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[SHORT COMMUNICATION]

Preliminary Note on the Embryonic Development of *Eucorydia yasumatsui* Asahina (Insecta: Blattaria, Polyphagidae)*

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Polyneoptera, comprising 11 orders, is a significant group for reconstructing the groundplan and evolution of Neoptera. However, for Polyneoptera, the ordinal divergence of which can be traced back to ancient times, it is difficult to reconstruct not only the interrelationship between each order but also their groundplan, as there are more than 20 phylogenetic hypotheses on the interordinal relationships within the group itself, without any consensus (Klass, 2009). In Polyneoptera, an assemblage of the Blattaria, Mantodea and Isoptera or the Dictyoptera is well supported to be monophyletic. However, the interrelationship between the three ordinal constituents of Dictyoptera has not been clarified despite numerous morphological and molecular phylogenetic studies, and a polyphyletic status has been often suggested for Blattaria (Klass, 2009). Thus, Blattaria may be the most significant group for reconstructing the groundplan and evolution of Dictyoptera. Against such a background, we have started a comparative embryological study of Blattaria. As a first step, we have tackled Polyphagidae, often regarded as one of the basalmost clades in Blattaria, using a Japanese polyphagid, *Eucorydia yasumatsui* Asahina. Establishing a culture (Fujita *et al.*, 2011), we could constantly collect eggs. This preliminary note reports our recent findings on the embryonic development of *E. yasumatsui*.

Eucorydia yasumatsui cockroaches were collected in Iriomote and Ishigaki Islands, Okinawa Prefecture. Out of oothecae deposited, the eggs were taken in physiological saline, and fixed in Bouin's fixative. The egg structures were examined with a differential interference contrast microscope (LEICA DM6000-B). For the examination of embryonic development, the eggs were stained with a DNA specific fluorescent dye, DAPI (4', 6-diamidino-2-phenylindole dihydrochloride, 10 μg/ml), and observed under UV-excitation with a fluorescence stereomicroscope (LEICA MZ FL Ⅲ).

Eggs are ellipsoidal about 1.5 mm long, provided with 10 to 15 funnel-shaped micropyles on the ventral side of the posterior region (Fig. 1). A small embryo forms on the posterior ventral side of the egg (Fig. 2A). The amnioserosal fold has already started to be produced at this stage. The embryo elongates and migrates on the egg surface towards the anterior to the middle of the ventral side (Fig. 2B). At this stage, the amnioserosal folds are closed, completely covering the embryo. Soon the segmentation (Fig. 2C) and formation of appendages (Fig. 2D) occur. With progressive development, the embryo elongates, with its abdomen folding (Fig. 2D, E). After a while, the amnioserosal folds open and are withdrawn. The embryo appears again on the egg surface, and the serosa is concentrated in the region just posterior to the developing head, forming the secondary dorsal organ (Fig. 2F). The definitive dorsal closure proceeds, and the embryo acquires its definitive form (Fig. 2G). Throughout development, the embryo does not suffer from a reversion of the anteroposterior axis.

Micropyles are reported to be found in a group in *Blatta orientalis* (=*Periplaneta orientalis*) and *Blattella germanica* (Blattellidae) (Kadyi, 1879; Wheeler, 1889). Our study revealed that in *Eucorydia yasumatsui*, 10 to 15 micropyles are located in a small area on the ventral side of the egg. It was reported that in Isoptera, micropyles occur on the dorsal side of the eggs (Mukerji, 1970). However, isopteran embryos are known to suffer from a reversion of the dorsoventral axis because of rotation of the embryos (Striebel, 1960), hence, it is concluded that the isopteran micropyles originally take their position on the "ventral side" of the egg. Thus, we may assert that the micropyles on the "ventral side" of the egg should be part of the groundplan of the Blattaria and Isoptera or Blattodea (=Blattaria + Isoptera). In Mantodea, Iwaikawa and Ogi (1982) reported that eggs of *Tenodera aridifolia* have one micropyle at the center of the egg's anterior pole, encircled by several micropyles arranged circularly. Therefore, the micropyles of Mantodea and Blattodea seem to differ in their localization and arrangement. However, there

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are some points to be re-examined in Iwaikawa and Ogi's description: for example, according to them, the mantodean micropyles have an extraordinarily wide opening about 4 μm. A careful re-examination of the egg structure for mantodeans is needed.

In *Eucorydia yasumatsui*, a small embryo forms on the

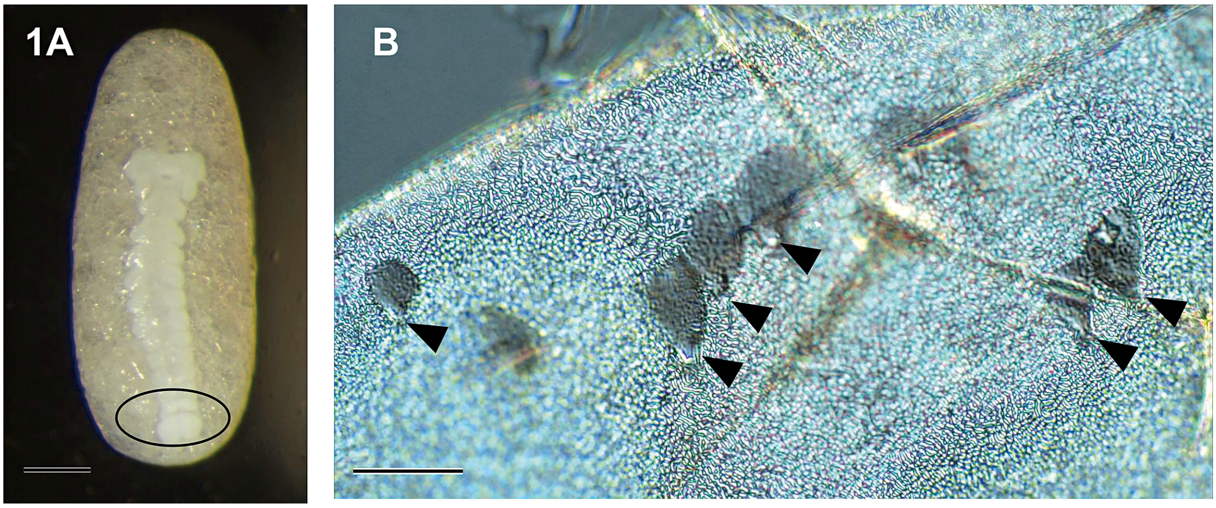

Fig. 1 Eggs of *Eucorydia yasumatsui* Asahina. A. Micropyles are located on the ventral side of the egg's posterior region as encircled. B. Micropyles (arrowheads), posterior to the bottom, differential interference contrast microscopy. Bars = A: 200 μm; B: 10 μm.

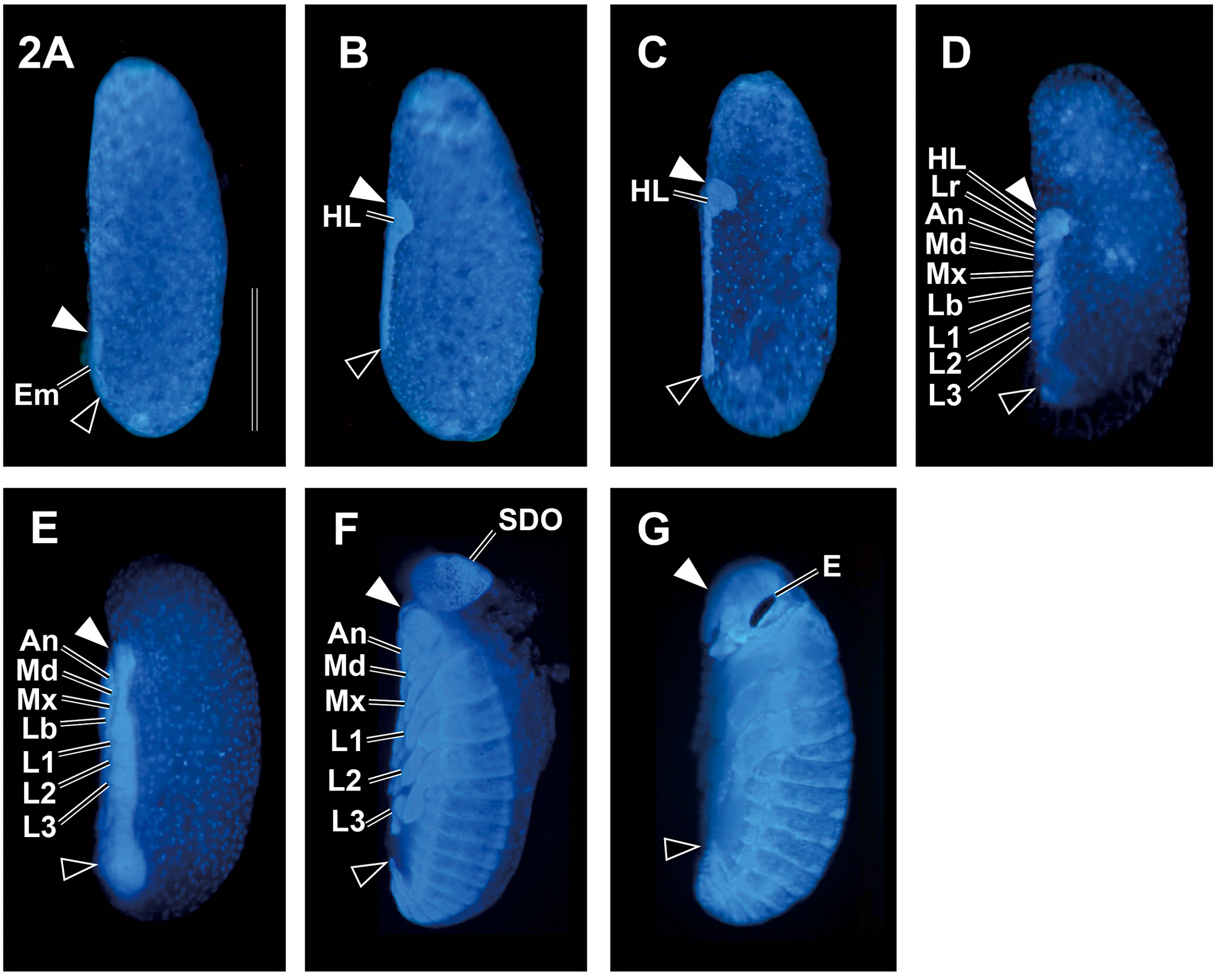

Fig. 2 Outline of the embryonic development of *Eucorydia yasumatsui* Asahina, A to G. White and black arrowheads respectively show the anterior and posterior ends of the embryo. A. Formation of the embryo. B. Elongation of the embryo. C. Segmentation. D. Formation of the appendages. E. Curvature of the abdomen. F. Formation of the secondary dorsal organ and definitive dorsal closure. G. Acquisition of the definitive form.
An: antenna, E: eye, Em: embryo, HL: head lobe, L1–3: pro-, meso- and methathoracic legs, Lb: labium, Lr: labrum, Md: mandible, Mx: maxilla, SDO: secondary dorsal organ. Bar = 500 μm.

ventral surface of the egg, and gradually elongates, accompanied by a sequential segmentation. The embryogenesis of this insect is categorized into the short germ band type. The embryo develops on the original position on the egg's ventral side without changing its anteroposterior axis throughout development. That is, the blastokinesis in Polyphagidae is of the "non-reversion type". *Blattella germanica* (Blattellidae) and *Blabera cranifer* (Blaberidae), both of which are members of Blaberoidea, undergo blastokinesis of the "non-reversion type" (Wheeler, 1889; Bulliére, 1969; Tanaka, 1976). On the other hand, *Periplaneta* spp. (Blattidae) and *Blatta orientalis* (Blattidae), which are included in the Blattoidea, have blastokinesis of the "reversion type", in which reversion of the embryo's axis occurs (*e.g.*, Heymons, 1895; Lenoir-rousseaux and Lender, 1970). In the type of blastokinesis, Polyphagidae resembles not the Blattidae but the Blattellidae and Blaberidae. This may be favorable to McKittrick's (1964) classification system, in which Blattaria are divided into two groups, Blaberoidea composed of Polyphagidae, Blattellidae and Blaberidae, and Blattoidea including Blattidae. That is, two types of blastokinesis, a "reversion type" and a "non-reversion type", may be understood as being characteristic of Blattoidea and Blaberoidea, respectively. As for other members of Dictyoptera, Isoptera (Knower, 1900; Striebel, 1960) and Mantodea (Görg, 1959) are respectively known to show blastokinesis of the "reversion type" and "non-reversion type". Understanding that Isoptera and Blattoidea exhibit blastokinesis of the "reversion type" and Mantodea and Blaberoidea that of the "non-reversion type" may be intriguing in the phylogenetic reconstruction of Dictyoptera.

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