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Morphogenesis of Adult Traits during the Early Development of *Mespilia globulus* Linnaeus, 1758 (Echinodermata: Echinoidea)

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Chisato Kitazawa, Chiaki Kobayashi, Mami Kasahara, Yuki Takuwa, and Akira Yamanaka (2012) Morphogenesis of adult traits during the early development of *Mespilia globulus* Linnaeus, 1758 (Echinodermata: Echinoidea). *Zoological Studies* **51**(8): 1481-1489. We observed the development of *Mespilia globulus* L. (Temnopleuridae) and *Hemicentrotus pulcherrimus* A. Agassiz, 1863 (Strongylocentrotidae) and focused on the formation of adult traits by light and scanning electron microscopy. In *M. globulus*, a primary pore canal constructing a part of the adult water vascular system appeared at the left and right coelomic sacs in the prism stage. The left and right primary pore canals respectively elongated to the left lateral and right dorsal ectoderm and opened to the outside, and then the right one disappeared until the 2-armed larval stage. On the other hand, the primary pore canal of prisms of *H. pulcherrimus* formed on the left side only. We first confirmed the existence of different types of asymmetrical primary pore canal formation at the prism stage in sea urchins. In 2-armed larvae of *M. globulus*, a cell mass was formed by invagination and pinching off of the left oral ectoderm. At the 6-armed larval stage, the developing cell mass attached to the hydrocoel, and together they formed an adult rudiment. In contrast, the left larval ectoderm of 6-armed larvae of *H. pulcherrimus* began to invaginate between the postoral and postdorsal arms and formed an amniotic cavity. The amniotic cavity had attached to the hydrocoel by the 8-armed larval stage for adult rudiment formation. This is the 1st report describing how adult rudiment formation depends on the cell mass in the early larval stage of *M. globulus* larvae. From these results, we propose that asymmetrical primary pore canal formation is initiated in the embryonic stage with some modifications in formational modes among species, and that the heterochronical start of adult rudiment formation by the cell mass may allow a sufficient period to develop and protect the rudiment.
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Key words: Echinoid, *Mespilia globulus*, Primary pore canal, Cell mass, Adult rudiment.

Echinoderms drastically change their body plan from larvae to adults through metamorphosis. During this developmental process, it is necessary to form adult traits as the adult rudiment inside the larval body.

In sea urchins, the pore canal (hydroporic canal) is the earliest adult trait, which elongates from the left axocoel derived from the left coelomic sac and opens dorsally as the hydropore at the 4- or 6-armed pluteus larval stages (MacBride 1911 1914 1918, Okazaki 1975); it is called the primary pore canal (PPC). The PPC becomes a peripheral duct connecting the adult water vascular system and the madreporic pore for exchanging seawater (Okazaki 1975). Interestingly, it was reported that the PPC elongates from the left and right coelomic sacs to each larval lateral side in the prism stage to maintain the larval body width, and then only the right one disappears at the 4-armed larval stage in *Temnopleurus hardwickii* Gray, 1855 (Hara et al. 2003).

After a larva fully develops, an adult rudiment consisting of the larval ectoderm and hydrocoel derived from the middle part of the left coelomic

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sac forms at a later larval stage, and then the adult oral structures, including the tube feet, are formed within this organ (MacBride 1914, Dan 1957, Okazaki 1975). Adult rudiment formation is classified into at least 3 types by the formational processes during larval stages as shown below.

The 1st type involves an adult rudiment which directly forms on the surface of the larval left side without formation of special ectodermal organs. This type is observed in species of the most primitive order, the Cidaroida (Smith 1984, Kroh and Smith 2010), including *Eucidaris thouarsii* L. Agassiz & Desor, 1846 (Emlet 1988), *Phyllacanthus parvispinus* Tension Woods, 1878 (Parks et al. 1989), and *P. imperialis* Lamarck, 1816 (Olson et al. 1993).

The 2nd type of adult rudiment formation depends on the amniotic cavity (vestibule). Many sea urchin species, including *Hemicentrotus pulcherrimus* (Camarodonta: Echinidea: Odontophora: Strongylocentrotidae), belong to this type (MacBride 1914, Dan 1957, Okazaki 1975, Ishihara and Noguchi 1996). After the hydrocoel has formed, an amniotic cavity autonomously invaginates from the ectoderm into the larval body, attaches, and finally interacts with the hydrocoel to form the adult rudiment (Runström 1912 1918, Czihak 1965 1996, Kitazawa and Amemiya 2000, Minsuk and Raff 2002).

The 3rd type of adult rudiment formation depends on the cell mass (CM). This type of adult rudiment formation is observed only in prisms of *Genocidaris maculata* A. Agassiz, 1869 (Ubisch 1959), and 4-armed pluteus larvae of *T. hardwickii* (Fukushi 1959 1960). *Genocidaris maculata* belongs the family Trigonocidaridae (Camarodonta: Temnopleuroidea) (Kroh and Smith 2010). A spherical CM, formed by invagination and pinching off of the left larval ectoderm, attaches to the hydrocoel, and these complex organs comprise the adult rudiment (Ubisch 1959, Fukushi 1959 1960), suggesting that the CM may function as an organ that substitutes for the amniotic cavity (Ubisch 1959, Fukushi 1960). However, it is still not clear whether this 3rd type has systematic significance to echinoids.

Morphological features of the early development of *Mespilia globulus*, one member of the family Temnopleuridae (Camarodonta: Temnopleuridea) as *T. hardwickii* (Kroh and Smith 2010), have been well studied (Mortensen 1921, Onoda 1936, Okazaki 1975). Recently, details of morphological changes on the surface of the fertilized egg, blastula formation (Kitazawa et al. 2010), and stepwise gastrulation (Takata and Kominami 2004) during the early development of this species were analyzed. On the other hand, later development of the PPC and adult rudiment formation as adult traits of *M. globulus* have not been fully elucidated. Clarifying adult trait formation in this species may expand our understanding of adult traits of the Temnopleuridea.

In the present study, we observed formational processes of adult traits of *M. globulus* by light and scanning microscopy, compared them to those of *H. pulcherrimus*, and discuss evolutionary changes in adult rudiment formation in sea urchins.

MATERIALS AND METHODS

Fertilization and culturing of embryos and larvae

Adult sea urchins of *Mespillia globulus* and *Hemicentrotus pulcherrimus* were respectively collected in July-Sept. and Dec.-May from the Inland Sea (Setonai), Yamaguchi Prefecture, Japan. Adults of *M. globulus* were kept in laboratory aquaria at 24 ± 1°C, whereas those of *H. pulcherrimus* were kept in other aquaria at 18 ± 1°C. A small amount of 0.5 M KCl solution was injected into the body cavity to induce spawning of eggs and sperm in July-Dec. for *M. globulus* and Dec.-May for *H. pulcherrimus*. After washing several times in filtered seawater, eggs were fertilized by adding few drops of diluted sperm. The fertilized eggs were cultured in plastic dishes filled with artificial seawater (ASW; TetraMarin® Salt Pro, Tetra, Melle, Germany) and cultured until the 4-armed pluteus larval stage at 24°C for *M. globulus* and at 18°C for *H. pulcherrimus*.

Four-armed pluteus larvae were transferred to 50-ml plastic tubes filled with ASW but containing a small bubble to stir the seawater at 24°C for *M. globulus* and 19°C for *H. pulcherrimus*. A few drops of *Chaetoceros gracilis* Pantocsek, 1892 diatoms suspended in ASW were added to each tube as larval food according to the culturing method of Wray et al. (2004) with small modifications. The plastic tubes were horizontally shaken in a plastic tray fixed to a double shaker (NR-3, TAITEC, Saitama, Japan) at 0.71 times/min. The seawater in the bottles was replaced with new ASW every 2 d under a stereomicroscope (SZ61, Olympus, Tokyo, Japan), and then new food was added. Metamorphosing larvae were transferred to a high-glass dish filled with ASW and fed a piece of seashell coated with algae.

Light microscopy

Embryos, larvae, and juveniles were observed and photographed under a microscope (OPTIPHOT-2, Nikon, Tokyo, Japan) or stereomicroscope (SZ61, Olympus) using a digital camera (μ810, Olympus). Young adult sea urchins were photographed using the digital camera. The body width of *M. globulus*, as the distance from the outer wall of the archenteron under each coelom for embryos or from the outer wall of the stomach with the maximum diameter to the surface of the left or right ectoderm perpendicularly intersecting the body median line for larvae, was measured under the microscope.

Scanning electron microscopy (SEM)

An aliquot of embryos and larvae were fixed for SEM according to Kitazawa et al. (2004) with slight modifications. They were fixed with fixative including 0.6 M sucrose, cacodylate buffer (pH 7.4), and 1% osmium for 1 h on ice. After removal of the fixative, they were treated with a series of ethanol solutions and then preserved in a 70% ethanol solution. Another aliquot of embryos and larvae were fixed with 4% formalin ASW for about 1 h at room temperature (ca. 25°C), rinsed, and kept in a 70% ethanol solution.

Fixed specimens were dehydrated and gradually changed to 2-methyl-2-propanol (ethanol: 2-methyl-2-propanol ratios of 3:1, 1:1, and 1:3). After specimens had been washed with 2-methyl-2-propanol twice, they were freeze-dried in a freeze-dryer (BFD-21S, Vacuum Device, Ibaraki, Japan). Dried specimens were mounted on an aluminum stage with double-sided conductive aluminum tape and coated with gold by a fine ion sputter (E-1010, Hitachi High-Technologies, Tokyo, Japan). Specimens were observed and photographed under SEM (Miniscope TM-1000S, Hitachi High-Technologies).

RESULTS

Development after gastrulation in *Mespilia globulus*

After gastrulation, the morphogenesis of *M. globulus* was observed in detail. At 19.5 h post-fertilization (hpf) at 24°C, most embryos had developed to the late gastrula stage (Fig. 1A). Spicules had formed on the left and right ventral sides. The blind end of the archenteron had expanded, showing differentiation of the future coelomic sacs, and some secondary mesenchyme cells had migrated from the tip of the archenteron to the blastocoel. Around this stage, the surface of the dorsal ectoderm was still smooth (Fig. 2A). However, a primary pore canal (PPC) that extended from the left and right coelomic sacs to the ectoderm (Fig. 1B, Table 1) was identified in embryos at 22.5 hpf. The left PPC became thicker than the right one. By 24 hpf, the left and right PPCs had respectively extended from each coelomic sac to the left lateral side and dorsal side in most embryos (Fig. 1C), except for some specimens in which the left and right PPCs had symmetrically formed (Figs. 1D, 2B). The PPCs opened to the exterior of the ectoderm (hydropore) (Figs. 1C, 2B). However, after this period, 65% of observed embryos only possessed a PPC on the left side (Table 1). Also, some cells of the left oral ectoderm on the base between the left postoral and presumptive anterolateral arms had begun to invaginate (Fig. 2C).

When specimens had developed to the 2-armed pluteus larval stage at 37.5 hpf, almost all of the larvae only possessed a PPC on the left side (Fig. 1E, Table 1). At this stage, invagination of some cells on the oral ectoderm had formed a small spherical organ (Fig. 1E, Table 1) identified in larvae of *Genocidaris maculata* (Ubisch 1959) and *Temnopleurus hardwickii* (Fukushi 1959 1960). We also called the small spherical organ in *M. globulus* a cell mass (CM) according previous reports. After CM formation, the left oral ectoderm became smooth again. The CM enlarged and formed a hollow pouch (Fig. 1F). Up to this stage, body widths were 55.1 ± 1.6 μm on the left side and 54.0 ± 1.7 μm on the right side (n = 18) in prisms at 27 hpf, 39.1 ± 2.7 μm on the left side and 35.9 ± 4.1 μm on the right side (n = 11) in the 4-armed larval stage at 2 d post-fertilization (dpf), and 49.5 ± 2.4 μm on the left side and 45.1 ± 2.3 μm on the right side (n = 18) in the 4-armed larval stage at 4 dpf.

When larvae had developed to the early 6-armed pluteus stage, the diameter of the CM grew more rapidly (Figs. 1G, 3). At this stage, the left coelomic pouch divided into the axocoel, hydrocoel, and somatocoel along the anterior-posterior axis, and the hydrocoel began to enlarge. As a result, the space between the CM and hydrocoel became narrower (Fig. 1H). At the same time, the hydropore, an opening of the left PPC on the ectoderm, migrated to the right dorsal

side (Fig. 1I).

At 7 dpf, the CM had attached to the hydrocoel (Fig. 1J, K). The hypertrophic CM covered the hydrocoel, and the complex of the CM and hydrocoel formed a symmetrical 5-radial structure (Fig. 1L). Therefore, the complex of these organs was considered to correspond to an adult rudiment. At around 10 dpf, 5 moving primary podia were identified under the larval ectoderm (Fig. 1M). At this stage, a small pouch was identified at the tip of the anterior-most primary podium, as described as a "small process" in larvae of *T. hardwickii* (Fukushi 1960) (Fig. 1M, M'). The adult rudiment continued to cover the larval ectoderm until metamorphosis (Fig. 2D). Finally, juvenile spines and tube feet broke through the larval ectoderm, and then the larvae metamorphosed at about 30 dpf (Figs. 1N-P, 2E). For several months after metamorphosis, juveniles developed into young adult sea urchins (Fig. 1Q). In these serial observations, *Mespilia* larvae never formed an amniotic cavity or amniotic opening during development.

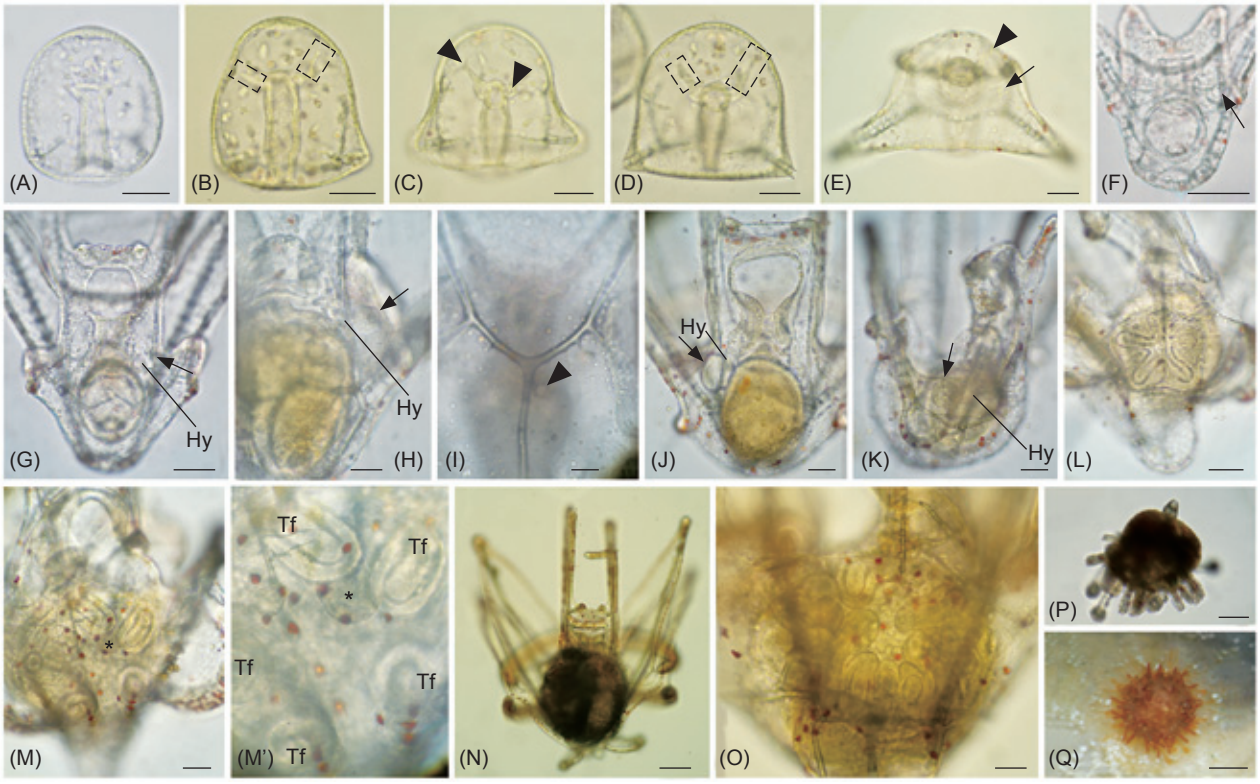

Fig. 1. Light micrographs of development after gastrulation of *M. globulus*. (M') Higher magnification of (M). (A) Dorsal view of a late gastrula at 19.5 h post-fertilization (hpf). The blind end of the archenteron has spread to form the future coelomic sacs. (B) Dorsal view of a prism embryo at 22.5 hpf. The primary pore canals (PPCs) at the left and right coelomic sacs of the embryos are elongated. (C, D) Dorsal (C) and oral (D) views of prisms at 24.4 hpf. The left and right PPCs are asymmetrically elongated to the left lateral side and dorsal side as shown in (C), except for some specimens in which the PPCs symmetrically formed as shown in (D). (E) Oral view of a 2-armed larva at 37.5 hpf. At this stage, the right PPC has become undetectable. The larva formed a cell mass (CM) by invagination and pinching off of the ectoderm at the base between the presumptive left postoral and anterolateral arms. (F) Ventral view of a 4-armed larva at 4 d post-fertilization (dpf). (G) Ventral view of an early 6-armed larva at 5 dpf. The CM and hydrocoel are larger and closer to each other. (H, I) Ventral (H) and dorsal (I) views of 6-armed larvae at 6 dpf. The CM has become larger and has formed a hollow pouch. At the same stage, the hydropore has migrated to the right dorsal side near the dorsal arch. (J, K) Dorsal (J) and left lateral (K) views of 8-armed larvae at 7 dpf. The CM has attached to and covered the hydrocoel. (L) Left lateral view of an 8-armed larva at 8 dpf. The complex of the CM and hydrocoel has formed an adult rudiment. The 5 lobes of the rudiment are detectable. (M) Left lateral view of an 8-armed larva at 11 dpf. The primary podia (tube feet) have grown under the larval ectoderm. A small process has budded from the anterior-most tip of the primary podium. (N, O) Dorsal (N) and left lateral (O) views of 8-armed larvae at 28 dpf. The tube feet are still completely covered by the larval ectoderm. (P) Lateral view of a metamorphosing larva at 27 dpf. The tube feet and spines project from the larval body. (Q) Dorsal view of a young adult sea urchin at about 5.5 mo post-fertilization. Arrowheads and arrows show a hydropore which is the opening of the PPC and CM, respectively. Asterisks indicate a small process. Squares of broken lines show the PPC. Hy, hydrocoel; Tf, tube foot. Scale bars = 50 μm for (A-P) and 1 mm for (Q).

Comparison of the formation of adult traits between *M. globulus* and *Hemicentrotus pulcherrimus*

Considering the evolutionary processes in the formation of adult traits of Camarodonta sea urchins, the development of *M. globulus* (Temnopleuridae) shown above was compared to that of *H. pulcherrimus* (Strongylocentrotidae), a typical sea urchin species that forms an amniotic cavity.

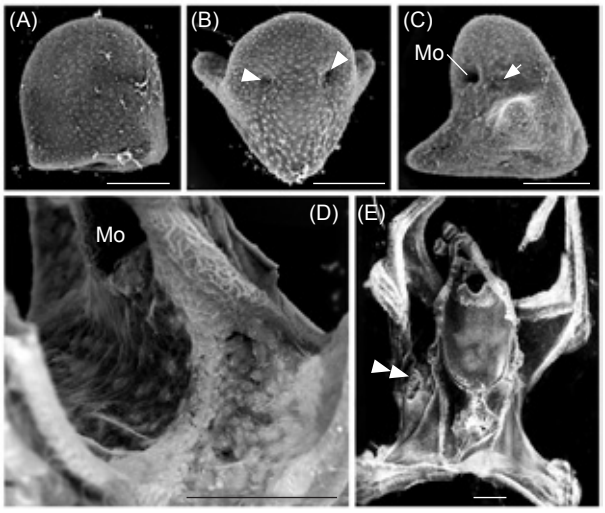

Fig. 2. Scanning electron micrographs of embryos and larvae of *M. globulus*. (A) Dorsal view of a prism embryo at 22 h post-fertilization (hpf). The dorsal ectoderm is still smooth. (B, C) Dorsal (B) and left oral (C) views of early 2-armed larvae at 24.3 hpf. The larvae have hydropores on the left and right dorsal sides. Also, the left oral ectoderm has begun to invaginate to form a cell mass (CM). (D) Oral view of a 6-armed larva at 14 d post-fertilization (dpf). This larva never formed an amniotic cavity. (E) Dorsal view of an 8-armed larva at 33 dpf. The left ectoderm between the postoral and postdorsal arms has burst open, and the tube feet are projecting from the opening. Arrowheads and arrows respectively show a hydropore and invagination of the ectoderm for CM formation. Double arrowheads show the amniotic opening. Mo, larval mouth. Scale bars = 50 μm.

We initially observed the formation of adult traits by *H. pulcherrimus.* Prism embryos of this species formed coelomic sacs at 25.5 hpf at 18°C (Fig. 4A), and then only the left coelomic sac had elongated into a PPC on the dorsal side by about 30 hpf (Fig. 4B-D). The PPC opened onto the surface of the left dorsal ectoderm in the 4-armed stage (Fig. 4E, F). Some cilia extended from the primary hydropore (Fig. 4F'). When these larvae were cultured at 19°C, the left ectoderm between the postoral and postdorsal arms began to invaginate forming an amniotic cavity in the 6-armed larval stage (at about 10 dpf). After the amniotic cavity had elongated and attached to the hydrocoel by the 8-armed larval stage (approximately 30 dpf), it covered the hydrocoel. The amniotic opening was always opened, even though the diameter temporarily became smaller (Fig. 4G, G'), and then became bigger again before metamorphosis. The tube feet projected from the amniotic opening, and then the larvae metamorphosed. The small process observed in larvae of *M. globulus* was not identified on the primary podia in this species (Fig. 4H, H'). Table 2 summarizes the features and timing of the developmental events in the formation of adult traits of *M. globulus* and *H. pulcherrimus*.

DISCUSSION

Results presented here provide new knowledge that *Mespilia globulus* forms a PPC at the left and right coelomic sacs in the prism stage and then loses the right PPC, and that this species directly forms the adult rudiment from the CM (Table

Table 1. Formational timing and direction of formation of the primary pore canal (PPC) and cell mass (CM) in *M. globulus*

Time after fertilization (h)	*n*	Directions of formation of the PPC/CM (%)										
		U/U	B/U	L/U	U/B	U/L	B/B	B/L	L/B	L/L	R/B	R/L
22.5	40	77.5	22.5	0	0	0	0	0	0	0	0	0
24-26.5	100	3.0	32.0	65.0	0	0	0	0	0	0	0	0
30.5	27	18.5	14.8	59.3	0	0	0	0	0	7.4	0	0
37.5	138	0.7	3.6	0	0	3.0	0.7	15.9	1.5	73.9	0.7	0
44.5-45	166	0	0	0.6	0	5.4	0	6.6	0	87.4	0	0
62	118	0	0	0	0.8	2.6	0	0.8	0	95.0	0	0.8

These data were based on 3 independent experiments. U, undetectable; B, both; L, left; R, right.

2), although these traits had never been mentioned in previous studies (Mortensen 1921, Onoda 1936, Okazaki 1975).

Formation of the PPCs

A previous report on *Temnopleurus hardwickii* revealed that PPCs symmetrically form on the left and right sides in prism embryos, and then the right one disappears at the 4-armed larval stage (Hara et al. 2003). It was also proposed that symmetrical PPC formation contributes to maintenance of a normal body width in larvae by experiments of removing the coelomic sacs in the stage before the PPCs form. In *M. globulus*, PPCs also initially formed in the left and right coelomic sacs. However, the left PPC became thicker and elongated more to the lateral side than the right one and then migrated to the dorsal side until the 6-armed larval stage, while the right one elongated to the dorsal side and then disappeared in the 2-armed larval stage (Fig. 1, Table 1). On the other hand, *Hemicentrotus pulcherrimus* only formed the left PPC in the prism stage, and it opened onto the

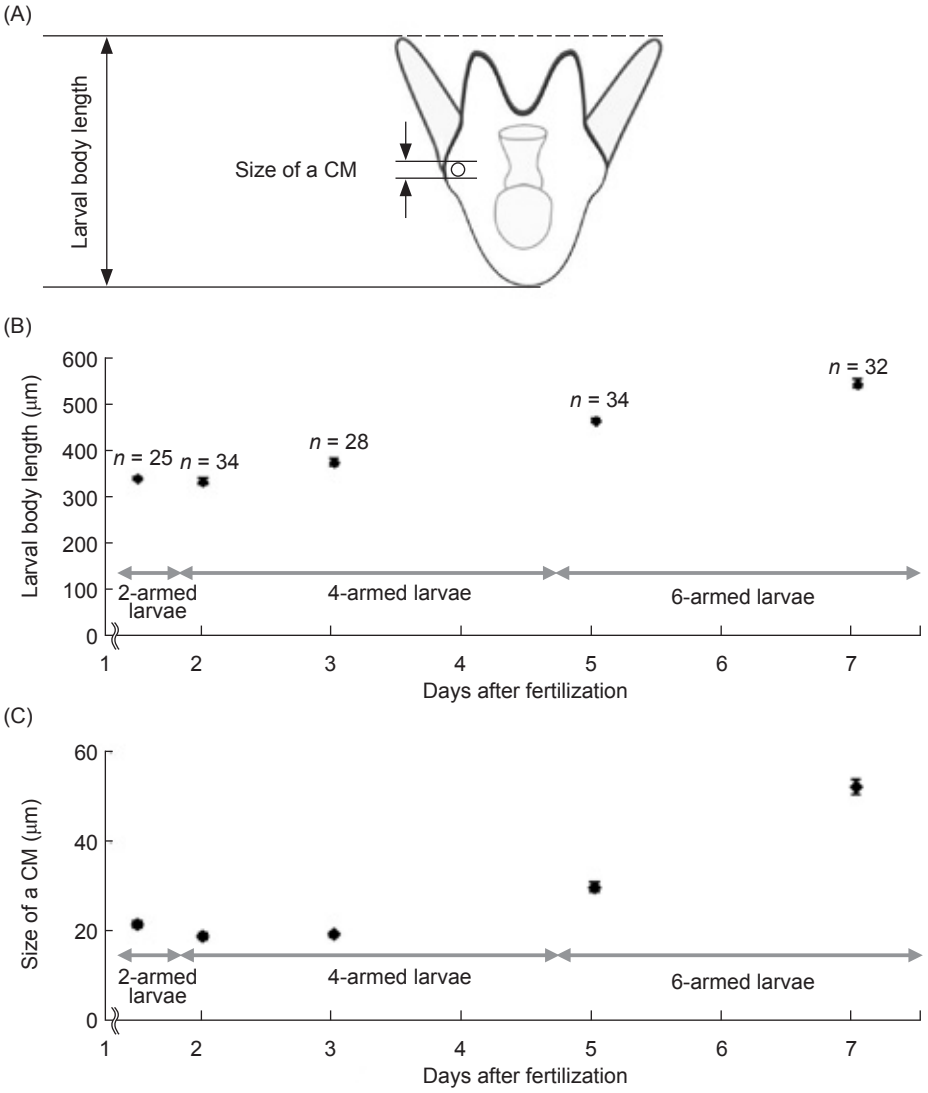

Fig. 3. Growth of the larval body length and the size of a cell mass (CM) during development of *M. globulus*. (A) Schematic diagram of a 4-armed larva. The length between the tip of the postoral arm and posterior end of the larval body was measured as the "larval body length," and the maximum diameter of the CM was measured as the "size of the CM". (B) Change in larval body length. (C) Change in the size of the CM. Data of (B) and (C) are from the same larvae. The standard error of the mean is shown on each dot. The measurement of these lengths was begun from 37.5 h post-fertilization when most larvae had formed a CM.

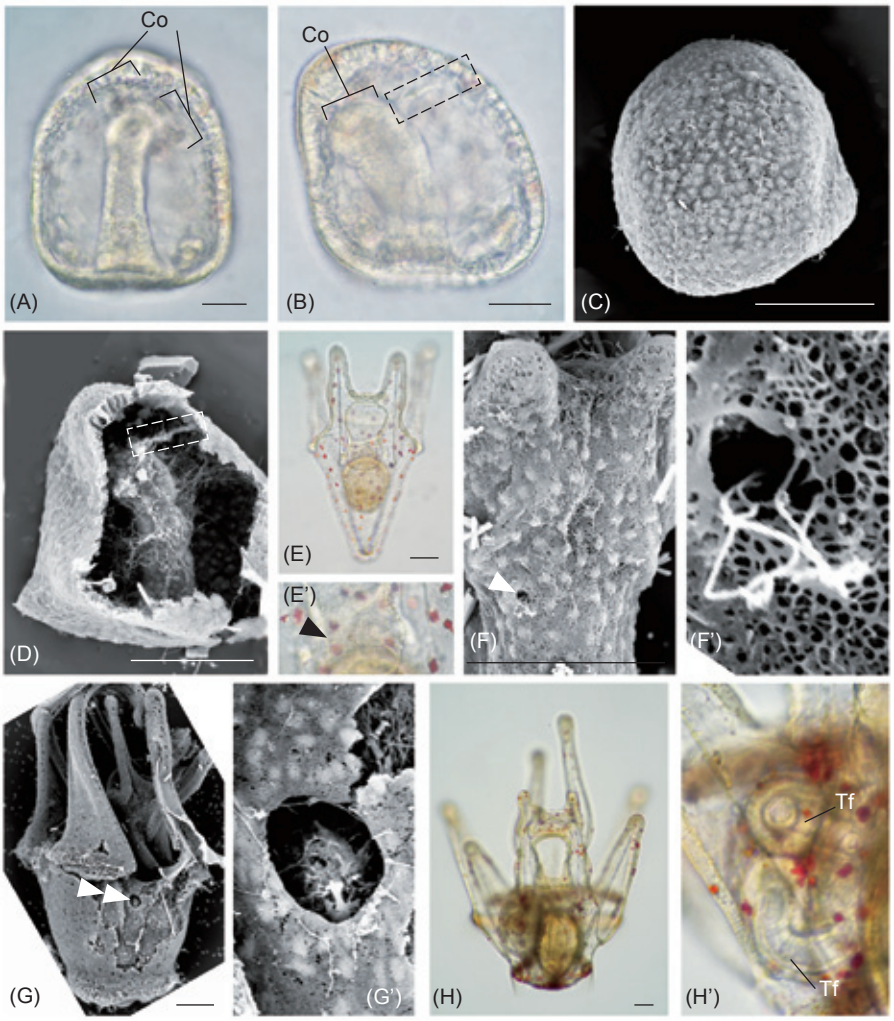

Fig. 4. Formation of adult traits in *Hemicentrotus pulcherrimus*. Photographs show light micrographs (A, B, E, H) and scanning electron micrographs (C, D, F, G). (E', F', G', H') Higher magnifications of (E, F, G, H). (D, G) Micrographs of specimens in which a part of the left ectoderm was removed with a piece of the cellophane tape before coating. (A) Dorsal view of a prism embryo at 25.5 h post-fertilization (hpf). The embryo has formed coeloms. (B) Left lateral view of a prism embryo at 29.7 hpf. The embryo has formed a primary pore canal (PPC) only on the left side. (C, D) Dorsal (C) and left lateral (D) views of the prism at 1.5 d post-fertilization (dpf). At this stage, the hydropore at the dorsal ectoderm has not opened, (C) but the PPC has reached the left dorsal ectoderm (D). (E) Dorsal view of a 4-armed larva at 5 dpf. The hydropore has opened. (F) Dorsal view of a 4-armed larva at 4 dpf. Some cilia are detectable from the hydropore (F'). (G) Left lateral view of an 8-armed larva at 17 dpf. The amniotic opening has remained open during formation of the adult rudiment. (H) Dorsal view of an 8-armed larva at 28 dpf. Tube feet have developed, but the small process cannot be identified. Arrowheads and double arrowheads respectively show a hydropore and an amniotic opening. Squares of broken lines show the PPC. Co, coelomic pouch; Tf, tube foot. Scale bars = 50 μm.

Table 2. Summary of the timing of developmental events with formation of adult traits in *M. globulus* and *H. pulcherrimus*

Developmental event	*M. globulus*	*H. pulcherrimus*
Beginning to form the primary pore canals (PPCs)	Prism stage	Prism stage
(direction of the PPC)	(both to the left)	(to the left)
Beginning to form the cell mass (CM) or amniotic cavity	2-armed larval stage	6-armed larval stage
(rudiment formational mode)	(CM)	(amniotic cavity)
Attachment of the CM or amniotic cavity to the hydrocoel	6-armed larval stage	Late 6-8-armed larval stage

left dorsal area (Fig. 4) in spite of previous reports that the PPC forms in the 6-armed larval stage (Ishikawa and Noguchi 1973, Ishihara and Noguchi 1996). These results suggest that PPC formation begins in an early developmental stage in these species, but the initial PPC formation on each side may be a common feature of Temnopleuridae sea urchins including *M. globulus* and *T. hardwickii*.

The present results also revealed that the mechanism for maintaining the larval body width may differ among these species. PPC formation only on the left side of *Hemicentrotus* embryos may mean that the PPC is not necessary to maintain the larval body width in this species. Among species with PPC formation on the left and right sides, there were also differences in features of PPC formation and the larval body width of *M. globulus*, which was much wider than that of *T. hardwickii* (about 22.5 μm in 2-armed larvae (Hara et al. 2003)). These results suggest that PPCs may be necessary to maintain the larval body width in *T. hardwickii* but not in *M. globulus*. Differences in the epithelial structure of blastulae among these species may also cause differences in maintenance of the larval body width, because blastulae of *M. globulus* maintain an abundant blastocoel of about 1.3-times that of *T. hardwickii*, even though thicknesses of the epithelium of the blastulae of these species are almost the same (Kitazawa et al. 2010).

Evolution of the formation of the adult rudiment depends on the CM

The present study newly revealed that *M. globulus* larvae also form a CM on the larval left side, and the CM forms the adult rudiment with the hydrocoel (Figs. 1, 2) as also seen in *T. hardwickii* (Fukushi 1959 1960) and *Genocidaris maculata* (Ubisch 1959). These 3 species belong to the same infraorder, the Temnopleuridea (Kroh and Smith 2010). An amniotic cavity is present during rudiment formation in another Temnopleuridea sea urchin, *Holopneustes purpurescens* A. Agassiz, 1872, belonging to the Temnopleuridae (Morris 1995). However, it is a directly developing species with accelerated and abbreviated formation of larval feeding organs and larval period (Morris 1995). Although Fukushi (1960) suggested that CM formation could be a common trait of the genus *Temnopleurus* based only on observations of *T. hardwickii*, the results reported here suggest that CM formation might only occur in indirect developing species that pass through a larval feeding period as in Camarodonta sea urchins of the Temnopleuridea.

It was previously thought that dependence of the formation of the adult rudiment in the amniotic cavity was widely present in many species within the Euechinoidea following divergence from the Cidaroidea (Emlet 1988, Parks et al. 1989). On the other hand, the CM may be considered a modification of the amniotic cavity, because it is thought that the Temnopleuridea, including species in which the formation of the adult rudiment depends on the CM, diverged after involvement of the amniotic cavity in the Euechinoidea (Smith 1984), and the CM is derived from the left larval ectoderm and forms the adult rudiment similar to what occurs with the amniotic cavity.

One of the reasons why the CM is introduced into the formational process of the adult rudiment instead of the amniotic cavity might be greater protection of the adult rudiment, and also of the larval body, because the surface of the larval body fuses and closes after invagination of the CM (Figs. 1, 3). Another reason is that the CM might cause formation of the adult rudiment extremely precociously as shown with *Mespilia* larvae which begin to form a CM in the 2-armed larval stage (Fig. 1), while *Hemicentrotus* larvae begin to form the amniotic cavity in the 6-armed larval stage (Table 2). It was reported that species with an amniotic cavity or CM develop faster than members of the Cidaroidea which require very long periods to form the adult rudiment (Parks et al. 1989), and the amniotic cavity is thought to be an organ for precocious formation of the adult rudiment (Emlet 1988). These results suggest that CM formation in an early larval stage may possibly ensure a sufficient period for adult rudiment formation by indirect developing Temnopleuridea sea urchins.

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