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Early Embryonic Development and External Features of Developing Embryos in the Primitive Moth, *Eriocrania sp.* (Lepidoptera, Eriocraniidae)*

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and

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Synopsis

The early embryonic development and external features of the developing embryo of an eriocranid moth, *Eriocrania sp.* (suborder Dacnonypha), are described. The eggs of this species are laid in the tissue of leaf buds of *Alnus inokumae*. The newly laid egg is elongated and ovoid, about 0.48 by 0.23 mm in size and later it progressively increases to about 0.62 by 0.35 mm. The egg period is about 7 days at the temperature of 15−20℃.

The periplasm is thicker than that of the most primitive lepidopteran *Neomicropteryx nipponensis* (suborder Zeugloptera), but thinner than that of the advanced ditrysian Lepidoptera. The thick blastoderm is formed by the occurence of cleavage furrows between the energids migrated into the periplasm as in observed in most lepidopteran species. The germ disk is very large, as in the case of the ditrysian species, and differentiates into large germ band *in situ*. The invagination of the germ disk into the yolk, as observed in the zeuglopteran and exoporian Lepidoptera, does not occur. Embryonic membranes are formed by the fusion of amnioserosal folds: this situation is assumed to be an archetype of the fault type in the ditrysian embryo. Yolk cleavage does not occur. The germ band develops on the yolk surface, as in the case of the zeuglopteran and exoporian eggs, but does not sinks into the yolk as in the ditrysian eggs. In spite of forming the superficial germ band, the embryonic membranes persist even after dorsal closure of the embryo; therefore, the secondary dorsal organ does not form. This situation is common to all

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other Lepidoptera except for the Zeugloptera in which the organ forms. In the young germ band, two temporary transverse furrows, which are similar to those observed in the dipteran embryos and can be termed a cephalic furrow and abdominal furrow, appear in the gnathal segments and anterior abdominal segments. These furrows are smoothed out when the germ band is fully elongated. Furrows of this kind have not been observed in any other lepidopteran embryos, thus being characteristic of the Dacnonypha. In the pre-revolution stages, hydropyle cells are present on the dorsal side of the egg. This feature is common only between the Zeugloptera and Dacnonypha.

The mode of the embryonic development of the eriocranid species, as a whole, has closer affinity to that of the advanced ditrysian Lepidoptera than to that of the most primitive Lepidoptera, *i. e.*, Zeugloptera.

Introdution

In the past decade, many descriptive embryological studies on several primitive lepidotperan species, such as *Neomicropteryx nipponensis* in the suborder Zeugloptera (Ando and Kobayashi, 1978; Kobayashi, 1983; Kobayashi and Ando, 1981, 1982, 1983, 1984), *Endoclita excrescens* and *E. sinensis* in the suborder Exoporia (Ando and Tanaka, 1976, 1980; Kobayashi *et al.*, 1981; Tanaka, 1980; Tanaka *et al.*, 1985), demonstrated that the mode of their embryonic development is largely different from that of the advanced suborder Ditrysia in some important respects, but is similar to that of the closely related order Trichoptera. These embryological data are expected to afford available means of considering the phylogenetic relationship among the lepidopteran suborders (Kristensen, 1984). However nothing has been known about the embryogenesis of other primitive Lepidoptera, such as the members of the suborder Dacnonypha.

In the present study we describe the early embryonic development and the external features of the developing embryo of an eriocranid species (*Eriocrania sp.* on *Alnus*, undescribed species) belonging to the Dacnonypha, and discuss their phylogenetic significance from the comparative embryological standpoint.

Materials and Methods

Moths of *Eriocrania sp.* emerge in late April to early May at the artificial experimental forest of *Alnus inokumae* in the Hokkaido Forest Experiment Station, Bibai City, Hokkaido, Japan. Immediately after copulation, the female moth inserts her ovipositor into a leaf bud of *A. inokumae*, and lays her eggs in the parenchyma of the leaf bud. In many cases, the moths laid two or three eggs at one time.

The eggs at various developmental stages were removed from the leaves and fixed in alcoholic Bouin's solution at room temperature. After fixation the embryos become easily observable through the thin transparent chorion. After observation the eggs were punctured with a fine needle, dehydrated, mounted in paraffin, sectioned at 6 to 8 μ m, and stained with Delafield's haematoxylin and eosin.

A scanning electron microscope was also used to observe the egg surface.

Observations

1. *Structure of newly laid egg*

The egg period of *Eriocrania sp.* is 160 to 170 hours (about 7 days) under the incubated condition maintained at 15－20℃ and about 100% relative humidity. In cool natural conditions, the period is prolonged to be about 10 days.

The newly laid egg is translucent white, long ovoid, and about 0.48 by 0.23 mm in size. As development proceeds, the size increases to 0.62 by 0.35 mm. The surface of the egg is almost smooth except the anterior pole in which some ridges are observed (Fig. 1). The ridges consists of an outer, large, and nearly circular and inner, small meshy ones. Five micropylar canals are arranged radially along the inner edge of the outer ridge. No aeropyles are observed on the egg surface.

The chorion is very thin (about 0.2 μm) and soft, so that the egg is brittle. In natural conditions, however, the egg is saved from damage, because it is protected from outer environment by being covered with the soft parenchyma. The vitelline membrane, expected to exist below the chorion, cannot be observed in this species. The periplasm is a thin layer (5－6 μm in thickness) of cytoplasm around the yolk mass, and stains lightly with haematoxylin (Fig. 2). The cytoplasmic island, which is a thickened area of periplasm, is lying about one-third of the way posterior to the anterior pole. The yolk mass is composed of two kinds of yolk spherules. The first type of spherules is numerous, varies in size (1－10 μm in diameter), and stains lightly with eosin; it is probably proteid yolk. The other type is not evident in paraffin sections, but can be seen as spherical vacuoles located among red-stained yolk droplets; this type is made of soluble fatty droplets. Polar granules are absent.

2. *Early embryonic development*

As stated before, the egg period of this species is 160 to 170 hours under the incubated conditions. For the convenience of description this period can be divided into 16 stages. The description of the following three early stages is chifly based on the histological observations of sections of eggs.

Stage 1 (0－8 hours, Fig. 3): Maturation division, fertilization, and cleavage. The maturation division is expected to occur in the cytoplasmic island, but the authors failed to observe its exact process and the subsequent ones, *i. e.*, fertilization and early cleavage. At the end of this stage, cleavage nuclei or energids migrate outwards and finally reach the periplasm almost simultaneously to form the syncytial blastoderm (Fig. 3), composed of about 400 nuclei. Some of the energids remain in the yolk to become the primary vitellophages.

Stage 2 (8－18 hours, Fig. 4): Blastoderm formation. After reaching the periplasm the nuclei undergo two or more divisions with the spindle parallel to the egg surface, and their number reaches about 2,000. Soon later, cell membranes appear between the nuclei and then cell membranes are formed basally between the each nucleus and the

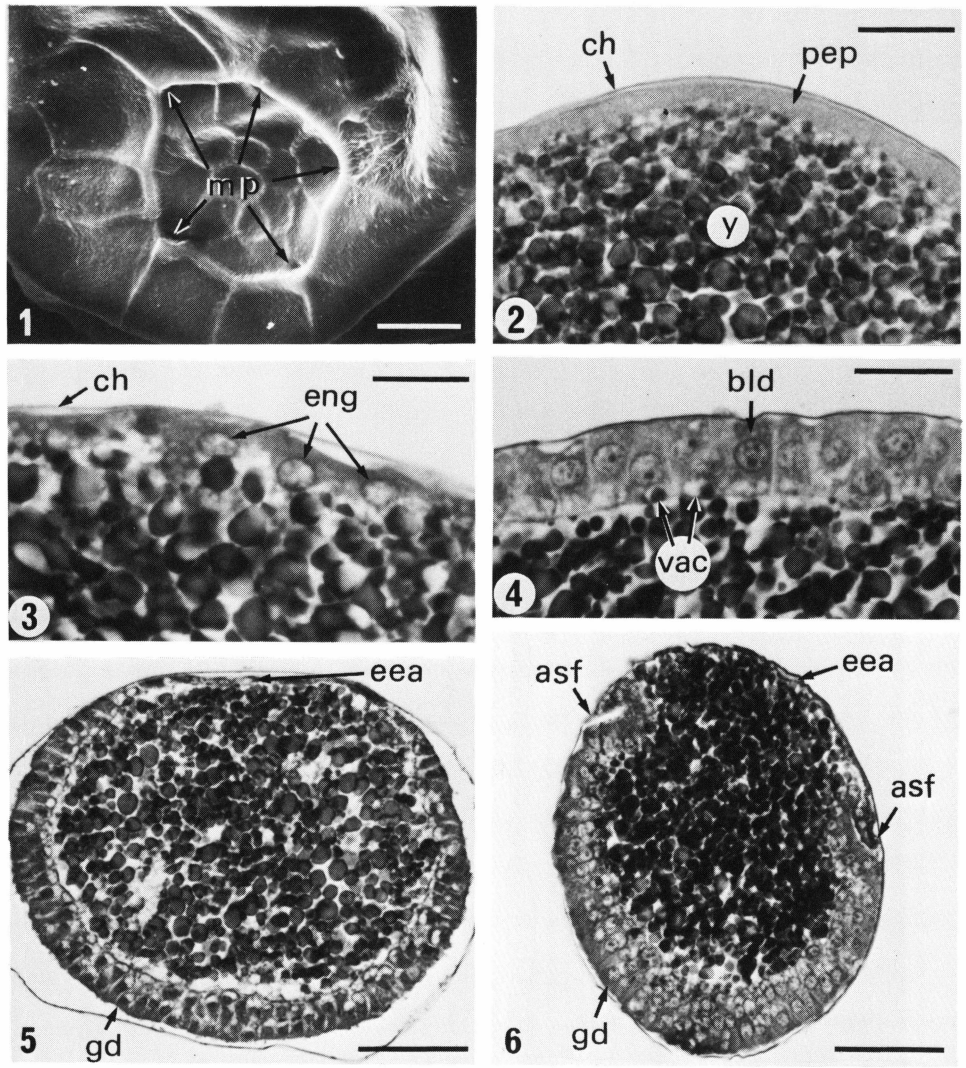


Fig. 1. Anterior pole of egg, showing micropylar region. Scale: 10 μm.
Fig. 2. Cross section of newly laid egg, showing periplasm. Scale: 20 μm.
Fig. 3. Cross section of about 8-hour-old egg (stage 1), showing energids reached the periplasm. Scale: 20 μm.
Fig. 4. Longitudinal section of about 15-hour-old egg (stage 2), showing blastoderm. Scale: 20 μm.
Fig. 5. Cross section of about 20-hour-old egg (stage 3), showing germ disk and extraembryonic area. Scale: 50 μm.
Fig. 6. Cross section of about 24-hour-old egg (stage 3), showing the developing amnioserosal folds. Scale: 50 μm.

asf, amnioserosal fold; bld, blastoderm; ch, chorion; eea, extraembryonic area; eng, energid; gd, germ disk; mp, micropyle; pep, periplasm; vac, vacuole; y, yolk.

yolk, thereby completing the cellular blastoderm (Fig. 4). At the time of its formation, the peripheral yolk granules are incorporated into the basal part of the blastodermal cells, so that the blastoderm becomes far thicker (about 15 μm) than the periplasm. A fine yolk membrane or secondary vitelline membrane could not be observed at the surface of the yolk. Some of the blastodermal cells migrate back into the yolk mass and become the secondary vitellophages.

Stage 3 (18−24 hours, Figs. 5−8): Formation of germ disk and appearance of amnio-serosal folds. When the blastoderm is complete, its cells are fairly uniform in size. At about 18 hours after oviposition, however, the cells, ranging from the ventral to the lateral side of the blastoderm, divide actively to become columnar. This broad and thickened area is the embryonic area or germ disk (Figs. 5, 7, 8), whereas the cells on the dorsal side decrease in thickness and increase in width without cell division. This thin dorsal area is the extra-embryonic area or presumptive serosa.

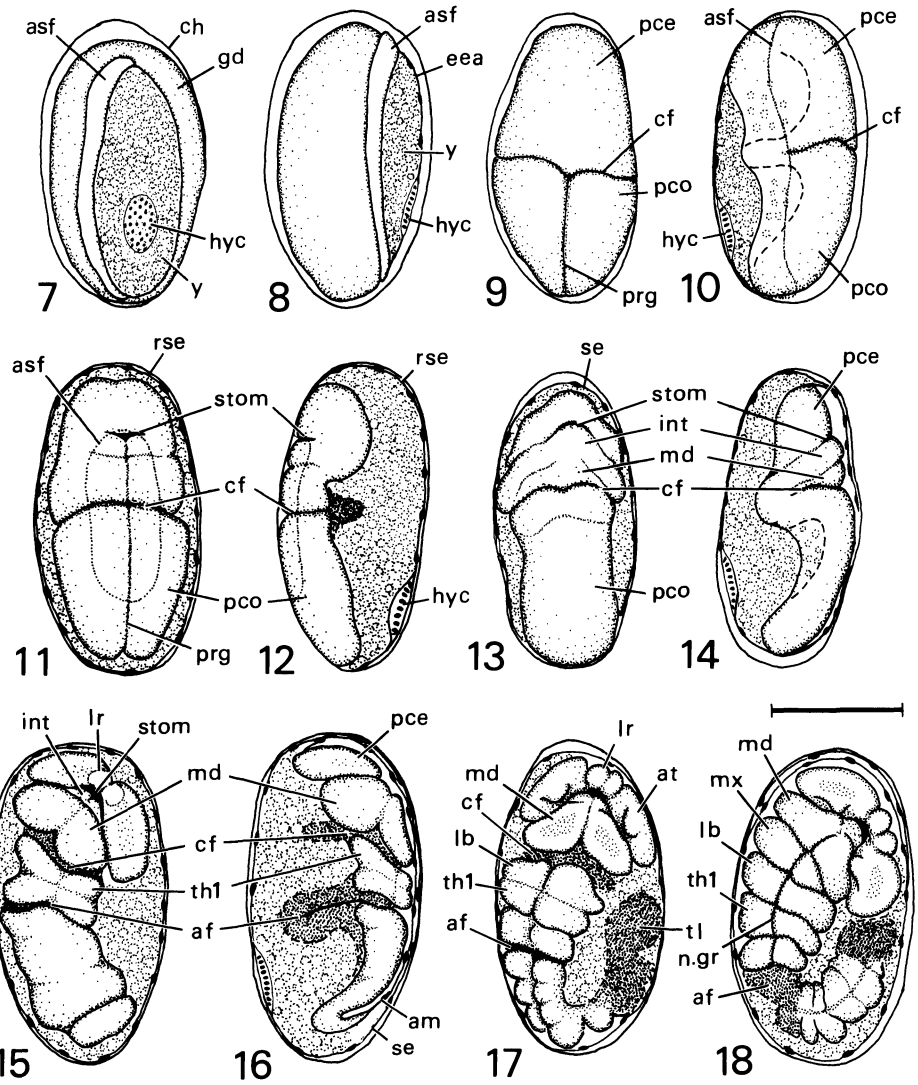
As the differentiation between the germ disk and the extra-embryonic area advances, a slightly thickened, circular area consisting of about 50 cells appears in the extra-embryonic region near the posterior pole (Figs. 7, 8, hyc). These cells then become columnar, about 15 μm tall, and have deeply stained basal nuclei. It is assumed that these columnar cells correspond to hydropyle cells found in the eggs of the grasshopper, *Melanoplus differentialis* (Slifer, 1938), and in many heteropteran species (Cobben, 1968; Mori, 1970).

Soon after formation of the germ disk, the amnio-serosal fold is formed along the margin of the germ disk. That is, the marginal tissue composed of columnar cells folds ventrally over the germ disk and begins to spread out towards the center of the germ disk, carrying the margin of the attenuated extra-embryonic area (Figs. 6−8, amf). The first sign of folding is usually seen along either half side of the germ disk, but soon later the fold arises along another half side too. The amnio-serosal folds extend so slowly that their lips open throughout this stage; these meet and fuse at the midventral part of the egg in stage 6, as described later.

3. *Changes in the external form of the embryos*

The description of the following stages is chiefly based on the external observations of the whole eggs. Detailed histological observations will be described in future papers.

Stage 4 (24−36 hours, Figs. 9, 10, 19, 20): Formation of cephalic furrow and primitive groove. At the beginning of this stage, the primitive groove appears along the ventro-midline of the posterior region of the germ disk to form the inner layer (Figs. 9, 19, prg, inl); thus the germ disk develops into a germ band *in situ*. Soon later, a long transverse furrow is formed near the center of the germ band (Figs. 8, 9, 20). This furrow may safely be called a cephalic furrow as in the case of the dipteran embryos, because its position corresponds to the future gnathal segments (maxillary and labial ones). Meanwhile, the primitive groove grows forward to the level of the cephalic furrow (Fig. 20).



Figs. 7, 8. Dorsal (Fig. 7) and lateral (Fig. 8) view of egg at stage 3.
Figs. 9, 10. Ventral (Fig. 9) and lateral (Fig. 10) view of egg at stage 4.
Figs. 11, 12. Ventral (Fig. 11) and lateral (Fig. 12) view of egg at early stage 5.
Figs. 13, 14. Ventral (Fig. 13) and lateral (Fig. 14) view of egg at late stage 5.
Figs. 15, 16. Ventral (Fig. 15) and lateral (Fig. 16) view of egg at stage 6.
Fig. 17. Ventral veiw of egg at stage 7.
Fig. 18. Ventral view of egg at stage 8.
af, abdominal furrow; am, amnion; asf, amnioserosal fold; at, antennal rudiment; cf, cephalic furrow; ch, chorion; eea, extraembryonic area; gd, germ disk; hyc, hydropyle cell; int, intercalary segment; lb, labial segment; lr, labral rudiment; md, mandibular segment; mx, maxillary segment; n. gr, neural groove; pce, protocephalon; pco, protocorm; prg, primitive groove; rse, rudimentary serosa; se, serosa; stom, stomodaeum; th1, first thoracic segment; tl, telson; y, yolk. Scale: 0.2 mm.

The amnio-serosal folds further extend from all margins of the germ band, but do not yet fuse each other in this stage.

Stage 5 (36−44 hours, Figs. 11−14, 21, 23): Differentiation of protocephalon and protocorm and appearance of stomodaeal invagination. In the early period of this stage, the large germ band becomes slightly slender and then the protocephalon and protocorm become discernible (Figs. 11, 12). The primitive groove grows further forward to the posterior region of the protocephalon.

In the middle of this stage, the stomodaeum invaginates at the posterior part of the protocephalon (Fig. 21). Four gnathal segments, *i. e.*, the intercalary, mandibular, maxillary, and labial one, are formed in the anterior part of the protocorm. However the maxillary and labial segments are situated in the invaginated part of the cephalic furrow; hence these are not observed from outside (Fig. 13, 14). As the development advances, the primitive groove disappears first in the posterior part of the protocorm, and then in the anterior part, and disappears completely by the end of this stage.

In this stage, the amnio-serosal folds extend rapidly to cover almost all the ventral surface of the germ band (Fig. 23), but their lips still open until the beginning of the next stage.

The yolk segmentation, which is observed in the embryos at this stage in all the ditrysian Lepidoptera, does not occur.

Stage 6 (44−54 hours, Figs. 15, 16, 22): Appearance of labral rudiments and abdominal furrow. At the beginning of this stage, the lips of the amnio-serosal folds meet and fuse at the midventral region of the egg; thus the amnion and serosa are completed. The protocephalon becomes large and bilobed, and labral rudiments appear as a pair of separate lobes at the anterior end of the protocephalon. The mandibular segment becomes large and is observed clearly from outside, but both the maxillary and labial ones are still kept within the cephalic furrow. As the mandibular segment develops, the intercalary one shifts anteriorly, and then degenerates gradually. Thus the segment becomes slightly observed only between the protocephalon and the mandibular segment.

The most remarkable feature in this stage is that the region corresponding to the future third thoracic segment to the third abdominal segment invaginates deeply into the yolk, thus the large transverse furrow is formed in the middle part of the protocorm (Figs. 15, 16, 22). We call it an abdominal furrow. Therefore, two transverse furrows are observed in the germ band; the anterior one is the cephalic furrow and the posterior one the abdominal furrow.

Stage 7 (54−66 hours, Fig. 17): Segmentation of the future thoracic and abdominal region. The posterior part of the germ band elongates rapidly towards the dorsal region of the egg; thus the germ band becomes dorsoventrally curved (Fig. 17). Accompanying elongation, the thoracic and abdominal segments become apparent, although the cephalic and abdominal furrows still remain. In most eggs, the elongated posterior abdominal segments (behind the seventh abdominal one) are slightly immersed in the dorsal part of the yolk mass.

About the middle of this stage, a pair of antennal rudiments is formed near the posterior border of the protocephalon.

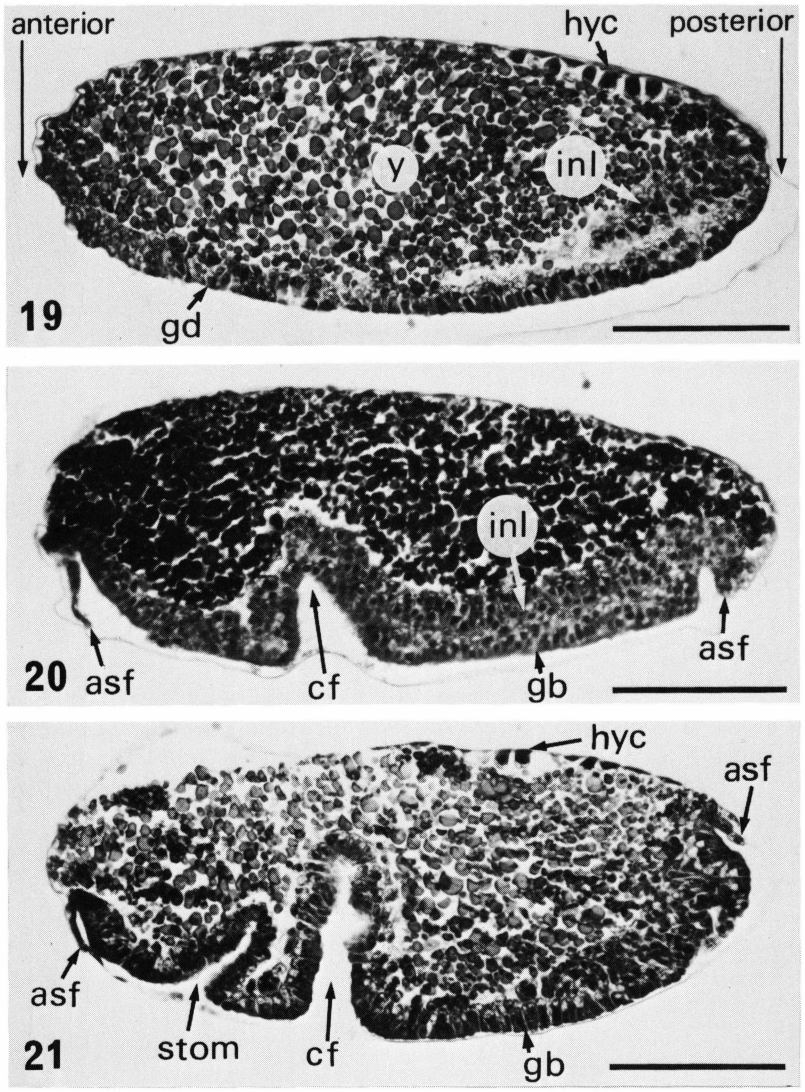


Fig. 19. Parasagittal section of about 28-hour-old egg (stage 4), showing germ disk, hydropyle cells, and inner layer. Scale: 100 μm.

Fig. 20. Sagittal section of about 35-hour-old egg (stage 4), showing cephalic furrow and amnioserosal folds. Scale: 100 μm.

Fig. 21. Parasagittal section of about 40-hour-old egg (stage 5), showing stomodaeal invagination. Scale: 100 μm.

asf, amnioserosal fold; cf, cephalic furrow; gb, germ band; gd, germ disk; hyc, hydropyle cell; inl, inner layer; stom, stomodaeum; y, yolk.

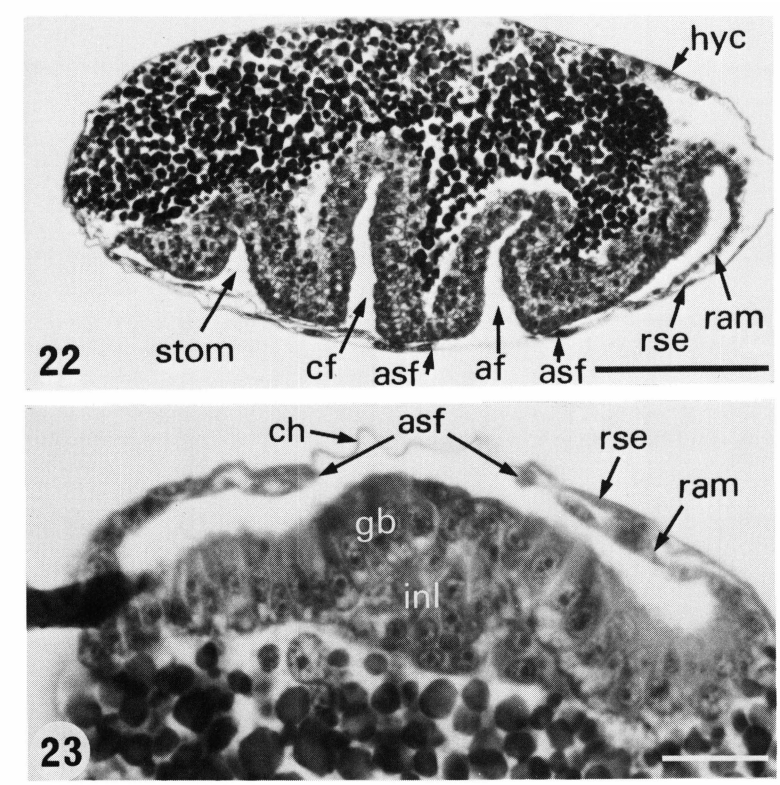


Fig. 22. Longitudinal section of about 50-hour-old egg (stage 6), showing abdominal furrow and amnioserosal folds just before their fusion. Scale: 100 μm.
Fig. 23. Cross section of about 40-hour-old egg (stage 5), showing amnioserosal folds extending over the ventral surface of protocorm. Scale: 20 μm
af, abdominal furrow; asf, amnioserosal fold; cf, cephalic furrow; ch, chorion; hyc, hydropyle cell; inl, inner layer; ram, rudimentary amnion; rse, rudimentary serosa; stom, stomodaeum.

In sections, it is observed that the proctodaeal invagination is formed in the last abdominal segment or telson.

Stage 8 (66 — 75 hours, Fig. 18): Formation of neural groove. The germ band or embryo widens and the neural groove appears along the ventro-midline of the germ band. The invaginated part of the cephalic furrow, *i. e.*, the maxillary and labial segments, come to the surface gradually, but the abdominal furrow still remains.

Stage 9 (75 — 85 hours, Fig. 24): Appearance of gnathal appendages. Both the labral and antennal rudiments become large and hemispherical. In the gnathal region, the rudiments of mandible, maxilla, and labium are formed as three pairs of swellings. The intercalary segment disappears completely. In most eggs, although the abdominal furrow becomes shallow, it still remains.

Stage 10 (85 — 93 hours, Fig. 25): Appearance of rudimentary thoracic appendages. The embryo further widens and a pair of rudimentary appendages appears in each thoracic segment, but these lack in the abdominal segments (Fig. 25). As the development advances, the invaginated part of the abdominal furrow, *i. e.*, the third thoracic to the third abdominal segment, comes to the surface gradually; thus the abdominal furrow disappears completely. The posterior end of the abdomen, however, is still immersed slightly in the yolk.

Stage 11 (93—100 hours, Fig. 26): Morphogenetic movement of the cephalo-gnathal region. In the early period of this stage, the neural groove disappears completely. The embryo further widens and thickens, and morphogenetic movement of the cephalo-

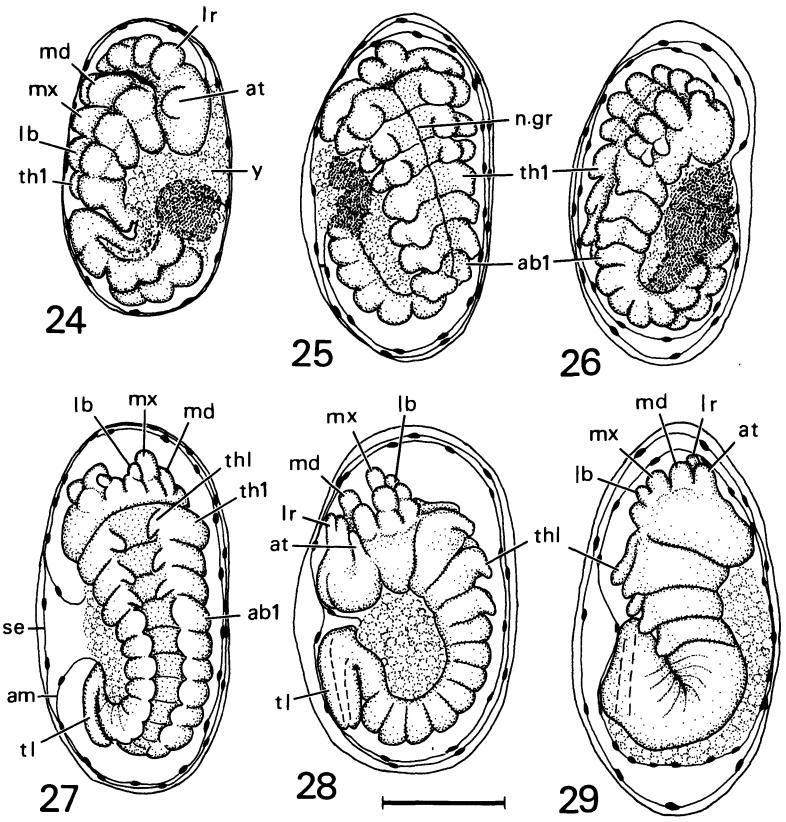


Fig. 24. Lateral view of egg at stage 9.
Fig. 25. Ventral view of egg at stage 10.
Fig. 26. Lateral view of egg at stage 11.
Figs. 27, 28. Ventral (Fig. 27) and lateral (Fig. 28) view of egg at stage 12.
Fig. 29. Lateral view of egg at stage 13 (revolution).
abl, first abdominal segment; am, amnion; at, antennal rudiment; lb, labial segment; lr, labral rudiment; md, mandibular segment; mx, maxillary segment; n. gr, neural groove; se, serosa; thl, thoracic leg rudiment; th1, first thoracic segment; tl, telson. Scale: 0.2 mm.

gnathal region to form the head begins. That is, the labral rudiments shift slightly posteriorward and come in contact with the mandibular ones. The antennal rudiments also shift to the outer side of the labral ones. Three gnathal segments contract slightly and unite each other (Fig. 26). At the end of this stage, each labial rudiment also moves towards the median line.

Stage 12 (100 — 108 hours, Figs. 27, 28): Pre-revolution stage. The embryo becomes widest and the morphogenetic movement of the cephalo-gnathal region further proceeds. That is, the labral rudiments fuse and become flat (Fig. 28). The mandibular rudiments become globose and the maxillary ones are two-segmented and project forwards. The labial rudiments moving towards the median line also become two-segmented and pointed at their extremities, and these finally fuse to form a single lobe. The antennal rudiments become slender and pointed. The thoracic appendages are fully developed, projecting posteriorly (Figs. 27, 28). The abdominal region consists of 10 segments inclusive of the telson bending sharply to the ventral side.

At the end of this stage owing to the increasing consumption of the yolk by the embryo, the yolk mass gradually decreases in volume and finally is left only on the dorsum of the embryo. Thus the embryo assumes a completely superficial position and is nearly C-shaped in lateral view (Fig. 28). In the sections of this stage, it is observed that the amnion spreads dorsally as paired folds across the dorsal surface of the yolk mass. The folds come together in the narrow space between the protocephalon and the telson. The inner side of the amnion formed from its inner fold provisionally covers the dorsal surface of the yolk mass, the remainder forms a closed envelope around the embryo.

Stage 13 (108—125 hours, Fig. 29): Revolution of the embryo. In this process the posterior abdominal segments are first turned ventrally, causing the abdominal end to shift anteriorwards and finally reach the level of the mesothorax (Fig. 29). As revolution proceeds, the integration of the cephalo-gnathal region also proceeds, and lastly the cephalo-gnathal region is turned ventrally, thereby causing the gnathal appendages surrounding the stomodaeal opening to project forwards. The amnion and serosa do not rupture and are retained during the revolution.

Stage 14 (125—145 hours, Figs. 30, 31): Progress of dorsal closure. After revolution the head is located just at the anterior pole of the egg (Fig. 30). The thoracic leg rudiments completely degenerates by the end of this stage (Fig. 31). The abdomen is composed of 10 segments, and its end gradually reaches the level of the head. Lateral walls of the embryo grow dorsally, and the envelope provisionally covering the yolk mass become replaced by these growing lateral walls. This process or dorsal closure begins first at the posterior region of the abdomen and then proceeds anteriorly (Fig. 31). Two embryonic membranes are left intact in this process.

Stage 15 (145—155 hours, Fig. 32): Completion of dorsal closure. Lastly the dorsal region of the mesothorax is enclosed by the lateral walls, and dorsal closure is completed at about 40 hours after revolution. After completion of dorsal closure, basic form of the first instar larva is established (Fig. 32). That is, the labrum and mandibles become

thin. The tips of the maxillae become sharp and segmented. The labium is located between the maxillae, thus being not observed from lateral side. The antennae become very small and pointed. The thoracic segments grow wide and stout.

The serosa is still intact even after completion of dorsal closure, but the amnion is not observed in this period. The latter is thought to be degenerated *in situ*. Thus the secondary dorsal organ is not formed.

Stage 16 (155 — 165 hours, Figs. 33, 34): Full-grown embryo just before hatching. At the beginning of this stage, the serosa disappears probably owing to the ingestion by the embryo.

The definitive form of the head capsule and mouth-parts are established (Fig. 33).

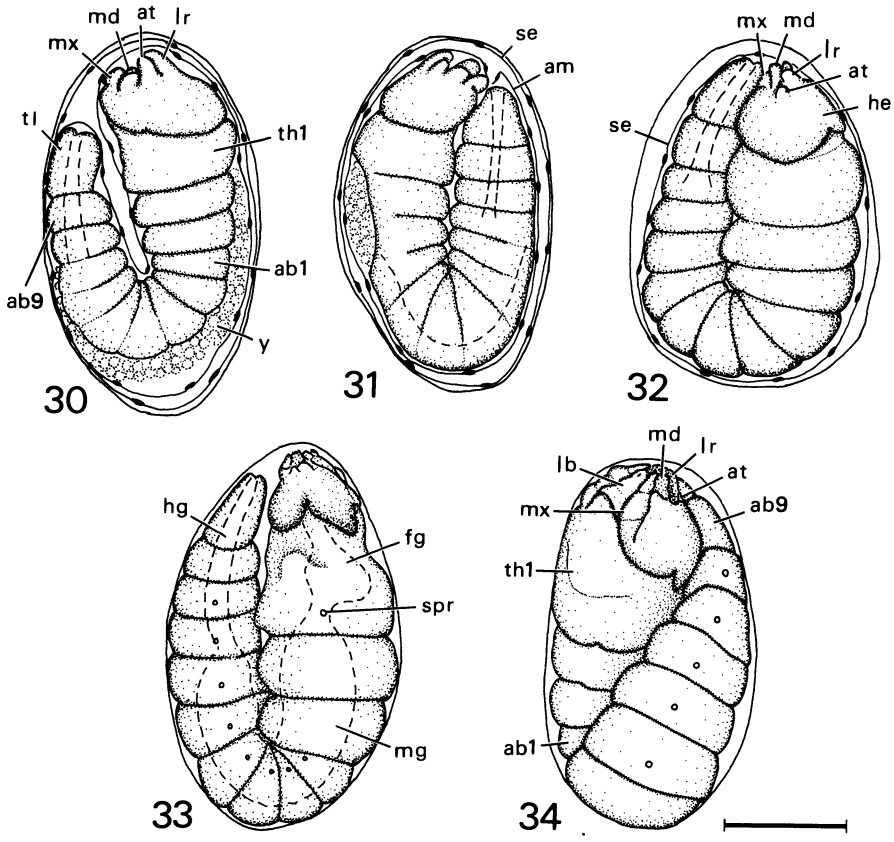


Fig. 30. Lateral view of egg at early stage 14.
Fig. 31. Lateral view of egg late stage 14.
Fig. 32. Lateral view of egg at stage 15.
Fig. 33. Lateral view of egg at stage 16.
Fig. 34. Lateral view of egg at late stage 16 (just before hatching).
abl, 9, first and ninth abdominal segments; am, amnion; at, antennal rudiment; fg, foregut; he, head; hg, hindgut; lb, labium; lr, labrum; md, mandible; mg, midgut; mx, maxilla; se, serosa; spr, spiracle; thl, first thoracic segment; tl, telson. Scale: 0.2 mm.

At first, the mandibles become sclerotized and pigmented at their distal end. Then the head capsule and other mouth appendages are also sclerotized and pigmented. A pair of spiracles becomes discernible on both sides of the prothorax and first eight abdominal segments.

Just before hatching, the embryo twists its head and thorax to the ventral side of the egg and its abdominal end to the dorsal side (Fig. 34). The mandibles begin their pulsatile movement.

On hatching, the anterior pole of the egg shell is ruptured by gnawing with the mandibles, and the newly hatched larva (Fig. 35) immediately eats into the tissue of the leaf.

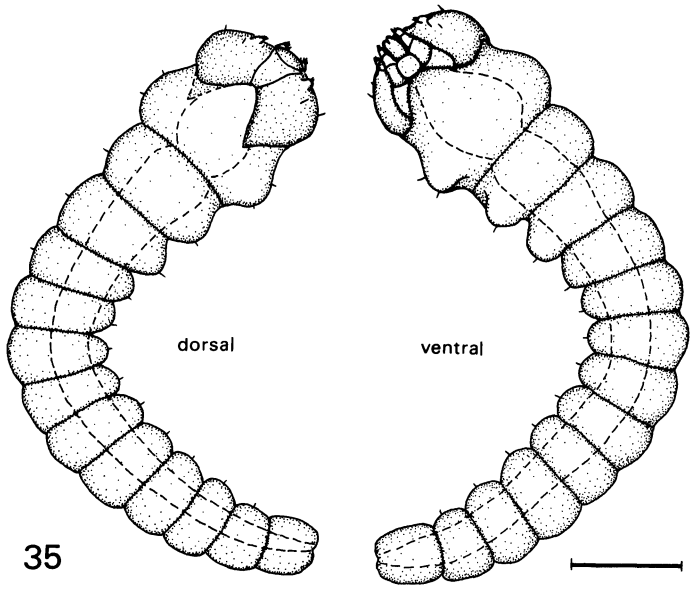


Fig. 35. Newly hatched larva. Scale: 0.2 mm.

Discussion

1. *Structure of newly laid egg*

Most eggs of the higher ditrysian Lepidoptera have a hard and dense chorion throughout which are found many aeropyles (Hinton, 1981). In these Lepidoptera, the chorion is highly impermeable to water or air, hence the eggs can survive both in dry and flooded environments without desiccation or drowning. On the other hand, in one of the most primitive Lepidoptera, *Neomicropteryx nipponensis* (Micropterygidae), the egg has a porous, spongy chorion and is covered with many hygroscopic gelatinous matrices which swell with surrounding water or moisture (Kobayashi and Ando, 1981, 1982). Since the eggs of this species cannot survive in a dry environment, both the presence of a spongy chorion and the occurrence of hygroscopic matrices are thought to be

advantageous for retaining moisture. A similar porous and spongy structure is also found in the eggs of other primitive Lepidoptera, such as the hepialid moths (*Hepialus humuli*, *Endoclita excrescens*), although these eggs lack the hygroscopic material (Hinton, 1981; Kobayashi, unpublished data). *Endoclita* eggs do not hatch out if these were not kept moist. The eggs of *Eriocrania* have a thin but dense chorion without spongy structure; hence, in this respect, these are similar to those of the ditrysian Lepidoptera. However *Eriocrania* eggs also do not survive, if these were taken out from the parenchyma of the leaf and kept in a dry environment. Therefore in the eggs of *Eriocrania*, instead of lacking such structure retaining water or moisture as observed in the micropterygid and hepialid species, it is presumed that the parenchyma surrounding the egg has a role in retaining water and supplying it to the egg. The possibility of need of water for the development of *Eriocrania* eggs will be supported by both the increase in egg volume during development and the presence of hydropyle cells as discussed later. Increase in egg volume is also reported in *N. nipponensis* and the monotrysian lepidopteran, *Nemophora raddei* (Kuroko, 1961) and *Nepticula castanopsiella* (Kobayashi, 1983).

It is well known that in general the eggs of most holometabolan insects have a well-developed periplasm and an extensive cytoplasmic reticulum, but less yolk. The eggs of hemimetabolous insects, on the other hand, are characterized by abundant yolk and a poorly developed periplasm. Almost all the known eggs of the ditrysian Lepidoptera have a well-developed, distinct periplasm (about 10−20 μm in thickness irrespective of egg size), and thus belong to the holometabolan type. On the contrary, the eggs of the zeuglopteran *Neomicropteryx* have a very thin, poorly developed periplasm, and are thus like the hemimetabolan type. The nature of the periplasm in this species is thought to reflect its primitive position in the systematics of the Lepidoptera. The periplasm of *Eriocrania* is thicker than that of *Neomicropteryx*, but thinner than that of the ditrysian species. Therefore the periplasm of this species shows an intermediate condition between the most primitive Lepidoptera and higher ones.

In the eggs of some ditrysian Lepidoptera such as *Orgyia antiqua* and *Amata fortunei*, regional differences in the distribution of yolk granules and of their stainability have been reported (Christensen, 1942; Tanaka, 1985). These differences in the yolk system are not observed in *Eriocrania* as in the case of other primitive lepidopterans, *Neomicropteryx* and *Endoclita*.

2. *Formation of blastoderm and germ disk*

According to Tanaka (1985), the mode of the blastoderm formation in the Lepidoptera is classified into three types. The first type is the formation of the blastoderm by the occurrence of cleavage furrows between the energids migrated into the periplasm, and is observed in most species of the Lepidoptera. In the second type the blastoderm is formed by protrusion of energids beyond the initial level of the periplasm, and this type is reported in *Bombyx mori* (Takesue *et al.*, 1980; Keino and Takesue, 1981) and *Amata fortunei* (Tanaka, 1985). In the third type protrusion of energids occurs beyond the initial level of the periplasm at first, but the boundaries between the energids are formed by cleavage furrows, and this type is observed in several lepidopteran species. The first type is widely known among many insects other than the Lepi-

doptera, but the second and third type are peculiar to the Lepidoptera. With respect to these modes of the blastoderm formation, *Eriocrania* belongs to the first type.

In addition to the mode of the blastoderm formation, another differences can be pointed out in thickness of the blastoderm between the zeuglopteran *Neomicropteryx* and non-zeuglopteran species. In the former, the completed blastoderm is very thin (5－10 μm), whereas in the latter, it is composed of cuboidal or tall columnar cells (above 15 μ m in thickness), and the cells often incorporate yolk granules located on the periphery of yolk mass into their basal part (Tanaka, 1970; Ando and Tanaka, 1980; Miya, 1984). The blastodermal cells of *Eriocrania* is thick and often contain several peripheral yolk granules. In this respect, *Eriocrania* eggs have a common character to many other lepidopteran eggs, but differ from those of *Neomicropteryx*.

In *Eriocrania* the broad area of the blastoderm ranging from the ventral to lateral side, develops into the germ disk composed of tall columnar cells. This situation of the germ disk is very similar to that observed in many ditrysian Lepidoptera in which the broad equatorial germ disk is established (Eastham, 1927; Johannsen, 1929; Tanaka, 1985; Miya, 1985). On the contrary, in other primitive Lepidoptera such as *Neomicropteryx* and *Endoclita*, a small circular germ disk is formed on the ventral side of the blastoderm (Kobayashi and Ando, 1981, 1982; Ando and Tanaka, 1980). Therefore, with respect to the size of a germ disk too, *Eriocrania* has a closer affinity to the higher, ditrysian Lepidoptera than to the primitive *Neomicropteryx* and *Endoclita*.

3. *Formation of germ band and embryonic membranes*

Concerning the mode of formation of the germ band and embryonic membranes in the Lepidoptera, two different types have been known. The first type is widely distributed in the ditrysian lepidopteran eggs. In this type, as shown in Fig. 36-F, the large germ disk is cut off along the margin of the extraembryonic area, then sinks slightly into the yolk (Huie, 1918; Eastham, 1927; Lautenschlager, 1932; Gross and Howland, 1940; Christensen, 1943, 1953; Rempel, 1951; Presser and Rutschky, 1957; Stairs, 1960; Okada, 1960; Bassand, 1965; Anderson and Wood, 1968; Tanaka, 1970; Miya, 1984). Next, the serosa is completed by the spread of the rudimentary serosal margin over the ventral surface of the germ disk. The amnion is formed independently by the reflex extension of the edge of the germ disk. As the germ disk sinks into the yolk, the yolk granules enter into the space between the completed amnion and serosa, and then the germ disk becomes somewhat compact to form a cup-shaped germ rudiment (Here the germ rudiment refers to the embryonic area in which the inner layer formation is not yet finished). We can call this type a fault type (F-type). The fault type is also observed in eggs of the monotrysian moth, *Nepticula castanopsiella* (Kobayashi, 1983), although its germ disk is not so large as that of the ditrysian Lepidoptera.

The second type is known only in the zeuglopteran *Neomicropteryx* and the exoporian *Endoclita*. In these primitive Lepidotpera, as shown in Fig. 36-C and D, the sac-shaped germ rudiment is formed by the deep invagination of the small circular germ disk into the yolk, although the depth of the invagination is slightly different between the two groups. The serosa and amnion are formed simultaneously by the fusion of the mouth of the sac-shaped germ rudiment. Formation of the germ rudiment and

embryonic membranes in this manner can be called an invaginate type (I-type), that is fundamentally different from the F-type.

In the present study, we could find out another type of formation of the germ band and embryonic membranes in *Eriocrania*. That is, the large germ disk differentiates into a large germ band *in situ* without its invagination or sinking into the yolk, and embryonic membranes are completed by the fusion of the lips of amnioserosal folds which extend slowly over the ventral surface of the developing germ band (Fig. 36-E). This type can be called an amnio-serosal fold type (AF-type).

In the closely related order Trichoptera, both the I- and AF-types have been observed (Fig. 36-A and B). In the annulipalpian trichopteran *Stenopsyche griseipennis* (Miyakawa, 1974), the germ rudiment is formed by the deep invagination of the germ disk (Fig. 36-A); thus the process is very similar to that of *Neomicropteryx*, and belongs to the I-type. On the other hand, in the integripalpian trichopteran *Neophalax concinnus* (Patten, 1884) and *Neoserierina crassicornis* (Akaike *et al.*, 1982), the germ disk does not invaginate, and the amnion and serosa are completed by fusion of amnio-serosal folds (Fig. 36-B); thus the state belongs to the AF-type. However in other integripalpian *Glyphotaelius admorsus* (Akaike *et al.*, 1982), a shallow invagination is observed when the germ rudiment is formed; hence the state is similar to that of *Stenopsyche*. Therefore both the AF- and I-types are present in the suborder Integripalpia.

To summarize, with respect to the formation of the germ rudiment and embryonic membranes in the trichopteran and lepidopteran suborders, three different types are observed: the I-type as in the Annulipalpia, Integripalpia in part, Zeugloptera, and Exoporia; the AF-type as in the Integripalpia in greater part and Dacnonypha; the F-type as in the Monotrysia and Ditrysia. The I-type is thought to be most primitive, because this type is observed in many hemimetabolous insects. The AF-type is very common in holometabolous insects, and is presumed to be derived from the I-type. The F-type is a highly specialized one observed only in Lepidoptera, and is probably derived from the AF-type. We believe that the existence of the I-type in the Annulipalpia, Integripalpia, Zeugloptera, and Exoporia is a reflection of the close relationship between the Trichoptera and Lepidoptera. In other words, the eggs of the common ancestor of these two orders are presumed to belong to the I-type as to formation of the germ rudiment and embryonic membranes. On the other hand, the existence of the AF-type in the Integripalpia and Dacnonypha can be regarded as an example of the parallel evolution in the embryogenesis of the Trichoptera and Lepidoptera.

4. *Hydropyle cells*

For a long while, the columnar serosal cells or hydropyle cells which are responsible for water absorption in the eggs were known only in hemimetabolous insects such as in locust, *Melanoplus differentialis* (Slifer, 1938; Slifer and Sekhon, 1963), and many heteropteran species (Cobben, 1968; Mori, 1970). However in recent years, we discovered the columnar serosal cells in the eggs of the zeuglopteran *Neomicropteryx* for the first time in the Holometabola (Kobayashi and Ando, 1982). Although there is no experimental evidence, we have assumed that the cells have a function similar to that of hydropyle cells in the eggs of locusts and Heteroptera, because the egg volume of

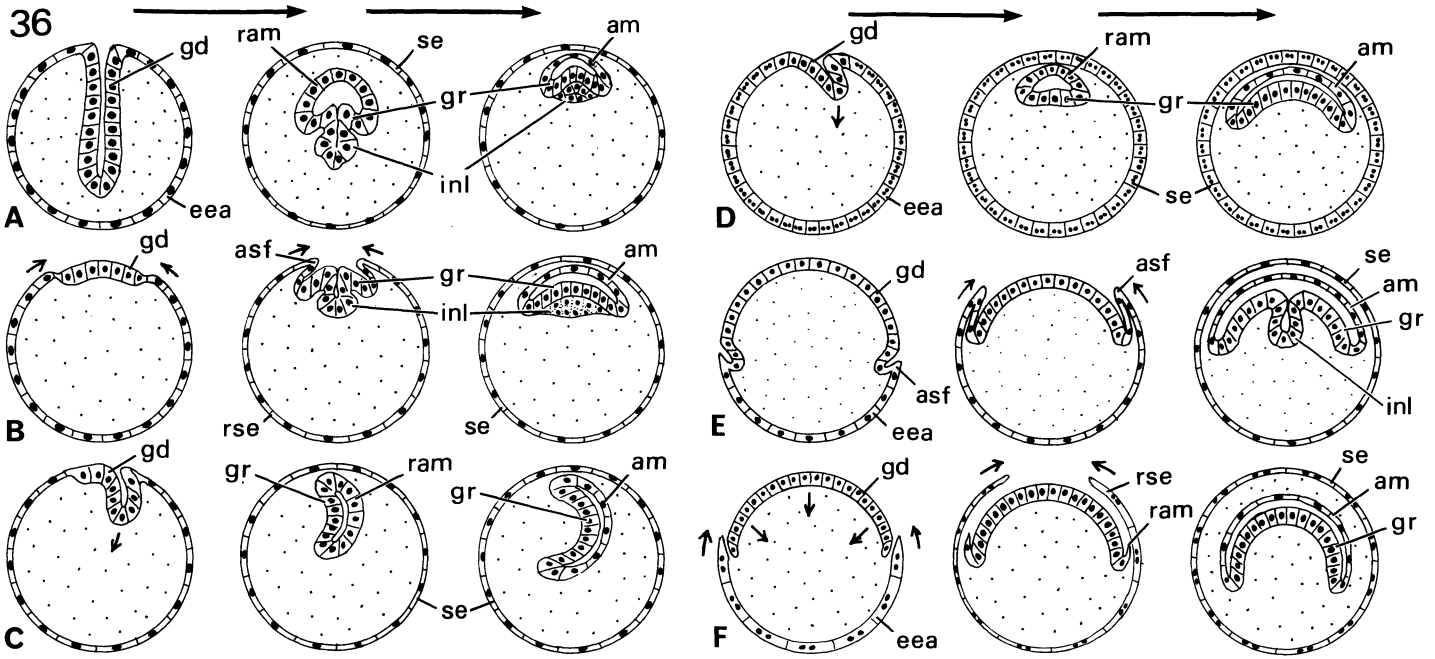


Fig. 36. Comparative diagram showing formation of germ rudiments and embryonic membranes in the trichoptera (A and B) and lepidopteran (C to F) suborders. A, Annulipalpia (cross section, modified from Miyakawa, 1973); B, Integripalpia (cross section, modified from Akaike *et al.*, 1982); C, Zeugloptera (longitudinal section, modified from Kobayashi and Ando, 1981); D, Exoporia (longitudinal section, modified from Ando and Tanaka, 1980); E, Dacnonypha (cross section); F, Monotrysia and Ditrysia (longitudinal section).

am, amnion; asf, amnio-serosal fold; eea, extraembryonic area; gd, germ disk; gr, germ rudiment; inl, inner layer; ram, rudimentary amnion; rse, rudimentary serosa; se, serosa.

Neomicropteryx increases during embryonic development in a semi-aquatic environment as stated before, and the columnar serosal cells adhere directly to the endochorion even after formation of the serosal cuticle. The columnar serosal cells of *Eriocrania* are also so similar to those of *Neomicropteryx*, locusts, and Heteroptera that these are presumed to have the same function as that of these insects; hence these can be termed hydropyle cells. In case of *Eriocrania*, the eggs are probably supplied with water through the hydropyle cells from the parenchyma surrounding eggs.

5. *Growth pattern of germ band or embryo*

As mentioned before, in ditrysian Lepidoptera yolk granules penetrate into the space between the amnion and serosa after their completion. As a result the germ rudiment becomes completely immersed in the yolk, and afterwards the embryo is kept immersed in the yolk until just before hatching. On the contrary, the germ band of the exoporian *Endoclita* is superficial in position from the beginning of germ band formation, although its germ rudiment is formed by the invagination of the germ disk into the yolk (Ando and Tanaka, 1980). In the zeuglopteran *Neomicropteryx* the sac-shaped germ rudiment is also immersed in the yolk, but as the germ band grows, it gradually appears on the egg surface beginning anteriorly. Just before revolution, it finally assumes a completely superficial position. In the young embryo of *Eriocrania*, although the parts of the cephalic and abdominal furrows and the abdominal end temporarily sink in the yolk, the embryo at the stage just before revolution assumes a completely superficial position. In all trichopteran species in which the embryonic development has been known, the embryos are completely superficial in position throughout their entire embryonic period (Patten, 1884; Miyakawa, 1973; Anderson and Lawson-Kerr, 1977). Therefore concerning the location of the embryo, the Trichoptera and the primitive Lepidoptera share a common character.

The most diagnostic feature in the development of the germ band of *Eriocrania* is occurrence of two transverse furrows, *i. e.*, the cephalic furrow and abdominal one. The formation of the cephalic furrow has been known in the embryos of several dipteran species such as *Drosophila melanogaster* (Ede and Counce, 1956), *Dacus tryoni* (Anderson, 1962), and *Culex tarsalis* (Rosay, 1959), but unknown in other insect orders. In these insects the cephalic furrow appears almost simultaneously with the onset of primitive groove formation and before the onset of germ band elongation. In *D. tryoni* and *C. tarsalis*, moreover, several temporary smaller furrows form behind the cephalic furrow during germ band elongation. As the posterior end of the germ band elongates rapidly towards the dorsal side of the yolk mass, these furrows are gradually smoothed out and finally effaced when the germ band is fully elongated. Although no satisfactory explanation of the exact mechanism of surface furrowing has been offered, Anderson (1972) has postulated that the cephalic furrow forms an anchor point for elongation, which takes place mainly behind the furrow, because elongation of germ bands always occurs only behind the furrow.

In *Eriocrania* both the cephalic and abdominal furrows also form before the onset of germ band elongation (stage 8), and are effaced by the time of its completion (stage 10); hence the situation is similar to that of the dipteran embryos, although segmenta-

tion of the germ band proceeds during elongation, but not after completion of elongation as in the dipteran embryos. Therefore, these temporary furrows of *Eriocrania* also may act as ancor points for elongation.

6. *Dorsal closure of embryo and fate of embryonic membranes*

In the monotrysian and ditrysian Lepidoptera (*Nepticula castanopsiella*: Kobayashi, 1983; *Diacrisia virginica*: Johannsen, 1929; *Pieris rapae*: Eastham, 1930), when the definitive dorsal closure is nearly completed just before revolution, the mid-dorsal body wall of the embryo is still open as a yolk stalk or umbilical passage; hence, the enclosed yolk is barely connected with the peripheral yolk by means of the umbilical passage. Soon after revolution in these species the passage closes and consequently the embryo lies free within the amniotic cavity. The amnion and serosa, therefore, do not rupture during revolution, and are intact even after dorsal closure. A small amount of yolk persists in the space between the amnion and serosa until just before hatching. In the exoporian *Endoclita*, the embryonic membranes are also intact even after dorsal closure, although completion of dorsal closure is slightly later than that of the species above (Kobayashi *et al.*, 1981). In this species, however, yolk granules do not invade the space between the amnion and serosa from the beginning of germ band formation (Fig. 36-D), so that the embryo is completely superficial in position before and after dorsal closure as stated before (Tanaka, 1980).

The amnion and serosa of the zeuglopteran *Neomicropteryx* likewise do not rupture at revolution, as do those of all other lepidopteran embryos. It is noteworthy, however, that the embryonic membranes of this species are ruptured and incorporated into the dorsal portion of the yolk just before completion of dorsal closure. These enclosed membranes turn into a secondary dorsal organ in the anterior part of the midgut, then degenerate there (Kobayashi and Ando, 1983).

The embryo of *Eriocrania* at stages before and after revolution (Figs. 28 — 30) is completely superficial on position, carrying the yolk mass in its dorsum; thus the situation is very similar to that of *Neomicropteryx*. In *Eriocrania*, however, the embryonic membranes do not rupture and are intact even after completion of dorsal closure. In this respect, *Eriocrania* has a character common to all other Lepidoptera except for the Zeugloptera. Although we cannot explain here the meaning of remains of embryonic membranes after dorsal closure, the acquisition of this character in *Eriocrania* must be an important step in the evolution of the lepidopteran embryogenesis.

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