GENETIC VARIATION AMONG SEA BASS POPULATIONS

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Abstract: Horizontal starch gel electrophoresis was used to investigate the genetic structure of sea bass populations. 146 individuals collected from coastal waters of China and 40 individuals from Tokyo Bay were examined. The proportions of polymorphic loci of sea bass populations varied from 0.2667 to 0.5333. The values of observed and expected heterozygosity were from 0.0211 to 0.0515 and from 0.0398 to 0.0797 respectively. There was a nearly complete replacement of alleles at *LDH**, *GPI-1**, *GPI-2** between Chinese and Japanese sea bass. The Nei's genetic distance among Chinese populations varied from 0.0004 to 0.0011 with an average of 0.0008. The Nei's genetic distance between the Chinese populations and Japanese population varied from 0.1870 to 0.1954 with an average of 0.1926. Isozyme analysis indicated that the genetic variation among Chinese populations was small, but that between Chinese and Japanese sea bass was much larger than that among Chinese populations.

Key words: Sea bass; Population; Variation; Isozyme
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Genus *Lateolabrax*, a single species *Lateolabrax japonicus* (Cuvier et Valenciennes) established by Bleeker, is a small genus confined to the coast of China, Japan and Korea, which belongs to Perciformes, Serranidae and was described as a second species *L. latus*[1,2]. It also has the distinctive characters of this genus such as slender body, the color pattern, the gently fork tail, the absence of canines on the jaws and the deep notch almost separating the spinouts from soft-rayed portions of the dorsal fin. It has been considered that Chinese sea bass is the same species as Japanese sea bass, *Lateolabrax japonicus*. Recently, there have been some discussions on the classification of them and the Chinese sea bass was referred to *Lateolabrax* sp. Yokogawa *et al*. examined the Japanese and the Chinese sea bass populations and concluded that the Japanese and Chinese sea bass should be classified as two independent species. Nakayama *et al*. studied the morphological difference in early stage rearing and considered that the Chinese and Japanese sea bass represent distinct species. Park *et al*. reported that the present two populations of Korea should be classified at subspecies level by isozyme analysis. Kim *et al*. also did some studies on the classification of the genus *Lateolabrax* by morphological analysis and they didn't conclude *L.* sp. as a subspecies but an independent species[3-6].

Chinese sea bass is one of the most important commercial species of mariculture in China. It is widely distributed along the Chinese coast, including the borders with Vietnam and Korea, which are mainly caught in waters less than 40 meters deep, and it is strictly a seasonal fish captured only in winter. They congregate in waters above the rocky seabed and feed on crustacean, worms, small fishes and sometimes algae. The young fish of Bohai Sea and Yellow Sea populations have been cultured in southern China. In aquaculture of Japan, the seeds of *L.* sp. have been mostly imported from Korea, Chinese mainland, Taiwan and Hong Kong since 1990. The effects of the existence of free living *L.* sp. in Japanese waters is of concern[7]. In China, a lot of studies have been carried out on growth, feeding, early stage development and other ecological characteristics of the sea bass[8-11], but the genetic structure of the sea bass popular-

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tions has not been examined yet. The present study was carried out to investigate the genetic variation and divergence among the populations.

1 Materials and Methods

1.1 Samples 146 individuals of Chinese sea bass and 40 individuals of Japanese sea bass were collected from Beihai (Guangxi province) in October 1999, Xiamen and Fuzhou (Fujian province) in November 1999, Zhoushan (Zhejiang province) and Weihai (Shandong province) in November 2000, and Tokyo Bay in April 1999 (Fig. 1). All samples were brought to the laboratory of Ocean University of China either alive or in a frozen state.

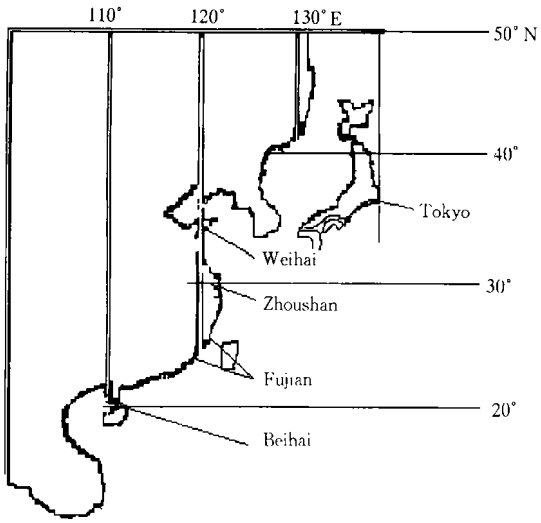

Fig. 1 A map showing the locations of Chinese and Japanese sea bass samples used in this study

1.2 Methods All samples to be analyzed for isozyme were homogenized with approximately equal volumes of frozen tissues and distilled water. After homogenation, the homogenate was centrifuged at 12000r/min for 12min at 4℃ and the supernatant was absorbed by filter paper and was used for electrophoresis. Horizontal starch gel electrophoretic techniques and staining procedure followed the method of the Japan Fisheries Resource Conservation Association and Pasteur et al[12].

A total of 12 enzymes were surveyed under TG-8 buffer system: alcohol dehydrogenase (ADH), glucose-6-phosphate isomerase (GPI), glucose-3-phosephate dehydrogenase (G3PDH), glucose-6-phosphate dehydrogenase (G6PD), hexokinase (HK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH), superoxide dismutase (SOD). The names, numbers, and abbreviations of the enzymes followed Shaklee et al (Tab. 1)[13]. The allele frequencies and observed heterozygosities for each locus were determined by direct census of the population data. Mean heterozygosity within populations was estimated from average values across all samples. A locus was considered to be polymorphic if the most common allele was equal or less than 0.99 at one or more localities. In order to estimate the degree of genetic divergence among the samples, the genetic distance between samples was calculated using Nei's formulas[14]. The dendrograms from the matrix of genetic distances were constructed using the UPGMA method.

Tab. 1 Names, numbers, tissues and abbreviations of enzymes used for electrophoresis

Enzyme	Enzyme abbreviation	Enzyme number	Tissue
Alcohol dehydrogenase	ADH	1.1.1.1	Liver
Glucose-6-phosphate isomerase	GPI	5.3.1.9	Muscle
Glucose-3-phosphate dehydrogenase	G3PDH	1.1.1.8	Muscle
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	Liver
Hexokinase	HK	2.7.1.1	Liver
Isocitrate dehydrogenase	IDHP	1.1.1.42	Muscle
Lactate dehydrogenase	LDH	1.1.1.27	Muscle
Malate dehydrogenase	MDH	1.1.1.37	Muscle
Malic enzyme	ME	1.1.1.40	Muscle
Phosphoglucomutase	PGM	5.4.2.2	Muscle
Sorbitol dehydrogenase	SDH	1.1.1.14	Liver
Superoxide dismutase	SOD	1.15.1.1	Liver

2 Results

Of the 12 enzymes assayed routinely, 6 loci (*G6PD-1**, *G6PD-2**, *HK**, *MDH-1**, *MDH-2**, *ME**) were monomorphic in all samples, while the others (*ADH**, *GPI-1**, *GPI-2**, *G3PDH**, *IDHP**, *LDH**, *PGM**, *SDH**, *SOD**) showed the allele frequencies were less than 0.99 in at least one sample. These isozymes obtained consistently and clearly in all samples, were considered useful genetic markers for population analysis (Fig. 2).

Genetic variability was estimated by the proportion of polymorphic loci and average heterozygosity. The average proportion of polymorphic loci (P^*) was from 0.2667 to 0.5333, and the average observed and expected heterozygosity was from 0.0211 to 0.0515 and from 0.0398 to 0.0797 respectively (Tab. 2). Nei's genetic distance (Nei'D) between all

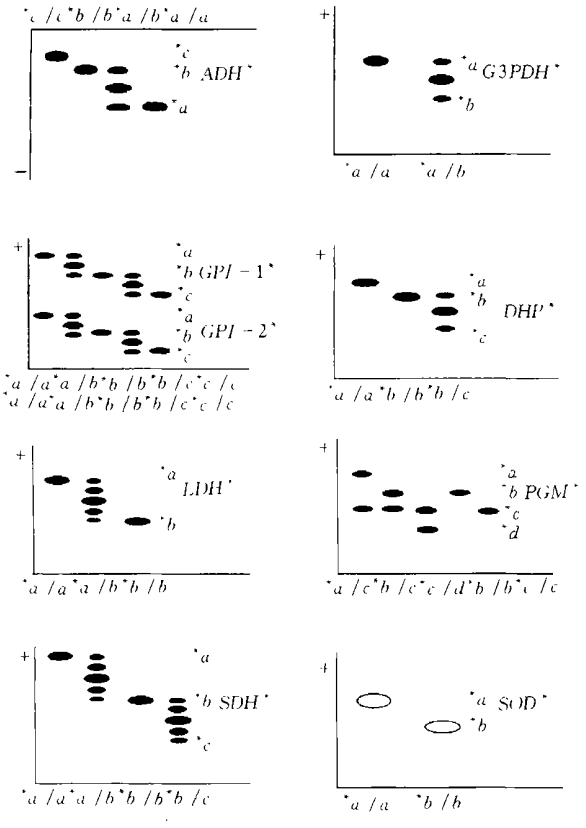

Fig. 2　Electrophoretic patterns of the nine polymorphic loci in sea bass populations

samples based on the 15 loci were given in Tab. 3. Isozyme analysis of genetic characters indicated a nearly complete replacement of alleles at *LDH**, *GPI-1**, *GPI-2** between Chinese and Japanese sea bass. The Nei's D among Chinese sea bass populations varied from 0.0004 to 0.0011 with an average of 0.0008, and that between Chinese and Japanese sea bass populations were from 0.1870 to 0.1954 with an average of 0.1926.

Tab. 2　Allele frequency, proportion of polymorphic loci, and average heterozygosity

Locus	Allele	Beihai (40)	Fujian (29)	Zhoushan (27)	Weihai (50)	Tokyo Bay (40)
*ADH**	-* *a*	0.0000	0.0000	0.0740	0.0000	0.0500
	-* *b*	1.0000	1.0000	0.9260	1.0000	0.8375
	-* *c*	0.0000	0.0000	0.0000	0.0000	0.1125
*GPI-1**	* *a*	0.0375	0.0172	0.0000	0.0500	0.0000
	* *b*	0.9625	0.9655	0.9815	0.9200	0.0750
	* *c*	0.0000	0.0172	0.0185	0.0300	0.9250
*GPI-2**	* *a*	0.0375	0.0172	0.0000	0.0500	0.0000
	* *b*	0.9625	0.9655	0.9815	0.9200	0.0750
	* *c*	0.0000	0.0172	0.0185	0.0300	0.9250

续表

Locus	Allele	Beihai (40)	Fujian (29)	Zhoushan (27)	Weihai (50)	Tokyo Bay (40)
*G3PDH**	* *a*	1.0000	1.0000	1.0000	1.0000	0.9625
	* *b*	0.0000	0.0000	0.0000	0.0000	0.0375
*G6PD-1**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*G6PD-2**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*HK**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*IDHP**	* *a*	0.0000	0.0345	0.0000	0.0000	0.0000
	* *b*	1.0000	0.9655	1.0000	1.0000	0.9875
	* *c*	0.0000	0.0000	0.0000	0.0000	0.0125
*LDH**	* *a*	1.0000	1.0000	1.0000	1.0000	0.0125
	* *b*	0.0000	0.0000	0.0000	0.0000	0.9875
*MDH-1**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*MDH-2**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*ME**	* *a*	1.0000	1.0000	1.0000	1.0000	1.0000
*PGM**	* *a*	0.0000	0.0000	0.0000	0.0100	0.0000
	* *b*	0.3500	0.3488	0.2780	0.2650	0.4625
	* *c*	0.6500	0.6522	0.7035	0.7250	0.5375
	* *d*	0.0000	0.0000	0.0185	0.0000	0.0000
*SDH**	* *a*	0.0000	0.0690	0.0000	0.0200	0.0375
	* *b*	0.9750	0.9310	1.0000	0.9800	0.9500
	* *c*	0.0250	0.0000	0.0000	0.0000	0.0125
*SOD**	* *a*	1.0000	1.0000	1.0000	0.9800	1.0000
	* *b*	0.0000	0.0000	0.0000	0.0200	0.0000
*P**		0.2667	0.3333	0.2667	0.3333	0.5333
Ho		0.0400	0.0245	0.0211	0.0247	0.0515
He		0.0405	0.0489	0.0398	0.0489	0.0797

3　Discussion

The average observed and expected heterozygosity of the sea bass populations were from 0.0211 to 0.0515 and from 0.0398 to 0.0797 respectively. Compared with other species of marine fishes[15—17], the *Ho* and *He* of Chinese sea bass and Japanese sea bass were at a middle level.

Nei's genetic distance calculated from allele frequencies of isozyme genes is a useful measurement in estimating the degree of genetic divergence. Nei summarized that the genetic distance was distributed around 0.01 between local races, around 0.1 between subspecies[14]. In our study, the Nei's D among Chinese sea bass populations varied from 0.0004 to 0.0011 and the average was 0.0008, which is within the range of distance that is usually observed among intraspecific populations. Isozyme analysis of genetic characters indicated a nearly complete replacement of alleles at *LDH**, *GPI-1**, *GPI-2** between Japanese and Chinese sea bass. The average Nei's D between Japanese and Chinese sea bass populations

was about 0.1926, which is about 240 times of that among Chinese sea bass populations. From the above, the genetic divergence among populations of Chinese sea bass populations was at a low level. Obvious genetic divergence may indicate that barrier of gene flow existed between Tokyo and Chinese populations. Yokogawa et al. examined the Japanese populations and a Chinese population (seeds of Yellow Sea sea bass population), and the average Nei's D was 0.1740, which was similar to our results[3]. It has been reported that the Nei's D among four selected Japanese sea bass populations was less than 0.0050. The Nei's D between the Ariake and other populations of Japanese sea bass was also less than 0.0100[18]. In comparison with our results, the genetic divergence among Japanese populations was higher than that of Chinese populations.

The genetic divergence indicated that the Chinese form might be a distinct species different from *Lateolabrax japonicus*. Recently, Gao et al. compared the sequences of cytochrome b gene (the length of sequence obtained here is 410 bp) and the results showed that there was about 7% sequence difference between Chinese and Japanese sea bass[19]. Due to overfishing and the change of ecological environment, it is thought that the Chinese sea bass suffered a great loss in abundance, and also their genetic diversity of wild stock were threatened. Further study among three *Lateolabrax* species and the populations is needed.

Tab. 3 Nei's genetic distance among *Lateolabrax* populations based on 16 loci

Location	Beihai	Fujian	Zhoushan	Weihai	Tokyo
Beihai	0.0000				
Fujian	0.0004	0.0000			
Zhoushan	0.0011	0.0010	0.0000		
Weihai	0.0008	0.0009	0.0009	0.0000	
Tokyo	0.1954	0.1931	0.1947	0.1870	0.0000

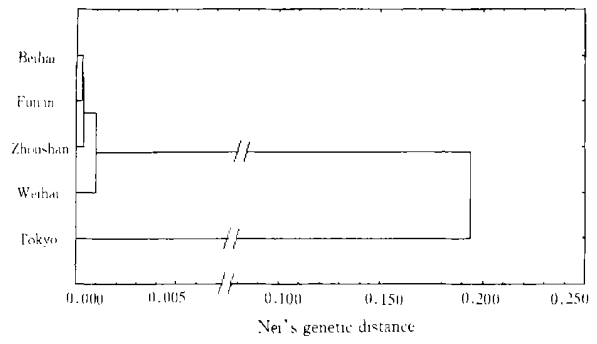

Fig. 3 Dendrogram constructed using UPGMA cluster analysis of Nei's genetic distance showing the relationship among the sea bass populations

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花鲈群体的遗传变异

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摘要: 采用水平淀粉凝胶电泳技术对花鲈群体的遗传结构进行了研究, 共检测了中国沿海花鲈 146 尾和 40 尾日本东京湾花鲈。其群体的多态位点比例为 0.2667—0.5333, 观测杂合度和预期杂合度分别为 0.0211—0.0515 和 0.0398—0.0797。中日花鲈在 *LDH**, *GPI-1**, *GPI-2** 基因位点上的等位基因接近完全置换。中国花鲈各群体之间的根井遗传距离为 0.0004—0.0011, 平均值约为 0.0080; 而中日花鲈间的根井遗传距离为 0.1870—0.1954, 平均值为 0.1926。以上结果表明中国花鲈群体间遗传变异很小, 中日花鲈间遗传变异远大于中国花鲈群体间的遗传距离。

关键词: 花鲈; 群体; 变异; 同工酶