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[SHORT COMMUNICATION]

Note on the Early Germ Band Stage in *Galloisiana yuasai* Asahina (Insecta: Notoptera)

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Notoptera, one of the Polyneoptera (Orthopteroidea), are apterous insects inhabiting alpine areas or caves of the Northern Circumpacific Region. The relationships among orders are highly controversial in Polyneoptera (cf. Kristensen, 1989). Notoptera share important morphological and anatomical characters with other Polyneoptera and resemble Protorthoptera in general features, making it quite important to any phylogenetic discussion of Polyneoptera (cf. Kristensen, 1991; Rentz, 1991). Comparative embryology is a most promising approach to phylogenetic analyses. However, there have been few embryological studies on Notoptera (Ando and Nagashima, 1982; Ando and Machida, 1987).

We here perform an embryological study of Notoptera using *Galloisiana yuasai* Asahina as material. In this report, we give a description of a pear-shaped early germ band in which we found several interesting features.

Figure 1 is a fluorescence micrograph (DAPI staining, UV-excitation) of the pear-shaped, early germ band obtained. The germ band is about 600 μm in length. A wide protocephalon and narrow protocorm are distinguishable. In the protocephalon, two or three segments are found to be differentiated, although this is not clear from the figure. The posterior part of the germ band seems to be densely cellulated. A groove is discernible along the median line in the middle of the germ band (a white arrowhead in Fig. 1).

This embryo was processed into sagittal sections (Fig. 2A). The mesoderm is observed to be already segregated in the dorsad of the embryo, most heavily in the protocorm (Fig. 2B). The embryo has started invagination from the posterior end, and the amnioserosal fold extends over the protocorm (Fig. 2B). The amnion has a two-cellular-layer thickness, like the ectoderm. The serosa, represented by a layer of cells with flattened nuclei, is rather thick in the amnioserosal fold. The invagination pore of the embryo appears as a dark slit (black arrowheads in Fig. 1). Large cells with a basophilic nucleus are observed to be segregated on the inner sides of both the embryo and serosa (Fig. 2A, B). They are identified as secondary york cells, and can be detected as large DAPI-philic spots (arrows in Fig. 1).

Groove along the median line in the middle of the germ band

To better understand the median groove observed in Figure 1, we examined an embryo in the germ disc stage, about 500 μm in diameter, when the amnioserosal fold has yet to form (Fig. 4). There is found a circular area about 100 μm in diameter, appearing as a dark spot showing low cellular thickness, approximately at the center of the germ disc, and a median groove about 200 μm in length runs back from the circular area. A transverse section of the same embryo through the groove reveals that the mesoderm is segregated dorsally to the groove (Fig. 5B, D). Accordingly, the groove can be recognized as a primitive groove involved in mesodermal formation, and the median groove observed in the early germ band (Fig. 1) may be regarded as the remnant of this primitive groove.

Another transverse section of the embryo shown in Figure 4 through the circular area anterior to the primitive

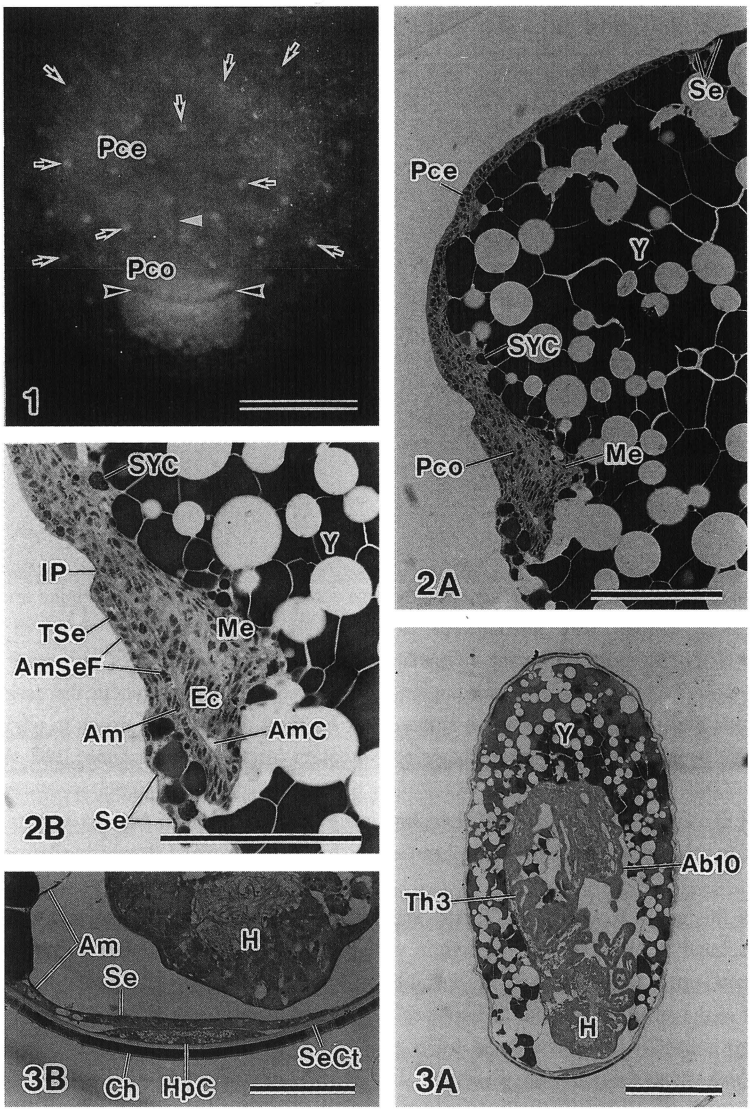

Fig. 1 Pear-shaped early germ band of *Galloisiana yuasai*, situated at the posterior pole of the egg. The egg, with its chorion partially dissolved and removed, was stained with DAPI (4',6-Diamidino-2-Phenylindole Dihydrochloride, diluted about 100 times with PBS) and observed under a fluorescense stereomicroscope (Leica MZ FL Ⅲ, UV-excitation). Black arrowheads, a white arrowhead, and arrows respectively show the structures identified as the invagination pore, primitive groove, and secondary york cells, in the sections of this embryo and others (cf. Figs. 2A, B, 5A–D). Pce: protocephalon, Pco: protocorm. Bar=200 μm.

Fig. 2 A. Sagittal section of the same *Galloisiana yuasai* germ band as shown in Figure 1. Anterior is towards the top. Sections were processed with a methacrylate resin Technovit 7100 in accordance with Machida *et al.* (1994), cut 4 μm thick and stained with haematoxilin and eosin. B. Enlargement of the posterior area of the germ band. Am: amnion, AmC: amniotic cavity, AmSeF: amnioserosal fold, Ec: ectoderm, IP: invagination pore, Me: mesoderm, Pce: protocephalon, Pco: protocorm, Se: serosa, SYC: secondary york cell, TSe: thickened serosa, Y: york. Bars=100 μm.

Fig. 3 A. Sagittal section of *Galloisiana yuasai* embryo in the diapause stage. Chorion was partially dissolved with antiformin. Sections were processed with a methacrylate resin Technovit 7100 in accordance with Machida *et al.* (1994), cut 4 μm thick and stained with haematoxilin, eosin, and fast green. B. Enlargement of the posterior pole of the egg. Serosal cuticle, which does not exist before anatrepsis (cf. Fig. 2B), is secreted beneath the chorion. Ab10: 10th abdominal segment, Am: amnion, Ch: chorion, H: head, HpC: hydropyle cell, Se: serosa, SeCt: serosal cuticle, Th3: mesothoracic segment, Y: york. Bars=A, 300 μm; B, 100 μm.

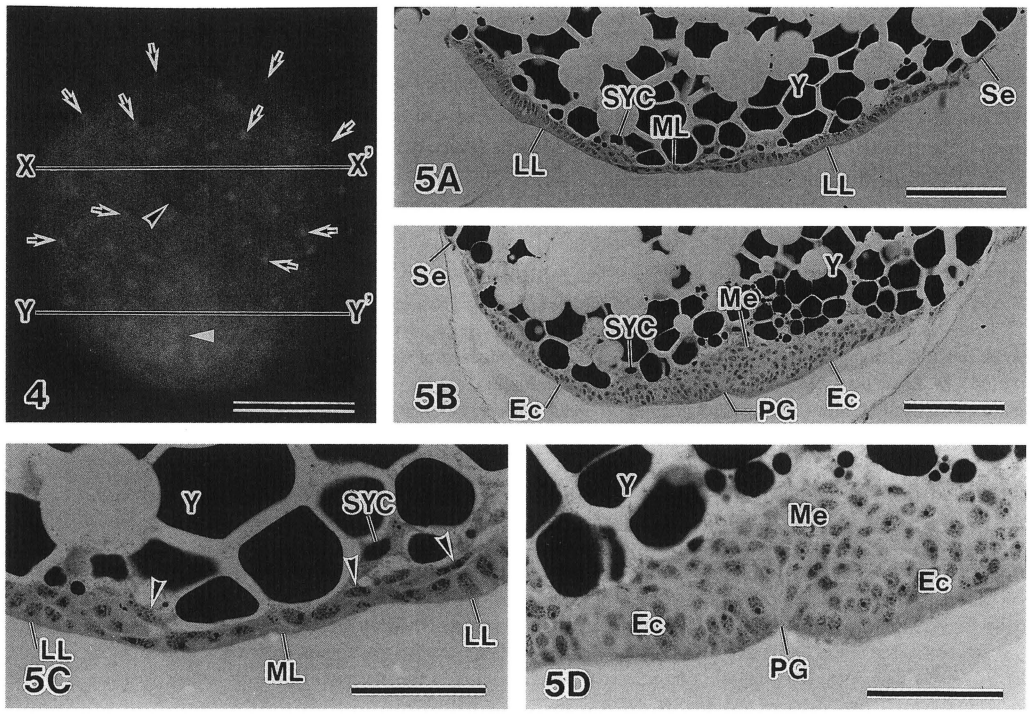

Fig. 4 Germ disc of *Galloisiana yuasai*. Methods used were the same as those in Figure 1. A white arrowhead and arrows respectively show the primitive groove and secondary york cells. A black arrowhead indicates a circular area appearing as a dark spot. Bar=200 μm.

Fig. 5 Transverse sections of the same *Galloisiana yuasai* germ disc as shown in Figure 4. Methods used were the same as those in Figure 2. A. Section through X–X' in Figure 4. B. Section through Y–Y' in Figure 4. C. Enlargement of A. Arrowheads show the freshly differentiated mesoderm. D. Enlargement of B. See text. Ec: ectoderm, LL: lateral lobes, Me: mesoderm, ML: middle lobe, PG: primitive groove, Se: serosa, SYC: secondary york cells, Y: york. Bars=A, B, 100 μm; C, D, 50 μm.

groove (Fig. 5A, C), reveals a tripartite organization: a middle lobe with lobes on either side. The former lobe seems to be pushed up dorsally by the latter two. This is reminiscent of a mesodermal segregation of the fault-type (the cut-off-and-sink type; cf. Johannsen and Butt, 1941). If the mesodermal segregation of *Galloisiana yuasai* is categorized as a fault-type, i) the middle and lateral lobes observed in Figure 5A, C could be respectively designated as the middle and lateral plates related to the mesodermal segregation, ii) the circular area could represent the median plate, and iii) the primitive groove running posteriorly from the circular area (Fig. 5B, D) could be regarded as the consequence of the fusion of lateral plates. However, the fault-type mesodermal segregation is only known in higher insect orders such as Homoptera, Lepidoptera and Hymenoptera, and has not been reported in Polyneoptera (cf. Johannsen and Butt, 1941). Notopteran mesodermal segregation needs to be examined in detail.

Amnioserosal fold

As mentioned above, the amnion and serosa in the newly formed amnioserosal fold are quite thick in *Galloisiana yuasai* (Fig. 2A, B). It is reported that a thick amnion is initially formed in the amnioserosal fold in some polyneopteran representatives, Plecoptera (Miller, 1940; Kishimoto and Ando, 1985) and Isoptera (Knower, 1900). Furthermore, in Plecoptera, the serosa is reported to thicken (Miller, 1940; Kishimoto and Ando, 1985), to form a special structure, which Miller (1940) called *grumulus*. Thus, the resemblance in the amnioserosal fold between *Galloisiana yuasai* and Plecoptera and Isoptera might reflect phylogenetic affinities.

Figure 3A is a sagittal section of an elongated *Galloisiana yuasai* embryo in the diapause stage. The hydropyle cells are observed to differentiate at the posterior pole of the egg. The serosal thickening observed in the amnioserosal

fold in the early germ band (Fig. 2B) may be the precursor of the hydropyle cells.

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