

Geographic differentiation between populations of the starfish *Asterina pectinifera*

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Introduction

The population genetic studies of echinoderms had not been carried out for long time in Japan, though Japan has rich echinoderm fauna. As we have been studying the molecular phylogeny of echinoderms (sea-urchins and starfish), the starfish *Asterina pectinifera* (Fig.1) was adopted as a target species for the population genetic studies, because the starfish has many merits as follows. The starfish is common species and one of the symbols of seashore animals in Japanese waters. Therefore, it has been widely used as experimental animal in the fields of developmental biology, biochemistry or physiology for long time in Japan. Further, the starfish is widely distributed in Japanese waters, and thus it is suitable for the investigation of the genetic differentiation and relationships among local populations of the species. We consider that such studies are important to understand the speciation of echinoderms. *Asterina pectinifera* is more abundant in northern Japan than southern Japan and it can be easily collected the large individual numbers in northern parts of Japanese waters. Accordingly, the starfish is also much suitable for population genetic study that requires many samples. To clarify the genetic differentiation and relationships among local populations of the species at molecular level, we adopted the following five local populations from northern Japan: three populations (Esashi in Hokkaido, Fukaura in Aomori Pref. and



Fig.1. *Asterina pectinifera* used in the present study.

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Atsumi in Yamagata Pref.) from northern part of Japan Sea, one population (Shimoburo of Shimokita Peninsula in Aomori Pref.) from the Tsugaru Strait and one population (Asamushi in Aomori Pref.) from Mutsu Bay. The map of these localities is shown in Fig. 2. The marine fauna and geographical conditions of these localities are different to one another. As evident from this figure (map), populations from Mutsu Bay and Tsugaru Strait are considerably isolated from other three populations in Japan Sea by land barriers of Tsugaru Peninsula and Shimokita Peninsula in Aomori Pref., and Oshima Peninsula in Hokkaido. In the previous paper, we reported the extent of genetic variation within these five local populations and the maintenance mechanism (Matsuoka and Asano, 2003).

In this study, we have attempted to clarify the genetic differentiation and relationships among these five local populations of the starfish *Asterina pectinifera* by allozyme analysis.

Materials and Methods

The starfish *Asterina pectinifera* was collected from five different localities in northern Japan showing in Fig.2. 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 snorkeling by the present authors. After collection, the pyloric caeca were cut off from fresh specimens and stored at -40°C until use. Enzyme electrophoresis and allozyme analysis were performed as described in Matsuoka and Asano (2003) and Matsuoka and Hatanaka (1991). Enzymes analyzed in this allozyme study were 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 amino transferase (AAT), alkaline phosphatase (ALK), peroxidase (PO), esterase (EST), amylase (AMY) and cytosol aminopeptidase (CAP).

Results and Discussion

From allozyme variation in 12 enzymes described in the section of materials and methods, 23 genetic loci were scored. The genetic variation and the maintenance mechanism in populations of *A. pectinifera* have already

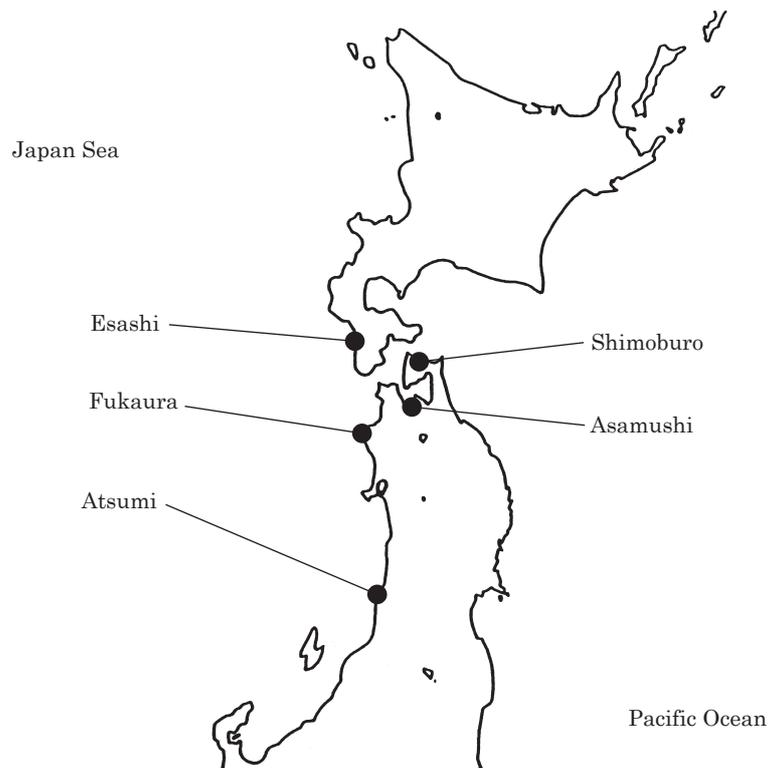


Fig.2. Map showing the geographic localities of five local populations of the starfish *Asterina pectinifera* in northern Japan examined in this study.

been reported and the allele frequencies data in 23 genetic loci are described in Matsuoka and Asano (2003). The statistically significant differences in the allele frequencies among five local populations were observed in the following seven genetic loci: *Mdh*, *Aat*, *Amy-2*, *Est-4*, *Lap-2*, *Lap-3* and *Po*. Fig.3 shows the allozyme variation in SOD.

To quantify the degree of genetic differentiation among five local populations, the genetic identity (I) and genetic distance (D) between each populations were calculated by the method of Nei (1972) from the allele frequencies data in 23 genetic loci. Table 1 shows the matrices of I and D values between all pairs of populations. One of the present authors (NM) has been studying the molecular phylogeny of echinoids from Japanese waters. As a part of serial studies, I reported the genetic identity (I) values between conspecific local populations of various echinoids. Table 2

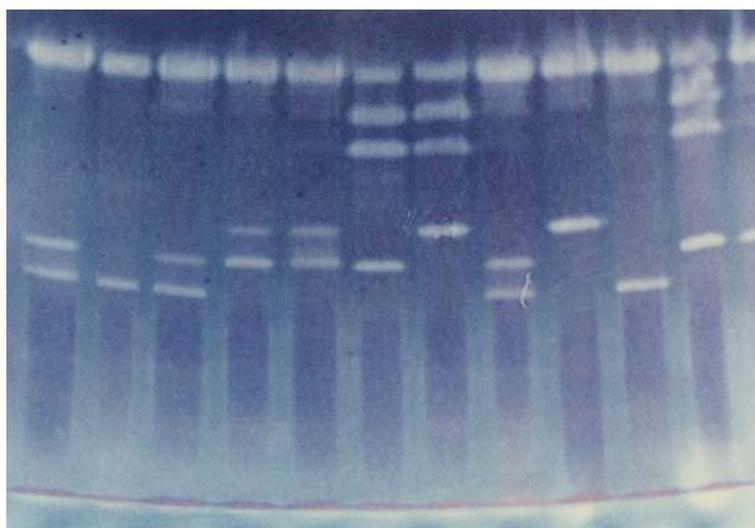


Fig.3. Allozyme variation of SOD in polyacrylamide gel electrophoresis. The bands in the faster zone showed the extensive allozyme variation. The genotypes of these band patterns from left to right are bc, cc, bc, ab, ab, bb, aa, bc, aa, cc, bb and bb. The a, b and c are alleles in the locus.

Table 1. Genetic identities (above diagonal) and genetic distances (below diagonal) between five local populations of *A. pectinifera*

Populations	1	2	3	4	5
1. Esashi	-	0.984	0.980	0.990	0.993
2. Shimoburo	0.016	-	0.990	0.974	0.985
3. Asamushi	0.020	0.010	-	0.960	0.986
4. Fukaura	0.010	0.026	0.041	-	0.985
5. Atsumi	0.007	0.011	0.014	0.015	-

Genetic identity (I) and genetic distance (D) were calculated by the method of Nei (1972).

Table 2. Genetic identities between conspecific local populations of various echinoid species and *Asterina pectinifera*

Species	No. of local populations	Genetic identity (I)	References
<i>Diadema setosum</i>	3	0.993-0.999	a
<i>Echinothrix calamaris</i>	2	0.985	a
<i>Asthenosoma ijimai</i>	2	0.917	b
<i>Stomopneustes variolaris</i>	2	0.955	c
<i>Strongylocentrotus nudus</i>	2	0.994	d
<i>Echinometra mathaei</i> (A-type)	2	0.990	e
<i>Anthocidaris crassispira</i>	6	0.933-0.992	f
<i>Echinostrephus aciculatus</i>	2	0.998	g
<i>Asterina pectinifera</i>	5	0.960-0.993	present study

a=Matsuoka (1989), b=Minokawa *et al.* (2002), c=Matsuoka and Nakamura (1991), d=Matsuoka *et al.* (1995), e=Matsuoka and Hatanaka (1991), f=Matsuoka and Suzuki (1989), g=Matsuoka and Suzuki (1987).

summarizes the species names, number of local populations examined, and the genetic identity (I) values between conspecific local populations in various echinoid species and *A. pectinifera* studied here. As evident from this table, the I values obtained in five local populations of *A. pectinifera* were comparable to those of various echinoid species belonging to the same echinoderms. Thorpe (1982) reported that there exists the positive correlation between the genetic identity (I) values and taxonomic ranks in many animal species, and that the I values between conspecific local populations are in the range of about 0.9-1.0. The present data were comparable to those of many other animals

To clarify the genetic relationships among five local populations, we constructed the molecular phylogenetic tree (Fig.4) for five local populations by using the UPGMA clustering method of Sneath and Sokal (1973). The molecular phylogenetic tree demonstrated that the five local populations are divided into two large clusters: one consists of three populations from Japan Sea (Fukaura, Atsumi and Esashi) and the other cluster consists of two populations from Mutsu Bay and Tsugaru Strait (Asamushi and Shimoburo). The genetic distance (D) between these two large clusters was $D=0.024$. The results are well consist with the geographical distribution of the five local populations of *A. pectinifera* (Fig.2 and Fig.5). Further, the allele frequencies data demonstrated the difference in allele compositions between the two large clusters. The two clusters are different from each other in allele frequencies of some genetic loci. For example, the "a" allele in *Mdh* locus was predominant in Mutsu Bay (Asamushi)- and Tsugaru Strait (Shimoburo)-populations, and the "b" allele in the same locus predominant in three Japan Sea (Esashi, Fukaura and Atsumi)-populations (Fig.5). As evident from Fig.2 and Fig.5, there exist the geographical barriers between these two clusters (populations from Japan Sea and those from Mutsu Bay and Tsugaru Strait): Oshima Peninsula in Hokkaido, Tsugaru Peninsula in Aomori Pref. and Shimokita Peninsula in Aomori Pref. The gene-flow between two clusters would be considerably limited by these geographical barriers.

According to Nei (1975), genetic distance (D) corresponds well with the divergence time (T) from the common ancestor, and T of two taxa can be estimated by $T=5 \times 10^6 D$ (years). From the application of this equation to the D values, it was calculated that the divergence time of the two large clusters was 12×10^4 (years).

We would like to discuss about the paleogeographical history of the Tsugaru Strait which exists between the two clusters with the divergence time of two large clusters estimated by the molecular clock. Based on the geological evidence, Ohshima (1976) estimated that the emergence time of the Tsugaru Strait might date back to $7-15 \times 10^4$ (about 10^5 years ago). The divergence time of 12×10^4 years ago between the two clusters which

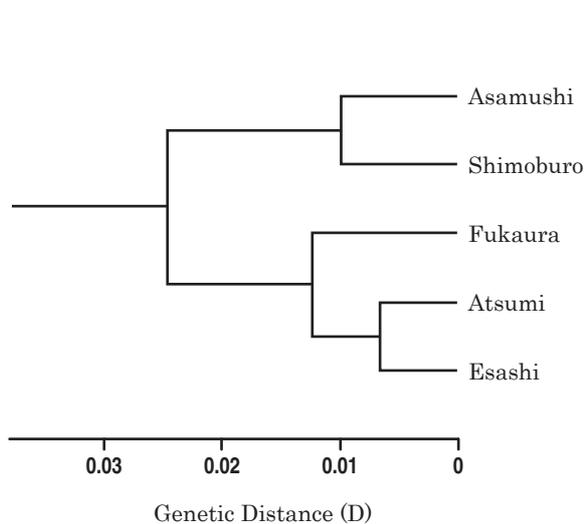


Fig.4. A molecular phylogenetic tree of five local populations of the starfish *Asterina pectinifera*. It was constructed from Nei's genetic distances (Nei, 1972) by the UPGMA clustering method of Sneath and Sokal (1973).

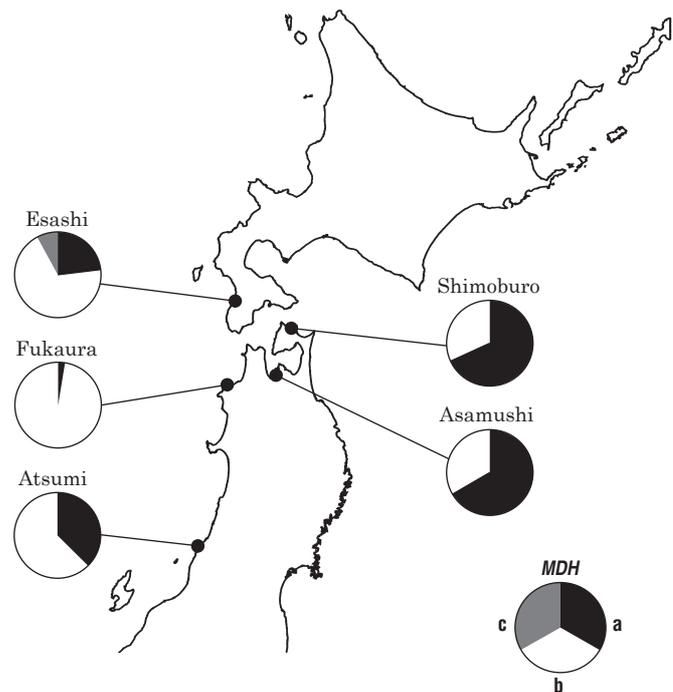


Fig.5. The circular graph showing the composition of allele frequencies at *Mdh* locus in five local populations of *Asterina pectinifera*.

was calculated from the genetic distances is in the range of the emergence time of the Tsugaru Strait estimated geologically ($7-15 \times 10^4$ years ago). Thus, it is suggested that the genetic divergence between the two clusters of local populations of *A. pectinifera* closely correlates with the emergence time of the Tsugaru Strait. Further, the geological evidence suggests that the outline of Mutsu Bay might be nearly like the present one about 10^5 years ago (Ohshima, 1976). From the molecular and geological evidence, we speculated the following possibility: There existed only one large population before the emergence of the Tsugaru Strait. The Mutsu Bay- and Tsugaru Strait-populations would be descended from the population in Japan Sea, and has accumulated the genetic differentiation from that time right up to the present.

In conclusion, the present allozyme study indicated the genetic differentiation between Mutsu Bay- and Tsugaru Strait- populations and Japan Sea-populations of the starfish *Asterina pectinifera*. This study would provide useful information for understanding the speciation of marine invertebrates.

Abstract

The genetic differentiation and relationships among five local populations of the common starfish *Asterina pectinifera* were studied by allozyme analysis of 12 enzymes. The localities of five local populations examined were as follows: Asamushi from Mutsu Bay; Simoburo from Shimokita Peninsula; Esashi, Fukaura and Atsumi from northern parts of Japan Sea. From allele frequencies data in 23 genetic loci scored, the genetic identities (I) and genetic distances (D) between populations were calculated by the method of Nei (1972). The genetic distance values obtained were comparable to those between conspecific local populations of other echinoderms and many other animals. To clarify the genetic relationships among five local populations, the molecular phylogenetic tree was constructed by the UPGMA clustering method. It indicated that the five local populations are divided into two large clusters: one consists of two populations (Asamushi and Simoburo) from Mutsu Bay and Tsugaru Strait, and the other consists of three populations (Esashi, Fukaura and Atsumi) from Japan Sea. This result was well consistent with the geographical distribution of five local populations. From the statistically significant difference of allele frequencies in some genetic loci, it was strongly suggested that the gene-flow between two clusters is considerably limited by the geographical barriers of three large peninsula (Tsugaru, Shimokita and Oshima Peninsula). The divergence time between two clusters estimated from molecular clock based on genetic distance was approximately consistent with the geological data of the emergence time of Mutsu Bay and Tsugaru Strait. The present allozyme study would provide useful information for elucidating the speciation of marine invertebrates.

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イトマキヒトデ地域集団の遺伝的分化

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和文要旨

海産無脊椎動物の地域集団間の遺伝的分化に関しては不明な点が多い。このような集団遺伝学的研究は海産無脊椎動物における種分化のメカニズムの解明に重要な意味を持つ。本研究では、日本近海の普通種である棘皮動物イトマキヒトデ (*Asterina pectinifera*) の5地域集団の遺伝的分化をアロザイム分析により調査した。分析した集団は、北海道・江差、青森県・深浦、山形県・温海、陸奥湾・浅虫、下北半島・下風呂の5地域集団である。12酵素のアロザイム分析により、23酵素遺伝子座が検出され、各遺伝子座における対立遺伝子頻度より集団間の遺伝距離を算出し、5地域集団の遺伝的分化および遺伝的關係を示す分子系統樹を作成した。その結果、5地域集団は大きく2つのグループに分かれた。1つは陸奥湾と津軽海峡の2集団（浅虫、下風呂）、他は日本海の3集団（江差、深浦、温海）からなる2つの大きなグループである。また、いくつかの遺伝子座における対立遺伝子頻度の統計学的有意差が2つのグループ間で観察されたことから、津軽半島・下北半島・渡島半島の地理的障壁により、日本海集団と陸奥湾・津軽海峡集団間で、かなり gene-flow（遺伝子拡散）が制限されている可能性が示唆された。本研究より、日本近海で広い分布域を有するイトマキヒトデは、地域集団間で確実に遺伝的組成の違いが蓄積されつつあることが判明した。今後、本研究は海産無脊椎動物、特に棘皮動物の種分化の解明に基礎的で有益なデータを提供することが期待される。