Genetic Divergence of Two Local Japanese Populations of the Echinothurioid Echinoid, *Asthenosoma ijimai*

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Introduction

The modes of embryonic development of echinoids are classified into two major categories, typical indirect and modified direct developers [22, 26]. The majority of species develop in a typical manner with a feeding larval stage, which is considered a primitive type of larval development [7]. On the other hand, modified modes of development without a feeding larval stage are considered as derived types of development [25]. These two developmental modes have been found in all living orders of echinoids except for the orders Echinothurioida and Diadematoida.

The order Echinothurioida is considered to be the most ancestral sea-urchins in the Euechinoids from the point of morphological and palaeontological observations [23, 24] Fig. 1. However, the larval development of all species belonging to Echiothurioida examined to date is thought to be by modified mode because of egg size [7. 19] and sperm morphology [2]. In addition, the normal development of an echinothurioid, *Asthenosoma ijimai*, was found to be by modified mode [1, 3]. A previous study of another echinothurioid, *Asthenosoma* sp., also found modified development [4]. It is curious that no species of the echinothurioid echinoids develop in a typical ancestral manner, even though the Echiothurioida are considered to be a ancestral order. To solve this paradox, we need to understand not only the larval development but also the phylogeny of Echinothurioida.

Recent molecular phylogenetic studies by allozyme analysis in echinoderms have helped clarify the phylogenetic relationships between species [9, 10, 11, 12, 14, 15, 17] and between local populations [8, 16, 21]. As a first step of the molecular phylogenetic study of the order Echinothurioida, we examined the genetic variation within and genetic differentiation between local populations of *Asthenosoma ijimai* Fig. 1. The echinothurioid echinoid, *A. ijimai* is distributed along the south coast of Japan from Sagami Bay to Kagoshima Bay and the west coast of Kyushu, and is a common Japanese species of this order. In this study, we report the genetic difference between two local populations, each located near the edge of the distribution area, and separated by about 900km, and allozyme variation within poppulations.
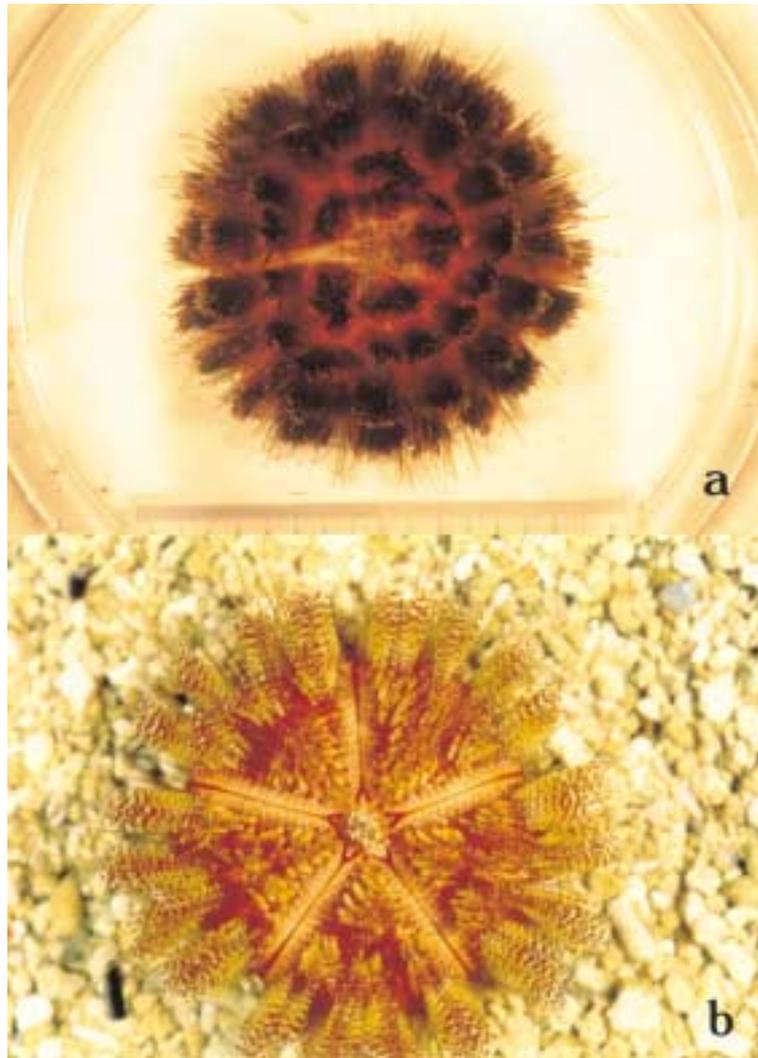
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Fig. 1. Sea-urchin species of the family Echinothuriidae.   a : *Asthenosoma ijimai* from Japan, b : *Asthenosoma* sp. from Okinawa Island.

Materials and Methods

Echinothurioid echinoid, Asthenosoma ijimai

Adult echinoid *Asthenosoma ijimai* were collected in two localities Fig. 2 .   Ten adult echinoids were collected in the vicinity of Misaki Marine Biological Station facing Sagami Bay, and 11 individuals were collected from Kagoshima Bay.   Each individual was dissected, and its gonads and intestine were isolated.   The isolated organs were washed several times with filtered sea water and were frozen at   80   until use.

Preparation of samples and gel electrophoresis

The procedures for preparation of samples, electrophoresis and staining gels were similar to previous studies 15 .   Briefly, about 0.2g of each tissue sample and 0.6ml of 50mM phosphate buffer pH 7.0 containing 0.1M KCl and 10mM EDTA were individually mixed and homogenized, using a polyethylene Potter-Elvehjem homogenizer in an ice-water bath.   The homogenized samples were centrifuged at 6,100× *g* for 10 min, yellowish lipid that covered the surface of the centrifuged samples was removed, and the clear supernatant was resolved by 7.5% polyacrylamide gel electrophoresis.   After electrophoresis, gels were stained for different enzymes as reported previously 13 .
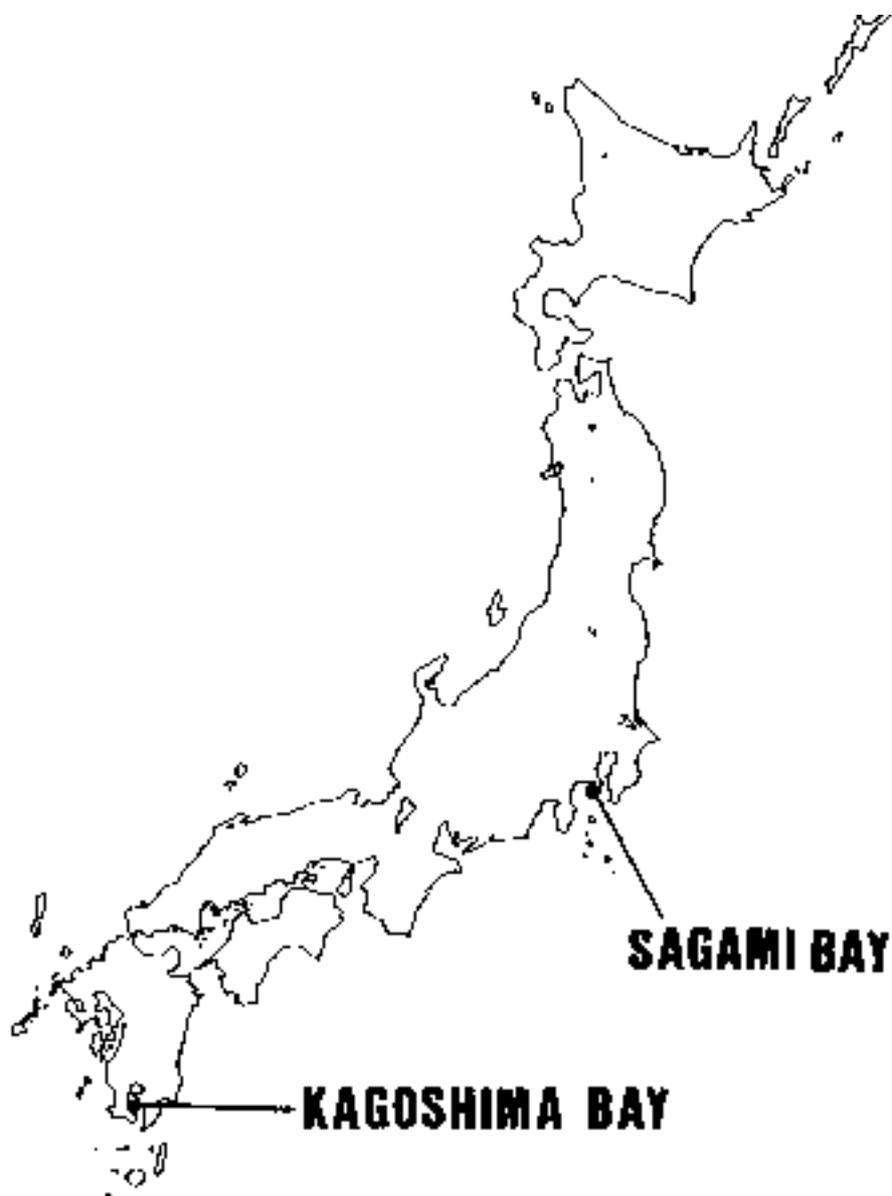
Fig. 2. Map showing the two local populations of the sea-urchin, *A. ijimai* of the family Echinothuriidae from Japan.

Results and Discussion

ALLOZYME PATTERNS

In the first step of a molecular phylogenetic study of echinothurioids, enzymes were resolved by gel electrophoresis.   From a total of 31 enzymes reported previously 13 , we detected 10 different enzymes ; alkaline phosphatase ALK , esterase EST , glucose 6 phosphate dehydrogenase G6PD , leucine amino peptidase LAP , malate dehydrogenase MDH , nothing dehydrogenase NDH , octanol dehydrognease ODH , phosphoglucoisomerase PGI , peroxidase PO and superoxide dismutase SOD .   All enzymes were assayed in the supernatant of intestinal homogenate, except for ALK and PGI which were assayed in gonad.

A total of 19 genetic loci were estimated based on the electrophoretic patterns Table 1 .   The major features of variation in each enzyme are summarized as follows.

ALK activity was detected as several bands in each individual.   These were assumed to be the products of three different loci Alk 1, Alk 2 and Alk 3 .   All ALK loci in the Sagami Bay population and two loci Alk 1 and Alk 3 in the Kagoshima Bay population were polyallelic.   The Alk 1 locus of the Sagami Bay

Table 1. Allele frequencies at 19 genetic loci coding for 10 different enzymes in two local populations of the echinothurioid echinoid Asthenosoma ijimai

Locus	Allele	Sagami	Kagoshima
Alk 1	a	0.50	0.18
	b	0.06	0
	c	0.22	0.09
	d	0.22	0.73
Alk 2	a	0.90	1
	b	0.10	0
Alk 3	a	0.75	0.65
	b	0.25	0.35
Est 1	a	0.63	0.58
	b	0.37	0.42
Est 2	a	0.45	0.41
	b	0.55	0.59
Est 3	a	0.30	0.36
	b	0.70	0.64
Est 4	a	1	1
Est 5	a	0.06	0.25
	b	0.61	0.10
	c	0.33	0.65
G6pdh 1	a	0.88	0.83
	b	0.12	0.17
Lap 1	a	0	0.44
	b	0.83	0.12
	c	0.17	0.44
Lap 2	a	0.90	0.91
	b	0.10	0.09
Lap 3	a	1	1
Mdh 1	a	0	0.04
	b	1	0.96
Ndh 1	a	0.40	0.36
	b	0.60	0.64
Odh 1	a	1	1
Pgi 1	a	1	1
Po 1	a	0.45	0.64
	b	0.22	0
	c	0.33	0.36
Sod 1	a	1	0.45
	b	0	0.55
Sod 2	a	0	0.04
	b	0.95	0.96
	c	0.05	0

population had four alleles.   The Alk 2 locus in the Kagoshima Bay population was monoallelic.   According to the band pattern, each ALK locus was interpreted as a multi-allelic system at a single locus coding for a monomeric protein.

Five loci were estimated from the EST band pattern.   All loci except Est 4 were polyallelic in each local population.   In Est 1 and Est 2, single- and double-banded phenotypes were observed in two populations. This variation was interpreted as a diallelic system at a single locus coding for a monomeric protein.   Est 3 exhibited one active band in each individual.   There were two distinct bands of different mobility in both populations, which were interpreted as the products of different alleles at a single locus.   The mobility of the active band of the Est 4 locus was identical in all individuals of the two local populations, indicating that this

locus was monomorphic.   According to the band pattern, Est 5 locus was interpreted as a triallelic system at a single locus coding for a monomeric protein.

The band patterns of G6PD exhibited only one locus.   G6PD exhibited one active band in each individual. In both populations, there were two bands of different mobility.   These two distinct band patterns were interpreted as the products of different alleles at a single locus.   No heterozygotes were found.

LAP showed high levels of variation in each local population.   The Lap 1 locus was interpreted as a triallelic system at a single locus coding for a monomeric protein.   Lap 2 showed two active bands interpreted as a diallelic system at a single locus coding for a monomeric protein.   Lap 3 exhibited a single band of strong activity and was monoallelic.   Most of the bands showed strong enzyme activities.   This might be because of the carnivorous tendency of the species.

MDH in the Sagami Bay population showed a single band and was monoallelic.   On the other hand, MDH in the Kagoshima Bay population exhibited single- and double-banded phenotypes.   The individuals of the Sagami Bay population exclusively had the MDH 1 b allele.   Both the Mdh 1 a and Mdh 1 b alleles were scored in the Kagoshima population.   However, the frequency of Mdh 1 a was only 4.0% Table 1 .

In NDH, single- and double-banded phenotypes were found in both local populations.   This variation was interpreted as a diallelic system at a single locus coding for a monomeric protein.

ODH exhibited a single band of activity.   This locus was monoallelic both within and between the two populations.   A similar relationship was found for the PGI locus.

PO showed a faint, single band in all individuals from both local populations.   From the band patterns, three and two different alleles were estimated in the Sagami Bay and Kagoshima Bay populations, respectively. No heterogeneous band pattern was found in either population.

From the band patterns of SOD, we estimated two loci.   Sod 1 exhibited an active band in each individual. In the Kagoshima Bay population, there were two bands of different mobility.   These two distinct band patterns were interpreted as the products of different alleles at a single locus.   Sod 2 showed single- and triple-banded phenotypes.   This variation was interpreted as a diallelic system at a single locus coding for a dimeric protein, with single-banded patterns corresponding to the homozygous state, and triple-banded patterns to the heterozygous state.

The allele frequencies for all loci in the two populations are given in Table 1.   The proportions of polymorphic loci   P in each population were 68.4% for the Sagami Bay population and 73.7% for the Kagoshima Bay population Table 2 .   The mean P value of the species *A. ijimai*   P 71.1% was considerably higher than that of the other echinoid species in Japanese waters reported previously   mean P value of 13 species   8.4%   12, 17 . The genetic mechanism responsible for this high level of genetic variation in *A. ijimai* remains unclear.

GENETIC VARIATION IN THE ECHINOTHURIOID ECHINOID A. IJIMAI

To quantify the degree of the genetic variation, the expected average heterozygosity per locus   H in each population was calculated.   The H values of the Sagami Bay and Kagoshima Bay populations of *A. ijimai* were 26.3% and 29.5%, respectively   Table 2 .   The H value of each population also strongly supported that the genetic variation in the species *A. ijimai* was considerably higher than that of other echinoid species studied previously   mean H value of 13 species   3.1%   12, 17 .

It has been suggested that there is a correlation between the environment and the H value.   Previous reports demonstrated that the H values in shallow water echinoids were relatively lower than that of the echinoderms living in the deep-sea   5, 6 .   The echinothurioid echinoid *A. ijimai*, however, lives in relatively shallow water 0 120m   7 , which suggests that there is no relationship between the depth of living habitat and H value.

The high H value might be related to the developmental process.   The mean H value of the 13 typical developing sea urchins, *Anthocidaris crassispina, Colobocentrotus mertensii, Echinometra mathaei, Echinostrephus aciculatus, E. molaris, Hemicentrotus pulcherrimus, Heterocentrotus mammillatus, Pseudoboletia maculata,*

Table 2.   Genetic variation in two local populations of the echinothurioid *Asthenosoma ijimai*

	Sagami Bay	Kagoshima Bay
Proportion of polymorphic loci : P %	68.4	73.7
Expected average heterozygosity per locus : H %	26.3	29.5

Pseudocentrotus depressus, *Strongylocentrotus intermedius*, *S. nudus*, *Toxopneustes pileolus* and *Tripneustes gratilla*, was 3.1% 12, 17 .   This value is markedly lower than that of *A. ijimai* determined in the present study   mean H value of two local populations of *A. ijimai* 7.9% .   *A. ijimai* is the only echinoid species with modified development on which the H value has been examined to date.   The examination of other species with the modified development would be necessary to confirm a relationship between the mode of larval development and the genetic variability.

A correlation between developmental mode and the value of the haploid genome size had also been suggested. The haploid genome size of a species with typical development, *H. pulcherrimus* 0.91 pg , was smaller than that of the modified developers *A. ijimai* 1.35 pg and *Araeosoma owstoni* 1.17 pg   27 .   One of the authors has reported the H value of *H. pulcherrimus* to be 0 12 .   These previous studies suggest that the H value correlates with the size of the haploid genome.

GENETIC DIRENGENCE BETWEEN TWO LOCAL POPULATIONS OF *A. IJIMAI*

Based on the allele frequencies for all loci in these two local populations given in Table 1, the genetic distance D between the two populations was calculated according to the procedure of Nei 20 .   The D value between the two populations D 0.087 was larger than that between the local populations of another echinoid reported previously   between the Fukaura population and Shirahama population of *Anthocidaris crassispina*, D 0.022   16 , and was slightly smaller than that between different types of *E. mathaei* that are considered to be a sibling species   type A and type C, D 0.115 ; type B and type D, D 0.145   15 .   The large D value between two populations of *A. ijimai* obtained in the present study and the slight difference in morphology between the two populations   Amemiya, unpublished observation   suggest that the two populations are on the way to become distinct species.

The vast genetic distance between two local populations of *A. ijimai* might be explained by the effect of larval life history strategy.   Typical developers usually produce a large number of small eggs.   The larvae of this type have a relatively long planktonic larval period.   This larval life history strategy of typical developers promotes the wide range of larval dispersal and gene exchange between widely separate populations, resulting in reduced genetic variation among the local population.   On the other hand, modified developers usually produce a small number of large eggs.   The larvae of this type have a relatively short planktonic period.   This larval strategy of modified developers promotes the narrow range of larval dispersal and relatively small amount of gene exchange between local populations resulting in a vast genetic difference.   The larvae of the Crown-of-thorns starfish, *Acanthaster planci*, produce a large number of eggs, have a relatively long planktonic period   10 days 6 weeks and show geonegative swimming behavior.   The D values between several local populations of *Acanthaster planci*, which separated wider than the two local populations of *A. ijimai* in the present study, were relatively lower   21 than that between the two local populations of *A. ijimai*.   This supports an effect of larval strategy on genetic distance.   In addition, it was reported that local populations of the modified developing sea-urchin, *Heliocidaris erythrogramma*, showed langer genetic divergence over relatively small geographic ranges than the typical developing congeneric species *H. tuberculata* 18 .   Taken together with our present results, these studies strongly suggest that the larval life history strategy could affect the genetic variation between local populations in a species.

Summary

A direct developing echinothurioid echinoid, *Asthenosoma ijimai*, is distributed along the south coast of Japan from Sagami to Kagoshima Bays and the west coast of Kyushu. This species is a common member of this order in Japan. As a first step towards understanding the evolutionary biology of the echinothurioids, genetic differences in two local populations of *A. ijimai* from Sagami and Kagoshima Bays were examined by allozyme analysis of 10 different enzymes. The average heterozygosity per locus H was 26.3% and 29.5% for the Sagami and Kagoshima populations, respectively. These values were considerably higher than those in other typical indirect developing echinoids reported to date. The high level of genetic polymorphisms might correlate with the developmental mode. The Nei's genetic distance D between the two local populations was 0.087. This value was higher than the average of data obtained between conspecific local populations, but slightly lower than that obtained between sibling species of other echinoid species. These results indicate a substantial degree of genetic divergence between these two local populations of this species.

Acknowledgements

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2

2   Nei 1972

D 0.087

2   10   2

26.3% 29.5%