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Variation in Embryonic Temperature Sensitivity among Groups of the Sea Urchin, *Hemicentrotus pulcherrimus*, which Differ in their Habitats

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ABSTRACT—The sea urchin, *Hemicentrotus pulcherrimus*, spawns during winter and spring in Kagoshima, Aomori and Kanagawa. The seawater temperature during the spawning season ranges from about 16 to 19°C at Sendai, Kagoshima, from about 12 to 15°C at Misaki, Kanagawa, and from about 5 to 7°C in Mutsu Bay, Aomori. Among the groups of this species differing in their habitats, optimal temperature ranges for development were found to differ significantly and the difference corresponded to the difference in seawater temperature during the spawning season. Despite the difference in the optimal temperature range for development among the groups, the relationship between developmental speed and temperature within the common optimal temperature range for these groups was nearly constant.

The thermotolerance of embryos produced by cross-fertilization between the group from Kagoshima and that from Aomori was also examined. These embryos showed maternal inheritance of embryonic thermotolerance.

INTRODUCTION

The sea urchin, *Hemicentrotus pulcherrimus*, is one of the most widely distributed indigenous echinoids in Japan. The northern limit of its distribution is around the southern coast of Hokkaido, while the southern limit is Kagoshima Prefecture except for the southernmost area including the Satsuma and the Osumi Peninsula.

This species usually spawns during winter and early spring; from January to early April at Sendai, Kagoshima, as well as at Misaki [19], and from January to early May in Mutsu Bay, Aomori. Although the spawning seasons are nearly the same in both areas, the seawater temperatures during the seasons are significantly different; from 16 to 19°C in Sendai, compared with 5 to 7°C in Mutsu Bay. The difference in seawater temperature between the areas during the spawning season is as high as 12°C.

In general, distribution and spawning season depend on species-specific embryonic thermotolerance [10]. A typical example is the thermotolerance of the sea urchin *Diadema setosum*. This sea urchin spawns from May to November in the Ryukyus, throughout the year near the equator [12, 22, 23, 26], while only for one or two weeks in late August at the northern limits of its distribution such as Sagami Bay and Suruga Bay [29]. The distribution and the span of the spawning season have been reported to depend on the seawater temperature being above about 25°C for at least one month in a year [20, 21]. In contrast, *H. pulcherrimus* spawns during winter and early spring irrespective of the latitudinal difference of its habitats. Therefore it seems probable that embryonic thermotolerance of this species may be different among individuals differing in their habitats.

The present study examined the difference in embryonic thermosensitivity among individuals of *H. pulcherrimus* inhabit at Sendai, Misaki and in Mutsu Bay.

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MATERIALS AND METHODS

Specimens of *H. pulcherrimus* from Mutsu Bay were supplied by Asamushi Marine Laboratory, Tohoku University. Specimens from Misaki, Kanagawa, were obtained from Misaki Marine Laboratory, University of Tokyo, and those from Sendai, Kagoshima, were gifts from Prof. Junzo Tsukahara, Kagoshima University. The sea urchins from Mutsu Bay were kept at 8 to 10°C, those from Misaki at 12°C, and those from Sendai at 20°C in a laboratory at Saitama University.

For comparison of embryonic temperature dependence of the species, other three other species of sea urchin, *Echinometra mathaei* (A type), *Anthocidaris crassispina*, and *Strongylocentrotus droebachiensis* were used. *E. mathaei* was collected in Okinawa and used in August. *A. crassispina* was supplied by Misaki Marine Laboratory and used in July. These two species were kept at room temperature in a laboratory at Saitama University. *S. droebachiensis* was collected in Puget Sound, Washington, USA, and used at Friday Harbor Laboratories, University of Washington.

Seawater temperatures were taken from data recorded at the Kagoshima Prefectural Fisheries Experimental Station, the Misaki Marine Biological Station, University of Tokyo, in Sagami Bay, and the Aomori Agriculture Center, Mutsu Bay.

Eggs and sperm obtained by injecting acetylcholine (0.1 M in seawater) into the body cavity were washed with artificial seawater (Jamalin U) and divided into groups. Each group was suspended in seawater in a 50-ml vessel, preincubated at a given temperature for 1 min., and then inseminated. The seawater temperature in each vessel was adjusted using temperature-controlled water baths (Aqua, Tokyo) with an accuracy of 0.3°C.

The optimal temperature for sea urchin embryogenesis was defined as the temperature at which the embryos were able to develop into gastrulae without any deformities [9, 10]. At temperatures which the embryos were able to develop into normal gastrulae, further developmental stages showed any morphological abnormalities. The upper and lower limits of the optimal temperature range

for the embryos were determined by culturing embryos at temperatures increasing and decreasing at intervals of 1°C.

At each temperature, the times after insemination for 50% of embryos to develop into the 2-cell, 4-cell, 8-cell and 16-cell stages were measured at counting intervals of 5 min.

Relative developmental speed was calculated from the formula defined as the ratio $(1/T_t)/(1/T_m)$, where T_t is the time required to attain a stage at a temperature within the optimum temperature range and T_m is the time required to attain the stage at the upper limit of the range [9].

RESULTS

Seawater temperatures during the spawning season of Hemicentrotus pulcherrimus

The spawning season of *H. pulcherrimus* in the three areas examined was from winter to spring. The seawater temperatures during the spawning season, however, were significantly different between Mutsu Bay and Sendai. As seen in Figure 1, the temperature during the spawning season in Mutsu Bay ranged from 5 to 7°C, while at Sendai it ranged from 16 to 19°C. The difference in seawater temperature between the two areas was as high as 12°C on average. This difference is significantly high in comparison with the range of seawater temperature during the spawning season in Mutsu Bay or at Sendai.

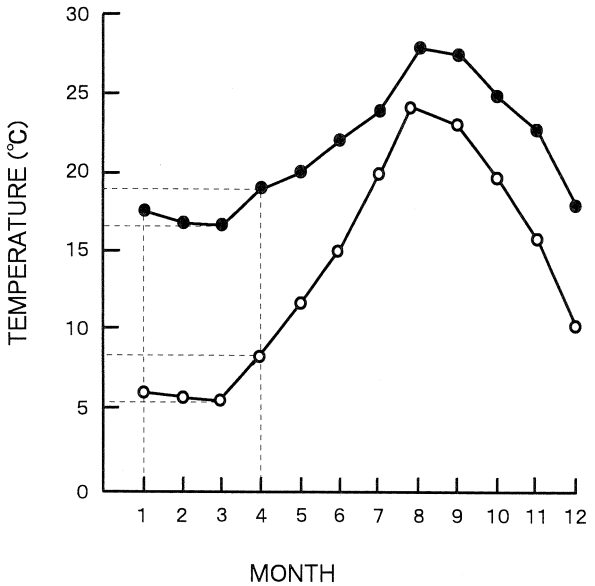

FIG. 1. Annual changes in seawater temperature at Asamushi, Mutsu Bay, Aomori, and at Sendai, Kagoshima. Dotted lines indicate the temperatures during the spawning season. Clear circles, Asamushi; solid circles, Sendai.

Embryonic temperature tolerance of H. pulcherrimus collected from Mutsu Bay and Sendai

The optimal temperature ranges for the embryogenesis of *H. pulcherrimus* collected from Mutsu Bay (Aomori group), Misaki (Kanagawa group) and Sendai (Kagoshima group) were examined by culturing the embryos from fertilized eggs to gastrulae at temperatures increasing and decreasing at intervals of 1°C in temperature-controlled water baths. Table 1 shows the temperature ranges optimal for development and the seawater temperature during the spawning season. It is clear that in each group, the ranges of seawater temperature during the spawning season were within the optimal temperature ranges. It is also evident that the optimal temperature range for the Aomori group was lower than that for the Kagoshima group. The differences of both the upper and lower limits of the optimal temperature range between the two groups were as high as 3°C.

TABLE 1. Temperature ranges optimal for development of *Hemicentrotus pulcherrimus* embryos and seawater temperature during the spawning season

Group	Temperature (°C)	
	Optimal range for development	Seawater temperature during spawning season
Kagoshima	8–24	16–19
Aomori	5–21	5– 7
Kanagawa	5–23	12–15

Developmental times and relative developmental speed of early cleavage stages

The timing of the 2-cell, 4-cell, 8-cell and 16-cell stages of embryos in the three groups was measured. Intervals between the 2-cell and 4-cell, 4-cell and 8-cell, and 8-cell and 16-cell stages were nearly the same at each temperature as shown in Table 2. Average cleavage times shown in Table 2 are the means of these three intervals at each temperature. Slight differences in developmental time were found among these groups, being especially conspicuous at lower temperatures within the common optimal temperature range. The embryos in the Aomori group developed considerably faster than those in the other two groups below 12°C, while the embryos developed only slightly more slowly above 20°C. Differences in the developmental time of early cleavages were also conspicuous at lower temperatures within the common optimal temperature range. However, these differences were relatively small in comparison with the developmental times of other species. Average early cleavage times for the Aomori and Kagoshima groups are plotted against temperature in Figure 2, together with plots of the cleavage times for other species, *Echinometra mathaei, Anthocidaris crassispina* [10] and *Strongylocentrotus droebachiensis* (unpublished data), for comparison. These results suggest that the temperature dependence of early cleavage time is fixed and species-specific.

Relative developmental speed in Table 3 was calculated based on the data in Table 2. T_m was taken as the time required to attain the stage at 24 °C, the upper limit of the

TABLE 2. Developmental times (min) of *H. pulcherrimus* embryos in the Aomori, Kagoshima and Kanagawa groups

Aomori group						
	Temperature (°C)					
Stage	5	10	12	15	20	21
2-cell	313± 3	150± 0	118± 3	93± 3	68± 3	63±3
4-cell	475± 5	238± 3	185± 0	145± 3	103± 3	98±3
8-cell	648± 3	318± 3	250± 0	193± 3	138± 3	133±3
16-cell	830± 5	395± 3	318± 3	243± 3	178± 3	168±3
Average cleavage time	172±14	82± 8	67± 2	50± 3	37± 4	35±0
Kagoshima group						
	Temperature (°C)					
Stage	8	10	12	15	20	24
2-cell	200± 5	158± 3	128± 3	93± 3	65± 0	55±0
4-cell	323± 3	250± 5	203± 3	145± 0	100± 0	85±0
8-cell	443± 3	333± 3	268± 3	193± 3	133± 3	118±3
16-cell	545± 5	425± 5	328± 3	248± 3	173± 3	143±3
Average cleavage time	115±15	89± 7	67±11	52± 5	36± 5	29±6
Kanagawa group						
	Temperature (°C)					
Stage	5	10	12	15	20	23
2-cell	343± 8	163± 8	128± 3	90± 0	65± 0	60±0
4-cell	513±13	245±15	188± 8	138± 3	97± 3	90±0
8-cell	703±13	325±15	260±15	193±12	135± 5	123±3
16-cell	880±40	413±25	330±15	240±10	170±10	155±5
Average cleavage time	182±15	83± 6	67± 9	50± 6	35± 4	32±2

Numbers indicate the average ± SD for two separate experiment

optimal temperature range for Kagoshima group. As is clearly seen in Table 3, relative developmental speed was stage-nonspecific, being dependent only on temperature. Averages of relative developmental speed in Table 3 are plotted in Figure 2. It was found that relative developmental speed against temperature for three groups could be plotted approximately on a quasi-linear curve.

Embryonic temperature sensitivities of embryos produced by cross-fertilization between the Kagoshima and Aomori groups

Cross-fertilized *H. pulcherrimus* embryos were made using eggs and sperm from the Aomori and Kagoshima groups. The optimal temperature range for Kagoshima (♀) ×Aomori(♂) embryos, i.e., eggs from the Kagoshima group fertilized with sperm from the Aomori group, was 8–24°C, the same as that of Kagoshima×Kagoshima embryos. Also the optimal temperature range for Aomori (♀)×Kagoshima (♂) embryos was 5–21°C, the same as that of Aomori× Aomori embryos. These results confirm that the thermotolerance of embryos is determined only by the eggs, and not

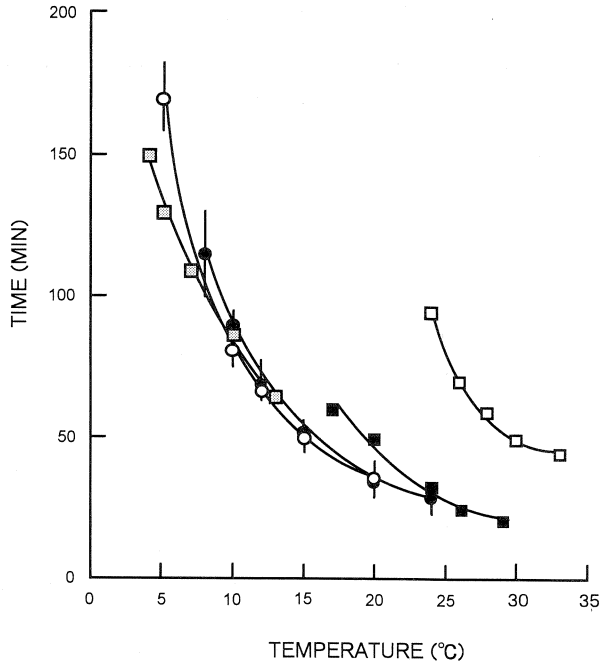

FIG. 2. Average early cleavage times of sea urchin embryos. Clear circles, *H. pulcherrimus*, Aomori group; solid circles, *H. pulcherrimus*, Kagoshima group; clear squares, *Echinometra mathaei*; solid squares, *Anthocidaris crassispina*; dotted squares, *Strongylocentrotus droebachiensis*.

by sperm, i.e., the thermotolerance is maternally inherited.

Developmental time of these embryos was also examined (Table 4). The average early cleavage times of 2-cell, 4-cell, 8-cell and 16-cell embryos of Kagoshima (♀)×Aomori (♂) were plotted on a curve representing the relationship between temperature and the average early cleavage time of Kagoshima×Kagoshima embryos (Fig. 4A). Also the average early cleavage time of Aomori (♀)×Kagoshima (♂) was plotted on a curve representing the relationship between temperature and the average early cleavage time of Aomori×Aomori embryos (Fig. 4B). Both results indicated that the temperature dependence of early cleavage time is also maternally inherited.

DISCUSSION

The spawning season of the sea urchin *H. pulcherrimus* is nearly constant, irrespective of the wide range of its habitats. The difference in seawater temperature between the northernmost and southernmost areas of its distribution during the spawning season is as high as 12°C, far higher than the range of local fluctuation of seawater temperature during the season. For embryos of this sea urchin living at Misaki, the range of optimal temperature was 5 to 21°C. The average seawater temperature during the spawning season in Sendai is near the upper limit of this range, while that during the season in Mutsu Bay is near the lower limit. In general, sea urchins spawn when the seawater temperature is completely within the optimal temperature range. *Diadema setosum*,

TABLE 3. Relative developmental speed of *H. pulcherrimus* embryos in the Aomori, Kagoshima and Kanagawa groups

Aomori	Temperature (°C)					
Stage	5	10	12	15	20	21
2-cell	0.18±0.00	0.37±0.00	0.47±0.01	0.59±0.02	0.81±0.04	0.87±0.04
4-cell	0.18±0.00	0.36±0.00	0.46±0.00	0.59±0.01	0.83±0.02	0.87±0.03
8-cell	0.18±0.01	0.37±0.01	0.47±0.01	0.61±0.02	0.85±0.03	0.89±0.03
16-cell	0.17±0.01	0.36±0.01	0.45±0.01	0.59±0.01	0.80±0.02	0.85±0.02
Average	0.18±0.01	0.37±0.01	0.46±0.02	0.60±0.03	0.82±0.06	0.87±0.06
Kagoshima	**Temperature (°C)**					
Stage	8	10	12	15	20	24
2-cell	0.28±0.01	0.35±0.01	0.43±0.01	0.59±0.02	0.85±0.00	1
4-cell	0.26±0.01	0.34±0.01	0.42±0.01	0.59±0.01	0.85±0.00	1
8-cell	0.27±0.01	0.35±0.01	0.44±0.01	0.61±0.02	0.88±0.03	1
16-cell	0.26±0.01	0.34±0.01	0.44±0.01	0.58±0.01	0.83±0.02	1
Average	0.27±0.02	0.35±0.02	0.43±0.02	0.59±0.03	0.85±0.04	1
Kanagawa	**Temperature (°C)**					
Stage	5	10	12	15	20	23
2-cell	0.16±0.01	0.34±0.02	0.43±0.01	0.61±0.00	0.85±0.00	0.92±0.00
4-cell	0.17±0.01	0.35±0.02	0.45±0.02	0.62±0.01	0.88±0.03	0.94±0.00
8-cell	0.17±0.01	0.36±0.02	0.45±0.03	0.61±0.04	0.87±0.04	0.96±0.03
16-cell	0.17±0.01	0.35±0.02	0.43±0.02	0.60±0.03	0.84±0.05	0.92±0.03
Average	0.17±0.02	0.35±0.04	0.44±0.04	0.61±0.05	0.86±0.07	0.94±0.04

Numbers were based on the data in Table 2 according to the formula $(1/T_t)/(1/T_m)$, where T_t is the time required to attain a stage at a temperature within the optimal temperature range and T_m is the time required to attain the stage at 24℃, the uppermost optimal temperature for embryos of the Kagoshima group. Numbers indicate the average± SD

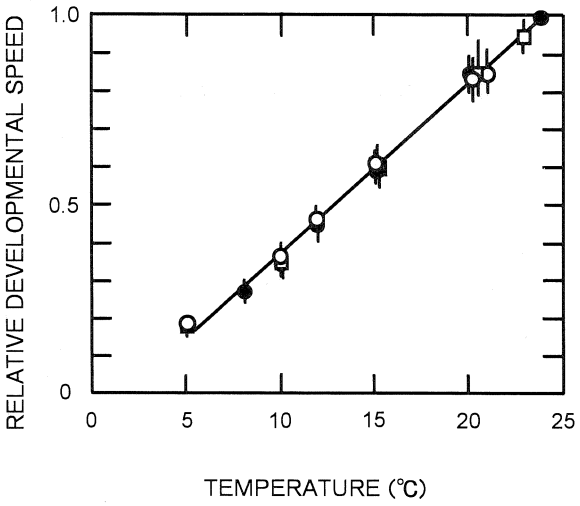

FIG. 3. Relationship between temperature and relative developmental speed of *H. pulcherrimus* embryos with different habitats. Clear circles, Aomori; solid circles, Kagoshima; clear squares, Kanagawa. Data based on Table 3. Vertical bars indicate SD.

TABLE 4. Developmental times (min) of *H. pulcherrimus* embryos produced by cross-fertilization between the Aomori and Kagoshima groups

Aomori (♀)×Kagoshima (♂)	Temperature (°C)				
Stage	5	10	12	15	19
2-cell	310	150	125	95	75
4-cell	470	235	190	145	115
8-cell	650	320	255	195	155
16-cell	830	410	320	250	195
Average cleavage time	173±16	87±4	65±0	52±4	40±0
Kagoshima (♀)×Aomori (♂)	**Temperature (°C)**				
Stage	9	11	14	20	
2-cell	175	140	115	65	
4-cell	275	220	170	105	
8-cell	380	300	230	140	
16-cell	480	380	285	175	
Average cleavage time	102±4	80±0	57±4	37±4	

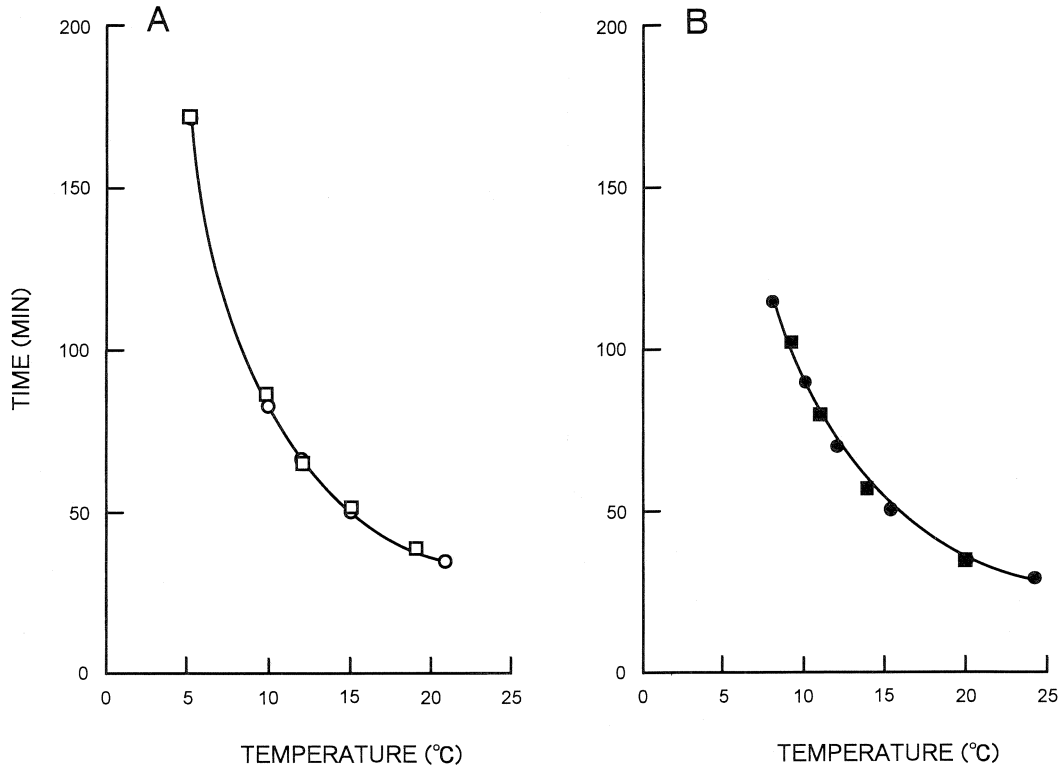

FIG. 4. Temperature dependence of average cleavage time of *H. pulcherrimus* embryos. Data are based on Table 4. (A) Clear squares, eggs from the Aomori group cross-fertilized with sperm from the Kagoshima group; clear circles, embryos from the Kagoshima group. (B) Solid squares, eggs from the Kagoshima group cross-fertilized with sperm from the Aomori group; solid circles, embryos from the Kagoshima group.

for example, spawns from late spring to autumn in the Ryukyus (T. Uehara, personal communication) and throughout the year in the tropics [12, 22, 23, 26], while at the northern limits of its distribution on the Pacific side of Japan, at sites such as Suruga Bay and Sagami Bay, the spawning season is restricted to one or two weeks in late August, when the seawater temperature is highest [10, 29]. It has been suggested that this sea urchin is distributed where seawater temperatures are above 25°C for at least one month in the year [20, 21, 23]. *H. pulcherrimus* is different from this species because its spawning season is nearly the same despite its different latitudinal distribution. The optimal temperature ranges for embryogenesis were found to differ significantly between the Kagoshima and Aomori groups. The difference was found to correspondent well to the difference in local seawater temperature during the spawning season. This phenomenon leads to the problem of whether the embryonic thermotolerance of this species is genetically fixed or whether this species is able to acclimatize its eggs to a given thermal environment. I am now examining whether this species is able to adapt its eggs to changes in external temperature.

The relationship between cleavage interval and temperature was also examined in this study. Within the common optimal temperature range for both the Aomori and Kagoshima groups, it was possible to plot the cleavage interval against temperature approximately on one curve. This suggests that the relationship between developmental time schedule and temperature for *H. pulcherrimus* embryos is not affected as much by differences in the thermal environment as by the optimal temperature range. It is noteworthy, however, that especially at lower temperatures within the optimal temperature range, the cleavage periods as well as the times of each stage were shifted; embryos of the Aomori group developed slightly faster than those of the Kagoshima group at lower temperatures. It is uncertain why the embryos from individuals acclimatized to cold seawater were able to develop slightly faster at lower temperatures.

An example contradictory to this case has been reported for *Paracentrotus lividus* [13, 14]. The reproductive season and embryonic thermosensitivity of this species have been reported to differ; individuals living in the Mediterranean have been considered to spawn several times throughout the year, although individuals living at Roscoff, in Brittany, spawn only once a year during the summer. Embryos produced from winter and summer eggs from individuals collected at Naples have been reported to show marked differences in thermosensitivity. It will be necessary to confirm whether each individual living in the Mediterranean is able to spawn several times throughout the year, or once at a different season in a year.

In this work, I also examined developmental time and

relative developmental speed. As is clear in Table 3, relative developmental speeds were stage-nonspecific, i.e., dependent only on temperature. I have already reported that this stage-nonspecificity of developmental times also holds for other stages, such as the times of onset of mesenchyme ingression and gastrulation [9]. The relationship between relative developmental speed and temperature is depicted in Figure 3. The curve shows that the relationship is quasi-linear. With regard to the dependence of developmental speed on temperature, several early embryology studies investigated whether the Arrhenius law could be applied, but it has become evident that the law can scarcely holds for this relationship, and that instead the relationship can be represented as a sigmoidal curve, the major proportion of which is linear [1, 2, 7, 16, 18, 24, 25, 27, 28]. Several equations have been proposed to describe the effect of temperature on developmental speed [3, 8, 15, 17]. However, the precision adopted for theoretical considerations is not always matched in experimental procedures, and these approaches have been unsuccessful in revealing the underlying mechanisms of the thermal effect on developmental speed [5].

The quasi-linear relationship between relative developmental speed and temperature suggests the existence of a structure which may be sensitive to temperature and suppress any exponential increase of biochemical reaction rates to quasi-linear thermal dependence. Such a structure may also determine species-specific optimal temperature ranges, as well as enabling embryos of individuals adapted to cold seawater to develop faster than those acclimatized to warm seawater at lower temperatures within an optimal temperature range. The cell membrane is one candidate for such a structure because of its delicate thermosensitivity [4–6]. I am now examining the possibility that membrane physicochemical properties may be relevant to embryonic temperature sensitivity.

The present work has also confirmed that embryonic thermotolerance is inherited maternally. Maternal inheritance of embryonic thermotolerance has been confirmed in an experiment using hybrids produced between *Dendraster excentricus* × *Strongylocentrotus purpuratus*, which differ in embryonic thermotolerance [11]. In comparison with these interspecific hybrids of different subclasses, the present material is thought to be more suitable for proving the maternal inheritance of embryonic thermotolerance.

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