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GENETIC DIVERSITY AT EACH HABITAT OF *PSEUDOLABRUS SIEBOLDI*, USUAL FISH SPECIES IN A WESTERN JAPAN COASTAL AREA

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ABSTRACT: *Pseudolabrus sieboldi* is a very common labrid fish in Japan coastal region, so the species wasn't being watched so much up to now. Therefore, the habitats of *P. sieboldi* have been developed easily. But it's pointed out that genetic isolation easily occurred due to being polygyny in their mating system. In order to investigate the genetic diversity of usual species, we sampled 6 populations of the species form western Japan coastal area, and studied them for allelic variation at 24 enzyme loci. In comparison with other sea fishes, the total genetic diversity is standard. Though sex ratio was inclined to females and inbreeding were easy to be performed, the gene diversity was not decreasing. In fact, there was inbreeding mating within the population. Moreover, standard genetic distance within populations along the Japan Sea is high and standard genetic distance within Pacific Ocean area is low. The fry of the species dispersed to the Pacific or the Japan Sea. When they grew adults, they returned to rocky shores for mating. The populations along the Pacific Ocean did not return exactly at their birth area. On the other hand, the populations along the Japan Sea returned more exactly at their birth area.

Keywords: Genetic Diversity, Genetic Distances, Coastal Conservation, Pseudolabrus sieboldi, Mating System

1. INTRODUCTION

Recently it's thought that the conservation of genetic diversity is very important [1]-[3]. Many researchers have pointed out the importance of the genetic conservation. The maintenance of genetic diversity is important for endangered species. Though the same investigation was also necessary to usual species for genetic diversity to be maintained, few studies were done up to now. Especially biology in the sea cannot observed because the sampling from the sea is very difficult. Despite the coast living fish, it is very hard to collect a sufficient number of fishes. That is why the research of the sea living fish genetic analysis has not been done. But the development of the seacoast has been done by reclamation work. To understand about the biological aspects it is necessary that the research of the genetic diversity of coastal area about usual species.

Pseudolabrus sieboldi is very common labrid fishes along rocky shores in the East Asian temperate region. Recently Mabuchi and Nakano showed the species separated form *P. eoethinus* [4], differing in coloration and morphological characters. Mabuchi *et al*., also investigated the genetic diversity among 3 populations [5]. They collected 5 fishes each population, total 15 fishes, and investigated. There were some differences observed two labrid fishes. But they couldn't analyze the population structure because of low numbers of species. Matsumoto *et al*., also reported the mating system of the species, and they are polygamous [6]. Males of polygamous species have territory. When the females come to one male's territory, he mates her. Then he can hybrid some females come to his territory. Then the cohort of the mating is half brothers and sisters with the same father. The largest male can have any numbers of sons and daughters at one time. Polygamous species restricts gene flow and caused population isolation.

Maruyama's personal observation (2001) revealed the larva of the species was observed at the Pacific Ocean and the Japan Sea, not at coastal area [7]. The phenomenon suggested the adult fishes breed at coastal area, the larva fishes go to Ocean and growing fishes returned to the coastal area. The phenomenon suggests the gene flow easily occurred in Ocean. The strategy of the migration caused population uniformity. The polygamous mating system cannot cause the population isolation.

In this study, we investigated the genetic structure and genetic diversity of *P. sieboldi* to know the genetic structure of usual species with 3 questions, 1) Is there a relationship between the location and genetic distance? 2) Is there a difference between Pacific Ocean side and Japan Sea side? 3) Do the adult fishes return to the birth area? The attention was paid at above-mentioned 3 points in our study.

2. MATERIALS AND METHODS

2.1 Study Site

The samplings were carried out on 6 locations of west Japanese seashore (Fig. 1). Specimens (from 26 to 31 fishes) were collected from 6 localities widely distant from each other: 30 samples Nobeoka Bay at Miyazaki Pref., 26 samples Senzaki Bay at Yamaguchi Pref., 30 samples Miho Bay at Shimane Pref., 31samples Seto Bay at Nagasaki Pref., 28 samples Tasoura Bay at Mie Pref. and 30 samples Miyagawa Bay at Kanagawa Pref. In order to survey the genetic diversity of 20 specimens needed at each location.

Every location is affected by different sea currents. Miyazaki is the Seto Inland Sea area (inner sea area), Yamaguchi, Shimane, and Nagasaki are the Japan Sea area (affected by Japan Sea Current). Mie and Kanagawa are the Pacific Ocean area (affected by Kuroshio Current).

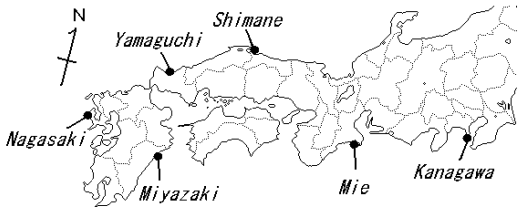

Fig. 1 Map of sampling localities.

2.2 The Study Fish

Pseudolabrus sieboldi Mabuchi and Nakano is a very common labrid fish in the coastal region in Japan (Fig. 2). They have white spits on the dorsal part of the body from initial to terminal phase (IP and TP, respectively). The species is known as the fish which does a sex change, and all larval fishes are being born as a female. Within a population the largest female exchanges her sex to male. A matured male makes the territory at sunken rocks and reproduces with a lot of females. Each male reproduces many sibs on one reproductive season.

Pair spawning occurred within TP male territories during the daytime. *P. sieboldi* spawned smaller rocks (ca. 0.5m in height) with seaweed and seagrass, whereas *P. eoethinus* usually above large rocks of 1m or more in height. The season and time of spawning of the species widely overlapped, spawning occurring from mid-November to mid-December. The two species have the same niche, the species differentiation occurred by the mating system.

(a) IP (b) TP

Fig. 2. A different phase of *P. sieboldi*, left side (a) is IP (initial phase female) and right side (b) is TP (terminal phase male).

2.3 Electrophoresis

All fresh fishes were collected from 26-31 individuals per population in 2004 and 2005. Fishes were kept on ice during transportation to the laboratory within a day. A part of liver tissues, fin tissues, tail tissues and muscle tissues were cut and homogenized being added with an approximately equal amount of 0.5M sucrose solution. And homogenized tissues were centrifuged at 10,000rpm at 5 minutes. The supernatants were subjected to horizontal starch gel electrophoresis. A tris-citrate buffer system no.5 (Soltis *et al*. [8]) was used. Starch gels were run for 2 hours at 80mA in a refrigerator at 4ºC. During the electrophoresis ice bag with water was laid above the starch gel in order to maintain a low temperature. After the run, the gel was sliced horizontally that have a thickness of 1mm. The following enzyme systems were examined: triose-phosphate isomerase (TPI), malate dehydrogenase (MDH), acid phosphate (ACP), mannose phosphate isomerase (MPI), Glycerophosphate dehydrogenase (GPD), 6-phosphogluconate dehydrogenase (6PG), phospho-glucose isomerase (PGI), malic enzyme (ME), Alcohol dehydrogenase (ADH), aldrase (ALD), menadione reductase (MR), phosphoglucomutase (PGM) and glucose 6 phosphate dehydrogenase (G6PDH). Muscle tissues were used to resolve the following 19 loci: tpi-1, tpi-2, mdh-1, mdh-2, mdh-3, mdh-4, acp-1, acp-2, mpi-1, mpi-2, gpd, 6pg, pgi, me, adh, ald, mr, pgm and g6pdh. Liver tissues were correspondingly used for 5 loci mdh, me-1, me-2, pgm and adh. We followed previous works [8][9][10] about staining.

2.4 The Statistical Analysis

The number of alleles per locus (*A*) that is the mean number of alleles at each locus, proportion of polymorphic loci (*P*) that is the rate of polymorphic loci to the total loci, and gene diversity (*h*) were calculated on each population. Additionally, total gene diversity [11] was calculated for species level. The population genetic structure was analyzed by initially calculating Nei's G_{ST} value [11]. Values for

standard genetic distance (D) was calculated for each pairwise comparison of all populations. The neighbor-joining method [12] based on D was used for constructing a phenogram for *P. sieboldi*.

3. RESULTS

Table 1 Allele frequencies of 24 polymorphic loci of 6 examined populations of *P. sieboldi*.

	allele	Miyazaki	Yamaguchi	Shimane	Nagasaki	Mie	Kanagaw
tpi-1	n	30	26	30	31	28	30
	a	0.717	1.000	0.767	0.919	1.000	0.933
	b	0.283	0.000	0.233	0.081	0.000	0.067
tpi-2	n	30	40	28	32	28	30
	a	0.767	0.860	0.750	0.969	0.964	1.000
	b	0.233	0.140	0.250	0.031	0.036	0.000
mdh-1	n	17	33	12	32	28	30
	a	0.294	0.500	1.000	1.000	1.000	0.917
	b	0.706	0.500	0.000	0.000	0.000	0.083
mdh-2	n	40	33	30	32	28	30
	a	0.957	0.500	0.667	1.000	1.000	0.983
	b	0.043	0.500	0.333	0.000	0.000	0.017
mdh-3	n	17	33	30	32	28	30
	a	1.000	0.500	0.700	0.031	1.000	0.967
	b	0.000	0.500	0.300	0.969	0.000	0.033
mdh-4	n	40	33	30	32	28	30
	a	0.723	0.500	0.617	0.953	1.000	0.950
	b	0.277	0.500	0.383	0.047	0.000	0.050
acp-1	n	40	33	30	32	28	30
	a	0.489	1.000	1.000	0.906	0.929	0.967
	b	0.447	0.000	0.000	0.094	0.071	0.033
	c	0.064	0.000	0.000	0.000	0.000	0.000
acp-2	n	40	33	30	32	28	30
	a	0.702	1.000	1.000	0.891	0.929	1.000
	b	0.298	0.000	0.000	0.109	0.071	0.000
mpi-1	n	40	33	12	25	28	30
	a	0.053	1.000	1.000	0.800	1.000	0.967
	b	0.798	0.000	0.000	0.040	0.000	0.033
	c	0.149	0.000	0.000	0.160	0.000	0.000
mpi-2	n	30	33	12	32	28	30
	a	0.217	1.000	1.000	1.000	1.000	0.967
	b	0.783	0.000	0.000	0.000	0.000	0.033
gpd-1	n	30	33	12	33	28	30
	a	0.667	0.409	0.917	1.000	0.929	0.900
	b	0.333	0.591	0.083	0.000	0.071	0.100
6pg-1	n	17	33	12	33	28	30
	a	0.706	1.000	1.000	1.000	0.929	0.967
	b	0.294	0.000	0.000	0.000	0.071	0.033
pgi-1	n	17	33	12	33	28	30
	a	0.206	0.227	0.583	1.000	0.768	0.983
	b	0.412	0.697	0.417	0.000	0.232	0.017
	c	0.206	0.076	0.000	0.000	0.000	0.000
	d	0.176	0.000	0.000	0.000	0.000	0.000
me-1	n	40	22	26	32	28	30
	a	0.633	0.636	0.673	1.000	1.000	1.000
	b	0.367	0.364	0.327	0.000	0.000	0.000
adh-1	n	17	33	12	32	28	30
	a	0.176	0.970	0.917	0.938	0.732	0.867
	b	0.824	0.030	0.083	0.063	0.268	0.133
ald-1	n	17	33	12	32	28	30
	a	0.588	1.000	0.875	1.000	1.000	0.967
	b	0.412	0.000	0.125	0.000	0.000	0.033
ms-1	n	17	33	12	32	28	30
	a	0.882	0.970	1.000	0.953	0.875	0.867
	b	0.118	0.030	0.000	0.047	0.125	0.133
pgm-1	n	17	33	30	32	28	30
	a	0.235	1.000	0.700	0.813	0.929	1.000
	b	0.647	0.000	0.300	0.188	0.071	0.000
	c	0.118	0.000	0.000	0.000	0.000	0.000
g6pdh-1	n	17	33	12	33	28	30
	a	0.059	1.000	1.000	1.000	1.000	0.967
	b	0.941	0.000	0.000	0.000	0.000	0.033

from muscle tissue

	allele	Miyazaki	Yamaguchi	Shimane	Nagasaki	Mie	Kanagawa
mdh-5	n	17	32	12	32	28	30
	a	1.000	1.000	0.875	0.125	0.804	1.000
	b	0.000	0.000	0.125	0.875	0.196	0.000
me-2	n	17	33	12	20	28	30
	a	0.265	1.000	1.000	0.850	1.000	0.933
	b	0.647	0.000	0.000	0.150	0.000	0.067
	c	0.088	0.000	0.000	0.000	0.000	0.000
me-3	n	17	33	12.000	32	28	30
	a	1.000	1.000	1.000	0.938	1.000	0.967
	b	0.000	0.000	0.000	0.063	0.000	0.033
pgm-2	n	17	33	12	27	28	30
	a	0.000	0.000	0.000	0.019	0.000	0.000
	b	0.118	0.000	0.000	0.111	0.018	0.000
	c	0.471	0.152	1.000	0.185	0.089	0.067
	d	0.235	0.848	0.000	0.593	0.893	0.900
	e	0.176	0.000	0.000	0.093	0.000	0.033
adh-2	n	17	32	12	32	28	30
	a	0.176	0.212	0.833	0.938	0.964	1.000
	b	0.824	0.789	0.167	0.063	0.036	0.000

from liver tissue data n: number of individuals

24 loci were scored (19 for muscle and 5 for liver) in Table1. 4 loci were scored (2 for fin and 3 for tail). The enzyme activity was very low level at fin and tails. Furthermore, all loci were similar to those of muscle. Then we did not use the result of fin and tails. All loci have at least two alleles in at least one population.

Fig.3 showed the 20 individuals data on Miyazaki population of starch gel condition staining MDH.

Fig. 3 Staining starch gel at Miyazaki population staining MDH.

Table 2 summarizes the resultant values of *A*, *P* and *h* for each population. *A* was from 1.5 to 2.2, *P* was from 0.5 to 0.9 and *h* was 0.09 to 0.21. Especially every genetic diversity value was very high in Miyazaki population.

Table 2. Mean a number of polymorphic loci (*A*), the proportion of polymorphic loci (*P*), and gene diversity within a population *(h*) at 24 loci for examined populations of *P. sieboldi.*

Population	*A*	*P*	*h*
Miyazaki	2.208	0.917	0.206
Yamaguchi	1.583	0.500	0.217
Shimane	1.542	0.542	0.092
Nagasaki	1.792	0.625	0.091
Mie	1.542	0.500	0.028
Kanagawa	1.792	0.750	0.019

And total gene diversity (H_T) of the species was

0.278. The levels of genetic diversity in *P. sieboldi* was high that of other fish species, for example, *Sonoran topminnow* of 0.03 to 0.116 [13].

The mean level of isozyme variation within populations and the total gene diversity of the species are given in Table 3. The resultant hierarchical analysis of population genetic structure is G_{ST}. G_{ST} of Pacific Ocean is 0.03, on the other hand, G_{ST} of Japan Sea is 0.31. It was ten times difference between the Japan Sea and the Pacific Ocean. That of the Japan Sea was higher than that of the Pacific Ocean.

Table 3. Inbreeding coefficient within a population (F_{IS}), Fixation index (F_{ST}) and inbreeding coefficient among total populations(F_{IT}).

Population	F_{IS}	F_{ST}	F_{IT}
Miyazaki	0.415	-0.262	0.262
Yamaguchi	-0.196	0.347	0.220
Shimane	0.504	0.336	0.670
Nagasaki	0.236	0.570	0.672
Mie	0.703	0.657	0.898
Kanagawa	0.754	0.716	0.930

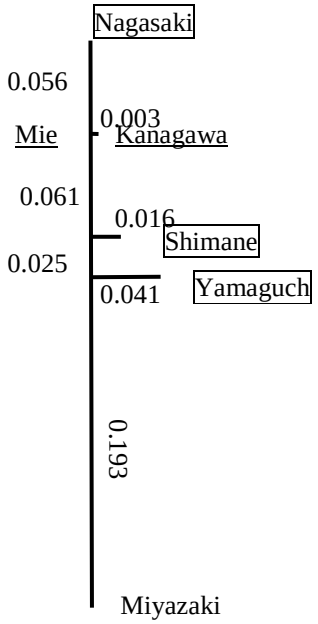

Fig. 4 Phenogram using the neighbor-joining method based Nei's (1987) standard genetic distance. Underline locations are the Pacific Ocean area, locations are the Japan Sea locations.

Fig. 4 showed the phenogram using the NJ methods based on Nei's standard genetic distance [12]. The standard value of the genetic distance is 0.01. If the genetic distance is under 0.01, the genetic diversity is very low. When the genetic distance is over 0.01, genetic diversity is caused by the mating system. The Fig. 3 showed population at the Pacific Ocean area differentiation is low, on the other hands, the Japan Sea area caused the differentiation. And Miyazaki population was far from other population. This area is very different from other sea current and holds high gene diversity. And there are populations of the Pacific Ocean coastal area among the Japan Sea coastal area. Kanagawa and Mie population belonged to the Japan Sea populations.

4. DISCUSSION

Mabuchi and Nakano investigated the genetic diversity of the species [5]. They were investigated by examination of partial sequences of the mitochondrially encoded 16S rRNA gene. Total 9 individuals representing three population were sequenced. Sequence differences within the species were 0.7% and the value was higher than that of *P. eoehinus.* There was some gene diversity among populations. But the sampling size was 5 every 3 populations (total number was 9). Then they cannot analyze the genetic structure of the species. They suggested the gene diversity occurred among the populations. In this study, we can collect over 25 individuals from 6 populations and analyze the genetic structure along the sea coast of Japan.

In comparison with other sea fishes, the total gene diversity is standard [1][2]. A mean number of polymorphic loci is from 1.0 to 3.6, the proportion of polymorphic loci is from 0.1 to 0.95. Despite the *P. sieboldin* is a usual species. the gene diversity is usual. They cannot maintain high gene diversity with the large number.

But there are some differences in gene diversity among populations. The gene diversity within a population (*h*) of Mie and Kanagawa (Pacific Ocean side) was lower than those of other population. The value of *h* is from 1/4 to 1/6 of the expected value. Moreover, the value of the fixation index at the Pacific Ocean side populations was higher than those of others. The value of the fixation index showed the mating system. When there is an inbreeding mating (kin-reproduction) in the population, the fixation index close to 1.0. If the random mating occurred, the value of the fixation index is 0. The value ranged from 0 to 1.0. The result showed that inbreeding occurred within a population and the effective population size is small at the Pacific Ocean side populations.

Furthermore, there was no genetic diversity within the Pacific Ocean area, they cannot return the borne area. Along the Pacific Ocean coast at Japan there are many industrial zoning districts, the vast sea zone was reclaimed by the Ministry of Land. Infrastructure and Transport. 85% of seashore was developed during some decades. Growing up labrid returned the borne area, but they cannot return the reclamation. The selection occurred by the

reclamation. Only the adult labrid that can be swimming until reaching suitable area can succeed the reproduction, so the gene flow is dependent on the construction. This is very severe selection, so many numbers of diversity must be extinct.

The inbreeding was expected from the mating system of the labrid species. But there was no inbreeding at other 4 populations. This suggested that there is a sufficient number of population along the Japan Sea and Set Inner Sea. This data is suited to the development of the Japanese industrial development or the geographical feature. There are many rocky habitats in the Japan Sea and the Seto Inland Sea. The adults of rabrid can easily detect the rocky seashore area near the born area.

Our data show another interesting fact. Standard genetic distance within populations along the Japan Sea is high (D=0.180). On the other hand, that within the Pacific Ocean side is low (D=0.000). It showed the gene flow easily occurred along the Pacific Ocean side but hardly occurred along the Japan sea side. And there was no relationship between real distance and genetic distance. From the phenogram populations of the Japan Sea side contains the Pacific Ocean side populations.

The fry of the species disperses to the Pacific Ocean or the Japan Sea. When the fry is grown up, they return along rocky shores for reproduction. The populations along the Japan Sea side return their birth area exactly because of the genetic distances over 0.01. But the populations along the Pacific Ocean side did not return to the birth area exactly due to the genetic distance value within 0.01, so there was high gene flow among populations.

The reason for the phenomenon is the climate. The typhoons attacked several times per year along the Pacific Ocean seashore area. So the fry of the rabrid moved easily at the typhoon. The reproductive season is late autumn after the typhoon, some gene flow occurred along the Pacific Ocean area. When the fry went to south sea area, the fry can ride the different ocean current. When the mixed occurred along the different current, the phylogeny of our study will be probable. The species try to return the birth area, but there is much trouble to prevent the return, climate, development, current and so on.

Our investigation showed the genetic diversity and phylogeny and mating system. There were some differences among the populations along the sea system even usual species. The interesting results showed but the systems of the difference among the population cannot be realized. In order to understand the systems of the labrid we must study ecology, the phenology and the life cycle of the species. It is necessary to understand the ecology that new tools for marking the individuals. The new technology in salinity durable will be needed.

5. CONCLUSION

We get three main conclusions.

1) There is no relationship between the real distance and genetic distance.
2) There is a difference between the Pacific Ocean side population and the Japan Sea side population. The Pacific Ocean side populations have low genetic diversity and easily occurred inbreeding. The Japan Sea side populations have high genetic diversity and isolated each population.
3) The matured individuals returned exactly to the Japan Sea side population, on the other hand, mixed return occurred at the Pacific Ocean side populations.
4) There was the difference among the population about genetic distance, mating system and genetic diversity at usual species. It is suggested that the local extinction occurred at usual species along the seashore.

Based on the conclusions, four proposals about the conservation activities are considered

1) There is a possibility of inbreeding in the polygamous mating system species, so the investigation should be done at usual species, because of low genetic diversity.
2) There is a possibility of local extinctions in usual species, the investigation should be done in every area before the development.
3) We should know the differences between the sea current and climate between the Japan Sea area, the Seto Inland Sea and the Pacific Ocean area.
4) We should know about the ecology of sea species because of the development with too much speed.

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