アカウニ×ムラサキウニの交雑と雑種幼生の形態

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Hybridization of *Pseudocentrotus depressus* egg and cryopreserved sperm of *Anthocidaris crassispina* and the morphology of hybrid larva

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Crossbreeding between *Pseudocentrotus depressus* egg and cryopreserved *Anthocidaris crassispina* sperm were performed, in order to obtain several information concerning the influence of maturation of egg and sperm donors on the morphological appearance of parental characters in hybrids. Hybrid larvae were reared up to metamorphosis at 20°C, and *Chaetoceros gracilis* was used as the food. Skeletal structure of hybrid larvae exhibited intermediate characters, such as incomplete compound basket body structure, structural differences in postoral rod, postero dorsal rod and posterior transverse rod. Utilization of cryopreserved sperm in the experiment, permitted the use of sperm collected during the breeding season of the paternal species. This in turn is thought to have enhanced the probability of producing intermediate characters in the hybrid larva.

Sea urchin is gaining importance in the field of aquaculture, as the demand for bigger and tasty varieties is increasing. Hybridization is an useful technique to produce the desired offspring. However, some of the aspects that are to be taken into consideration before venturing into the actual process of hybridization, are maturing seasons of the two parental species that are adopted, the quality of their eggs *etc.* Looking back at the earlier reports, it has been stressed that in the hybrid varieties of sea urchin maternal influence is dominant,[1–3] and Shearer *et al.*[4] expressed a possibility that the paternal gene did not function in individuals which indicated dominant maternal characters. Vernon[5] observed the seasonal morphological fluctuation of a hybrid produced with *Sphaerechinus granularis* ova and *Strongylocentrotus lividus* spermatozoa and explained that paternal or maternal character dominance in the offspring is dependent on seasonal variation in the quality of the sperm or ova.

Cryopreservation techniques come in handy to solve the problem of seasonal variation in the quality of sperm or ova. In this study, we attempted hybridization of two species of sea urchin such as *Pseudocentrotus depressus* and *Anthocidaris crassispina*. These two have different maturation seasons. Hybridization was carried out by using the cryopreserved sperms of *A. crassispina*. The influence of paternal and maternal characters in the offspring is assessed by the characters of the hybrid larvae.

Materials and Methods

Mature *Anthocidaris crassispina* were collected during August 1987 from Sensui-jima, Fukuyama city. *Pseudocentrotus depressus* were collected during November 1988 (provided by Kanagawa Prefectural Fisheries Experimental Station). These collection months were chosen as they fall within their respective natural breeding season. Sperms of *A. crassispina* were obtained by injecting 1 m*l* KCl into the body cavity. The sperms collected were cryopreserved following the method suggested by Kurokura *et al.*[6]

The cryopreservation of sperms yielded a result of 10% motility. Artificial insemination was carried out using these sperms and the freshly collected ova of *P. depressus*. Artificial insemination was carried out at a water temperature of 20°C and 2×10^5: 1 sperm, egg ratio.

Rearing of fertilized eggs and larvae were carried out in 5 liter beakers. The filtered sea water used in culture was collected from the same location as that of the parental female. *Chaetoceros gracilis* was used as the food. Food concentration was maintained at 5,000, 10,000 and 15,000 cells

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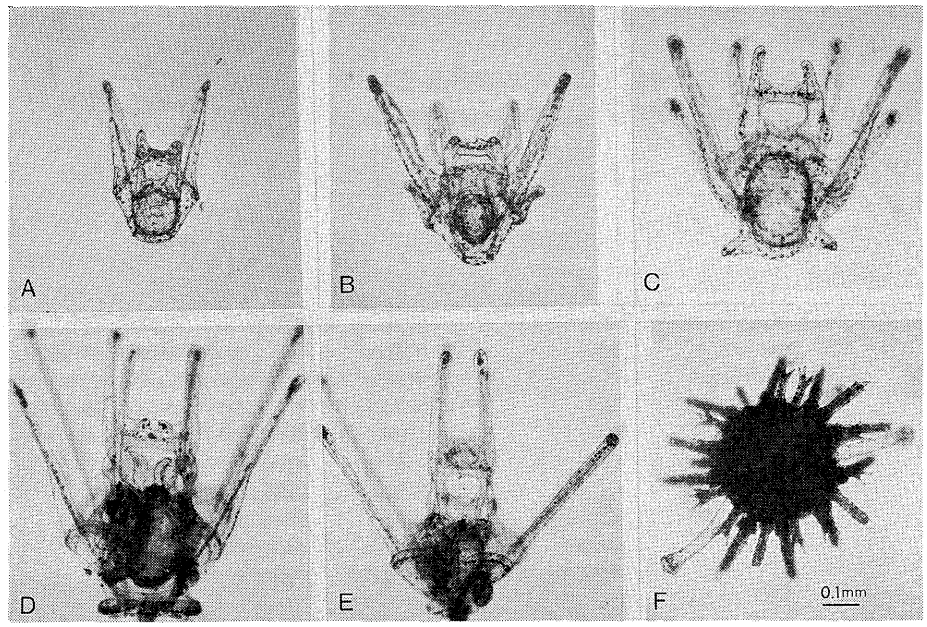

Fig. 1. *Pseudocentrotus depressus* (♀)×*Anthocidaris crassispina* (♂). A: Pluteus in the four armed stage, five day old, viewed from anal surface. B: Pluteus in the six armed stage, eight days old, viewed form anal surface. C: Pluteus in the eight armed stage, twelve days old, viewed from anal surface. D: Pluteus in the eight armed stage, fourteen days old, viewed from anal surface. E: Pluteus in the eight armed stage before metamorphosis, nineteen days old, viewed form anal surface. F: Young sea urchin, twenty four days old. (Scale bar equals 0.1 mm).

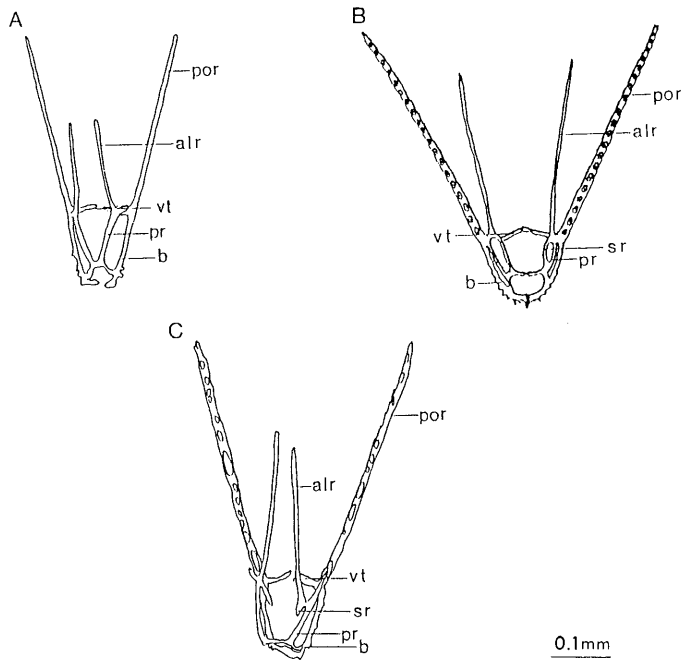

Fig. 2. The skeleton of four-armed larva. Notice the Postoral rod (por) and the recurrent rod (pr, sr) in hybrid larva and those in pure larvae. A: *Pseudocentrotus depressus*; B: *Anthocidaris crassispina* (redrawn from Onoda[9]); C: Hybrid larva in *P. depressus* (♀)×*A. crassispina* (♂). (Scale bar equals 0.1 mm).

alr: antero-lateral rod; b: body rod; por: postoral rod; pr: primary recurrent rod; sr: secondary recurrent rod; vt: ventral transverse rod.

per 1 m*l* of medium at four, six and eight armed stage period respectively by adjusting the food level every day. The concentration of larvae at the beginning of rearing was 5 individuals per 10 m*l*. The water inside the beaker was gently agitated by a propeller (1 round/1 second) and the temperature was maintained at 20°C.

The skeletons of 20 individuals at four, six and eight armed stage larvae were observed. In the case of older stage larvae which were difficult to observe owing to the opacity of the body, samples were treated with NaOH solution and sealed in glycerol to make them transparent.

Result

Fresh sperm and ova of *P. depressus* when inseminated produced nearly 100% fertilized eggs (personal observation). However when the cryopreserved sperm of *A. crassispina* was inseminated to fresh ova of *P. depressus* produced only 6.5% fertilized eggs. The larvae of the hybrid reached four armed, six armed and eight armed stage on the 3rd, 8th and 12th day after the insemination, respectively (Figs. 1, A, B and C). Pedicellaria appeared on the 14th day (Fig. 1, D) after the insemination. Completion of larval metamorphosis took 20 days. Majority of the hybrid plutei did not show any abnormality either in morphology or in movement. In the four armed larval

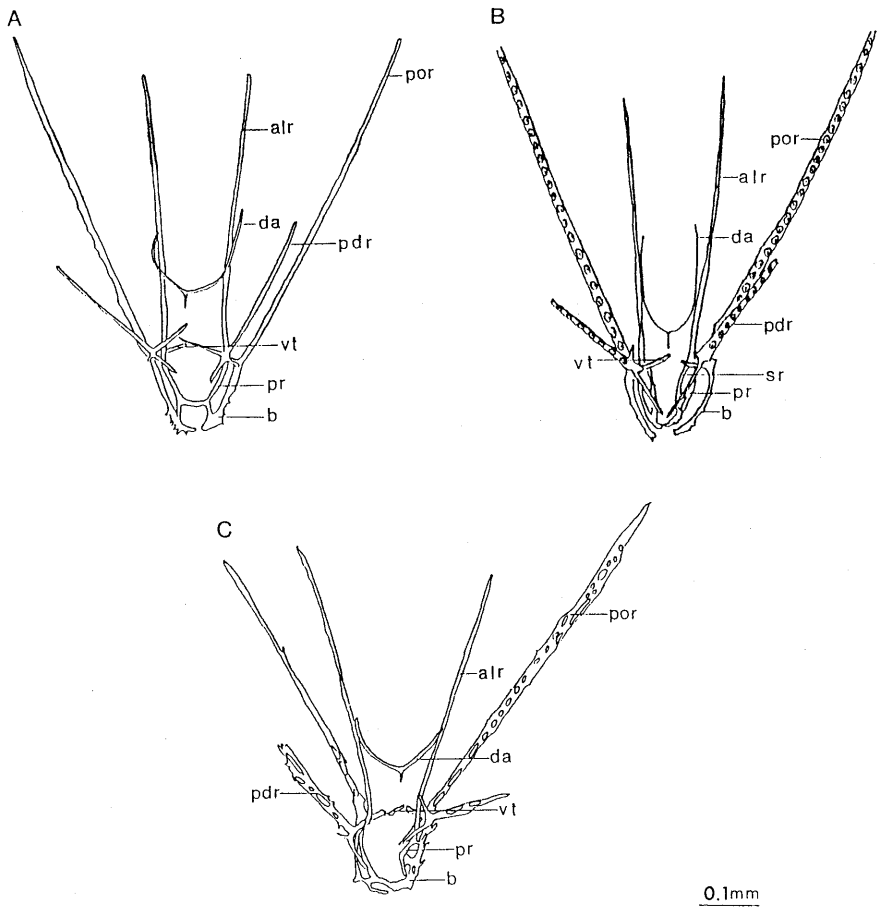

Fig. 3. The skeleton of six-armed larva. Notice the postero dorsal rod in the hybrid larva and those in pure larvae. A: *Psudocentrotus depressus*; B: *Anthocidaris crassispina* (redrawn from Onoda[8]); C: Hybrid larva in *P. depressus* (A)×*A. crassispina* (A). (Scale bar equals 0.1 mm).

alr: antero-lateral rod; b: body rod; da: dorsal arch; pdr: postero dorsal rod; por: postoral rod; pr: primary recurrent rod; sr: secondary recurrent rod; vt: ventral transverse rod.

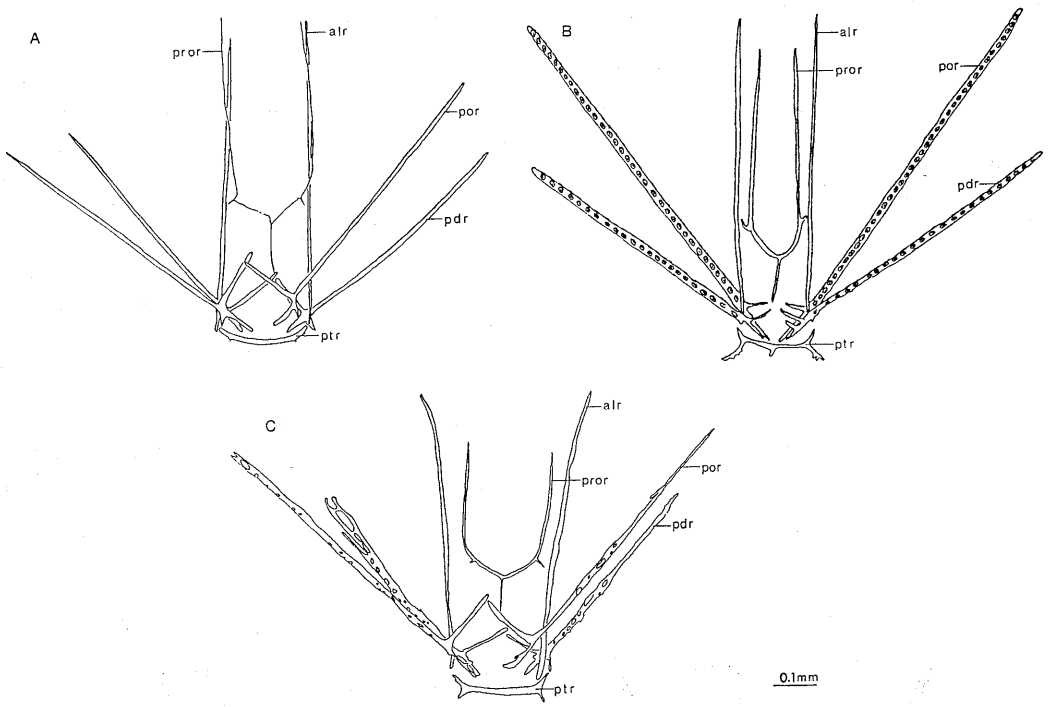

Fig. 4. The skeleton of eight-armed larva. Notice the posterior transverse rod (ptr) in hybrid larva and those in pure larvae. A: *Pseudocentrotus depressus*; B: *Anthocidaris crassispina* (redrawn from Onoda[8]); C: Hybrid larva in *P. depressus* (♀)×*A. crassispina* (♂). (Scale bar equals 0.1 mm).

alr: antero-lateral rod; b: body rod; da: dorsal arch; pdr: postero dorsal rod; por: postoral rod; pr: primary recurrent rod; pror: preoral rod; ptr: posterior transverse rod; sr: secondary recurrent rod; vt: ventral transverse rod.

stage wide variation in morphological characters was observed, which in later stages developed into forms with more or less similar morphology. Most of them exhibited intermediate characters between female *P. depressus* and male *A. crassispina*. Distinct paternal influence such as shape pigmentation, length and slenderness of the arm was seen in considerable larval forms.

The typical skeletons of hybrid pluteus at four, six and eight armed stage are illustrated and compared with those of *P. depressus* and *A. crassispina* pluteus in Figs. 2, 3, 4 and 5. At the four armed stage, pluteus of *P. depressus* has a simple basket structure with simple primary recurrent rod (pr) and its postoral rod (por) is a single calcareous rod (Fig. 2, A). On the other hand, four armed stage pluteus of *A. crassispina* has a compound basket structure with double recurrent rod, in which secondary recurrent rod (sr) are observed besides the primary recurrent rod (pr), and a lattice work structure which consists of parallel calcareous rods bound together by numerous cross bars (Fig. 2, B). In the four armed stage of hybrid

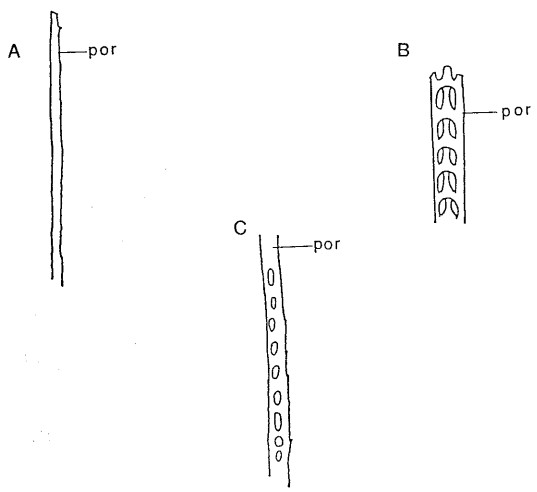

Fig. 5. Model postoral rod (por) structure of sea urchin larva. A: *Pseudocentrotus depressus*; B: *Anthocidaris crassispina*; C: Hybrid larva in *P. depressus* (♀)×*A. crassispina* (♂).

Table 1. Distinctive features of the plutei of the hybrid species compared with that of parental species.

species Distinctive feature	*Pseudocentrotus depressus*	*P. depressus* (♀) *A. crassispina* (♂)	*Anthocidaris crassispina*
Body skeleton	simple basket structure	incomplete compound basket structure	compound basket structure
Postoral rod	simple (single rod)	fenestrate (double rod)	fenestrate (triple rod)
Postero dorsal rod	simple (single rod)	fenestrate (double rod)	fenestrate (triple rod)
Recurrent rod	simple	incomplete double	double
Posterior transverse rod	not bifurcate	slightly bifurcate	bifurcate

variety incomplete secondary recurrent rod (sr) could be observed, which never touch the primary recurrent rod. A lattice work structure could be observed in the postoral rod of the hybrid, which is to differ to some extent from *A. crassispina*. The postoral rod (por) of the *P. depressus* is smooth and consists of a single rod (Fig. 5, A). *A. crassispina* possess a postoral rod consisting of three single rods which are joined together at short distance forming a fenestrate postoral rod (por) (Fig. 5, B). In the hybrid pluteus, the post oral rod consisted of two single rods (Fig. 5, C). In six armed stage, fenestrate postero dorsal rod were observed in the hybrid pluteus, whereas *P. depressus* has simple structural postero dorsal rod and *A. crassispina* has fenestrate postero dorsal rod (Fig. 3). Fig. 4 shows the skeletons of *P. depressus*, *A. crassispina* and hybrid pluteus in the eight arm stage before the appearance of pedicellaria. The posterior transverse rod (ptr) is not bifurcated in *P. depressus* where as in the case *A. crassispina* it is bifurcated and subdivided branches were pressent on the lower branch. The obtained hybrid has a bifurcated posterior transverse rod without any subdivisions on the lower branch.

Morphological characters of the hybrid pluteus and those of parental species are summarized in Table 1.

Discussion

Previous work on the hybridization between the female *P. depressus* and male *A. crassispina*, reported stronger maternal influence. However, they did not explain the morphology of the hybrid larva[3]. Even in hybridization of sea urchin with other parental combinations, than that of the present one, dominance of maternal influence has been emphasized. Matsui[1] observed elimination of chromosomes during the second cleavage of the hybrid embryo between male *Echinarachnius parama* and female *Arbacia punctulata* and hybrid larvae possessed dominant maternal character. From this, he supposed that the eliminated chromosomes had originated from paternal chromosomes. Shearer *et al.*[4] also pointed out the possibility that paternal chromosomes did not function in the hybrid of sea urchin which represent dominantly the maternal characters.

In the preliminary karyotype observation of hybrid in the present experiment, a large acrocentric chromosome was observed.* Karyotype of *A. crassispina* is not available yet. However, at this point it is interesting to note the observation of Yamanaka *et al.*[7]. According to them *P. depressus* did not possess large acrocentric chrosomes. These observations lead to a conclusion that large acrocentric chromosomes originate from the paternal species in this case. The morphological observations of larval skeleton in the present study showed intermediate characters between *P. depressus* and *A. crassispina*, this in turn supports the possibility of the above mentioned karyotype to be present in the hybrid. Existence of the large acrocentric chromosome in *A. crassispina* if be confirmed in a future study, the possibility of the gynogenesis in the hybrid will be denied.

Hybrid character is often influenced by the environmental condition in which insemination of the sperm and incubation of the embryo are carried out. The selection mechanism also depends upon the severity of competition among

* J. G. Wu: Study on Cryopreservation of Sea Urchin Sperm and it's Utilization in Hybridization. Master Thesis, University of Tokyo (1989).

the inseminated spermatozoa and the mortality during embryonic development. Vernon[5] observed the seasonal changes in the characters of the hybrids between *Sphaerechinus granularis* ova and *Strongylocentrotus lividus* sperm. He reported that the morphological characters of hybrid plutei showed larger resemblance to those in maternal species of *Sphaerechinus granularis* when crossbreeding were performed in summer, the season of the minimum maturity of the parental species (*St. lividus*) when compared to the those of the hybrids which were produced by the crossbreeding in spring season. He concluded that maturity of the genital gland of the parental species plays a major role in the determination of the characters in the hybrids. Crossbreeding experiment carried out earlier to this were generally performed in the maturation season of the maternal species. The experimental condition are also set to relate closely to that of maturity season of the female in nature. This could possibly have resulted in strong maternal dominance in the hybrid. In the present experiment, though the conditions were chosen with a view of having an ideal condition for the female, the sperm utilized for hybridization, were cryopreserved and also came from the parent stock which were collected during their maturity season. Insemination and larval rearing were carried out at 20°C in the maturation season of maternal species and sea water used as the rearing medium was collected from the sampling site of the maternal individuals during the same season. Hence, in the present work, hybridization was carried out with desirable sperm and ova, which could have brought out intermediate characters in the hybrid. Vernon[5] did not propose reliable mechanisms in which gonadal maturation levels influenced the characters of hybrid, and even in the present study the larval characters among the hybrids which were produced by the sperm preserved at different maturation level of the gonad is not compared. Hence these conclusions should be tested further. Another important aspect for further investigation would be the effect of cryopreservation on the quality of the sperm, as this can play a very vital role in the determination of hybrid characters. Summarizing the importance of this work it can be said that paternal and maternal characters in the hybrid larvae can be observed equally in the hybridization between *P. depressus* ova and cryopreserved sperm of *A. crassispina.*

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