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Relationships among Endopterygotan Orders: A Comparative Embryological Approach[*]

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Endopterygota (Holometabola) consist of the following 11 orders; Neuroptera, Megaloptera, Raphidioptera, Coleoptera, Strepsiptera, Diptera, Mecoptera, Siphonaptera, Trichoptera, Lepidoptera, and Hymenoptera. Although the phylogenetic relationships among these orders are not completely resolved, based on the condition of appendages of female abdominal ends, Endopterygota are often divided into two major lineages, neuropteroid orders and mecopteroid ones; the former contains Neuropterida (Neuroptera, Megaloptera, and Raphidioptera) and Coleoptera, and the latter is composed of Diptera, Mecoptera, Siphonaptera, Trichoptera, and Lepidoptera (Beutel and Pohl, 2006). Strepsiptera and Hymenoptera are the orders whose relationships to these two lineages are obscure. On the other hand, some recent results of molecular phylogenetic analyses have denied the monophyly of the neuropteroid orders and that of mecopteroid orders (Whiting, 2002a). Consequently, the ambiguity of endopterygotan phylogeny has increased, and the controversy on this subject has been heated.

The embryology of endopterygotan insects has been well known in such major orders as Coleoptera, Diptera, Lepidoptera, and Hymenoptera, but embryological information is scanty in such small orders as Raphidioptera and Siphonaptera (Table 1). Even in the major orders mentioned above, however, Lepidoptera is the only order whose embryogenesis is fairly known throughout the higher taxa at the levels of suborders or superfamilies (Kobayashi *et al*., 2003; Kobayashi, 2006). In this order, we have found out that the mode of embryogenesis is highly diversified among the higher taxa. That is, there are considerable differences in the mode of embryogenesis between the primitive lineages and the higher ones especially in early embryogenesis such as the formation of the germ disk (Ando and Tanaka, 1976, 1980; Ando and Kobayashi, 1978; Kobayashi and Ando, 1981, 1982, 1983, 1984, 1987, 1988; Kobayashi and Gibbs, 1990, 1995; Kobayashi, 1996, 1998). Similar diversification has been recognized in Trichoptera (Miyakawa, 1973, 1974; Akaike *et al*., 1982; Kobayashi and Ando, 1990) and Mecoptera (Suzuki, 1990). Such diversification within an order provides a useful tool for inferring the phylogeny of higher taxa within an order from the comparative embryological standpoint, but, conversely, diversification makes it

Table 1 The amount of embryological information in endopteryogotan orders and major embryonic features in each order

Endopterygotan order	Embryological work	Diversification of embryogenesis within an order	Germ type	Polar granules or pole cells	Pleuropodium	Secondary dorsal organ
Neuroptera	several	−	L	+	+	+
Raphidioptera	few	?	L?	+	+	+
Megaloptera	a few	−?	L	+	+	+
Coleoptera	many	+	S, I, L	−, +	−, +	−, +
Strepsiptera	several	+	?	?	+	−
Diptera	many	+?	L	+	−	−, +
Mecoptera	several	+	S?, L	−, +	−	−
Siphonaptera	a few	−	L	+	−	+
Trichoptera	several	+	S?, I	−	+	+
Lepidoptera	many	+	S?, I, L	−	−, +	−, +
Hymenoptera	many	+	L	+	−	−

S: short, L: long, I: intermediate, +: present, −: absent.

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difficult to extract embryonic groundplans of an order. For this reason there is no article intentionally discussing the relationships among endopterygotan orders from the comparative embryological standpoint other than that of Kobayashi and Ando (1988) which refers to the sister group relationship between Trichoptera and Lepidoptera. On the contrary, for example, in four suborders of Coleoptera, the largest order in Metazoa, the embryology is little known except for the largest suborder Polyphaga; thus, we have little information on the 'comparative' embryology of Coleoptera. Hence, in future approaches, it will be expected to obtain useful embryological data for the evaluation of the relationships among orders or among higher taxa within an order by clarifying the mode of embryogenesis of *primitive groups* in major orders such as Coleoptera, Diptera, and Hymenoptera.

In this perspective, we have undertaken the comparative embryology of the suborder Adephaga which is considered to be a primitive group of Coleoptera (Klausnitzer, 1975; Beutel, 1997). In the process of examining the embryogenesis of a carabid beetle, *Carabus insulicola*, we have found the possibility that a pair of minute lateral projections appearing temporarily in the first eight abdominal segments of the young embryo is homologous with the abdominal tracheal gills of *Sialis* and *Protohermes* in the closely related order Megaloptera (Fig. 1) (Miyakawa, 1979; Ando *et al*., 1985). Since the structure similar to these projections has not been found in any other orders and also in the suborder Polyphaga of Coleoptera, the minute lateral projections, or the vestigial tracheal gills, might be a candidate of synapomorphy connecting Adephaga and Megaloptera. If the speculation is true, the embryogenesis of *Carabus* will provide a new datum for arising questions about each monophyly of Neuropterida and Coleoptera. The vestigial appearance of the projections in the embryo of this terrestrial Adephaga also may provide the ground for supporting the aquatic origin of this suborder that includes many aquatic families (Bradley, 1947). In order to answer these questions or subjects, future embryological works of Adephaga should be extended to the embryogenesis of some aquatic Adephaga such as the Gyrinidae and the Dytiscidae. In the Gyrinidae, the larvae are aquatic and have tracheal gills like those of the megalopteran larvae, but inversely the larvae of the Dytiscidae have no lateral tracheal gills. Moreover, it might be a common feature in the neuropteroid insects (including parts of Coleoptera) that the segmentation of the anterior part of the embryo progresses before the completion of the germ band from the germ disk (Heider, 1889; Bock, 1939; Kamiya and Ando, 1985); then detail observations should be needed on this subject.

In the so-called mecopteroid insects, only the sister group relationship between Trichoptera and Lepidoptera has been strongly supported by both morphological and molecular data. As mentioned before, the relationship is also supported by embryological data, although the mode of extension of developed silk glands derived from labial glands is the sole embryonic synapomorphy connecting the two orders (Kobayashi and Ando, 1988). Other mecopteroid orders, that is, Diptera, Mecoptera, and

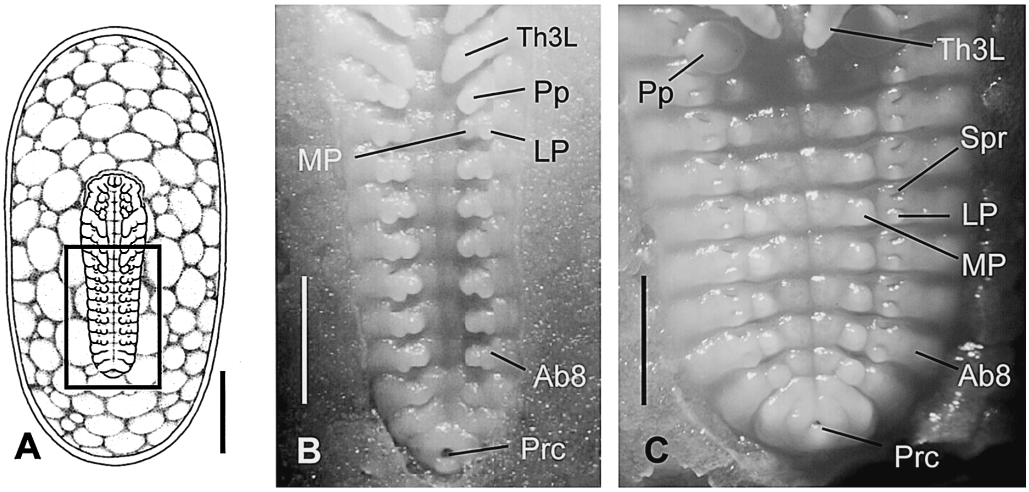

Fig. 1 Appearance of 'lateral projections' (LP) on the first eight abdominal segments in the ground beetle *Carabus insulicola* (Coleoptera, Adephaga). A. Ventral view of the egg at stage 6. B. Higher magnification of the rectangle in Figure A, showing 'medial projections' (MP) and 'lateral projections'. Medial projections are homologous with pleuropodia (Pp). Lateral projections are unknown in other coleopteran embryos, and might be homologous with abdominal tracheal gills of megalopteran larvae, because their tracheal gills are formed from the similar projections located laterally to pleuropodia. C. Abdominal region of the embryo at stage 7. As the embryo widens, medial and lateral projections are degenerated, and completely disappear in the next stage. Pleuropodia, however, develop into large discoidal glands. It should be noticed that vestigial lateral projections are located posteriorly to spiracular invaginations (Spr) in each segment. This suggests that the lateral projection belongs to the subcoxal region from which tracheal gills appear in megalopteran larvae.
Ab8: eighth abdominal segment, Prc: proctodaeum, Th3L: metathoracic leg. Scales = A: 1 mm; B, C: 0.5 mm.

Siphonaptera are often combined in the monophyletic taxon Antiliophora (having a sperm pump). The complete absence of the pleuropodium is presumed to be embryonic synapomorphies supporting this taxon, but it is not confirmatory, because the embryonic development of Tipulomorpha, one of the primitive dipteran group, and of the Nannochoristidae, the most primitive family of Mecoptera, is almost unknown. Moreover, in recent years, it has been pointed out that Siphonaptera is the sister group of the family Boreidae of Mecoptera (Whiting *et al*., 1997; Whiting, 2002b), but the embryological evaluation of this relationship has not been done, because embryonic information of the former is very scanty.

In Hymenoptera, comparing the suborders Symphyta and Apocrita, although the mode of embryogenesis of the latter is more specialized than that of the former, it is also difficult to infer the phylogenetic position of this order from embryonic features. However, taking into account the absence of pleuropodia and the secondary dorsal organ in both suborders, it is assumed that the Hymenoptera is closer to the mecopteroid orders than to the neuropteroid orders.

The females of Strepsiptera are permanently endoparasitic in host insects such as Orthoptera, and the embryonic development proceeds in the female's hemolymph (viviparity) (Brues, 1903; Noskiewicz and Poluszyński, 1927). Consequently the embryogenesis of Strepsiptera is fairly specialized, and this also makes it difficult to assume the relationship to other endopterygotan orders from embryonic features. The presence of pleuropodia and the formation of the secondary dorsal organ, though primitive characters, might be in favor of the traditional view that the Strepsiptera is closer to the Coleoptera than to the Diptera.

In summary, although it is considerably difficult to infer the strict phylogeny of Endopterygota only from embryological data in their present state of studies, two major branches of the neuropteroid and mecopteroid lineages except the Hymenoptera and Strepsiptera, as a whole, will be acceptable from the mode of embryogenesis. In Coleoptera and Diptera, comparative embryological studies should be extended to their primitive lineages, in order to detect the possible diversification of embryogenesis. Small orders, such as Megaloptera, Raphidioptera, Siphonaptera, deserve detailed embryological studies, because our embryological knowledge on these taxa is classical or entirely lacking. We have still many subjects to do in the comparative embryology of Endopterygota.

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