Low Genetic Differentiation with High Genetic Variability Observed in Common Coastal Starfish Asterina pectinifera around Japan Inferred from Isozyme Analysis

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Low Genetic Differentiation with High Genetic Variability Observed in Common Coastal Starfish *Asterina pectinifera* around Japan Inferred from Isozyme Analysis

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Key words : Isozymes, genetic variability, *Asterina pectinifera*, starfish, genetic differentiation

Abstract

Genetic variability and geographic population structure in population of the common coastal starfish *Asterina pectinifera* were examined in nine localities around Japan through isozyme analysis. Surveying 28 enzymes by using starch gel electrophoresis, it was found that eight loci controlling seven enzymes were useable as genetic markers. Genetic variability of this species estimated from the 8 loci followed; the proportion of variant loci ranged from 0.63 to 1.00 with a mean of 0.80, expected average heterozygosity was from 0.176 to 0.208 with a mean of 0.184, and mean number of alleles per locus ranged from 2.5 to 3.8 with a mean of 3.0. The genetic variability of this species was comparatively high among aquatic animals. In order to evaluate genetic differences among the nine localities around Japan, the allelic and genotypic homogeneity tests were carried out and Nei's genetic distance was calculated between every pair of the nine localities. Significant differences among localities showed at only a few loci, but genetic distances were low between any pair of localities. Thus, genetic differentiation has not occurred among localities around Japan in *A. pectinifera.*

Introduction

Starfish *Asterina pectinifera*, which lives on rocky coastal shores and sandy floors in shallow water, is one of the most common species on coastal area around Japan (Oguro 1995). Starfish, including the species, are commonly recognized as a nuisance mainly because of the damage to commercial bivalves and getting tangled in fishing nets. Since they do not have industrial value, few studies have been carried out for biological/genetic resources. However, the starfish is considered to be one of the important constituent members as a scavenger in the coastal ecological system. Therefore, the population structure should be analyzed for conservation of coastal environment and sustainable yield of the starfish. It should be difficult, however, to carry out such a study by ecological observation like other marine animals. Genetic approach using genetic markers is effective for such a study.

In a genetic study on population structure, Matsuoka et al. (1995) examined the starfish *A. pectinifera* sampled from Mutsu Bay and Fukaura in Aomori prefecture using 23 allozyme loci as genetic marker detected by polyacrylamide gel eletrophoresis (PAGE), and described the samples were genetically divergent despite only 20 or 30 km away from each other. And Matsuoka and Asano (2003) reported genetic variation of this species in the northern part of Japan using same method. However, their sample sizes were too small to detect genetic variability and genetic divergence. Moreover, estimation of genetic characteristics based on PAGE reported by them could not be compared with the results from starch gel electrophoresis as previously reported (Yamashita et al. 2005). The aims of this study are (1) to detect isozyme marker genes by using starch gel electrophoresis, (2) to estimate genetic variability, and (3) to quantify the genetic difference and population structure of the starfish *A. pectinifera* around Japan.

Materials and Methods

Starfish *Asterina pectinifera* examined in this study were collected from 9 localities around Japan

Table 1. Sampling sites, dates and the shape of specimens (mean ± standard deviation)

locality name	prefecture	n*1	sampling date	R*2(mm)	r*3(mm)	wet weight(g)
Yoichi	Hokkaido	34	Aug 2001	47.6 ± 10.9	29.4 ± 7.3	45.6 ± 26.4
Usujiri	Hokkaido	39	Jun 2000	38.4 ± 4.8	22.6 ± 3.5	21.1 ± 7.4
Asamushi	Aomori	137	Dec 2000	55.2 ± 7.3	35.1 ± 5.4	57.0 ± 21.1
Rikuzentakata	Iwate	84	Nov 2000	49.9 ± 8.1	31.0 ± 5.6	42.4 ± 19.7
Onagawa	Miyagi	50	Oct 2000	51.6 ± 7.5	30.0 ± 4.9	49.2 ± 21.1
Izumisano	Osaka	100	Aug 2001	43.5 ± 6.6	25.8 ± 4.0	32.1 ± 14.1
Kure	Hiroshima	58	Oct 2001	40.7 ± 5.8	25.4 ± 17.9	22.4 ± 9.3
Namerikawa	Toyama	98	Oct 1999	49.5 ± 4.6	27.4 ± 3.3	51.3 ± 12.4
Miyazu	Tyoto	46	Jul 2001	41.5 ± 5.5	25.2 ± 4.4	30.4 ± 12.4

*1, number of animals; *2, radius length; *3, interradius length

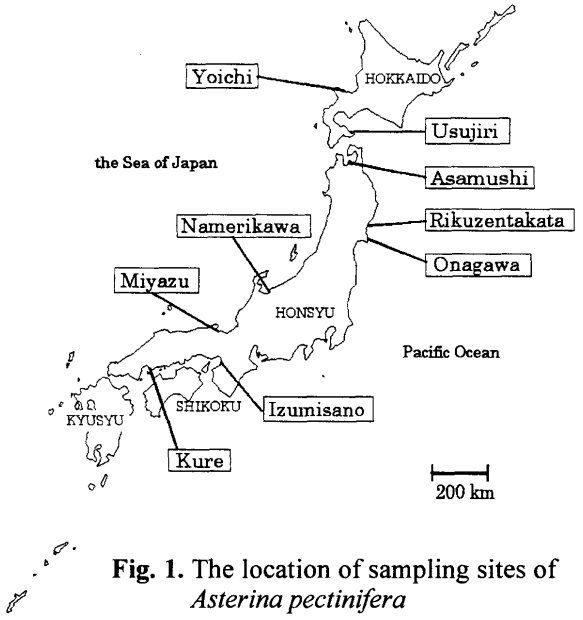

Fig. 1. The location of sampling sites of *Asterina pectinifera*

from April 1999 to October 2001 as shown in Fig. 1 and Table 1. Collected samples were kept cool and transferred to our laboratory where they were kept alive under running seawater until dissection.

Isozymes were detected by using horizontal starch gel electrophoresis following the method of Fujio and Ikeda (1999). The tube feet, pyloric caeca and rectal sac were used to detect isozymes expressed in these tissues. The procedure and condition of electrophoresis were the same as previously reported (Yamashita et al. 2005).

A total of 28 enzymes were examined, as shown in Table 2. When isozymes were encoded by several loci, these loci were numbered in order of decreasing anodal mobility. Alleles were named according to the mobility of their products relative to the most common allele in the samples of Onagawa, which was assigned a value of *100*. Chi-square tests were carried out to examine whether observed genotype numbers accorded with those expected under Hardy-Weinberg equilibrium (HWE).

Genetic variability was measured by using the average number of alleles per locus (A/L), the proportion of polymorphic loci (P), the proportion of variant loci less than polymorphism (V), and observed and expected average heterozygosity (Ho and He). Here, the polymorphic locus was defined as the locus that had the maximum allele frequency of $\leqq$ 0.95. The observed heterozygosity was a direct count of heterozygous individuals in the sample, and expected heterozygosity was calculated as $1-\Sigma\Sigma x_{ij}^2/n$, where x_{ij} was the frequency of j-th allele of i-th locus and n was equal to number of loci examined.

The extent of genetic differences among localities was estimated using several measures. The gene diversity coefficient (G_{ST}) was calculated as $(H_T-H_S)/H_T$, where H_T (total genetic diversity) was the expected average heterozygosity in the total population and H_S (mean genetic diversity per area) was the mean of the expected average heterozygosities within localities. Nei's genetic distances (Nei 1972) between localities were calculated. The above-mentioned measures were calculated using Microsoft excel. Tests to examine genetic differences among localities were carried out using a population genetic software package, Genepop ver. 3.1 (Raymond and Rousset 1995). With regard to these tests, the null hypothesis was H_0 : "the allelic (sub-option 1 or 2 in option 3) and genotypic (sub-option 3 or 4 in option 3) distribution is identical across localities." Tests were performed for each locus.

The genetic relationship among the localities was estimated constructing dendrogrms by unweighted

Table 2. Examined enzymes, expected loci as genetic marker and condition of electrophoresis.

	enzyme name	abbreviation	EC. number	buffer*	pH	expected loci
1	Acid phosphatase	ACP	EC. 3.1.3.2			
2	Adenylate Kinase	AK	EC. 2.7.4.3			
3	Alcohol Dehydrogenase	ADH	EC. 1.1.1.1			
4	Aldolase	ALD	EC. 4.1.2.13			
5	Alkaline Phosphatase	ALP	EC. 3.1.3.1			
6	Aspartate Aminotransferase	AAT	EC. 2.6.1.1			
7	Creatine Kinase	CK	EC. 2.7.3.2			
8	Diaphorase	DIA	EC. 1.6.-.-			
9	Esterase	EST	EC. 3.1.1.1			
10	Fructose-1,6-Diphosphatase	FDP	EC. 3.1.3.11			
11	Fumarate Hydratase	FH	EC. 4.2.1.2			
12	Galactose Dehydrogenase	GAD	EC. 1.1.1.48			
13	Glucose-6-Phosphate Dehydrogenase	G6PD	EC. 1.1.1.49			
14	Glucosephosphate Isomerase	GPI	EC. 5.3.1.9			
15	Glutamate Dehydrogenase	GDH	EC. 1.4.1.3			
16	Glycerol-3-Phosphate Dehydrogenase	αGPD	EC. 1.1.1.12	T-C	7	*αGpd*
17	Hexokinase	HK	EC. 2.7.1.1	T-C	7	*Hk*
18	Isocitrate Dehydrogenase	IDH	EC. 1.1.1.42			
19	Lactate Dehydrogenase	LDH	EC. 1.1.1.27			
20	Leucine Aminopeptidase	LAP	EC. 3.4.11.1			
21	Malate Dehydrogenase	MDH	EC. 1.1.1.37	T-C	8	*Mdh-1,2*
22	Malic Enzyme	ME	EC. 1.1.1.40			
23	Mannose-6-Phosphate Isomerase	MPI	EC. 5.3.1.8	T-C	8	*Mpi*
24	Octanol Dehydrogenase	ODH	EC. 1.1.1.73			
25	Phosphoglucomutase	PGM	EC. 2.7.5.1			
26	6-Phosphogluconate Dehydrogenase	6PGD	EC. 1.1.1.44	T-C	7	*6Pgd*
27	Solbitol Dehydrogenase	SDH	EC. 1.1.1.14	T-C	8	*Sdh*
28	Superoxide Dismutase	SOD	EC. 1.15.1.1	T-C	7	*Sod*

* T-C: tris citerate buffer

pair-group method with averaging (UPGMA) (Sneath and Sokal 1973) and neighbor joining method (NJ) (Saitou and Nei 1987) using the PHYLIP computer package (Felsenstein 1993).

Results

Genetic control of isozymes

A total of 28 enzymes were examined in the tube feet, pyloric caeca and rectal sac. Among them, tissue specific patterns were not obtained, but comparatively clear zymograms were observed in the tube feet. Using the tube feet, enzyme activity was observed in 21 enzymes except ADH, FH, GAD, G6PD, GDH, LDH and ODH. But distinctly clear band patterns were not necessarily observed in all active enzymes. Finally, eight loci coding seven enzymes, namely *αGpd*, HK, MDH, MPI, *6Pgd*, SDH and SOD, were estimated as genetic markers as shown in Table 2.

Typical zymograms of these markers were shown in Fig. 2. Six enzymes with exception of MDH, showed single active zone on the anodal area, then these were controlled by single locus. MDH

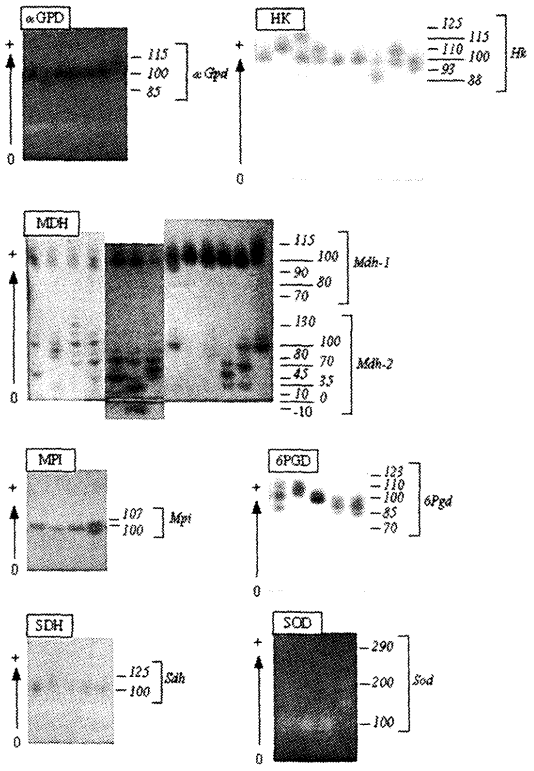

Fig. 2. Electrophoretic band patterns of isozymes in *Asterina pectinifera*

Table 3. Allele frequencies in nine localities for *Asterina pectinifera*. Numbers in parenthesis are sample size.

locus	allele	Yoichi	Usujiri	Asamushi	Rikuzen-takata	Onagawa	Izumisano	Kure	Nameri-kawa	Miyazu
αGpd	*115*	0.015	0	0.036	0.012	0.010	0.010	0.026	0.065	0.033
	100	0.985	1.000	0.960	0.982	0.990	0.990	0.974	0.935	0.967
	85	0	0	0.004	0.006	0	0	0	0	0
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Hk	*125*	0	0.013	0	0	0	0	0	0	0
	115	0	0.013	0.011	0	0.010	0	0	0	0.011
	110	0.088	0.077	0.146	0.190	0.120	0.175	0.224	0.175	0.120
	100	0.838	0.858	0.777	0.750	0.840	0.760	0.724	0.755	0.782
	93	0.059	0.013	0.062	0.060	0.020	0.060	0.052	0.070	0.087
	88	0.015	0.026	0.004	0	0.010	0.005	0	0	0
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Mdh-1	*115*	0	0	0	0.006	0.010	0	0	0	0
	100	0.985	0.974	0.974	0.982	0.980	0.980	0.957	0.985	1.000
	90	0	0.013	0.004	0.006	0.010	0.000	0.034	0.015	0
	80	0.015	0.013	0.015	0.006	0	0.020	0.009	0	0
	70	0	0	0.007	0	0	0	0	0	0
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Mdh-2	*130*	0.029	0	0.047	0.065	0.050	0.050	0.026	0.050	0.011
	100	0.455	0.462	0.529	0.476	0.530	0.445	0.473	0.495	0.511
	80	0	0	0	0	0.010	0	0	0	0
	70	0.397	0.410	0.328	0.351	0.280	0.430	0.362	0.315	0.380
	45	0.074	0.064	0.033	0.036	0.070	0.025	0.078	0.050	0.054
	35	0.015	0.038	0.033	0.036	0.050	0.045	0.052	0.060	0.033
	10	0.015	0.026	0.015	0.012	0	0	0	0.005	0
	0	0	0	0.015	0.018	0.010	0.005	0.009	0.020	0.011
	-10	0.015	0	0	0.006	0	0	0	0.005	0
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Mpi	*107*	0	0	0.007	0	0	0	0	0	0
	100	1.000	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
6Pgd	*123*	0	0	0.004	0	0	0	0	0	0
	110	0.088	0.038	0.026	0.042	0.070	0.040	0.009	0.065	0.065
	100	0.794	0.757	0.839	0.767	0.840	0.840	0.724	0.790	0.848
	85	0.118	0.179	0.124	0.179	0.080	0.115	0.267	0.145	0.087
	70	0	0.026	0.007	0.012	0.010	0.005	0	0	0
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Sdh	*125*	0	0	0.004	0.018	0.010	0.005	0	0	0
	100	1.000	1.000	0.996	0.982	0.990	0.995	1.000	1.000	1.000
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)
Sod	*290*	0	0	0	0.006	0	0	0.009	0	0
	200	0.059	0.051	0.018	0.018	0.030	0.030	0.026	0.015	0.065
	100	0.941	0.949	0.982	0.976	0.970	0.970	0.965	0.985	0.935
		(34)	(39)	(137)	(84)	(50)	(100)	(58)	(100)	(46)

Table 4. Genetic variability in 9 localities for *Asterina pectinifera*. A/L, allels per locus; P, proportion of polymorphic loci; V, proportion of variable loci less than polymorphic; Ho, observed heterozygosity; He, expected heterozygosity; R, provability of recognition of individuals. Fractions in parenthesis are (number of genotypic combination)/(sample size).

	No. of loci	A/L	P	V	P+V	Ho	He	R
Yoichi	8	2.8	0.50	0.25	0.75	0.195	0.179	0.706 (24/34)
Usujiri	8	2.9	0.50	0.13	0.63	0.173	0.176	0.744 (29/39)
Asamushi	8	3.8	0.38	0.62	1.00	0.190	0.181	0.547 (52/95)
Rikuzentakata	8	3.5	0.38	0.50	0.88	0.193	0.196	0.655 (55/84)
Onagawa	8	3.3	0.38	0.50	0.88	0.140	0.166	0.660 (33/50)
Izumisano	8	2.9	0.38	0.50	0.88	0.183	0.176	0.729 (51/70)
Kure	8	2.8	0.38	0.38	0.76	0.198	0.208	0.672 (39/58)
Namerikawa	8	2.8	0.50	0.25	0.75	0.189	0.197	0.650 (65/100)
Miyazdu	8	2.5	0.50	0.13	0.63	0.168	0.176	0.808 (21/26)
average	8	3.0	0.43	0.36	0.80	0.181	0.184	0.686

showed various band patterns in two separate zones. Therefore, it was estimated that MDH was encoded at two loci (*Mdh-1*, *Mdh-2*).

Genetic variability of isozymes

Allele frequencies of each locality were given in Table 3. The results of chi-square tests for all loci of each locality showed only three significant deviations of genotypic frequencies from those expected under HWE (*Hk* at Rikuzentakata and *6Pgd* at Yochi and Miyazu). After pooling the rare alleles, chi-square tests indicated no significant deviation, and this species were under condition of HWE.

All of eight loci were genetically variable at no less than one locality. And *Hk*, *Mdh-2* and *6Pgd* were polymorphic in all examined localities. Genetic variability was estimated based on eight marker gene as shown in Table 4. The mean number of alleles per locus (A/L) ranged from 2.5 to 3.8, with a mean of 3.0. The proportion of polymorphic loci (P) was 0.38 to 0.50 with a mean of 0.43. The proportion of variable loci less than polymorphic (V) was distributed from 0.13 to 0.62 with a mean of 0.36. The observed average heterozygosity (Ho) ranged from 0.140 to 0.198 with a mean of 0.181 and the expected one (He) did from 0.166 to 0.208 with mean of 0.184.

Genetic differentiation

In order to estimate an extent of genetic differences among examined all localities, a homogeneity test of genetic composition was carried out and the G_{ST} value was calculated (Table 5). In tests using allele frequencies, *αGpd* and *6Pgd* showed a significant difference at the significance level (α) = 0.01, and *Hk* at α = 0.05. Genotype frequencies also showed a significant difference at *αGpd* ($P < 0.01$) and *6Pgd* ($P < 0.05$). G_{ST} value was 0.010.

Next, a number of loci showing a significantly different allele frequencies and Nei's genetic distance between every pair of localities are shown in Table 6. Significant differences of allele frequencies were shown in 16 pairs out of 36, but the number of loci showing a significant difference was small (0 or 1) in most pairs. Also, a significant difference was not observed in any pair of localities at *Mdh-2* which was the most variable locus examined in this study. The minimum genetic distance was 0.00077 between

Table 5. The results of tests of genetic differentiation among all samples by GENEPOP and the value of G_{ST}. "Genic" and "genotypic" show the tests about distribution of alleles and genotypes, respectively. (*, P<0.05; **, P<0.01)

	genic	genotypic
locus	P-value	P-value
αGpd	0.0420 *	0.0246 *
Hk	0.0326 *	0.0508
Mdh-1	0.1944	0.1288
Mdh-2	0.3526	0.3290
Mpi	0.7621	0.4385
6Pgd	0.0070 **	0.0154 *
Sdh	0.4163	0.3536
Sod	0.1763	0.3026
G_{ST}	0.010	

Table 6. Nei's genetic distance among localities where *Asterina pectinifera* were sampled (blow diagonal), and number of loci that showed significantly different allele friquency between localities at $\alpha = 0.05$ (above diagonal).

	Yoichi	Usujiri	Asamushi	Rikuzen takata	Onagawa	Izumisano	Kure	Namerikawa	Miyazu
Yoichi	-	0	0	0	0	0	2	0	0
Usujiri	0.00108	-	1	1	0	1	1	2	0
Asamushi	0.00237	0.00341	-	0	0	0	1	0	1
Rikuzentakata	0.00264	0.00303	0.00125	-	0	0	0	1	0
Onagawa	0.00229	0.00356	0.00126	0.00297	-	0	2	2	0
Izumisano	0.00193	0.00307	0.00164	0.00136	0.00345	-	1	2	0
Kure	0.00548	0.00444	0.00395	0.00148	0.00666	0.00388	-	1	1
Namerikawa	0.00282	0.00405	0.00077	0.00089	0.00211	0.00209	0.00274	-	1
Miyazdu	0.00121	0.00304	0.00114	0.00252	0.00187	0.00149	0.00563	0.00186	-

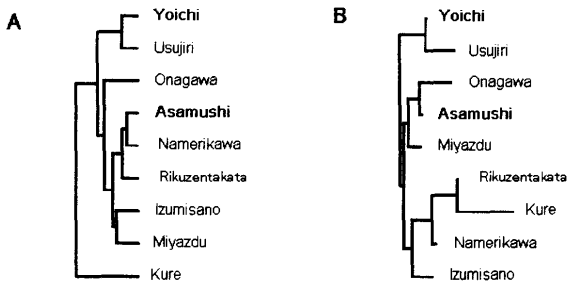

Fig. 3. Dendrograms constructed by UPGMA(A) and NJ(B) based on Nei's genetic distance among 9 localities

Asamushi and Namerikawa, the maximum was 0.00666 between Onagawa and Kure, and average was 0.00263.

In order to reveal genetic relationship among 9 localities, two kind of dendrograms were constructed through UPGMA and NJ methods based on the genetic distance matrix (Fig. 3). Clear geographical relationship was not observed in both dendrograms.

Discussion

Detection of isozymes

In the present study, 8 loci were estimated from substrate-specific 7 enzymes as genetic marker for *A. pectinifera*. Matsuoka et al. (1995) and Matsuoka and Asano (2003) estimated 23 loci from 12 enzymes extracted from pyloric caeca of *A. pectinifera* using PAGE. However, non-substrate-specific enzyme loci, i.e. three loci from alkaline phosphatase (ALK), four from leucine amino peptidase (LAP), two from amylase (AMY) and two from nothing dehydrogenase (NDH), were included. As non-substrate-specific enzymes were often controlled by several loci, careful attention should be paid to using these enzymes as genetic marker. Except these loci, eleven loci controlling eight enzymes were estimated by them. And a critical difference exists between their estimation and our result. They reported MDH to be a monomeric enzyme but the authors showed to be dimeric enzyme based on the evidence of typical phenotypes of variation on the gel (see Fig. 2). Even if the experimental conditions differ, the enzyme structure does not change. Therefore, it is unable to compare our results with the data from Matsuoka et al. (1995) and Matsuoka and Asano (2003). In other starfish, in *Linckia laevigata* 7 loci from 6 enzymes were estimated (Williams and Benzie 1993), in *Acanthaster planci* 14 from 12 (Nishida and Lucas 1988) and 9 from 7 (Benzie and Stoddart 1992), in *Leptasterias epichlora* and *L. hexactis* 14 from 12 (11 from 10 except non-substrate-specific enzymes) (Kwast et al. 1990), and in *Asterias amurensis* 12 from 10 (Yamashita et al. 2005). These numbers of estimated loci in starfish species were not so many. From the facts mentioned above, it is likely to be harder to detect isozymes in starfish than fish or mollusca. Indeed, the activity of some examined enzymes rapidly disappeared even though the tissues were frozen at -30 ℃ . This would be one of the factors that causes the detection of isozymes difficult in starfish species.

Genetic variability

Comparing the genetic variability of *A. pectinifera* with other marine animals, the values in the present study were higher than that averaged from 41 fish species ($P = 0.194 \pm 0.023$, $He = 0.059 \pm 0.007$)

reported by Fujio and Kato (1979) and from 25 shellfish (P = 0.412±0.030, He = 0.147±0.011) (Fujio et al. 1983). In other starfish, average heterozygosity (He) was reported as 0.402, 0.199, 0.255, 0.164 and 0.254 in *Linckia laevigata* (Williams and Benzie 1993), in *Acanthaster planci* (Nishida and Lucas 1988, Benzie and Stoddart 1992), and in *Leptasterias epichlora* and *L. hexactis* (Kwast et al. 1990), respectively. Compared with these reports about other aquatic animals, relatively high genetic variability was found in the starfish including *A. pectinifera.*

Soul (1976) and Nei and Graur (1984) showed the correlation between genetic variability and effective population size. Assuming that the isozyme markers used in the present study are neutral against natural selection, the fact that such high genetic variability exists in *A. pectinifera* population suggests this species have considerably large effective population size. As a practical matter, *A. pectinifera* inhabits broadly around coastal area of not only Japan but east Asia.

Genetic uniformity of A. pectinifera population

G_{ST} of *A. pectinifera* was 0.010, that is, the genetic diversity derived from the difference among localities is only 1% and most of the variability exists within locality. The results of homogeneity tests of allele and genotype frequencies suggest that the genetic composition of this species is not quite homogeneous through the whole sampling range. But in the loci showing significantly different allele and/or genotype frequencies, a single common dominant allele always existed across the all localities examined in the present study. And there is no cline of allele frequencies along geographical locations. With regard to Nei's genetic distance, the values were small, independent of geographic distance. Nei (1975) summarized that the genetic distance estimated from various animals using protein polymorphism was approximately the order of 1.0 between species, 0.1 between subspecies and 0.01 among local races. Compared with the report, the values in the present study were much smaller than upper limit among local races. With regard to dendrograms, although the topologies are not altogether identical between UPGMA and NJ trees, this result paradoxically suggests that the genetic composition should be very similar among localities and there shouldn't be enough difference to divide them genetically. Thus, the extent of genetic divergence of *A. pectinifera* is quite small, in other words, this species has uniform genetic composition across most coastal area of Japan.

Contrary to our results, Matsuoka et al. (1995) reported Nei's genetic distance was no less than 0.041 between Mutsu Bay and Fukaura, both of which are located near by Asamushi in Aomori prefecture, that value is over ten times larger than the average shown in the present study. Their result substantially depended on the genetic difference estimated from the loci expected from MDH and LAP. However nevertheless their estimation about the loci of these enzymes was obscure as mentioned above.

This study showed that *A. pectinifera* was under HWE in all localities examined and had low genetic differences with high genetic variability. The dispersal during the planktonic larval phase for about one month (Komatsu and Oguro 1988) seems to bring about the genetic homogeneity like this. In marine animals, there are many reports suggesting that presence (and its period) or absence of floating property (dispersal potential) of egg, larva and/or juvenile is closely related to gene flow and the important factor to form population structure (Hunt 1993, Doherty et al. 1995, Arndt and Smith 1998 and Bernardi 2000). The genetic uniformity throughout extensive geographical range was reported in other asteroid, *Linckia laevigata* (Williams and Benzie 1993, 1996). They suggested the larval life remaining in the water column during 28 d of that species caused high gene flow and the genetic composition to be homogeneous. Williams and Benzie (1993) showed the negative correlation between the dispersal ability and genetic differentiation. Taking account of the length of planktonic larval phase in *A. pectinifera*, which is considerably long among aquatic animals with pelagic larval phase, the results in the present study agree with their opinion. In order to maintain homogeneous genetic composition extensively, gene flow must have been continuous and as large scale as a great number of individuals exchange among local areas. The effective population size of *A. pectinifera* is expected to be very large because of its high genetic variability, so that probably the scale of gene flow will be large.

The results shown in the present study is the first information about the genetic variability and divergence of *A. pectinifera* sampled from a wide range of coastal area around Japan. That data will be the basic information for maintenance of the starfish population in coastal fishing places and aquaculture areas.

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