Notes on Structure of the Ovary and Oogenesis in *Triops longicaudatus* (Notostraca, Branchiopoda, Crustacea)

Hitoshi ANDO and Toshiki MAKIOKA

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Two different types of the ovaries are seen in the arthropods; the one in many mandibulates such as the insects, myriapods and some crustaceans, and the other in most chelicerates including xiphosurans and arachnids (Makioka, 1987, 1988). One of the most remarkable differences between these two types of the ovaries is found in the localities of the growing oocytes. In the mandibulates, the growing oocytes in the germarium enter the ovarian lumen, where they grow further and become mature. In the chelicerates, however, these oocytes do not enter the ovarian lumen, but protrude into the haemocoel followed by the epithelial stalks, and they grow further on their stalks.

In some notostracan crustaceans, it has been known that the ovary has some features like those in the chelicerates, such as the growing oocytes protruding into the haemocoel (Longhurst, 1955; Trentini and Scanabissi, 1978; Ogata, 1981). We have been studying some ovarian features of a notostracan, *Triops longicaudatus*, comparing with those in the chelicerates and in some other mandibulates to discuss the evolution of these features.

Adult females of *T. longicaudatus* were collected from a rice field in Saitama, Japan, in June, 1990. These specimens were kept in some shallow aquaria and were fed some tubificid oligochaetes.

Mature females were dissected under a stereomicroscope. The ovaries were fixed with Bouin's solutions or the "Kryofix" fixative (MERCK Co.) and prepared into serial paraffin sections $4-7 \mu m$ thick. These sections were stained with Delafield's haematoxylin and eosin or PAS-haematoxylin.



Fig. 1 Diagrammatic representation of right ovary in Triops longicaudatus. g: germarium, ob: ovarian branch, oc: folliculated oocyte, od: oviduct, ot: ovarian trunk.

Paired ovaries extended along both side of the alimentary canal. An ovary consisted of a long tubular ovarian trunk and many tubular ovarian branches repeatedly ramifying and mostly filling the haemocoel (Fig. 1). No tissue connection was observed between the left and the right ovaries. An oviduct extended laterally from the middle portion of the ovarian trunk and turned forward to be connected with the genital pore opening on each of the 11th thoracopods.

A germarium including young germ cells and somatic cells was located on each terminal of the ovarian branch (Figs. 1, 2). An oocyte and three nurse cells formed a four-celled cluster covered by a thin follicular epithelium (Fig. 2). The follicles containing the four-celled clusters grew in the germarium, and then the largest follicle protruded from the germarium into the haemocoel (Fig. 2). In the follicle on the tip of each ovarian branch, the oocyte grew further, consuming the nurse cells and accumulating yolk granules (Figs. 3, 4). The nurse cells completely disappeared during the vitellogenic stage, and the germinal vesicle of the oocyte was broken down at the end of the vitellogenesis (Fig. 5). The maximum diameter of the mature eggs was about 250μ m.

The cytoplasm of epithelial cells of the ovarian branches was strongly stained with haematoxylin, but not with PAS. The liquid egg shell-material similar in stainability to the epithelial cells was accumulated in the lumens of the ovarian branches possibly by the secretion of epithelial cells.

The germarium separating the folliculated mature egg and the lumen of each ovarian branch was put aside to make way for the ovulation (Fig. 5). The egg was ovulated from the follicle into the lumen through the narrow passage (Fig. 6). The liquid egg shell-material surrounded each ovulated egg in the lumen. The eggs with a hardened shell were transported into the ovarian trunk (Fig. 6) and then into the oviduct.

A growing cycle of the follicle in T. longicaudatus is schematically represented in Fig. 7.



- Fig. 2 Young follicle with an oocyte and three nurse cells (four-celled cluster), stained with haematoxylin and eosin (H-E). Scale=50µm. fe: follicular epithelium, g: germarium, nc: nurse cell, ob: ovarian branch, oc: oocyte.
- Fig. 3 Growing follicle with a vitellogenic oocyte and reduced nurse cells, stained with PAShaematoxylin (PAS-H). Scale=50µm. ep: epithelium of ovarian branch, lu: lumen of ovarian branch.



- Fig. 4 Follicle with ripe oocyte, stained with H-E. Germarium put aside prior to ovulation. Young follicle protruding outward from germarium. Scale=50µm. g: germarium, ob: ovarian branch, oc: oocyte, yf: young follicle.
- Fig. 5 Mature egg migrating from follicle into ovarian branch, stained with H-E. Scale=50µm. e: mature egg.
- Fig. 6 Ovulated egg surrounded with shell material partly hardened, stained with PAS-H. Scale= 100µm. od: oviduct, sm: shell material.



Fig. 7 Diagrammatic representation of growing cycle of follicle. 1. Young follicle with four-celled cluster in germarium. 2. Follicle with four-celled cluster protruding into haemocoel. 3. Growing follicle with vitellogenic oocyte and reduced nurse cells, and young follicle with four-celled cluster newly protruding outward. 4. Mature egg just before ovulation. Germarium put aside to make passage for ovulation. 5. Ovulated egg in lumen of ovarian branch. e: mature egg, ef: empty follicle, ep: epithelium of ovarian branch, fe: follicular epithelium, g: germarium, lu: lumen of ovarian branch, nc: nurse cell, oc: oocyte, yf: young follicle.

T. longicaudatus seems to have both ovarian features common to those in many other mandibulates and to those in many chelicerates. The former features are, for example, 1) the tubular ovary tapered off toward the terminals, 2) the germarium located in the terminal of each ovarian branch, and 3) oocytes accompanied by nurse cells. The latter features are also listed such as, 1) oocytes located on the tips of the ovarian branches protruding into the haemocoel, and 2) the ovarian lumen containing no growing oocytes, but only ovulated eggs.

Among these ovarian features of T. longicaudatus, the former features should be basic and primary, and the latter ones seem to appear only on the basis of the former ones. In particular, the oocytes protruding into the haemocoel are located on the tips of the ovarian branches, not on their epithelial stalks like those in the chelicerates. Consequently, we consider that the latter features are of secondarily acquired during the evolution of notostracans.

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