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# Genetic Variation in Northern Japanese Populations of the Starfish *Asterina pectinifera*

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**ABSTRACT**—The starfish *Asterina pectinifera* of the family Asterinidae is endemic Japanese species and commonly found in Japanese waters. In order to examine the degree of genetic variation and the maintenace mechanism of polymorphism within population, we studied the allozyme variation in five northern Japanese local populations of the starfish by electrophoresis. The species showed much higher genetic variability than many other shallow water echinoderms. Based on other allozyme studies and the ecological data, it was suggested that the high genetic variation of the starfish was closely related to the population size. Additionally, the relation between the degree of enzyme variation and the quaternary structure of enzymes was also examined, and the results suggested the close relation between the enzyme variability and functional constraints.

**Key words:** echinoderm, allozyme variation, population genetics

## INTRODUCTION

Since the technique of enzyme electrophoresis was introduced into the population genetics, many workers have reported on the levels of genetic variation in natural populations of many animal groups (Nei,1987). However, biochemical surveys of genetic variation in echinoderms at protein level are very limited when compared with those of land animals such as insects or mammals(Matsuoka *et al*.,1993).

In addition to biochemical systematics of echinoderms (Matsuoka,1985,1986,1987,1989,1990,1992; Matsuoka and Hatanaka,1991,1998; Matsuoka and Inamori,1996,1999; Matsuoka and Suzuki, 1987,1989), we are also much interested in allozyme surveys of the genetic variation within populations of echinoderms (starfish or sea-urchins), and also in the maintenance mechanism of enzyme polymorphism within population. Echinoderms are well suited in some respects of population genetics: Many echinoderms are abundant and form large dense populations which can be sampled extensively without decreasing their natural population densities. Furthermore, many of them have the broad geographical distributional ranges. In addition, the Japanese waters are diversified in biogeochemical conditions and the marine fauna, and have been well known to be a rich yield for marine invertebrates. In particular,the echinoderm fauna is very abundant and the numbers of endemic species and genera found in the Japanese waters are the greatest in the world (Shigei,1974). Therefore, we have good conditions for undertaking the studies to clarify the genetic structure of natural populations of echinoderms.

Among many echinoderm species from Japanese waters, the endemic Japanese starfish *Asterina pectinifera* is well suited to surveys of genetic variation within populations. It is one of very common shallow water echinoderms and easily collected in large numbers in many localities of the Japan Sea and the Pacific Ocean of Japan, and therefore, it is one of the symbols of the seashore animals. The starfish has been widely studied in developmental biology, biochemistry or physiology.

We consider it is important to find out, using allozyme electrophoresis, how much genetic variation has accumulated within echinoderm population. Such an investigation provide useful information in the field of population genetics of marine invertebrates. Further,in recent years, the genetic analysis of populations of marine invertebrates is a matter of concern and interest in the field of research about experimental animals: when the starfish or sea-urchin is used as experimental animals in the field of developmental biology, biochemistry or physiology, it would be desired that the genetic structure of their populations is clarified. The present study would also contribute to the elucidation of such problems. In this paper, we report the genetic variation within the starfish *Asterina pectinifera* from northern Japan.

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## MATERIALS AND METHODS

### Materials

The starfish examined in this study was *Asterina pectinifera* of the family Asterinidae. It was collected from five different localities in northern Japan. The locality and number of individuals examined were as follows: Esashi in Hokkaido, 13; Shimoburo of Shimokita Peninsula in Aomori Pref.,11; Asamushi of Mutsu Bay in Aomori Pref., 28; Fukaura in Aomori Pref.,30; Atsumi in Yamagata Pref.,12. These specimens were collected by snorkering by the present authers. These five localities are shown in Fig. 1. After collection, the pyloric caeca were cut off from fresh specimens and stored at −45°C until use.

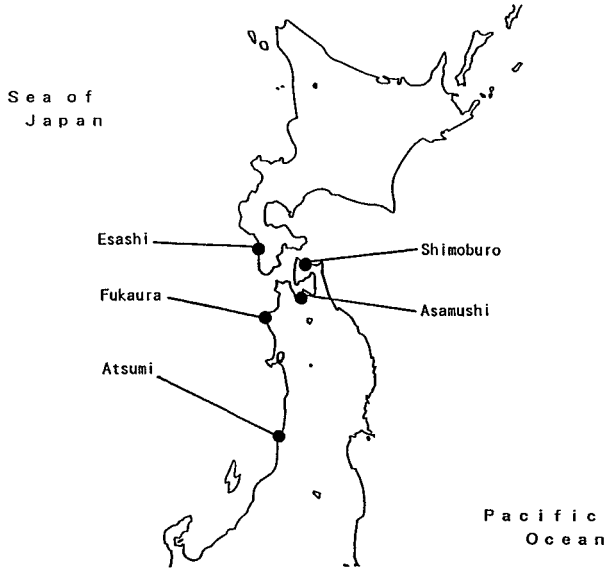



**Fig. 1**. Map showing the geographic localities of five populations of the starfish *Asterina pectinifera* in northern Japan.

### Allozyme electrophoresis

Allozyme electrophoresis was performed on 7.5% polyacrylamide slab gels by the method as described previously (Matsuoka, 1985):0.5g of tissue was individually homogenized in 3 vols of 20 mM phosphate buffer, pH 7.0, containing 0.1M KCl and 1mM EDTA by using a small polyethylene homogenizer of the Potter-Elvehjem type in an ice water bath. After centrifugation at 6,000 × g for 10 min, 0.01–0.10 ml of the clear supernatant was used for electrophoretic analyses of enzymes. Electrode buffer was 0.38M Glycine-tris buffer, pH 8.3. After electrophoresis, the gels were stained for the following 12 different enzymes: malate dehydrogenase (MDH), nothing dehydrogenase (NDH), xanthine dehydrogenase (XDH), hexokinase (HK), glucose-6-phosphate isomerase (GPI),superoxide dismutase (SOD),aspartate aminotransferase (AAT), alkaline phosphatase(ALK), peroxidase (PO), esterase (EST), amylase (AMY), and cytosol aminopeptidase (CAP). Stain recipes for these enzymes have been described previously (Matsuoka and Hatanaka, 1991).

## RESULTS AND DISCUSSION

Twenty-three genetic loci for five local populations of *A. pectinifera* were inferred from the allozyme variation observed in 12 different enzymes. The allele frequencies for all genetic loci are given in Table 1. Twelve loci (*MDH, XDH,*

**Table 1.** Allele frequencies at 23 genetic loci in the five local populations of the starfish *Asterina pectinifera*.

| Locus | Esa | Shi | Asa | Fuka | Atsu |
|---|---|---|---|---|---|
| *MDH* | a(0.23) | a(0.68) | a(0.66) | a(0.02) | a(0.37) |
| | b(0.69) | b(0.32) | b(0.34) | b(0.98) | b(0.63) |
| | c(0.08) | | | | |
| *NDH-1* | a | a | a | a | a |
| *NDH-2* | a | a | a | a | a |
| *XDH* | a | a(0.96) | a | a | a |
| | | b(0.04) | | | |
| *HK* | a | a | a | a | a |
| *PGI* | a | a | a | a | a |
| *SOD-1* | a(0.96) | a(0.96) | a(0.90) | a(0.95) | a(0.92) |
| | b(0.04) | b(0.04) | b(0.10) | b(0.05) | b(0.08) |
| *SOD-2* | a(0.15) | a(0.13) | a(0.20) | a(0.20) | a(0.21) |
| | b(0.54) | b(0.55) | b(0.52) | b(0.58) | a(0.67) |
| | c(0.31) | c(0.32) | c(0.28) | c(0.22) | c(0.12) |
| *AAT* | b(0.85) | b | a(0.02) | b | b |
| | c(0.15) | | c(0.98) | | |
| *ALK-1* | a | a | a(0.98) | a | a |
| | | | b(0.02) | | |
| *ALK-2* | a | a | a | a | a |
| *ALK-3* | a | a | a | a | a |
| *EST-1* | a | a | a | a | a |
| *EST-2* | a | a | a | a | a |
| *EST-3* | a | a | a | a | a |
| *EST-4* | a(0.40) | a(0.64) | a(0.67) | a(0.54) | a(0.37) |
| | b(0.60) | b(0.36) | b(0.33) | b(0.46) | b(0.63) |
| *PO* | b | b(0.96) | a(0.02) | b(0.90) | a |
| | | c(0.94) | b(0.98) | c(0.10) | |
| *AMY-1* | a | a | a | a | a |
| *AMY-2* | a(0.20) | b(0.50) | a(0.06) | a(0.17) | a(0.46) |
| | b(0.60) | c(0.50) | b(0.62) | b(0.48) | b(0.50) |
| | c(0.20) | | c(0.32) | c(0.33) | c(0.04) |
| | | | | d(0.02) | |
| *CAP-1* | a | a | a | a | a |
| *CAP-2* | a(0.42) | a(0.36) | a(0.77) | a(0.17) | a(0.50) |
| | b(0.58) | b(0.64) | b(0.23) | b(0.83) | b(0.50) |
| *CAP-3* | a(0.04) | b | a(0.04) | b | a(0.08) |
| | b(0.96) | | b(0.96) | | b(0.92) |
| *CAP-4* | a | a | a(0.94) | a(0.93) | a |
| | | | b(0.06) | b(0.07) | |

Alleles are correspondingly lettered from “a”, this being the allele of the lowest electrophoretic mobility. The value in parenthesis represents the frequency of each allele in population. Esa=Esashi; Shi=Shimoburo;Asa=Asamushi;Fuka=Fukaura; Atsu=Atsumi.

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*SOD-1, SOD-2, AAT, ALK-1, PO, EST-4, CAP-2, CAP-3, CAP-4* and *AMY-2*) were polymorphic and the other 11 loci were monomorphic. In general, enzymes such as SOD, EST,CAP and AMY were highly polymorphic in various echinoderms examined previously (*eg.*,Matsuoka and Hatanaka,1991).Namely,as mentioned in late section, non-specific substrate enzymes trend to show high variability. When a large number of loci are examined for studying genetic variation within populations, the amount of variation is usually measured by the proportion of polymorphic loci (*P*) and average heterozygosity per locus (*H*). These are appropriate measures of genetic variation. Table 2 summarizes the extent of genetic variation in five populations. The number of alleles per locus was in the range of 1.39-1.57 with a mean of 1.46. The proportion of polymorphic loci (*P*) was in the range of 30.4–47.8% with a mean of 36.5%, and the expected average heterozygosity per locus (*H*), in the range of 10.6–13.0% with a mean of 12.0%.

In a previous paper, we compiled on the extent of genetic variation in echinoderms reported until now (Matsuoka *et al*.,1993). The *H* values in five populations of *A. pectinifera* were much higher than those of other shallow water echinoderms. Generally, the *H* values of shallow water echinoderms are in the range of 0–6%, showing that *A. pectinifera* had much higher genetic variability. The difference in the degree of genetic variablity might be due to the difference of the population size: Several workers noted that small populations often have a lower heterozygosity than large populations. For example, Selender *et al*. (1971) showed that the Santa Rosa Island (off the Gulf Coast of the Florida panhandle) population of *Peromyscus polionotus*, of which the size is known to be 12,000, has a heterozygosity of 1.8%, compared with the heterozygosity of 8.6% in the Florida population. The cave populations (200–500 individuals) of the characid fish *Astyanax mexicanus* in Mexico also have a very low heterozygosity compared with the nearby surface population (Avise and Selender,1972). Later, Nei (1983) and Nei and Graur (1984) examined the relationship between average heterozygosity and population size for 77 different species. As a result, they found a significant correlation between them. From these evidence,it is safely considered that the starfish *A. pectinifera* showing higher genetic variability has much larger population size than other shallow water echinoderms. In fact, the species is very commonly found in the Japanese waters and is one of the most abundant starfish species in the sea around Japan. The larger population size would make it possible to maintain higher genetic variability within population. Previously, we reported that in echinoderms deep-sea species generally showed higher genetic variability than shallow water species (Matsuoka *et al*., 1993). For example, *Distolasterias nipon* in deep-sea of Mutsu Bay in Aomori Pref. showed relatively high genetic variation (*H*=17%) (Matsuoka, *et al*., 1993). Additionaly, Ayala *et al*., (1975) reported that all of four starfish species from deep-sea showed high genetic variation (*H*=10–20%). Further, Ayala and Valentine (1974) found that the ophiuropid, *Ophinomusium lymani* from deep-sea also showed the high genetic variation(*H*=17%). Kimura (personal communication) who proposed the neutral mutation theory (Kimura,1983) suggested that the species from deep-sea would have larger population size than those from shollow water species. In order to confirm the difference of genetic variation between deep-sea and shallow water, further extensive population genetic surveys in various marine invertebrates are required.

Several authors found one factor which is clearly related to heterozygosity at the protein level. It is quartenary structure of proteins (number of protein subunit). Zouros (1976) compared the amounts of electrophoretic variability of loci coding for multimeric enzymes (consisting of more than one polypeptide subunit) with those of loci coding for monomeric enzymes in four animal and two plant species. In all cases, he found that heterozygosities were lower for multimers than for monomers. He also found that within a given species or population, multimeric enzymes were less polymorphic than monomeric enzymes of similar function. That monomeric proteins show, on the average, singnificantly higher levels of hetrozygosity (and also higher polymorphism) than multimeric proteins has been confirmed by other workers (Ward, 1977; Harris *et al*., 1977). In this study,we examined the relationship between heterozygosity (*H* values) and the quaternary structure of enzymes analysed in five local populations of *A. pectinifera* (Table 3). As evident in Table 3, the monomer proteins showed the higher heterozygosity (*H* value) than the dimer proteins in all of five local populations. These results are well consistent with the

**Table 2.** Genetic variation in five local populations of the starfish *Asterina pectinifera.*

| Parameter | Esa | Shi | Asa | Fuka | Atsu |
|---|---|---|---|---|---|
| No. of genetic loci scored | 23 | 23 | 23 | 23 | 23 |
| No. of alleles per locus | 1.48 | 1.39 | 1.57 | 1.48 | 1.39 |
| Proportion of polymorphic loci: *P*(%) | 34.8 | 34.8 | 47.8 | 34.8 | 30.4 |
| Average heterozygosity per locus: *H*(%) | 13.0 | 11.7 | 12.5 | 10.6 | 12.1 |

Esa=Esashi; Shi=Shimoburo; Asa=Asamushi; Fuka=Fukaura; Atsu=Atsumi.

**Table 3.** Relationship between enzyme heterozygosity and quaternary structure in five local populations of the starfish *Asterina pectinifera*.

| Population | Enzyme Heterozygosity (*H*: %) | |
|---|---|---|
| | Monomer | Dimer |
| 1.Esashi | 16.0 | 2.4 |
| 2.Shimoburo | 13.8 | 5.7 |
| 3.Asamushi | 15.2 | 6.2 |
| 4.Fukaura | 14.8 | 3.2 |
| 5.Atsumi | 17.0 | 5.1 |

Monomer loci: HK, AMY-1, AMY-2, CAP-1, CAP-2, CAP-3 and CAP-4.
Dimer loci: XDH, PGI and SOD-1.
The indentification of monomer and dimer loci was inferred from the electrophoretic band patterns of enzymes.

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data obtained by the other workers (Nei,1987). These results strongly support the neutral theory of Kimura (1983). Namely, structural constraints for proper function must be greater for multimers (due to intersubunit interactions) than for monomers, and therefore when other things being equal, the fraction of mutations that are neutral (*i.e.*,not harmful) is smaller for multimeric enzyme loci than for monomeric ones.

In comparison between allozyme analysis and mtDNA analysis, Nei (1987) suggested that the resolving power of the latter is not necessarily higher than that of the former. This is particularly so when the restriction enzyme technique is used. According to the estimation of Nei (1987), electrophoresis is expected to survey about 100 nucleotides per locus. If we examine 30 loci by electrophoresis, it is equivalent to studying 3,000 base pairs at mtDNA level. Therefore, the resolving power of allozyme analysis is not lower than mtDNA analysis. Murphy *et al*. (1996) claimed that in phylogenetic and population genetic studies many molecular characters should be used and that the enzyme loci are the important molecular characters. The number of molecular characters adopted in protein electrophoresis is more enough than that of mtDNA study. Protein electrophoresis is one of the powerful techniques of estimating genetic variation within population.

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