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Notes on the Postembryonic Development of Gonads in a Baetid Mayfly, *Cloeon dipterum* (Linnaeus) (Insecta: Ephemeroptera)

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Introduction

The gonad of *Cloeon dipterum* (Linnaeus) has a short bridge-like structure by which lateral gonoducts are connected with each other. This results in an "H" form appearance of the gonad in this mayfly (Takahashi and Tsutsumi, 2001). An "H" form appearance of the gonad has also been found in both males and females in other baetid mayflies (Saftoiu, 1995; Takahashi, unpublished data), but such a form of the gonad has not been reported in other families of mayflies (cf. Soldán, 1979; Landa and Soldán, 1985). Therefore, this gonad structure seems to be characteristic of baetid mayflies.

Herein, we describe the postembryonic development of gonads in *C. dipterum* in order to shed light on the origin of the short bridge-like structure.

Materials and Methods

Larvae of *Cloeon dipterum* were collected from experimental water tanks and ponds in the campus of Fukushima University, Fukushima Prefecture, Japan. They were anesthetized by submerging them in 70% ethanol for a short time (10–20 sec). For light microscopy, the heads and legs were removed from their bodies in a fixative (Bouin's solution). The fixed larvae were then dehydrated in a graded ethanol–*n*-butanol series and embedded in paraffin. Serial sections of 7 μm in thickness were stained with Mayer's acid haemalaum and eosin (H–E staining).

For electron microscopy, the heads and legs were removed from their bodies in a physiological saline (Ringer's solution), and they were fixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde) followed by 1% osmium tetroxide. They were then dehydrated in a graded acetone series, embedded in low-viscosity epoxy resins Quetol 651 (Nisshin EM, Tokyo), and cut into ultrathin sections. These sections were double-stained with uranyl acetate and lead citrate and observed under a transmission electron microscope JEM 1010 (JEOL, Tokyo) at 80 kV. Semi-thin sections of 1 μm in thickness were stained with toluidine blue O (toluidine blue staining) and observed under a light microscope.

Results and Discussion

Younger larval stage (ca. 2.5 mm in body length)

The gonad in this larval stage lies on the alimentary canal in the second to third abdominal segments and measured about 100×50 μm. Though the gonads of the insect are generally paired (cf. Büning, 1994), the gonad in this larval stage of *Cloeon dipterum* was not paired and contains many germ cells and somatic cells (Fig. 1a). These germ cells have large spherical nuclei, few organelles in the cytoplasm, and some huge mitochondria. These germ cells seem to be gonial cells (oogonia or spermatogonia), and some of them are connected with each other by cytoplasmic bridges (Fig. 1b). Most of the somatic cells are localized in the peripheral region of the gonad.

Morphological differences between female and male gonads are not observed in this stage. The cytoplasmic bridges between sister germ cells are found, however, it is well known that sister germ cells are generally connected by cytoplasmic bridges to make a cluster in both male and female germ lines of metazoans (Fawcett, 1971; cf. Tsutsumi *et al.*, 1993). Therefore, male gonads can not be distinguished from female ones by this characteristic.

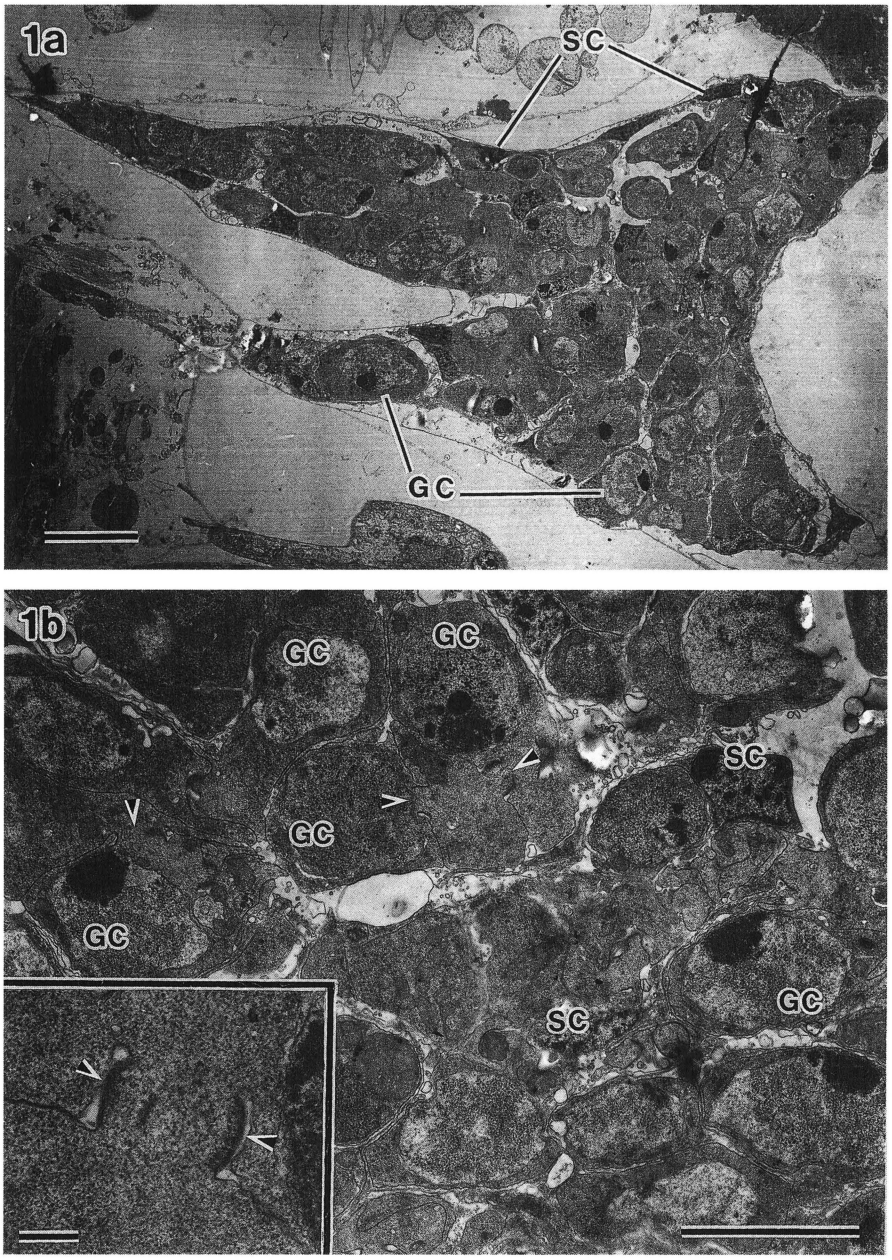

Fig. 1 TEM images showing the gonad of a young larva with a body length of 2.5 mm. a. Horizontal section of the gonad. Note that the gonad is not paired. b. Enlargement of central region of the gonad. Note that cytoplasmic bridges (arrowheads) by which germ cells (GC) are connected with each other. Inset: enlargement of cytoplasmic bridge (arrowheads). SC: somatic cells. Scales=a, 10 μm; b, 5 μm; inset, 0.5 μm.

Half-grown larval stage (ca. 3.0–6.5 mm in body length)

The gonad in this larval stage gradually increases in size anteriorly and posteriorly. However, the median part of the gonad which is located in the second or third abdominal segment remains not to enlarge, and this gonadal part changes into an intergonadal short bridge-like structure filled with many germ cells and a few somatic cells (Fig. 2). Even after the completion of the testis or ovary, this bridge-like structure does not change its location in the second or third abdominal segment.

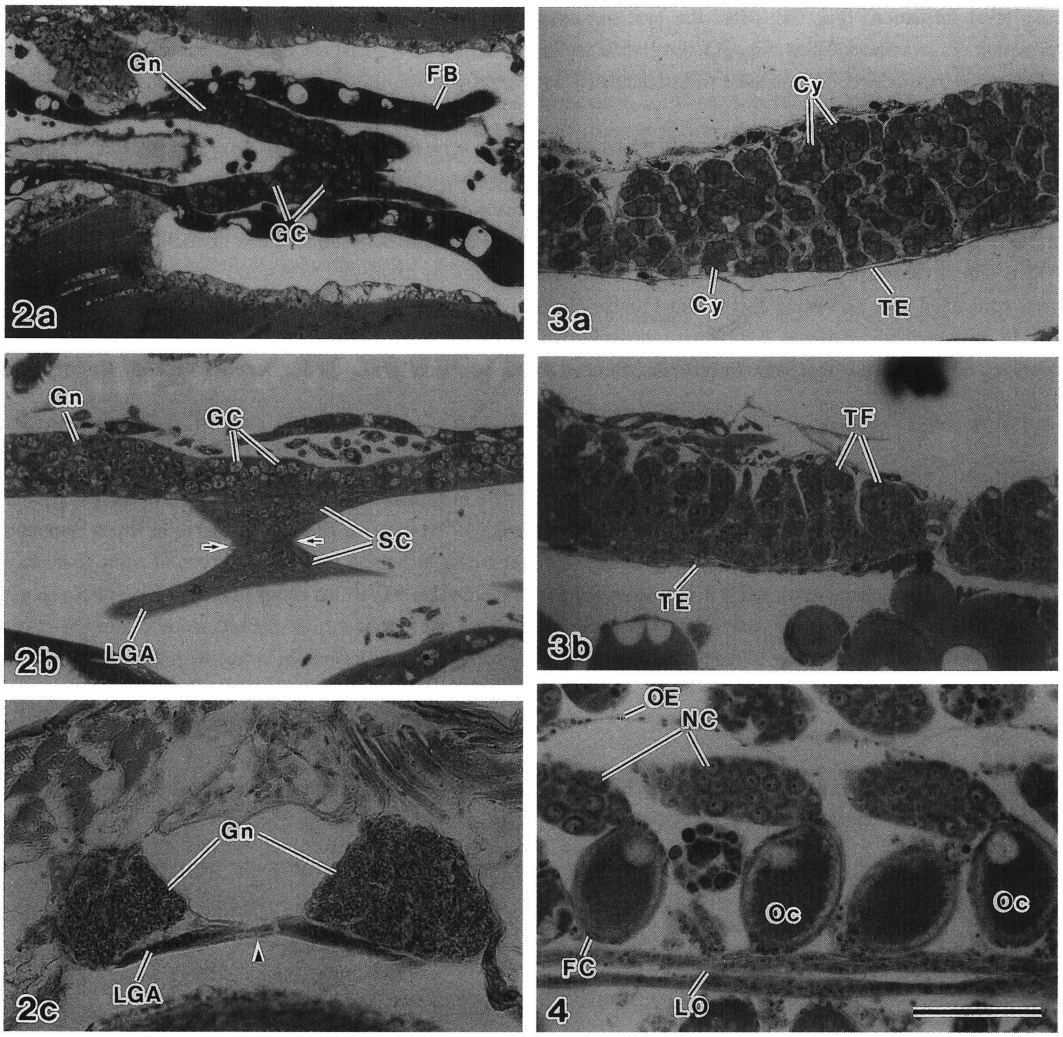

Fig. 2 Horizontal (a, b) and cross (c) sections of gonads of half-grown larvae with body lengths of 3.0–4.0 mm (a), 4.0–5.0 mm (b) (toluidine blue staining) and 5.0–6.0 mm (c) (H–E staining). In b, not shown one of the paired gonads (Gn) connected by the anlage of a short bridge-like structure (arrows) in this section. In c, arrowhead shows the short bridge-like structure by which the anlagen of gonoducts are connected with each other.

Fig. 3 Sections of testicular anlagen of older larvae with a body lengths of more than 6.8 mm (toluidine blue staining). a. Many cysts of germ cells (Cy) have formed in the testicular anlage. b. The testicular anlage is dividing into several testicular follicles (TF).

Fig. 4 Sagittal section of the ovary of an older larva with body length of 6.5 mm (H–E staining).

FB: fat body, FC: follicle cells, GC: germ cells, LGA: lateral gonoduct anlage, LO: lateral oviduct, NC: nurse cells, Oc: oocytes, OE: ovarian epithelium, SC: somatic cells, TE: testicular epithelium. Scale=50 μm.

In larvae with body lengths of more than *ca.* 4.0 mm, the bridge-like structure does not contain germ cells and comes to be filled only with somatic cells (Fig. 2b). Afterwards, as a result of proliferation of germ cells, the larval gonad continues to increase in size to about 300×70 μm, and the gonadal parts connected with each other by the short bridge-like structure differentiate into lateral gonoduct anlagen (Fig. 2c).

Older larval stage (over ca. 6.5 mm in body length)

The smallest larva in which testicular follicles had been formed was *ca.* 6.8 mm in body length (Fig. 3). The formation of testicular follicles is recognized by the enclosure of some sister spermatogonia or spermatocytes by cyst

cells (cyst formation) (Fig. 3a). After the cyst formation, the gonad is divided into several compartments by the formation of testicular follicles (Fig. 3b). The larval testis just before emergence of the imago have increased to about 1,000×150 μm in size. The gonadal parts connected with each other by the short bridge-like structure differentiate into the seminal ducts.

On the other hand, the smallest larva in which ovarioles had been formed was *ca.* 6.5 mm in body length (Fig. 4). In their ovarioles, nurse cells have already differentiated, then we could not confirm whether the differentiation of nurse cells precedes ovariole formation. After the oogonia have differentiated into nurse cells and oocytes, the latter starts to grow remarkably, and each oocyte becomes ensheathed by a monolayered follicular epithelium (Ikigame *et al.*, 2000). The larval ovary just before emergence of the imago increases to about 1,000×200 μm in size. The gonadal parts connected with each other by the short bridge-like structure differentiate into the lateral oviducts.

In some individuals whose body length increased to 7.0 mm, however, no differentiation of testicular follicles or ovarioles was observed. Testicular follicles or ovarioles had been absolutely formed in larvae with body lengths of *ca.* 7.2 mm.

Adult males of baetid and some leptophlebiid mayflies have eyes divided into a lower rounded portion and a stalked upper portion (Peters and Campbell, 1991). In the male of *C. dipterum*, these characteristic adult compound eyes are visible under the head cuticle of the older larva (Kuriki, 1976) (Fig. 5). The smallest larva in which compound eyes had developed was *ca.* 6.5 mm in body length. However, cyst formation was not observed in some individuals with developed compound eyes, while it was observed in some individuals in which compound eyes had not developed. These findings indicate that the compound eyes in adult males are not always formed simultaneously with the cyst of male germ cells. Both testicular follicles and compound eyes had absolutely formed in larvae with body lengths of *ca.* 7.2 mm. Thus, the female and male gonads in *C. dipterum* are difficult to distinguish until the larval body length has reached *ca.* 7.2 mm.

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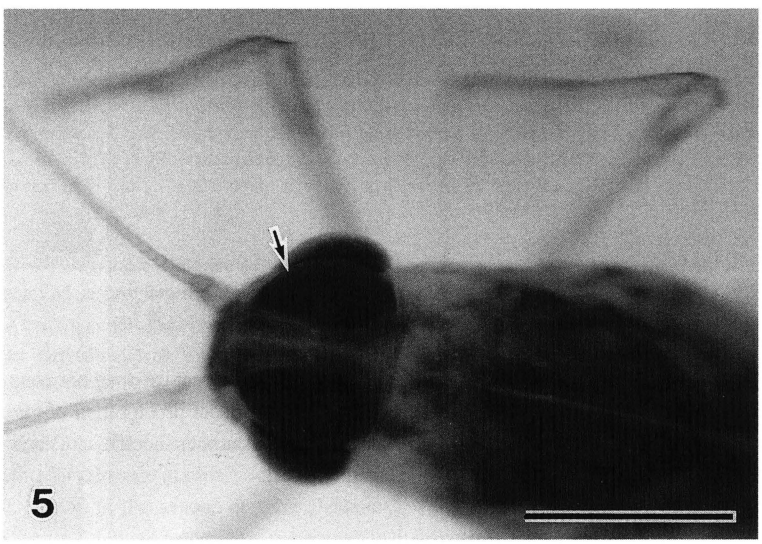

Fig. 5 A male older larva in which imaginal compound eyes are forming. Note that the upper portions of the imaginal compound eyes (arrow) are visible under the larval cuticle. Scale=1 mm.

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