Biochemical Genetic Variations in Black Seabream

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The samples of three lots of black seabream *Acanthopagrus schlegelii* (BLEEKER) collected from the coastal waters of Okayama, Osaka, and Aichi were used for the screening of polymorphism in eighteen enzymes, sarcoplasmic proteins, and hemoglobins. Among 44 loci examined, 16 loci of the ten enzymes and sarcoplasmic proteins were polymorphic. The observed numbers of phenotypes in respective loci well agreed with the Hardy-Weinberg expectations. The genetic model of each polymorphic enzyme was discussed. Most of the polymorphic loci were dimer, except for phosphoglucosmutase, esterase-2, sarcoplasmic protein-1, and sarcoplasmic protein-2 which were monomer, and sarcoplasmic protein-3 which was tetramer.

The genic variability in this species was relatively high compared with other marine fishes. According to the chi-square values for heterogeneity between two locations, Okayama was considered to be different from the other two locations. Based on the low values in F-statistics (F_{ST}) and genetic distance (D), we concluded that the differentiation among localities was very small.

In recent years, mariculture in Japan has been making a rapid progress. The black seabream *Acanthopagrus schlegelii* (BLEEKER), is one of the important marine fish cultivated widely. Artificial breeding of this species has been applied successfully for fish seed mass production. The fish seed has been used not only for stocking of the cage culture, but also for releasing to the natural waters for conservation of the sea resources.

Electrophoresis of various proteins or enzymes has been applied to biological research of both basic and applied interests.¹⁾ In Japan, the electrophoretic method has been widely used for genetic population study of some freshwater fish, marine fish, and anadromous fish. Since MOAV *et al.*²⁾ proposed to use the electrophoretic genetic markers to fish breeding study, these markers were applied for the breeding study of common carp *Cyprinus carpio*,³⁻⁴⁾ coho salmon *Oncorhynchus kisutch*,⁵⁾ and nibe croaker *Nibea mitsukurii*, and red seabream *Pagrus major*.⁶⁾

In this study, the electrophoretic patterns of polymorphic enzymes and proteins examined in black seabream will be shown using three lots of samples collected from the coastal waters of Okayama, Osaka, and Aichi. The results may be important as the basic knowledge for the future development in the breeding study of this species.

Materials and Methods

Black seabream samples were collected from

the coastal waters of Okayama Pref. (80 specimens), Osaka Pref. (32 specimens), and Aichi Pref. (72 specimens) during August to November 1980. The fish samples were stored at -20°C until used. The skeletal muscle, liver, heart, and eye were dissected for electrophoretic analysis. The preparation of enzyme and protein samples and horizontal starch-gel electrophoretic procedures are given by TANIGUCHI and NUMACHI.⁷⁾ Two buffer systems used were tris-citric acid (T-C) pH 8.0 and citric acid-aminopropylmorpholine (C-APM) pH 6.0.

The enzymes examined here were as follows: Adenylate kinase (AK, E.C. 2.7.4.3), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), aspartate aminotransferase (AAT, E.C. 2.6.1.1), creatine kinase (CK, E.C. 2.7.3.2), esterase (EST, E.C. 3.1.1.3), fumarate hydratase (FH, E.C. 4.2.1.2), glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49), glutamate dehydrogenase (GuDH, E.C. 1.4.1.2), glucosephosphate isomerase (GPI, E.C. 5.3.1.9), α -glycerophosphate dehydrogenase (α -GDH, E.C. 1.1.1.8), D(-)-3-hydroxybutyrate dehydrogenase (HBDH, E.C. 1.1.1.30), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), phosphoglucosmutase (PGM, E.C. 2.7.5.1), 6-phosphoglucosmutase dehydrogenase (6-PGD, 1.1.1.44), and sorbitol dehydrogenase (SDH, E.C. 1.1.1.14). The list of enzymes and proteins, tissue assayed, buffer systems used, and

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Table 1. The list of enzymes and proteins examined, organs assayed, buffer systems used, and references for staining method

Enzyme and protein	Organ	Buffer	Staining method
Adenylate kinase (AK)	Heart	C-APM	SHAW and PRASAD ⁹⁾
Alcohol dehydrogenase (ADH)	Liver	T-C	TANIGUCHI <i>et al.</i> ¹⁰⁾
Aspartate aminotransferase (AAT)	Muscle	C-APM	TANIGUCHI and NUMACHI ⁷⁾
Creatine kinase (CK)	Liver	C-APM	SHAW and PRASAD ⁹⁾
Esterase (EST)	Liver	T-C	SHAW and PRASAD ⁹⁾
Fumarate hydratase (FH)	Muscle	T-C	SHAW and PRASAD ⁹⁾
Glucose-6-phosphate dehydrogenase (G-6-PDH)	Muscle	T-C	SHAW and PRASAD ⁹⁾
Glucosephosphate isomerase (GPI)	Muscle, heart	C-APM	SHAW and PRASAD ⁹⁾
Glutamate dehydrogenase (GuDH)	Liver	T-C	SHAW and PRASAD ⁹⁾
α -Glycerophosphate dehydrogenase (α -GDH)	Muscle, liver	T-C	NUMACHI ¹¹⁾
Hemoglobin (HEM)	Blood	C-APM	Amido black 10 B
D(-)3-hydroxybutyrate dehydrogenase (HBDH)	Liver	T-C	SHAW and PRASAD ⁹⁾
Isocitrate dehydrogenase (IDH)	Muscle, liver	C-APM	TANIGUCHI and NUMACHI ⁷⁾
Lactate dehydrogenase (LDH)	Muscle, heart, eye	T-C	NUMACHI ¹¹⁾
Malate dehydrogenase (MDH)	Muscle	T-C	NUMACHI ¹¹⁾
Malic enzyme (ME)	Muscle, heart	T-C	AYALA <i>et al.</i> ¹²⁾
Phosphoglucumutase (PGM)	Liver	C-APM	SHAW and PRASAD ⁹⁾
6-Phosphogluconate dehydrogenase (6-PGD)	Liver	C-APM	SHAW and PRASAD ⁹⁾
Sarcoplasmic protein (SP)	Muscle	pH 3.5-9.5	LUNDSTROM ¹³⁾
Sorbitol dehydrogenase (SDH)	Muscle, liver	T-C	SHAW and PRASAD ⁹⁾

T-C: tris-citric acid pH 8.0.

C-APM: citric acid-aminopropyl morpholine pH 6.0.

staining references are presented in Table 1.

Isoelectric focusing was applied to the special separation of sarcoplasmic protein. For the materials and procedure of isoelectric focusing see TANIGUCHI *et al.*⁹⁾

Results and Discussion

Polymorphism and Its Genetic Control

Eighteen enzymes, sarcoplasmic proteins, and hemoglobins (Table 1) encoded by 44 loci were examined by electrophoretic method. Among 44 loci, 16 loci were polymorphic. The remaining 16 loci of enzymes, 9 loci of sarcoplasmic proteins, and 3 loci of hemoglobins were monomorphic. Diagramatic representation of relative electrophoretic mobility of the polymorphic enzymes are provided in Fig. 1. The brief description of the polymorphic enzymes are as follows:

ADH: This enzyme moved to the cathodal zone which is considered to be controlled by three alleles of one locus. The heterozygous type has three bands, so that the protein structure should be a dimer in sub-unit composition. The slower and the faster homozygous types and type AC did

not appear in all of the samples examined.

CK: The one locus with two alleles model was presumed for this enzyme which appeared in the anodal zone. The heterozygous type has three bands and the homozygous one band. No fast homozygous band (phenotype A) was detected in the samples tested this time.

EST: Esterase enzyme appeared in the anodal zone which supposed to be consisted of two loci, namely *Est-1* and *Est-2*. Each locus has two bands for heterozygous type and one band for homozygous type, so that these two loci must be a monomer. Since one band (phenotype C) of *Est-1* was located in the *Est-2* zone, it was rather difficult to distinguish the phenotypes C, CB, and CA from each other. The banding pattern of *Est-1* indicated that this locus is controlled by three alleles, and *Est-2* locus by two alleles.

α -GDH: Two loci were detected in this enzyme, liver specific isozyme (α -Gdh-1) and muscle specific isozyme (α -Gdh-2) which both appeared in the anodal zone. The α -Gdh-1 locus was presumed to be controlled by three alleles, and α -Gdh-2 by two alleles. The heterozygous type of both locus has three bands, so that this enzyme

Fig. 1. Isozyme patterns of polymorphic enzymes in black seabream. As for the SP pattern see TANIGUCHI *et al.*⁸⁾ Phenotypes in parenthesis were not yet found. The start line is shown by "O".

may be dimeric in protein structure.

IDH: This enzyme was encoded by three alleles of one locus in the anodal zone. The heterozygous type has three bands, so that this enzyme should dimeric in sub-unit composition. The phenotypes A, AC and C were not yet detected in all of the samples examined here.

MDH: This enzyme was detected in the anodal zone, and consisted of three loci named *Mdh-1*, *Mdh-2*, and *Mdh-3*. Polymorphism was found at *Mdh-1* and *Mdh-3* loci, and each locus has three alleles type. The heterozygous type has three bands as dimeric structure in sub-unit composition.

6-PGD: The relative mobility and genetic model of 6-PGD were more or less similar to IDH.

PGM: This enzyme was controlled by two alleles. The heterozygous type showed two bands, so that this enzyme should be monomeric in molecule structure. Under the electrophoretic conditions applied, one band of heterozygous type was located in the anodal zone and the other in the cathodal zone, very near the origin.

GPI: There were two loci of GPI, *Gpi-1* in

the anodal zone and *Gpi-2* in the cathodal zone. The bands appearing near the origin was located in the middle zone between *Gpi-1* and *Gpi-2* and supposed to be heterodimer. *Gpi-1* was a non-polymorphic locus, but *Gpi-2* was polymorphic under the control of three alleles with banding pattern as dimeric structure.

SDH: The polymorphism of SDH enzyme was detected in the liver, and appeared as one locus controlled by two alleles. Under the electrophoretic conditions used, we could not ascertain the band number of heterozygous type.

SP: Three loci in sarcoplasmic isoelectric focusing pattern were polymorphic loci, namely *Sp-1*, *Sp-2*, and *Sp-3*. The *Sp-1* locus was of a monomeric structure. The *Sp-2* also was presumed to be a monomeric structure, but has an unusual number of band: the homozygous type has two bands and the heterozygous type has four bands. The heterozygous type of *Sp-3* locus has five bands, so that this locus was considered to be related to a tetrameric structure. For the detailed discussion see TANIGUCHI *et al.*⁸⁾

The observed number and their HARDY-WEINBERG expectation of phenotypes are presented in Table 2. This table also shows the allelic frequencies and chi-square values for the differences between observed and expected number of phenotypes of each polymorphic enzyme and each locality. The data in Table 2 indicated that the differences between observed numbers and the HARDY-WEINBERG expectations were not significant at $P < 0.05$ in the respective polymorphic loci examined here. These facts support the validity of the genetic model proposed for each polymorphic enzymes, and suggest that those black seabream samples from each location were collected from the single Mendelian population.

Genic Variability

The genic variability of black seabream from each locality are presented in Table 3. There was no clear difference on the genic variability among localities, except the small difference which may be due to the difference in sample number. Number of allele per locus ranged from 1.272 in Osaka to 1.386 in Okayama. It was relatively high compared with the ayu, *Plecoglossus altivelis*¹⁴⁾ and nearly similar to the red seabream, *Pagrus major*.¹⁵⁾

The proportion of polymorphic loci ranged from 0.250 to 0.341 (average 0.303). It was higher than some other members of order Percida¹⁶⁾ but a little lower than red seabream.¹⁵⁾ On the other hand the average value of hetero-

Table 2. Observed number of phenotypes, their HARDY-WEINBERG expectations in parenthesis and allelic frequencies at polymorphic loci in black seabream

Locus	Locality	Phenotype						N	Allelic frequency			χ^2
		A	AB	AC	B	BC	C		A	B	C	
<i>Adh</i>	Okayama	0	0	0	78 (78.0)	2 (2.0)	0 (0.0)	80	0	0.987	0.013	0.013
	Osaka	0 (0.0)	1 (1.0)	0 (0.0)	30 (30.0)	1 (1.0)	0 (0.0)	32	0.016	0.969	0.016	0.000
	Aichi	0 (0.0)	2 (2.0)	0 (0.0)	69 (69.0)	1 (1.0)	0 (0.0)	72	0.014	0.979	0.007	0.003
<i>Ck</i>	Okayama	0 (0.1)	4 (3.9)	—	56 (56.1)	—	—	60	0.033	0.967	—	0.071
	Osaka	0	0	—	32	—	—	32	0	1.000	—	0
	Aichi	0 (0.0)	1 (1.0)	—	71 (71.0)	—	—	72	0.007	0.993	—	0.004
<i>Est-1</i>	Okayama	51 (50.4)	4 (4.0)	21 (22.2)	0 (0.1)	1 (0.9)	3 (2.5)	80	0.794	0.031	0.175	0.294
	Osaka	19 (18.0)	1 (1.5)	9 (10.5)	0 (0.0)	1 (0.4)	2 (1.5)	32	0.750	0.031	0.219	1.334
	Aichi	32 (30.0)	5 (6.5)	24 (26.5)	1 (0.3)	3 (2.8)	7 (5.8)	72	0.646	0.069	0.285	2.158
<i>Est-2</i>	Okayama	31 (33.8)	42 (36.4)	—	7 (9.8)	—	—	80	0.650	0.350	—	1.893
	Osaka	15 (14.4)	13 (14.1)	—	4 (3.4)	—	—	32	0.672	0.328	—	0.198
	Aichi	29 (27.3)	30 (33.5)	—	12 (10.3)	—	—	71	0.620	0.380	—	0.761
α - <i>Gdh-1</i>	Okayama	—	—	—	27 (26.4)	11 (12.2)	2 (1.4)	40	—	0.813	0.188	0.380
	Osaka	—	—	—	28 (28.1)	4 (3.8)	0 (0.1)	32	—	0.938	0.063	0.142
	Aichi	0 (0.0)	1 (0.9)	0 (0.1)	54 (55.1)	17 (14.9)	0 (1.0)	72	0.007	0.875	0.118	1.469
α - <i>Gdh-2</i>	Okayama	80	0	—	0	—	—	80	1.000	0	—	0
	Osaka	32	0	—	0	—	—	32	1.000	0	—	0
	Aichi	71 (70.0)	1 (1.0)	—	0 (0.0)	—	—	72	0.993	0.007	—	0.014
<i>Gpi-2</i>	Okayama	0 (0.0)	1 (1.0)	0 (0.0)	78 (78.0)	1 (1.0)	0 (0.0)	80	0.006	0.988	0.006	0.013
	Osaka	0	0	0	32	0	0	32	0	1.000	0	0
	Aichi	0	0	0	72	0	0	72	0	1.000	0	0
<i>Idh</i>	Okayama	0 (0.2)	7 (6.7)	0 (0.0)	72 (72.2)	1 (0.9)	0 (0.0)	80	0.044	0.950	0.006	0.002
	Osaka	0 (0.0)	1 (1.0)	0 (0.0)	30 (30.0)	1 (1.0)	0 (0.0)	32	0.016	0.969	0.016	0.033
	Aichi	0 (0.0)	1 (1.0)	—	71 (71.0)	—	—	72	0.007	0.993	—	0.004
<i>Mdh-1</i>	Okayama	1 (0.1)	3 (4.8)	—	76 (75.1)	—	—	80	0.031	0.969	—	0.061
	Osaka	0	0	—	32	—	—	32	0	1.000	—	0
	Aichi	0 (0.0)	1 (1.0)	—	71 (71.0)	—	—	72	0.007	0.993	—	0.004
<i>Mdh-3</i>	Okayama	71 (70.3)	8 (9.4)	—	1 (0.0)	—	—	80	0.938	0.063	—	0.035
	Osaka	29 (29.0)	3 (2.9)	—	0 (0.1)	—	—	32	0.953	0.047	—	0.077
	Aichi	71 (71.0)	1 (1.0)	—	0 (0.0)	—	—	72	0.993	0.007	—	0.004

Table 2. Continued

Locus	Locality	Phenotype						N	Allelic frequency			X ²
		A	AB	AC	B	BC	C		A	B	C	
6-Pgd	Okayama	—	—	—	67 (67.5)	13 (11.9)	0 (0.5)	80	—	0.919	0.081	0.626
	Osaka	—	—	—	26 (26.3)	6 (5.4)	0 (0.3)	32	—	0.906	0.094	0.432
	Aichi	0 (0.0)	1 (0.9)	0 (0.1)	57 (57.8)	14 (12.5)	0 (0.7)	72	0.007	0.896	0.097	0.973
Pgm	Okayama	39 (39.0)	1 (1.0)	—	0 (0.0)	—	—	40	0.975	0.025	—	0.000
	Osaka	32	0	—	0	—	—	32	1.000	0	—	0
	Aichi	72	0	—	0	—	—	72	1.000	0	—	0
Sdh	Okayama	71 (71.3)	9 (8.5)	—	0 (0.3)	—	—	80	0.944	0.056	—	0.006
	Osaka	32	0	—	0	—	—	32	1.000	0	—	0
	Aichi	66	6	—	0	—	—	72	0.958	0.042	—	0.136

Table 3. Summary of genic variability in black seabream based on electrophoretic analysis of 44 loci. The numbers in parenthesis are calculated based on 1% criterion for polymorphism.

Items	Locality		
	Okayama	Osaka	Aichi
No. of samples	80	32	72
No. of loci examined*	44	44	44
No. of polymorphic loci	16 (16)	11 (11)	14 (9)
Proportion of polymorphic loci	0.341 (0.341)	0.250 (0.250)	0.318 (0.205)
No. of alleles per locus	1.386 (1.386)	1.272 (1.272)	1.386 (1.136)
Heterozygosity: Observed (H ^o)	0.072	0.057	0.069
Expected (H ^e)	0.071	0.057	0.066
H ^o /H ^e	1.014	1.000	1.045

* 12 loci of sarcoplasmic proteins and 3 loci of hemoglobins were included.

zygosity in black seabream (0.066) was relatively similar to the result obtained by FUJIO and KATO,¹⁶⁾ to rainbow trout population,¹⁾ and to red seabream.¹⁵⁾

Table 3 shows the relatively high genic variability in black seabream. Because the genic variability in the qualitative characters is an indicator of the genic variability throughout the genomes of the population, the high average of heterozygosity of this species shows the possibility of high additive genetic variance in quantitative traits which are important for genetic improvement in the future breeding study.

The values of H^o/H^e were higher than 1 ranging from 1.000 to 1.014, so that the possibility

of inbreeding in wild black seabream population could not be considered.

Geographical Differentiation

The differentiations among localities were tested by chi-square values for heterogeneity (Table 4). In some alleles, differences between two locations were significant in some cases, such as alleles A and C of *Est-1*, *Idh* and *Mdh-3* loci for Okayama vs. Aichi, and also α -*Gdh-1* and *6-Pgd* for Okayama vs. Osaka. These differences found in some loci between Okayama and other locations indicated that the population of Okayama may be independent.

F-statistics (F_{ST})¹⁷⁾ computation for each polymorphic enzyme are provided in Table 5. The average value of F_{ST} in black seabream was 0.0094, ranging from 0.005 to 0.0327. This average value of F_{ST} in black seabream was smaller than those of blue gill *Lepomis macrochirus*¹⁸⁾ and milkfish *Chanos chanos*.¹⁹⁾ This small value of F_{ST} indicated that there were very small differentiation among localities in the population of black seabream examined here.

The values of genetic distance (D)²⁰⁾ were as follows: Okayama vs. Osaka 0.0018; Okayama vs. Aichi 0.0012; and Osaka vs. Aichi 0.0016. The D values between localities of black seabream were lower than milkfish,¹⁸⁾ and far smaller than chum salmon *Oncorhynchus keta* population of North America²¹⁾, *Coregonus albula* populations in Finland,²²⁾ blue gill populations in Florida Lakes,¹⁸⁾ endemic arctic char *Salvelinus alpinus* populations from eastern United States and Canada,²³⁾ and skipjack tuna *Katsuwonus pelamis* populations of Pacific Ocean.²⁴⁾ The very small

Table 4. Chi-square values for heterogeneity tests in polymorphic loci between two localities of black seabream. Significant values were marked by asterisks

Locus	<i>Adh</i>		<i>Ck</i>	<i>Est-1</i>			<i>Est-2</i>	α - <i>Gdh-1</i>	α - <i>Gdh-2</i>	<i>Idh</i>
	<i>B</i>	<i>C</i>		<i>A</i>	<i>B</i>	<i>C</i>				
Okayama vs. Osaka	0.916	0.034	2.181	0.512	0.000	0.574	0.970	4.851*	0.000	0.377
Okayama vs. Aichi	0.325	0.239	2.453	8.293**	2.357	5.200*	0.298	1.599	1.115	4.890*
Osaka vs. Aichi	0.205	0.351	0.447	2.202	1.189	0.991	0.518	1.830	0.447	1.841

Table 4. Continued

Locus	<i>Mdh-1</i>	<i>Mdh-3</i>	<i>6-Pgd</i>	<i>Pgm</i>	<i>Gpi-2</i>	<i>Sdh</i>	<i>Sp-1</i>	<i>Sp-2</i>	<i>Sp-3</i>
Okayama vs. Osaka	2.046	0.204	6.275*	0.806	0.807	3.751	0.069	1.851	0.651
Okayama vs. Aichi	2.341	6.707**	0.476	1.808	1.812	0.344	0.007	0.384	1.693
Osaka vs. Aichi	0.447	3.746	0.053	0.000	0.000	2.764	0.102	0.825	0.037

*: $P < 0.05$ **: $P < 0.01$ **Table 5.** F-statistics (F_{ST}) values based on allelic frequencies in black seabream

Locus/allele	F_{ST}	Locus/allele	F_{ST}
<i>Adh</i> A	0.005	<i>Mdh-1</i> A	0.014
B	0.003	<i>Mdh-3</i> A	0.015
C	0.001	<i>6-Pgd</i> A	0.005
<i>Ck</i> A	0.015	B	0.001
<i>Est-1</i> A	0.020	C	0.001
B	0.008	<i>Pgm</i> A	0.017
C	0.012	<i>Gpi-2</i> A	0.004
<i>Est-2</i> A	0.002	B	0.002
α - <i>Gdh-1</i> A	0.005	C	0.004
B	0.024	<i>Sdh</i> A	0.033
C	0.024	<i>Sp-1</i> A	0.001
α - <i>Gdh-2</i> A	0.005	<i>Sp-2</i> A	0.008
<i>Idh</i> A	0.011	<i>Sp-3</i> A	0.004
B	0.011		
C	0.006		

values of D in black seabream show that those populations are in a very close phenetic relationship.

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