Embryogenesis of *Ephemera japonica* McLachlan (Insecta: Ephemeroptera)

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Introduction

The Ephemeroptera is an important group for understanding the ground plan of the Pterygota and insect evolution which is still highly controversial (cf. Hennig, 1969; Kristensen, 1975; Boudreaux, 1979), because it is widely accepted as a pterygote representative closest to the pterygote ancestors. For the phylogenetical studies, the comparative embryological approach is one of the most promising methods. The embryology of the Ephemeroptera has been studied by Joly (1876), Heymons (1896a, b, c), Murphy (1922), Ando and Kawana (1956), Wolf (1960), Bohle (1969), and Tsui and Peters (1974), but the details still remain unclear. We have started a comparative embryological study of the Ephemeroptera, using *Ephemera japonica* McLachlan (suborder Schistonota, family Ephemeridae) (*e.g.*, Tojo and Machida, 1996). Here, we describe the outline of embryogenesis of the species.

Results and Discussion

The egg period of *Ephemera japonica* ranges from 15 to 17 days at room temperature (20±2℃). Herein, we describe its embryonic development, dividing it into 13 stages.

Stage 1 Egg cleavage (Fig. 1)

Egg cleavage is of typical superficial type. The first five cleavages are synchronized, each cleavage occuring at an interval of approximately nine hours.

Stage 2 Blastoderm formation (Fig. 2)

Cleavage nuclei arrive at the egg periphery after eight cleavages, and the syncytial blastoderm (blastema) is formed. Soon, the cell membrane appears between the blastoderm cells, and the cellular blastoderm (blastoderm *s. str.*) is completed.

Stage 3 Germ disc formation (Fig. 3)

Even in the newly formed blastoderm, the posterior half embryonic and anterior half extra-embryonic areas can be distinguished from each other in cellular size. The posterior cells concentrate to the posterior pole of the egg, to form the germ disc. The anterior cells become more flattened, and they form the future serosa.

Stage 4 Pear-shaped embryo (Fig. 4A, B)

The germ disc starts to elongate backwards and a pear-shaped germ band is formed, in which the anterior broad protocephalon and the posterior protocorm differentiate.

Stage 5 Start of invagination of germ band (Anatrepsis I) (Fig. 5A, B)

The germ band further elongates, and its caudal end starts to proceed into the yolk. At the same time, the amnion starts to be produced from the embryonic margin, and the amnioserosal fold is formed. The amnio-serosal fold is exclusively derived from the posterior area, hardly from the anterior area.

Stage 6 S-shaped embryo (Anatrepsis II) (Fig. 6A, B)

With the active cell proliferation, the embryo further elongates and deeply invaginates into the yolk. The caudal end of embryo reaches the middle of the egg long axis, and the embryo acquires an S-shape.

At this stage, the posterior amnioserosal fold extends anteriorly to the cephalic level, and the anatrepsis is completed. The ventral side of embryo is completely covered by the amnioserosal fold or the amnion. The inner layer or mesoderm differentiates all over the dorsal side of embryo.

Stage 7 Longest embryo (Fig. 7)

The embryo further elongates with its posterior half bending three times, and it acquires its maximum length, reaching 3/4 of the egg long axis from the posterior egg pole. With the bendings, the posterior half of embryo or the future abdomen is folded and divided into four regions (regions I to IV from the anterior to posterior). In a cross section, the regions I and II and the regions III and IV are respectively connected with each other by the amnion, and all of regions I to IV are dorsally lined with the inner layer.

Stage 8 Segmentation of embryo (Fig. 8)

The segmentation of embryo commences. It proceeds from the head to the rear, and simultaneously appendage rudiments appear in the cephalic and thoracic segments.

In regions I and II of the abdomen which has been divided into four regions, the first to fifth and the sixth to eleventh abdominal segments develop respectively. Later in this stage, neuroblasts and neuropiles are clearly observed in all the first to eleventh abdominal segments. On the other hand, no attributes suggestive of segmental nature are found in regions III and IV.

Stage 9 Proctodaeum formation (Fig. 9)

The appendages of the head and thorax are segmented. Regions III and IV of the abdomen fuse with each other to form the proctodaeum. The proctodaeum itself is soon enclosed by region II. This enclosure of region II is the definitive dorsal closure.

The caudal filament develops at the apex of region IV. A pair of cerci develop at the eleventh abdominal segment which is the extremity of region II. With the progression of definitive dorsal closure, the cerci move from the original ventro-lateral to the dorso-lateral positions, and they reach the same level as the caudal filament.

Stage 10 Revolution (Katatrepsis) (Fig. 10)

The amnioserosal fold tears near the labrum, and the embryo appears on the egg surface. The embryo, which is slightly shortened temporarily, moves along the ventral surface of the egg towards the anterior pole, with its head oriented ahead, and the antero-posterior axis of the embryo is reversed.

Stage 11 Post-revolution I (Fig. 11)

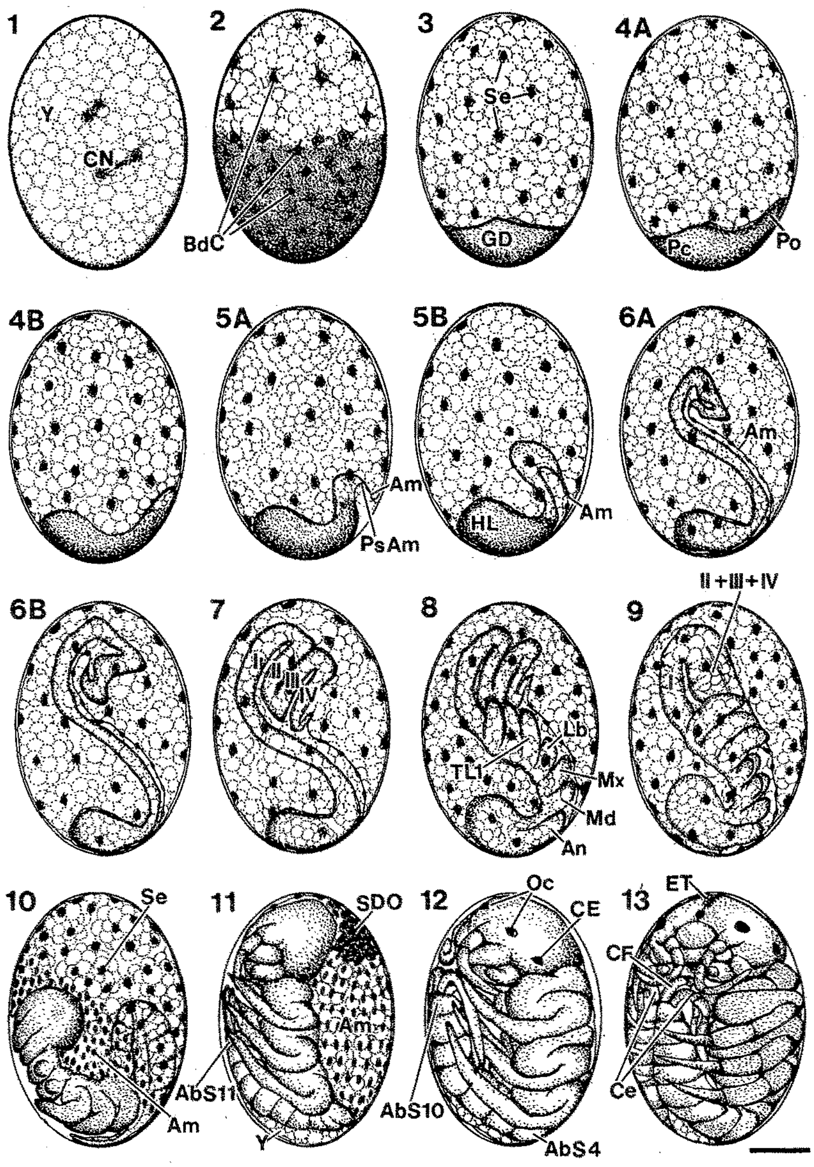
The serosal cells are condensed to the antero-dorsal part of the egg, to form the secondary dorsal organ at the back of the head. With the progressive condensation and withdrawal of serosal cells, the amnion replaces the serosa and finally spreads over the dorsal yolk as a provisional dorsal closure.

Stage 12 Post-revolution II (Fig. 12)

The secondary dorsal organ which formed at the previous stage sinks into the yolk and disappears. The appendages of the head and thorax further develop. The definitive dorsal closure proceeds anteriorwards. A pair of cerci and caudal filament elongate, and they become segmented. Later in this stage, compound eyes and three ocelli are clearly observed.

Stage 13 Post-revolution III (Fig. 13)

The definitive dorsal closure is completed, and the embryo acquires its definitive form. The larval cuticle is



Figs. 1–13 Embryonic development of *Ephemera japonica* McLachlan. 1. Stage 1 (Egg cleavage). 2. Stage 2 (Blastoderm formation). 3. Stage 3 (Germ disc formation). 4. Early (A) and late (B) stage 4 (Pear-shaped embryo). 5. Early (A) and late (B) stage 5 (Start of invagination of germ band). 6. Early (A) and late (B) stage 6 (S-shaped embryo). 7. Stage 7 (Longest embryo). 8. Stage 8 (Segmentation of embryo). 9. Stage 9 (Proctodaeum formation). 10. Stage 10 (Revolution). 11. Stage 11 (Post-revolution I). 12. Stage 12 (Post-revolution II). 13. Stage 13 (Post-revolution III). Figures 1 to 12: lateral views, Figure 13: ventro-lateral view. I–IV: abdominal regions I to IV, AbS4, 10, 11: 4th, 10th and 11th abdominal segments, Am: amnion, An: antenna, BdC: blastoderm cell, CE: compound eye, Ce: cercus, CF: caudal filament, CN: cleavage nucleus, ET: egg tooth, GD: germ disc, HL: head lobe, Lb: labium, Md: mandible, Mx: maxilla, Oc: ocellus, Pc: protocephalon, Po: protocorm, PsAm: presumptive amnion, SDO: secondary dorsal organ, Se: serosa, TL1: proleg, Y: yolk. Scale = 50 μm.

secreted, and a sclerotized egg tooth is visible on the frons.

The germ disc of *Ephemera japonica* is formed with the concentration of cells at the embryonic area which is broadly defined at the posterior half of blastoderm. Germ disc formation in a similar manner is also observed in other ephemeropterans, *i.e., E. strigata* (Ando and Kawana, 1956), *Baetis rhodani* and *B. vernus* (Bohle, 1969). Thus, this type of germ disc formation should be regarded as an important character of the ephemeropteran embryogenesis.

The germ type of *Ephemera japonica* can be categorized into the typical short germ type (cf. Krause, 1939), which is characterized by the sequential proliferation of segments from the anterior to posterior. Because the short germ type dominates in the primitive pterygotes (*e.g.,* Odanata: Ando, 1962; Plecoptera: Kishimoto and Ando, 1985) as well as in ectognathan apterygotes (cf. Sander, 1984), this may be regarded as an ancestral feature. As a result of elongation of the germ band, the embryo of *E. japonica* acquires the same S-shape as in odonatans and plecopterans.

In the longest-embryo stage, the abdomen of *Ephemera japonica* is folded and divided into four regions. From regions I and II all the first to eleventh segments are derived. Regions III and IV fuse with each other to form the proctodaeum (cf. Tojo and Machida, 1996). This manner of abdominal formation may be regarded as the most basic type in the Pterygota (Tojo and Machida, submitted), as found in another palaeopteran group or the Odonata (Ando, 1962).

The caudal filament has been interpreted as the elongation of eleventh abdominal tergum (cf. Heymons, 1896a, b; Snodgrass, 1935; Matsuda, 1976). However, previously Tojo and Machida (1996) revealed that the caudal filament of *Ephemera japonica* originates from the posterior extremity of region IV and has its origin away from the eleventh abdominal segment, with regions III and IV or the precursors of proctodaeum interposing between, and they considered that the caudal filament should be correlated not to the eleventh abdominal segment but to the telson.

Acknowledgements: This work was supported by Grants-in-Aid from the Showa Seitoku Foundation and from the Ministry of Education, Science and Culture of Japan (08640881), to R. M. Contribution No. 154 from the Sugadaira Montane Research Center, University of Tsukuba.

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