Morphological and Genetic Differences between Japanese and Chinese Threeline Grunt *Parapristipoma trilineatum*

(日本産イサキと中国産イサキの形態的および遺伝的差異)

Kōji YOKOGAWA

Kagawa Prefectural Fisheries Experimental Station

(横川浩治 (香川県水産試験場))

Abstract

In order to ascertain the biological features of Chinese threeline grunt *Parapristipoma trilineatum*, which currently is being cultured in western Japan, morphological and genetic comparisons with it and the Japanese form were made. Significant morphological differences between the two forms included differing body depth, body width, caudal peduncle depth, interorbital width proportions, scales above lateral line and scales below lateral line counts, and condition factor. Genetic analysis by isozyme detected 16 enzymes and 2 non-enzymic proteins, and 36 loci were presumed. Allelic frequencies showed no significant differences by chi-square tests between the two forms at any loci. The genetic distance (D value) between the two forms was 0.0008, a figure within the intrapopulation level. Although the given results seem to show that the considerable morphological differences between the two forms were not due to the genetic factors but the environmental ones, some prior reports suggest that the morphological differences are not due to the latter. In order to elucidate the discrepancy, further studies on more genetic markers for the two forms necessitate.

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Recently in Japan, many species of fishes and shellfishes are being earnestly imported from foreign countries as aquacultural seeds[1]. During such importation, the threeline grunt *Parapristipoma trilineatum* was introduced from China and cultured at some localities in western Japan. It is said that the Chinese form of this species (Fig. 1-B) grows more rapidly than the Japanese form, hence, the reason for the Chinese form to be greatly valued. However, the biological features of the Chinese threeline grunt are unknown, necessitating their examination. Thus in this study, morphological and

Corresponding author : Kōji Yokogawa, 75-5 Yashimahigashimachi, Takamatsu, Kagawa, 761-0111 Japan;
e-mail: yokogawa@shikoku.ne.jp

Fig. 1. General aspects of Japanese and Chinese forms of threeline grunt *Parapristipoma trilineatum*. A-Japanese form, B-Chinese form.

genetic comparisons were made as the examples between the Japanese and the Chinese threeline grunts.

Materials and Methods

Japanese threeline grunt (hereafter called Japanese form) were obtained from coastal waters of Tsubakidomari, Anan, Tokushima Prefecture, on August 17th and 23rd, 1995, by angling. Chinese threeline grunt (hereafter called Chinese form) were sampled from cultured fish of Kamosho nursery, Shido, Kagawa Prefecture, on August 16th, 1995. Although they were from China, the original locality was uncertain. Number of the specimens examined was 42 in each form, sizes (total lengths) being 160.6-229.5 mm (average 191.3 mm) in the Japanese form, and 146.8-185.1 mm (average 164.2 mm) in the Chinese form, respectively.

The specimens were preserved in refrigerators at either -80 or -20℃ prior to genetic examination. Isozymes detected by horizontal starch-gel electrophoresis were used as genetic markers. After the

Table 1. Morphometric measurements of Japanese and Chinese forms of *Parapristipoma trilineatum*, with analysis of variance and *t* test results between the two forms

Form	Japanese		Chinese		F	*t*
	Average	Range	Average	Range		
Total length[1]	124.18	118.21-128.95	121.07	117.87-124.46	2.998***	7.232***
Fork length[1]	116.83	111.14-122.57	116.02	113.94-117.59	4.543***	2.166*
Pre-anus length[1]	63.62	59.41- 67.95	65.08	62.69- 68.11	1.637	4.126***
Body depth[1]	31.80	27.34- 35.76	38.02	34.95- 40.35	1.581	20.184***
Body width[1]	15.00	13.33- 16.86	19.75	17.88- 21.68	1.362	27.288***
Caudal peduncle depth[1]	9.62	8.66- 10.35	10.43	9.91- 10.93	1.835*	11.987***
Caudal peduncle length[1]	23.52	19.70- 25.86	23.08	20.44- 24.79	2.775***	1.664
Pre-dorsal length[1]	31.50	29.06- 35.44	33.95	30.24- 37.57	1.328	6.962***
Dorsal fin spine length[1]	10.25	8.10- 12.25	8.72	6.56- 10.25	1.528	7.295***
Dorsal fin ray length[1]	7.10	5.32- 9.01	6.38	4.82- 8.48	1.437	4.001***
Anal fin spine length[1]	11.25	9.37- 14.43	10.41	8.57- 12.01	1.537	3.991***
Anal fin ray length[1]	10.66	7.87- 13.29	8.96	7.04- 10.46	1.984*	8.381***
Pectoral fin length[1]	24.72	19.76- 27.76	22.18	19.35- 24.80	2.203**	8.563***
Pelvic fin length[1]	20.28	16.42- 22.94	18.74	17.01- 20.81	2.577**	6.118***
Head length[1]	28.26	23.95- 30.26	28.70	25.29- 30.08	2.031*	1.992*
Snout length[2]	28.24	24.74- 33.85	28.64	25.68- 31.47	2.564**	0.982
Orbital diameter[2]	29.45	27.17- 34.53	29.07	25.52- 32.45	1.101	1.018
Interorbital width[2]	30.10	23.97- 36.78	34.32	29.55- 37.60	1.899*	9.082***
Sub-orbital width[2]	14.75	10.22- 26.78	14.97	11.06- 20.05	1.748*	0.388
Upper jaw length[2]	33.04	27.86- 40.53	32.23	28.80- 36.61	2.146**	1.836
Lower jaw length[2]	34.11	28.77- 41.03	33.82	29.87- 37.23	1.700*	0.685
Dorsal fin spines	14.12	13 - 17	14.19	14 - 16	1.699*	0.620
Dorsal fin rays	16.79	15 - 19	17.43	15 - 19	1.128	3.652***
Anal fin spines	3.00	3 - 3	3.00	3 - 3		
Anal fin rays	7.90	7 - 9	7.81	7 - 9	1.637	0.882
Pectoral fin rays	17.81	17 - 19	18.05	17 - 19	1.136	2.088*
Pelvic fin spines	1.00	1 - 1	1.00	1 - 1		
Pelvic fin rays	5.00	5 - 5	5.00	5 - 5		
Pored scales on lateral line	56.21	53 - 59	55.86	52 - 60	1.722*	1.150
Scales above lateral line	15.83	14 - 19	18.74	15 - 21	2.219**	9.715***
Scales below lateral line	23.38	20 - 26	25.36	22 - 29	1.563	6.618***
Gill rakers (upper limb)	14.86	12 - 18	15.10	10 - 18	1.076	0.743
Gill rakers (lower limb)	24.14	22 - 28	24.21	22 - 27	1.623	0.273
Gill rakers (total)	39.00	34 - 44	39.31	34 - 44	1.231	0.719
Vertebrae (abdominal)	9.97	9 - 11	10.07	10 - 11	2.701**	1.242
Vertebrae (caudal)	16.00	15 - 17	15.95	15 - 16	1.050	1.000
Vertebrae (total)	26.00	25 - 27	26.02	25 - 27	2.175**	0.318
Condition factor	22.48	18.88- 27.66	35.45	31.17- 39.76	1.011	32.547***

[1] Percentage of standard length.
[2] percentage of head length.

* Significant at 5% level.
** Significant at 1% level.
*** Significant at 0.1% level.

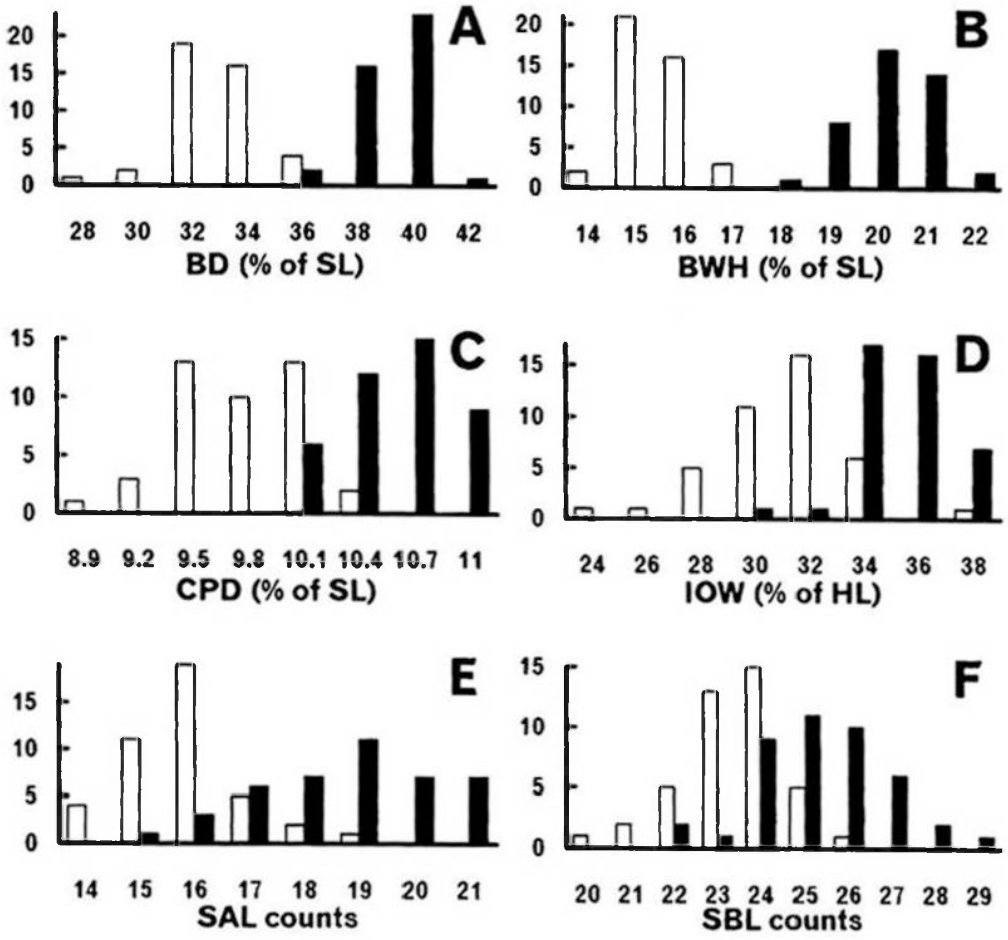

Fig. 2. Frequency distributions of significant morphological characters in Japanese and Chinese forms of threeline grunt *Parapristipoma trilineatum*. Open bars-Japanese form, dark bars-Chinese form. Longitudinal axes indicate number of individuals. A-Body depth (BD), B-Body width (BWH), C-Caudal peduncle depth (CPD), D-Interorbital width (IOW), E-Scales above lateral line (SAL), F-Scales below lateral line (SBL).

electrophoretic experiments, the specimens were fixed in 10% formalin for subsequent recording of morphological and meristic data.

The morphological and genetic analyses were according to methods of Yokogawa and Seki[2]. In addition, a condition factor (CF) for each individual was calculated with the usual method ($CF = BW/SL^3 \times 10^4$, where BW : body weight in g; SL : standard length in cm). Also, the principal component analysis (PCA) with the total morphological data by the usual method was introduced to compare the morphology of the two forms synthetically.

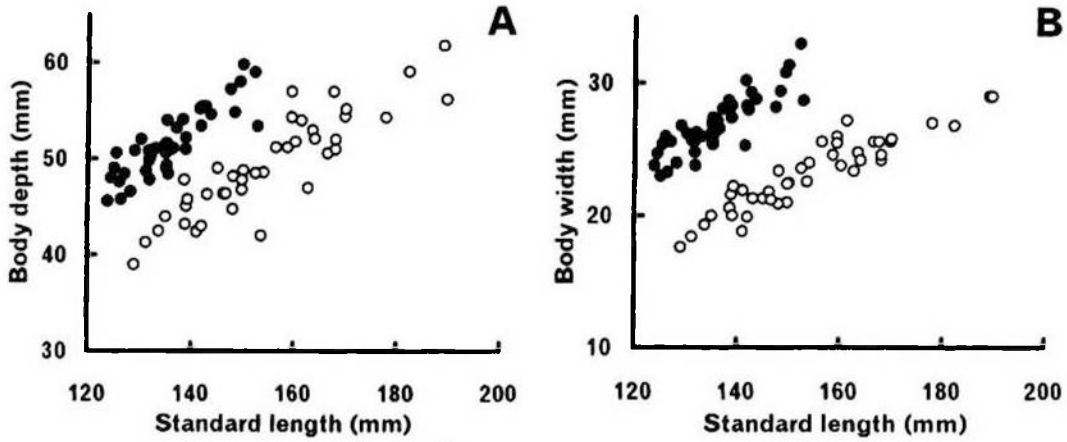

Fig. 3. Relative growth of some morphological characters for Japanese and Chinese forms of threeline grunt *Parapristipoma trilineatum*. A-Relationships between standard length (SL) and body depth (BD), B-Relationships between SL and Body width (BWH). Open circles-Japanese form, dark circles-Chinese form.

Results

Morphological characters

Results of the all the morphological characters examined, together with the results of analysis of variance (F) and *t*-test (*t*) of the average values between the two forms are shown in Table 1. Significant differences between the two forms were recognized in either the F or the *t* values at most of the characters (Table 1).

Histograms showing frequency distributions of some significant characters are given in Fig. 2. The distribution of the two forms are clearly distinct for each character, in both mode and range, and in general, the values of the Chinese form are greater than in the Japanese form (Fig. 1). In particular, the histograms of the two forms at the body width (BWH) showed no-overlapping of each other, also the body depth (BD) showing almost no-overlapping (Fig. 2). Figure 3 shows relationships between these characters and the standard length, both indicating clear differences of relative growth even in the same body size.

Figure 4 shows plotting of principal component scores of the individuals by the principal component analysis (PCA) using all the morphological data except the numbers of anal fin rays, pelvic fin spines and rays which gave no variance. The Japanese and the Chinese individuals were distinctly separated by the principal component 1, indicating considerable morphological differences with each other (Fig. 4).

Genetic characters

The electrophoretic study resulted in 16 enzymes and 2 non-enzymic proteins being detected, with 36 loci presumed (Table 2).

Initially, fitness for the Hardy-Weinberg law was examined by chi-square tests on the polymorphic loci. Neither form showed any significant difference (5%) at any loci, both corresponding well with the Hardy-Weinberg equilibrium and therefore being regarded as representing simple Mendelian populations.

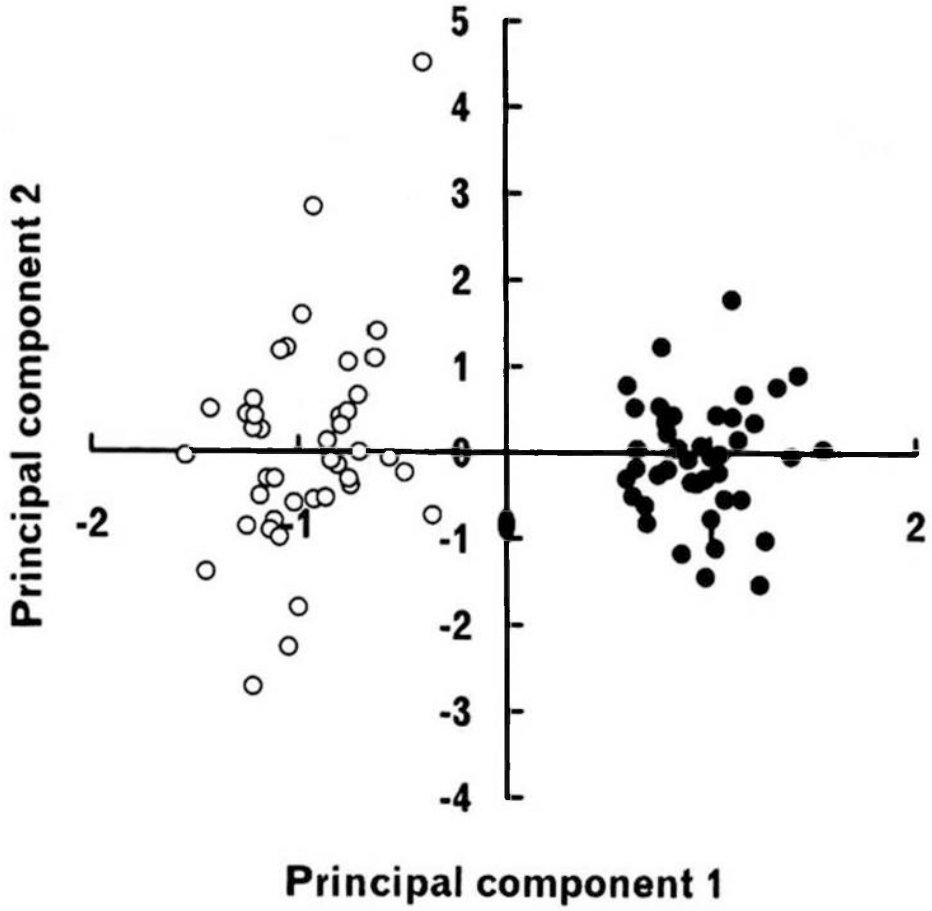

Fig. 4. Principal component analysis for Japanese and Chinese forms of threeline grunt *Parapristipoma trilineatum*. Open circles-Japanese form, dark circles-Chinese form.

Values indicating genetic features of the two forms are shown in Table 3. Values of the allele numbers per locus and the proportions of polymorphic loci resulted in totally the same in the two forms (Table 3). However the Ho/He ratio differed from each other. That of the Japanese form was over 1, indicating an excess of heterozygotes, whereas that of the Chinese form was rather less than 1, indicating an excess of homozygotes (Table 3). This might suggest some differentiation of genetic structure between the two forms.

Allelic frequencies of the 36 loci examined in the two forms, together with chi-square hetero values to indicate statistical differentiation between the allelic frequencies of the two forms, are shown in Table 4. Since the chi-square values did not reach the significant level (5%) at any loci, it could be regarded that there were no differences in the

Table 2. Enzymes, protein and tissues examined

Enzyme or protein name	Enzyme number	Locus	Subunit structure	Tissue
Aspartate aminotransferase	2.6.1.1	*AAT-1**	Dimeric	Liver
		*AAT-2**	Dimeric	Liver
Alcohol dehydrogenase	1.1.1.1	*ADH**	Dimeric	Liver
Esterase	3.1.1.-	*EST-1**		Heart
		*EST-2**	Monomeric	Liver
Fructose biphosphate aldolase	4.1.2.13	*FBALD-1**		Muscle
		*FBALD-2**		Muscle
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**		Liver
		*G3PDH-2**		Liver
		*G3PDH-3**		Muscle
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	Dimeric	Muscle
		*GPI-2**	Dimeric	Muscle
Hemoglobine		*HB-1**		Heart
		*HB-2**		Heart
		*HB-3**		Heart
		*HB-4**		Heart
		*HB-5**		Heart
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	Tetrameric	Liver
		*IDDH-2**	Tetrameric	Muscle
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*IDHP-1**	Dimeric	Liver
		*IDHP-2**	Dimeric	Muscle
Lactate dehydrogenase	1.1.1.27	*LDH-1**		Liver
		*LDH-2**	Tetrameric	Muscle
Malate dehydrogenase	1.1.1.37	*MDH-1**	Dimeric	Muscle
		*MDH-2**	Dimeric	Muscle
		*MDH-3**		Muscle
Malic enzyme (NADP+)	1.1.1.40	*MEP-1**		Muscle
		*MEP-2**	Tetrameric	Muscle
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**		Liver
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Dimeric	Muscle
Phosphoglucomutase	5.4.2.2	*PGM**	Monomeric	Muscle
General protein		*PROT-1**		Muscle
		*PROT-2**		Muscle
		*PROT-3**		Muscle
Superoxide dismutase	1.15.1.1	*SOD**	Dimeric	Liver
Xantine dehydrogenase	1.1.1.204	*XDH**		Liver

Table 3. Genetic features of Japanese and Chinese forms of *Parapristipoma trilineatum*

		Japanese	Chinese
Alleles / Locus		1.333	1.333
P*		0.083	0.083
P		0.250	0.250
P+P*		0.333	0.333
Average	Ho	0.039	0.033
Heterozygosity	He	0.038	0.038
	Ho / He	1.034	0.887

P* : Polymorphism less than 0.95.
P : Polymorphism over 0.95.
Ho : Observed heterozygosity.
He : Expected heterozygosity.

allelic frequencies of the two forms at any loci (Table 4).

The genetic distance (D value)[3] between the two populations, calculated from the allelic frequencies, was 0.0008, a figure within the intrapopulation level[4].

Discussion

The results found here showed that although the Chinese threeline grunt considerably differed morphologically from the Japanese form, genetically it was quite similar to the Japanese form. Yoshimatsu *et al.*[5,6] also examined the morphological and genetic differences between the Japanese and the Chinese form of this species with the genetic marker of mitochondrial DNA. They detected statistically significant morphological differences between the two forms at 14 characters, while their genetic examination by base sequence analysis of the D-loop in the cytochrome-b region showed no difference between the two forms. Their morphological and genetic results corresponded with those in this study.

It is difficult to explain on the inconsistency between the morphological and the genetic differences in the two forms. Supposing the two forms were genetically completely uniform, the morphological differences could have been caused by environmental factors. Actually, it is known that the morphological characters of fishes can be affected by the environmental factors, in particular the meristic characters can be altered by water temperature or salinity in their larval stage[7-9].

However, it may be somewhat difficult to regard the extreme morphological differences between the two forms detected in this study as variation due to environmental factors. Because Yoshimatsu *et al.*[5,6]'s fish samples of the Japanese and the Chinese forms were both artificially produced and bred by them in the same environmental condition, showing the considerable morphological differences. This suggests that the morphological differences between the two forms are not due to the environmental factors.

The other biological characteristics such as ecology of the two forms are not known very well. Murata *et al.*[10] compared growth of the two forms

Table 4. Allelic frequencies of Japanese and Chinese forms of *Parapristipoma trilineatum*, with results of χ^2 heterogeneity between the two forms

Locus	Allele	Frequency		χ^2 hetero	P[1]
		Japanese	Chinese		
*AAT-1**	*100 A	1.000	0.988	0.982	0.322
	*70 B	0.000	0.012		
*AAT-2**	*-70 B	0.000	0.012	0.982	0.322
	*-100 A	1.000	0.988		
*ADH**	*40 B	0.024	0.000	2.075	0.354
	*-100 A	0.963	0.988		
	*-425 C	0.012	0.012	–	
*EST-1**	*100 A	1.000	1.000		
*EST-2**	*100 A	0.988	0.988	0.000	0.990
	*85 B	0.012	0.012		
*FBALD-1**	*-100 A	1.000	1.000	–	
*FBALD-2**	*-100 A	1.000	1.000	–	
*G3PDH-1**	*100 A	1.000	1.000	–	
*G3PDH-2**	*100 A	1.000	1.000	–	
*G3PDH-3**	*100 A	1.000	1.000	–	
*GPI-1**	*110 B	0.012	0.000	2.000	0.368
	*100 A	0.988	0.988		
	*85 C	0.000	0.012		
*GPI-2**	*-75 B	0.012	0.000	1.006	0.316
	*-100 A	0.988	1.000		
*HB-1**	*-100 A	1.000	1.000	–	
*HB-2**	*-100 A	1.000	1.000	–	
*HB-3**	*-100 A	1.000	1.000	–	
*HB-4**	*-100 A	1.000	1.000	–	
*HB-5**	*-100 A	1.000	1.000	–	
*IDDH-1**	*-100 A	1.000	0.976	1.880	0.170
	*-170 B	0.000	0.024		
*IDDH-2**	*-60 C	0.063	0.019	2.480	0.290
	*-100 A	0.771	0.712		
	*-140 B	0.167	0.269		
*IDHP-1**	*130 B	0.024	0.000	2.074	0.150
	*100 A	0.976	1.000		
*IDHP-2**	*150 C	0.012	0.000	2.000	0.368
	*100 A	0.988	0.988		
	*55 B	0.000	0.012		
*LDH-1**	*100 A	1.000	1.000	–	
*LDH-2**	*100 A	1.000	0.988	1.006	0.316
	*-900 B	0.000	0.012		
*MDH-1**	*115 B	0.012	0.000	1.006	0.316
	*100 A	0.988	1.000		
*MDH-2**	*100 A	1.000	0.988	1.006	0.316
	*65 B	0.000	0.012		
*MDH-3**	*-100 A	1.000	1.000	–	
*MEP-1**	*115 B	0.143	0.167	3.300	0.192
	*100 A	0.833	0.750		
	*75 C	0.024	0.083		
*MEP-2**	*100 A	1.000	1.000	–	
*MPI**	*100 A	1.000	1.000	–	
*PGDH**	*115 B	0.024	0.000	2.024	0.155
	*100 A	0.976	1.000		
*PGM**	*200 E	0.000	0.012	4.568	0.335
	*100 A	0.750	0.833		
	*-100 B	0.214	0.107		
	*-200 C	0.024	0.036		
	*-300 D	0.012	0.012		
*PROT-1**	*100 A	1.000	1.000	–	
*PROT-2**	*100 A	1.000	1.000	–	
*PROT-3**	*-100 A	1.000	1.000	–	
*SOD**	*100 A	0.988	1.000	1.031	0.310
	*-10 B	0.012	0.000		
*XDH**	*100 A	1.000	1.000	–	

[1] Risk percentage for chi-square value.

produced artificially and bred in the same conditions. They obtained the final results of average body size at 551 days breeding after hatching out, whereby that is, the Japanese form was TL 14.5 cm and BW 40.7 g, and the Chinese form was TL 22.2 cm and BW 169.4 g. This experiment showed that the Chinese form had much greater growth rate than the Japanese form. The growth rate of the bred Japanese form by Murata *et al.*[10] was similar to that of some Japanese wild threeline grunt populations as summarised by Watanabe and Okazaki[11] except for Tokushima population which showed excellent growth perhaps due to the high water temperature. This leads the assumption that the Chinese form grows similar to Murata *et al.*[10]'s results in the natural waters in China. Thus, it may be natural to consider that there are some genetic differences ruling the growth between the Japanese and the Chinese forms. Similarly, spotted sea bass *Lateolabrax* sp. from China, which has been treated conspecifically as Japanese sea bass *L. japonicus* grows more rapidly than the latter[12,13], thereby reflecting the genetic differentiation between the two species as shown in the isozymic loci[2].

Inclusive of the difference in growth, the considerable morphological differences between the two forms may reflect the genetic differences, which have not been detected yet. Although the allelic frequencies of the two populations showed no differences (Table 4), the Ho/He ratio differed from each other (Table 3). Although the isozyme analysis is considered to survey the genome DNA evenly, the examined area is likely to be very small in comparison to the total DNA size. Hence, if there are some differences in any area of the genome DNA, there may be a probability that they cannot be detected.

For example, Izuka *et al.*[14] made population genetic analysis of three forms (SHIROIKA, AKAIKA, KUAIKA) of oval squid *Sepioteuthis lessoniana* around the Japanese archipelago by using the genetic marker of 13 isozymic loci, detecting no significant genetic differences in the SHIROIKA populations. But thereafter, Yokogawa and Ueta[15] reexamined the same subject with 34 isozymic loci, detecting a complete replacement of alleles between the Japan Sea and Pacific groups of SHIROIKA at the *LDH-4** locus which was excluded in the former study. This example shows that the detecting sensibility of genetic difference depends on the number of loci (area size in the total DNA) examined.

In the case of the mitochondrial DNA examined by Yoshimatsu *et al.*[5,6], it can hardly represent the total genetic features because it is a rather restricted region in the total DNA. Therefore, the absence of genetic differences in the mitochondrial DNA between the Japanese and the Chinese threeline grunts may not always represent the total genetic features of the two forms.

Thus, a hypothesis that there are undetected regions which code the morphological characters might be likely to explain the morphological differences between the two forms. But anyway, further genetic studies such as genome DNA analysis is necessary in order to reveal the problem.

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