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Embryonic Thermosensitivity of the Ascidian, *Ciona savignyi*

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ABSTRACT—Embryonic temperature sensitivity of the solitary ascidian, *Ciona savignyi*, was examined with special reference to acclimatization to seasonal change in seawater temperature. This ascidian spawns throughout the year; its life span is completed within six months and depends on the cumulative environmental temperature. The optimal temperature range for development from early cleavage stage to metamorphosis in embryos produced by individuals raised in warmer seasons differed significantly from that of individuals raised in colder seasons. Within the common optimal temperature range, developmental times at any given temperature were the same for both groups of embryos. The thermal acclimation of ascidian embryos is discussed and compared with that of echinoid embryos.

INTRODUCTION

Most marine poikilotherms have a specific breeding season and there have been several reports on the relationship between embryonic thermosensitivity of marine ectothermic animals such as echinoids and their breeding seasons (Fox, 1936; Mortensen, 1937; Pearse, 1970; Stephens, 1972). For echinoids, the close relationship between embryonic thermosensitivity and breeding season has been examined in detail, and it has been established that environmental temperature is one of the most important factors determining the echinoid breeding season (Fujisawa 1989; Fujisawa and Shigei, 1990). Most echinoids spawn during the season when seawater temperature is within the range of optimal temperature for their embryogenesis. At Misaki (Sagami Bay, Kanagawa Prefecture, Japan), several species of sea urchin spawn during seasons which differ species-specifically. The limited spawning seasons of these species are well adjusted, depending on their species-specific embryonic thermotolerance (Fujisawa, 1989).

Several species of solitary ascidians such as *Ciona intestinalis* and *C. savignyi*, however, spawn throughout the year around the sea coast at Misaki. Unlike echinoids, the life span of these poikilothermic animals is less than six months and its duration depends on the cumulative ambient temperatures experienced during the animal's lifetime (Nomaguchi, 1974). Two possibilities may be envisaged concerning the embryonic temperature sensitivity of the ascidians. One is that the range of optimal temperature for the ascidian embryos is wide enough to enable them to develop normally at any season of the year. Considering their short life span compared with that of echinoids, it is also possible that the range of optimal temperature for the embryos shifts according to the season in which the individuals are growing. We examined the embryonic temperature sensitivity of *C. savignyi* living on the seashore around Misaki, and found that the range of embryonic thermotolerance of this species was adjusted to the annual change in seawater temperature.

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MATERIALS AND METHODS

Animals

The solitary ascidian, *Ciona savignyi*, was used. Specimens were collected in Sagami Bay (Kanagawa Prefecture, Japan) and bred according to the standard methods (Nomaguchi, 1974). Eggs were collected by Pasteur pipette from the oviduct of mature individuals and immediately washed once with seawater. Sperm was collected from the sperm duct of other adults. The eggs were fertilized in Petri dishes by mixing with diluted sperm suspension. The embryos were allowed to develop at 20°C. The tadpole-like larvae usually hatched and began to swim 16 hr after insemination. The larvae were kept in the dark in order to facilitate adherence to the substrata of the dishes. The dishes to which the larvae had adhered in order to metamorphose were tied with fishing line to a mesh basket in order to prevent them from being eaten by predators, and the basket was hung from a raft in the sea 1.5 m below the surface. The raft was moored at Aburatubo-Moroiso Inlet near the Misaki Marine Biological Station of the University of Tokyo. Individual ascidians matured sexually about two months after fertilization in summer and after about three months in winter.

Thermosensitivity of the embryo

The gametes used in this experiment were obtained from two groups of adults: those that had grown and matured from July to

Table 1. Optimal temperature range for development of *Ciona savignyi* embryos and seawater temperature during the periods when the gametes matured

Parents from which embryos were obtained	Range of seawater temperature during the period when the gametes matured (°C)	Range of optimal temperature (°C) for	
		early cleavage	metamorphosis
Summer group	26 – 22	14 – 27	15 – 25
Winter group	17 – 12	10 – 20	12 – 20

September (summer group) and those that had grown and matured from late October to the following December (winter group). The gametes from the summer group were obtained in September and those from the winter group in December and the following January. The mean seawater temperature was 24°C (26-22°C) in September and 14°C (17-12°C) from December to the following January. Gametes were collected with a Pasteur pipette from oviducts and sperm ducts of anaesthetized adults which had been illuminated overnight. The eggs were inseminated and cultured in a vessel at a given temperature. The temperature in the vessel was adjusted using temperature-controlled water baths (AQUA, Tokyo) with an accuracy of 0.3°C.

The optimal temperature for embryogenesis was defined as the temperature at which the embryos were able to develop to the stage of metamorphosis without any deformity. The upper and lower limits of the optimal temperature range were determined by culturing embryos at temperatures increasing and decreasing at intervals of 1°C.

The times of the first and the second cleavages were determined by counting samples fixed with 1% formalin in seawater at intervals after insemination. The times at which 50% of the fertilized eggs had divided into two and four cells were taken as the times of the first and second cleavages, respectively. The times of both hatching of the larvae and metamorphosis of the juveniles adhering to the substratum of the vessel were determined by observation at intervals of one hour.

Seawater temperature

Seawater temperature in the inlet was measured at a depth of 1 m below the surface twice every day at 10:00 a.m. and 4:00 p.m.

RESULTS

Optimal temperature range for early cleavages

The embryos obtained from mature individuals that had grown from summer to early fall (summer group) divided normally at temperatures within the range from 14 to 27°C. At 13°C, only about 60% of the eggs were able to divide, and at temperatures below 12°C all eggs remained undivided. At 28°C, division was abnormal and at temperatures higher than 29°C, all the eggs were unable to divide. In contrast, the embryos from individuals that had grown from late fall to winter (winter group) were able to divide normally at temperatures from 10 to 20°C. At temperatures below 9°C, the eggs were unable to divide. At 21°C, less than 50% of the eggs divided and cleavage was abnormal. The optimal temperature range for early cleavage thus differed significantly between the two groups of embryos. This shift in optimal temperature range for early development corresponded with the thermal environment in which the gametes matured in the parent gonads. Embryos from individuals grown from summer to early fall were adapted to higher temperatures, while those from individuals grown from late fall to winter were acclimatized to lower temperatures.

We also examined the optimal temperature range for further development to hatching and metamorphosis. Embryos from individuals grown from summer to early fall were able to hatch and metamorphose normally within the temperature range from 15°C to 25°C. At 26°C, all embryos hatched into swimming larvae but they were abnormal and were unable to metamorphose into juveniles. The optimal temperature range for the development of the winter group embryos, however, was from 12°C to 20°C. At 11°C, the embryos developed into normal larvae but were unable to metamorphose. The above results are summarized in Table 1.

Developmental times of early cleavages and hatching

The timing of the first and the second cleavages of both groups of embryos was measured. Figure 1 shows an example of the first and second cleavage rates (%) after insemination. Curves I and II indicate the changes in the rates of the first

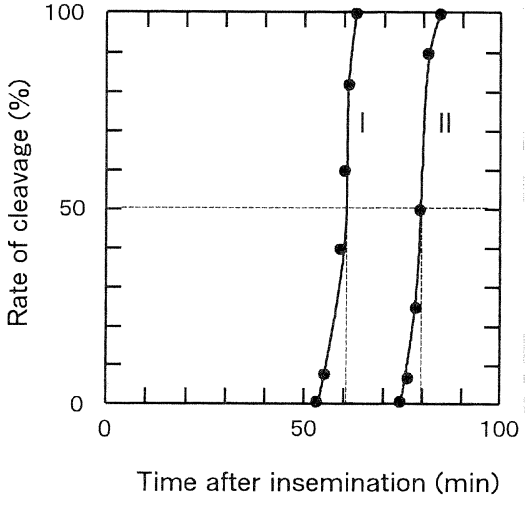

Fig. 1. Changes in the rate (%) of the first and second cleavages of embryos of the ascidian *Ciona savignyi* with time at 20°C. The embryos in this example were obtained from adults that had grown and matured in summer. Aliquots of embryos were fixed in 4% formalin in seawater at intervals of one or two minutes and counted the number of cleavages. Curve I indicates the change in the rate of the first cleavage and curve II that of the second cleavage. From these curves the times of the first and second cleavages, defined as the time for 50% of the embryos to cleave, were determined.

Table 2. Early cleavage times (min) of *C. savignyi* embryos

Embryos from the parents grown from late fall to winter						
	Temperature (°C)					
Cleavage	10	11	13	15	18	20
First	211 ± 3	180	124	88 ± 3	66	55 ± 2
Second	273 ± 4	237	173	126 ± 4	92	77
Embryos from the parents grown from summer to early fall						
	Temperature (°C)					
Cleavage	15	18	20	23	25	27
First	91	68	59 ± 1	51	45 ± 1	45
Second	121	90	79 ± 1	71	66	65

Numbers indicate the average ± SD for duplicate experiment.

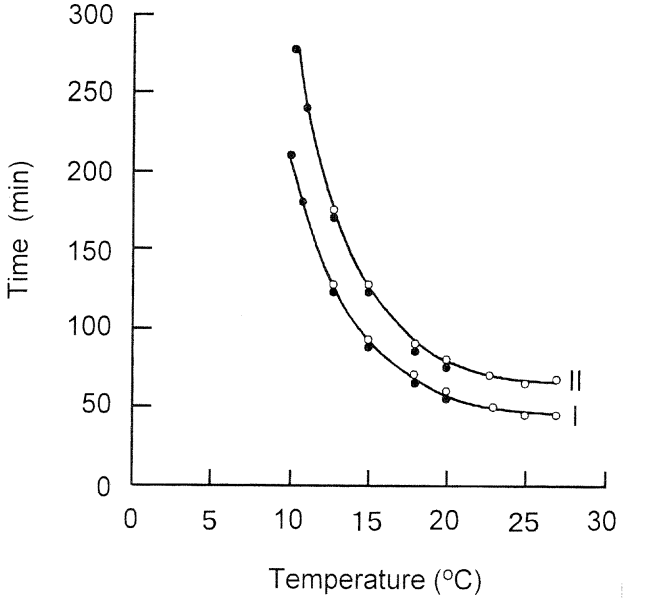

Fig. 2. Times of the first and second cleavages and temperature. Clear circles, embryos from adults that had matured in summer; solid circles, embryos from adults that had matured in winter. Curve I represents the times of the first cleavage and curve II those of the second cleavage.

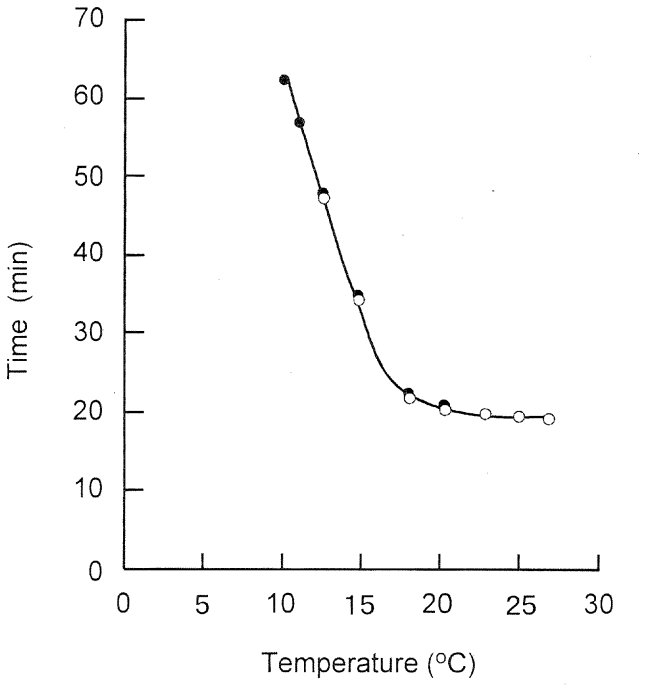

Fig. 3. Duration of the early cleavage cycle and temperature. Clear circles, embryos from adults that had matured in summer; solid circles, embryos from adults that had matured in winter. Duration of cleavage cycle based on the data in Table 2.

and second cleavages, respectively. From these curves, the times of the first and the second cleavages (50%) were estimated to be 60 and 80 min, respectively. The duration of the early cleavage cycle, 20 min, was calculated as the interval between these times. The times of the first and the second cleavages of embryos in both groups are shown in Table 2 and are plotted in Fig. 2. The relationship between temperature and duration of the early cleavage cycle for both groups of embryos is shown in Fig. 3. As clearly seen in Figs. 2 and 3, the times of the first and second cleavages and duration of the early cleavage cycle decreased as incubation temperature increased, and it can be seen that, within the common optimal temperature range from 12°C to 20°C, the times of these cleavages and the duration of the cycle of both groups of embryos were nearly the same, irrespective of the season in which the parents grew and matured. It can also been seen in Fig. 3 that the minimum duration of the early cleavage cycle (maximum speed) was attained at 20°C. At temperatures higher than this, the duration did not shorten further, although the times of the first and second cleavages shortened gradually at increasing temperatures.

Time of hatching was also measured in both groups of embryos (Table 3). As in the case of the first and the second cleavages, the times of hatching were constant within the

Table 3. Hatching times (hr) of *C. savignyi* embryos

Embryos from the parents grown from late fall to winter								
Temperature (°C)	10	11	12	13	14	16	18	20
Time	48	46	42	40	30	24	20	16
Embryos from the parents grown from summer to early fall								
Temperature (°C)	15	16	18	20	23	25	27	
Time	27	24	20	16	16	16	16	

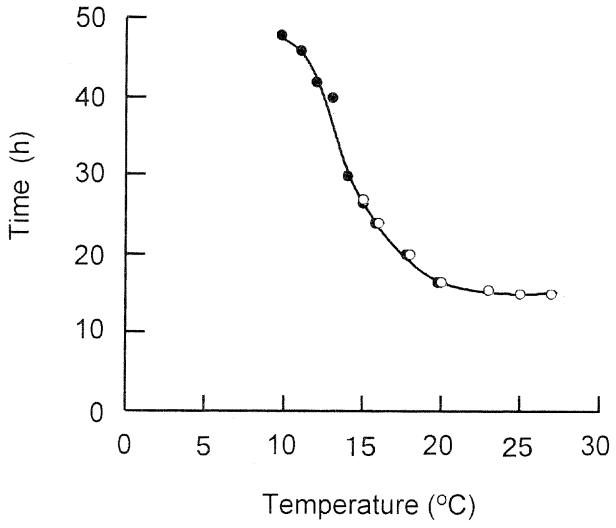

Fig. 4. Time of hatching and temperature. Clear circles, embryos from adults that had matured in summer; solid circles, embryos from adults that had matured in winter.

common optimal temperature range (from 12°C to 20°C) for both groups (Fig. 4). It was also noted that the minimum time of hatching was attained at 20°C, i.e., the time did not shorten further at temperatures higher than 20°C.

DISCUSSION

Various species of sea urchin adapt their spawning season to the species-specific embryonic thermotolerance (Fujisawa 1989; Fujisawa and Shigei, 1990). At Misaki, common species of echinoids spawn in different seasons; *Hemicentrotus pulcherrimus* spawns in winter and spring, while *Anthocidaris crassispina* and *Clypeaster japonicus* do so in summer, and *Pseudocentrotus depressus* in fall and early winter. The close correlation between the seawater temperature in the spawning seasons and the species-specific embryonic thermotolerance has been precisely confirmed. In general, embryonic thermotolerance of echinoids is species-specifically fixed, hence the breeding season has to be flexibly adjusted according to changes in seawater temperature. The seashore around Misaki is the northern limit of the distribution of the sea urchin, *Diadema setosum*, and the breeding time at this locality lasts for only about one week in late August. The seawater temperature is at its highest in early September. This species spawns throughout over half the year in tropical sea such as the coasts around the Ryukyus (Mortensen, 1937; Pearse, 1970). This sea urchin is a typical example of the close correlation between breeding season and species-specifically-fixed embryonic thermotolerance.

Contrary to this example, the embryonic thermotolerance of *H. pulcherrimus* has been found to be flexible to some extent (Fujisawa, 1995). The breeding season of this sea urchin is nearly the same, irrespective of wide latitudinal differences in its distribution, and hence irrespective of differences in seawater temperature during the season. A difference in the embryonic thermotolerance of both groups of embryos has been confirmed. It has not yet been examined whether the difference in embryonic thermotolerance of both groups is genetically fixed or whether it depends on flexible acclimatization to the thermal environment.

In contrast with these echinoids, the solitary ascidian, *Ciona savignyi*, spawns all the year round at Misaki. Moreover, the life span of the animal is two to six months depending on the season in which it grows and matures, hence adults that have matured in summer are different from individuals that have matured in winter. For this ascidian to be fertile throughout the year, the embryos must be sufficiently eurythermal to tolerate a broad range of annual seawater temperature change, or for each individual to be able to adjust the optimal temperature range of embryos to changes in seawater temperature during the season when the animals breed and grow. The present results suggest that mature adults were able to adjust the embryonic thermotolerance of there gametes, especially of eggs, to allow them to develop normally at the temperatures at which they grew.

Thermal tolerance of several marine poikilotherms, such as the anthozoan *Actinia equina* (Dregolskaya, 1962, 1967), the bivalve *Mytilus galloprovincjalis* (Ushakov, 1968) and the polychaete *Nereis diversicolor* (Ivleva, 1967), is in part dependent on their thermal history (Vernberg *et al.*, 1963; Schlieper, 1966, 1967; Kinne, 1970). Watts *et al.* (1982) have suggested that larval thermotolerance of the asteroid *Echinaster* may be related to past influences of the thermal environment on previous generations.

In both groups of ascidian embryos, the relationship between developmental time and temperature was nearly the same, i.e., at a temperature within the common optimal temperature range the time of development of a stage was

constant, irrespective of any difference in thermotolerance between them. Similar results was also found with *H. pulcherrimus* embryos. No difference was found between the developmental times of early cleavages, hatching and onset of gastrulation at any temperature within the common optimal temperature range in individuals of this species living at the northern and southern limits of its geographical range (Fujisawa, 1995).

In the present experiment, the temperature range in which the ascidian embryos hatched, metamorphosed and developed further into adults without any abnormal morphology was usually narrower than that in which the embryos cleaved normally. The optimal temperature for this species of ascidian was therefore defined as the temperature at which the embryos were able to develop into adults without any deformity.

From the present results with ascidian embryos, it seems probable that animals such as ascidians and echinoids have a regulatory mechanism to adapt their gametes to the environmental temperature during the period of gonadal growth. The gametes which are acclimatized are thought to be the eggs, because embryonic thermotolerance is determined by eggs in general (Fujisawa, 1993). We are now examining the flexibility of ascidian embryonic thermotolerance.

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