Vitellogenesis of an ovoviviparous mayfly, *Cloeon dipterum* (Linnaeus) (Insecta: Ephemeroptera)

Junko IKIGAME, Ayuki TAKAHASHI and Tadaaki TSUTSUMI

Biological Laboratory, Faculty of Education, Fukushima University, Fukushima, Fukushima 960–1296, Japan

Introduction

The main deutoplasmic substances in the insect oocyte are proteid yolk globules, lipid yolk droplets, and carbohydrate (glycogen) particles (Bonhag, 1958; Gillott, 1995; Büning, 1998). Though it has been shown that there are no proteid yolk globules in the oocyte of some parasitic (King *et al.*, 1971) or viviparous insects (Hagan, 1951; Couchman and King, 1978; Dhileepan and Ananthakrishnan, 1986) that obtain their nourishment from the body fluid of their hosts or their maternal hemolymph, these three chemical components of the yolk can be found in almost every insect oocyte.

In the baetid mayfly *Cloeon dipterum*, however, no eosinophilic proteid yolk globules are found in the ooplasm or cytoplasm of nurse cells during vitellogenesis, although it is neither parasitic nor viviparous. This mayfly is well known as an ovoviviparous species (Degrange, 1959; Soldán, 1979). Vitellogenesis of this species has been briefly described (Soldán, 1979), but the description does not include yolk composition. In the present study, we describe the vitellogenesis of this mayfly, with special reference to the chemical components of yolk.

Materials and Methods

Larvae of *Cloeon dipterum* (Linnaeus) were collected from experimental water tanks and ponds in the campus of Fukushima University, Fukushima Prefecture, Japan. Subimagines and imagines were obtained through rearing of the larvae. 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 materials 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 or periodic acid–Schiff (PAS) and Mayer's acid haemalaum.

For electron microscopy, ovaries dissected out of female bodies were fixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde) followed by 1% OsO₄. The ovaries were then dehydrated in a graded ethanol series, mediated with methyl glycidyl ether (QY–2, Nisshin EM, Tokyo, Japan), embedded in low-viscosity epoxy resins (Quetol 651, Nisshin EM), and cut into ultrathin sections. These sections were double-stained with uranyl acetate and lead citrate, and they were observed under a transmission electron microscope JEOL JEM 1010 at 80 kV. Semi-thin sections of 0.5 μm in thickness were stained with toluidine blue O and observed under a light microscope.

Results

Older larval stage (Fig. 1)

The commencement of vitellogenesis is approximately coincident with formation of wing buds. In older larvae with wing buds, the vitellarium of each ovariole is formed by only one (or rarely two) previtellogenic oocyte (*ca*. 50×35 μm in size) with many nurse cells. These nurse cells cluster to form a tropharium, but they do not form a syncytium. Among these nurse cells, there are a few somatic interstitial cells. Each oocyte is ensheathed by a monolayered follicular epithelium.

The lipid droplets are deposited in the periplasm of previtellogenic oocytes. No yolk components other
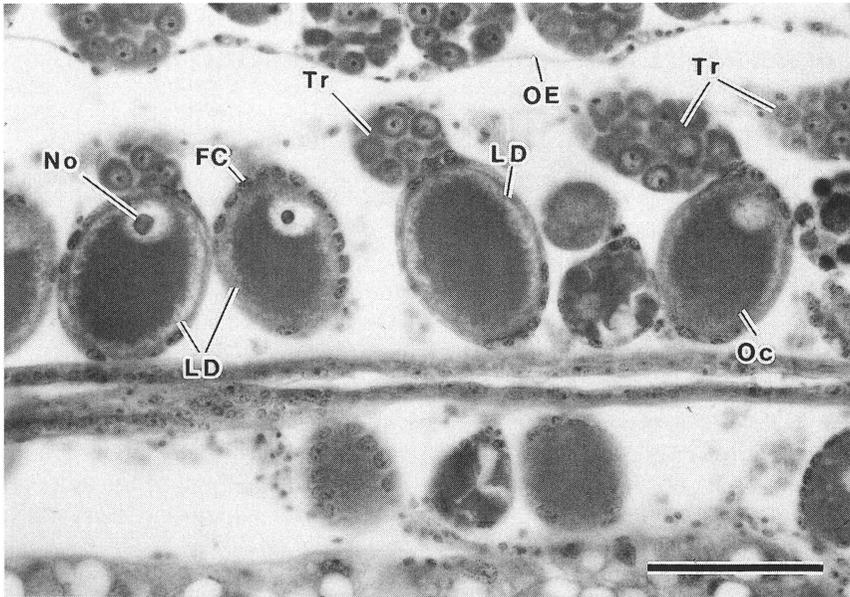
Fig. 1 Horizontal section of the ovary in the older larval stage of *Cloeon dipterum*. FC: follicular cell, LD:
lipid droplet, No: nucleolus, Oc: oocyte, OE: ovarian epithelium, Tr: tropharium. Scale = 50 μm.

than lipid droplets are accumulated in the ooplasm in this stage.

Subimaginal stage (Figs. 2, 3)

Some time after emergence of the subimagine, the germinal vesicles of oocytes break down. The nurse cells detach from the oocyte and begin to degrade. The follicular cells are flattened due to stretching of the follicular epithelium caused by growth of the oocyte. In the cytoplasm of the follicular cells, a large amount of rough endoplasmic reticulum appears, and formation of the egg membranes begins in the vitellogenic oocytes (Fig. 3).

The lipid droplets coalesce with each other, resulting in a remarkable increase in their size (*ca.* 19.5 μm in diameter), and they disperse throughout the ooplasm. In addition to lipid droplets, the second component of yolk appears in the ooplasm in this stage. This component is small granules (*ca.* 3.6 μm in diameter) characterized by the absence of a unit membrane, a higher electron density than that of the lipid droplets (Fig. 3), a higher stainability with toluidine blue O and PAS, and weak staining with both Mayer's acid haemalaum and eosin.

On the other hand, eosinophilic proteid yolk globules, usually found in insect vitellogenesis, are not yet observed in the vitellogenic oocytes (*ca.* 105×65 μm in size) in this stage.

Imaginal stage (Fig. 4)

When the imagine emerges, the egg membranes are nearly completed. The egg membranes are very thin (*ca.* 90 nm in thickness) and composed of a vitelline membrane (*ca.* 20 nm in thickness) and chorion (*ca.* 70 nm in thickness). After completion of these membranes, the follicular epithelium degenerates.

In the ooplasm, lipid droplets decrease in size to *ca.* 15.5 μm in diameter, while PAS-positive granules remarkably increase in number. Thus, the ooplasm is filled with these two yolk components. When the oocytes reach their definitive size (*ca.* 120×60 μm), the nurse cells fully degenerate. These mature oocytes remain in the membranous remnants of the ovariole sheath (basal lamina) (Fig. 4), but they do not descend into the oviduct at this stage.

In this stage, eosinophilic small spheres appear within the fat body around the ovaries (Fig. 4). However, eosinophilic proteid yolk globules that are each surrounded with a unit membrane and filled inside
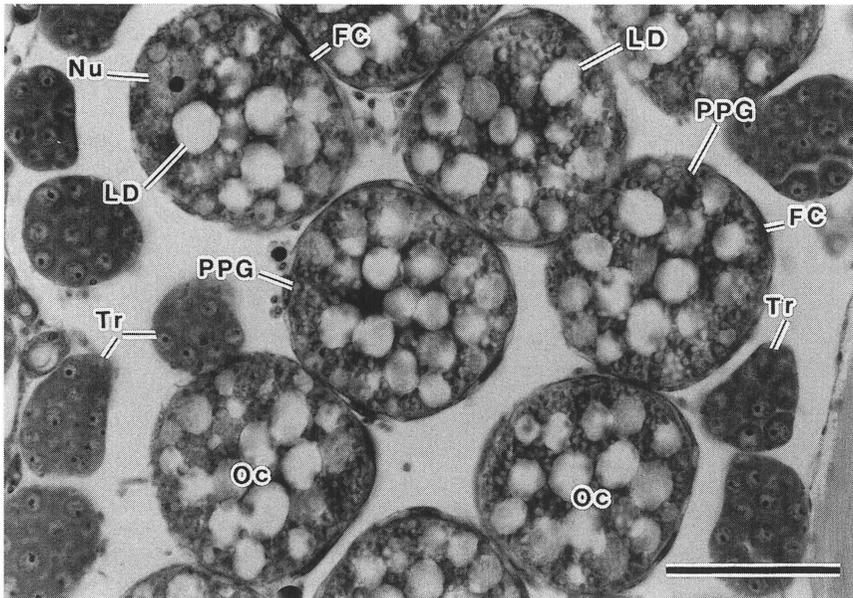
Fig. 2 Horizontal section of the ovary in the subimaginal stage of *Cloeon dipterum*. FC: follicular cell, LD: lipid droplet, Nu: nucleus, Oc: oocyte, PPG: PAS-positive granule, Tr: tropharium. Scale = 50 μm.
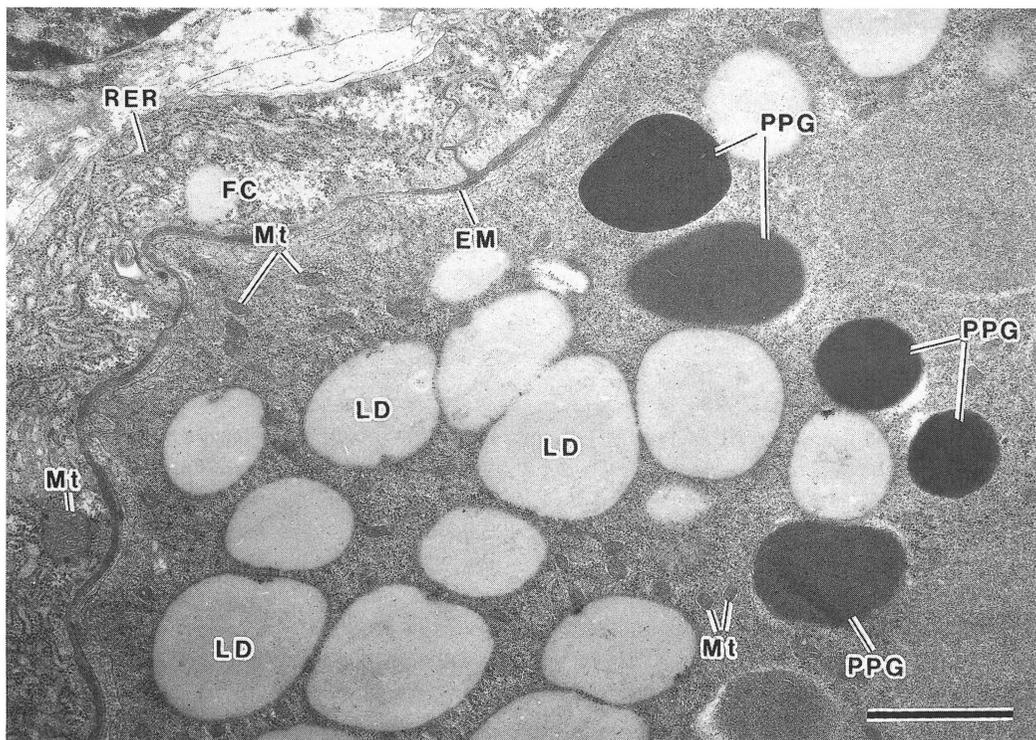

Fig. 3 Peripheral region of late vitellogenic oocyte in the subimaginal stage of *Cloeon dipterum*. EM: egg membrane, FC: follicular cell, LD: lipid droplet, Mt: mitochondria, PPG: PAS-positive granule, RER: rough endoplasmic reticulum. Scale = 1 μm.
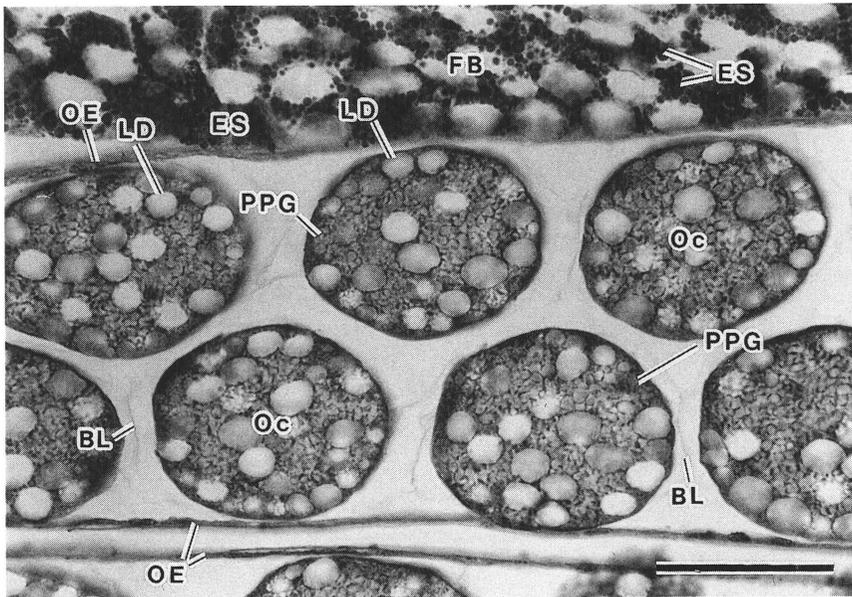
Fig. 4 Horizontal section of the ovary in the imaginal stage of *Cloeon dipterum*. Note the eosinophilic
spheres (ES) within the fat body (FB) adjacent to the ovary. BL: basal lamina, LD: lipid droplet,
Oc: oocyte, OE: ovarian epithelium, PPG: PAS-positive granule. Scale = 50 μm.

with electron dense material are not deposited in the ooplasm, even at one week after emergence of the
imagine.

Discussion

Soldán (1979) described the oogenesis and ovarian development of *Cloeon dipterum* in the postembryonic
stages, and found some unique features of oogenesis: very short vitellarium housing only one or rarely two
growing oocytes and no descent of mature oocytes into oviducts. These unique features may be due to
ovoviviparity in this species, as Soldán (1979) pointed out.

Soldán (1979) paid no attention to cells other than oocytes, *e. g.*, nurse cells or follicular cells. In the
present study, we confirmed that the oocyte is accompanied with many nurse cells. Therefore, the ovarian
type of *C. dipterum* is meroistic, but since an obvious nutritive cord was not observed, it is still not clear
whether or not the subtype of the ovary is telotrophic, as it is in other ephemeropteran species (cf.
Gottanka and Büning, 1993).

The present study revealed that the chemical components of the yolk deposited in the ooplasm of *C.
dipterum* were lipid droplets and PAS-positive granules, and typical proteid yolk globules were not detected.
This lack of accumulation of eosinophilic proteid yolk globules is unusual not only in mayflies but in all
insects. The proteid yolk globules that are known in most insect species [*i. e.*, 1) eosinophilic, 2) filled with
high electron dense material, and 3) surrounded by a unit membrane] were certainly not deposited, but
PAS-positive granules were weakly stained with eosin. This suggests that these granules may be
protein-carbohydrate complexes, which are known to exist in the cockroach *Periplaneta americana* (Nath *et
al.*, 1958) and in other animals (Wourms, 1987). Moreover, the appearance of eosinophilic small spheres
within the fat body adjacent to the ovaries in the imaginal stage may imply that the precursor of yolk
proteins is also synthesized in the fat body in *C. dipterum*, as it is in other insects (cf. Gillott, 1995; Oishi
et al., 1995).

We may presume that an eosinophilic proteid yolk precursor produced in the fat body is secreted into
the hemolymph and then taken up by the oocyte and deposited in the ooplasm as a particle containing a
protein-carbohydrate complex. The deposited proteid component of yolk in this mayfly, however, is different

from the eosinophilic type found in almost all insect oocytes. Further studies on pinocytosis in the oocyte in the imaginal stage will be useful for testing our hypothesis.

References

Bonhag, P.F. (1958) Ovarian structure and vitellogenesis in insects. *Annu. Rev. Entomol.*, **3**, 37–160.

Büning, J. (1998) The ovariole: structure, type, and phylogeny. *In* F.W. Harrison and M. Locke (eds.), *Microscopic Anatomy of Invertebrates, Vol. 11C*, pp. 897–932. Wiley-Liss, New York.

Couchman, J.R. and P.E. King (1978) Germarial structure and oogenesis in *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). *Int. J. Insect Morphol. Embryol.*, **8**, 1–10.

Degrange, C. (1959) L'ovolarviparité de *Cloëon dipterum* (L.). *Bull. Soc. Entomol. Fr.*, **64**, 94–100.

Dhileepan, K. and T.N. Ananthakrishnan (1986) The pleuropodium in the embryos of two species of viviparous sporophagous species of tubuliferan thrips (Thysanoptera: Insecta). *Curr. Sci.*, **55**, 1105–1108.

Gillott, C. (1995) *Entomology, 2nd ed.* Plenum Press, New York.

Gottanka, J. and J. Büning (1993) Mayflies (Ephemeroptera), the most "primitive" winged insects, have telotrophic meroistic ovaries. *Roux's Arch. Dev. Biol.*, **203**, 18–27.

Hagan, H.R. (1951) *Embryology of the Viviparous Insects*. The Ronald Press Company, New York.

King, P.E., N.A. Ratcliffe and M.R. Fordy (1971) Oogenesis in a braconid, *Apanteles glomeratus* (L.) possessing an hydropic type of egg. *Z. Zellforsch.*, **119**, 43–57.

Nath, V., B.J. Gupta and B. Lal (1958) Histochemical and morphological studies of the lipids in oogenesis. I. *Periplaneta americana*. *Q. J. Microsc. Sci.*, **99**, 315–332.

Oishi, K., M. Sawa and M. Hatakeyama (1995) Developmental biology of the sawfly, *Athalia rosae* (Hymenoptera). *Proc. Arthropod. Embryol. Soc. Jpn.*, **30**, 1–8.

Soldán, T. (1979) The structure and development of the female internal reproductive systems in six European species of Ephemeroptera. *Acta Entomol. Bohem.*, **76**, 353–365.

Wourms, J.P. (1987) Oogenesis. *In* A.C. Giese *et al.* (eds.), *Reproduction of Marine Invertebrates, Vol. 9, General Aspects: Seeking Unity in Diversity*, pp. 49–178. Blackwell Scientific Publications and Boxwood Press, California.