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Authors: Furuya, Hidetaka, and Tsuneki, Kazuhiko

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[REVIEW]

Biology of Dicyemid Mesozoans

Hidetaka Furuya* and Kazuhiko Tsuneki

Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

ABSTRACT—We reviewed recent advances of some aspects on the biology of dicyemid mesozoans. To date 42 species of dicyemids have been found in 19 species of cephalopod molluscs from Japanese waters. The body of dicyemids consists of 10−40 cells and is organized in a very simple fashion. There are three basic types of cell junction, septate junction, adherens junction, and gap junction. The presence of these junctions suggests not only cell-to-cell attachment, but also cell-to-cell communication. In the development of dicyemids, early stages and cell lineages are identical in vermiform embryos of four genera, *Conocyema*, *Dicyema*, *Microcyema*, and *Pseudicyema*. Species-specific differences appear during later stages of embryogenesis. In the process of postembryonic growth in some species, the shape of the calotte changes from conical to cap-shaped and discoidal. This calotte morphology appears to result from adaptation to the structure of host renal tissues and help to facilitate niche separation of coexisting species. In most dicyemids distinctly small numbers of sperms are produced in a hermaphroditic gonad (infusorigen). The number of eggs and sperms are roughly equal. An inverse proportional relationship exists between the number of infusorigens and that of gametes, suggesting a trade-off between them. Recent phylogenetic studies suggest dicyemids are a member of the Lophotrochozoa.

Key words: cell lineages, dicyemid mesozoans, life history traits, cell junctions, morphological adaptation

INTRODUCTION

Dicyemid mesozoans (phylum Dicyemida) are endosymbionts that typically are found in the renal sac of benthic cephalopod molluscs. The dicyemid bodies consist of only 8 to 40 cells, which are the fewest in number of cells in metazoans except for aberrant myxozoans, and are organized very simply. They have neither body cavities nor differentiated organs. E. Van Beneden (1876) proposed the name "Mesozoa" for the dicyemids as an intermediate between Protozoa and Metazoa in body organization. Subsequently, Hyman (1940, 1956) and Lapan and Morowitz (1975) also considered the dicyemids to be truly primitive multicellular organisms. However, several zoologists regarded the simple organization of dicyemids is the result of specialization of parasitism (Nouvel, 1947; Stunkard, 1954; Ginetsinskaya, 1988). Recent molecular studies have revealed that dicyemids might not be truly primitive animals deserving the name of “mesozoans” (Katayama *et al*., 1995; Kobayashi *et al*., 1999), but it still remains to be explored how such a simple body organization has been brought about.

The renal sac of cephalopods is a unique environment providing living space for a diversity of parasites. The fluid-filled renal coelom provides an ideal habitat for the establishment and maintenance of dicyemids (Hochberg 1982, 1983, 1990). Vermiform individuals live specificially within the renal sac. They insert the distinct anterior region termed a “calotte” into renal tubules or crypts of the renal appendages of the host (Ridley, 1968; Furuya *et al*., 1997). Dicyemids are subjected to a number of selecting pressures due to their unique habitats. In terms of morphological and ecological adaptation, this microenvironment could afford a space for a simple natural experiment.

Here we review recent advances on the biology of dicyemids, paying main attention to the progresses made after a previous review (Furuya *et al*., 1996).

LIFE CYCLE

The life cycle of dicyemids consists of two phases of different body organization (Fig. 1): (1) the vermiform stages, in which the dicyemid exists as a vermiform embryo formed asexually from an agamete, and as a final form, the nem-

* Corresponding author: Tel. +81-6-6850-5807;
FAX. +81-6-6850-5817.
E-mail: hfuruya@bio.sci.osaka-u.ac.jp

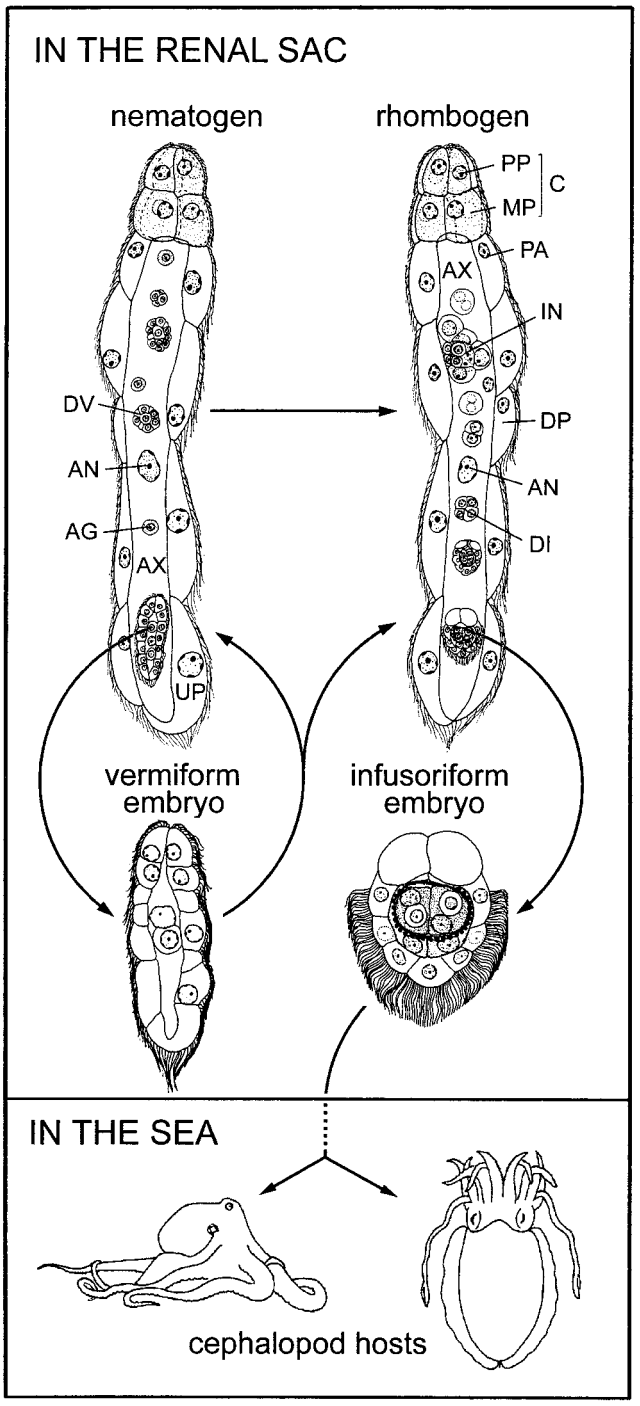

Fig. 1. Life cycle of the dicyemids. The dashed line indicates an unknown process involved in the infection of a new cephalopod and development into adult forms. In vermiforms (nematogen, rhombogen, vermiform embryo), a large cylinderical axial cell is surrounded by peripheral cells. Four to ten anterior peripheral cells (propolars and metapolars) form a calotte. The other peripheral cells are diapolars. Two posterior diapolars are somewhat specialized as uropolars. The development of infusorigens, gametegenesis around the infusorigen and development of two types of embryo all proceed within the axial cell cytoplasm. AG, agamete; AN, axial cell nucleus; AX, axial cell; C, calotte; DI, developing infusoriform embryo; DP, diapolar cell; DV, developing vermiform embryo; IN, infusorigen; MP, metapolar cell; PA, parapolar cell; PP, propolar cell; UP, uropolar cell.

atogen or rhombogen (Fig. 2a, b), and (2) the infusoriform embryo which develops from a fertilized egg produced around the hermaphroditic gonad called the infusorigen (Fig. 2c, d). The infusorigen itself is formed from an agamete. The name “dicyemids” is derived from the fact that they produce two types of embryo in the life cycle. A high population density in the cephalopod kidney may cause the shift from an asexual mode to a sexual mode of reproduction (Lapan and Morowitz, 1975). Vermiform stages are restricted to the renal sac of cephalopods, whereas the infusoriform embryos escape from the host into the sea to search for a new host. Infusoriform larvae actively swim *in vitro* for a few days (McConnaughey, 1951). However, it remains to be understood how infusoriform larvae develop into vermiform stages in the new host.

DICYEMID FAUNA IN JAPAN

Dicyemids are distributed in a variety of geographical localities: Okhotsk Sea, Japan sea, Western and Eastern North Pacific Ocean, New Zealand, North Indian Ocean, Mediterranean, Western North and Eastern Atlantic Ocean, Gulf of Mexico, and Antarctic Ocean (Hochberg, 1990). About 104 species of dicyemids have so far been reported in at least 40 species of benthic cephalopods of the world. The first record of dicyemids in Japan was made by Nouvel and Nakao (1938). They described *Dicyema misakiense* Nouvel and Nakao, 1938 from *Octopus vulgaris* Lamarck, 1798, and *D. orientale* Nouvel and Nakao, 1938 from *Sepioteuthis lessoniana* Lesson, 1830. Later, Nouvel (1947) described *D. acuticephalum* Nouvel, 1947 from *O. vulgaris* and identified a dicyemid species from *Sepia esculenta* Hoyle, 1885 as *Pseudicyema truncatum* Whitman, 1883, which had been described earlier in Europe. We have been surveyed Japanese cephalopod and 42 species of dicyemids including described ones have been recognized from 19 species of cephalopods (Table 1). Among them, two dicyemids were described from *O. vulgaris* and *O. minor* (Sasaki, 1920) as *Dicyema japonicum* Furuya and Tsuneki, 1992 and *Dicyema clavatum* Furuya and Koshida, 1992, respectively (Furuya *et al*., 1992a). Later, 14 dicyemid species were described from six species of cephalopods (Furuya, 1999). A dicyemid species from *Sepia esculenta,* once identified as *P. truncatum* by Nouvel (1947), differs from *P. truncatum* in Europe in distribution, host species, length of vermiform stages and infusoriform embryos, and thus described as a new species, *P. nakaoi*.

In general, dicyemids are found in benthic cephalopods, namely, octopuses and cuttlefishes. However, a few species of dicyemids were reported in squids, *Sepioteuthis lessoniana* (Nouvel, 1947) and *Loligo* sp. (Kalavati and Narasimhamurti, 1980). Such cases have been considered to be rather exceptional. Recently, we also have found two undescribed dicyemid species from two species of squids, *S. lessoniana* and *Todarodes pacificus* (Table 1). Host species of dicyemids might not be necessarily restricted to the benthic

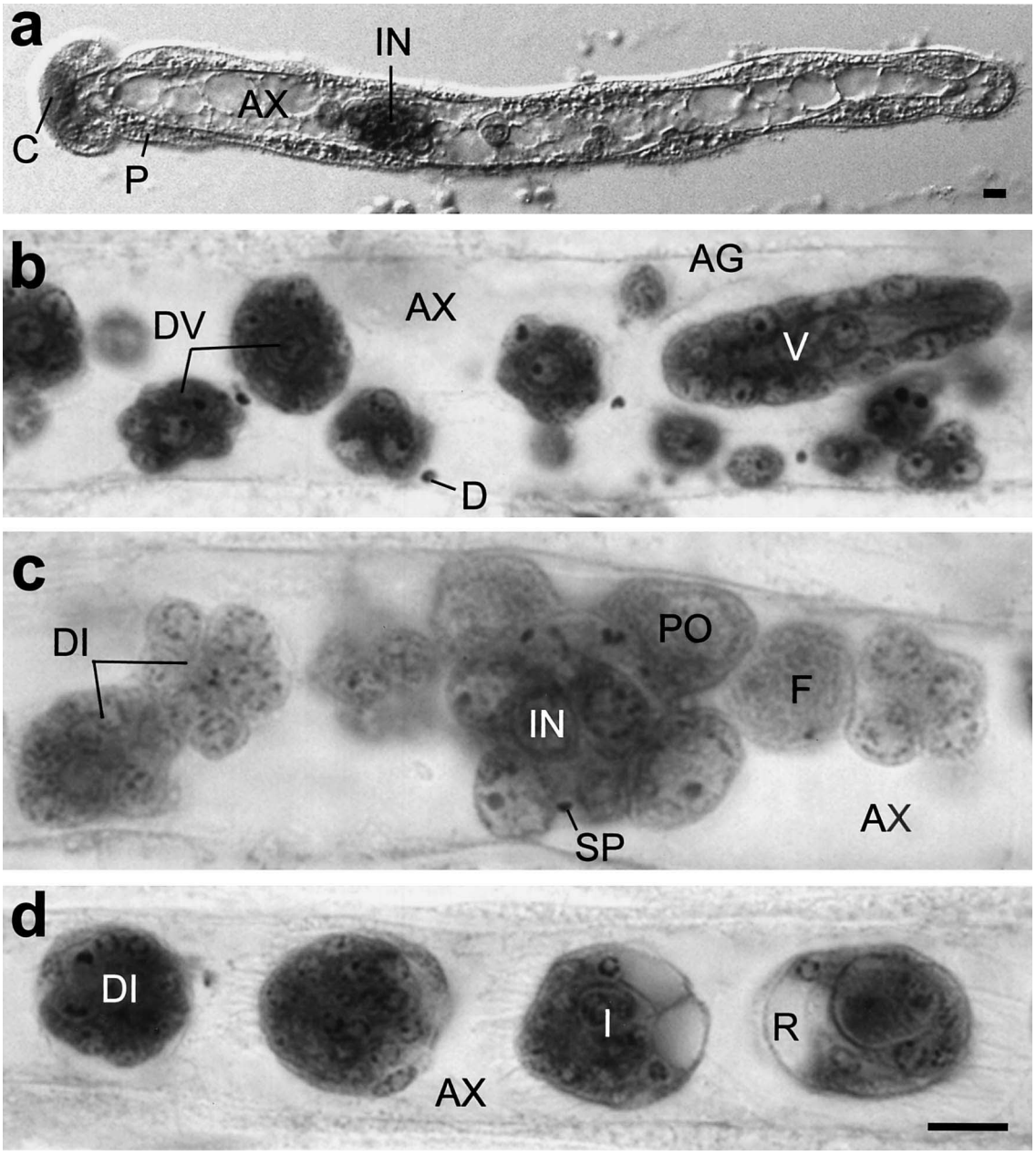

Fig. 2. Light micrographs of *Dicyema japonicum* stained with Ehrlich's hematoxylin. (**a**) Whole body of a rhombogen. (**b**) Part of a nematogen. (**c, d**) Part of a rhombogen. Bars represent 10 μm. The scale bar of (**d**) applies to (**b**) and (**c**). AG, agamete; AX, axial cell; C, calotte; D, degenerating cell; DI, developing infusoriform embryo; DV, developing vermiform embryo; F, fertilized egg; I, infusoriform embryo; IN, infusorigen; P, peripheral cell; PO, primary oocyte; R, refringent body; SP, sperm; V, vermiform embryo.

cephalopods. Most species of dicyemids are host specific, although more than one species of dicyemids are usually found in each cephalopod species or individual.

Many potential cephalopod host species still remain to be examined in Japan. More than 70 potential host species have been reported to occur in Japanese waters and only 19 species have so far been surveyed. Besides the Japanese waters, eight species of dicyemids have been described recently in several species of cephalopods from a variety of geographical localities, the Mediterranean (Furuya and Hochberg, 1999), the Gulf of Mexico (Furuya *et al*., 2002a), the Northwestern Atlantic Ocean (Canada) (Furuya *et al*., 2002b), the Weddell Sea (Czaker, 1994), and the Scotia Sea (Furuya and Hochberg, 2002). Future survey for dicyemids from various cephalopod species in each local region will clarify the world dicyemid fauna and host-dicyemid specificity patterns.

BODY ORGANIZATION AND JUNCTIONAL COMPLEX

Vermiform stages, namely, vermiform embryos, nematogens, and rhombogens, are similar in shape (Fig. 1). On

the surface of the kidney (renal appendage), individuals of vermiforms insert their heads into renal tubules (Hochberg, 1990; Furuya *et al*., 1997). The surface of the dicyemid body possesses numerous cilia and the folded structure, which is believed to contribute to absorb nutrients more efficiently from urine (Bresciani and Fenchel, 1965; Ridley, 1968; Furuya *et al*., 1997). The body of vermiform stages consists of a central cylindrical cell called the axial cell and a single layer of 8 to 30 ciliated external cells called the peripheral cells (Fig. 2a). The number of peripheral cells is species specific and constant. At the anterior region, 4 to 10 peripheral cells form the calotte, of which cilia are shorter and denser than in more posterior peripheral cells (Fig. 1). The calotte shape varies, depending on the species, and might be resulted as an adaptation to attach to the various regions of host renal tissues (Furuya *et al*., 2003a).

Infusoriform embryos mostly consist of 37 or 39 cells (Short, 1971; Furuya, 1999), which are more differentiated than those of vermiform stages (Matsubara and Dudley, 1976; Furuya, 2002; see Fig. 3). Internally, there are four large cells called urn cells, each containing a germinal cell that probably gives rise to the next generation (Fig. 3c). At the anterior region of embryo, there is a pair of unique cell called the apical cell (Figs. 2, 3), each containing a refringent body composed of magnesium inositol hexaphosphate (Lapan, 1975). The external cells are mostly ciliated.

The bodies of vermiform stages might be simplified as a reflection of their specialization in their parasitic habitat

Table 1. Dicyemids found from nineteen species of Japanese cephalopods

Cephalopods	Dicyemids
Octopus hongkongensis	*Dicyemennea gyrinodes* Furuya, 1999
	Diyemennea trochocephalum Furuya, 1999
	Dicyemennea ophioides Furuya, 1999
Octopus fangsiao	*Dicyema colurum* Furuya, 1999
	Dicyema erythrum Furuya, 1999
	Dicyema sp. R
	Dicyema sp. S
	Dicyema sp. T
Octopus minor	*Dicyema clavatum* Furuya and Koshida, 1992
	Dicyema sphyrocephalum Furuya, 1999
	Dicyema dolichocephalum Furuya, 1999
Octopus vulgaris	*Dicyema acuticephalum* Nouvel, 1947
	Dicyema misakiense Nouvel and Nakao, 1938
	Dicyema japonicum Furuya and Tsuneki, 1992
Octopus dofleini	*Dicyemennea* sp. V
	Dicyemodeca anthinocephalum Furuya, 1999
Octopus sasakii	*Dicyema* sp. O
	Dicyema sp. Q
Octopus aegina	*Dicyema* sp. E
	Dicyema sp. M
	Dicyema sp. N
Octopus areolatus	*Dicyema* sp. F
	Dicyema sp. G
Euprymna morsei	*Dicyema* sp. H
Sepia lycidas	*Dicyema lycidoeceum* Furuya, 1999
	Pseudicyema nakaoi Furuya, 1999
Sepia esculenta	*Dicyema rhadinum* Furuya, 1999
	Pseudicyema nakaoi Furuya, 1999
	Dicyema hadrum Furuya, 1999
	Dicyemennea minabense Furuya, 1999
	Dicyemennea mastigoides Furuya, 1999
Sepia latimanus	*Dicyemennea* sp. B
Sepia kobiensis	*Dicyema* sp. I
	Pseudicyema sp. P
Sepia madokai	*Dicyema* sp. J
	Pseudicyema sp. K
Sepia peterseni	*Dicyema* sp. L
Metasepia tullbergi	*Dicyemennea* sp. U
Sepiella japonica	*Dicyema* sp. C
Sepioteuthis lessoniana	*Dicyema orientale* Nouvel and Nakao, 1938
	Dicyema sp. A
Todarodes pacificus	*Dicyemennea* sp. D

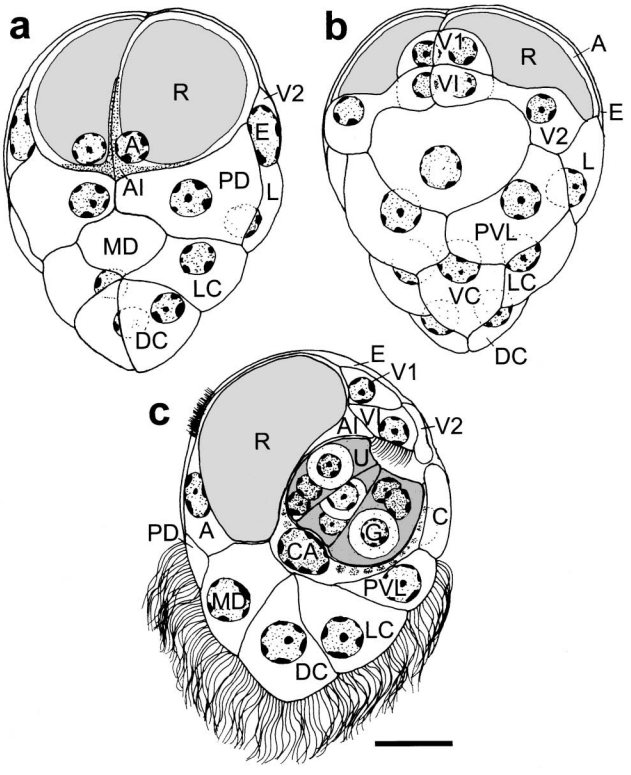

Fig. 3. Sketches of infusoriform embryos of *Dicyema acuticephalum*. (**a**) Dorsal view. (**b**) Ventral view. (**c**) Sagittal section. Bar represents 5 μm. A, apical cell; AI, apical internal cell; C, couvercle cell; CA, capsule cell; DC, dorsal caudal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; MD, median dorsal cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell.

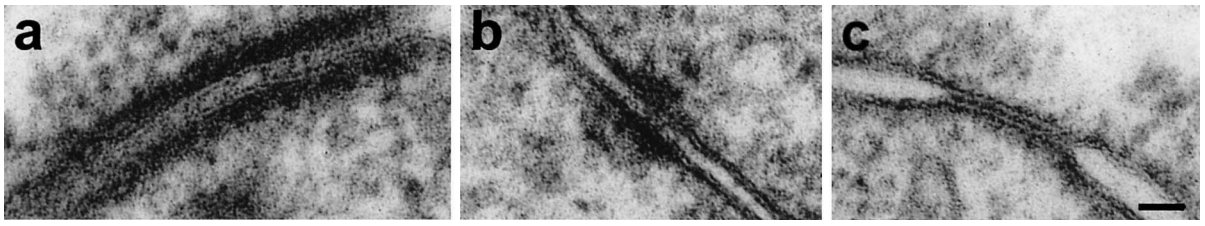

Fig. 4. Junctional complex in the infusoriforms of *Dicyema acuticephalum*. (**a**) Possible septate junction between two posteroventral lateral cells. Some septa-like structures can be seen in the intercelllar space. (**b**) Adherens junction between two capsule cells. (**c**) Gap junction between posteroventral lateral cell and capsule cell. Bar represents 10 nm.

Table 2. Junctional complex in primitive multicellular animals

	sponge[1]	placozoan[2]	dicyemid[3]	orthonectid[4]	turbellarian[5]
septate junction	+	+	+	+	+
adherens junction	+	+	+	+	+
desmosome	–	–	–	–	+
gap junction	?	-	+	?	+

1 Green and Bergquist (1979, 1982), Harrison and Vos (1991)
2 Grell and Ruthmann (1991)
3 Revel (1988), Furuya *et al.* (1997)
4 Kozloff (1969, 1971), Slyusarev (1994)
5 Rieger *et al.* (1991)

composed of renal tubules (Nouvel, 1947). By contrast, infusoriform embryos seem to represent the true level of organization because they become free-swimming organisms (Furuya *et al*., 1997). Nevertheless, the body organization of infusoriform embryos cannot be regarded as achieving the grade of tissue level.

The most significant synapomorphy of multicellular animals is their multicellularity, which is characterized by cell interactions that are mediated by intercellular junctions and a variety of adhesion molecules. The fine structure of the dicyemid, *Dicyema acuticephalum*, was studied with special attention to intercellular junctional complexes between various kinds of cell (Furuya *et al*., 1997). Three types of intercellular junction, zonula adherens, macula adherens, and gap junction, were found at vermiform stages and in infusoriform embryos (Fig. 4). The zonula adherens was observed between adjacent peripheral cells at vermiform stages, between adjacent external cells of infusoriform embryos, and between members of groups of internal cells that cover the urn. These zonulae adherentes possibly belong to the septate junction because of the presence of fine septa-like structures in the intercellular space (Fig. 4a). The macula adherens was seen between a peripheral cell and an axial cell at vermiform stages. In infusoriform embryos, these junctions were observed between various types of cell, excluding urn cells (Fig. 4b). Using a freeze-fracture method, Revel (1988) observed gap junctions at vermiform stages. Furuya *et al*. (1997) revealed the distribution of gap junctions between various kinds of cell at vermiform stages and in infusoriform embryos (Fig. 4c). Coordinated ciliary movement among peripheral cells is essential for body movement of dicyemids, and gap junctions might mediate such coordination among multiciliary cells.

Gap junctions are seen in all eumetazoans with the exception of anthozoans and scyphozoans (Mackie *et al*., 1984). Primitive multicellular animals with monociliary cells (sponges and placozoans) do not have gap junctions (Table 2). In sponges, however, Green and Bergquist (1979) suggested the presence of intercellular communicating channels, and Grell and Ruthmann (1991) demonstrated several patterns of organized behavior in placozoans, suggesting the presence of some communication system.

Cell junctions of dicyemid mesozoans are rather well developed. In dicyemids, extracellular materials, such as basal laminae and collagen fibrils, are absent in observations using a transmission electron microscope. In the electron immunocytochemical study, however, Czaker (1998, 2000) reported fibronectin-, laminin-, and typeIV collagen-like protein molecules are present in dicyemids. These molecules show a similar pattern of distribution, although the intensity differs. They distribute along the folds of ruffled surface of peripheral cells, the septate boundary between peripheral cells, and the inner surface of peripheral cell membrane of the vermiforms. Czaker (2000) considers these distributions of the extracellular matrix molecules seem to reflect a very primitive situation of these molecules having not yet reached their definitive position outside the cell.

DEVELOPMENTAL PROCESSES AND CELL LINEAGES IN VERMIFORMS

The pattern of cell divisions and cell lineages during the development of two types of embryo and a functionally her-

maphroditic gonad, the infusorigen, in *Dicyema japonicum* were summarized in the previous review (Furuya *et al*., 1996). Recently we described patterns of cell division and cell lineages of the vermiform embryos in four species belonging to four genera: *Conocyema polymorpha*, *Dicyema apalachiensis*, *Microcyema vespa*, and *Pseudicyema nakaoi (*Furuya *et al*., 2001). In embryogenesis of each species, cell divisions proceed without variation and result in a fully formed embryo with a definite number and arrangement of cells. The process of development of vermiform embryos is very simple and seems to be programmed similarly to that of infusoriform embryos and infusorigens (Furuya *et al*., 1992b, 1993). The early development is conservative and may be summarized as follows: (1) the first cell division produces prospective cells that generate the anterior peripheral region of the embryo; (2) the second cell division produces prospective cells that generate the posterior peripheral region plus the internal cells of the embryo; (3) in the lineage of prospective internal cells, several divisions ultimately result in the death of one of the daughter cells. Developmental processes to the 7-cell stage are almost identical in vermiform embryos of the four genera examined (Fig. 5). In

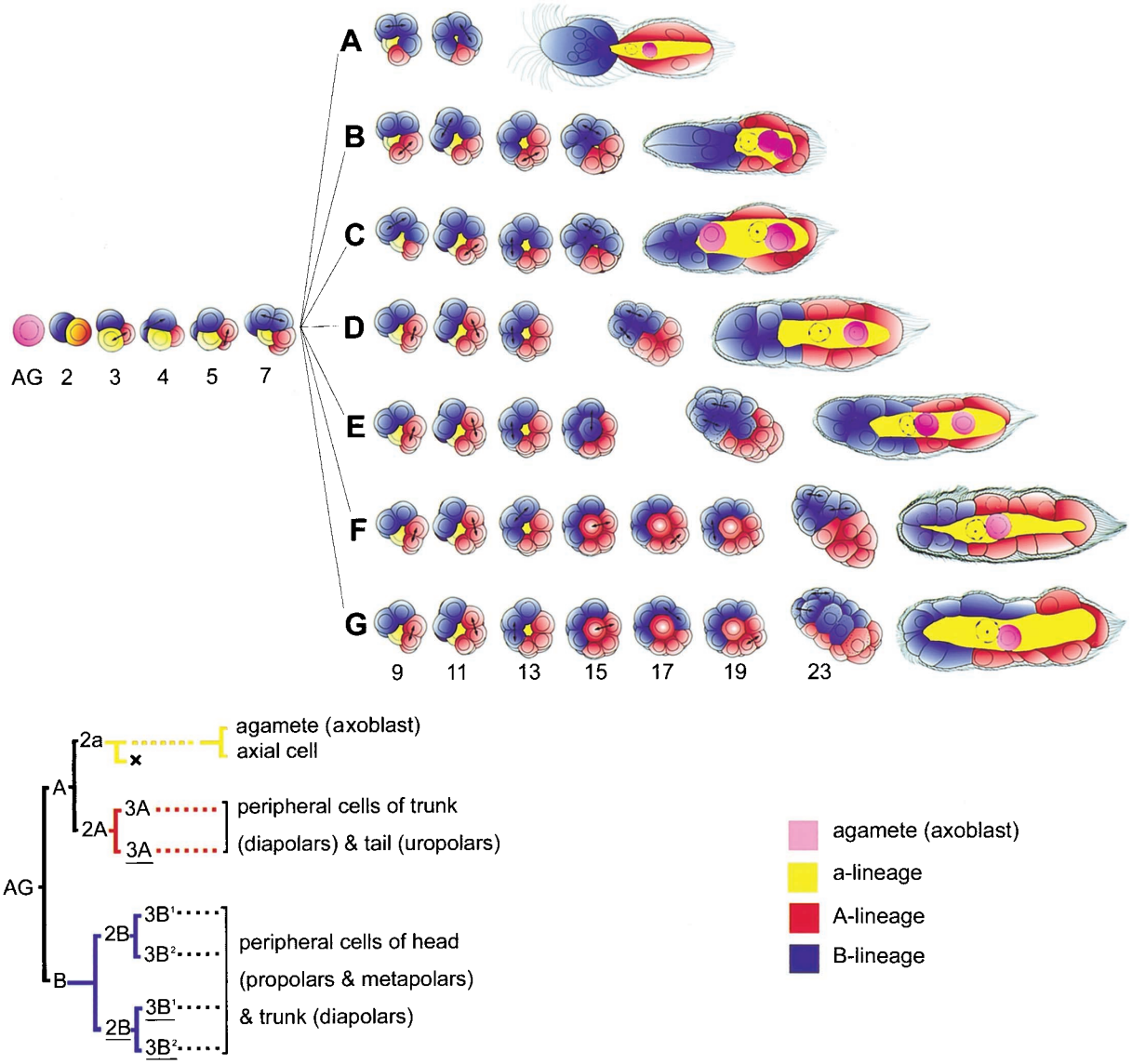

Fig. 5. Developmental processes of vermiform embryos in several species of dicyemids (modified from Furuya *et al*., 2001). **A**, *Microcyema vespa*. **B**, *Conocyema polymorpha*. **C**, *Dicyema apalachiensis*. **D**, *D. acuticephalum* with 16 peripheral cells. **E**, *D. acuticephalum* with 18 peripheral cells. **F**, *D. japonicum*. **G**, *Pseudicyema nakaoi*. The developmental patterns and cell lineages from an agamete (AG) to 7-cell stage are identical among the species. The numerals in the bottom row represent cell number stages in the development. Arrows in the developing embryos indicate daughter cells that were produced by the proceeding division. A common cell lineage in all the vermiform embryos is indicated at left lower corner. At the first division, an agamete (AG) divides to produce two daughter cells, A and B. Cell A divides into two daughter cells. Cell 2a is a mother cell for both an axial cell and agamete. Descendants of cell 2A form the peripheral cells of both trunk and tail. Descendants of cell B form the peripheral cells of both the head and anterior trunk. A cross (x) indicates that a smaller cell formed by unequal division degenerates and does not contribute to the formation of the embryo.

contrast, distinct species-specific differences appear in the order and number in terminal divisions of peripheral cells. Thus, the number of peripheral cells is fixed and hence species-specific. Generic differences appear in the number of cells that contribute to the calotte during the final stage of embryogenesis. Distinct morphological features typically emerge following a final cell division or after the embryo escaped from the axial cell of the adult. Subsequent processes, proceeding without cell divisions, are cell differentiation in the head region and cell elongation in the trunk region.

On the basis of cell lineage, a simple cladogram was constructed (Fig. 6). Cell lineages from an agamete to the 7-cell stage were almost identical among species (bar 1). The terminal of B-cell lineage varies among species. In the family Conocyemidae, a calotte is formed with a tier of polar cells (bar 2A), whereas in the Dicyemidae a calotte consists of two tiers of polar cells, propolars and metapolars (bar 2B). Thus, the tree indicates that two clusters initially separate to form two families. In *Microcyema* a calotte and peripheral cells form a syncytium (bar 3A), but in *Conocyema* a calotte is cellular and diapolars are present (bar 3B). In *Dicyema japonicum*, the calotte is formed in $3B^1$- and $3B^2$-cell lineages (bar 4A), while in *D. acuticephalum*, *D. apalachiensis*, and *Pseudicyema nakaoi* the calotte is formed only in $3B^1$-cell lineage (bar 4B). The orientation of propolars to metapolars separates *Pseudicyema* from *Dicyema.* In *Pseudicyema* propolars are obliquely oriented to metapolars (bar 5B). In *Dicyema* propolars are located perpendicularly above metapolars (bar 5A). In *D. acuticephalum*, cell death occurs both in $3B^1$- and $3B^2$-cell lineages (bar 6A), but in *D. apalachiensis* it occurs only in $3B^2$-cell lineage (bar 6B).

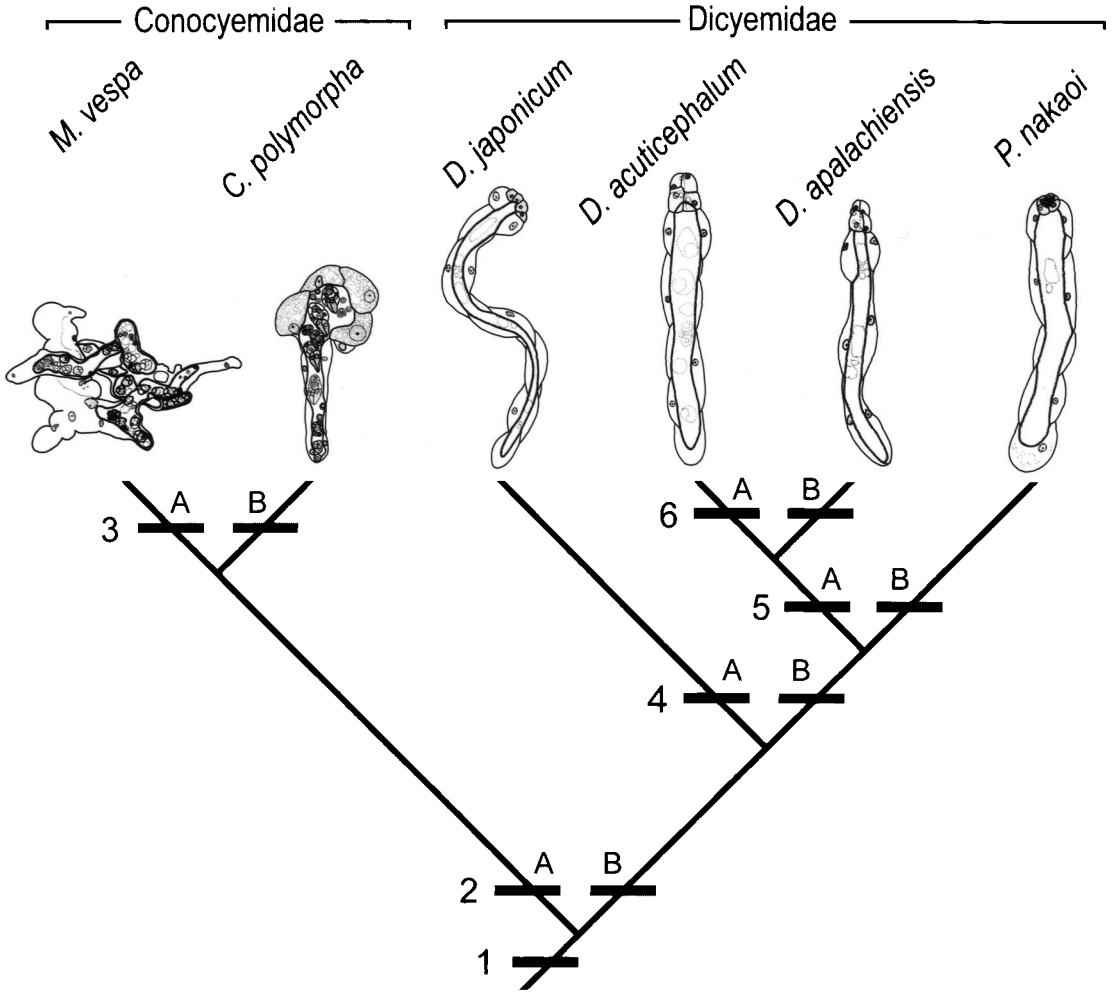

Fig. 6. Cladogram of six species of dicyemids based on cell lineages of the vermiform embryos (modified from Furuya *et al*., 2001). These dicyemids might have been derived from an ancestor that had a basic cell lineage as shown in Fig. 5. Modifications in cell lineages might result in diversity of morphology. Bars represent modifications in cell lineages. (1) Early development as shown in Fig. 5. (2A) Calotte is formed with a tier of polar cells. (2B) Calotte is formed with two tiers of polar cells; propolars and metapolars present. (3A) Calotte forms a syncytium; diapolars absent. (3B) Calotte is cellular; diapolars present. (4A) Calotte is formed from both $3B^1$- and $3B^2$-cell lineages. (4B) Calotte is formed only from $3B^1$-cell lineage. (5A) Propolars are located perpendicularly above metapolars. (5B) Propolars are obliquely oriented to metapolars. (6A) Cell death occurs both in $3B^1$- and $3B^2$-cell lineages. (6B) Cell death occurs only in $3B^2$-cell lineages; both $4A^1$- and $4A^2$-cells undergo no further divisions. Sketches indicate the shape of the whole body of adult stage.

Based on the above criteria, separation of the dicyemids into two families may be justified; however, the generic state of *Pseudicyema* apparently warrants further study.

MORPHOLOGICAL ADAPTATION OF CALOTTE: NICHE SEPARATION AND CONVERGENCE

The majority of the dicyemid species studied were found to be host-specific. Typically, two or more species of dicyemids live in each host species or each host individual (Table 1). When dicyemid species co-occur, their calotte shapes are distinctly different. Four basic types of calotte shapes are recognized among 61 species of dicyemid (Furuya *et al*., 2003a, see Fig. 7a). A conical calotte (Type I) is by far the most typical configuration observed. Dicyemids with a discoidal calotte (Type III) frequently are found together with species having cone-shaped calottes (Fig. 7b). Cap-shaped calottes (Type II) appear to be intermediate in shape between the conical and discoidal type, and tend to occur in the cephalopods when more than two species of dicyemids are present (Fig. 7c). Dicyemids with irregular shaped bodies and calottes (Type IV) occur when more than three species coexist (Fig. 7d). When more than two dicyemid species were present in a single host individual, calotte shapes were dissimilar as a rule. It is a common phenomenon that calotte shapes in dicyemid species from different host species more closely resemble each other than those of dicyemids observed within the same host species. Species of dicyemids that possess similar calotte shapes are very rarely found together in a single host individual, and in all such cases one species is much dominant. In *Octopus joubini*, two dicyemid species, *Dicyema apalachiensis* and *D. hypercephalum*, are very similar in calotte shape and were never found together in 17 host individuals examined. In the Japanese *O. vulgaris*, two species of dicyemids possess similarly shaped calotte, namely, *D. acuticephalum* and *D. misakiense.* In over 150 octopuses examined these two species have never been found together (Furuya *et al*., 2003a). In these cases, the most adaptive species for the habitat possibly becomes a dominant and niche-occupying species. In a host individual, interspecific competition between dicyemids may result when they have similar calotte shapes, and these dicyemids tend to infect different host individuals.

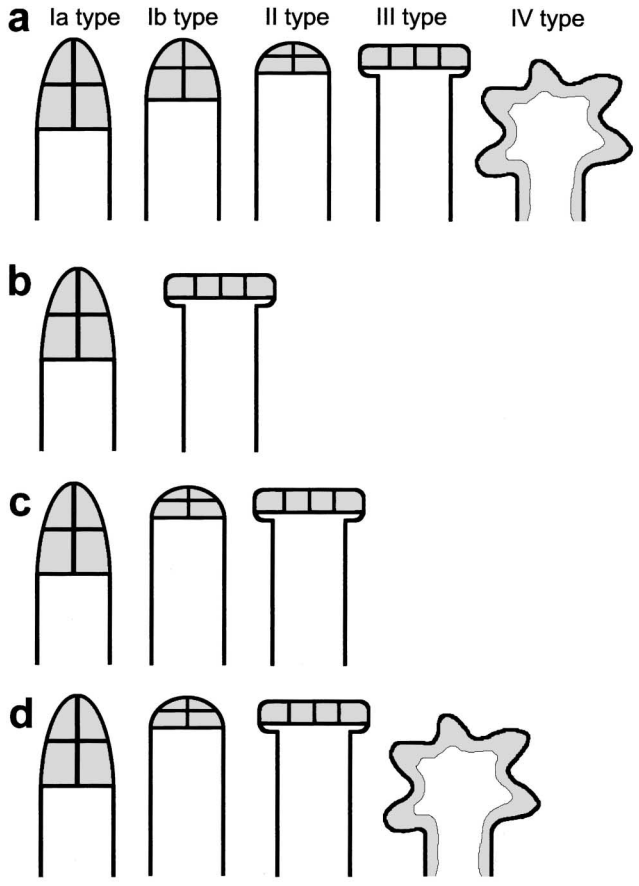

Fig. 7. Calotte shapes found in vermiform individuals. (**a**) Stylized drawings of the main three types of regular calotte configurations and a type of irregular configulation. Conical type is divided into two subtypes (Type Ia and Ib). Type II, cap-shaped; Type III, discoidal; Type IV, irregular. (**b**) When two species of dicyemids are present, two distinct calotte shapes, conical and discoidal, are usually observed. (**c**) When three species of dicyemids were present, three types of calotte configurations are usually observed, conical, cap-shaped and discoidal. (**d**) When more than four species of dicyemids are present, one species is characterized by its rare irregular shaped calotte.

Different species of dicyemids appear to be able to coexist in the renal sac without competition, when their calottes are different in shape. For instance *Dicyemennea abreida* (conical) and *Dicyemodeca deca* (discoidal) show a high prevalence of co-occurrence. Species of dicyemids with different calotte shapes, for example, *D. misakiense* (conical) and *D. japonicum* (discoidal) inhabit different regions of the renal organs (Furuya *et al*., 2003a). In general, dicyemids with conical calottes (Type I) insert the anterior region of the body into crypts or folds in the renal appendages. In contrast, dicyemids with cap-shaped (Type II) or disc-shaped calottes (Type III) attach to the broad, flat or gently rounded, surfaces of the renal appendages. Interspecific competition is most likely avoided by the habitat segregation in dicyemids that possess different calotte shapes.

Dicyemids that have similar calotte shapes, e. g. *D. acuticephalum* and *D. misakiense,* never have been found together as noted earlier. However, when *D. japonicum* is present, these two species are able to coexist in the same renal habitat. The presence of *D. japonicum* may reduce the competition between *D. acuticephalum* and *D. misakiense*. Similarly, the presence of unusual dicyemids with irregularly shaped calottes and bodies (Type IV) may reduce competition between dicyemids with various calotte configurations. Although it is unknown how Type IV dicyemids attach to the renal organs, most likely they adhere to the surface of the renal appendages, as do dicyemids with discoidal calottes.

Calottes typically are conical in shape in vermiform

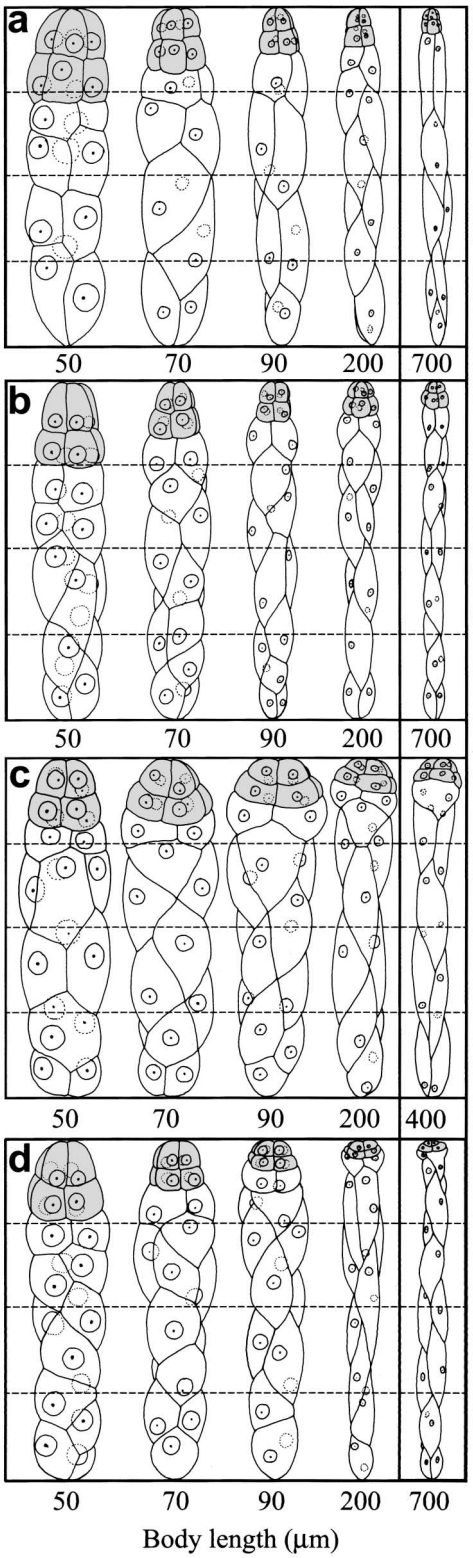

Fig. 8. Allometric growth of vermiforms in four species of dicyemids (modified from Furuya *et al*., 2003a). Individuals of different body lengths are sketched at an equal height. (**a**) *Dicyema acuticephalum* (Type Ia). (**b**) *D. misakiense* (Type Ib). (**c**) *D. clavatum* (Type II). (**d**) *D. japonicum* (Type III). Shaded areas indicate calottes. In each species the individual on the left represents a fully formed embryo found within the axial cell of the parent nematogen. Others represent stages after eclosion.

embryos of almost all species and in adult vermiform stages of many species (Figs. 5, 8). This shape is formed simply by proportional cell enlargement. In the process of ontogenetic growth from embryo to adult, the shape of the calotte changes from conical to cap-shaped and discoidal. The cap-shaped calotte appears to be intermediate between what might be termed the plesiomorphic or primitive condition (conical configuration) and a more advanced or apomorphic discoidal configuration. Consequently, various morphological characters might have been selected to reduce competition in each different host species as a result of heterochrony.

In Japan, *D. misakiense* and *D. japonicum* often are found together in the same host individual. Nouvel and Nakao (1938) did not differentiate them as distinct species because of their general morphological similarities. Cell lineages in both vermiform larvae and infusorigens, and the number and types of cells in infusoriform larvae are identical (Furuya *et al*., 1992a, 1993, 1994, 2001). The principal difference between these species exists in calotte shape and an intermediate shape is never found (Furuya *et al*., 1992b). As far as morphological characters are concerned, these two species are considered to be closely related, but different species. In such a sympatric, congeneric species of dicyemids, morphological character displacement might occur to increase differences between species.

LIFE HISTORY TRAITS

There are relationships among several life history traits of the dicyemids (Furuya *et al*., 2003b; See Fig. 9). Individual adults of dicyemids spend all of their life in the renal organs of cephalopod hosts. The renal sacs of larger cephalopod hosts may provide more living space and more nutrients for the dicyemids, which in turn might allow for larger sized dicyemids. However, the correlation between adult body size and host size is not significant. In the case of dicyemids, body size is likely determined by several factors related to habitat structure: the volume of the renal coelom, the diameter of the renal tubules, and the depth of the crypts or folds in the surface of the host renal appendages. In addition, lineage-specific factors may affect dicyemid body size. For example, the bodies of *Dicyemennea* are larger than those of the other genera.

In contrast to the size of vermiform embryos, there is a correlation between the size of infusoriform embryos and host body size (measured by mantle length of octopuses). This suggests that infusoriform embryo size is adapted to octopus size although it is not clear what character is directly correlated with infusoriform size. One possible character limiting embryo size is a renal pore diameter, because infusoriform embryos escape through the pore during elimination of urine. Another possiblity is the size of the site where infusoriform embryos first enter a new host, although it is unknown whether infusoriform embryos infect new hosts directly or not.

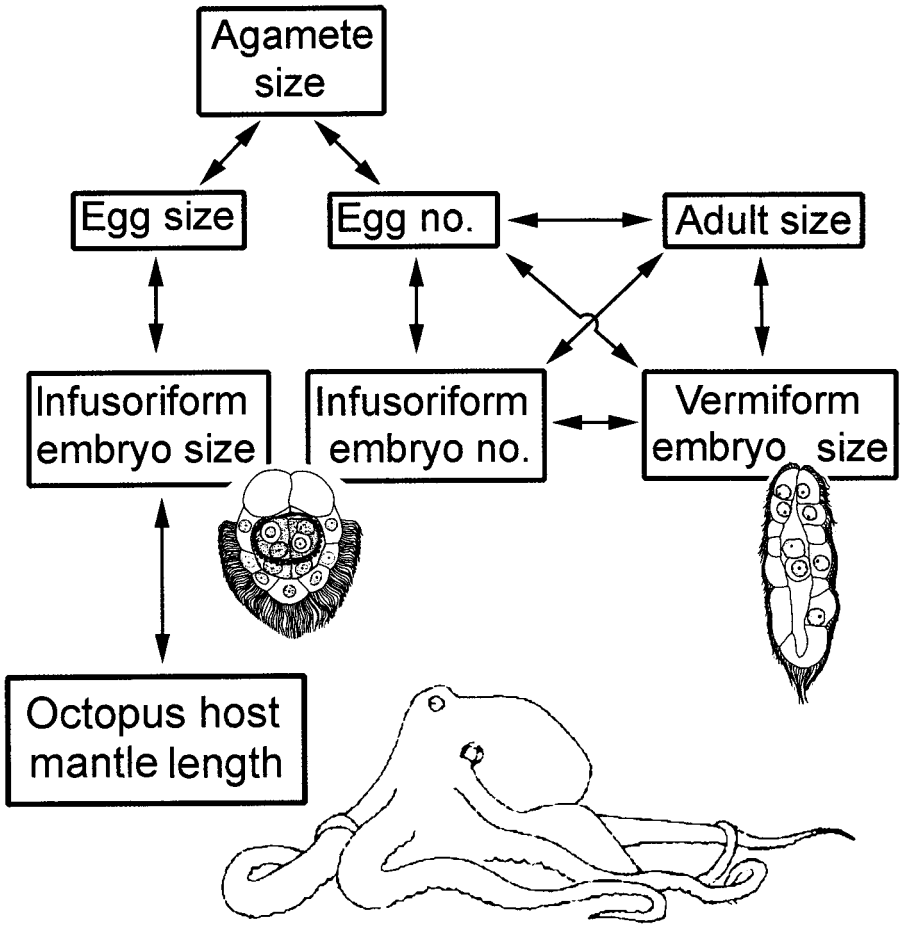

Fig. 9. Relationships among life history characters of dicyemids (modified from Furuya *et al*., 2003b). Arrows indicate positive correlations at the level $P<0.05$. An agamete is a germ cell and generates two different reproductive types, adult vermiforms and infusorigens. The agamete size is regarded as a representative of cell size of dicyemids and may be significant for reproductive traits. Its size is correlated with both egg size and egg number, thus likely exerting considerable influence on several life history characters.

As a general feature of invertebrates, body size is positively correlated with fecundity, both within and across species (Poulin 1995). In dicyemids, larger bodies usually consist of a larger number of somatic cells. Somatic cell number is a significant character in multicellar organisms of which body is composed of a small number of cells. In dicyemids, a positive correlation is found between body size and somatic (peripheral) cell number (Fig. 9). In vermiform stages, the somatic cells are produced by a fixed number of cell divisions during embryogenesis and the number of cells is species specific (Furuya *et al*., 1994; 2001). Thus body size is positively correlated with the number of somatic cell divisions. Therefore, large dicyemid species with a large number of somatic cells may have a higher capacity for cell divisions than small ones. In terms of cell production, there seems to be a positive correlation between the number of cell divisions and fecundity. Peripheral cell number of vermiform stages actually is positively correlated with several life history characters involved in reproduction.

Alteration of sexual and asexual modes of reproduction occurs in the life cycle of all dicyemids. The relationship between adult size and embryo number varies with each mode of reproduction. In the sexual mode, which produces infusoriform embryos, adult body size is positively correlated with embryo number (fecundity). Infusoriform embryos represent the dispersal stage and high fecundity may have evolved to increase the number of new hosts infected. In contrast, the asexual mode of reproduction, which produces vermiform embryos, does not show a positive correlation between adult body size and embryo number. The size of full grown vermiform embryos just prior to eclosion is proportional to adult size and is species-specific. A trade-off between number and size of vermiform embryos does not appear to be present. This may be due to differences in the role of each embryo type (dispersal to another host vs. multiplication of individuals within the renal sac).

In dicyemids, fecundity of a single individual is not high relative to that reported for other endoparasite taxa. However, the total reproductive capacity per population of dicyemids may nearly equal fecundity in other groups of endoparasites. In the case of dicyemids, low fecundity per individual is compensated for by an increase in adult popu-

lation size in the renal sac through asexual multiplication. Asexual reproduction is functionally associated with an increased capacity for reproductive potential in a limited habitat, where genetic diversity related to sexual reproduction is not required. The cephalopod renal sac represents such a habitat, where it may not be necessary to differentiate distinct reproductive strategies. A continuous nutrient supply can maintain asexual multiplication of adult vermiforms until the population attains a very high density in the renal sac. Embryogenesis in the axial cell of adults also reduces loss of embryos during development. In addition, vermiform embryos may rapidly develop and grow to reproductive size due to their small number of somatic cells. Consequently a relatively large number of dispersal larvae are produced as observed in other endoparasite taxa.

REPRODUCTIVE STRATEGY IN HERMAPHRODITIC GONAD

The number of hermaproditic gonads, infusorigens, observed in the axial cell of a parent rhombogen is positively correlated with the adult body size. The maximum number per parent individual is species-specific. This suggests that the number of infusorigens depends on the volume of cytoplasmic space in the axial cell. Large dicyemids with many infusorigens manifest high fecundity of embryos.

The number of both types of gametes per infusorigen is different in each species. An inverse relationship is found between the number of infusorigens per adult and the number of gametes per infusorigen (Fig. 10). There seems to be a trade-off between infusorigen number and gamete number. Two distinct types are recognized: (1) large numbers of infusorigens with a small number of gametes: (2) small numbers of infusorigens with a large number of gametes. In the dicyemids of similar adult sizes, in the end there may be little difference in total number of gametes produced by these two types. The costs producing gametes also seem to be nearly equal.

In addition to the two types mentioned above, there are a few species in which there is a positive correlation between the number and size of infusorigens, namely, a large number of infusorigens that produce a large number of gametes (Fig. 10, Type 3). This third type is found in only two middle- to large-sized species and may not be realized as a distinct strategy. Additionally, there are dicyemids with a few infusorigens and a relatively small number of gametes. These are small dicyemids and are probably species with progenesis.

Dicyemids, thus, are marine invertebrates that do not produce large numbers of gametes. In particular, a very few sperm are produced. In *Dicyema sullivani*, the number of sperm may even be smaller than the number of oocytes (McConnaughey, 1983). We discovered that nearly 10% of all species examined produce sperm fewer in number than eggs (Furuya *et al*., 2003b). The number of eggs and sperms were roughly equal (means of the number of sperm:

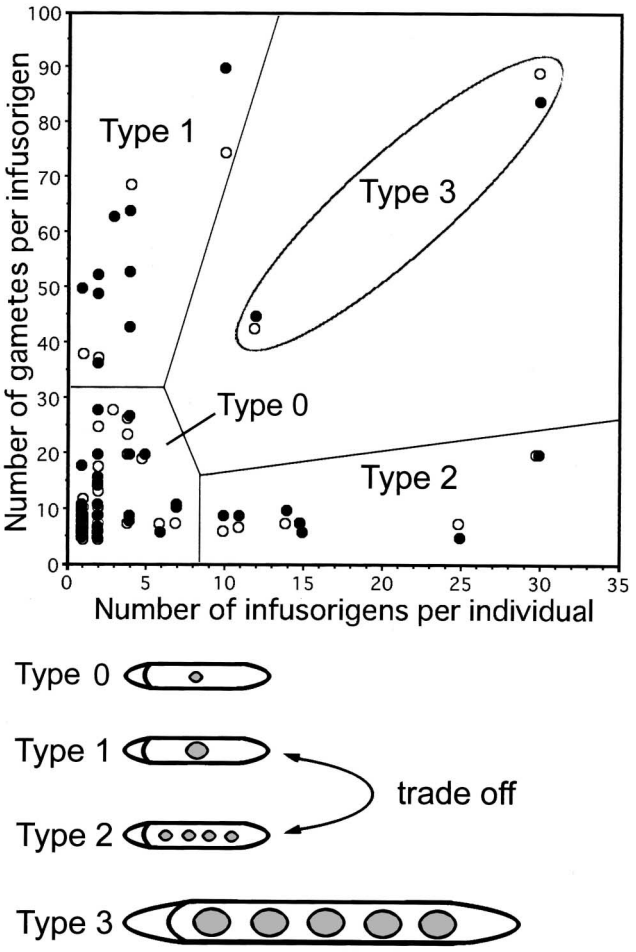

Fig. 10. Relationships between the number of sperm-line cells (●) and the number of egg-line cells (○). Type 0 represents species that produce small numbers of infusorigens with the small number of gametes. Type 1 represents species that produce small numbers of infusorigens with the large number of gametes. Type 2 represents species that produce large numbers of infusorigens with the small number of gametes. Type 3 represents species that produce large numbers of infusorigens with the large number of gametes.

egg =1: 1.58). The rates of development of both sperm and oocytes appear to be similar (Furuya *et al*., 1993). As a consequence a few oocytes probably remain unfertilized due to the disproportional ratio of both gametes. Because of the unique organization of hermaphroditic gonads, spermatogenesis proceeds within the cytoplasm of an infusorigen's axial cell. The number of sperm is possibly constrained by the cytoplasmic space.

ADAPTATION OF LIFE CYCLE

Dicyemids most likely evolved from free-living ancestors (Hyman, 1940; Nouvel, 1947; Stunkard, 1954). The complicated diphasic life cycle of dicyemids probably evolved as a parasitic adaptation to the renal organs of cephalopod hosts. One of the remarkable characters that makes the life cycle complicated is asexual reproduction. It

is observed in many endoparasitic groups, namely, protozoans (Grell, 1956; Hochberg, 1990; Smyth, 1994), cestodes (Hyman, 1940, 1949; Stunkard 1975; Rohde, 1993), trematodes (Hyman, 1940, 1949; Stunkard, 1975; Rohde, 1993), and orthonectids (Kozloff, 1990). Because of the similarity in alteration of sexual and asexual generations, some workers previously postulated a close phylogenetic relationship between trematodes and dicyemids (Stunkard, 1954; Bogolepova, 1963; Ginetsinskaya, 1988). However, differences are apparent in the pattern of asexual reproduction between trematodes and dicyemids. Asexual reproduction or parthenogenesis in trematodes occurs in the body cavity of various larvae in different developmental stages. In contrast asexual reproduction in dicyemids occurs within the cytoplasm of the parent's axial cell. In orthonectids asexual reproduction occurs within the cytoplasm of the host cells where germinal cells multiply to form male and female adult individuals (Kozloff, 1997). Comparisons of nucleotide sequences of 18S rRNA in the dicyemids and orthonectids have shown that the two groups have separate origins (Pawlowski *et al*., 1996). Thus asexual reproduction in all three groups of parasites seems to develop independently in each lineage. In these endoparasites, asexual reproduction appears to be an adaptation for similar niches in different hosts.

In aquatic animals, taxa with small adults are commonly brooders with embryos held on or in the adult body. However, in species with larger adults, offspring typically are either not cared for or are released at an earlier stage (Strathmann, 1990). Adult dicyemids are small in size and embryos are formed within the adult body. Full grown embryos are released. This essentially equates with brooding. Brooding is common among colonial animals that are composed of many small modules (Strathmann and Strathmann, 1982), although brooding style is diverse in bryozoans, pterobranch hemichordates, compound ascidians, and several kinds of hard and soft corals. A population or community of dicyemids formed in the renal sac is similar to a colony, although individuals are monozoic.

In dicyemids, the community may develop from a small number of individuals (one or few) at the initiation of the infection of the renal sac because success of infection of new non-gregarious hosts is apparently low at the level of an individual infusoriform. Dicyemids are occasionally found in only one of the two renal sacs in a host octopus. In some instances two different dicyemids species are detected, one in the right and the other in left renal sac of the host (Furuya *et al*., 1992b). These cases suggest that only a small number of propagules may infect an individual host. Subsequent asexual multiplication forms a large population in the renal sac. Under such conditions, cross-fertilization is of little advantage. Thus, self-fertilization via a hermaphroditic gonad might be settled for in dicyemids.

A very short planktonic larval stage also is typical in colonial benthic animals (Strathmann, 1990). Infusoriform larvae actively swim close to the dish bottom for only a few days *in vitro*. In the anterior region of an embryo, there is a pair of unique cells called the apical cells, each containing a refringent body composed of hydrated magnesium salt of inositol hexaphosphate. Its high specific gravity imparts a negative buoyancy to the dispersal larvae. McConnaughey (1951) and Lapan (1975) suggested the role of refringent bodies is to help the larvae remain near the sea bottom, where they can encounter benthic cephalopods. Dicyemids eventually enter the excretory organ and apparently do not move when once attached. The analogy between colonial animals and dicyemids can be attributed to their sedentary life styles.

PHYLOGENETICAL RELATIONSHIPS

Contradictory to van Beneden's original naming, Nouvel (1947) considered dicyemids were degenerates from metazoans such as trematodes, because of the adaptation for the parasitic life style. Analysis of the G+C content of nuclear DNA suggested a close relationship between ciliate protazoans and dicyemids (Lapan and Morowitz, 1974). Phylogenetic analyses using nucleotide sequences of 5S rRNA also suggested that dicyemids diverged earlier than other primitive metazoans such as sponges, cnidarians, and flatworms (Hori and Osawa, 1987). To the contrary, the analyses using nucleotide sequences of 18S rDNA suggested the dicyemids are rather degenerate triploblastic animal (Katayama *et al*., 1995). They suggest that dicyemids are a sister group to nematodes, myxozoans and acoel turbellarians. Recently, Hox gene sequence data were analysed to investigate the phylogenetic affinity of dicyemids (Kobayashi *et al*., 1999). The homeodomain sequence showed that the dicyemid Hox gene (*DoxC*) is a member of the "middle (central)" group of Hox (or Hox-like) genes (see Brooke *et al*., 1998), and identity is highest with *Antp* and its orthologues. The middle group of Hox genes has only been reported from triploblasts; no cnidarian genes fall into this group (Martinez *et al*., 1998). The dicyemid Hox gene encodes a spiralian peptide motif assigning it to the Lophotrochozoa including turbellarians, nemerteans, annelids, and brachiopods.

If dicyemids were degenerated and specialized metazoans, this raises a question how the transition from the metazoan body organization to such a simple body organization occurred. It is plausible that progenesis was caused by precocious sexual maturation at an early developmental stage of dicyemids. This progenesis might truncate the life cycle. The cephalopods might be originally intermediate hosts, and the final host species were possibly predacious marine vertebrates such as *Mosasaurus*. Subsequently, progenesis might excluded the final host from the life cycle. This process might be related somehow to the extinction of these Mesozoic vertebrates.

Dicyemids have several unique features. For instance, germ line cells are incorporated in the cytoplasm of certain cells throughout the life cycle; agametes (axoblasts) in the axial cell of vermiforms, spermatogonia in the axial cell of

infusorigens, and a germ cell in an urn cell. The mitochondrial COI, COII, and COIII genes are encoded on three small, separate circular DNA molecules (minicircles) in *Dicyema misakiense* (Watabnabe *et al*., 1999). The extrachromosomal circular DNAs appear during early embryogenesis in *Dicyema japonicum* (Noto *et al*., 2003). These particular features in dicyemids do not necessarily have phylogenetic information, but are of significant implication for understanding diversification of primitive multicellar animals.

The phylogenetic affinity of dicyemids has been one of the main questions in the biology of dicyemids. Although recent studies have revealed that they might not be truly primitive animals deserving the name of "mesozoans" (Katayama *et al*., 1995; Kobayashi *et al*., 1999), they are still one of the most interesting and puzzling groups of lower invertebrates.

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