 1, Fig. 4). At the 5th oocytes, they become regular and increase in thickness up to a maximum of 2 μm (Table 1).

Stage 2 (6th oocyte): Late previtellogenic oocytes in the vitellarium. The ellipsoidal oocytes increase in volume more than in the previous stage (Table 1). In the ooplasm, the mitochondrial accumulations around the germinal vesicle begin

Table 1. Dimensions of developing oocytes and follicular cells in *Oligotoma japonica*

Number of oocyte	Oocyte Length × Width (μm)	Germinal vesicle Diameter (μm)	Follicular cells	
			Height (μm)	Shape
1	30× 30	23	Irregular (0.3–0.9)	Flattened
2	70× 35	23	I. (0.4–0.8)	Flattened
3	80× 40	25	I. (0.4–1.0)	Flattened
4	90× 50	30	I. (0.4–1.6)	Flattened
5	100× 70	33	2.0	Flattened
6	130× 85	37	4.0	Flat./Cuboid
7	240×150	55	13.0	Columnar
8	1150×600	Break down	21.0	Cuboid
			14.0	Flattened



Figs. 7–8. Diagrammatic representation of follicular cells surrounding terminal 8th oocyte. —— 7, Cuboidal follicular cells (FC) at vitelline membrane formation (stage 4); 8, flattened follicular cells (FC) at choriogenic stage 5. CH, Chorion; VM, vitelline membrane; YG, yolk globules. All scales: 20 μm.

to disperse uniformly, and several fatty yolk globules (max. 3 μm in diameter) appear (Fig. 5). The flattened follicular epithelium, in which a few mitotic figures are observed, thickens and their cells become cuboidal (Table 1, Fig. 5).

Stage 3 (7th oocyte): Vitellogenic oocytes in the penultimate region of the vitellarium. Rapid accumulation of the proteid yolk globules progresses from the periphery to the center of oocytes. With the progress of the yolk formation, the oocytes become more ellipsoidal and the germinal vesicles also grow to the maximum volume (Table 1). Late at this stage, the proteid and fatty yolk globules of various sizes (max. 20 μm in diameter) can be observed in the ooplasm (Fig. 6). The follicular cells early in this stage quickly increase in number and in thickness, and become columnar (Table 1, Fig. 6). Similar rapid development of the follicular cells surrounding early vitellogenic oocytes has been observed in other insects with panoistic ovarioles; for example, *Periplaneta* (ANDERSON, 1964), *Locusta* (LAUVERJAT *et al.*, 1984), *Galloisiana* (MATSUZAKI *et al.*, 1979), *Carausius* (DUTKOWSKI & GRZELAKOWSKA, 1965), and others.

Stage 4 (8th oocyte): Following yolk formation, the vitelline membrane is formed in the space between the oocyte and follicular epithelium. The 8th oocytes situated in the most posterior portion of ovariole are filled with numerous yolk globules, and attain to the maximum volume (ca. 1150×600 μm). The follicular cells also increase in volume and attain to maximum thickness, and change their shape to a cuboid (Table 1, Fig. 7). The germinal vesicles of the oocytes break down in this stage. The vitelline membrane (0.5–0.8 μm thick) is completed late in this stage.

Stage 5 (8th oocyte): The oocytes maintain maximum volume and the follicular cells produce the chorion after the vitelline membrane is completed. With the progress of the chorion formation, the follicular cells change their shape and become flattened (Table 1, Fig. 8). The thickness of completed chorion is about 10 μm and about 15 μm in the operculum region.

As described above, the mode of oogenesis in *O. japonica* is approximately similar to those of the panoistic ovarioles in other orthopteran insects. Details of the developing oocyte and follicular cell ultrastructure will be dealt with in a forthcoming paper.

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